IN VITRO EFFECTS OF AQUEOUS EXTRACT FROM *ARTOCARPUS TAKOOCHA* ROXB ON TEGUMENTAL SURFACE OF *HAPLORCHIS TAICHUI*

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Abstract. The tegumental surface of the adult intestinal fluke, *Haplorchis taichui*, after *in vitro* incubation in Tyrode's solution containing 25, 50, 75 and 100% of aqueous extract of *Artocarpus takoocha* for 1, 6, 12 and 24 hours, was monitored by Scanning Electron Microscopy (SEM). The effect on worm motility was studied in contemporizing period. The adult worms stopped their motility immediately after being incubated with 25, 50, 75 and 100% of aqueous extract of *A. takoocha*. Observed under a light microscope, most worms showed bubbling from the oral sucker, ventral sucker and excretory pore. After exposure to the solution, SEM observation showed large blebs protruding from the oral sucker, ventral sucker and excretory pore. The surface damage was composed of numerous small blebs, followed later by the disruption of tegument. The severity of damage increased due to the increasing of aqueous extract of *A. takoocha* and incubation periods.

INTRODUCTION

Artocarpus lakoocha Roxb is a large deciduous tree reaching 30 meters in height. It is commonly found in northern and northeastern Thailand. The light brown powder (Puag-Haad) is the product of aqueous extract of the wood chips of *A. lakoocha* Roxb by boiling, then slow evaporation, followed by cooling. Consequently, the powder that floats on top is separated by straining.

Puag-Haad is well known as a traditional medicine in northern Thailand. It has been commonly used for tapeworm infection. Treatment with 5 grams of Puag-Haad in 39 patients with tapeworm infection of Charoenlarp *et al* (1981) showed that 32 patients had a good response in eliminating the tapeworm. The side effects were vomiting and nausea in seven of them. Other studies have shown that 3 grams of Puag-Haad powder had a good result in taeniasis treatment and had less side-effects (Charoenlarp *et al*, 1989).

The chemical of purified Puag-Haad is 2:4:3´:5´ tetrahydroxystibene, reported by Mongolsuk *et al* (1957). Reportedly, Puag-Haad is not effective in other kinds of worms. Our research aimed to study the effect on *Haplorchis taichui* (a minute intestinal fluke), whose endemic area covers the northern part of Thailand and still constitutes a public health problem. SEM study was undertaken to provide data on surface changes during exposure to different doses of Puag-Haad.

MATERIALS AND METHODS

Gallus gallus domesticus, the experimental definitive host, was infected with metacercaria of *Haplorchis taichui* obtained from naturally infected cyprinoid fish collected from Mae Ngud Somboonchol Dam, Mae Taeng district in Chiang Mai Province. Metacercarial cysts were isolated by 1% acid pepsin solution for 2 hours at 37˚C. The digested material was passed through graded sieves and rinsed with 0.85% NaCl, then examined for metacercariae under a stereomicroscope. At the end of five days post-infection, adult flukes were collected and washed many times with 0.85% NaCl. Thirty adult worms were incubated in small Petri dishes containing 5 ml of Tyrode's solution and various concentrations (25, 50, 75 and 100%) of a aqueous extract of *Artocarpus takoocha* at 37˚C. In the control group, aqueous extract of *A. takoocha* was omitted from the incubating medium. At 1, 6, 12 and 24 hours samples were collected and fixed in 2.5% glutaraldehyde for 24 hours, at 4˚C and post fixed with 1% osmium tetroxide. They were then processed for SEM, using conventional methods and observed with the JEOL JSM-5400LV scanning electron microscope (SEM).

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RESULTS

Stereomicroscopic observation

Under the stereomicroscope, the superficial morphology of the whole body of *H. taichui* in the control group (Fig 1A) exhibited a normal shape. Meanwhile, in all treatment groups, most worms showed bubbling from all of the body parts (Fig 1B).

SEM observations

Control group. Under SEM, the tegument of parasites (after various incubation times) retained their normal structure as shown in Fig 2A-D. The whole

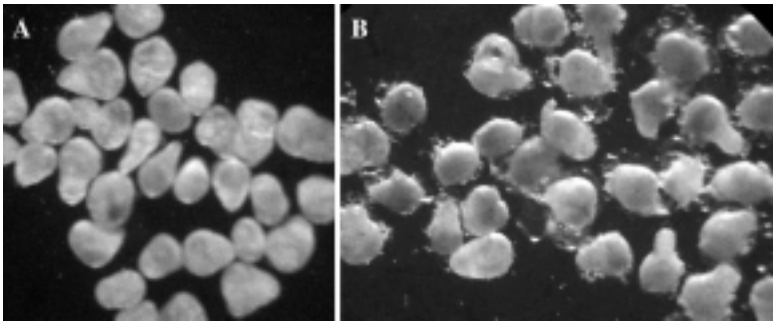

Fig 1- Stereomicroscope showing superficial morphology of the whole body of *H. taichui.* A- In control group; B- In 25 % of aqueous extract of *A. takoocha* after 1-hour incubation, the micrographs showing the bubbling from the oral sucker, ventral sucker and excretory pore.

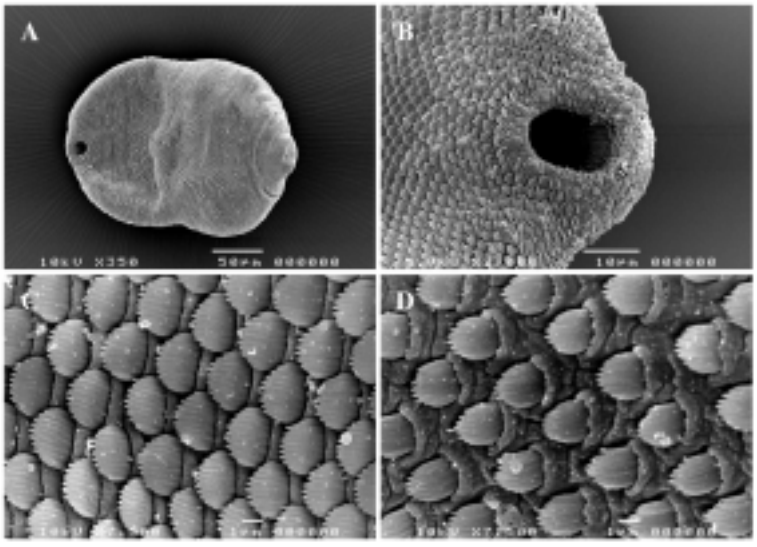

Fig 2- SEM micrographs of the tegumental surface of adult *Haplorchis taichui* incubated for 6 hours in Tyrode's solution as control group; A- ventral surface. B- oral sucker showing densely spines; C- the anterior surface covered with a large pectinate spines; D- the posterior part of the body showing the small spines and decrease in number.

body surface was covered with scale-like spines. The spines on the dorsal surface were similar to that of the ventral side in shape and size. The number of points on the spine tips decreased from the anterior to the posterior part of the body.

Treatment with aqueous extract of *Artocarpus takoocha* groups. Flukes recovered after 1-hour *in vitro* incubation in aqueous extract of *A. lakoocha* at a concentration of 25% showed tegumental surface change in all parts. A number of irregular swellings, or blebs, was present over both the ventral surface and dorsal surface, but was particularly concentrated in the anterior regions (Fig 3A). High magnification of the anterior tegumental surface (Fig 3B) displayed lesions of disruption.

Flukes recovered after 6-hour *in vitro* incubation in aqueous extract of *A. lakoocha* at a concentration of 25% featured large blebs, protruding from the oral sucker and excretory pore (Fig 3C-E). Some specimens at the ventral sucker exhibited big blebs and disruption at the end. The tegumental surface change around the oral sucker showed disruption of the tegument (Fig 3F).

Flukes recovered after 12-hour *in vitro* incubation in aqueous extract of *A. takoocha* at a concentration of

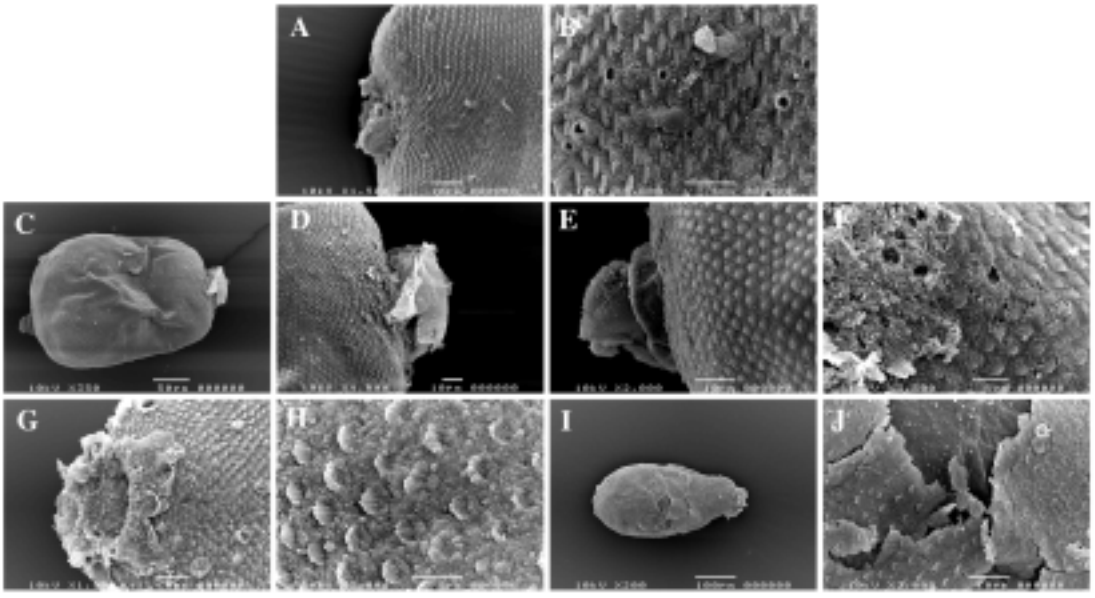

Fig 3- SEM micrographs of the tegumental surface of adult *Haplorchis taichui* incubated in Tyrode's solution containing 25 % of aqueous extract of *A. takoocha*. After 1-hour incubaton: A- a disrupt of a big bleb at the oral sucker and tegument surface; B- the swelling of tegument and hole after the disrupt of bleb at the tegumental surface. After 6-hour incubation: C- the whole body, a big bleb at the oral sucker and excretory pore; D- a big bleb at the oral sucker; E- the posterior region of the body showing a big bleb at the excretory pore; F- a lesion after the disrupt of bleb at the tegumental surface surround the oral sucker. After 12-hour incubation: G- a disrupt of a big bleb and the lesions in the tegument of oral; H- a swelling of tegument and destroyed spines; I- a whole body with lift slap tegumental surface; J- the dorsal surface showing a lesions on the mid body.

25% displayed blebbing at the oral sucker (Fig 3G). The surface had a more roughened, flaky appearance. The ultrastructure around the spines was swollen so that their tips were indistinct and projected out of the whole body (Fig 3H). The damage to other parts of the fluke was similar to that described for the 6-hour time period, except that it was more severe.

After 24-hour incubation in aqueous extract of *A. takoocha* at a concentration of 25%, there were many large, flattened blebs in the area around the oral sucker, a number of which appeared to have burst, causing lesions. Only one of the flukes displayed tegumental sloughing over a large area of the mid body (Fig 3I-J).

The condition of the flukes after incubation in aqueous extract of *A. takoocha* at a concentration of 50, 75, and 100%, with times of exposure 1, 6, 12 and 24 hours, damage to the fluke was similar to that described for incubation in aqueous extract of *A. takoocha* at a concentration 25% (1, 6, 12, and 24 hours), except that it was more severe (Figs 4A-H, 5A-F, and 6-D). More concentration and high exposure time showed that the body surface became more wrinkled than others (Figs 5A, 5C-E, and 6A-D).

DISCUSSION

The present study showed that aqueous extract of *A. takoocha* caused progressively severe tegumental surface damage in *Haplorchsi taichui*. Surface changes observed after 1, 6, 12, and 24-hours incubation in 25, 50, 75, and 100% of aqueous extract of *A. lakoocha* were similar, except that the damage was more severe, depending on concentration of solution and time of exposure. The evidence of surface changing was blebbing and disruption. Early change led to more severe damage with loss of the tegument. This would facilitate penetration of the aqueous extract of *A. takoocha* into the deeper-lying tissues of the flukes. The damage of tegument in this study was the same as the effect of the antihelminthic drug, praziquantel. Apinhasmit and Sobhon (1996) observed the effect of praziquantel on the adult tegument of *Opisthochis viverrini*. Tegumental changes were similar; that is, blebbing and distruption lesions led to sloughing. In contrast, the effect on *O. viverini* was the posterior part while our solution caused more damage to the anterior part. This finding might be associated with the various physiological properties of the tegument

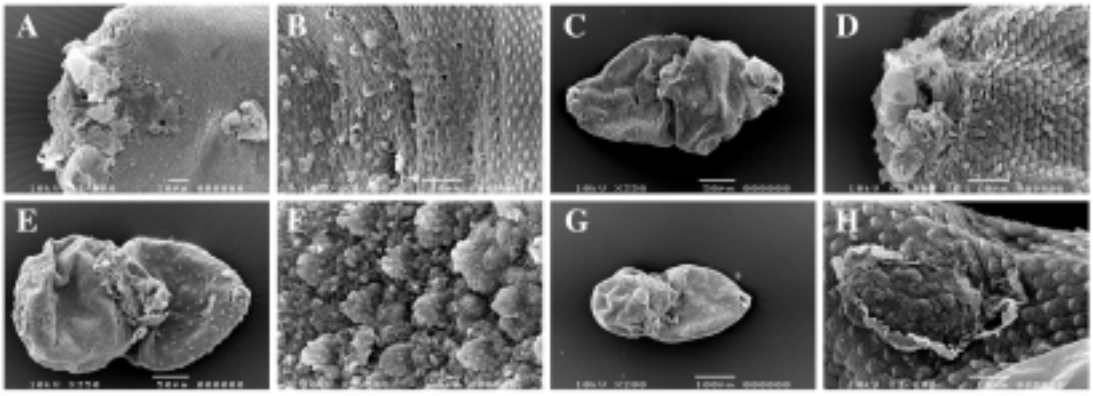

Fig 4- SEM micrographs of the tegumental surface of adult *Haplorchis taichui* incubated in Tyrode's solution containing 50 % of aqueous extract of *A. takoocha*. A and B showing lesions at the oral sucker (A) and tegumetal surface around the oral sucker (B) after incubation 1 hour; C and D showing a winkle body (C) and the lesion of disrupt of big bleb at the oral sucker (D) after 6-hours incubation; E and F the tegumental surface showing a winkle body (E) and numerous of lesion in the anterior surface, high magnification of disorder of spines (F) after 12-hours incubation; G and H showing a winkle body with a numerous of lesion of bleb (G) and the lesion on the mid body surface (H) after 24-hours incubation.

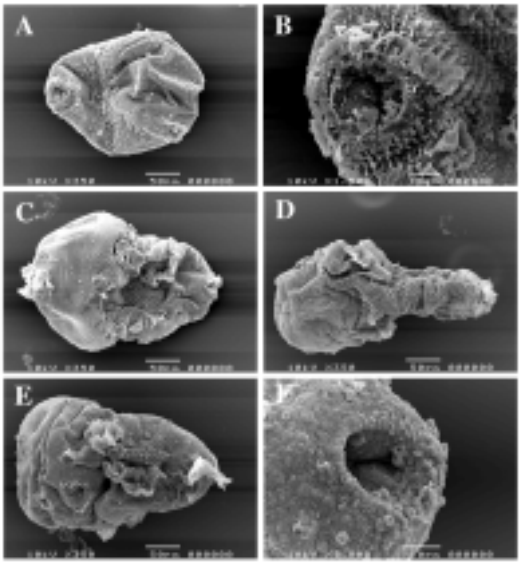

Fig 5- SEM micrographs of the tegumental surface of adult *Haplorchis taichui* incubated in Tyrode's solution containing 75% of aqueous extract of *A. takoocha* showing a winkle body (A) and the lesion of disrupt of big bleb at oral sucker (B) after 1-hour incubation; C- showing a winkle body and the lesion of bleb disruption after 6-hour incubation; D- showing a winkle body and the lesion of bleb disruption after 12-hour incubation; E-F showing a winkle body and the lesion of bleb disruption (E); the destroy of papillae and spine around the oral sucker (F) after 24-hour incubation.

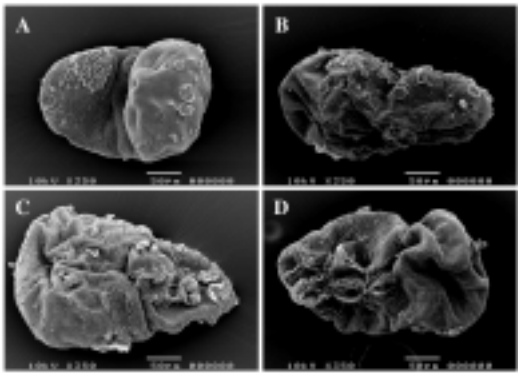

Fig 6- SEM micrographs of the tegumental surface of adult *Haplorchis taichui* incubated in Tyrode's solution containing 8 mg/ml of aqueous extract of *A. takoocha*. A-D showing a winkle body and the lesion of bleb disrubtion after 1-hour (A), 6-hour (B), 12-hour (C), and 24-hour (D) incubation.

and the chemicals in aqueous extract of *A. takoocha* Roxb. Moreover, the effect of praziquantel on *Fibricola seoulensis* gave a similar phenomenon: huge blebbing and disruption at the ventro-lateral margins of the forebody and dorsal junctional area between forebody and hindbody (Lee, 1985). This demonstration supported the different sensitive areas and patterns of the drug. To date, studies on the effects of medicinal plant extracts on the tegumental surface of flukes is not present. The result of this study proved the

antihelminthic property of this plant. Details regarding exposure time and the minimum concentration of aqueous extract of *A. takoocha* that causes damage of tegument warrant further study.

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REFERRENCES

Apinhasmit W, Sobhon P. *Opisthorchis viverrini*: effect of praziquantel on the adult tegument. *Southeast Asian J Trop Med Public Health* 1996;27:304-11.

Charoenlarp P, Radomyos P, Harinasata T. Treatment of taeniasis with Puag-Haad: A crude extract of *Artocarpus takoocha* wood. *Southeast Asian J Trop Med Public Health* 1981;12: 568-70.

Charoenlarp P, Radomyos P, Bunnag D. The optimum dose of Puag-Haad in the treatment of taeniais. *J Med Assoc Thai* 1989;72:71-3.

Lee SH. *In vitro* effects of praziquantel on *Fibticola seoulensis*. *Korean J Parasitol* 1985;26: 41-51.

Mongolsuk S, Robertson A, Tower R, 2:4: 3´:5´ Tetrahydroxystibene from *Artocarpus takoocha. J Chem Soc* 1957;2:2231-3.