Original Article

In Vitro and *In Vivo* effect of Chia oil (*Salvia hispanica* L.) on *Haplorchis taichui*

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Abstract

Haplorchis taichui is an intestinal heterophyid fluke that is pathogenic to humans. This parasite can mature in the small intestine of birds and mammals. The purpose of this study was to investigate the effect of chia seed oil (*Salvia hispanica* L.) on *H. taichui in vitro* and *in vivo*. The activity of adult *H. taichui* worms incubated in Tyrode's solution containing praziquantel (10µg/ml) or chia oil (1 or 10 mg/ml) for 1, 6, 12 and 24 hours was assessed by observation under a stereo-microscope. Syrian hamsters infected with 200 *H. taichui* metacercariae were treated with 25mg/kgBW praziquantel in a single dose or 100mg/kgBW chia oil for 3 days. The number of worms recovered from the small intestine was determined and morphology was examined by scanning electron microscopy (SEM). All the worms were inactive and contracted after 1h exposure to 10 µg/ml praziquantel. Twenty percent of the worms were inactive after exposure to 1 mg/ml of chia oil for 12 h and 100% of the worms were inactive after exposure to 10 mg/ml chia oil for 1 h. The average number of adult *H. taichui* worms recovered from the intestines of hamsters treated with chia oil was 60 ± 11.57 and SEM analysis showed time and concentration dependent blebbing, swelling and sloughing of the tegument surface. There were no worms recovered from the hamsters treated with praziquantel. The blebbing and sloughing results of chia oil on the *H. taichui* covering looked similar to those of praziquantel.

Keywords: *Haplorchis taichui*, chia oil, *Salvia hispanica* L., Heterophyiasis

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Introduction

The family Heterophyidae are intestinal flukes. One member of the family is *Haplorchis taichui* (*H. taichui*), which is endemic in Southeast and East Asia, inhabiting the small intestine of birds and mammals (Abdi *et al*., 2017; Chai *et al*., 2009; Eom *et al*., 2014; Toma *et al*., 1999). *H. taichui* can also infect humans via the oral route through the consumption of cyprinoid fish contaminated with metacercariae. Heterophyiasis can cause acute abdominal pain, mild intestinal discomfort, mucousy diarrhea and colicky pains (Tantachamrun and Kliks, 1978; Toledo *et al*., 2006). The eggs of heterophyid flukes may also be deposited in the brain, spinal cord, heart and other organs and in some patients lesions in the myocardium have led to heart failure (Africa *et al*., 1935). In the first reported heterophyid infection of a human in Thailand, *H. taichui* was found in a man in Udonthani Province in 1971 (Manning *et al*., 1971). The drug for treating heterophyiasis is praziquantel, which is administered as a single dose at 25 mg/kgBW (Chai *et al*., 2000; Waikagul *et al*., 2005). However, praziquantel has unwanted side effects such as headache, nausea, abdominal pain, vomiting, bloody stools and fever (Erko *et al*., 2012).

Recently, the use of traditional medicinal plants in the fight against parasites has been applied in many regions of the world (Abdel-Ghaffar *et al*., 2011; Aukkanimart *et al*., 2015; Rungruang and Boonmars, 2009; Wonkchalee *et al*., 2012). Chia (*Salvia hispanica* L.) is a biennial plant that belongs to the family *Lamiaceae*, native to southern Mexico and northern Guatemala (Ayerza and Coates, 2007). The chia seed has been reported as an important source of oil (30% - 40% dry weight), dietary fiber (20% - 30%), protein (15% - 30%), as well as polyphenolic compounds and minerals (Ixtaina *et al*., 2011; Ixtaina *et al*., 2008; Marineli *et al*., 2014; Reyes-Caudillo *et al*., 2008). Several studies have reported that the consumption of chia seeds has health benefits, improving biological markers related to glucose homeostasis, dyslipidemia, cardiovascular disease and inflammation (Chicco *et al*., 2009; Vuksan *et al*., 2010). From a previous study, chia oil has been reported in antifungal activity and antibacterial activity (Elshafie *et al*., 2018). Chia oil significantly decreased the mycelium growth (*Aspergillus fumigatus*, *Penicillium expansum*, *Monilinia laxa*, and *Monilinia fructigena*) and was found more antibacterial activity against gram positive bacteria than gram negative strains (Elshafie *et al*., 2018). However, antiparasitic activity has not been reported. We therefore investigated the trematocidal activity of chia oil *in vitro* and in an *in vivo* hamster model.

Materials and Methods

Preparation of H. taichui metacercariae: Cyprinoid fish, collected from an area in the northeastern past of Thailand endemic for *H. taichui*, were blended thoroughly with 0.25% pepsin in 0.85% sodium chorlide solution and 0.15% hydrochloric acid in a ratio of 1:3 by volume, incubated in a shaking water bath at 37 °C for 1 h, and then filtered through a sieve using 0.85% NaCl as a diluent. *H. taichui* metacercariae were selected from the residue under a stereo-microscope.

Preparation of chia oil: Chia seeds were obtained from the Faculty of Agriculture, Khon Kaen University. Chia seeds were ground into powder and extracted with ethanol for 7 days at room temperature. After extraction, samples were filtered and filtrates were evaporated at 50°C with a rotary evaporator (Rotavapor R-200/205, BUCHI, Flawil, Switzerland). Chia oil was collected following centrifugation at 3,000 rpm for 10 minutes and kept at -20°C until use.

In vitro treatment: To prepare the adult *H. taichui* worms, Syrian hamsters were infected with 200 *H. taichui* metacercariae. After seven days the hamsters were anesthetized with diethyl ether and the small intestines were collected. Adult *H. taichui* worms were collected from the small intestines, washed in Tyrode's solution, and separated into 5 groups (60 worms/group): i) normal control (Tyrode's soluton), ii) diluent control (distilled water), iii) positive control (praziquantel 10µg/ml), iv) chia oil 1mg/ml, and v) chia oil 10mg/ml. Active worms (3 replicates of 5 worms) were placed in 2 ml of test solution in 24-well plates and incubated at 37°C for 1, 6, 12 and 24 h. The worms were then washed 3 times in phosphate buffered saline before observation under a stereo-microscope where any movement within 15 sec was recorded as "active". For scanning electron microscopy (SEM), worms were fixed in 2.5% (w/v) glutaraldehyde at 4°C.

In vivo treatment: Syrian hamsters were infected with 200 *H. taichui* metacercariae (Songsri *et al*., 2018) and divided into 3 groups (5 hamsters/group): i) control (no treatment), ii) positive control (praziquantel 25mg/kgBW in a single dose), iii) chia oil treatment (chia oil 100mg/kgBW 3 days). The hamsters were maintained with unlimited food and water in the animal unit at the Faculty of Medicine, Khon Kaen University. After treatment, the hamsters were weighed and anesthetized and adult worms were collected from the small intestine and fixed in 2.5% (w/v) glutaraldehyde at 4°C. The small intestines were sectioned and stained with hematoxylin and eosin (H&E). The experimental protocols were approved by the Animal Ethics Committee of Khon Kaen University (ACUC-KKU-16/2560).

Scanning electron microscopy (SEM): After fixation, worms were washed with distilled water 3 times for 15 mins/time and stained with 1% osmium for 90 mins. The worms were washed again with distilled water 3 times for 15 min/time and dehydrated with increasing concentrations of acetone (20%, 40%, 60%, 80% and 100% acetone 3 times for 15 min/time). After dehydration, the worms were dried with a Leica CPD300 critical point dryer, mounted on a stub, coated with gold and photographed with a scanning electron microscope (Carl Zeiss, AURIGA®).

Statistical analysis: The data are expressed as mean ± standard deviation (SD). Statistical analysis was by one-way analysis of variance (ANOVA) using SPSS statistical software, version 16.0. The statistical significance between groups was determined at the threshold of $P<0.05$.

Results

In vitro treatment: Sixty active *H. taichui* worms were treated in each group. After one hour exposure to 10 µg/ml praziquantel, all worms were inactive and contracted. Chia oil reduced *H. taichui* activity in a time and concentration dependent manner. Exposure of the worms to 1 mg/ml of chia oil for 12 h resulted in 20% of worms becoming inactive and all worms were inactive after exposure for 24 h. Exposure to 10 mg/ml chia oil for 1 h rendered all the worms inactive . Inactive worms in the chia oil treatment groups appeared bloated, not contracted as they appeared in the praziquantel treatment group (Fig 1). In the control groups (Tyrode's solution and diluent) all worms remained active at 24 h (Table 1).

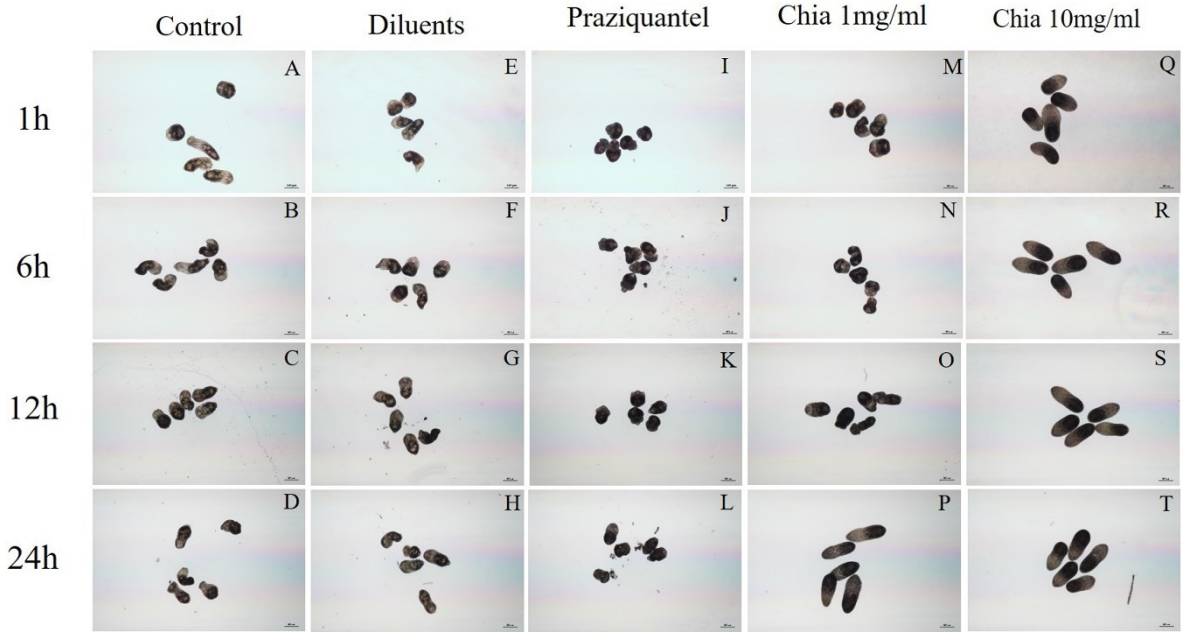

Figure 1 *H. taichui* adult worms after incubation with solutions for 1 h (first row), 6 h (second row), 12 h (third row), and 24 h (fourth row). A-D: Control (Tyrode's solution), E-H: Diluent control (distilled water), I-L: Praziquantel (10µg/ml), M-P: Chia oil 1 mg/ml, Q-T: Chia oil 10 mg/ml. (magnification 40x)

Table 1 Percentage of inactive worms treated with praziquantel and chia oil concentrations 1 and 10 mg/ml at different times post-incubation.

Groups	Percentage of inactive worms (%) after incubation (±SD)			
	1h	6h	12h	24h
Control	0±0	0±0	0±0	0±0
Diluent control	0±0	0±0	0±0	0±0
Praziquantel 10µg/ml	100±0	100±0	100±0	100±0
Chia oil 1mg/ml	0±0	0±0	20±17.89	100±0
Chia oil 10mg/ml	100±0	100±0	100±0	100±0

* inactive (>10 sec no movement).

Scanning electron microscopy examination of morphology: Exposure to chia oil damaged the *H. taichui* tegumental surface and the degree of damage increased with the concentration and exposure time. The morphology of the tegumental surface of *H. taichui* exposed to Tyrode's solution or diluent control appeared normal and was covered with numerous lamina-like spines and sensory papillae. The oral (OS) and ventral (VS) suckers appeared smooth and intact (Fig 2).

***Treatment with 10µg/ml praziquantel*:** After exposure to 10µg/ml praziquantel for 1 h, all the worms were inactive and contracted. The SEM images show extensive blebbing of the tegument (Fig 3 A-D) and the formation of lesions where the tegument was sloughing off the surface (Fig 3C). In addition, the areas around the oral and ventral suckers were swollen (Fig 3A and C).

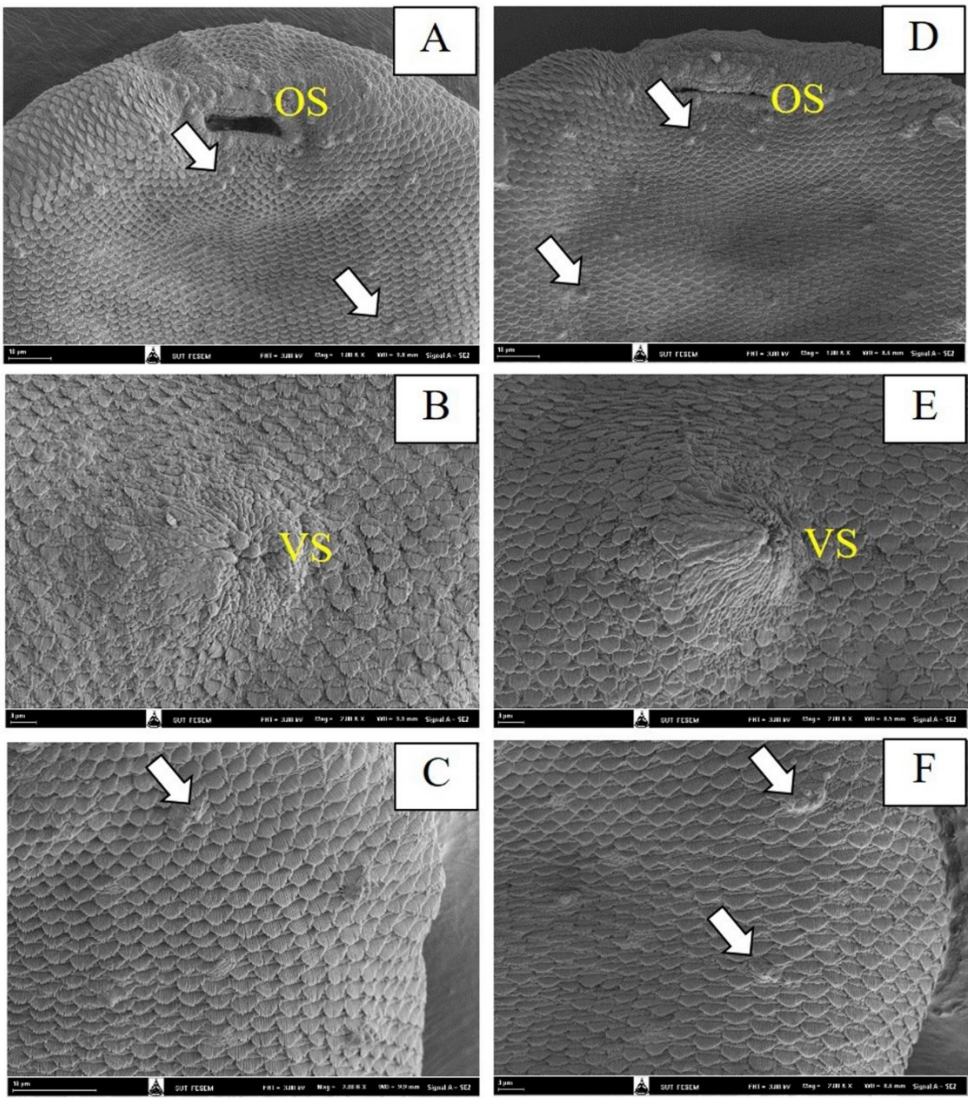

Figure 2 Tegumental surface of adult *Haplorchis taichui* after incubation with Tyrode's solution (A-C) or diluent (D-F).OS; oral sucker, VS; ventral sucker. Arrows indicate sensory papillae.

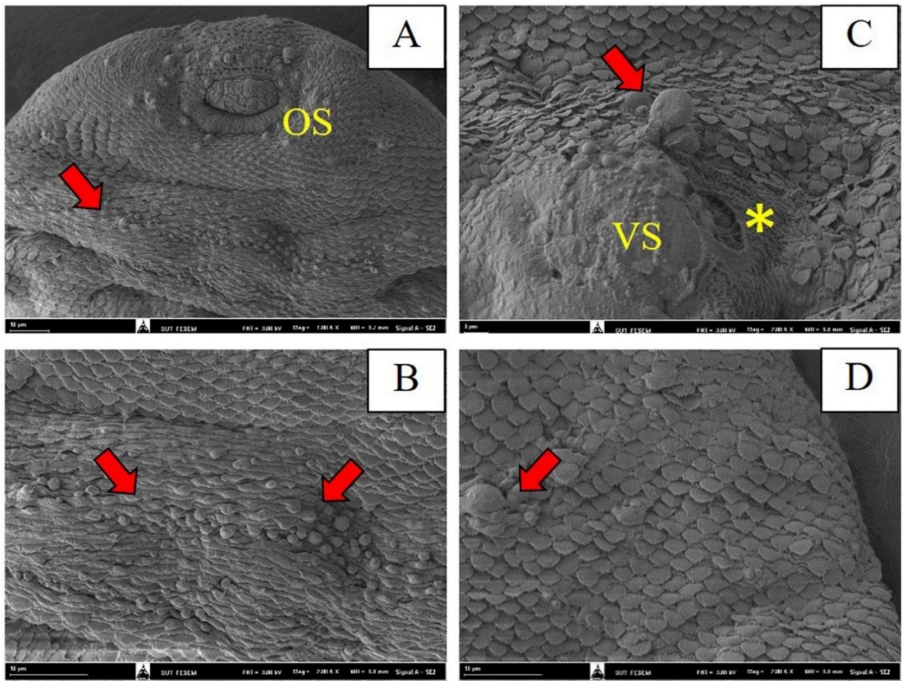

Figure 3 Tegumental surface of adult *H. taichui* after 1 h incubation with 10µg/ml praziquantel. OS; oral sucker, VS; ventral sucker. Arrows indicate blebbing, a star indicates sloughing.

Treatment with 1 mg/ml chia oil: The tegumental surfaces of active worms examined by SEM after treatment with 1 mg/ml chia oil for 1 h appeared normal, with spines and sensory papillae visible (Fig 4A). After longer exposures (12 h), the tegument surface appeared uneven, with some swollen and some sunken areas (Fig 4C and D). The tegument surfaces of inactive worms collected after exposure to 1 mg/ml chia oil for 24 h appeared swollen with extensive blebbing and and sloughing of the tegument from the surface (Fig 4E and F)

Treatment with 10 mg/ml chia oil: After 1 h, the tegument examined by SEM after treatment with 10 mg/ml chia oil, appeared swollen and to be blebbing in areas of the spines (Fig 5A and B). In worms after 24 h, the oral and ventral sucker and sensory papillae surfaces showed blebbing and the tegument surface appeared swollen and to be sloughing (Fig 5).

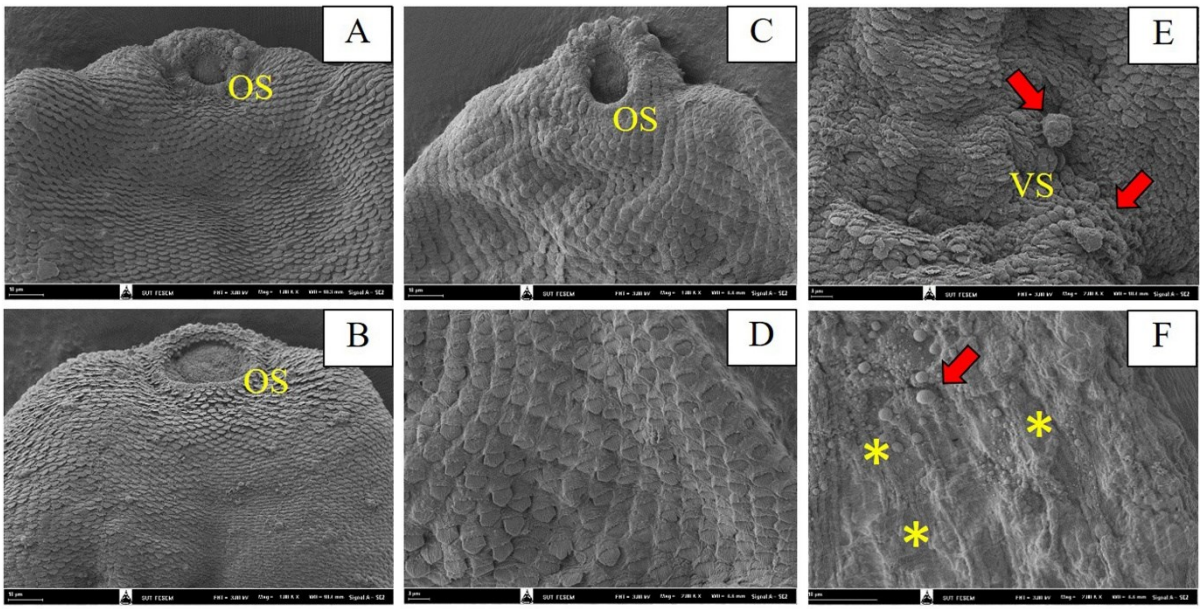

Figure 4 Tegumental surface of adult *H. taichui* after incubation with 1 mg/ml chia oil for 1h (A), 6 h (B), 12 h (C and D) and 24 h (E and F). OS; oral sucker, VS; ventral sucker. Arrows indicate blebbing, stars indicate sloughing.

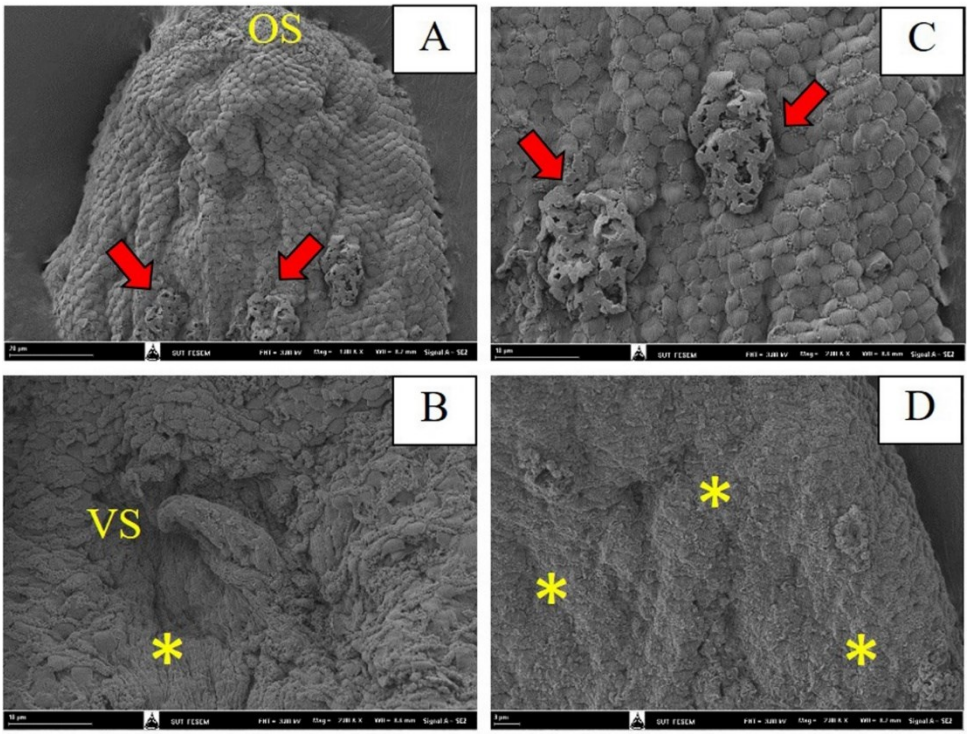

Figure 5 Tegumental surface of adult *Haplorchis taichui* after incubation with 10 mg/ml chia oil. OS; oral sucker, VS; ventral sucker, Arrowed; sensory papillae blebbing, Star; sloughing.

In vivo treatment: This experiment is designed for studying the effect of chia oil against *H. taichui* infecting Syrian hamsters. End points were differences in the numbers of worms recovered from the host and pathological changes to the small intestine. To determine the effect of chia oil on the viability of *H. taichui*, the number of active adult worms that were collected from each hamster was counted. The number of worms recovered from infected hamsters treated with chia oil was lower than that from the untreated group ($p < 0.05$). There were no worms recovered from any of the infected hamsters in the praziquantel treatment group (Fig 6).

Scanning electron microscope examination of morphology: The scanning electron micrographs of the worms recovered from the untreated control showed no changes in morphology (Fig 7A). The oral and ventral suckers were smooth and not swollen (Fig 7B and C) and the tegumental infoldings were intact with visible spines and sensory papillae (Fig 7D). Worms recoved from infected hamsters treated with chia oil showed gross morpholoigical changes (Fig 7E) with blebbing around both the oral and ventral suckers (Fig 7F and G) and profound erosion of the tegument that exposed the spines (Fig 7H).

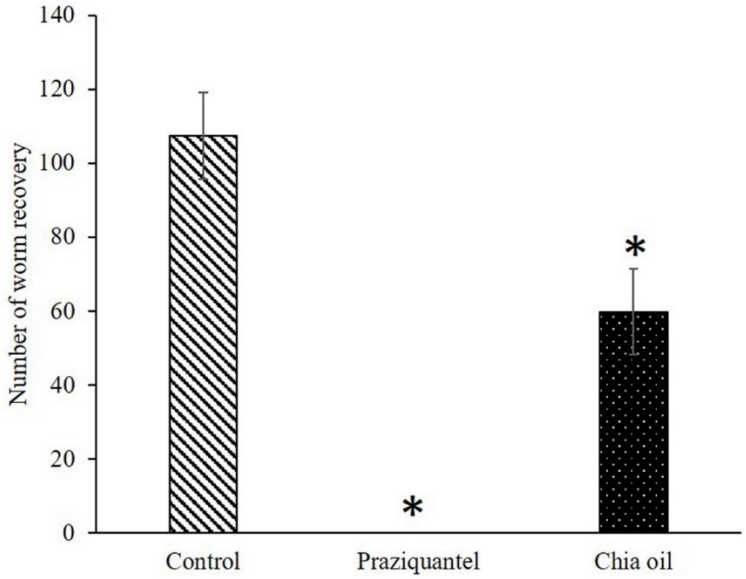

Figure 6 Number of active worms recovered from *H. taichui* infected Syrian hamsters. Control (no treatment), Praziquantel (25mg/kgBW in a single dose), and Chia oil (100mg/kgBW 3 days). *indicates $p < 0.05$.

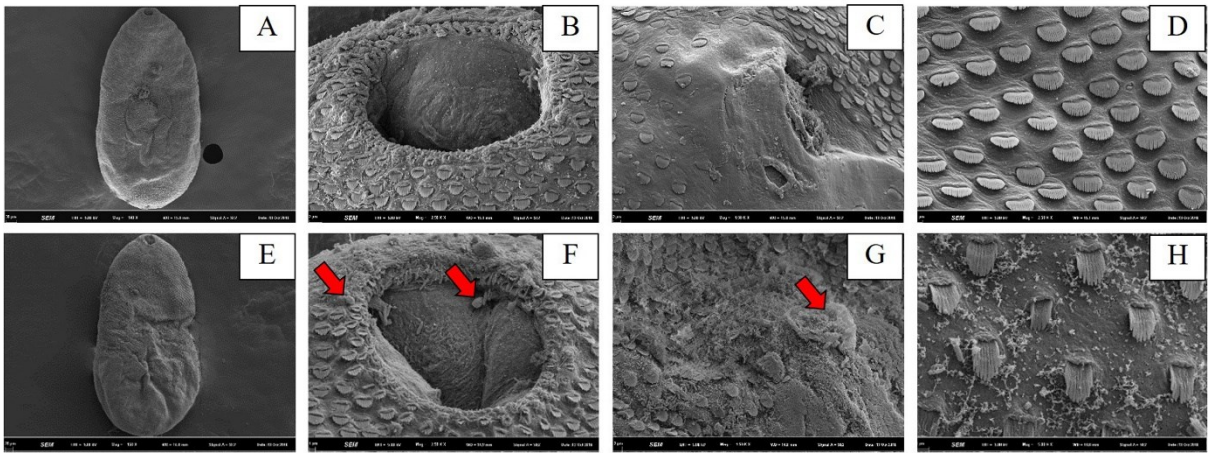

Figure 7 Tegument surface of adult *H. taichui* recovered from infected Syrian hamsters: A-D untreated control group; E-H treatment with chia oil 100mg/kgBW 3 days. Arrows indicate blebbing.

Discussion

The present study showed that oil extract from chia seed (*Salvia hispanica* L.) caused tegumental surface damage to *H. taichui*. Tegumental changes started with surface swelling and blebbing and then the disruption of the blebs led to the formation of lesions and the tegument beginning to slough off the surface. These effects were dependent on exposure time and concentration. The effect of *Citrullus colocynthis* against *Orthocoelium scoliocoelium* (Trematoda: Digenea) was revealed in a wide-scale deformity in the tegumental architecture with breakage and detachment (Swarnakar and Kumawat, 2014). The blebbing and sloughing effects of chia oil on the *H. taichui* tegument resembled those of praziquantel, the standard antihelminthic drug. The aqueous extracts of *Artocarpus takoocha* weres found in the anti-helminthic

activity against *Haplorchis taichui* by induced numerous small blebs and disruption of the tegument surface (Wongsawad *et al*., 2005). Similarly, after 24 h incubation with *Nigella sativa* oil 1 mg/ml, the *Fasciola gigantica* tegumental surface became swollen and the spines appeared sunken (Shalaby *et al*., 2012). Praziquantel exerts its anti-helminthic activity by increasing calcium influx, which causes the muscles of parasites to contract (Martin *et al*., 1997). In the present study, chia oil appeared to have the opposite effect, causing the body of the worm to appear stretched, instead of contracted. It could be that chia oil acts by activating chloride channels causing muscle cells to hyperpolarize, which relaxes the muscles and worms with abnormal tegumental surface are more easily excreated from hosts (Martin, 1985). *H. taichui* develop from metacercariae to adult stage within 7 days (Sukontason *et al*., 2001). Therefore, this study chose to treat after 7 days infection to determine the effect of chia oil on adult worms. We can conclude that chia oil has activity against adult *H. taichui* worms, which is elicited through damage to the tegument surface. Based on the current research findings, chia oil can be considered a promising natural alternative that could be potentially used in controlling some intestinal parasite. However, a combined treatment of chia seed oil and praziquantel will be more effective.

Conflict of interest: The authors declare that they have no conflict of interests.

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References

Abdel-Ghaffar F, Semmler M, Al-Rasheid KA, Strassen B, Fischer K, Aksu G, Klimpel S, Mehlhorn H. 2011. The effects of different plant extracts on intestinal cestodes and on trematodes. Parasitol Res. 108(4):979-984.

Abdi M, Nibret E, Munshea A. 2017. Prevalence of intestinal helminthic infections and malnutrition among schoolchildren of the Zegie Peninsula, northwestern Ethiopia. J Infect Public Health. 10(1):84-92.

Aukkanimart R, Boonmars T, Sriraj P, Songsri J, Laummaunwai P, Waraasawapati S, Boonyarat C, Rattanasuwan P, Boonjaraspinyo S. 2015. Anthelmintic, anti-inflammatory and antioxidant effects of *Garcinia mangostana* extract in hamster opisthorchiasis. Exp. Parasitol. 154:5-13.

Chai JY, Han ET, Shin EH, Sohn WM, Yong TS, Eom KS, Min DY, Um JY, Park MS, Hoang EH, Phommasack B, Insisiengmay B, Lee SH, Rim HJ. 2009. High prevalence of *Haplorchis taichui*, *Phaneropsolus molenkampi*, and other helminth infections among people in Khammouane province, Lao PDR. Korean J Parasitol. 47(3):243-247.

Chicco AG, D'Alessandro ME, Hein GJ, Oliva ME, Lombardo YB. 2009. Dietary chia seed (*Salvia hispanica* L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. Brit J Nutr. 101(1):41-50.

Elshafie HS, Aliberti L, Amato M, De Feo V, Camele I. 2018. Chemical composition and antimicrobial activity of chia (*Salvia hispanica* L.) essential oil. Eur Food Res Technol. 244(9): 1675-1682.

Eom KS, Yong TS, Sohn WM, Chai JY, Min DY, Rim HJ, Jeon HK, Banouvong V, Insisiengmay B, Phommasack B. 2014. Prevalence of helminthic infections among inhabitants of Lao PDR. Korean J Parasitol. 52(1):51-56.

Ixtaina VY, Martínez ML, Spotorno V, Mateo CM, Maestri DM, Diehl BW, Nolasco SM, Tomás MC. 2011. Characterization of chia seed oils obtained by pressing and solvent extraction. J. Food Compos. Anal. 24(2):166-174.

Ixtaina VY, Nolasco SM, Tomas MC. 2008. Physical properties of chia (*Salvia hispanica* L.) seeds. IND CROP PROD. 28(3):286-293.

Marineli RD, Moraes EA, Lenquiste SA, Godoy AT, Eberlin MN, Marostica MR. 2014. Chemical characterization and antioxidant potential of Chilean chia seeds and oil (*Salvia hispanica* L.). Lwt-Food Sci Technol. 59(2):1304-1310.

Martin R. 1985. γ-Aminobutyric acid-and piperazine-activated single-channel currents from *Ascaris suum* body muscle. Br J Pharmacol. 84(2):445-461.

Martin R, Robertson A, Bjorn H. 1997. Target sites of anthelmintics. Parasitology. 114(7):111-124.

Reyes-Caudillo E, Tecante A, Valdivia-Lopez MA. 2008. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. Food Chem 107(2):656-663.

Rungruang T, Boonmars T. 2009. *In vivo* antiparasitic activity of the Thai traditional medicine plant--*Tinospora crispa*--against *Plasmodium yoelii*. Southeast Asian J Trop Med Public Health. 40(5):898-900.

Swarnakar G, Kumawat A. 2014. *In vitro* anthelmintic effect of *Citrullus colocynthis* on tegument of amphistomes *Orthocoelium scoliocoelium* (Trematoda: Digenea). Int J Curr Microbiol App Sci. 3(6): 571-582.

Shalaby H, Abu El Ezz N, Farag T, Abou-Zeina H. 2012. In vitro efficacy of a combination of ivermectin and Nigella sativa oil against helminth parasites. Global Veterinaria 9(4):465-473.

Songsri J, Boonmars T, Boonjaraspinyo S, Aukkanimart R, Sriraj P, Boueroy P, Wongchalee N. 2018. Syrian hamster (*Mesocricetus auratus*) as an animal model for *Haplorchis taichui* infection: biology, morphology, and pathology. THAI J VET MED. 48(3), 479-486.

Sukontason K, Sukontason KL, Boonsriwong N, Chaithong U, Piangjai S, Choochote W. 2001. Development of *Haplorchis taichui* (Trematoda: Heterophyidae) in *Mus musculus* mice. Southeast Asian J Trop Med Public Health. 32:43-47.

Toma A, Miyagi I, Kamimura K, Tokuyama Y, Hasegawa H, Selomo M, Dahlan D, Majid I,

Hasanuddi I, Ngatimin R, Mogi M, Kuwabara N. 1999. Questionnaire survey and prevalence of intestinal helminthic infections in Barru, Sulawesi, Indonesia. Southeast Asian J Trop Med Public Health. 30(1):68-77.

Vuksan V, Jenkins AL, Dias AG, Lee AS, Jovanovski E, Rogovik AL, Hanna A. 2010. Reduction in postprandial glucose excursion and prolongation of satiety: possible explanation of the long-term effects of whole grain Salba (*Salvia Hispanica* L.). Eur J Clin Nutr. 64(4):436-438.

Wongsawad C, Wongsawad P, Luangphai P, Kumchoo K. 2005. In vitro effects of aqueous extract from *Artocarpus takoocha* Roxb on tegumental surface of *Haplorchis taichui*. Southeast Asian J Trop Med Public Health. 36 Suppl 4:112-116.

Wonkchalee O, Boonmars T, Aromdee C, Laummaunwai P, Khunkitti W, Vaeteewoottacharn K, Sriraj P, Aukkanimart R, Loilome W, Chamgramol Y, Pairojkul C, Wu Z, Juasook A, Sudsarn P. 2012. Anti-inflammatory, antioxidant and hepatoprotective effects of *Thunbergia laurifolia* Linn. on experimental opisthorchiasis. Parasitol Res. 111(1):353-359.