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Effects of AIBL on *Oncomelania hupensis*, the intermediate snail host of *Schistosoma japonicum*: An enzyme histochemical study

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ABSTRACT

Objective: To explore the effect of AIBL on *Oncomelania hupensis*, the intermediate snail host of *Schistosoma japonicum*. **Methods:** The enzyme histochemical profiles of cholinesterase, cytochrome oxidase, lactate dehydrogenase, nitric oxide synthase, and succinate dehydrogenase in the soft tissues of *Oncomelania hupensis*, the intermediate host snail of *Schistosoma japonicum*, were analyzed before and after treatment with the active ingredient of *Buddleia lindleyana* (AIBL), a potent and safe plant molluscicide. **Results:** Treatment with AIBL induced a notable decrease in the activities of the five enzymes ($P<0.01$). **Conclusions:** The results indicate that AIBL impairs the activities of the enzymes, thereby influencing the transfer of neurotransmitter and energy supply in *Oncomelania hupensis* and ultimately harming their various physiological functions, which are considered to cause death of the species.

1. Introduction

Schistosomiasis is a major tropical disease worldwide. Attacking and breaking down the transmission cycle of *Schistosomiasis japonica* are important strategies to control schistosomiasis[1-3]. Niclosamide is the only effective chemical molluscicide for the rapid control of snails[4]. Unfortunately, this compound is expensive and has side effects on non-target organisms[5]. Therefore, the molluscicidal properties of numerous plant extracts have been studied[6-10]. *Buddleia lindleyana* (*B. lindleyana*) is a potent and safe botanical molluscicide. The n-butanol fraction of its leaf has been reported to exhibit significant activity against *Oncomelania hupensis* (*O. hupensis*) with an LC_{90} value of 40.47 mg/L (72 h). Research has also shown that the active ingredient of *B. lindleyana* (AIBL) has a concentration of 86.24% acacetin-7-rutinoside. However, the biological mechanism of action of AIBL remains to be investigated. Therefore, studying the activities of enzymes in the soft tissues of healthy snails and the changes that could occur resulting from AIBL is necessary. For this reason, we subjected five important enzymes, namely, succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), cytochrome oxidase (CCO), cholinesterase (CHE), and nitric oxide synthase (NOS), to enzyme histochemical analysis.

2. Materials and methods

2.1. *O. hupensis*

O. hupensis snails relatively uniform in size (8-10 mm) were collected from the beach of Yangtze River near Zhenjiang in Jiangsu Province, China, and acclimatized in the laboratory at room temperature [(25±1)℃] for 24 h. Next, 100 active adult snails with seven to eight spirals were randomly divided into two groups for subsequent experiments. The snails were placed on a mud disc (22 cm ×30 cm) and then moved to a biochemical incubator at a temperature of (25±1) ℃. Snails in the experimental group

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were soaked in AIBL at a dose of 4.64 mg/L. Twenty-four hours later, their shells were washed with dechlorinated water to remove any residual AIBL. Snails in the control group were soaked in distilled water, after which they were cracked open[11]; their shells were then removed to manipulate the soft tissues under a microscope. Undamaged soft tissues were selected for the following experiments. For CCO, LDH, SDH, and NOS, the fresh soft tissues were frozen at a temperature of −25 ℃. After freezing for 30 min, 15-μm cryostat sections were cut using a freezing microtome (Leica CM1850, Germany) and placed on 24 mm×24 mm coverslips. For CHE, the tissues were fixed in calcium formaldehyde (4 g of calcium chloride added to 100 mL of 4% formaldehyde solution), cooled to 4 ℃ for 24 h, washed with distilled water, and then cut into sections.

2.2. Enzyme histochemical techniques

The activity of CHE was determined using the "direct-coloring" thiocholine method[12], the activities of CCO and LDH were determined as described by Zhong[13], the activity of NOS was determined according to the method of Ben and Li[14], and the activity of SDH was determined following the method of Nachlas *et al*[15]. After rinsing with double distilled water, air drying, dehydrating in a series of ethanol, and dimethylbenzene hyalinization, sections were enveloped with neutral balata for microscopic examination. The coloration of the tissues from the sections was observed under a microscope. The gray density values of the tissues were determined using Image Processing FR-988 (Smart Scape 2002, China).

3. Results

3.1. CHE

The coloration of CHE, which was located in the pellicle and ganglia of the snails, was higher in the control group than in the experimental group (Figure 1). The mean gray density values between these groups significantly differed (P<0.01) (Table 1).

3.2. CCO

CCO was located in the buccal mass and liver of the snails. Its coloration was higher in the control group than in the experimental group (Figure 2), and significant differences in the mean gray density values between the groups were observed (P<0.01) (Table 1).

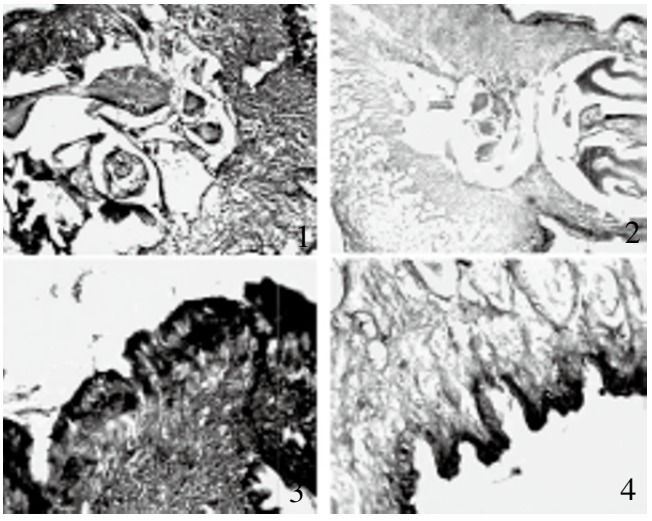

Figure 1. CHE.
(1) in the ganglia of *O. hupensis* snails from the control group; (2) in the ganglia of *O. hupensis* snails from the experimental group; (3) in the pellicle of *O. hupensis* snails from the control group; (4) in the pellicle of *O. hupensis* snails from the experimental group.

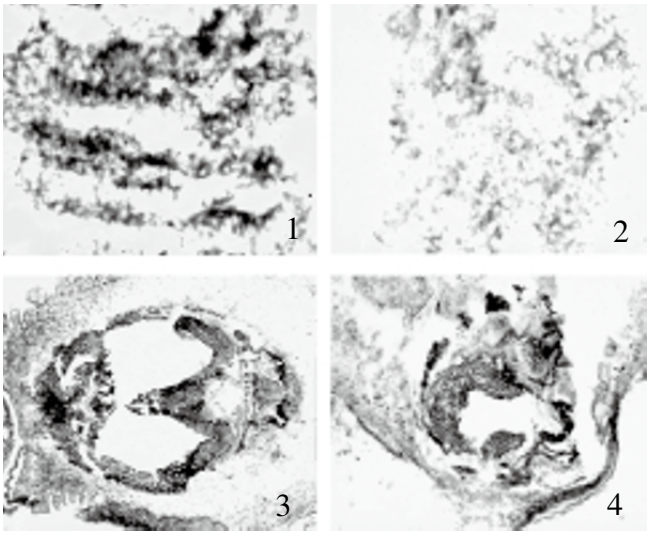

Figure 2. CCO.
(1) in the liver of *O. hupensis* snails from the control group; (2) in the liver of *O. hupensis* snails from the experimental group; (3) in the buccal mass of *O. hupensis* snails from the control group; (4) in the buccal mass of *O. hupensis* snails in the experimental group.

Table 1
Mean gray density values of staining tissues of *O. hupensis* in experimental and control groups (mean ± SD).

Groups	CCO		LDH		SDH		NOS		CHE	
	Buccal mass	Liver	Muscular fiber	Buccal mass	Muscular fiber	Buccal mass	Muscular fiber	Pharyngeal canal	Pellicle	Ganglia
Control group	0.21±0.06	0.25±0.05	0.28±0.07	0.32±0.06	0.09±0.04	0.22±0.05	0.47±0.06	0.08±0.03	0.02±0.13	0.20±0.11
Experimental group	0.40±0.13*	0.53±0.05*	0.57±0.08*	0.60±0.10*	0.49±0.08*	0.48±0.11*	0.58±0.07*	0.33±0.09*	0.06±0.04*	0.36±0.07*

* P<0.01 *vs*. control.

3.3. LDH

LDH was located in the buccal mass and muscular fibers of the foot muscle of the snails. The coloration in these areas was higher in the control group than in the experimental group (Figure 3). The differences in mean gray density values between the groups were significant ($P<0.01$) (Table 1).

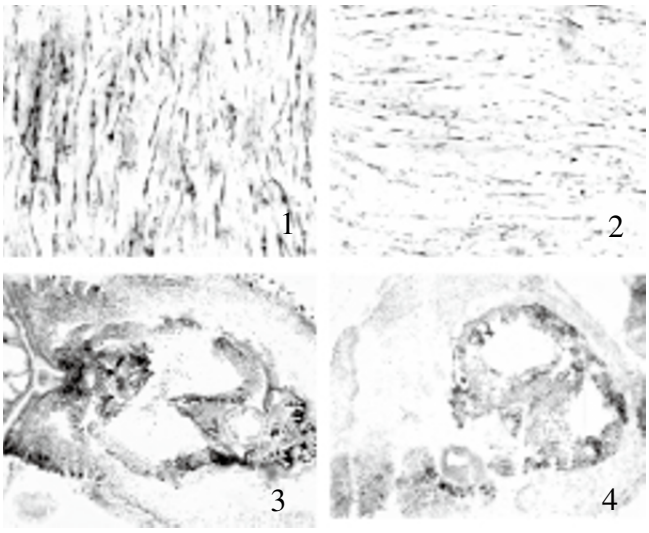

Figure 3. LDH.
(1) in the muscular fiber of *O. hupensis* snails from the control group; (2) in the muscular fiber of *O. hupensis* snails in the experimental group; (3) in the buccal mass of *O. hupensis* snails from the control group; (4) in the buccal mass of *O. hupensis* snails in the experimental group.

3.4. NOS

The activity of NOS was high in the muscular fiber and pharyngeal canal of the snails in the control group, with the coloration in which being much stronger than that in the experimental group ($P<0.01$) (Figure 4, Table 1).

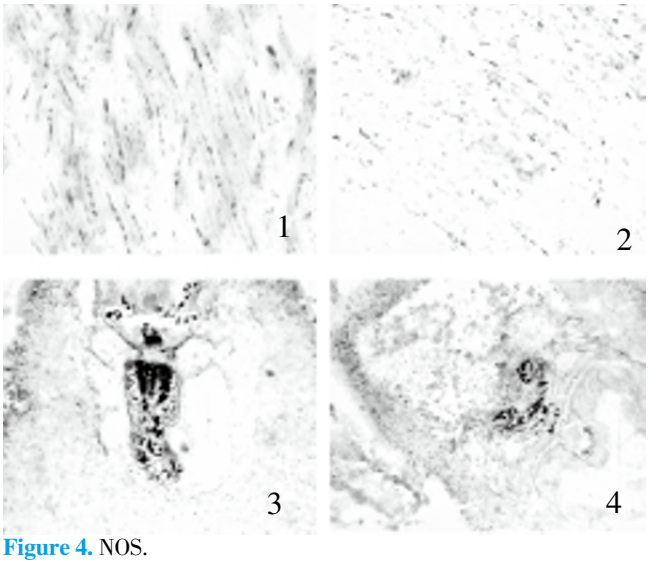

Figure 4. NOS.
(1) in the muscular fiber of *O. hupensis* snails from the control group; (2) in the muscular fiber of *O. hupensis* snails in the experimental group; (3) in the pharyngeal canal of *O. hupensis* snails from the control group; (4) in the pharyngeal canal of *O. hupensis* snails in the experimental group.

3.5. SDH

The activity of SDH was strong in the muscular fiber and buccal mass of the snails in the control group. The coloration in the said group was much higher than that in the experimental group ($P<0.01$) (Figure 5, Table 1).

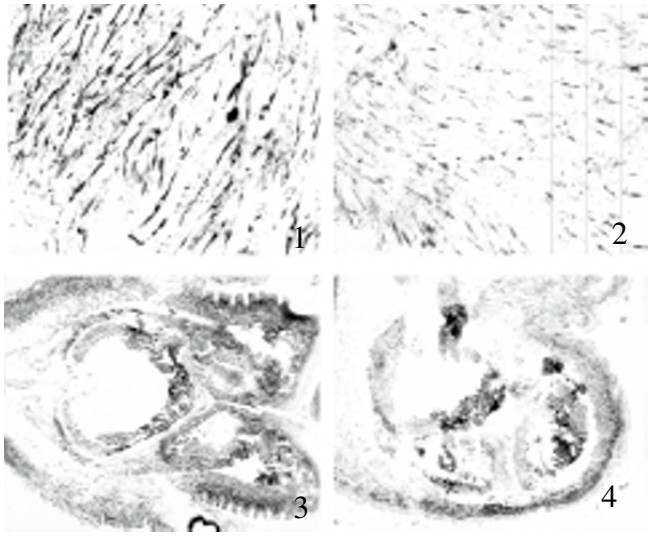

Figure 5. SDH.
(1) in the muscular fiber of *O. hupensis* snails from the control group; (2) in the muscular fiber of *O. hupensis* snails fron the experimental group; (3) in the buccal mass of *O. hupensis* snails fron the control group; (4) in the buccal mass of *O. hupensis* snails fron the experimental group.

4. Discussion

Inhibition of the activity of CHE results in the accumulation of diacetylcholine, which is a neurotransmitter; leads to functional incapacitation of the voix pedis muscle, which is the foundation of movement, respiration, and palmus; and accelerates the death of snails[16-18]. This study showed that the active principle significantly lowered the activity of AChE as well as hindered the transfer of neurotransmitters, muscular paralysis, and loss of tension and coherence in the locomotor activity of the snails.

CCO, LDH, and SDH are widely distributed in a snail's body, where aerobic metabolism and the tricarboxylic acid cycle exist, as confirmed by the ultrastructure of its mitochondria. CCO, a kind of marker enzyme, is located in the mitochondrial inner membrane and associated with cellular aerobic metabolism[16,18]. LDH is the representative enzyme existing in the cells of all organisms and correlated with anaerobic glycolysis[16,18]. SDH is an important dehydrogenase located in crista mitochondriales that is closely associated with the respiratory chain; it catalyzes the reaction between succinic acid and fumaric acid[18-25]. The active principle significantly weakened the activities of CCO, LDH, and SDH; influenced energy metabolism and energy provision; inhibited aerobic oxidation and anaerobic glycolysis; accumulated poisonous metabolites; and damaged cellular function and the framework of the tissues. NOS is widely distributed in the central nervous system,

heart wall, branchial ducts, and canalis pharyngeus of snails[26, 27]. NO, a kind of messenger molecule and effector molecule in biosystems, generally participates in physio accommodation and patho accommodation. NOS is responsible for the synthesis of NO, and NO has been confirmed to directly participate in nerve-muscular movement, reproduction, and cardiovascular regulation. The active principle significantly lowered the activity of NOS and reduced information transfer capabilities among nerves, muscular locomotor activity, peripheral nervous activity, and glandular organic externalization dominated by nerves, which influenced the metabolism, physiological functions, and biochemical functions of the snails. All the above-described changes were correlated with their death.

The results of enzyme histochemistry showed that the active principle decreased the activities of CHE, CCO, LDH, NOS, and SDH; affected the neurotransmitter and energy supply; led to physiological function disorder or loss; and ultimately caused the death of the snails.

Conflict of interest statements

We declare that we have no conflict of interest.

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