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Molluscicidal Activity of *Nerium indicum* Mill, *Pterocarya stenoptera* DC, and *Rumex japonicum* Houtt on *Oncomelania hupensis*[1]

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Objective To evaluate the molluscicidal activities of three Chinese plants *N. indicum* Mill, *P. stenoptera* DC, and *R. japonicum* Houtt, and to clarify the molluscicidal mechanism. **Methods** N-butanol extracts and water extracts of the three plants were obtained. The reactions of EST isozyme, glycogen and total protein of snails to the plant extracts were studied. **Results** EST electrophoresis showed that EST was an important antidotal enzyme system and reacted strongly to environment. EST changed greatly during the whole exposure period so that it could be viewed as a pathological index of toxicity. Extracts decreased the glycogen content of the snails' soft tissues greatly, and also the protein content. **Conclusion** All extracts show strong molluscicidal activity. The LD_{50} value of the water extract of *N. indicum* Mill is as low as 13.2 mg/L. EST can be viewed as a pathological index of toxicity. The energy metabolism abnormity is the key reason for the molluscicidal activities. The biochemical mechanism needs further research.

Key words: *Nerium indicum* Mill; *Pterocarya stenoptera* DC; *Rumex japonicum* Houtt; Active components

INTRODUCTION

Snail is the only intermediate host of *Schistosoma japonicum* which is the species in China causing schistosomiasis. It is an efficient way to annihilate snails for the control of schistosomiasis. Sodium pentachlorophenate and niclosamide are the most widely used synthetic molluscicides in China at present[1-3]. But they are highly toxic towards non-target organisms and destroy ecosystem. In recent years, much attention has been given to the study of plant molluscicides because they might provide a cheap, biodegradable and effective control way in rural areas of developing countries where schistosomiasis is endemic[4-5]. *N. indicum* Mill, *P. stenoptera* DC, and *R. japonicum* Houtt are widely distributed in China and used as traditional Chinese medicines. Some reports show that they are toxic to amphibians and particularly *P. stenoptera* DC has been used as a folk herb to control snails for many years. We conducted laboratorial evaluation of molluscicidal activity of the three plants against the snail *O. hupensis*, and tried to clarify the toxicological mechanism of their molluscicidal activity.

MATERIALS AND METHODS

Plants

Three plants *N. indicum* Mill, *P. stenoptera* DC, and *R. japonicum* Houtt were collected locally and identified by the herbarium of Biology Department, Hubei University, and voucher specimens were deposited there.

Snails

Adult snails *O. hupensis* (9-11 mm in length) were collected in Dongting Lake, Hunan Province. The snails were kept in our laboratory at 21℃ for one month before being used in experiments.

Preparation of Plant Extracts

The leaves of *N. indicum* Mill and *P. stenoptera* DC, and the roots of *R. japonicum* Houtt were used in experiments. After shade drying, the plant materials

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were ground into fine powder. N-butanol and distilled water were added to the powder. Seven days after extraction at room temperature twice, the same crude extract was combined. Excess solvent was removed with a vacuum rotary evaporator. From 100 g dry plant materials, we got 4.0908 g, 1.8692 g and 1.6814 g n-butanol extracts of *N. indicum* Mill, *P. stenoptera* DC, and *R. japonicum* Houtt, respectively, and 13.0700 g, 11.6830 g and 9.2486 g water extracts of *N. indicum* Mill, *P. stenoptera* DC, and *R. japonicum* Houtt, respectively.

Molluscicidal Assay

For each plant extract, 5 different concentrations (20, 40, 60, 80, and 100 mg/L for the extracts of *N. indicum* Mill; 200, 400, 600, 800, and 1000 mg/L for the extracts of *P. stenoptera* DC and *R. japonicum* Houtt) were used. Ten snails were put into a nylon mesh bag (mesh size 2 mm) and immersed into 400 mL dechlorinated water solution of a known concentration. After 48 h exposure and 24 h recovery period in dechlorinated tap water, mortality was checked. No response to a needle probe under dissecting microscope was the evidence of death. The data were subjected to the probit analysis, the LD_{50} and LD_{90} values were calculated. Experiments were conducted at 21℃.

Esterase (EST) Isozyme Electrophoresis Assay

For each plant extract, 50 snails were treated with LD_{50} concentration. Dead snails were removed to avoid contaminating aquarium water during the whole experiment. After exposed for 8 h, 16 h, and 48 h, several living snails were chosen randomly for EST assay. The chosen snails were crushed. The soft tissues were taken out and phosphatic buffer solutions were added (1 drop/snail). The ice-based homogenates were centrifuged at 8000 rpm for 10 min at 0℃. Supernatant was placed in EDTA-coated tubes and stored at -20℃ for electrophoresis. Ten μL each sample (2 replicates) was electrophoresed with a Hoefer vertical electrophoresis system SE600. Polycrylamide gel electrophoresis (PAGE) was carried out with 20 mA constant current until the tracing dye reached the bottom of the gel. The chemicals were purchased from Sigma. The separating gel consisted of 7.5% (acrylamide+ bisacrylamide) and 0.04 mol/L Tris-HCl, pH 8.8. The stacking gel consisted of 3.75% (acrylamide+ bisacrylamide) and 0.01 mol/L Tris-HCl, pH 6.8. The buffer was Tris-glycine buffer, pH 8.3. After electrophoresis, gels were stained, washed, fixed and then photoed. Acetic acid (7.5%) was used as stationary phase.

Glycogen (Gn) Assay

As we described in *Esterase (EST) Isozyme Electrophoresis Assay*, 50 snails were treated with LD_{50} concentration for each plant extract. After exposure for 48 h, the soft tissues of living snails were taken out and weighed before and after being dried in oven, and then ground into fine powder. Two mL 30% KOH was added to 10 mg sample in a test tube and incubated in boiling water for 20 min. Ten mL ethanol was added after cooling. Glycogen was obtained after centrifuge. Glycogen content was measured by anthrone colorimetric method.

Total Protein (TPr) Assay

We used the same powder samples as in *Glycogen (Gn) Assay*. KND-04 Kjeldahl nitrogen determination device was used (TPr)%=6.25×N%.

RESULTS

Molluscicidal Activity

The LD_{50} and LD_{90} values for different extracts are shown in Table 1. N-butanol extract and water extract of *N. indicum* Mill showed strong molluscicidal activity. The LD_{50} value of the water extract was as low as 13.2 mg/L. The extracts of *R. japonicum* Houtt and *P. stenoptera* DC also showed strong molluscicidal activity.

TABLE 1

LD_{50} and LD_{90} (mg/L, 95% CI) of Extracts

	N-butanol Extract of *N. indicum* Mill	N-butanol Extract of *P. stenoptera* DC	N-butanol Extract of *R. japonicum* Houtt	Water Extract of *N. indicum* Mill	Water Extract of *P. stenoptera* DC	Water Extract of *R. japonicum* Houtt
LD_{50}	16.2 (13.1-20.1)	505.1 (397.2-642.7)	398.1 (331.1-467.7)	13.2 (10.6-16.5)	359.5 (316.2-407.4)	90.0 (69.2-117.5)
LD_{90}	89.0 (71.9-110.2)	3477.8 (2753.3-4365.2)	1479.1 (1288.2-2818.4)	79.0 (63.1-98.9)	1009.7 (891.2-1148.2)	708.0 (549.5-912.0)

EST Electrophoresis Assay

EST electrophoresis profiles are shown in Figs. 1A, 1B, and 1C. In early stage of toxicosis (Fig. 1A), enzyme activity of the treated samples was higher than that of the control. New enzyme bands appeared and color turned deeper. In middle stage (Fig. 1B) and late stage (Fig. 1C) of toxicosis, enzyme activity of the treated samples was lower than that of the controll, until enzyme bands completely disappeared.

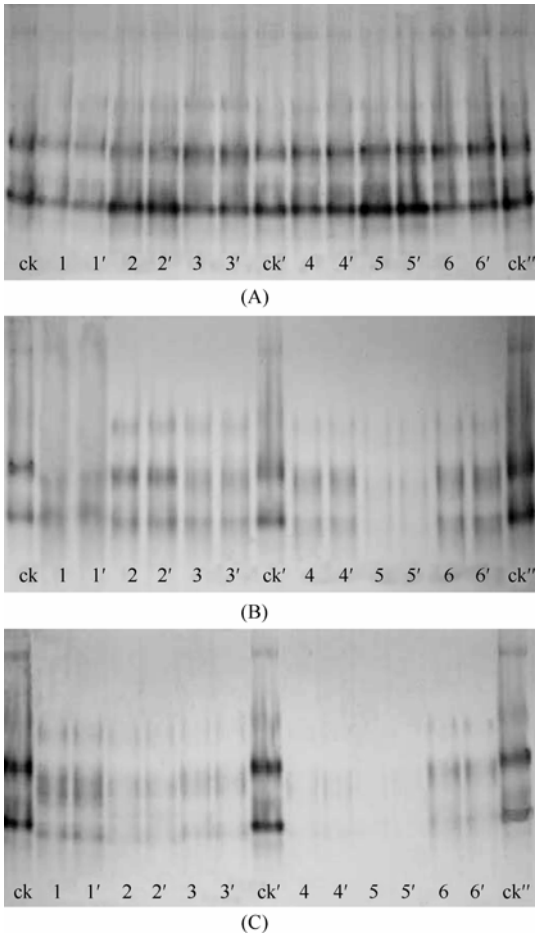

FIG. 1. EST lsozyme profiles after treated for 8 h (A), 16 h (B), and 48 h (C). ck, ck', ck'': samples controlled; 1, 1': samples treated with n-butanol extract of *N. indicum* Mill; 2, 2': samples treated with n-butanol extract of *P. stenoptera* DC; 3, 3': samples treated with n-butanol extract of *R. japonicum* Houtt; 4, 4': samples treated with water extract of *N. indicum* Mill; 5, 5': samples treated with water extract of *P. stenoptera* DC; 6, 6': samples treated with water extract of *R. japonicum* Houtt.

Glycogen Assay

Average wet and dry weights of soft tissues are shown in Table 2. Water content showed no obvious changes before and after the treatment. The results of glycogen assay are shown in Table 3. Glycogen content decreased greatly after the treatment, ranging from 9.6% to 33.6%. The decreasing rate accorded with the molluscicidal activity. That is, the extracts with stronger molluscicidal activity could decrease the glycogen content more.

Total Protein Assay

The results of total protein assay are shown in Table 4. Protein content also decreased after the treatment, ranging from 13.9% to 23.0%. Effect of the extracts of *P. stenoptera* DC was stronger than that of the others. No relationship was found between molluscicidal activity and protein content decreasing rate.

DISCUSSION

Our research group has published several papers about the molluscicidal activities of the three plants that can be viewed as potential plant moluscicides in the past several years[6-7]. The molluscicidal activities of the extracts of *N. indicum* Mill are stronger than those of the extracts of *P. stenoptera* DC and *R. japonicum* Houtt. Especially, the LD_{50} value of the water extract of *N. indicum* Mill is as low as 13.2 mg/L. Although the molluscicidal activities of these plant extracts are lower than those of the synthetic molluscicides (the LD_{50} of sodium pentachlorophenate or niclosamide is around 0.1 mg/L), plant molluscicides are much safer to human beings and environment. EST is an important detoxification enzyme system *in vivo* and also one of the enzymes showing the strongest reaction to environmental stimulation. EST change significantly during the whole exposure period and can be regarded as a pathological index of toxicosis of snails. The results

TABLE 2

Weight of Snail Soft Tissues

	1	2	3	4	5	6	Ck
Average Wet Weight (mg/Snail)	12.93	12.32	13.56	13.33	12.29	13.18	13.31
Average Dry Weight (mg/Snail)	3.00	3.07	3.11	2.98	3.00	3.22	3.02
(Average Dry Weight)/ (Average Wet Weight) (%)	23.2	24.9	22.9	22.4	24.4	24.4	22.7

TABLE 3

Glycogen Content in Snail Soft Tissues

	1	2	3	4	5	6	Ck
Content of Dry Powder (%)	7.51	9.83	9.15	8.92	10.22	9.17	11.31
Decreasing Rate (%)	33.6	13.1	19.1	21.1	9.6	18.9	—

TABLE 4

Total Protein Content in Snail Soft Tissues

	1	2	3	4	5	6	Ck
Content of Dry Powder (%)	29.11	27.11	29.32	28.22	26.23	28.64	34.05
Decreasing Rate (%)	14.5	20.4	13.9	17.1	23.0	15.9	—

of EST electrophoresis assay accorded with the normal pathological reaction. In an early stage, toxic extracts stimulated the expression of EST to increase the detoxification ability of liver. In middle and late stages, because of the effect and time surpassing the physiological limen of normal functions *in vivo*, substance metabolism and energy metabolism chaos appeared, and synthesis of EST was restrained. In the dying organisms, synthesis of EST almost ceases. All plant extracts can decrease the protein content in snail soft tissues, but not so significantly when compared with the glycogen content. Actually, in many cases, molluscicides cannot decrease the protein content in snail soft tissues[8], suggesting that the abnormity of energy metabolism might be the major factor for the molluscicidal activity. Toxicological mechanism deserves further research.

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