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Ultrastructural observations on *Raillietina echinobothrida* exposed to crude extract and active compound of *Securinega virosa*

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ABSTRACT

Securinega virosa has been used traditionally by the natives of Mizoram, India, to control intestinal worm infections. In the present study, the crude ethanol extract of the plant and its active component virosecurinine were tested *in vitro* on *Raillietina echinobothrida* to evaluate its potential anthelmintic efficacy and ultrastructural changes. The test parasites were exposed to different concentrations of the plant extract, active compound virosecurinine and reference drug praziquantel. Scanning and transmission electron microscopic observations on the paralyzed worms revealed wide scale destruction of the tegument with intense vacuolization of the syncytium and swellings of the basal lamina accompanied by deformities in the cell organelles. Extensive structural alteration of tegument indicates that the plant extract and its active component alter membrane permeability of the parasite leading to paralysis and subsequent death, as confirmed by *in vitro* tests.

1. Introduction

Continued use of synthetic drugs against helminths has led to the widespread emergence of drug resistance (Waller, 1997). This has made alternative anthelmintic strategies involving phytoproducts much needed as control systems in the present day world. Traditional medicine practice among the natives of Mizoram (Northeast Indian state) has relied upon the treatment with leaves of *Securinega virosa* (Family: Euphorbiaceae) against intestinal worm infections. The aqueous juice of the leaves is made into paste with tobacco and used to control worm infections (Kirtikar and Basu, 1975). The plant has many reported uses ranging from anthelmintic and anti-diabetic to antidiarrhoeal and sedative (Ross, 2005; Magaji et al., 2007, 2008; Tanko et al., 2008). Although the majority of the evidence on antiparasitic activity of plants has been traditionally based on anecdotal observations, presently there are an increasing number of controlled experimental studies that aim to verify, validate and quantify in a scientific manner such plant activity (Roy et al., 2007, 2008, 2009; Dasgupta et al., 2010; Challam et al., 2010). Thus, the purpose of the present investigation is to study the *in vitro* effects of *S. virosa* leaf extract and its active compound virosecurinine on *Raillietina echinobothrida*, the most prevalent helminth parasite of domestic fowl in Northeast India (Dasgupta, 2010). The parasite induces nodule formation throughout the intestine of the host, leading to huge losses to the poultry industry in India (Kumar et al., 2007).

2. Materials and methods

2.1. Preparation of extracts

Leaves of *S. virosa* were collected from different villages around Aizawl, Mizoram, India. After thorough washing in water, the leaves were shade-dried and grounded into powder. Ethanol extract of 100 g of dry powder was prepared by the reflux and filtration method as described earlier (Roy et al., 2008). The active principle of *S. virosa*, namely, virosecurinine, was obtained from Pharmeks, Moscow.

2.2. In vitro experiment

Live *R. echinobothrida* were collected in 0.9% physiological buffered saline (PBS, pH 7.2) from the intestine of freshly sacrificed fowl. The worms were incubated in 5, 10 and 25 mg crude ethanol extract/ml of PBS and 1 mg virosecurinine/ml of PBS at 39 ± 1 °C. Dimethyl sulphoxide (DMSO) was added to each concentration to give a final solvent concentration of 0.1%. Controls were prepared by incubating the worms in the culture medium (PBS) with 0.1% DMSO only. Praziquantel (PZQ) was obtained from Chandra Bhagat Pharma Pvt. Ltd. (Mumbai, India) and used as the reference cestocidal drug at concentrations of 0.01and 0.001 mg/ml of PBS. Complete loss of visible movements in the parasite was taken as a

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Table 1
Efficacy of crude extract of *S. virosa* and reference drug praziquantel: effect on *R. echinobothrida*[*] ($n = 6$).

Test material	Concentration (mg/ml)	Paralysis (h)	Death (h)
S. virosa	5	11.63 ± 0.25	13.70 ± 0.18
	10	6.7 ± 0.39	6.9 ± 0.16
	25	2.95 ± 0.22	3.8 ± 0.2
	50	1.05 ± 0.45	2.1 ± 0.29
Virosecurinine	1	1.25 ± 0.15	8.9 ± 0.01
Praziquantel	0.01	0.5 ± 0.01	7.3 ± 0.15
	0.001	3.0 ± 0.14	9.8 ± 0.21

[*]Control worms survived for 72 ± 0.06 h in the incubation medium. $p < 0.05$ *vs.* control value, Student's *t*-test. Values are mean ± SEM.

sign of paralysis, and death was ascertained by dipping the paralyzed worms in warm PBS, which evoked movements in the live worms. Only worms paralyzed with 25 mg dosage of the plant extract were used for further analytical studies as paralysis was induced at a time span comparable with that of the reference drug PZQ (Table 1).

2.3. Scanning electron microscopy

One set each from control worms, treated with the crude plant extract, virosecurinine and PZQ, was fixed in neutral buffered formalin at 4 °C for 4 h. The fixed specimens were dehydrated through ascending grades of acetone and then air-dried in tetramethylsilane following the method of Dey et al. (1989) and Roy and Tandon (1991). After mounting the samples on metal stubs and gold coating, the surface architecture of the parasites was studied using a JEOL JSM 6360 scanning electron microscope operated at 15 kV.

2.4. Transmission electron microscopy

Control worms, parasites exposed to the phytochemicals and commercial drug were fixed in modified Karnovsky's fixative, post-fixed in 1% OsO_4 buffered with 0.2 M sodium cacodylate for 1 h, dehydrated through graded acetone and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate, and viewed in a JEM 100 CXII (Jeol) transmission electron microscope operated at 80 kV.

2.5. Statistical analyses

All data for *in vitro* experiment expressed in mean ± SEM for five replicates in each group. Comparison of the mean values of the experimental groups against the control groups was made using unpaired Student's *t*-test, and values of $p < 0.05$ were considered significant in all cases.

3. Results

R. echinobothrida incubated in alcoholic extract of *S. virosa*, active compound virosecurinine and the reference drug PZQ, revealed a decline in the motility of

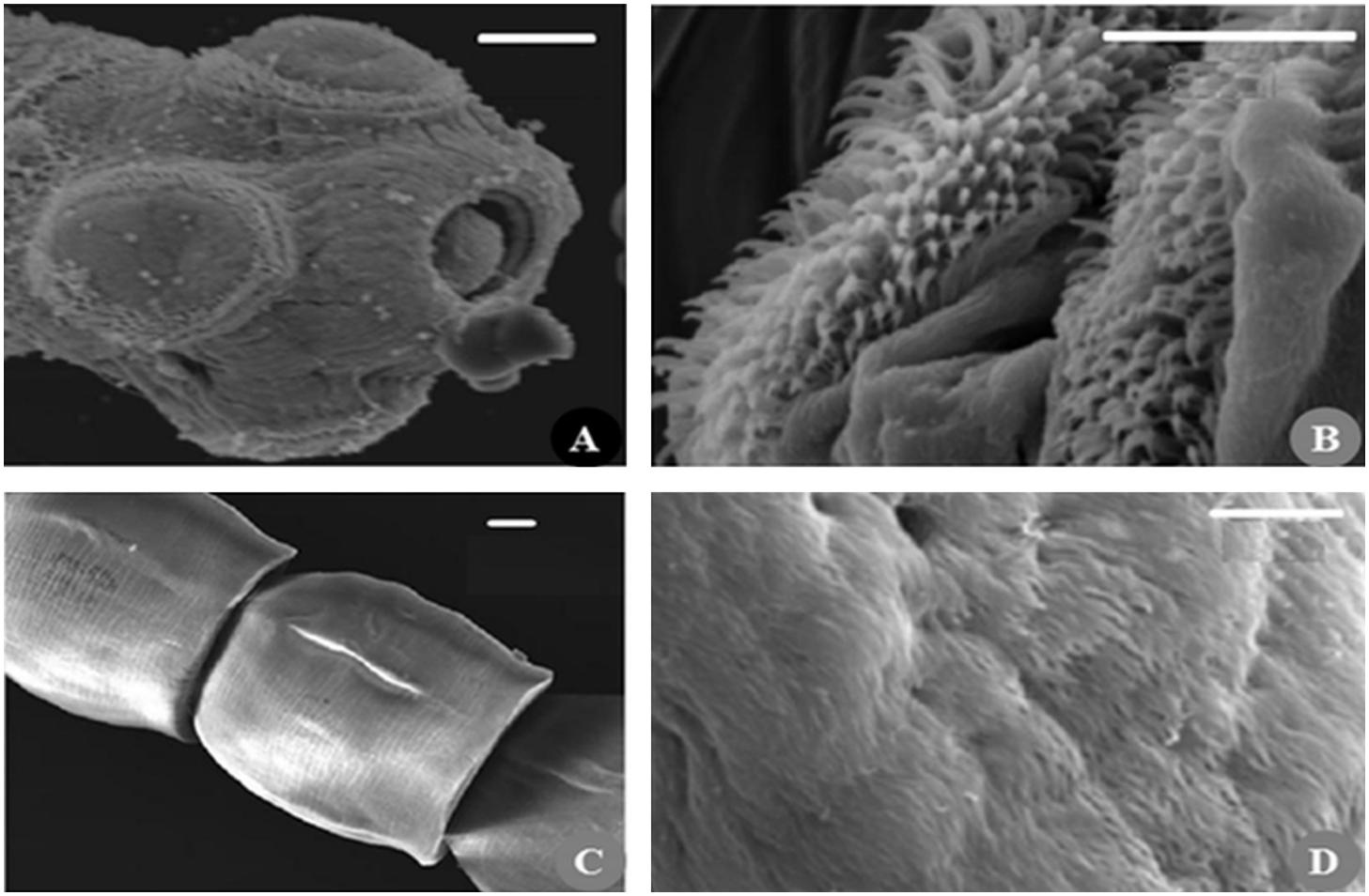

Fig. 1. Scanning electron micrographs of control *R. echinobothrida*: (A) Scolex showing retractable rostellum and four suckers (20 μm). (B) Sucker rim showing circlets of hooks that are broad at the base and tapering and bent toward the ends (10 μm). (C) Mature proglottids showing smooth tegument at low magnification (200 μm). (D) Enlarged view of a portion of proglottid showing layers of microtriches gently sloping downwards (5 μm).