

In vitro flucikidal effect of leaf extract of *Cannabis sativa* Linn. on the trematode *Fasciolopsis buski*

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Received 24 November 1995; revised 3 April 1996

The efficacy of crude extract of *Cannabis sativa* leaf on the motility and morphology of *Fasciolopsis buski* was studied using scanning electron microscopy. Treatment of the worms *in vitro* with 5, 10, and 20 mg of crude extract per ml of phosphate buffered saline caused paralysis, taking 1-1.3, 0.6-0.8 and 0.4-0.6 hr, respectively. SEM observations on treated flukes revealed deformation of the surface architecture, particularly of papillated ventral surface. Deep scars were also observed both on the dorsal and ventral surface. The leaf extract of the plant was thus found to be effective against intestinal giant fluke.

Among digenetic trematodes, *Fasciolopsis buski* gains considerable importance from veterinary and public health point of view in North-East India¹⁻³. Unhygienic and poor status of living in association with large-scale uncontrolled piggeries is supposed to be responsible for prevalence of this infection among human population in the region^{1,4}. To control this fluke infection several effective drugs are now available. However, the local tribals people of the region consume and also feed to their animals, parts of several wild plants as traditional cure for the disease⁵. The present investigation is an attempt to see *in vitro* efficacy of one such plant, i.e., *Cannabis sativa* against *F. buski*.

Fresh leaves of *C. sativa* were collected from the jungles in and around Shillong during July, 1994. They were dried in an oven at 50°C. About 20 g of dry leaves were ground and put in a reflux flask having 500 ml capacity with 200 ml rectified spirit. After reflux for 8 hr at 60°C, the solution was filtered out and dried overnight at 60°C.

Adult *F. buski* were collected in 0.9% phosphate buffered saline (PBS) from a freshly slaughtered pig. The flukes were then incubated at 37° ± 1° C with 5, 10 and 20 mg of extract/ml of PBS (3 replicates for each concentration) in 1% dimethylsulfoxide (DMSO). Control incubation consisted of flukes in PBS with 1% DMSO only.

Oxyclozanide B.P. was used as the reference flucikidal drug in concentrations similar to those used for the crude extract.

Time taken for complete inactivation of the flukes was recorded and death was confirmed by dipping such worms in slightly warm water. Soon after paralysis at a concentration of 20 mg/ml of PBS, the treated material along with one set of control was fixed in 10% cold buffered formalin at 4°C for 4 hr followed by dehydration in acetone and air-dried in tetramethylsilane. The gold-coated specimens were viewed under Jeol JSM-35 CF electron microscope at electron accelerating voltage of 10 kV.

The effect of different concentrations of the leaf extract and oxyclozanide on adult *F. buski* is shown in Table 1. The control worms maintained in PBS showed physical activity up to 18-22 hr, following which they became immobilized. In contrast, the flukes treated with 5, 10 and 20 mg extract/ml of PBS, became paralysed at 1-1.3, 0.6-0.8 and 0.4-0.6 hr, respectively. Flukes incubated in the medium containing Oxyclozanide showed considerably

Table 1—Effect of *Cannabis sativa* leaf extract and oxyclozanide on motility of *Fasciolopsis buski**

Conc. of extract and drug/ml PBS (mg)	Paralysis (hr)	
	<i>C. sativa</i>	Oxyclozanide
5	1.0-1.3	5.0-5.3
10	0.6-0.8	3.0-3.5
20	0.4-0.6	1.5-2.0

*Controls survived for 18-22 hr in PBS at 37°C.

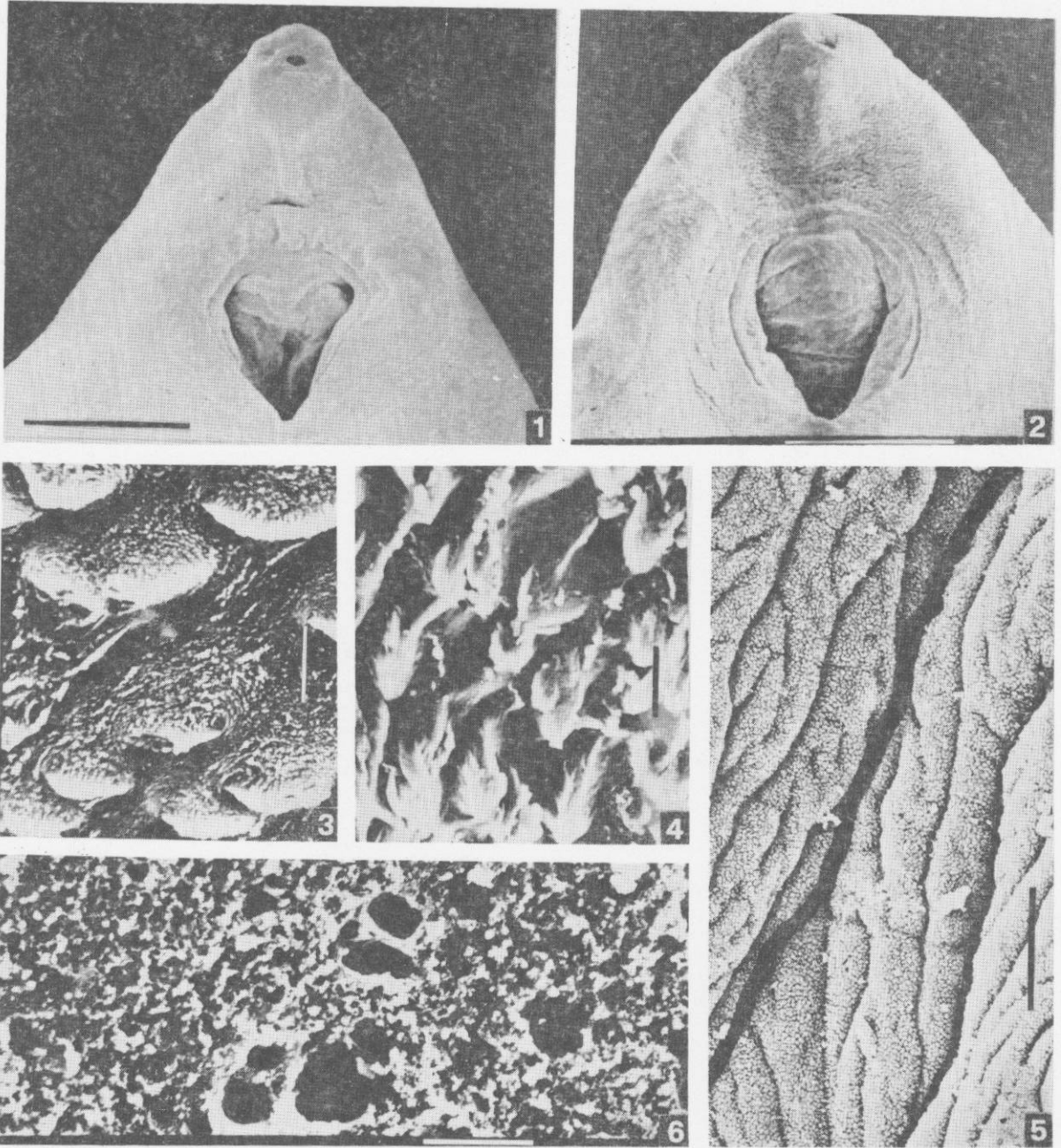
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slower acquisition of a paralytic state.

Scanning electron microscopic observations of the surface topography of the untreated controls revealed a normal contour with radial corrugations

of the oral and ventral sucker rim and presence of scale-like papillae on the ventral surface (Figs. 1, 3 and 5). In contrast, the worms incubated with 20 mg extract/ml of PBS showed deformity of the entire



Figs 1-6—*Fasciolopsis buski* - Scanning electron micrographs of normal (1, 3, 5) and treated (2, 4, 6) flukes. (1) Anterior region of body showing normal contour of oral and ventral sucker (scale bar = 1mm). (2) Anterior region of body showing deformed oral and ventral sucker (scale bare =1mm). (3) A portion of ventral surface, showing normalscales (scale bar = 10 μm). (4) A portion of ventral surface, showing distorted scale (scale bar = 10 μm). (5) A portion of dorsal surface, of normal fluke (scale bar = 10 μm). (6) A portion of dorsal surface of treated fluke (scale bar = 10 μm).

tegumental surface, particularly the rim of the oral and ventral sucker; cracks and scar-like formations were evident throughout the dorsal and ventral surface. Scale-like papillae of the ventral surface were deformed and the normal surface foldings on the dorsal surface of the fluke were not evident (Figs 2, 3 and 6).

The results of the present investigation reveal that the crude extract of *Cannabis sativa* causes destructive, degenerative and necrotic alterations in the tegumental surface of *F. buski* as early as 25 min after treatment at a concentration of 20 mg extract/ml of PBS. Moreover, the crude extract is found to be more lethal than the commercial flucikide, oxyclozanide. The crude extract of the number of plants e.g., *Zingiber officinalis*, *Zanthoxylum alatum* and *Lysimactia clethroides* etc. have been tested against *Schistosoma* spp. and *F. buski* and their anthelmintic efficacy has been established on the basis of the lethal effect on the flukes⁶⁻⁹.

Worms treated with 20 mg/ml of PBS were selected for morphological observations in the present study because of the early lethal effect of the dose compared to other concentrations. The normal body surface of the fluke is provided with scales, papillae and ridges as observed earlier^{10,11}. However, parasites treated with the leaf extract show total deformation and disruption of the suckers, tegumental papillae and scales which are vital structures in terms of sensory function, absorption of nutrients and for anchorage of the worm. Similar to the present findings, destruction of attachment organs like suckers, and hooks was also observed in *Dactylogyrus extensus* treated with Praziquantel¹². Mehlhorn *et al.*¹³ also reported disorganization and vacuolization of the absorptive tegumental surface in several digenetic flukes viz. *Clonorchis sinensis*, *Opisthorchis viverrini* and *Schistosoma japonicum* when exposed to Praziquantel¹⁴. Bricker *et al.*¹⁴ are of the opinion that contraction

and vacuolization in the tegumental surface of the parasites are closely related to the level of Ca^{2+} concentration of the media used.

It is observed in the present investigation that the flukes treated with various concentrations of the leaf extract of *C. sativa* became paralysed, followed by death. Thus the leaf extract seemingly exerts irreversible changes on the neuromuscular system of the worm and seems to have a vermifugal effect, with its active constituents perhaps operating transtegumentally.

Acknowledgement

This study was supported by an award of Research Associateship to BR from CSIR and DRS programme of UGC, New Delhi to the Department of Zoology. Thanks are due to the Head, Regional Sophisticated Instrumentation Centre, North-Eastern Hill University for providing necessary SEM facilities.

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