Parasitol Res (1996) 82:248–252

ORIGINAL PAPER

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B. Roy · V. Tandon

Effect of root-tuber extract of *Flemingia vestita*, a leguminous plant, on *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski*: a scanning electron microscopy study

Received: 21 June 1995 / Accepted: 29 August 1995

Abstract The tegumental surface of *Artyfechinostomum sufrartyfex* as viewed under the scanning electron microscope revealed the presence of double rows of spines in the collar. The dorsal surface (6–8 rows) and the ventral surface are provided with posteriorly directed spines. The normal body surface of *Fasciolopsis buski* shows posteriorly directed scales throughout the ventral surface; the dorsal surface is free of any scales but has domed, coarsely distributed papillae. When treated in vitro with ethanol root-tuber extract of *Flemingia vestita*, an indigenous medicinal plant in Meghalaya, India, at a concentration of 5, 10, and 20 mg/ml phosphate-buffered saline (PBS), *A. sufrartyfex* became paralyzed within 1.1–1.4, 0.8–1.0, and 0.3–0.5 h, respectively. Following similar treatment, *F. buski* took 3.0–3.6, 1.5–2.0, and 0.6–0.8 h, respectively, to reach a paralytic state. Oxyclozanide B.P. was used as the reference drug and paralyzed the worm, taking slightly less time than the crude extract for both species of flukes. Stereoscanning observations on the tegumental surface of treated (20 mg extract/ml PBS) *A. sufrartyfex* revealed sloughing off of most of the spines or their deformation as well as wrinkles and rupture of the general tegument. Severe tegumental alterations and deformities were also displayed by *F. buski* exposed to 20 mg extract/ml PBS.

Introduction

Plant products provide and are gaining importance as an alternative to current medical practices involving chemotherapy (Didier et al. 1988; Robinson et al. 1990). For many reasons, the latter may not be accessible to the masses in developing and underdeveloped countries. In Northeast India, Meghalaya in particular, many indigenous plants are used for their medicinal value (Rao 1981). *Flemingia vestita* Benth and Hooker (family Leguminoseae), locally known as Soh-phlang, is widely used by the native tribal population of Meghalaya, who consume the pulpy tuberous roots for their anthelmintic activity against intestinal worm infections. In a preliminary study, the whole root-tuber crude extract has been reported to be effective against *Ascaris suum* in vitro (Yadav et al. 1992). The active principles of the root-tuber peels have been isolated by Rao and Reddy (1991); these are isoflavones and have been identified as genistein (0.25), fomononetin (0.035%), pseudobaptigenin (0.015%), and daidzein (0.01%).

The present study aimed at testing the efficacy of root-tuber extract of *F. vestita* on *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski*, both of which are intestinal digenetic flukes occurring in pigs but also having a zoonotic potential. It pertains to scanning electron microscopic observations on the surface fine topography and its alterations induced by the plant crude extract on in vitro exposure. Microtopographical details of *F. buski* have previously been studied (Roy and Tandon 1993), whereas those of *A. sufrartyfex* are reported herein for the first time.

B. Roy · V. Tandon (✉)
Department of Zoology, North-Eastern Hill University, Shillong 793 022, India

Materials and methods

Preparation of crude extract

The edible root tubers of *Flemingia vestita* Benth and Hooker were collected from neighboring villages of Shillong, India, during November 1993, and their peels were dried at 50° C in an oven. Dried peels (about 100 g) were ground and put in a reflux flask having 1 l capacity with 500 ml rectified spirit. After reflux for 8 h at 60° C, the solution was filtered and dried overnight at 60° C; 26 g crude extract was obtained from 100 g dried peels.

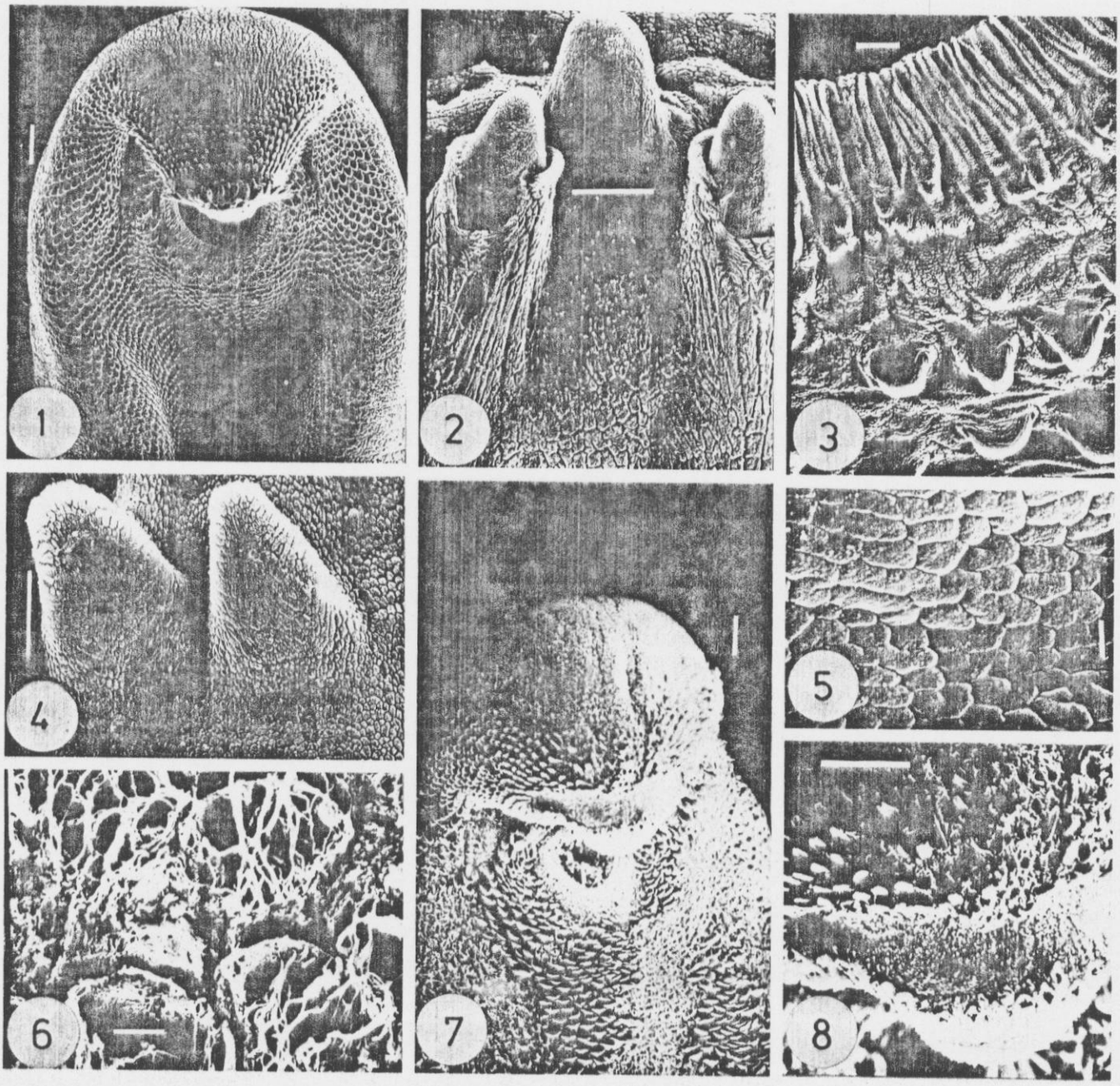
Experimental flukes and bioassay

Adult *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski* were collected from the intestine of a locally reared pig at an abattoir in Shillong. After a washing in phosphate-buffered saline (PBS), the flukes were incubated at 37±1° C with varying concentrations of

the extract, viz., 5, 10, and 20 mg/ml PBS (three replicates for each concentration) in 1% dimethylsulfoxide (DMSO). Control incubation consisted of flukes in PBS with 1% DMSO only. Oxyclozanide B.P., a wide-spectrum flukicide,was used as the reference drug at concentrations similar to those used for the crude extract.

Scanning electron microscopy

Flukes were fixed in 10% neutral buffered formalin at 4° C for 4 h, dehydrated in acetone, and air-dried in tetramethylsilane (Roy and Tandon 1991). The gold-coated specimens were viewed under a Jeol JSM-35CF electron microscope at an electron-accelerating voltage of 10–15 kV.



Figs. 1–8 *Artyfechinostomum sufrartyfex* – scanning electron micrographs of normal (**Figs. 1–5**) and treated (**Figs. 6–8**) flukes

Fig. 1 Anterior half of a whole worm, ventral view. Bar=100 µm

Fig. 2 Collar spines in a closer view, revealing the socketed and nonsocketed types. Bar=10 µm

Fig. 3 Portion of the ventral sucker rim and adjoining ventral surface. Bar=10 µm

Fig. 4 Dorsal spines in an enlarged view. Bar=10 µm

Fig. 5 Dorsal surface (general tegument). Bar=1 µm

Fig. 6 Portion of the ventral surface, showing pits and disrupted tegument. Bar=10 µm

Fig. 7 Anterior end of a worm (ventral view). Bar=100 µm

Fig. 8 Enlarged view of the collar region, showing deep pits in areas of spines and scales. Bar=100 µm

Results

Artyfechinostomum sufrartyfex

The fluke exhibits inward curling of the lateral edges of its body in the postacetabular region, with the tip being bent toward the ventral side (Fig. 1). The oral collar is armed with a double row of spines that are about 10 µm wide and have a smooth surface; whereas all the spines of the first row are lodged in deep sockets, a few spines in the second row are nonsocketed (Fig. 2). On the dorsal surface immediately posterior to the collar, there are 6–8 rows of tegumental spines, about 23 µm long and 15 µm wide, which also extend to the dorsolateral sides and merge with the spines of the ventral surface (Fig. 1). These spines are posteriorly directed conical elevations having bluntly rounded tips and cobblestone-like surface corrugations (Fig. 4). The rest of the dorsal surface is devoid of spines and has a corrugated mosaic-like texture similar to that of the spine surface (Fig. 5). The whole ventral surface is provided with a dense covering of posteriorly directed scale-like spines having broadly rounded tips and arranged in an overlapping fashion (Figs. 1, 3). The surface texture of these spines also resembles that of the general tegument (Fig. 5). The tegument of the rim of the ventral sucker has prominent radial folds and craters and appears to be very finely corrugated (Fig. 3).

Fasciolopsis buski

The ventral surface of the fluke is provided with scales. These scales are more densely packed in the post-ventral sucker region and are posteriorly directed, their bluntly rounded tip showing rough edges (Figs. 9, 10). They measure about 10 µm in length and 25 µm in width. Coarsely distributed button-shaped, nonciliated papillae

Figs. 9–12 *Fasciolopsis buski* – scanning electron micrographs of normal (**Figs. 9, 10**) and treated (**Figs. 11, 12**) flukes

Fig. 9 Portion of the ventral sucker and its adjoining area. Bar=100 µm

Fig. 10 Tegument behind the ventral sucker region, showing scales. Bar=10 µm

Fig. 11 Portion of the ventral sucker and its adjoining area. Bar=100 µm

Fig. 12 Tegument behind the ventral sucker region, showing disrupted tegument with pits. Bar=10 µm

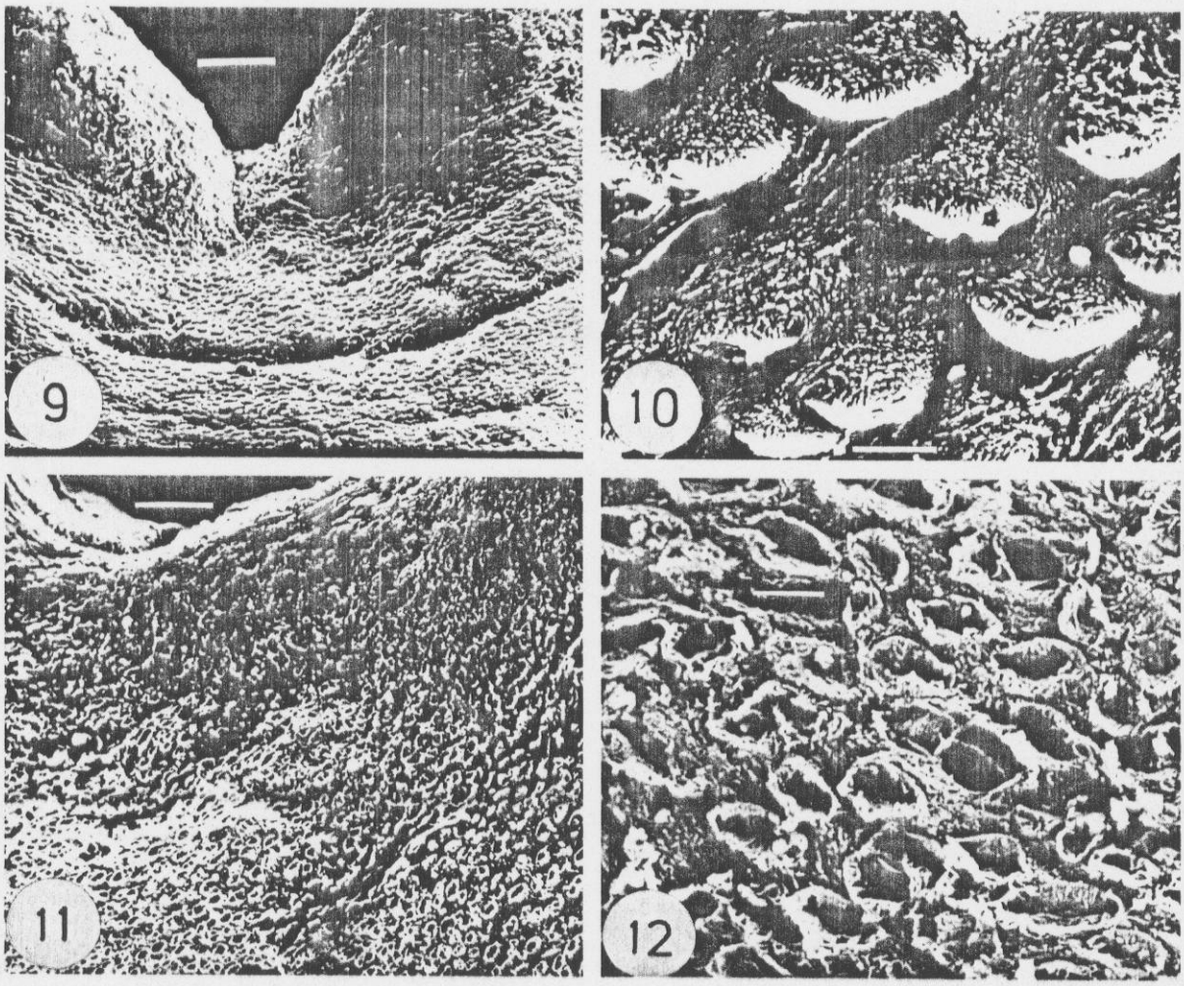


Table 1 Dose-dependent effects of *Flemingia vestita* (peel or root tuber) and oxyclozanide on *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski* (*P* Paralysis, *D* death)

Parasite	Crude extract drug		Longevity (in hours) at concentration			Control (in PBS)
			5 mg/ml	10 mg/ml	20 mg/ml	
A. sufrartyfex	*F. vestita*	P	1.1–1.4	0.8–1.0	0.3–0.5	18–20
		D	3.0–4.0	1.5–2.5	1.0–1.5	
	Oxyclozanide BP	P	1.0–1.2	0.6–0.8	0.3–0.4	
		D	2.5–3.0	1.5–1.8	1.0–1.2	
F. buski	*F. vestita*	P	3.0–3.6	1.5–2.0	0.6–0.8	18–22
		D	5.6–6.5	3.7–4.0	1.6–2.2	
	Oxyclozanide BP	P	1.5–3.0	1.2–1.5	0.5–0.6	
		D	5.0–5.3	3.0–3.5	1.5–2.0	

are present on the dorsal surface of the body and in the region of the oral sucker.

Treated flukes

The survival time of *A. sufrartyfex* and *F. buski* in different concentrations of the crude root-tuber peel extract of *F. vestita* and oxyclozanide is depicted in Table 1.

On exposure to the crude extract of root tuber at a concentration of 5, 10, and 20 mg/ml PBS in vitro, tegumental lesions in *A. sufrartyfex* could be observed after 1.1–1.4, 0.8–1.0, and 0.3–0.5 h, respectively. Lesions were common on both the dorsal and the ventral surface; the spiny surface area, particularly of the collar and ventral sucker region, was most severely affected. Treatment at a concentration of 20 mg/ml revealed uprooting of most of the collar spines, leaving holes apparent (Figs. 7, 8). The spines remaining attached at their location showed deformity. All the dorsal spines showed changes in their shape and surface architecture. Similar to the dorsal spines, ventral scale-like spines also showed changes in shape and texture, with no distinct surface feature being as evident in untreated control worms (Fig. 6). The rim of the ventral sucker revealed wrinkles and deformation of its muscular structure; most of the spines surrounding the sucker got sloughed off, leading to deep lesions (Fig. 6). The spine-free surface area also showed wrinkle and crack formation with loss of the cobblestone-like surface texture.

F. buski treated with the extract at 20 mg/ml PBS exhibited wrinkles and pit formation on the tegument (Fig. 11). Whereas there was no trace of papillae, extensive pits/vacuoles were evident (Fig. 12), indicating complete destruction and disorganization of the tegument.

Discussion

The body surface of the adult *Artyfechinostomum sufrartyfex* is provided with spines and scales, which conforms to the common topographical plan of echinostomatid flukes. Scale-like spines with blunt, rounded tips have been reported in *Echinostoma revolutum*, the common echinostomid fluke of domestic fowl (Tandon and Roy 1995). However, socketed spines in the collar and the nature (surface architecture), arrangement, and distribution of dorsal and ventral spines in *A. sufrartyfex* are distinctly different from those of *E. revolutum* (Fried and Fujino 1984; Tandon and Roy 1995). Furthermore, the sensory papillae present at the oral rim of the latter species are lacking in the former. The presence of circum-oral papillae has been reported for many intestinal flukes, also in *Gastrodiscoides hominis*, an amhistomid intestinal fluke of swine hosts (Tandon and Maitra 1983; Jones 1986).

In conformity with earlier observations (Roy and Tandon 1993), *Fasciolopsis buski* has densely packed scales on its ventral surface.

The results of in vitro treatment with the extract clearly indicate that the tegument of *A. sufrartyfex* and *F. buski* is extremely sensitive to the peel extract of the root tuber, which has the potential to paralyze and kill the flukes in vitro. Moreover, the crude extract proved to be almost as lethal as the commercial flukicide oxyclozanide. The crude extracts of a number of plants, viz., *Zingiber officinale, Zanthoxylum alatum,* and *Lysimactia clethorides*, among others, have been tested against *Schistosoma* sp. and *F. buski* and their anthelmintic property in terms of a lethal effect has been established (Soh et al. 1980; Singh et al. 1982; Adewunmi et al. 1990).

In the present study, flukes treated with the extract at 20 mg/ml PBS were selected for scanning electron microscopic observation because of the early lethal efficacy of this dose as compared with other concentrations. Although the deleterious effects on the surface topography of both fluke species were found to be of a similar nature, the damage caused was more severe in the giant fluke *F. buski*, which showed extensive wrinkles and deformation of the rim of the oral and ventral suckers and complete loss of the ventral scales and the circum-oral and dorsal papillae. Suckers, tegumental papillae, scales, and spines are vital structures both in terms of sensory absorption of nutrients and for anchorage, and these structures are among the first to be affected and altered by the crude ex-

tract in both species of flukes. Destruction of attachment organs (suckers) and loss of hooks have also been noted in *Dactylogyrus extensus* following treatment with praziquantel (Schmahl and Mehlhorn 1985).

Similar to the present observations, pits and vacuole formation have also been observed in several other digeneans such as *Clonorchis sinensis, Opisthorchis viverrini*, and *S. japonicum* and in the monogeneans *D. vastator* and *D. extensus* following treatment with praziquantel in vitro (Mehlhorn et al. 1983; Schmahl and Mehlhorn 1985). In digenetic flukes, the site of origin of vacuoles was found to be the basal lamina (Mehlhorn et al. 1983), whereas in *Diclidophora* spp., vacuoles originated from the surface of the tegument (Schmahl and Mehlhorn 1985).

Vacuolization and contraction in the parasite body surface are closely related to the level of Ca^{2+} concentration in the media used (Bricker et al. 1982). Disturbances in ion flux across the membrane, leading to changes in the tegumental integrity in different trematodes on treatment with praziquantel, are well established (Bricker et al. 1982; Mehlhorn et al. 1983; Schmahl and Mehlhorn 1985). Sobhon et al. (1986) showed that treatment of *O. viverrini* with amoscanate caused severe swelling and pit formation, leading to total disruption of the surface tegument, and suggested that the drug may have caused an imbalance in osmosis, thus resulting in impaired ion transfer.

Severe deleterious effects on the tegumental surface may be related to loss of spontaneous movement, detachment from the host's surface, and death. The peel extract of root tubers of *Flemingia vestita* thus seems to have a vermifugal/vermicidal effect, its active constituents perhaps operating transtegumentally. Further ultrastructure and biochemistry studies relating to the effect of the active principles of this plant material are desirable and are likely to explain its mode of action.

Acknowledgements This study was financially supported by the Council of Scientific and Industrial Research, New Delhi. The authors are grateful to the Head of RSIC, North-Eastern Hill University, Shillong, for providing SEM facilities.

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