

# Flukicidal activity of *Alpinia nigra* (Zingiberaceae) against the trematode, *Fasciolopsis buski*, in humans

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**Key words:** *Fasciolopsis buski*, *Alpinia nigra* extract, trematode infection

## Abstract

The *in vitro* effect of the traditional medicinal plant *Alpinia nigra* (Gaertn.) Burt. on the adult *Fasciolopsis buski* was determined. The flukes were incubated in 2.5, 5 and 10 mg of crude alcoholic extract of the shoots per ml of phosphate buffered saline at a temperature of 37 ± 1°C. This caused paralysis and death, taking 9.5–10.5, 6.0–6.8 and 4.0–4.5 h, respectively. Scanning electron microscopy of the surface topography of the extract-treated flukes revealed deformed body contours and suckers. The ventral papillae which have a distinct size and shape also showed deformity accompanied by deep scar formation at the base of each papilla.

## Introduction

*Fasciolopsis buski*, a digenetic trematode, is of considerable importance from the medico-veterinary point of view because of its zoonotic nature, particularly in north-east India (Buckley, 1939; Roy and Tandon, 1992a,b) where the pig acts as a reservoir host for human infection. Due to deleterious side effects (Sackey *et al.*, 1991; Abdalla *et al.*, 1994), and resistance developed by most of the helminths against prescribed drugs (Martin and Mckenzie, 1990; Coles and Bruce, 1990; Scott and Armour, 1991; Sangster and Bjorn, 1995), attempts are now being focused on natural products as an alternative means to control helminthiasis (Didier *et al.*, 1988; Robinson *et al.*, 1990; Tandon *et al.*, 1997).

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*Alpinia nigra* is one such plant, in which the aqueous extract of fresh shoots is consumed against intestinal worms by Tripuri tribes residing in the remote areas in a region having no proper modern medical facilities available to them.

The purpose of the present study was to evaluate the trematocidal efficacy of *Alpinia nigra* against *Fasciolopsis buski*, the giant fluke of the intestine. Scanning electron microscopy (SEM) emerged as an important tool to investigate the surface alteration induced by drugs in the helminth parasites (Roy and Tandon, 1996, 1997).

## Materials and methods

The edible fresh shoots of *Alpinia nigra* (Gaertn.) Burt. were collected from the jungle in and around Dharmanagar, Tripura, India, during June 1996. They were cut into pieces and dried in an oven at 50°C. About 25 g of dry shoots were ground and put in a 500 ml Reflux flask with 200 ml rectified spirit. After reflux for 8 h at 60°C, the solution was filtered out and dried overnight at 60°C.

Live, adult *F. buski* were collected in 0.9% phosphate buffered saline (PBS) from the intestine of a freshly slaughtered pig at an abattoir in Shillong. After washing in fresh PBS, the flukes were incubated at 37 ± 1°C with 2.5, 5 and 10 mg of extract/ml of PBS (three replicates for each concentration) in 1% dimethylsulphoxide (DMSO). Control incubation consisted of flukes in PBS with 1% DMSO only. Oxyclozanide was used as the reference trematocidal drug in concentrations in correspondance with those used for the alcoholic crude extract.

The time taken for complete paralysis and death of the flukes was confirmed by dipping the worms in slightly warm water. Immediately after paralysis at a concentration of 20 mg/ml PBS the treated material along with one set of control specimens were fixed in 4% cold buffered formalin at 4°C for 4 h followed by dehydration in acetone and air-dried in tetramethyl silane (TMS) as described earlier (Roy and Tandon, 1991). The gold-coated specimens were viewed under a Jeol JSM 35 CF scanning electron microscope at an accelerating voltage of 10–15 kV.

## Results and discussion

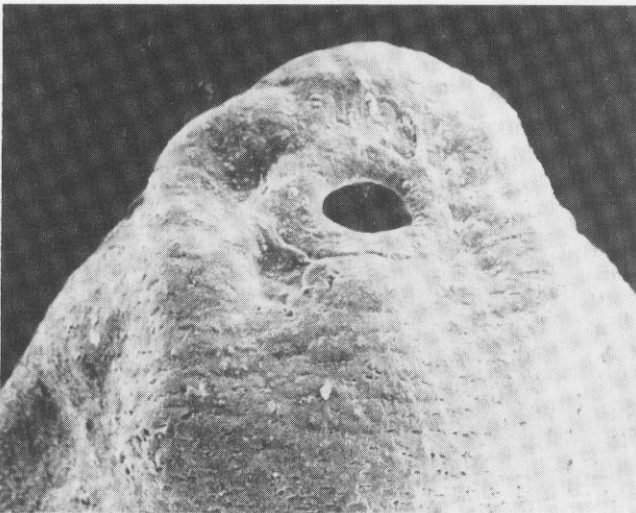
The effect of different concentrations of the crude alcoholic extract of shoots and oxyclozanide on the adult *F. buski* is shown in Table 1. The flukes incubated in the medium having 2.5, 5 and 10 mg of extract per ml of PBS became paralysed at 9.5–10.5, 6.0–6.8 and 4.0–4.5 h of incubation, respectively. In contrast, the control worms maintained in PBS showed physical activity up to 22 h following which they became immobilized. The flukes incubated in the medium containing oxyclozanide showed the most rapid paralysis. The parasites incubated with 10 mg of extract per ml of PBS were selected for stereoscan observations because of early lethal effects of the dose compared with other low concentrations.

**Table 1** Effect of *A. nigra* shoot extract and oxyclozanide on *F. buski*

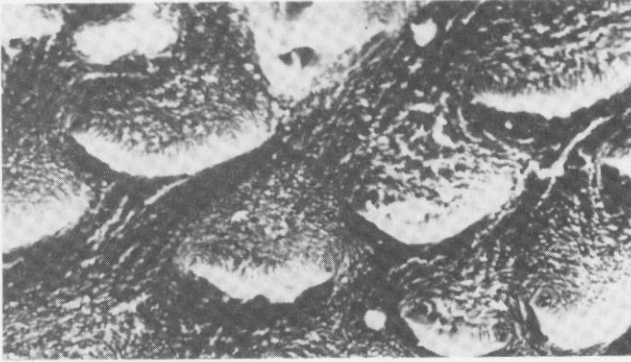
Concn of extract or drug (mg/ml)	Paralysis (h):		Normal and extract (10 mg) treated scales	Size ( $\mu$ m):	
	<i>A. nigra</i>	Oxyclozanide		Length	Width
2.5 mg	9.5–10.5	8.2–9.0	Normal	9–11	15–25
5.0 mg	6.0– 6.8	5.0–5.4	Treated	5–7	10–18
10.0 mg	4.0– 4.5	3.0–3.6	Tegumental scar	15–20	6–12

Stereoscan observations on the untreated control flukes revealed normal body contours (Figure 1) with scale-like papillae having distinct shapes and ridges, on the ventral surface (Figure 2). The rim of both the oral and ventral sucker revealed radial corrugations. In contrast the flukes treated with 10 mg of the shoot extract per ml of PBS manifested a deformed body, particularly at the anterior sucker, with a shrunken and wrinkled surface tegument (Figure 3). Scar

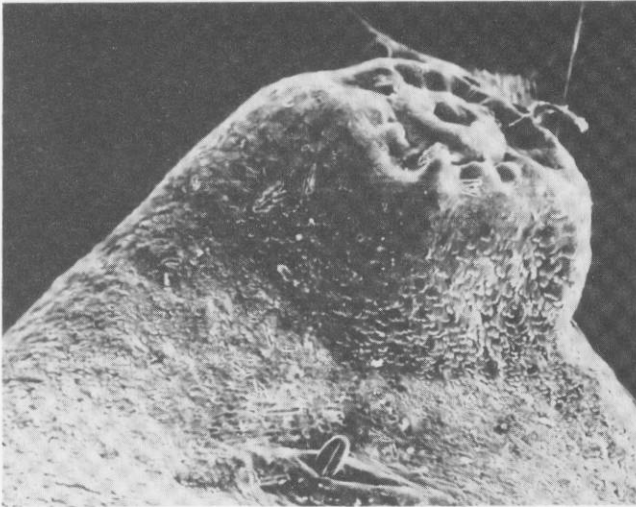
**Figures 1 to 5** *F. buski* showing scanning electron micrographs of normal (Figures 1 and 2) and treated (Figures 3 to 5) flukes.



**Figure 1** Anterior region of the body showing normal contour of oral sucker.  $\times 200$ .



**Figure 2** A portion of the ventral surface showing normal scales. x2,000.



**Figure 3** Anterior region of the body showing deformed oral sucker and anterior region. x140.



**Figure 4** A part of the ventral surface showing deformed tegumental scales at low magnification. x1,000.



**Figure 5** Deformed scales and tegumental scar at high magnification. x2,000.

formation was evident in the general body surface particularly at the base of each papilla. The extent (depth and diameter) of scarring was dependent on the time of incubation and the concentration of the extract used. The shape and size of the papillae changed markedly after treatment (Figures 3 and 4).

*Alpinia* shoots are regarded as common vegetables by Tripuri tribes in north-east India, and a concentrated aqueous extract of shoots is used as a traditional medicine to cure intestinal helminth infection. A crude extract of different plants, viz *Zingiber officinale*, *Zanthoxylum alatum*, *Lysimactia clethroides*, *Flemingia vestita* and *Canabis sativa*, were tested against *Schistosoma* sp *Fasciolopsis buski* and *Artyfechinostomum sufrartyfex*. Their trematocidal property in terms of a lethal effect has been established previously (Soh *et al.*, 1980; Singh *et al.*, 1982; Adewunmi *et al.*, 1990; Roy and Tandon, 1996, 1997; Tandon *et al.*, 1997).

The present study clearly indicates that the surface tegument of *F. buski* is sensitive to the crude extract of *Alpinia nigra*, which has the potential to paralyse and kill the worm *in vitro*. The normal microtopography of the fluke is provided with scale-like papillae and ridges having a distinct size and shape (Smith and Halton, 1983; Roy and Tandon, 1993). However, the treated flukes developed deformed scales with total disruption of the tegument. The severity of the disruption (scar formation) was dose and time dependent.

Destruction of the surface tegument was noticed in several trematodes of public health significance, viz *Clonorchis sinensis*, *Opisthorchis viverrini*, *Schistosoma japonicum* and *Metagonimus yokogawai*, when treated with the drug praziquantel *in vitro* and *in vivo*

(Mehlhorn *et al.*, 1983). However, the nature of the destruction was different from the present observations. Praziquantel initiated a sudden vacuolization of the tegument followed by extensive lesions leading to complete destruction of the tegument and suckers (Mehlhorn *et al.*, 1983). Destruction of suckers and hooks were noted in *Dactylogyrus extensus* following treatment with praziquantel (Schmahl and Mehlhorn, 1985). Vacuolization and pit formation was also recorded in *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski* when treated *in vitro* with root tuber peel extract of *Flemingia vestita* (Roy and Tandon, 1996). In digenetic trematodes the site of origin of vacuoles was found to be the basal lamina (Mehlhorn *et al.*, 1983), whereas in monogeneans it was the surface of the tegument where the vacuoles originated (Schmahl and Mehlhorn, 1985).

Disruption of tegumental integrity leading to vacuolization or pit formation are the result of disturbances in ion ( $\text{Ca}^{++}$ ) flux across the parasite membrane, and this was established in different trematode species (Bricker *et al.*, 1982; Mehlhorn *et al.*, 1983; Schmahl and Mehlhorn, 1985). Indeed, Sobhon *et al.* (1986) suggested that disruption of the surface tegument in *Opisthorchis viverrini* due to treatment with amoscanate was due to an osmotic imbalance resulting in impaired ion transfer. It seems that the crude extract of *A. nigra* probably causes changes in the permeability of the tegument leading to surface distortion and scar formation in *F. buski*.

In the present work, different concentrations of *A. nigra* extract were almost equally effective against *F. buski* when compared with the reference drug oxiclozanide. The severe deleterious effects caused by the shoot extract on the surface tegument may account for the loss of spontaneous movement, ultimately leading to death. However, further ultrastructural and biochemical studies on the fluke relating to the effect of the active principles of this plant are warranted to explain its mode of action.

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