In vitro anthelmintic activity of fresh tuber extract of *Flemingia vestita* against *Ascaris suum*

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SUMMARY. *Effects of crude extract of* F. vestita *fresh tubers on the motility and histomorphology of* A. suum *were studied by means of light and scanning electron microscopy after* in vitro *exposure to the extract. It emerged that the worms treated with 0.36% of the tuber extract became paralysed within 5 to 8 h. In the scanning electron microscopy, wrinkles and cracks on lips and body cuticle, and histologically perceptible disorganization of the somatic muscle layer were evident. In comparison, incubation with similar concentrations of the reference drug Mebex resulted in much faster acquisition of a paralytic state. The present* in vitro *data indicate that the tuber extract of this plant affects the motility and histopharmacology of* Ascaris *worms and functions as anthelmintic.*

INTRODUCTION

A recent estimate of the incidence pattern of some of the common diseases for India shows that the ascariasis affects well over 16% (nearly 140 million people) of the total population of the country. [1] Poverty, poor living status and insufficient hygiene, in general, are the factors found to be associated with the problem of worm infections. [2] In context of Meghalaya, a north-eastern state of India, recent studies revealed that besides the said factors, the ambient climatic conditions (*i.e.*, a high-rainfall and moderate temperature) and existence of large-scale piggeries are other factors unique to this region, which favour the risk of infection to community people for major part of the year. [3, 4]

There are now safe and effective drugs available for the treatment of ascariasis. However, an undeniable fact is that the majority of local people do not and cannot have access to these because of inadequate public health facilities in this area. The natives, particularly those of rural areas, are known to consume parts of several wild plants believing them to be workable against intestinal parasitic infections. [5] In the present study, we were interested to scientifically authenticate the efficacy of one such relatively commonly consumed plant, namely *Flemingia vestita*, against *Ascaris suum*. We report here our findings related to the motility and histomorphology, following *in vitro* exposure of the worms to different concentrations of the crude extract of the root tubers of this plant.

EXPERIMENTAL

Fresh tubers of *Flemingia vestita* were purchased in November from local markets in Shillong, India. They were washed gently with deionized water, peeled off thickly and the peel along with the pulp was allowed to boil for 3 h in deionized water in the ratio 1:3. The solution obtained was centrifuged at 5000 rpm for 25 min and the supernatant was extracted with ethyl acetate; sodium sulphate was used to dehydrate the ethyl acetate extract. A total of 6.64 kg of fresh tubers was extracted in this manner and yielded 7.20 g of ethyl acetate extract.

Live *Ascaris* worms were collected in 0.9% physiological buffered saline (PBS) from pigs slaughtered at local abattoirs. After thorough washing in PBS the worms were maintained

Treatment	Paralysis* (in hours)	
	F. vestita	Mebox
0.06%	43 - 50	4 - 6
0.18%	16 -18	3 - 4
0.36%	5 - 8	2

* Worms incubated in control medium showed physical activity till 78 to 102 h.

Table 1 - Effects of *Flemingia vestita* tuber extract and Mebox on motility of *Ascaris suum in vitro.*

in a climatic chamber at 37 ± 1°C. Each concentration was tested against two batches of six worms that are maintained separately in two Petri dishes containing 100 ml of medium. Dilutions were made in 1% dimethylsulphoxide (DMSO) in PBS, and 5 ml (final concentration 0.05%) of DMSO was added to the medium to give 0.06, 0.18, and 0.36 mg/ml concentrations of the tuber extract. Mebox® (mebendazole), a broad spectrum anthelmintic, wa used as reference drug. The latter was also dissolved in 1% DMSO and tested at concentrations similar to those of tuber extract. For each such concentration one Petri dish containing 5 ml of 1% DMSO (0.05%) in the medium served as control; the experiments were repeated for two times.

The efficacy of the extract was evaluated in terms of motility and histomorphological changes of the worms. Time taken for complete inactiveness of the worm was recorded and death was confirmed by dipping such worms in slightly warm water. Soon after the worms got paralysed, a set of those treated with 0.36% tuber extract along with the ones of controls were picked up and fixed in Bouin's solution or 5% buffered formalin. The Bouin's fixed material was processed for microtomy; the serial paraffin sections were cut at 6-7 μm thickness and stained with haematoxylin and eosin, whereas the worms fixed in formalin were subjected to scanning electron microscopic (SEM) studies by standard techniques. [6]

RESULTS

The worms maintained in PBS without the tuber extract showed physical activity till a period of 78 to 102 h, following which they became immobilized. In contrast, the worms incubated in the medium containing 0.06, 0.18, and 0.36% tuber extract became paralysed at 43 to 50, 16 to 18, and 5 to 8 h of incubation, respectively. Worms incubated in the medium containing Mebex showed considerably faster acquisition of a paralytic state (Table 1).

The surface fine topography of the worms incubated in the control medium appeared to be normal (Figs. 1,3); the three prominent lips have a smooth cuticle and appeared anchored to one another as if in a grasping state; the cuticle of the anterior region of the body reveals quite distinct and neatly arranged regular transverse striations. In contrast, in the worms incubated with 0.36% of plant extract, the lips appeared separated from each other and loosely compressed (Figs. 2,4) with prominently wrinkled surface; at the anterior extremity the body cuticle shows cracks and slight disorganization of the cuticular striations. Histologically, an irregular disorganization of the muscle layer was noticeable in the worms treated with 0.36% of the extract (Fig. 6).

DISCUSSION

The crude and/or purified extract of a number of plants *viz. Trigonella foenum-graecum, Zanthoxylum limonella,* etc., have been tested *in vitro* against

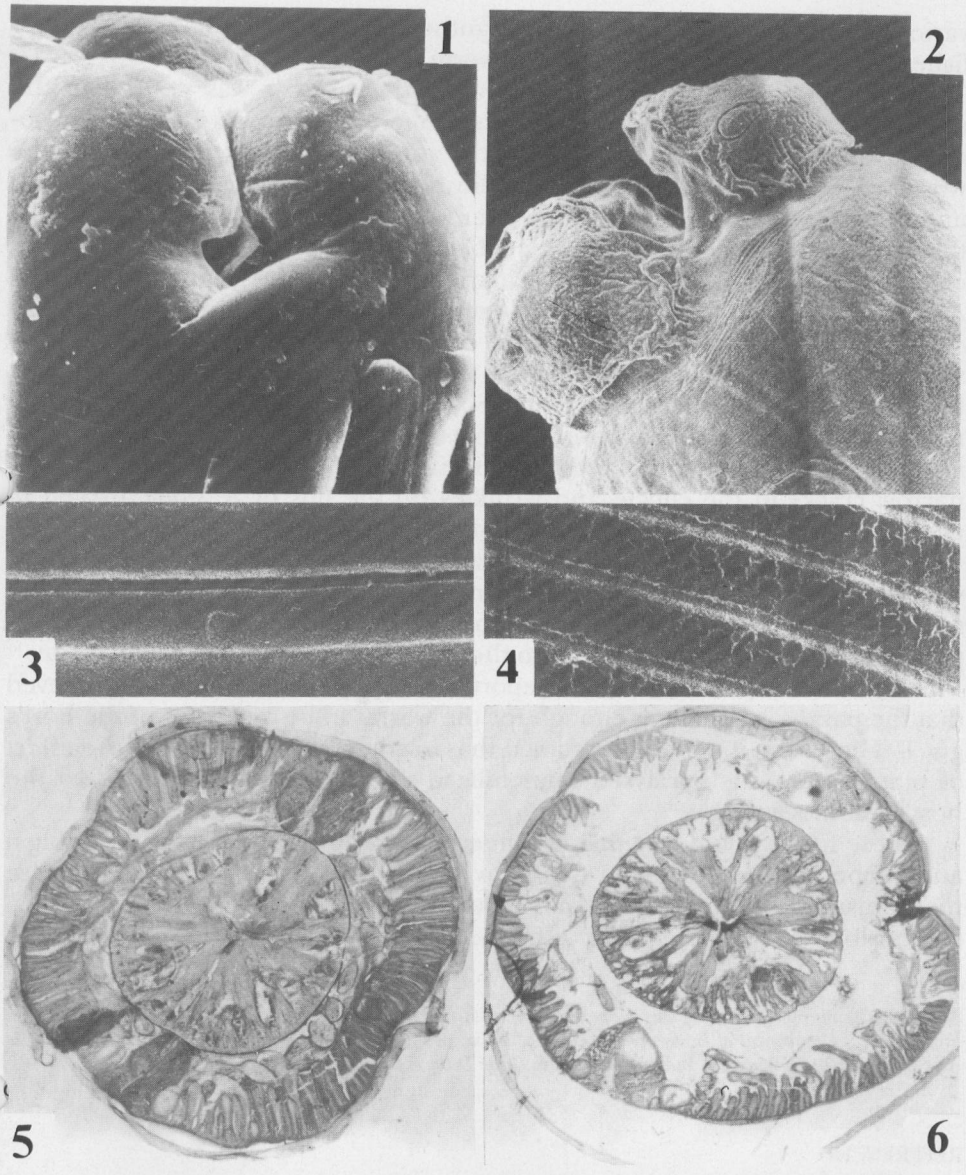

Fig. 1-6 - *In vitro* anthelmintic activity of tuber extract of *Flemingia vestita* on *Ascaris suum*.

Fig. 1-4 - Scanning electron micrographs.

Fig. 1 - Untreated control, anterior end showing three lips in grasping state. X 150.

Fig. 2 - Anterior end, tuber extract treated worm, showing collapsed surface of lips and wrinkled cuticle posterior to lip region. X 150.

Fig. 3 - Untreated control, pattern of cuticular ridges at anterior extremity. X 1800.

Fig. 4 - Treated worm, cuticular pattern at anterior extremity; note the distinct cracks and disorganization of transverse striations. X 2000.

Fig. 5-6. Light micrographs.

Fig. 5 - Normal transverse section through pharyngeal region. X 4.

Fig. 6 - Transverse section of the extract treated worm; note an irregular disorganization of muscle layer. X 4.

various helminth parasites and their anthelmintic efficacy has been established on the basis of lethal effect on worm parasites. [7, 8]

In the present investigation since an early paralysis was noticed in the tuber extract concentration of 0.36%, the same therefore was selected for observing the effects of the extract on the morphology and histology of the worms. A remarkable decrease of motility and contraction confined to to the anterior extremity and lips observed after incubation for 5 to 8 h with 0.36% of the extract indicates that the *F. vestita* tubers affect the motility and bring about changes in the body surface, particularly of the lip and cephalic region of *Ascaris* worms. The water extract of stem and leaves of *Trigonella foenum-graecum*, grown in Iran, when tested at 30% concentration against *Syphacia obvelata* showed mortality within 2 to 4, whereas the worms in control medium survived for about 10 h. [7] Similarly, Kaur and Sood [9] reported disorganization of the muscle layer and vacuolization of the intestinal epithelium in *Haemonchus contortus* exposed *in vitro* to tetramisole. Also in a SEM study [10] the appearance of narrow cracks in the cuticle and wrinkles on the lips of *A. suum*, treated *in vitro* with piperazine, has been reported.

During the present investigation it was found that the worms treated with various concentrations of the tuber extract became paralysed though they did not show any mortality for sometime to follow. This indicates that the extract possibly exerts a reversible action on the neuromuscular system of the worm. [11] The action of piperazine is also reported to be reversible and it is believed that the paralysis lasts long enough for the worms to be swept out of the host's gut. [12] Therefore, it may be presumed that in routine practice under the effect of plant tubers it is paralysed/immobilized worms that are evacuated by the host's gut movements.

These findings indicate that the tuber extract of *F. vestita* acts as vermifugal and support the need for an exhaustive investigation to establish its precise mechanism of action together with separating and identifying the principle(s) responsible for its anthelmintic efficacy.

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