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Anthelmintic Efficacy of *Flemingia vestita* (Fabaceae): Genistein-induced Alterations in the Ultrastructure of the Tegument in the Cestode, *Raillietina echinobothrida*

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To investigate the anthelmintic efficacy of *Flemingia vestita*, an indigenous leguminous plant of Meghalaya, the crude extract of its root-tuber peel and active chemical component, genistein, were tested in respect of the tegumental ultrastructure of the fowl tapeworm, Raillietina echinobothrida. Alterations and deformity in the structure of the tegument were revealed in the treated worms. Alterations in the contour of microtriches and disorganization of the tegumental region were conspicuously evident; the parasite exposed to the crude root-tuber peel extract showed deformed microtriches. The tegument, inner subtegumental region and muscle layers were the sites predominantly affected by the genistein treatment; severe distortion and disorganization occurred in the region of microtriches, and the inner subtegumental region showed pronounced vacuolization in comparison to control. The reference drug, praziquantel, also caused deformity in the parasite, somewhat at par with the genistein treatment.

Key words : Anthelmintic; *Flemingia vestita*; Genistein; Ultrastructure; Tegument; Cestode; *Raillietina echinobothrida*

Plant products provide and are gaining importance as an alternative to current medicinal practices involving chemotherapy (Didier *et al.*, 1938; Robinson *et al.*, 1990). Flemingia vestita Benth and Hooker (Family Fabaceae) is an indigenous medicinal plant of Meghalaya (North-East India). Its fleshy tuberous roots are consumed unpealed and raw to cure intestinal worm infections. Anthelmintic efficacy of this plant has been tested using several parameters. In-vitro treatment of the adult trematodes, viz., *Fasciolopsis buski* and *Artyfechinostomum sufrartyfex*, with the crude extract of the root-tuber peel of *F. vestita* induces paralysis and pronounced tegumental damage and disruption in the flukes (Roy and Tandon, 1996). While the crude extract of the root-tuber peel seems effective against trematode and cestode parasites, it did not show any effect on the viability of the nematode parasites (Tandon *et al.*, 1997). The major active component of the peel which has been identified to be genistein (Reo and Reddy, 1991) induces paralysis and deformity in the surface fine topography of the cestode, *R. echinobothrida* (Tandon *et al.*, 1997). Genistein was also shown to cause alterations in the activity of acetylcholinesterase and tegumental enzymes viz., acid phosphatase, alkaline phosphatase, adenosine triphosphatase and 5' -nucleotidase in this parasite (Pal and Tandon,1998, in press).

As an effect of anthelmintic drug action, at structural and cellular levels, alterations were significantly observable in the tegument of helminth parasites (Gonnert and Andrews, 1977; Grzywasz, 1980; Imai *et al.*, 1981; Mehlhorn *et al.*, 1981; 1983; Schmahl and Mehlhorn, 1985; Schmahl and Taraschewski, 1987; Bogoyavlenskii *et al.*, 1988; Zheng and Zhang, 1988; Xiao *et al.*, 1989). Destructive, degenerative and necrotic alterations to the absorption surfaces of *Fasciolc hepatica* were prominent after treatment with luxabendazole (Gorchilova *et al.*, 1990) and also with the deacetylated (amine) metabolite of diamphinethide (Anderson and Fairweather, 1995). Jiang and Xia (1992) noted ultrastructural alterations in *Paragonimus heteroticemus* treated with praziquantel and albendazole. Xu et al. (1994) reported tegumental damages in adult Schistosoma japonicum after *in vivo* treatment with levo-praziquantel.

The present study was set out to examine the internal changes that occur in the tegument of the cestode, *R. echinobothrida,* following treatment with genistein and may lead to the damage visible internally in the parasite.

MATERIALS AND METHODS

Drugs: The root-tuber peel extract and genistein were obtained from *F. vestita* following the procedure previously described by Tandon *et al.* (1997). Synthetic genistein (Sigma code no. G6649) was also used besides the pure genistein extracted from the plant material. Praziquantel was used as the reference drug.

Experimental parasites and treatment: The adult cestodes, *R. echinobothrida* (Megnin, 1888) were collected from the intestine of domestic fowl in 0.9% phosphate buffered saline

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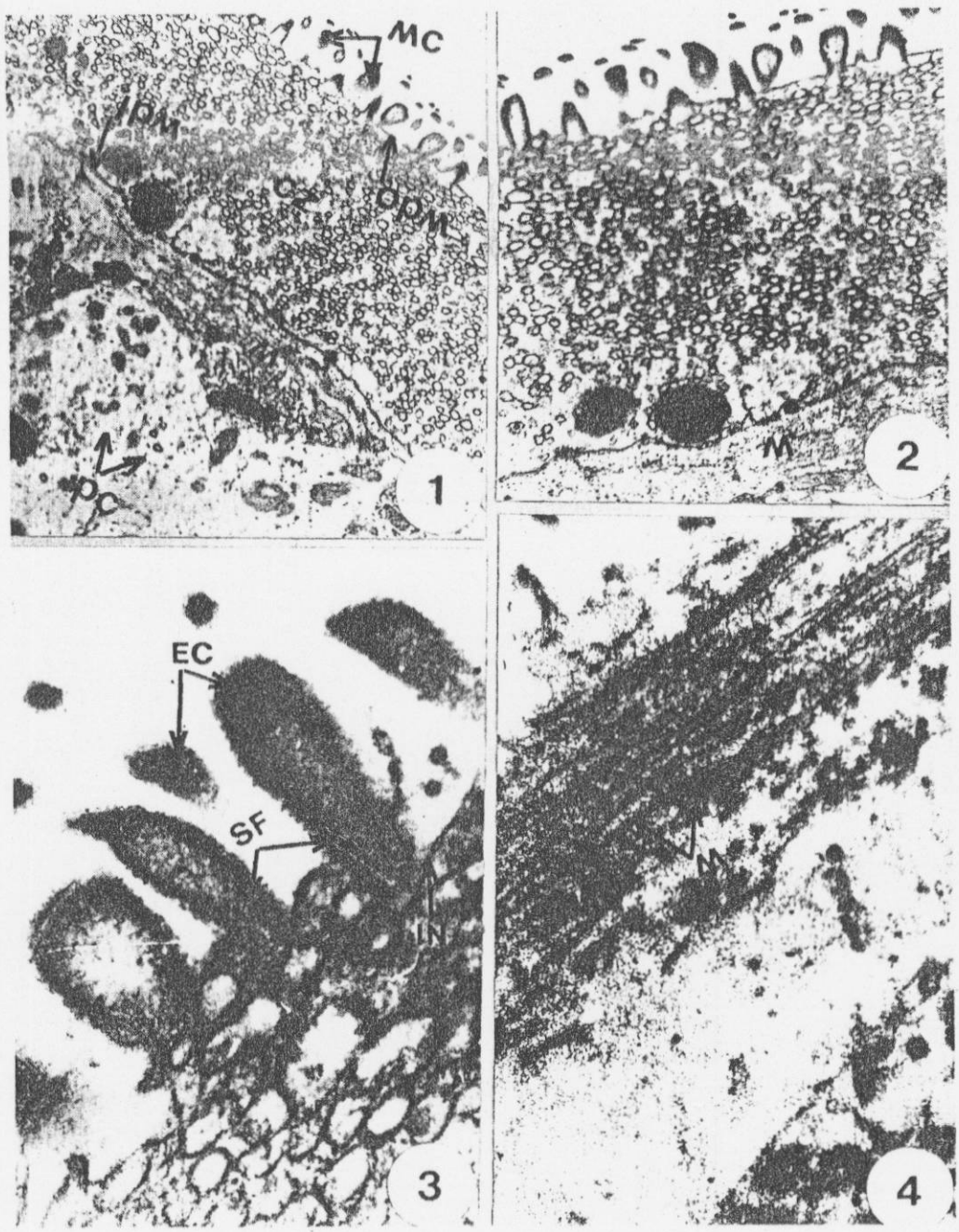

Fig. 1-4: Transmission electron micrographs of R. echinobothrida (control). Fig. 1,2: Tegument in ultrathin section, showing microtriches (MC), outer plasma membrane (OPM), cytoplasmic zone (CZ), inner plasma membrane (IPM), muscle components (M) and parenchymal cell (PC). X 7,800 and 12,500, respectively. Fig. 3: Microtriches as seen at higher resolution. The electron-dense cap (EC), the shaft (SF) and invaginations (IN) of outer plasma membrane are clearly seen. X 33,750. Fig. 4.: Muscular components, at higher resolution. X 50,000.

(PBS, pH 7-7.3), from freshly slaughtered hosts at local abattoirs in Shillong. The worms were incubated at 37 ± 1°C for treatment with 50 mg/ml crude extract, 0.5 mg/ml genistein and 0.01 mg/ml praziquantel, all made in dimethyl sulphoxide (DMSO) as per the dosages determined previously as causing paralysis of the worm within reasonable time of incubation (Tandon *et al.*, 1997). Three replicates for each incubation medium were used. After exposure to the treatment the paralyzed worms were processed for ultrastructural studies along with one set of control specimens maintained in 1% DMSO in PBS.

Transmission electron microscopy: The paralyzed cestode

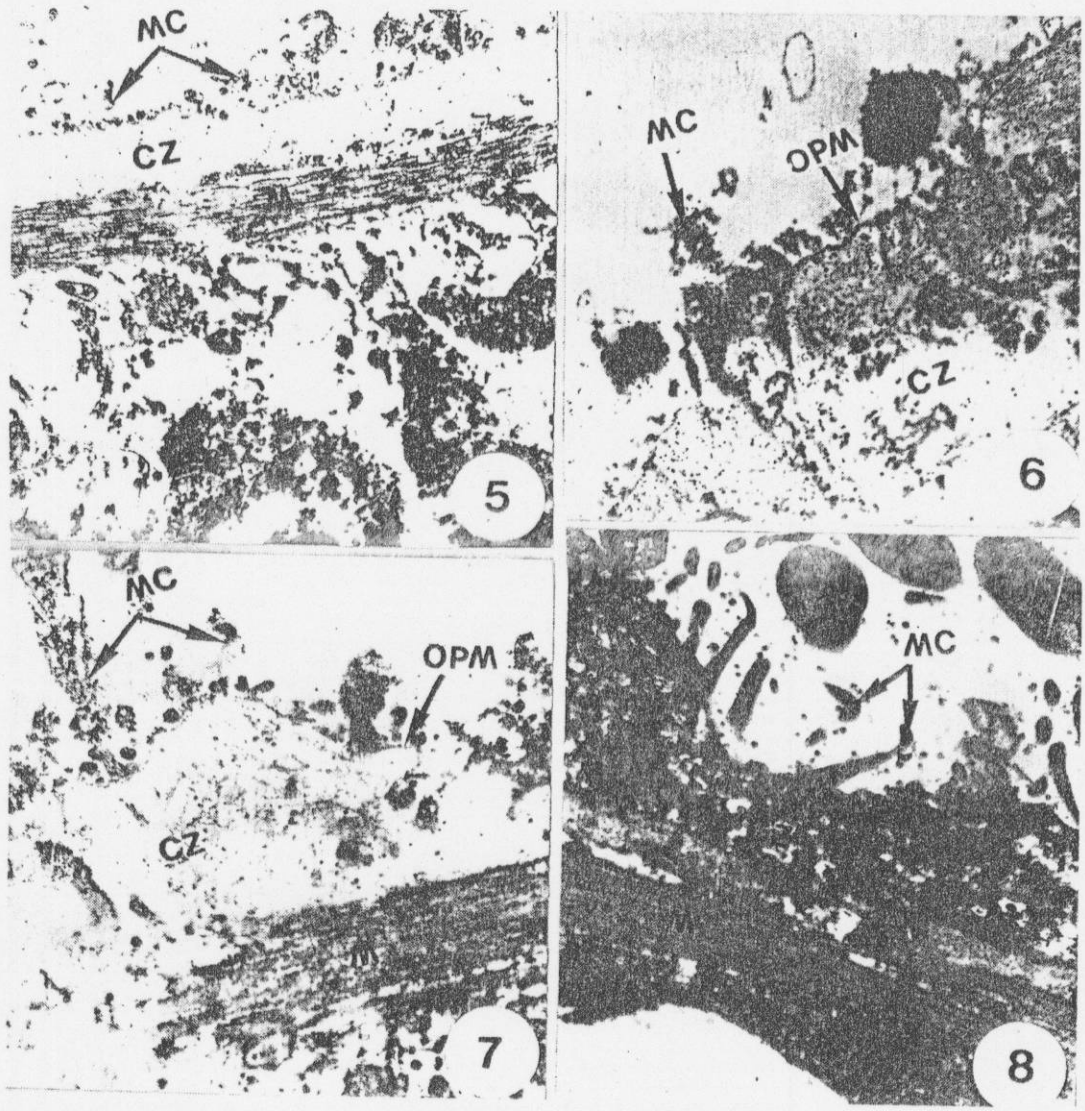

Fig. 5-8: R. echinobothrida exposed to test materials - transmission eletron micrographs. Fig. 5,6: Sectuib through the tegument of the parasite after treatment with crude root peel extract. Complete disorganization of the microdtriches, cytoplasmic zone and moscular components is evident. X 7,800 and 12,500, respectively. Fig. 7: Section through the tegument of the parasite after treatment with genistein. Severe distortion of tegument with disorganization of the muscutlature is revealed. X 17,500. Fig. 8: Same as Fig. 7, after treatment with praziquantel. X 12,500.

material was fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2 for 4 h. The samples were washed for 1 h in cacodylate buffer and postfixed in 1% osmium tetraoxide buffered in 0.1M sodium cacodylate for 1 h. All processing was undertaken at 4°C. After three washes, samples were dehydrated through graded acetone, transferred to propylene oxide, and embedded in araldite. Sections were cut on a LKB-2988 Bromma microtome, placed on 300 mesh copper grids and stained with uranyl acetate and lead citrate and examined with a JEOL-JEM-100 CX II transmission electron microscope.

RESULTS

Control: Histologically, the body of the cestode is covered with a thin tegument. Ultrastructurally, the body surface is elaborated by the presence of a cytoplasmic zone consisting of numerous ovoid vesicles and bordered externally and internally by an outer and inner plasma membrane, respectively. The outer plasma membrane has got inpushings towards the inner side in the form of small invaginations; it is also in continuity with the outer covering of the microtriches. Each microthrix is elongated and distinguished into two parts, an electron-dense cap and an electron-lucent shaft. The cytoplasmic zone is followed by a musculature zone where muscle cells are observed. The ultrastructural observations in the controls are prsented in Fig. 1-4.

Treated Worms: After treatment with genistein severe alterations were identifiable under transmission electron microscope, especially in the tegument of the parasite. The first sign of damage is vacuolization in the tegumental region. Conspicuous vacuolization of the tegument became obvious after 20 min of incubation in media containing crude extract. and was more pronounced after 60 min of incubation, indicating a time-dependent effect of genistein. Furthermore, the microtriches were affected at their apices, their surface coat was reduced to a thin layer and all the ovoid vesicles were destroyed and large holes were observed. The external plasma membrane was heavily damaged and formed distorted pieces. The subtegumental region showed severe distortion with disorganization of the cytoplasmic zone and tegumental musculature. Changes were also visible in the praziquantel-treated parasite; after treatment with 0.01 mg/ml for 0.47 hr (when paralysis set in) dramatic alterations in the tegument were observable somewhat at par with the genistein-treated parasite. Ultrastructural changes in the treated worms are presented in Fig. 5-8.

DISCUSSION

The present study demonstrates that the genistein component of *F. vestita* has a marked deleterious effect on the fowl tapeworm, *R. echinobothrida*. In the treated worm, alterations in the contour of microtriches and disorganization of the tegumental region were conspicuous; while the microtriches exhibited deformity and clumping, the tegumental region showed pronounced vacuolization and loss of muscular components in comparison with the control. In *Taenia taeniformis* and *Hymenolepis nana*, Borgers *et al.* (1975) and Verheyen et al. (1976) reported an increase of undefined secretory substances in the golgi areas. Isatin in combination with bunamidine produced hypervacuolization of the tegumental cytoplasmic syncytium in the secondary cysts of Echinococcus multilocularis (Hart *et al.*, 1977). Becker *et al.* (1981) also reported vacuolization in the syncytial zone as an effect of praziquantel on several species of cestodes including *E. multilocularis*. Tegumental alterations and severe vacuolization on exposure to flukicidal drugs have been observed in several species of trematodes (Schmahl and Taraschewski, 1987; Zheng and Zhang, 1988; Gorchilova *et al.*, 1990; Jiang *et al.*, 1990; Jiang and Xia, 1992; Schmahl, 1993; Stitt and Fairweather, 1993; Xu *et al.*, 1994); the extent of damage induced was reported to increase with exposure time. Similar changes were also noticed in the tegument of cestode parasites (Imai *et al.*, 1981; Delabre-Defayolle *et al.*, 1989; Perez *et al.*, 1994). Whereas in digenetic trematodes the vacuoles were found to originate from the basal lamina (Mehlhorn *et al.*, 1993), in the monogenean Dic!idophora spp., the site of their origin was the surface of the tegument (Schmahl and Mehlhorn, 1985). Changes also occurred in the tegumental cells, which were indicative of a disruption in the synthesis and release of tegumental secretory bodies. The ultrastructural changes in the tegument are linked to a possible mode of action of the drug as an inhibitor of protein synthesis (Anderson and Fairweater, 1995). Vacuolization and contraction in the parasite body surface have been attributed to the levels of Ca^{2+} concentration in the media used (Bricker *et al.*, 1982; Xiao *et al.*, 1984), imbalance in osmosis and alterations in the transmembranous ion flux consequent to treatment with the drug (Schmahl and Mehlhorn, 1985; Sobhon *et al.*, 1986). Disruption of the cuticular interface and / or intestinal epithelium and degenerative changes even in the subcuticular region have been reported in several nematode species exposed to anthelmintics in vitro (Kaur and Sood, 1983; Bogoyavlenskii *et al.*, 1988; Semenkov and Akil'zhanov, 1988; Xiao *et al.*, 1989; An, 1990; Storte *et al.*, 1990; Mackenstedt *et al.*, 1993; Rothwell and Sangster,1996).

Perhaps genistein, the chemical component in the root-tuber peel of *F. vestita*, might bring about permeability changes in the tegument of the worm. The deleterious alterations in the tegumental architecture of *R. echinobothrida* may be responsible for the loss of spontaneous movement and paralysis and hence detachment from the host's gut. The genistein component of F. vestita, thus, seems to have a vermifugal action.

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