

Electron Microscopic Observations on the Alterations of Tegumental Surface of *Raillietina echinobothrida* Treated With Root-Peel Extract of *Potentilla fulgens*

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KEY WORDS *Potentilla fulgens*; anthelmintic; electron microscopy

ABSTRACT *Potentilla fulgens*, a member of the family Rosaceae, has been known since ancient times in Northeast India for its antiparasitic, antitumour and antidiabetic properties. The crude ethanolic extract of the plant was assayed against *Raillietina echinobothrida*, the intestinal cestode parasite of domestic fowl to authenticate the putative anthelmintic efficacy and cestocidal potential in particular of the plant. Treatment of the worm in-vitro with 25, 50 and 100 mg of crude extract per ml of phosphate buffered saline caused paralysis leading to death taking 4.02–4.20, 3.05–3.75 and 2.05–2.83 h respectively. Scanning electron microscopic observations on treated worms revealed deformation of surface architecture, particularly in scolex and surface microtriches. Severe distortion and disorganization of the tegumental and subtegumental layer along with cytoplasmic organelle was also observed under transmission electron microscope. The possible use of the plant as a potential anthelmintic against cestode parasite is discussed. *Microsc. Res. Tech.* 75:1000–1005, 2012. © 2012 Wiley Periodicals, Inc.

INTRODUCTION

According to World Health Organization, 80% of the world's population depends on plants for their primary health care (Farnsworth et al., 1985). India is one of the worlds 12 regions having the largest biodiversity. It has 45,000 plant species of which 15,000–20,000 possess proven medicinal value (Das et al., 2006). *Potentilla fulgens* L. (Family: Rosaceae), locally known as “Lynniang” in Meghalaya, India has been known since ancient times for its curative properties. Aqueous extract of root-peel of the plant have been used in traditional medicine for the treatment of cancer, diabetics, bacterial and parasitic infections (Rosangkima and Prasad, 2004; Syiem et al., 2002). The genus *Potentilla* is reported to contain high amount of tannins, and a lesser extent of polyphenols, triterpenoids and flavonoids (Tomczyk and Latte, 2009). In the course of screening of antiparasitic ethnomedicinal plants of Northeast India, it was observed that though the plant is known as antiparasitic, particularly in remote areas of Meghalaya, no scientific record is available about the antiparasitic efficacy of the plant. In India, chickens play an important role in the provision of animal protein for the population, as both poultry meat and eggs are affordable sources. However, *Raillietina echinobothrida* infecting the poultry make them unfit for human consumption, further, heavy infections may cause death of young birds apart from the loss of egg production in laying chickens (He et al., 1990; Ojok, 1993). Therefore, the present study was undertaken to investigate the ultrastructural alterations induced by crude ethanol root peel extract of *P. fulgens* in *Raillietina echinobothrida*, an economically important cestode parasite of poultry (Eslami et al., 2009; Magwisha et al., 2002).

MATERIALS AND METHODS

Preparation of Extract and Treatment

Fresh roots of *P. fulgens* were collected from local market in Shillong, India. They were washed with distilled water, peeled and dried at 50°C in an oven. After grinding in an electrical grinder, crude ethanol extract of the root peel was prepared through reflux and filtration method as described earlier (Roy et al., 2009). Adult *R. echinobothrida* were collected in 0.9% phosphate buffered saline (PBS) from freshly sacrificed domestic fowl at local abattoirs, exposed to different concentrations of crude plant extract, viz. 25, 50, and 100 mg per ml of PBS (three replicates for each concentration) having five parasites at each concentration in 0.1% dimethyl sulfoxide (DMSO). Praziquantel (PZQ), a broad-spectrum reference drug was used at concentrations of 0.01 and 0.001 mg/mL. Control incubation consisted of worms in PBS with 0.1% DMSO only. Time taken for complete inactivation of the parasites was recorded and the death was confirmed by dipping the worms in slightly warm water. Totally, 50 mg dosage was selected for further studies as alterations of surface topography were clearly visible at this concentration of extract treatment.

All data are presented as means plus or minus the standard error (SE) of the mean. Comparison of the mean values between the treated and control groups was made using student's *t*-test, and probability value considered significant at $P < 0.05$.

Electron microscopy

Soon after paralysis at a dose of 50 mg extract/mL PBS, scolex region and mature proglottids of treated

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 Received 23 June 2010; accepted in revised form 26 October 2010
 DOI 10.1002/jemt.20972

Published online 9 March 2012 in Wiley Online Library (wileyonlinelibrary.com).

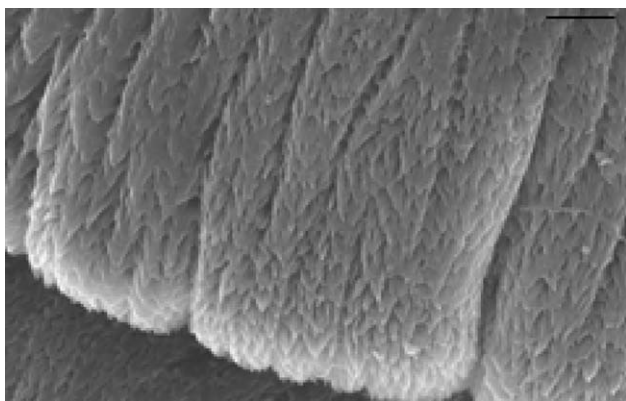


Fig. 1. Scanning electron micrograph of *R. echinobothrida* - Control. Typical surface topography with densely packed arrays of microtriches covering the entire body. Scale bar 5 μm .

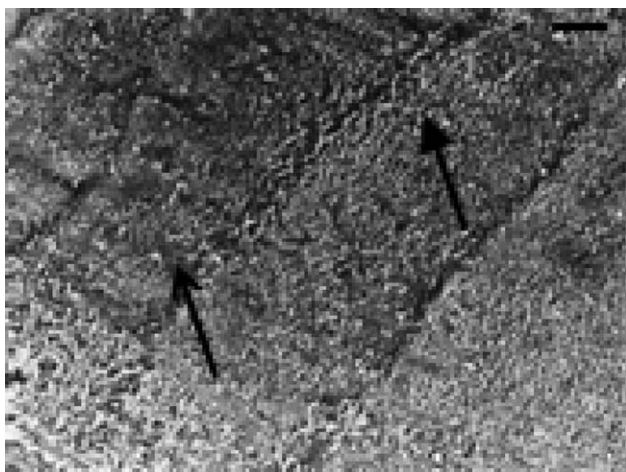


Fig. 2. Scanning electron micrograph of *R. echinobothrida* treated with crude alcoholic extract of *P. fulgens*. Parasite tegument shows irrevocable destruction with clumping (arrow) and erosion (open arrow) of microtriches. Scale bar 10 μm .

and also control worms were fixed in modified Karnovsky's fixatives, followed by postfixation in 2% OsO_4 buffered in 0.2M sodium cacodylate. For scanning electron microscopy, fixed specimens were dehydrated through increasing grades of acetone followed by air drying after treatment with tetramethyl silane as detailed by Roy and Tandon (1991), gold coated and viewed using JSM-6360 scanning electron microscope at an electron accelerating voltage of 10–15 kV. For transmission electron microscopy (TEM), fixed specimens were dehydrated in graded series of acetone, cleared in propylene oxide, and embedded in araldite, as per the standard procedure (Roy et al., 2009). Ultrathin sections were viewed in a JEM 100CX II (JEOL) transmission electron microscope operated at 80 kV.

RESULTS

Both the plant extract and praziquantel showed dose-dependent lethal cestocidal efficacy. *R. echinobothrida* exposed to 25, 50, and 100 mg of *P. fulgens* (root-peel) extract per milliliter of PBS showed early paralysis followed by death in 4.02–4.20, 3.05–3.75, and 2.05–

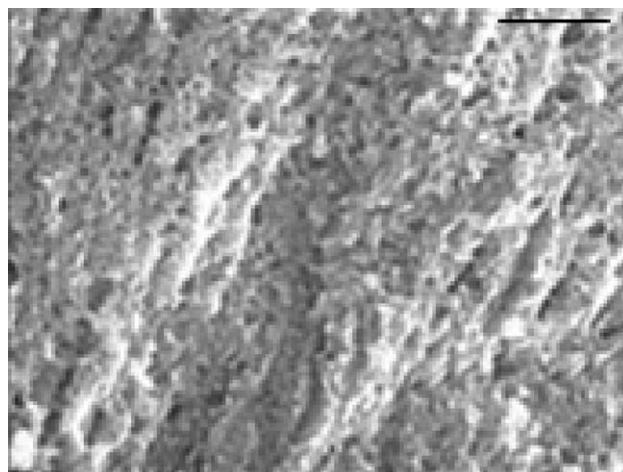


Fig. 3. Scanning electron micrograph of *R. echinobothrida* treated with PZQ. Tegument shows intense clumping of microtriches. Scale bar 20 μm .

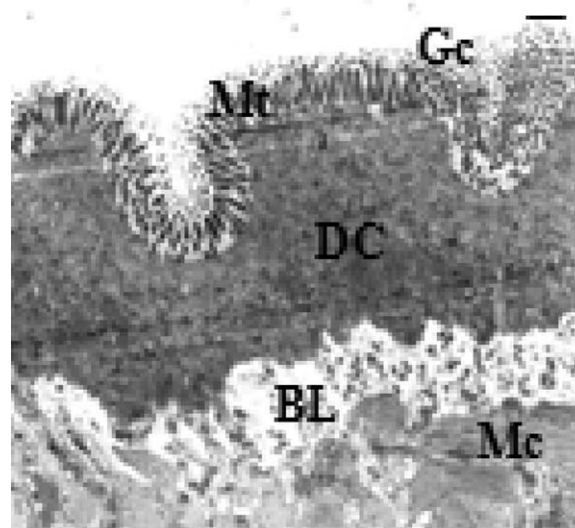


Fig. 4. Transmission electron micrograph of *R. echinobothrida* - Control. Tegument with glycocalyx coat (Gc), microtrich layer (Mt); distal cytoplasm (DC) electron-dense with tegumental discs, nondisrupted basal lamina (BL), and well organized subtegumental muscle blocks (Mc). Scale bar 0.5 μm .

2.83 h, respectively. In contrast, the control worms maintained in PBS showed physical activity up to 72 h. Parasites incubated in the medium containing praziquantel showed more rapid paralysis.

Morphologically, control *R. echinobothrida* showed normal surface topography with densely packed arrays of microtriches covering the entire body (Fig. 1). Treated worms revealed deformed scolex and irrevocable destruction of microtriches and general tegumental surface leading to total alteration of surface architecture (Fig. 2). Filamentous nature of the microtriches was lost and they were clumped in the form of irregularly distributed clustered protuberances on the surface of the tegument on treatment with PZQ (Fig. 3).

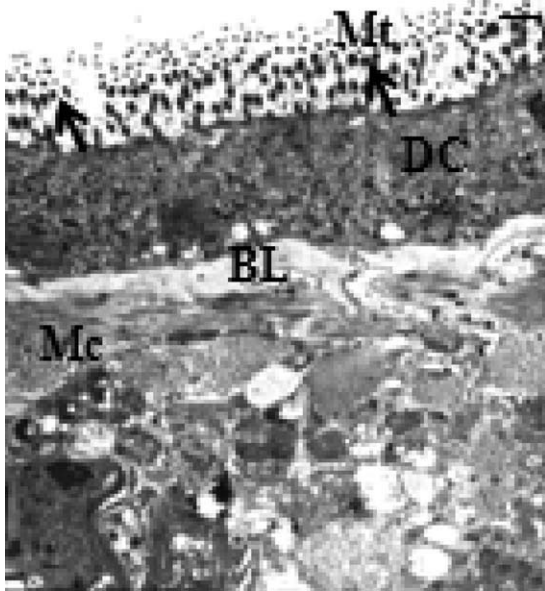


Fig. 5. Transmission electron micrograph of *R. echinobothrida* treated with crude alcoholic extract of *P. fulgens*. Magnified view of the tegument showing disruption of microtrich layer and erosion of the same at some places (arrows); thinning of basal lamina and fragmentation of muscle blocks. Scale bar 0.5 μ m.

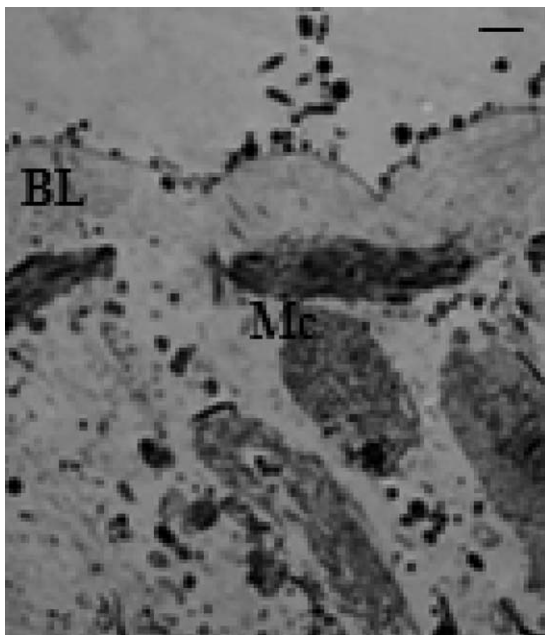


Fig. 6. Transmission electron micrograph of *R. echinobothrida* treated with PZQ. Section showing loss of tegument along with release of distal cytoplasm to the exterior. Scale bar 0.5 μ m.

Transmission electron microscopic observations on the tegument of control cestode revealed normal architecture with presence of numerous microtriches with pointed electron dense cap. Extensive glycocalyx can be seen as flocculent material over the microtriches. Below, in the main syncytial layer of tegument (distal cytoplasm), numerous dense disc-shaped bodies can be

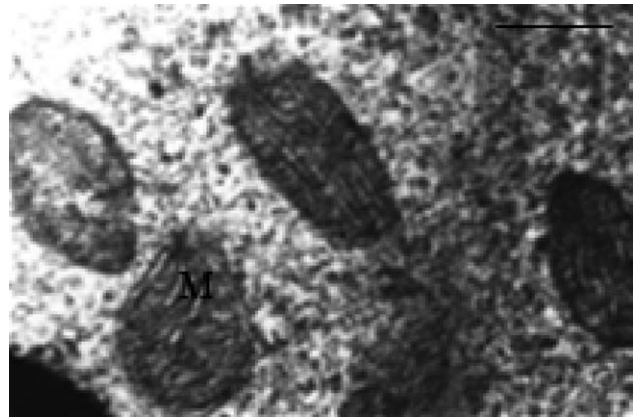


Fig. 7. Transmission electron micrograph of *R. echinobothrida* - Control. Electron-dense mitochondrial (M) matrix with prominent cristae. Scale bar 0.5 μ m.

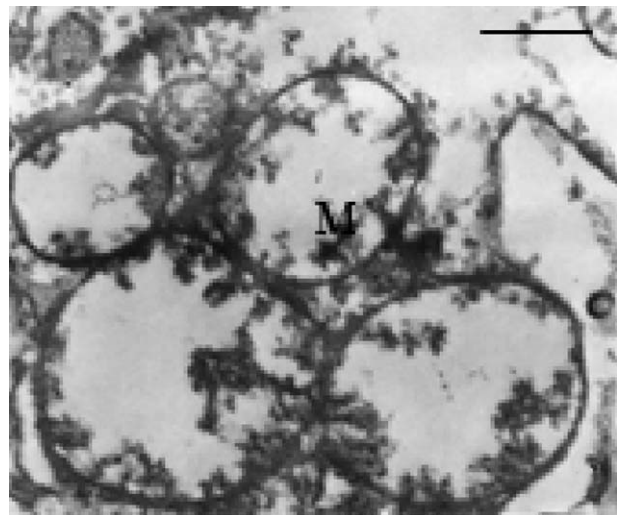


Fig. 8. Transmission electron micrograph of *R. echinobothrida* treated with crude alcoholic extract of *P. fulgens*. Remnants of cristae were not much disturbed but a lot of vacuolization could be seen in-between throughout the subtegumental region and also in the distal cytoplasm (Fig. 5). PZQ brought about a loss of tegument along with release of materials to the exterior (Fig. 6).

seen. Basal lamina marks the joining point between the distal cytoplasm and the subtegumental region. Densely stacked circular and longitudinal muscle layers lie just beneath the basal lamina (Fig. 4). Plant-extract treated tegumental surface was observed to have deformed microtriches. The distal cytoplasm became more condensed and shrunk up. Basal lamina though present, was very thin and cracked up at many places. The circular and longitudinal muscle blocks were not much disturbed but a lot of vacuolization could be seen in-between throughout the subtegumental region and also in the distal cytoplasm (Fig. 5). PZQ brought about a loss of tegument along with release of materials to the exterior (Fig. 6).

Mitochondria of control cestodes had normal surface contour and were concentrated mostly in the distal cytoplasm (Fig. 7). Extract-treated parasite showed a

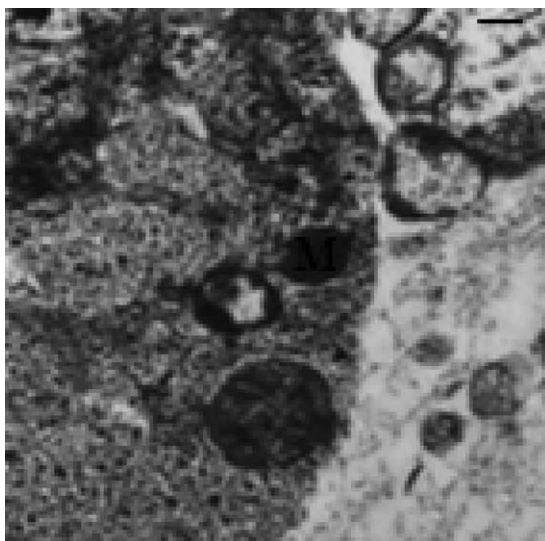


Fig. 9. Transmission electron micrograph of *R. echinobothrida* treated with PZQ. Treated parasite shows abundance of vesiculated mitochondria in tissue. Scale bar 0.5 μ m.

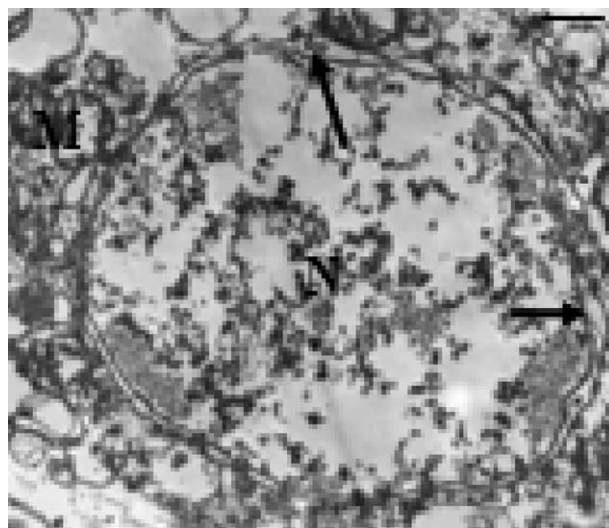


Fig. 11. Transmission electron micrograph of *R. echinobothrida* treated with crude alcoholic extract of *P. fulgens*. Nucleus (N) showing swelling of perinuclear space (arrows) and loss of nuclear material. Acristate mitochondria (M) in the cytoplasm around the nucleus. Scale bar 0.5 μ m.

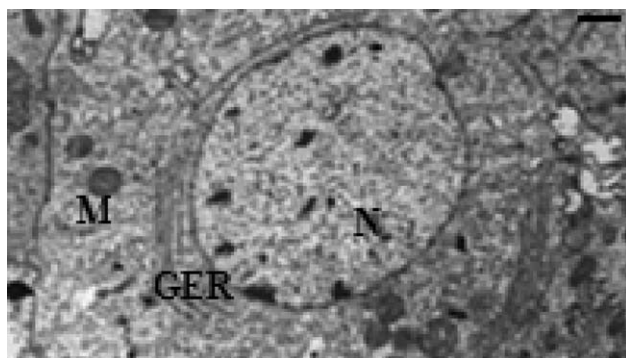


Fig. 10. Transmission electron micrograph of *R. echinobothrida* - Control. Tegumental cyton showing a typical nucleus with a double layered membrane. Mitochondria (M) and other cell organelles like granular endoplasmic reticulum (GER) can also be seen to be in abundance within the cytoplasm. Scale bar 0.5 μ m.

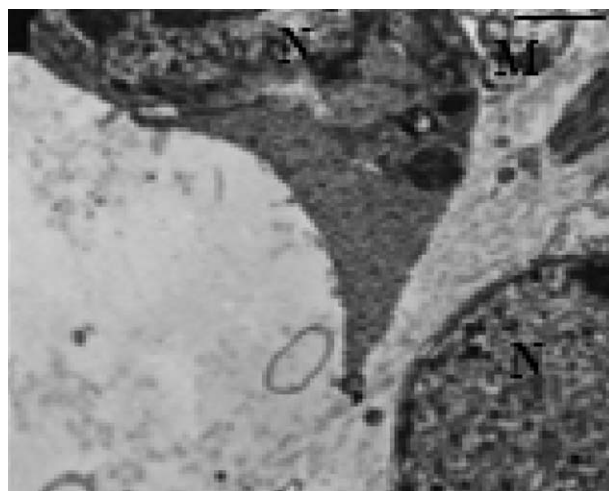


Fig. 12. Transmission electron micrograph of *R. echinobothrida* treated with PZQ. Loss of connections between the subtegumental cells of the parasite can be seen. Scale bar 0.5 μ m.

large number of morphologically irregular mitochondria having no cristae or inner matrix (Fig. 8). There was an abundance of slightly vesiculated mitochondria within the tissue of parasites treated with PZQ (Fig. 9).

Subtegumental cells of control worms showed typical double membraned nuclei, continuous with granular endoplasmic reticulum (GER). The material inside the cells as well as in the nuclei was dense and granular. Numerous mitochondria could also be seen within the cells (Fig. 10). *P. fulgens* treated subtegumental cells showed slightly swollen nuclei with puffed perinuclear space. Little remnants of nuclear material and GER were seen. Cellular mitochondria were mostly acristate, though abundant (Fig. 11). On treatment with PZQ, the nuclei did not show much damage, though slight loss of connections between the subtegumental cells were noted (Fig. 12).

DISCUSSION

On assessment of the survivability and electron microscopy of the treated parasites, it became apparent that the root-peel extract of *P. fulgens* indeed possessed anthelmintic property against the test parasite *R. echinobothrida*. The dose-dependent effect of the plant extract indicated potent necrotic impact on the cestode. Although the time taken for each concentration to effectively kill the parasite is comparatively longer than that for the reference drug, the results are highly significant. Similar to the present observation, evaluation of anthelmintic efficacy through observations on the viability of the worms upon exposure to extract of several botanicals like *Zinziber officinale*, *Mallotus*

philippinensis, *Acacia auriculiformis*, *Psidium guajava*, *Strobilanthes discolor*, *Trifolium repens*, *Artemisia ambrosifolia*, *Xanthoxylum rhetsa*, *Solanum myrsicanthum*, *Carica papaya*, *Hedera helix*, *Acacia oxyphylla*, and *Cocos nucifera*, were well documented by several workers (Adewunmi et al., 1990; Akhtar and Ahmed, 1992; Chakraborty et al., 1995; Eguale et al., 2007; Goto et al., 1990; Ghosh et al., 1993, 1996; Oliveira et al., 2009; Roy et al., 2007a; Tangpu and Yadav, 2003; Tangpu et al., 2006). Likewise, cestocidal properties of *Flemingia vestita* and its active component genistein, *Acacia oxyphylla*, *Millettia pachycarpa*, and *Lysimachia ramosa* were established through scanning and transmission electron microscopic observations on *R. echinobothrida* (Challam et al., 2010; Dasgupta et al., 2010; Roy et al., 2007a,b, 2008, 2009; Tandon et al., 1997).

In vitro exposure of *R. echinobothrida* to various concentrations of the crude ethanol extract of the plant causes large scale tegumental and subtegumental damage. The basal lamina of the tegument is speculated to act as site for active mitochondrial biogenesis (Smyth, 1996). However, in extract-treated parasites, a progressive thinning and disappearance of basal lamina along with disappearance of mitochondrial cristae and matrix as observed in the present investigation are indicative of the derogatory effect of the plant on the worm. In a similar experiment, erosion of basal lamina and disappearance of mitochondria was also observed in cestode parasites treated with crude ethanol extract of *Acacia oxyphylla* and its acetone fraction (Dasgupta et al., 2010). Reduction in GER, swelling of nuclear membrane, disruption of chromatin material and vacuolization in nucleus, as observed in the *P. fulgens*-treated cestode, are indicative of protein synthesis inhibition (Stitt and Fairweather, 1996).

Presence of densely packed mitochondria in the distal cytoplasm as well as in the subtegumental cells as observed in the present investigation was also recorded earlier in metabolically active tegumental syncytium of trematode and cestode parasites (Roy et al., 2008, 2009; Smyth, 1996). Generally, mitochondria are tubular in shape and circumstantial evidence suggests that there is an intricate structure-function relationship between mitochondrial morphology and energy-producing activity (Bach et al., 2003; Mannella et al., 1997; Nakada et al., 2001). Therefore, changes in mitochondrial morphology as observed in the treated parasites, could disrupt the local energy supply (McConville et al., 2008; Yoon, 2005), leading to paralysis and death of parasites.

The tegumental surface of *R. echinobothrida* thus seems to be a target site for the active components of *P. fulgens* to exert its vermifugal or vermicide effect. Though the active component of the plant responsible for anthelmintic activity is not known, the most pharmacologically active components of the genus *Potentilla* are high amount of tannins and to a lesser extent of triterpenes (Tomczyk and Latte, 2009). Tannins are reported to be relatively stable in the digestive tract of animals and are rarely have toxic effect (Haring et al., 2007). Condensed tannins with proven anthelmintic activity have been reported from several anthelmintic plants (Cenci et al., 2007; Kahiya et al., 2003), and are known to inhibit endogenous enzyme activities (Iqbal et al., 2002; Wang et al., 1996).

ACKNOWLEDGMENTS

The authors are grateful to the Head, SAIF, North-Eastern Hill University for providing SEM and TEM facilities. Infrastructural facilities provided by UGC-DSA program to the Department of Zoology and UPE-Biosciences program to the School of Life Sciences, NEHU, Shillong are also acknowledged. The authors are also extremely thankful to the two anonymous reviewers who helped immensely in the improvement of the manuscript.

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