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Anthelmintic Efficacy Of *Flemingia vestita* (Fabaceae): Genistein Induced Effect On The Nervous Components In Two Digenetic Trematodes

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The anthelmintic efficacy of crude root peel extract of *Flemingia vestita* (having usage in traditional medicine in Meghalaya) and its active component, genistein was tested *in vitro* with regard to the esterase activity in the swine digenetic flukes, *Fasciolopsis buski* and *Artyfechinostomum sufrartyfex*. Followig the histochemical localisation of non-specific esterases (NSE) and specific cholinesterase (ChE) the nervous system *in toto* could be traced in both the parasites and the arrangement of nerves in the central and peripheral nervous system (CNS, PNS), notably the paired cerebral ganglia, dorsal cerebral commissure, the posterior longitudinal nerve cords and the subtegumental nerve plexus visualised. Following an exposure to the plant crude extract or genistein *in vitro*, a sharp decline in the visible stain intensity was observable associated with the cholinergic components in both the digenean parasites, suggesting a reduced esterase activity in these sites. The plant-derived components seem to be causative in bringing about alteration in the activity of the specific ChE (which is acetylcholinesterase in these parasites).

Key words : Anthelmintic, *Flemingia vestita*, genistein, esterase activity, nervous system, trematode parasite, *Fasciolopsis*, *Artyfechinostomum*

The tuberous roots of *Flemingia vestita* (Fabaceae) have an anthelmintic usage in traditional medicine system practised in Meghalaya (North-east India). The crude extract of the root peel and its major active component, genistein, were shown to induce flaccid paralysis and pronounced tegumental damage and disruption in the cestode, *Raillietineaechinobothrida* in an *in vitro* exposure (Tandon *et al.*, 1997). The plant-derived components also caused alterations in the activity of tegumental enzymes (Pal and Tandon, 1998a). A pronounced decline was also recorded in the activity of the non specific esterases (NSE) and cholinesterases (ChE) in the treated cestode (Pal and Tandon, 1998b). Esterases, acetylcholinesterase (AChE) in particular, are closely associated with the cholinergic components of the central and peripheral nervous system in trematodes, and their role in nervous co-ordination has been established (Maule *et al.*, 1993; Halton and Gustafsson, 1996). A reduced esterase activity in the cestode following treatment with the root peel extract/genistein indicated towards cholinergic neurotransmitters as the plausible target of drug action.

For ascertaining the anthelmintic efficacy of active components of *F. vestita, in vitro* treatment of the adult trematodes, *Fasciolopsis buski* and *Artyfechinostomum sufrartyfex,* with the crude root peel extract showed gradual decline in the motility leading to paralysis and deformity in the tegumental architecture of the flukes (Roy and Tandon, 1996). We further studied alterations in the NSE and ChE activity in these parasites under the influence of the plant-derived materials. Localisation in toto of the cholinergic components of the nervous system in the two fluke species with the histochemical demonstration of the NSE and ChE activities forms the basis of this communications.

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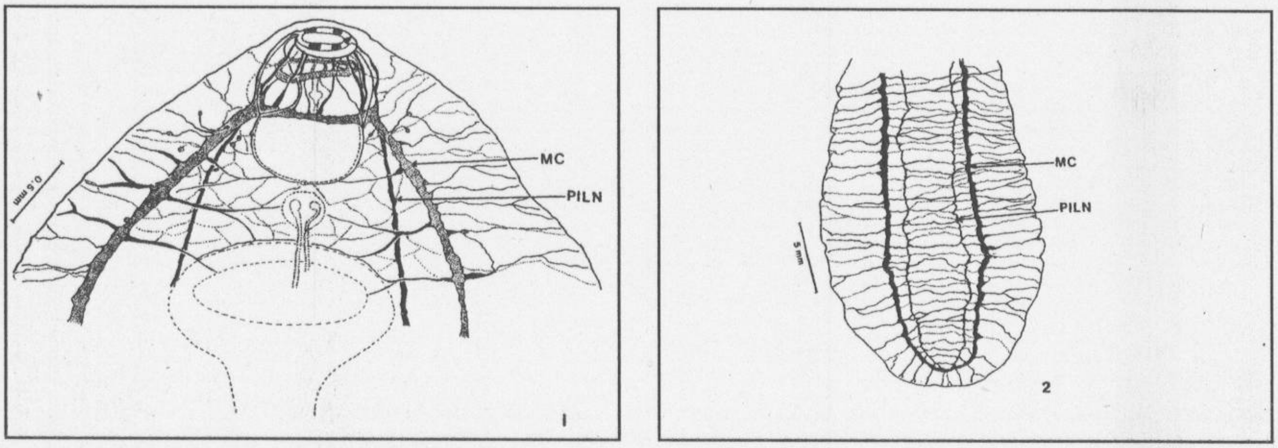
MATERIALS AND METHODS

Test material: The root peel extract and active component, genistein, were obtained from the tuberous roots of *F. vestita* as per the earlier described procedure (Tandon *et al.*, 1997); synthetic genistein (SIGMA, G 6649) was also used.

Treatment of Parasites: Adult *F. buski* and *A. sufrartyfex* were collected from the intestines of freshly slaughtered pigs at local abattoirs in 0.9% phosphate buffered saline (PBS). The flukes were incubaeted at 37$\pm$1°C for treatment with 20 mg/ml crude extract or 0.5 mg/ml genistein in PBS with 1% DMSO; at these concentrations the treated parasites showed paralysis within a shorter time than when lower doses of the test materials were used (Roy and Tandon, 1996). Immediately after the onset of the paralytic state in them, the flukes were processed for histochemical preparation along with the control specimens (maintained in 1% DMSO in PBS).

Histochemical processing: The live flukes were washed in PBS, flattened between two glass slides or under gentle pressure of a thin cover glass and fixed in 10% neutral buffered



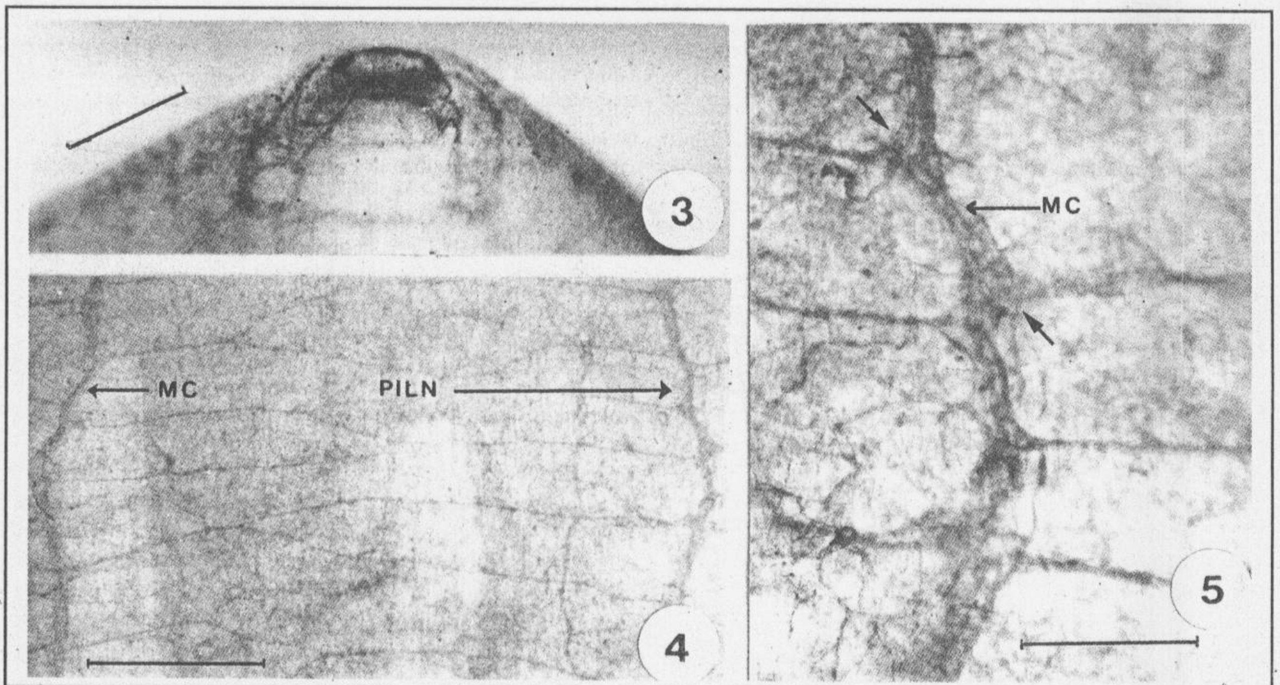
Figs. 1, 2 : Schematic representation of the nervous organization in *F. buski*: 1. the anterior part of the worm 2. In the posterior half of the body

formalin (NBF) at 4°C. Staining for NSE was done following Holt and Withers (1952) method using 5-bromoindoxyl acetate as the substrate in the incubation medium as per the procedure described earlier (Lyngdoh and Tandon, 1992). For ChE, acetylthiocholine iodide was used as the substrate following the method of Gomori (1952) with slight modifications as described by Rahemo and Gorges (1987). The NSE and ChE were visualised by the deep indigo and brown staining, respectively.

RESULTS

The organisation *in toto* of the cholinergic components of the nervous system could be visualised by localising reactivity for NSE and ChE. The cholinergic neuronal components are found organised in two recognisable parts- the central nervous system comprising the bilobed brain and paired main nerve cords (MCs), and the peripheral consisting of all the minor nerves and the nerve plexuses.

Fasciolopsis buski (Figs. 1-7): The two cerebral ganglia



Figs. 3-7 Photomicrograph of nervous system in *Fasciolopsis buski.*
Fig. 3 Anterior end depicting the cephalic nervous concentration (bromoindoxyl acetate, scale bar = 0.4 mm).
Fig. 4 Midventral region, showing the main nerve ords (MCs), PILNs and nerve net (acetylthiocholine iodide, scale bar = 2mm).
Fig. 5 A portion of the MC in magnified view; branching off points (arrows) of transverse nerves are evident, (acetylthiocholine iodide, scale bar = 0.5 mm).

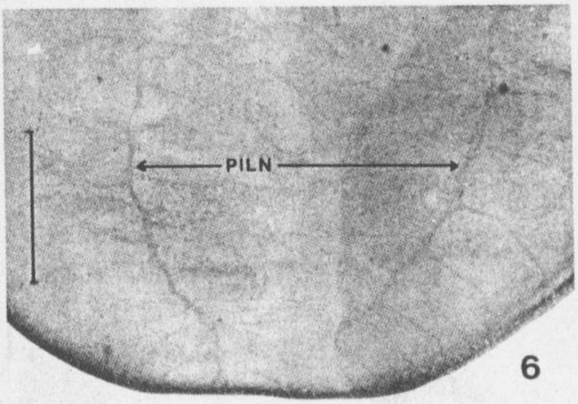


Fig. 6 Course of the PILNs and their posterior termination (bromoindoxyl acetate, scale bar = 2 mm).

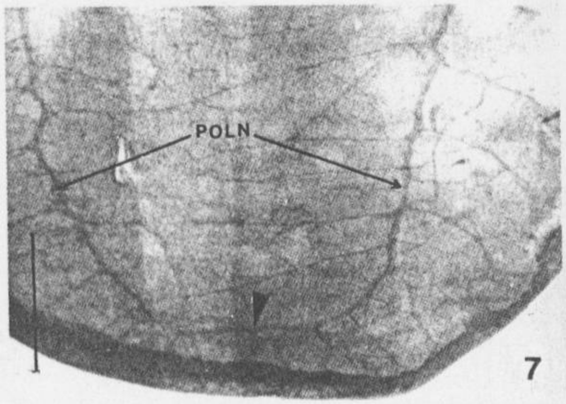
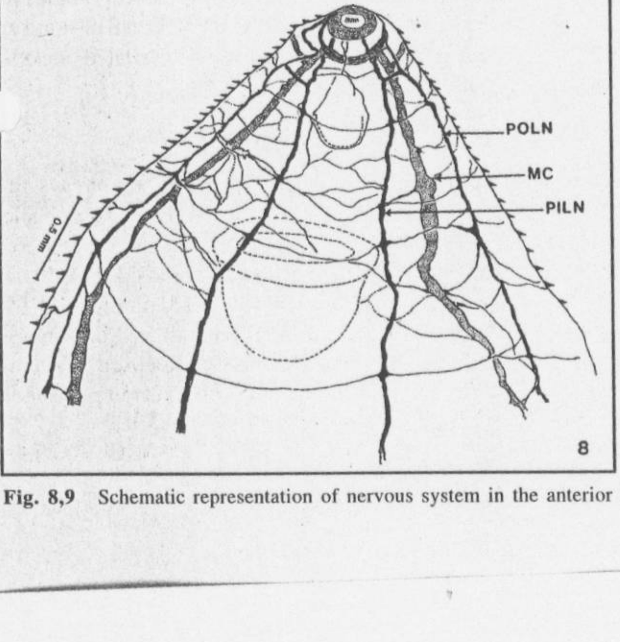


Fig. 7 POLN of either side joining medially (arrowhead) near posterior extremity of the worm (acetylthiocholine iodide, scale bar = 2 mm).

connected by a stout transverse commissure lying in the pre-pharyngeal region constitute the brain from which emanate several nerves anteriorly and posteriorly. Of the anterior nerves there are three moderately thick nerves given out from each cerebral ganglion which run up to and innervate the oral rim. Two more small nerves arising from either ganglion run anteriad and along with fine tributaries from other anterior nerves contribute to the formation of a sub-oral ring commissure. Besides these nerves, from a single locus on the ventro-median facet of each ganglion emanate three fine nerves, of which two proceed anteriorly up to the oral rim and one runs a circumpharyngeal course to innervate the pharynx. The post cephalic MCs comprise a pair each of postero-outer (POLN) and postero-inner (PILN) nerves. The POLNs are multi fibre-thick nerves as compared to PILNs. While the latter are deep seated in the parenchyma the former are more superficially lodged. All these nerves run parallel to the body till the posterior region where (as observed in acetylthiocholine iodide preparations) the nerves of the two sides converge medially and join to form a semicircular commissure. Along their length these nerves give out lateral tributaries. An extensive nerve plexus with randomly placed cell bodies connects all the posterior longitudinal nerves. Both the oral and the ventral sucker display a rich innervation of fibres and so does the circumgenital pore region. Fine branches from the posterior longitudinal nerves or their connectives innervate the various organ systems and form submuscular, somatogastric, reproductive and excretory nerve plexuses. Fine nerve terminals penetrate the tegument.

Artyfechinostomum sufrartyfex (Figs. 8-13): The paired cerebral ganglia are conspicuous and are connected to each other by a thick band-like commissure that lies immediately posterior to the oral sucker. Of the anterior nerves, there are three nerves arising from the anterior or antero-lateral facet of each cerebral ganglion and these terminate in the oral sucker rim. The posterior longitudinal nerves comprise a pair of MCs and two pairs of thinner nerves. Of the latter, one pair is of the outer longitudinal nerves (POLNs), each of which runs outer to the MC of its side and parallel to the lateral body margins. The POLNs however do not run the entire length of the body



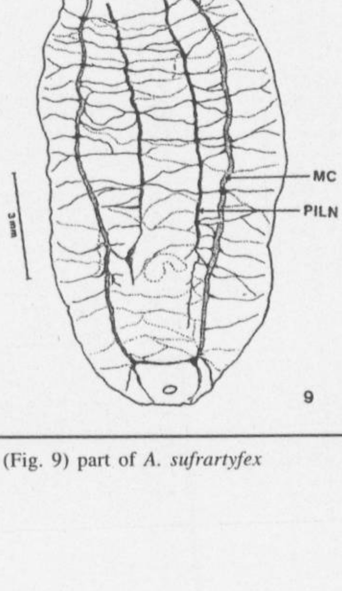


Fig. 8,9 Schematic representation of nervous system in the anterior (Fig. 8) and posterior (Fig. 9) part of *A. sufrartyfex*

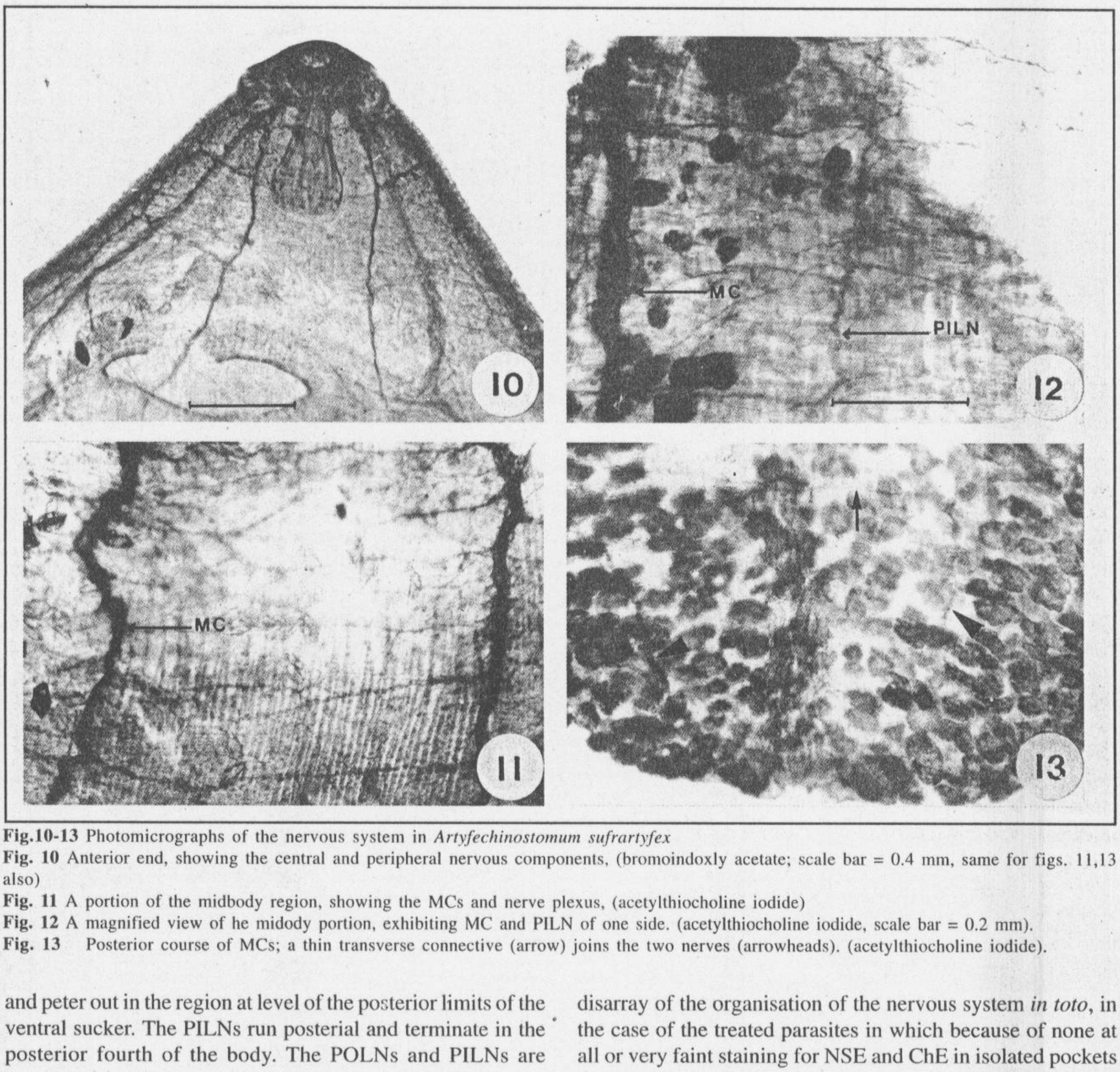


Fig.10-13 Photomicrographs of the nervous system in *Artyfechinostomum sufrartyfex*

Fig. 10 Anterior end, showing the central and peripheral nervous components, (bromoindoxly acetate; scale bar = 0.4 mm, same for figs. 11,13 also)

Fig. 11 A portion of the midbody region, showing the MCs and nerve plexus, (acetylthiocholine iodide)

Fig. 12 A magnified view of he midody portion, exhibiting MC and PILN of one side. (acetylthiocholine iodide, scale bar = 0.2 mm).

Fig. 13 Posterior course of MCs; a thin transverse connective (arrow) joins the two nerves (arrowheads). (acetylthiocholine iodide).

and peter out in the region at level of the posterior limits of the ventral sucker. The PILNs run posterial and terminate in the posterior fourth of the body. The POLNs and PILNs are connected by three conspicuous connectives in the region between the oral and ventral sucker. Besides, numerous fine branches from these nerves on either side in the post-acetabular region, innervate the various organs and exhibit an anastomosing pattern. The MCs which are median nerves on either side run up to the posterior extremity of the body; in the region just anterior to the excretory pore, a thick transverse connective joins the two MCs. Tributaries emanating from the MCs and other longitudinal nerves constitute a fine nerve net and many terminate in the subtegumental region.

Treatment: On treatment with crude root peel extract of *F. vestita* and genistein with doses of 20 mg/ml and 0.5 mg/ml, respectively *in vitro*, alterations in the NSE and ChE activities in the two fluke species were observed. There was a complete disarray of the organisation of the nervous system *in toto*, in the case of the treated parasites in which because of none at all or very faint staining for NSE and ChE in isolated pockets the course of the nerves was indiscernible.

DISCUSSION

Conforming to the basic orthogonal plan of nervous system in flatworms (Kotikova, 1991; Gustafsson, 1995; Halton and Gustafsson, 1996; Reuter *et al.,* 1988), the cholinergic nervous system in *F. buski* and *A. sufrartyfex* exhibits a rectilinear configuration of longitudinal nerve cords connected by numerous transverse connectives at intervals and presenting a somewhat ladder-like pattern. Minor variations are shown in the number of longitudinal nerve cords and overall pattern of the subtegument and submuscular nerve plexuses.

Following treatment with the plant root peel extract or genistein, there was no or very faint staining for cholinergic components in histochemical preparations of both *F. buski* and *A. sufrartyfex*. The specific choline found closely associated both with the CNS and PNs in the two species is acetylcholine (ACh) as revealed by the use of acetylthiocholine iodide as the substrate. The presence of ACh has been demonstrated in a number of adult digenetic trematodes and their post molluscan larval stages (Gustafsson, 1987; Sukhdeo and Sukhdeo, 1988; Magee *et al.,* 1989, 1991; Mckay *et al.,* 1990, 1991; Niewiadomska *et al.,* 1996; Humphries *et al.,* 1997). ACh is regarded as an inhibitory motor neurotransmitter in flatworms (Holmes and Fairweather, 1984) and there is evidence that the ACh receptors in platyhelminth parasites have different pharmacological properties from their counterparts in mammalian hosts (Mellin *et al.,* 1983; Mansour, 1984). In the latter, as in nematode parasites ACh is known to act as an excitatory neurotransmitter for motor activity (Bryant and Behm, 1989). AChE is the enzyme responsible for removing ACh at the synapses. Inhibitory effect of AChE on the motility has been demonstrated in schistosomes (Bennett and Depenbusch, 1984), the frog lung fluke, *Haplometra cylindracea* (Mckay *et al.,* 1990) and the cestode, *Hymenolepis diminuta* (Sukhdeo *et al.,* 1984, Thompson *et al.,* 1986). Many compounds inhibit AChE; in the drug-treated parasites temporary paralysis and consequent loss of hold by the anchoring structures may ensue, thus causing the worms to detach and shift from their normal site (Bennett and Depenbusch, 1984). Treatment with crude peel extract of *F. vestita* and genistein *in vitro* caused alterations in the AChE activity in the cestode parasite (Pal and Tandon, 1998a). A similar decline in the AChE activity in the treated fluke species in the present studies also suggests an antagonistic effect of *F. vestita* -derived phytochemicals on the soft-bodied helminth parasites.

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