ANTI-ACETYLCHOLINESTERASE ACTIVITY OF ROOT HAIR OF CAREX BACCANS ON THE TREMATODE ARTYFECHINOSTOMUM SUFRARTYFEX

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ABSTRACT
The current study was carried out to test the root hair extract of Carex baccans, a traditionally used medicinal plant by the natives (Jaintia tribes) of Northeast India, for its anthelmintic efficacy on the trematode Artyfechinostomum sufrartyfex. The test parasites were exposed to different concentrations of the root hair extract of the plant and praziquantel, a broad-spectrum anthelmintic at 37±1°C. Control parasites were incubated in PBS only. Immediately after attaining paralysis, the parasites were processed for histochemical analysis. Motility and mortality data obtained from the study showed a positive dose dependent relationship. A significant reduction in acetylcholinesterase activity in the parasites treated with different concentration of plant extract was observed compared to control parasites. Considerable inhibitions in the acetylcholinesterase activity in the treated parasites are suggestive of an efficient vermicidal activity of the root hair extract of C. baccans against A. sufrartyfex.

Keywords: Carex baccans, Artyfechinostomum sufrartyfex, anthelmintic, acetylcholinesterase

INTRODUCTION
Helminths are a group of worms that use different parts of host’s body, including lumen of the gut, as the normal habitat for their adult forms, causing high rate of mortality in both the human as well as domestic animals (Suleiman et al. 2005). Helminthiasis is mainly treated chemotherapeutically with the help of synthetic anthelmintics. However development of anthelmintic resistance due to their long term and continuous application has worsened the scenario (Waller, 1998). It has been observed that a tradition of ethnic medicinal system exists worldwide which can rescue the world from its present helminth problems (Roy et al. 2010, 2012a,b; Dasgupta et al. 2013; Giri et al. 2013). Many drugs presently prescribed by physicians are either directly isolated from plants or are artificially modified form of natural products (Wang et al. 2007). Studies on plants and plant extracts as potential anthelmintics have revealed that different parts of many such plants viz. the latex of Carica papaya, root tuber peel of Flemingia vestita, leaves of Spilenthes oleraceae, root peel of Millietia pachycapa, stem bark of Acacia oxyphylla, shoot of Alpinia nigra, leaves of Securenega virosa and root tubers of Potentilla fulgens are effective against nematode, cestode and trematodes (Satrija et al. 1994; Roy and Tandon, 1996; Pal et al. 1997; Roy, 2001; Roy et al. 2008, 2009; Dasgupta and Roy, 2010). The anthelmintic efficacy of some plant derived compounds has been found to be comparable with those of commonly used anthelmintics (Agaie and Onyeyili, 2007; Dasgupta et al. 2013). Nerve and muscle systems in helminth parasites interact in a highly co-ordinated manner to control movements associated with alimentation, reproduction, locomotion and attachment to the host. Acetylcholine causes an increase in the rhythmic frequency of muscle cell depolarizations. The action of acetylcholine at the neuromuscular synapse is terminated by acetylcholinesterase. For long time it has been known that neuromuscular junction in
parasites is susceptible to chemotherapeutic attack, and that compromising this aspect of parasite biology is sufficient to cure many parasite infections. Carex baccans is an anthelmintic medicinal plant in Meghalaya, the root tuber of which showed anthelmintic property when treated against cestode parasite Raillietina echinobothrida (Challam et al. 2012). The present study deals with the assessment of acetylcholinesterase activity in the treated and control trematode Artyfechinostomum sufrartyfex, to assess the damage encountered by the ethanolic root-hair extract of C. baccans in the parasite’s nervous system.

MATERIALS AND METHODS
Preparation of the Plant crude extract:
The fresh root hairs of C. baccans were collected from Jaintia Hills, Meghalaya, India (25.3594°N/92.3813°E). After washing in distilled water, the root hairs were shade-dried and grounded by motor-driven grinder. The powder was then refluxed in 90% ethanol for 12 h at 60°C, and the solution was filtered through Whatman filter paper No. 1. The collected solution was evaporated to dryness at 50°C to recover the plant extract as dry powder, which was stored at 4°C till further use.

Test parasites:
Live mature Artyfechinostomum sufrartyfex were collected from the intestine of naturally infected pig in 0.9% phosphate buffered saline (PBS; pH 7.2).

In vitro Experiment:
After washing in PBS, the test parasites were subjected to treatment with different concentrations of the plant extract viz., 1mg, 5mg, 10mg and 25mg/ml of PBS dissolved in 0.01% DMSO and incubated at 37±1°C. Control parasites were incubated in PBS having 0.01% DMSO. A broad-spectrum anthelmintic PZQ was used as the reference drug (WHO, 2009) at a concentration of 1mg/ml of PBS. Immediately after attaining paralysis the parasites were processed for biochemical and histochemical tests. All tests were carried out on parasites treated with 25mg of plant extract since this concentration showed early paralysis compared to other doses.

Histochemical Staining:
Acetylcholinesterase (AChE) was histochemically localized following Gomori (1952) as described by Pearse (1968) using 10-12µm thick sections fixed in cold formalin (4°C) and stained with an incubation medium (copper sulphate, glycine, magnesium chloride, sodium hydroxide, sodium sulphate, maleic acid; pH 6.6) for 30 minutes at 37±1°C. The enzyme activity was determined through observation of light black deposit in the stained section using light microscope.

RESULTS AND DISCUSSION
Following the exposure to different concentrations of the plant extract, the parasites contracted sharply for some time and then went into a relaxed state and continued in the same state till they attained a condition of flaccid paralysis, which was followed by death after some interval of time. The controls of A. sufrartyfex survived for a period of 10-12 h, whereas, worms treated with a concentration of 1mg, 5mg, 10mg and 25mg/ml of PBS showed paralysis taking 4.00-4.16, 2.50-2.75, 1.50-1.75 and 1.15-1.50 h, respectively. Motility and mortality data obtained from the study showed a positive dose dependent relationship i.e. higher the dosage the faster paralysis and death attained as indicated in Table I.

Histochemically the control sections showed intense AChE activities in the somatic musculature (SM) (Fig. 1; 1 and 2). Histological sections of plant extract exposed parasites showed a general reduction in the stain intensity in the somatic muscular (Fig.1; 3 and 4). However, no activity of AChE was detected in the tegument (T) and subtegument (ST), in both control and plant extract exposed parasites (Table II). The results of histochemical tests are demonstrated by the displayed figures revealing diminished stain intensity in the extract treated trematode compared to high stain intensity in the control samples.
Plants of the genus *Carex* (Family: Cyperaceae) have attracted recent attention as food additives and nutraceutical source because they contain high levels of bioactive polyphenols (Li *et al.* 2009). The inhibition data of parasite motility with different concentration of root hair extract of *C. baccans* showed dose-dependent efficacy with higher dosages require for early paralysis and death. Histochemical observations indicated towards affective anti-acetylcholinesterase activity of the plant. The inhibition of AChE has been proposed as biomarker for the neurotoxicity (Rickwood and Galloway, 2004). Similar to our observations a reduction in AChE activity in cestode and trematode parasites following *in vitro* exposure to anthelmintics or potential anthelmintics of plant origin has also been reported by several other workers (Pal and Tandon, 1998; Veerakumari and Priya, 2006). The association of neural elements with the circular and longitudinal muscles allows locomotion of parasites. Therefore, inhibition of acetylcholinesterase activity leads to mortality of parasites by interrupting with their vital activities like feeding and movement affecting neuromuscular function.

Similar to the present observations, plants like *Alpinia nigra* and *Flemingia vestita* were also found to be active against enzymes related to neurotransmitter in different helminth parasites (Kar *et al.* 2001; Swargiary and Roy, 2011). Sigurdsson and Gudbjarnason (2007) studied two medicinal herbs of Iceland, *Angelica archangelica* and

**Table I:** Motility and mortality of *Artyfechinostomum sufrartyfex* on exposure to different concentrations of the crude ethanolic extract of *Carex baccans* and praziquantel

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentrations (mg/ml)</th>
<th>Paralysis (h)</th>
<th>Death (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>7.15 - 8.00</td>
<td>10.00 - 12.00</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>1</td>
<td>4.00 - 4.16</td>
<td>5.15 - 5.50</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.50 - 2.75</td>
<td>3.00 - 3.15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.50 - 1.75</td>
<td>2.50 - 3.00</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.15 - 1.50</td>
<td>2.15 - 2.75</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>1</td>
<td>1.00 - 1.15</td>
<td>2.00 - 2.50</td>
</tr>
</tbody>
</table>

**Fig. 1.** Histochemical localization of acetylcholinesterase activity in the model test parasite *Artyfechinostomum sufrartyfex* (all Scale Bars = 100 µm). 1, 2: Control; 3, 4: *Carex baccans* exposed. T: Tegument; ST: Sub-tegument; SM : Somatic musculature.

**Table II:** Histochemical localization of acetylcholinesterase (AchE) in control and ethanolic extract of *Carex baccans* exposed *Artyfechinostomum sufrartyfex*

<table>
<thead>
<tr>
<th>Test material (mg/ml of PBS)</th>
<th>Localization and intensity of enzyme activity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>AchE</td>
</tr>
<tr>
<td></td>
<td>T</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td><em>C. baccans</em> (25)</td>
<td>-</td>
</tr>
</tbody>
</table>

T: Tegument; ST: Sub-tegument; SM: Somatic musculature
++++ = Intense activity; ++ = Mild activity; - = No activity
Geranium sylvaticum against the AChE activity and were proved to be effective with IC\textsubscript{50} values of 2.20 mg/ml and 3.56 mg/ml, respectively. Similarly, Mukherjee et al. (2007) established anti-acetylcholinesterase activity of six Indian medicinal plants namely Andrographis paniculata, Centella asiatica, Evalvulus alsinoides, Nardostachys jatamansi, Nelumbo nucifera and Myristica fragrans.

Parasites have modified biosynthetic pathways compared to the mammalian system (Bryant and Behm, 1989). This difference in the systems of the host and the parasite allows for identification of specific target sites in the parasite. Compounds from Flemingia vestita exert effect on acetylcholinesterase, cholinesterase and non-specific esterases of Fasciolopsis buski (Kar and Tandon, 2000) as well as affects the esterase activity of the cestode R. echinobothrida (Pal and Tandon, 1998). Similarly, Coryvinol, an active component isolated from Corydalys incise (Papaveraceae) showed significant AChE inhibitory effects with an IC\textsubscript{50} value of 30.6 µM (Kim, 2002).

Active compound of C. baccans responsible for inhibition of AChE activity leading to paralysis and death of parasites is not known. Therefore, it is important to isolate and identify the active principle of the plant responsible for anthelmintic activity and its possible mode of action in helminth parasites.

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