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SHORT COMMUNICATION

In vivo anthelmintic activity of *Carex baccans* and its active principle resveratrol against *Hymenolepis diminuta*

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Abstract Anthelmintic resistance against most of the commercial drugs is a great threat to humans as well as the veterinary live stocks. Hence, new treatment strategies to control helminth infections are essential at this hour. *Carex baccans* Nees has been traditionally used by Jaintia tribes in Northeast India to get rid of intestinal worm infections. Therefore, the present study was conducted to evaluate in vivo cestocidal activity of root tuber extract of *C. baccans* and its active component resveratrol against the zoonotic cestode *Hymenolepis diminuta* in the experimental model rat. The cestocidal activity was determined by monitoring the eggs per gram (EPG) counts in faeces of different treated groups. The result showed that the highest dose of the plant extract (50 mg/kg) and resveratrol (4.564 mg/kg body weight) has significant anthelmintic efficacy against *H. diminuta*. Crude extract of the plant as well as resveratrol reduced EPG count (56.012 and 46.049 %) and also resulted in decreased worm burden by 44.287 and 31.034 %, respectively. The efficacy of the crude extract and resveratrol can be compared to the reference drug praziquantel. The results exhibits considerable cestocidal potential of root tuber crude extract of *C. baccans* and resveratrol and justify its folklore use.

Keywords *Carex baccans* · Resveratrol · *Hymenolepis diminuta* · Cestocide · Traditional

Introduction

Helminth infections have afflicted humans and livestock since ancient times (Cox 2002) and are among the most common factors that limit veterinary animal production worldwide. Though over the last century medicinal chemistry has revolutionized the health-care practices in many parts of the globe, a large section of the population in developing countries still relies on traditional practice of herbal remedy. The World Health Organization (WHO 2002) has encouraged the research on medicinal plants by considering the fact that certain traditional knowledge on medicinal plants could contribute to the new pharmaceutical products to combat against diseases that affects the populations of Third World countries. The traditional use of medicinal plants as anthelmintics has become relevant at a present scenario of resistance developed by helminth parasites against most of the anthelmintic drugs (Abdel-Ghaffar et al. 2011).

Genus *Carex* (family Cyperaceae) contains over 2000 species distributed worldwide which consists of grasses and sedges. *Carex baccans* Nees is one such traditionally used medicinal plant of Meghalaya, where the Jaintia tribe uses the root tuber extract of the plant to get rid of intestinal helminth infection. In vitro exposure of cestode *Raillietina echinobothrida* to the crude alcoholic extract of the plant showed effective anthelmintic potential of the plant (Challam et al. 2012). Chemical analysis of different species of *Carex* revealed the presence of resveratrol oligomers and other stilbene derivatives (González-Sarrías et al. 2011). Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is one such compound that has a wide range of bioactivity including anticancer, antioxidant, antiaging, antimicrobial, anti-inflammatory and neuroprotective properties (Baur and Sinclair 2006). In vitro anthelmintic potential of resveratrol has also been proved in *Trichinella spiralis* and *R. echinobothrida* (Ozkoc et al. 2009; Giri and Roy 2014); however, no report pertaining to in vivo efficacy of the compound is available. Since in vitro findings are not always translatable to the in vivo situation, the present study is designed to investigate the in vivo therapeutic

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efficacy of root tuber extract of *C. baccans* and its active compound resveratrol against *Hymenolepis diminuta* developed in rat.

Materials and methods

Collection and preparation of plant extracts

The root tubers of *C. baccans* Nees (Cyperaceae) were collected from different parts of Meghalaya, India (25.5700° N/91.8800° E), cleaned in distilled water and dried in shade. The methanol extract preparation is made following the methods as described earlier (Giri et al. 2013).

Active component and reference drug

Resveratrol (RESV; R5010) and praziquantel (PZQ; P4668) were obtained from Sigma Chemicals (St. Louis, USA). Other chemicals used were of analytical grade and obtained from HiMedia (Mumbai, India). Milli-Q water was used to prepare phosphate-buffered saline (PBS; pH 7.2). Plant extract and PZQ solutions were prepared fresh in PBS (0.9 %) before administration to the experimental animals.

Experimental animals

Male and female Albino rats (150–200 g) were procured from recognized sources and maintained under standard environmental conditions, fed with rodent diet (Pranav Agro Industries Ltd., Delhi) and water ad libitum. The animals were acclimatized for 15 days in the laboratory prior to start of the experiments. Animal faecal sample examination was undertaken during this period to ensure that the animals were free from any intestinal worm infections. All the experiments were performed according to the guidelines of CPCSEA (1998), with approval from the Institutional Animal Ethics Committee, North-Eastern Hill University, Shillong.

Maintenance of *H. diminuta* infection

The life cycle of *H. diminuta* was maintained in the laboratory by cyclical passage through Wistar rats and the flour beetle, *Tribolium confusum* (intermediate host), as described by Dixon and Arai (1991). Briefly, the gravid segments of tapeworm were scratched smoothly on to the filter papers inside a small beaker and then beetles were allowed to feed on flour for 72 h. These beetles were then maintained at room temperature for at least 12–14 days for the cysticercoid larva to develop. Cysticercoids were collected by dissecting the beetles and inoculated to uninfected rats for development of infection. After 18–20 days, eggs of *H. diminuta* were detected in the faeces of rats, which were mixed with flour powder and fed to the beetles to continue the life cycle of the worm.

In vivo experiment

The plant extract was tested against the mature stages of *H. diminuta* in rats. The animals were divided into eight groups having six animals per group (total number of animals=48). Each animal was then orally infected with five cysticercoids and maintained in a separate cage. Plant extract, resveratrol and PZQ (the reference drug) solutions were prepared fresh in 0.9 % PBS. The first group of animals served as untreated control and given only the vehicle. The animals belonging to groups 2, 3 and 4 were treated with 10, 25 and 50 mg root tuber crude extract/kg body weight of rat, respectively. Similarly, groups 5, 6 and 7 were treated with 1.141, 2.282 and 4.564 mg resveratrol/kg (5, 10 and 20 mM/kg) body weight. The eighth group of animals was treated with 5 mg PZQ/kg body weight of rat. For the adult form of parasite, the root tuber extract, resveratrol and PZQ were given on days 21–23 post inoculation of the cysticercoids and the eggs per gram (EPG) count of the animals was performed between days 18–20 (pre-treatment period) and days 33–35 a week later (post-treatment period). The percentage reduction in the EPG counts was calculated and noted down. Finally, on day 39, all animals were sacrificed and the number of worms in their intestine was counted to determine the percentage reduction in worm counts.

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. The significance of difference was accepted at $P \leq 0.05$. All results were reported as mean±SEM ($n=6$).

Results and discussion

The present study demonstrated a clear dose-dependent cestocidal activity of *C. baccans* root tuber extract and RESV against the rat tapeworm *H. diminuta* (Fig. 1). Three-day single-dose treatments comprising of 10, 25 and 50 mg crude root tuber extract of the plant/kg body weight of rat achieved 6.832, 24.099 and 44.803 % reduction in worm burden, respectively (Fig. 2). However, when treated with RESV at doses of 1.141, 2.282 and 4.564 mg/kg body weight, there was 3.395, 20.641 and 30.993 % reduction in worm recovery, respectively. Animals exposed to 5 mg/kg body weight showed

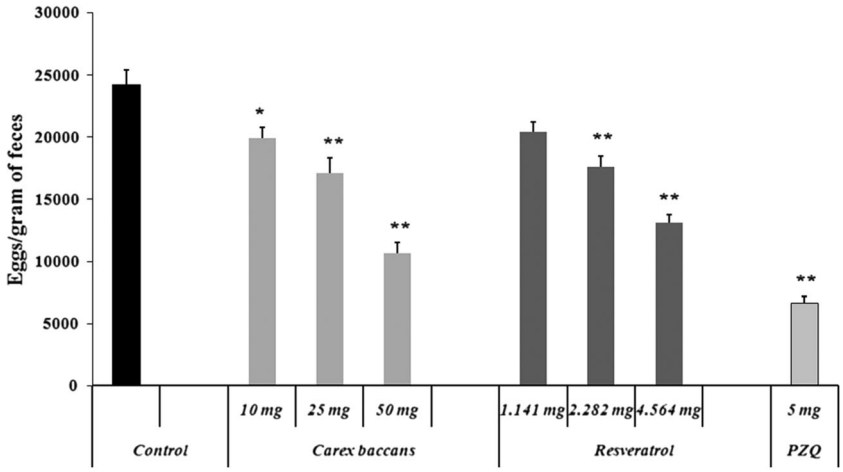

Fig. 1 Effects of *C. baccans* root tuber extract, RESV and drug PZQ on *H. diminuta* worms in vivo (n=6). *P≤0.05; **P≤0.001, significant values vs. control

68.994 % reduction in the worm recovery. Reduction in EPG counts and recovery of worms at necropsy have earlier been used as criteria to assess the anthelmintic efficacy of different traditionally used plants (Iqbal et al. 2006; Tritten et al. 2012).

As per an estimate, about 80 % of population in developing countries and about 65 % of the rural population in India relay on plant-based medicine for their primary health-care need (WHO 2002). There are numerous Indian medicinal plants which have been investigated for their potential anthelmintic efficacy, and a good number of them have shown profound activity against different helminth parasites (Challam et al. 2012; Yadav and Tengjongmongla 2012). Yadav and Tangpu (2008) tested the in vivo efficacy of *Adhatoda vasica* and found that 800 mg/kg double doses of the extract reduced EPG count by 79.57 % and worm recovery rate by 16.60 %, which were better than treatment with 5 mg/kg single dose of praziquantel. Similarly, the leaf extract of *Clerodendrum colebrookianum* possesses a dose-dependent efficacy against the larval, immature and adult stages of *H. diminuta* when tested in vivo. However, the efficacy of the extract was most effective against the adult stages of the parasite (Yadav and Tengjongmongla 2012). Sapaat et al. (2012) studied the anthelmintic activity of papaya seeds against *H. diminuta* in rats and showed the profound activity at doses of 0.6 and 1.2 g/kg body weight by reducing the EPG by 96.8 and 96.2 % and the worm burden by 90.77 and 93.85 %, respectively. In vitro anthelmintic activity of *C. baccans* and RESV has already been proved against *R. echinobothrida* (Challam et al. 2012; Giri and Roy 2014). Similarly, Hu et al. (2010) investigated the in vivo anthelmintic activity of *Bacillus thuringiensis*-derived crystal protein Cry5B against mice chronically infected with *Heligmosomoides bakeri* and *Ancylostoma ceylanicum* and revealed the reduction in EPG and in worm burden to an extent of 98 and 70 % at doses of 90 and 100 mg/kg body weight, respectively. Similarly, drugs tribendimidine showed a significant decrease in *Necator americanus* infection better than those achieved by the metabolite dADT (Xue et al. 2010).

The present in vivo study revealed the anthelmintic potential of *C. baccans* and RESV on the model parasite *H. diminuta*. As the compound RESV is well tolerated by host animal at higher concentration and has a wide range of biological activity (Williams et al. 2009), the transformation of the present results into clinical practice seems desirable.

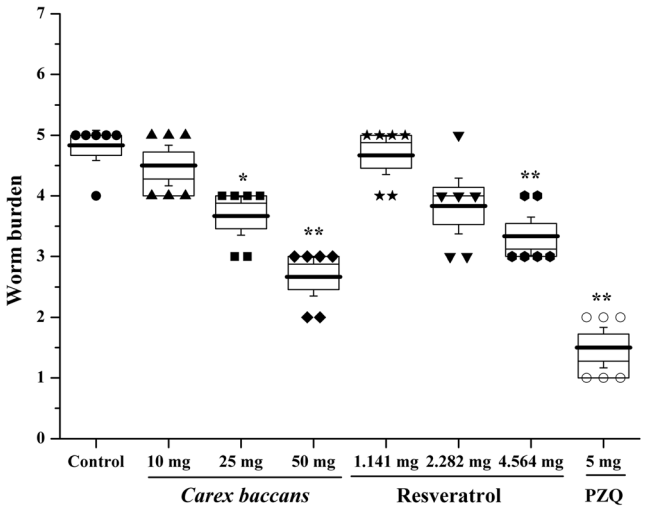

Fig. 2 Effects of *C. baccans* root tuber extract, RESV and drug PZQ against *H. diminuta* infections in rats. Worm burden is shown in *box plots with the median line*, where *each dot* represents the number of parasite (n=6). *P≤0.05; **P≤0.001, significant values vs. control

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