In vitro cestocidal activity of Persicaria hydropiper (L.) Delarbre, a traditionally used anthelmintic plant in India

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Research Article

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Abstract

This study was aimed to investigate the anthelmintic effects of *Persicaria hydropiper*, a traditionally used anthelmintic plant in India. The *in vitro* anthelmintic efficacy of methanol leaf extract of *P. hydropiper*was assessed on the basis of paralysis and mortality of *Raillietina echinobothrida*, the intestinal cestode parasite of domestic fowl, following exposure to 10 mg/ml, 20 mg/ml and 30 mg/ml concentrations of extract. The effects of extract were also observed on the body surface of parasite by scanning electron microscopy (SEM). The results indicated that *in vitro* anthelmintic efficacy of extract is dose-dependent. The highest efficacy was observed by 30 mg/ml concentration of extract, in which the mortality of parasites occurred in 4.79 ± 0.17 h, in comparison to control, where the parasites showed survival till 45.63 ± 0.18 h. The SEM observations of extract-treated parasites revealed notable impairment in scolex, with distorted suckers and eroded spines. Also, the tegument was observed shrunken with impaired microtriches. The results indicate that *P. hydropiper* leaves possess significant anthelmintic efficacy and justify their use in traditional medicine against intestinal worms.

Introduction

About 1.5 billion people are estimated to be infected with soil-transmitted helminths (WHO 2022), which are considered as one of the most widespread human pathogens. In the quest to find safe and effective anthelmintic drugs, a keen interest in ethnomedicinal studies have emerged in recent years. Plants and/or their secondary metabolites may serve as alternative anthelmintics, which can surmount manifold regulatory challenges and consequences caused by synthetic anthelmintics. Owing to these merits, several medicinal plants have been explored for their anthelmintic properties (Tandon et al. 2011).

Persicaria hydropiper (L.) Delarbre (Polygonaceae), called locally as “nhachü”, is an annual herb of about 40-70 cm height. It is found distributed in temperate and tropical Asia, Northern Africa, Australia and in many regions of Europe (Huq et al. 2014), besides in the Northeast India (Hazarika and Sarma 2006). In the traditional medicine of Pakistan, *P. hydropiper* has been used for a long time against inflammation, rheumatoid arthritis, epilepsy, headache, colic pain, fever, chill, joint pain, oedema and infectious diseases (Ayaz et al. 2015). Recent experimental studies on this plant have also established it to possess diverse biological activities, including anticholinesterase, antioxidant and gastroprotective activities (Ayaz et al. 2017). Ayaz et al. (2014) has reported the anthelmintic activity of whole plant extract of *P. hydropiper*, using adult earthworms (*Pheretima posthuma*) and roundworms (*Ascaridia galli*), as test organisms. It can be argued here that the anthelmintic data generated using earthworms as test organisms, possess only limited scope, as earthworms are the free-living organism, with a different structural and functional body organization than parasitic roundworms (Tandon et al. 2011).

During our recent ethnomedicinal studies, it was revealed from field surveys that the leaves of *P. hydropiper* (Fig. 1) are used by the Angami tribe of Nagaland, India as a common remedy to treat the intestinal worms. The present study was initiated to investigate the anthelmintic properties of *P. hydropiper* leaves, using *Raillietina echinobothrida,* the intestinal cestode parasite of domestic fowl. The

motility and/or mortality of the worms and alterations in body surface of parasite, as evident by scanning electron microscopy, following exposure of parasites to methanol leaves extract of plant, were the parameters of present study.

Materials And Methods

The fresh leaves of plant were collected from their natural habitats in Nagaland (India), and identified by Dr. N. Odyuo at Botanical Survey of India, Eastern Regional Centre, Shillong. The voucher specimen (No. NEHU – 12099) of plant material has been deposited in Department of Zoology, NEHU, Shillong. The leaves were washed thoroughly in distilled water, dried under shade, grounded into fine powder and extracted in methanol. The extract was stored at 4 °C until used for testing.

In vitro anthelmintic assay

Adult live worms of *R. echinobothrida* were collected from the intestines of freshly slaughtered domestic fowl from local markets in Shillong. The worms were washed thoroughly in phosphate buffered saline (PBS). The active worms (n=6) were then transferred in petri dishes (triplicates) containing 10 mg/ml, 20 mg/ml and 30 mg/ml concentrations of *P. hydropiper* leaf extract, with simultaneous maintenance of control in only PBS. Praziquantel (PZQ), at 1 mg/ml, was used as reference drug. The treated and control group of worms were maintained inside an incubator at 37 ± 1 °C. The efficacy of extract/praziquantel was assessed on the basis of physical motility of test worms, as evident by their paralysis and mortality (Vijaya and Yadav 2016).

Scanning electron microscopy

With the onset of paralysis, the worms from control medium, and those exposed to 30 mg/ml concentration of extract and PZQ were picked up and fixed in neutral buffered formalin (NBF) at 4 °C. The fixed specimens were dehydrated in ascending grades of acetone, air-dried in tetramethylsilane, gold coated and finally viewed in JEOL JSM-6360 SEM.

Statistical analysis

All data are represented as mean ± standard error mean (SEM), assessed through one-way ANOVA, followed by post hoc tests using GraphPad Prism. $P \leq 0.001$ was considered statistically significant.

Results

In vitro anthelmintic assay

The plant extract showed dose-dependent anthelmintic efficacy (Fig. 2). At 10 mg/ml and 20 mg/ml concentrations of extract, the mortality of worms was recorded in 6.08 ± 0.18 h and 5.13 ± 0.10 h, respectively. However, at 30 mg/ml concentration of extract, the mortality of cestodes was attained quite

early, i.e., in 4.79 ± 0.17 h. Worms exposed to reference drug PZQ showed mortality in 4.80 ± 0.12 h. On the other hand, the worms maintained in control medium revealed physical activity till 45.63 ± 0.18 h.

Scanning electron microscopy

SEM of control parasites revealed normal contour of the body, with suckers bearing short and pointed hooklets (Fig. 3a, b) and thickly-packed normal architecture of microtriches (Fig. 3c). In contrast, the worms exposed to 30 mg/ml concentration of extract showed profound impairment in scolex, with distorted suckers and eroded spines (Fig. 3d). The tegument was noted to be shrunken (Fig. 3e) and microtriches were disrupted, showing a wide-spread damage (Fig. 3f). PZQ also revealed detrimental effects on the scolex of parasites (Fig. 3g), wherein the body surface of parasite showed prominent furrows (Fig. 3h) and microtriches were clustered (Fig. 3i).

Discussion

In the present study, the *P. hydropiper* leaf extract showed a dose-dependent efficacy on adult *R. echinobothrida*. A similar dose-dependent efficacy of *Lysimachia ramosa* leaves extract, with varying concentrations of 5 – 50 mg/ml, on intestinal helminths, *Ascaris suum*, *Fasciolopsis buski*, *R. echinobothrida* was reported by Challam et al. (2010), wherein the highest concentration (50 mg/ml) of extract showed mortality of *R. echinobothrida* in 4.51 ± 0.15 h. However, in the present study only 30 mg/ml concentration of *P. hydropiper* extract showed a comparable efficacy with that of study by Challam et al. (2010). Similarly, Tandon et al. (1997) studied the cestocidal properties of *Flemingia vestita* tuber peel extract in different concentrations (0.5 – 50 mg/ml) and reported the mortality of worms at the highest concentration (50 mg/ml) in 6.5 ± 0.4 h, which is yet again on slightly higher side as compared to mortality of parasites observed by 30 mg/ml concentration of extract in the present study. Challam et al. (2012) and Kundu et al. (2015) also reported the cestocidal activities of *Carex baccans* and *Senna occidentalis* and observed mortality of worms in 4.13 ± 0.06 h and 12.82 ± 0.24 h, at the highest dose of extract, which was 50 mg/ml and 80 mg/ml, respectively. The comparison of data from the above-mentioned related studies suggests that worms exposed to 30 mg/ml of *P. hydropiper* leaf extract attain mortality in relatively short time, i.e., only in 4.79 ± 0.17 h, which indicates that *P. hydropiper* is a comparatively more effective anthelmintic plant, even at the lower concentration of extract in comparison to other traditionally used anthelmintic plants, like *L. ramosa*, *S. occidentalis*, *C. baccans* or *F. vestita.*

The cestode tegument has been established as the principal target site of different classes of synthetic drugs or natural anthelmintics. In the present study, the treatment of worms with plant extract revealed destruction in the attachment organs as well as in tegument of parasites. A damaged tegument can cause plausible nutrient deficit and may also distort the microtriches which in turn may lead to loss of holding ability of the parasites to its host, eventually leading to paralysis and death of the parasite (Roy and Giri 2017). SEM effects similar to the present study have also been reported on part of the standard cestocidal drug praziquantel, which brings out its action by eliminating worms from the host due to paralysis of musculature and tegumental damage (Chan et al. 2017). Several other workers have

documented the potency of anthelmintic plants by denoting the topographical and structural alterations in the tegument of cestodes. For example, *R. echinobothrida,* on exposure to ethanolic extract of *Securinega virosa* (25 mg/ml) revealed a contorted body with shriveled scolex. The suckers appearing as shortened, with hooks mostly broken, and clumped microtriches have also been reported by Dasgupta et al. (2013). Structural changes on the tegument of *Hymenolepis diminuta* due to ethanolic extract of *Senna alexandrina* at 40 mg/ml have also revealed irreversible destruction over the general body surface. In the latter study, the suckers were found to be constricted and the velvety appearance of microtriches was lost (Kundu et al. 2017).

Conclusion

This study indicates that leaves of *P. hydropiper* possess significant anthelmintic efficacy and justify their use in traditional medicine against intestinal-worms. Further detailed studies are needed to investigate the mechanism of action and toxicity profile of this plant.

Statements And Declarations

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Conflict of Interests

Keleni-i Nagi has no conflict of interest. Bishnupada Roy has no conflict of interest. Arun K. Yadav has no conflict of interest.

Author Contributions

This study was designed by B. Roy and A. K. Yadav and executed by Keleni-i Nagi. First draft was written by Keleni-i Nagi and finalised by B. Roy and A. K. Yadav. All listed authors have read and approved the final manuscript.

Ethical statement

This article does not contain any studies involving animals. This article also does not contain any studies involving human participants.

Consent to participate: Not applicable

Consent for publication: Not applicable

Data availability: All data generated during this study are included in this article.

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Figures

Figure 1

Persicaria hydropiper. Leaves with inflorescence

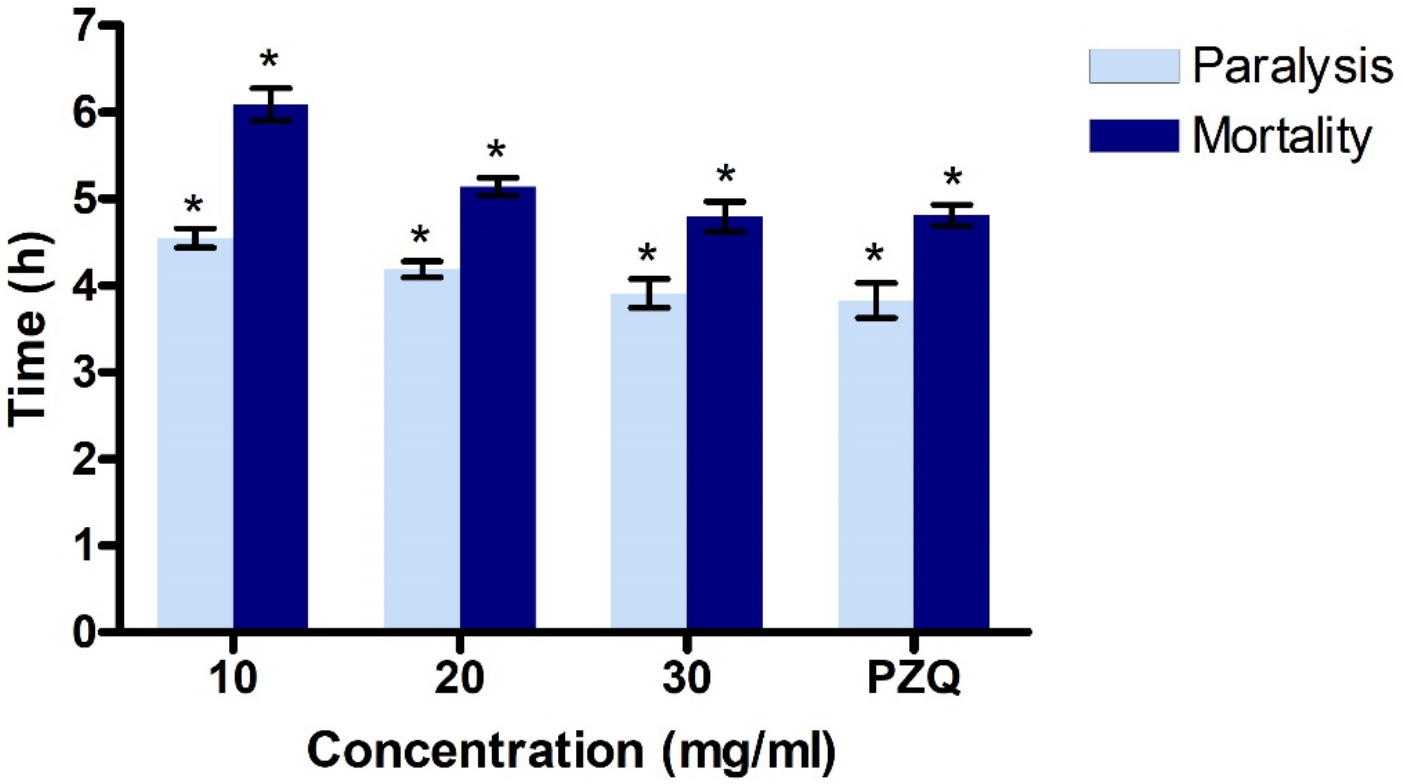

Figure 2

In vitro anthelmintic effects of *P. hydropiper* leaves extract and reference drug PZQ against *R. echinobothrida*. The worms maintained in control medium revealed physical activity till 45.63 ± 0.18 h. Data are represented as mean ± SEM. Values are significant at $*p \leq 0.001$ control vs. treated worms

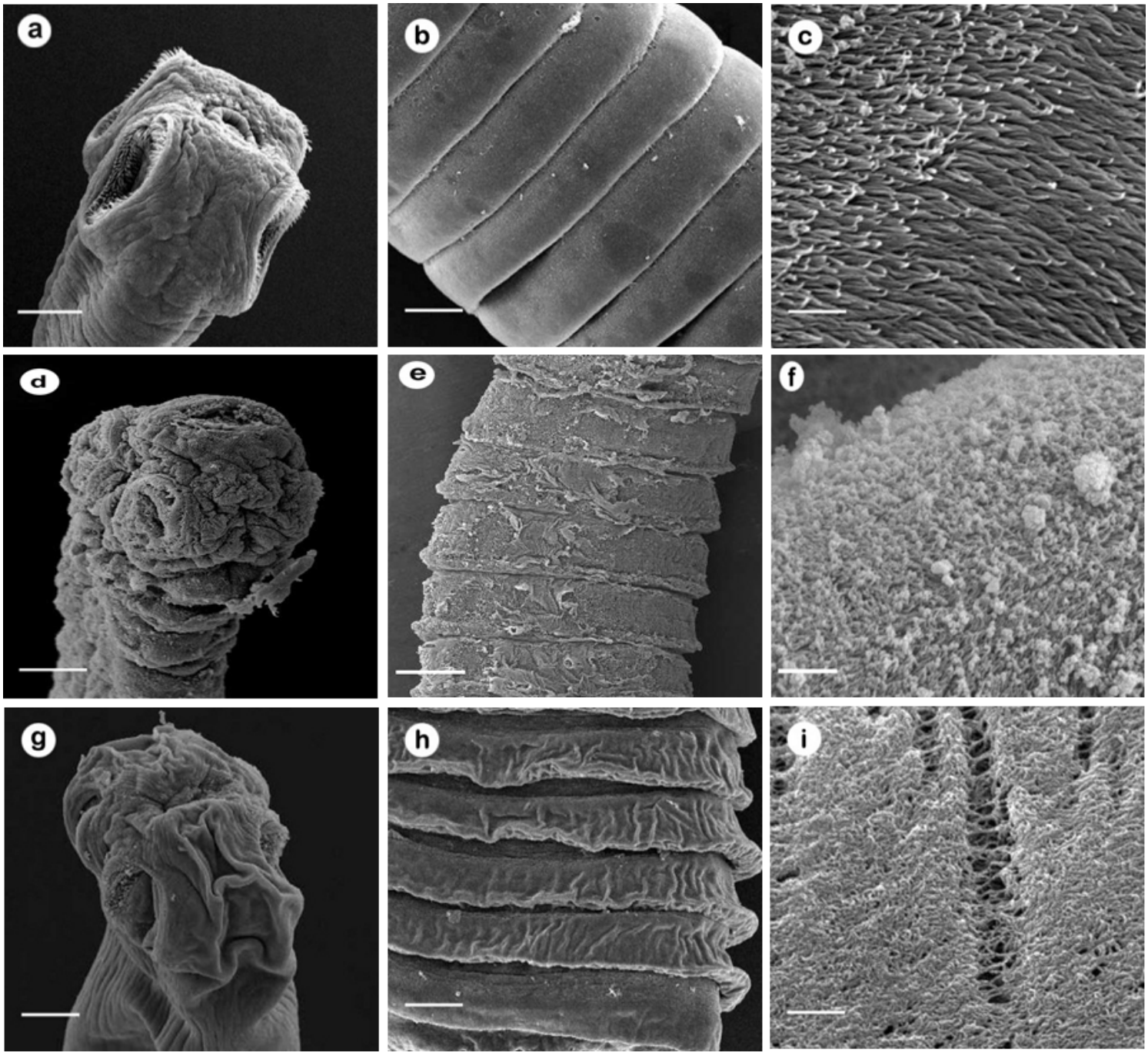

Figure 3

Scanning electron micrographs of *Raillietina echinobothrida*. **a–c** Control worms. **a**Scolex with suckers and spiny hooklets. **b**Normal pattern of segments. **c** Magnified image of tegument, showing thickly-packed microtriches. **d–f** *Persicaria hydropiper* leaf extract exposed worms (30 mg/ml). **d** Damaged scolex with complete destruction of suckers and hooklets. **e** Distorted segments. **f**Magnified image showing disrupted microtriches. **g–i**Praziquantel exposed worms. **g** Shrunken scolex with damaged suckers and hooklets. **h** Shrivelled segments. **i**Magnified image showing clumped microtriches (Scale bars: a and d = 50 µm; b, g, h = 100 µm; e = 200 µm; c and f = 2 µm;i = 5 µm)

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