Ethnopharmacological communication

Anticestodal activity of *Adhatoda vasica* extract against *Hymenolepis diminuta* infections in rats

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**Abstract**

**Aim of this study:** *Adhatoda vasica* Nees has been commonly used in the indigenous system of medicine of Naga tribes in India for curing intestinal worm infections. In this study the anticestodal efficacy of *Adhatoda vasica* leaf extract was evaluated using *Hymenolepis diminuta*-rat experimental model.

**Materials and methods:** The anticestodal efficacy of leaf extract was determined by monitoring the eggs per gram (EPG) of faeces counts and percentage worm recovery rates following treatment with methanol leaf extract of this plant to different groups of rats harbouring immature and mature *Hymenolepis diminuta* infections.

**Results:** The result indicated 800 mg/kg double dose of extract has profound efficacy against mature worms, where the EPG count was reduced by 79.57% and percentage worm recovery rate by 16.60%. These effects were better than treatment with 5 mg/kg single dose of praziquantel, the standard drug. In case of efficacy against immature worms, the extract showed a significant reduction in worm recovery rate (from 100% in control to 20.00% at 800 mg/kg dose of extract).

**Conclusion:** The study shows that the leaf extract of *Adhatoda vasica* possesses significant anticestodal efficacy and supports its use in the folk medicine.

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1. **Introduction**

1.1. Plant

*Adhatoda vasica* Nees. (Acanthaceae), with the common name 'vasaka', is a shrub widespread throughout the tropical regions of Southeast Asia, including India. Fresh leaves of *Adhatoda vasica* were collected in March 2003 from Paoyi village, Manipur, India and duly authenticated. A voucher specimen of the collected material has been deposited in the herbarium of Department of Zoology, NEHU (No. AKY-214).

1.2. Uses in traditional medicine

Extract of *Adhatoda vasica* leaves has been used for the treatment of various diseases and disorders in Ayurved and Unani medicine. It has been used as an herbal remedy for allergen-induced bronchial obstruction (Amin and Mehta, 1959), asthma (Dorsch and Wagner, 1991), tuberculosis (Barry et al., 1955) and possesses hepatoprotective activity (Bhattacharyya et al., 2005). In the Naga tribes of northeastern part of India, the leaf decoction of *Adhatoda vasica*, locally called 'sorukni', have a long history of traditional medicinal use, where it is used to get rid of intestinal worm infections. The present study is therefore designed to investigate the anticestodal efficacy of leaf extract of *Adhatoda vasica* as claimed by local people, using *Hymenolepis diminuta*-rat model.

1.3. Previously isolated class of constituents

The chemical examination of *Adhatoda vasica* revealed to contain alkaloids, glycosides, phenolic components and sterols (Lateef et al., 2003). The major constituents identified, however, are two alkaloids: vasicine and vasicinone (Das et al., 2005).

2. **Materials and methods**

2.1. Preparation of extract

The shade-dried leaves were powdered and extracted with methanol at 40 °C by Soxhlet fractional method (Yadav et al., 1992). The crude extract was evaporated to dryness and stored at +8 °C until used. The yield of final extract was 1.67% (w/w).
For assessing efficacy against immature worms, all the doses were given to the rats on days 8–10 post-inoculation (p.i.) of cysticercoids. From day 18 p.i., EPG count was done by collecting fresh faeces from each cage, using modified McMaster method (Anonymous, 1977) for 3 days (days 18–20 p.i.). Follow-up examination of EPG was done on days 28–30 p.i., 10 days following the first EPG count. Finally an autopsy was performed by sacrificing the animals on day 31 to determine the worm recovery rate (%) (Rim et al., 1980).

For evaluation of efficacy against mature worms, the treatment of animals was done for 5 days (days 21–25 p.i.) and EPG counts were undertaken for three consecutive days (days 18–20 p.i.) before treatment and for 3 days after treatment (days 26–28 p.i.). Animals were autopsied on day 29 and worm recovery rates (%) were calculated.

2.7. Statistical analysis

Results are expressed as mean ± S.E.M. Student’s t-test was used to determine the levels of significance of results. P values less than 5% were considered significant. The median lethal dose (LD50) value was calculated using SPSS software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Acute toxicity

The leaf extract when orally given to the rats at doses from 100 to 3200 mg/kg showed no mortality or any adverse signs with regard to body weight, body temperature, and food and water uptake up to 72 h post-treatment. The LD50 of extract was recorded as 3755.62 mg/kg, p.o.

3.2. Anticestodal efficacy of Adhatoda vasica leaf extract

With respect to efficacy of Adhatoda vasica against immature worms, the 800 mg/kg dose of leaf extract when given on days 8–10 p.i., showed moderate reduction in EPG counts between days 18–20 p.i. and days 28–30 p.i. as compared to control. The extract, however, showed a significant reduction in worm recovery rate (from 100% in control to 20.00% at 800 mg/kg dose of extract) as compared to control (Table 1). Treatments of plant extract against mature worms, however, revealed a significant reduction in the EPG counts, when com-

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### Table 1

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>EPG (mean ± S.E.M.)</th>
<th>Percentage difference in EPG between A and B</th>
<th>No. of worms recovered/rat (mean ± S.E.M.)</th>
<th>Percentage worm recovery rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30950 ± 1257</td>
<td>−2.82</td>
<td>5.00 ± 0.0</td>
<td>100.00</td>
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<tr>
<td>Extract</td>
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<td></td>
</tr>
<tr>
<td>200 × 1 × 3</td>
<td>10150 ± 1407</td>
<td>−19.33</td>
<td>1.83 ± 0.40</td>
<td>36.00</td>
</tr>
<tr>
<td>200 × 2 × 3</td>
<td>9922 ± 1293</td>
<td>−39.87</td>
<td>2.17 ± 0.17</td>
<td>43.40</td>
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<tr>
<td>400 × 1 × 3</td>
<td>6795 ± 2134</td>
<td>−1.92</td>
<td>1.17 ± 0.40</td>
<td>23.40</td>
</tr>
<tr>
<td>400 × 2 × 3</td>
<td>8522 ± 2053</td>
<td>−26.29</td>
<td>2.00 ± 0.52</td>
<td>40.00</td>
</tr>
<tr>
<td>800 × 1 × 3</td>
<td>5522 ± 1732</td>
<td>−28.39</td>
<td>1.00 ± 0.26</td>
<td>20.00</td>
</tr>
<tr>
<td>800 × 2 × 3</td>
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<td></td>
<td></td>
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<tr>
<td>Praziquantel</td>
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<td></td>
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</tr>
<tr>
<td>5 × 1 × 3</td>
<td>3567 ± 1449</td>
<td>−24.56</td>
<td>0.83 ± 0.31</td>
<td>16.60</td>
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<td>5 × 2 × 3</td>
<td>4367 ± 2041</td>
<td>−8.39</td>
<td>0.67 ± 0.33</td>
<td>13.40</td>
</tr>
</tbody>
</table>

*α* Administration of extract on days 8–10 post-inoculation with five cysticercoids per rat.

*b* No. of animals in each group, n = 6.

*c* *p* < 0.001 vs. control value; Student’s *t*-test.
pared to the control as well as pretreatment values. The 800-mg/kg extract-treated group showed significant reduction (16.60%) in worm recovery rates at autopsy as compared to 100% worms recovery in the control group. At the same dose, the reduction in EPG counts as well as worm recovery rate was better than treatment with 5 mg/kg single dose of praziquantel (Table 2).

4. Discussion

The present study was designed to evaluate the anticestodal efficacy of Adhatoda vasica leaf extract against the immature and mature stages of Hymenolepis diminuta. In order to investigate the effects of extract on immature stages the treatment was done on days 8–10 p. i. of cysticercoids, when the larval cestode is understood to attain the immature stage. The mean EPG in the extract-treated groups maintained an uniformity during the entire post-treatment period but the same showed a significant reduction as compared to the control group. The worm recovery rate showed a 100% recovery of worms in the control as compared to the extract-treated groups which showed a dose-dependent decrease in the worm recovery rates. Treatment with the standard drug praziquantel, however showed much better efficacy as compared to the leaf extract.

*Hymenolepis diminuta* attains the adulthood by 15–18 days p.i. in the host. Treatments of leaf extract (on days 21–25 p.i.) against the adult stages of parasite indicated dose-dependent decline in EPG counts as well as in recovery of worms. The 800 mg/kg extract-treated group showed the minimum recovery of surviving worms (16.60%) as compared to the control (100% recovery of worms). Reduction in EPG counts and/or recovery of worms at necropsy have previously been used as criterion to assess the anthelmintic efficacy of plants (Lateef et al., 2003; Iqbal et al., 2006). Interestingly, in contrast to our findings, the crude aqueous extract of *Adhatoda vasica* roots showed only mild anthelmintic activity against mixed species of gastrointestinal nematodes in sheep (Lateef et al., 2003).

The anticestodal activity emerged from this study may be attributed due to the presence of two major alkaloids, vasicine and vasicinone and glycosides in *Adhatoda vasica* (Lateef et al., 2003; Das et al., 2005). Several studies have reported that plant secondary metabolites, particularly the alkaloids, glycosides, saponins and tannins are the active compounds which confer the antiparasitic effects of plants (Athanasiadou and Kyriazakis, 2004). For instance, Akhtar and Ahmad (1992) reported that glycosides isolated from *Mollatus philippinensis* show significant anticestodal activity against gastrointestinal cestodes in goats. Thus a further study seems desirable to evaluate the various ingredients of Adhatoda vasica for anthelmintic efficacy. However, on the basis of considerably high LD$_{50}$ value and acute toxicity profile it may be suggested that the plant extract is non-toxic in nature.

In conclusion, the results of this study indicate that leaves of Adhatoda vasica possess anticestodal efficacy, which validates its use in folk medicine.

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