Research article

Efficacy and structural effects of *Acacia pennata* root bark upon the avian parasitic helminth, *Raillietina echinobothrida*

Kholhring Lalchhandama

Department of Zoology, Pachhunga University College, Mizoram University, Aizawl 796 001, Mizoram, India

**A R T I C L E  I N F O**

Article history:
Received 21 August 2012
Accepted 3 December 2012
Available online 11 January 2013

Keywords:
Anthelmintic
Albendazole
Medicinal plants
Scolex
Tegument

**A B S T R A C T**

*Introduction:* *Acacia pennata* (L.) Willd. is a well-known shrub in the south and southeast Asian regions, where the natives use it for a wide range of purposes from medicines to culinary cuisines. Among the Mizo tribes of India the plant is used in the treatment of gastrointestinal infections.

*Methods:* To validate this traditional practice, a defined set of poultry tapeworm, *Raillietina echinobothrida*, was treated with varying concentration viz. 1, 2, 5, 10, and 20 mg/mL, of the methanol extract of the root bark. Similar treatment was concurrently performed for a broad-spectrum drug albendazole, in corresponding concentrations.

*Results:* Assessment of the survival indicated that the plant extract caused concentration-dependent effects comparable to that of albendazole, with significant mortality (*P* < 0.5 by student’s *t*-test) of the test worms at all concentrations tested in comparison with those of the control group. Morphological observations using scanning electron microscope revealed that the tapeworms in the 20 mg/mL plant extract treatment group exhibited profound structural damages. The scolex became massively shrunken, with the special attachment organs reduced to crooked appendages. Overall deformation of the tegument was apparent throughout the body surface. All the body segments shrivelled up, de-flated and wrinkled into fluffy folds. The fine hairy microtriches completely disappeared, and replaced by an irregular mass of conglomerate tissues.

*Conclusion:* The study shows that *A. pennata* has an anthelmintic property that requires further investigations as to the nature and mode of action of the active compounds.

Copyright © 2013, Phcog.Net, Published by Reed Elsevier India Pvt. Ltd. All rights reserved.

1. Introduction

In spite of an enormous development in the discovery and understanding the pharmacology of anthelmintic drugs, helminth infestations continue to be the most debilitating factor of economic losses in animal industry all over the world, and the persistent cause of human morbidity and other health problems. To compound the situation, helminthiasis has not been ameliorated to any significant degree largely due to the rapid evolution of anthelmintic resistance among different parasitic helminths virtually to all types of conventional chemotherapeutic drugs, old and new. The consequence will be undesirably alarming if the crisis is not properly checked. To cope up with the inevitable dilemma, a large body of scientific documents has posited that a number of medicinal plants does indeed indicate potent anthelmintic activities, and use of well-established plants are highly advocated. However, many of the traditionally acclaimed therapeutic plants are experimentally revealed to have negligible credibility for clinical and veterinary applications, and scientific evidence for the effectiveness of many in use remains obscure.

*Acacia pennata* (L.) Willd. is a leguminous perennial climbing shrub belonging to the family Mimosaceae, and is native to Bangladesh, Bhutan, India, Myanmar, Sri Lanka and Thailand. It is a common cure for indigestion, especially in infants. The extract of the stem bark is applied as antidote to snake venom and fish poisoning. It is also used as antiseptic for scalding of urine and for curing bleeding gums. The root bark can be used as antiflatulent and to cure stomach pain. It is also used in the treatment of bronchitis, cholera and asthma. A decoction of the leaves is used for general treatment of body aches, headache and fever. The extracts of the dried leaves were experimentally demonstrated to possess analgesic and anti-inflammatory properties in laboratory mice. Burmese and Thai people have long used the shoots in various culinary preparations. In addition, based on the Thai traditional usage, the leaf extract was shown to have mild antimicrobial and antioxidiant activities.
solution was hour before experimental assay, different concentrations property.11,12 The present study is thus an attempt to vindicate the had been shown to exhibit strong anthelmintic Acacia oxyphylla obtained, which was then refrigerated at 4°C until further use. An
lane following the procedure already described elsewhere.15,16 After coating with gold in a fine-coat ion sputter, JFC-1100 (JEOL) and mounted on metal stubs, electron micrographs were generated using a LEO 435 VP scanning electron microscope at an electron accelerating voltage of 20 kV.

2.2. Chemicals and drug

All the chemicals used were of standard analytical grades, obtained either from Merck or S.D. Fine Chemicals Limited, India. The reference drug albendazole (Zentel®) was a product of GlaxoSmithKline Pharmaceutical Limited, India. Similar concentrations to those of the plant extract were prepared for albendazole using the same media.

2.3. Recovery and in vitro treatments of tapeworms

Live fowls (Gallus domesticus L.) were sacrificed using an overdose of anaesthesia and upon necropsy, live tapeworms, R. echinobothrida, were recovered from the intestines. The tapeworms were collected in PBS and then incubated at 37 ± 1°C. Actively alive tapeworms with more or less the same body size were selected and introduced into the different plant extract and drug media. One group of tapeworms was maintained in a medium containing only PBS with 1% DMSO to serve as control. Each experimental assay consisted of 5 replicates. Motility and mortality of the worms were observed, and duration of survival was recorded as previously described.12,13 Death was substantiated by dipping the tapeworms in tepid PBS (~45°C) that induced movement in sentient worms.

2.4. Scanning electron microscopy

A set of tapeworms was selected from the control and plant extract treated groups, thoroughly washed in PBS, and then immediately fixed in 4% cold-buffered formaldehyde at 4°C for 12 h. After post fixation in 1% buffered osmium tetroxide for 1 h, the specimens were dehydrated through ascending concentration of acetone and then air-dried after treatment with tetramethylsilane following the procedure already described elsewhere.15-18 After coating with gold in a fine-coat ion sputter, JFC-1100 (JEOL) and mounted on metal stubs, electron micrographs were generated using a LEO 435 VP scanning electron microscope at an electron accelerating voltage of 20 kV.

Table 1
Anthelmintic efficacy of the methanol extract of A. pennata root bark and albendazole on the survival of R. echinobothrida.

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Concentration (mg/mL)</th>
<th>Time (h) taken for deatha</th>
<th>df</th>
<th>t value</th>
<th>Probability levelb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>57.70 ± 1.37</td>
<td>8</td>
<td>20.93281</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>A. pennata extract</td>
<td>1</td>
<td>34.25 ± 2.99</td>
<td>8</td>
<td>33.44</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>19.97 ± 2.12</td>
<td>8</td>
<td>33.44</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.28 ± 1.92</td>
<td>8</td>
<td>48.94</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>06.41 ± 1.35</td>
<td>8</td>
<td>59.49</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Albendazole</td>
<td>1</td>
<td>03.89 ± 1.07</td>
<td>8</td>
<td>69.07</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.65 ± 1.56</td>
<td>8</td>
<td>48.53</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>05.94 ± 1.23</td>
<td>8</td>
<td>62.66</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>03.61 ± 0.86</td>
<td>8</td>
<td>74.69</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>02.95 ± 1.03</td>
<td>8</td>
<td>77.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

a Values are expressed as mean ± SD (n = 5).
b P value considered significant when <0.05 in comparison with control group; level of confidence at 95%.

Among the Mizo tribes of northeast India, the tender root bark, young leaves and shoots are regularly taken as seasonal delicacy and medicine for intestinal infection.10 A closely related species Acacia oxyphylla had been shown to exhibit strong anthelmintic property.11,12 The present study is thus an attempt to vindicate the use of A. pennata as an anthelmintic against a parasitic tapeworm, Raillietina echinobothrida Mégnin.

2. Materials and methods

2.1. Preparation of plant extract

The fresh roots of A. pennata were collected from the nearby forest of Aizawl, India. The specimens were identified by the botanists at the Department of Botany, Pachhunga University College, India, where voucher specimen (PUC-BOT-A 039) is maintained. The barks were peeled off, vigorously washed with deionized water, chopped off into small pieces, and dried in a hot air oven at 50°C. The dried parts were pulverized to fine powder and then air-dried after treatment with tetramethylsilane.

The resulting extract treated groups, thoroughly washed in PBS, and then immediately fixed in 4% cold-buffered formaldehyde at 4°C for 12 h. After post fixation in 1% buffered osmium tetroxide for 1 h, the specimens were dehydrated through ascending concentration of acetone and then air-dried after treatment with tetramethylsilane following the procedure already described elsewhere.15-18 After coating with gold in a fine-coat ion sputter, JFC-1100 (JEOL) and mounted on metal stubs, electron micrographs were generated using a LEO 435 VP scanning electron microscope at an electron accelerating voltage of 20 kV.

![Fig. 1. Scanning electron micrograph of normal R. echinobothrida showing the anterior end of the body called the scolex which bears holdfast organs such as four semicircular suckers and a rostellum.](Image)

![Fig. 2. Scanning electron micrograph of normal R. echinobothrida showing a sucker under high magnification; a sucker is lined with rows of sharply pointed spines.](Image)
2.5. Data analysis

All data are presented as means plus or minus the standard deviation (SD) of the mean. Comparison of the mean values between the treated and control groups was made using unpaired Student’s t-test, and the level of probability value considered significant when $P < 0.05$.

3. Results

The tapeworms maintained in a control medium that consisted of only PBS with DMSO survived well up to 57.70 ± 1.37 h. Observations on the efficacy of the methanol extract of *A. pennata* root bark and albendazole in terms of mortality of *R. echinobothrida* are shown in Table 1. The results indicate that both the plant extract and the reference drug exhibited dose-dependent lethal effects. Treatment of the worms with 1, 2, 5, 10 and 20 mg/mL of both the plant extract and albendazole showed significant efficacy on the survival of the tapeworms. In comparison, albendazole indicated a slightly higher level of potency to the plant extract at all concentrations. Survival of the tapeworms directly related to concentrations of the drug and the plant extract; the higher the concentration the shorter the survival, and vice versa.

For scanning electron microscopic descriptions the tapeworms treated with 20 mg/mL of the plant extract were chosen as the most extensive alterations were shown at this concentration in comparison with the control worms. Normal *R. echinobothrida* is a typical tapeworm with an elongated, flattened, ribbon-like segmented body, having a knob-like anterior end called the scolex. The scolex bears four bulging suckers surrounding a circular opening called rostellum (Fig. 1). These suckers and rostellum constitute the special attachment organs of the parasite to the host’s intestinal wall. They are specifically lined with prickly spines (Fig. 2). The body proper called strobila is composed of a chain of conjoined segments called proglottids (Fig. 3). The entire body covering, the tegument, is completely covered with delicate hairs called microtriches, giving the overall surface a velvety appearance (Fig. 4).

The tapeworms treated with the plant extract showed extensive deformity throughout the entire body surface. The scolex is represented by a severe shrinkage with a number of unusual tegumental folds and the prominent suckers were contracted (Fig. 5). Spines demarcating the suckers were sharply crooked and appeared defunct (Fig. 6). The tegument appeared totally disintegrated so much so that the proglottids look like wrinkled textile...
R. echinobothrida obstruction in complete sloughing off of the scolex surface and metabolic destruction on the tegument, entire removal of microtriches, bark extract of in the tapeworm extract showing total obliteration of the tegument and shrinkage of the body of the tapeworm, where they directly cause disruption of the tegumental and muscle layers by binding specifically to β-tubulins, thereby, inhibiting assembly and functioning of the cellular motor proteins. The tegument or cuticle is the fundamental interface of the helminth body with its environment, and responsible for selective absorption of nutrients, secretory activities and sensory perception, rendering it specifically susceptible to anthelmintic agents. Consequently, the tegument is the primary target of anthelmintic agents.

Formation of numerous blebs on the tegument, rostellar disorganization and loss of the microtriches were observed for pure albendazole and its sulphoxide combination therapy on the human tapeworm, Echinococcus granulosus. Albendazole and praziquantel combination treatment of E. granulosus and Mesococctoides corti resulted in the loss of sucker concavity, loss of microtriches and destruction of the tegument. Damaging effects described for albendazole, flubendazole and nitazoxanide are highly comparable and typified by reductions in number and length of the microtriches, rostellar degeneration, formation of blebs on the tegument, loss of hooks and destruction of microtriches and vesiculation in E. granulosus and Echinococcus multilocularis. Albendazole also caused complete tegumental disintegration, extensive shrinkage and obliteration of microtriches in R. echinobothrida.

It can therefore be concluded that the present study clearly provides the rationale behind the traditional usage of the extract of A. pennata root bark as an anthelmintic. Structural changes on the morphology, damages in the suckers and microtriches, and distortion of the fine tegument of tapeworms are the hallmark effects of anthelmintic drugs. Similar structural changes observed in the present investigation, suggests that the plant extract acts trans tegumentally to exert anthelmintic effects. However, the active chemical component of the plant extract and the precise mode of action at cellular level are beyond comprehension from the present study, and remain to be further investigated.

Conflicts of interest

The author has none to declare.

References


