Pharmacognosy Research [Phcog Res.]
Vol 1, Issue 4, Jul-Aug, 2009 Page 179-184
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 14 May, 2009
Modified: 02 June, 2009
Accepted: 02 June, 2009

PHCOG RES.: Research Article

Cestocidal Activity of *Acacia caesia* stem bark on *Raillietina echinobothrida*

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ABSTRACT

The effects of the methanol extract of *Acacia caesia* Linnaeus (Mimosaceae) stem bark were examined on the avian gastrointestinal cestode, *Raillietina echinobothrida* Megnin. *In vitro* treatments of the cestodes with different concentrations, viz, 0.5, 1, 2, 5, 10, and 20 mg ml^{-1}, of the plant extract indicated that the plant extract caused dose-dependent paralytic and mortality effects similar to that of albendazole, with significant mortality ($P < 0.05$) when compared to the control. However, the plant extract showed significant lethal effect only at the higher concentrations such as 5, 10, and 20 mg ml^{-1} while albendazole was effective at all concentrations tested. Scanning electron microscopy of the cestode treated with 20 mg ml^{-1} of the plant extract showed profound morphological alterations which were the deliberate hallmark effects of anthelmintic drugs. Devastating obliteration of tegumental surface, focal erosion and degeneration of the microtriches of the proglottids, and distortion of suckers on the scolex were clearly discernible. The plant extract thus showed profound anthelmintic effects and apparently acted trans-tegumentally to cause morphological damages.

Keywords: *Acacia caesia;* albendazole; anthelmintic; cestode; *Raillietina echinobothrida;* scanning electron microscopy; tegument

INTRODUCTION

Successful control of intestinal helminths remains the major conundrum in livestock agriculture throughout the world. Tremendous developments in the discovery and understanding of the pharmacology of anthelmintic drugs have not ameliorated the global crisis of helminth infestations. Rapid evolution of anthelmintic resistance virtually to all types of chemotherapeutic drugs has become the paramount threat to animal industry (1,2). Moreover, global appreciation of organic farming also constrains further dissemination of chemotherapeutics, as synthetic anthelmintics are proven to have detrimental effects on non-target organisms and the environment in general (3).

Therefore, a general stagnation in the application and development of chemotherapy has led to an increased vigour for research into alternative therapeutic agents to combat unimpeded helminth infections. One major field is the evaluation of traditional helminthic remedies. Traditional medicines are rich in the use of plants, and farmers all over the world are known to employ different plants to manage helminthiasis in their livestock. Thus pharmacology has drawn its attention to reviving and assessing traditional practices (4,5).

Acacia caesia Linnaeus is a leguminous perennial climbing shrub belonging to the family Mimosaceae, and is native to south-east Asia. In different parts of India, the tender leaves are widely used in culinary preparation, and the prickly stem for fencing agricultural fields. The stem bark forms copious froth when rubbed with water and thus used as soap, while its decoction as lice killer (6).

The juicy extract from the stem bark is well-known among the Mizo tribes of north-east India as remedy to

gastrointestinal infections. The crude extract of the stem bark indeed showed profound anthelmintic effects on *Raillietina echinobothrida*, causing extensive damage in the worm's tissue such as in the tegumental layer, muscle tissue and parenchyma (7), and also inhibition of vital enzymes (8). The plant extract also caused similar deleterious effect in the nematode, *Ascaridia galli* (9).

The present study is, therefore, an attempt to further investigate the cestocidal effects using the methanol extract, and to explicate the possible primary route of action.

MATERIALS AND METHODS

Preparation of Plant Extract

The fresh stems of *A. caesia* were collected from the nearby forest of Aizawl, Mizoram, India. Authentication, cataloguing and preparation of the crude extract of the plant were reported elsewhere (7). The stem barks were peeled off, thoroughly washed with deionized water and dried in a hot air oven at 50°C. The dried parts were pulverized in mortar and then refluxed with methanol (100 g l^{-1}) for 8 h at 60°C. The solution obtained was filtered through Whatman filter paper (No. 1) and then evaporated to complete dryness at 50°C. The methanol extract thus obtained was a deep green powdered precipitate, which was then refrigerated at 4°C until further use. The net yield from such extraction was 1.35%.

Chemicals and Drug

All the chemicals and reagents used were standard analytical grades, obtained either from Merck or S.D. Fine Chemicals Limited, India, except where otherwise stated. Methanol was supplied by Qualigens, India, and the reference anthelmintic drug, albendazole was a product of GlaxoSmithKline Pharmaceutical Limited, India.

Recovery and in vitro Treatments of Parasites

Live fowls (*Gallus domesticus* Linnaeus) obtained from the local poultry vendor at Aizawl, Mizoram, India, were sacrificed at the Department of Zoology, Pachhunga University College, and live *R. echinobothrida* were recovered from the intestines. The worms were collected in 0.9% neutral phosphate-buffered saline (PBS, pH 7-7.3) and then incubated at 37 ± 1°C in a glass-chambered automated incubator. One hour prior to experimental treatment, varying concentrations, viz, 0.5, 1, 2, 5, 10, and 20 mg ml^{-1}, of the plant extract were prepared by dissolving in PBS supplemented with 1% dimethylsulfoxide (DMSO). Similar concentrations were prepared for albendazole by serial dilution from the commercial dosage (20 mg ml^{-1}) using PBS with DMSO. The cestodes were introduced into the different media. One group of worms was maintained in a medium containing only PBS with 1% DMSO as control. Each experimental assay consisted of 5 replicates.

Motility and mortality of the worms were observed, time taken for paralysis and death was recorded, as previously described (7,10). Paralysis was defined as complete loss of spontaneous motor activity upon physical stimulation of the worms. Dipping the parasites in tepid PBS (~45°C) induced movement in sentient worms; if no movement occurred upon such stimulation, death was confirmed.

Scanning Electron Microscopy

Cestodes were selected from the control and 20 mg ml^{-1} treated groups. They were fixed in 10% cold-buffered formaldehyde at 4°C for 12 h. After post fixation in 1% buffered osmium tetraoxide for 1 h, they were dehydrated through ascending concentration of acetone up to pure acetone. They were then treated with tetramethylsilane for 10 min and air-dried under room temperature. After coating with gold in a fine-coat ion sputter, JFC-1100 (JEOL) and mounted on metal stubs, the specimens were observed using a LEO 435 VP scanning electron microscope at an electron accelerating voltage of 20 kV.

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 probability value considered significant at $P < 0.05$.

RESULTS

Motility and Survival Effects

Observations on the effects of the methanol extract *A. caesia* stem bark and albendazole in terms of motility and mortality of *R. echinobothrida* are shown in Table 1. The results indicate that both the plant extract and the reference drug exhibited dose-dependent paralytic and lethal effects on the cestode. The worms maintained as control in a medium containing only 0.9% PBS with 1% DMSO survived very well up to 54.78 ± 0.7 hours. Treatment of the worms with 0.5, 1, 2, 5, 10 and 20 mg ml^{-1} of the plant extract showed loss of motor activity at 30.00 ± 0.5, 20.47 ± 0.3, 15.65 ± 0.4, 10.15 ± 0.6, 5.25 ± 0.4 and 3.77 ± 0.5 h, respectively, and complete loss of life at 54.35 ± 0.4, 52.83 ± 0.7, 52.08 ± 0.6, 21.20 ± 0.5, 10.92 ± 0.5 and 5.90 ± 0.6 h, respectively. However,

significant mortality was observed only at 5 mg ml^{-1} and above concentrations of the extract in comparison to the control. Albendazole was significantly effective at all concentrations tested causing lethal effect at 27.10 ± 0.7, 18.25 ± 0.4, 12.98 ± 0.5, 5.72 ± 0.4, 3.22 ± 0.3 and 1.85 ± 0.4 h, respectively. Thus, albendazole exhibited more potent anthelmintic efficacy in comparison to the plant extract, though the dose-dependent activities were highly similar.

Table 1. Dose-dependent effects of the methanol extract of A. caesia stem bark and albendazole on the viability of R. echinobothrida.

Test group	Dosemg ml^{-1}	Time in hour taken for	
		Paralysis	Death
Control	0		54.78 ± 0.7*
Methanol extract of *Acacia caesia*	0.5	30.00 ± 0.5	54.35 ± 0.4*
1	20.47 ± 0.3	52.83 ± 0.7*	
2	15.65 ± 0.4	52.08 ± 0.6*	
5	10.15 ± 0.6	21.20 ± 0.5*	
10	5.25 ± 0.4	10.92 ± 0.5*	
20	3.77 ± 0.5	5.90 ± 0.6*	
Albendazole	0.5	17.07 ± 0.6	27.10 ± 0.7*
1	12.94 ± 0.6	18.25 ± 0.4*	
2	9.62 ± 0.6	12.98 ± 0.5*	
5	3.40 ± 0.4	5.72 ± 0.4*	
10	1.32 ± 0.2	3.22 ± 0.3*	
20	1.12 ± 0.3	1.85 ± 0.4*	

Values are expressed as mean ± SD (n = 5); * Student's *t*-test, significantly different at $P < 0.05$ in comparison with the control.

Morphological Structure and Changes

For scanning electron microscopy, the cestodes treated with 20 mg ml^{-1} of the extract were chosen as the most extensive alterations were shown at this concentration in comparison with the control worms. The cestode body is whitish in colour and greatly elongated terminating into a knob-like anterior end termed the scolex. The scolex bears four bulging suckers located radially around the proximal end of the scolex. Centrally around the four suckers is located a circular opening called rostellum (Fig. 1A). Each circular sucker is marked with 12 rows of spines along its rim (Fig. 1B). The body proper called strobila is an elongated ribbon-like structure composed of a chain of conjoined segments called proglottids (Fig. 1C).

The entire body covering called the tegument is completely covered with cascades of posterior-directed

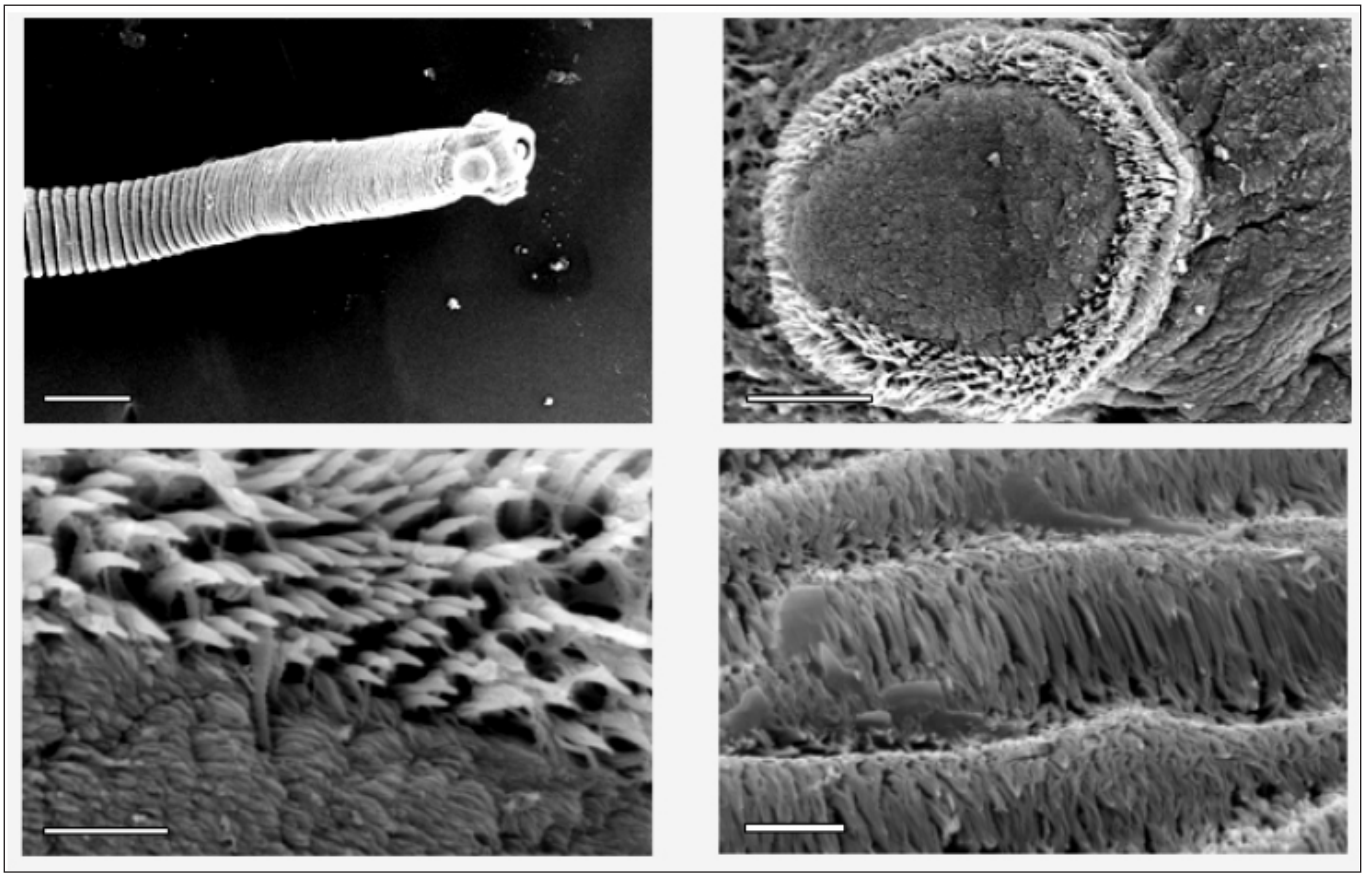

Figure 1: Scanning electron micrographs of an untreated control R. echinobothrida. A. The strobila composed of a series of proglottids with apical scolex (bar = 20 µm). B. A sucker with its marginal rim of rows of spines (bar = 2 µm). C. The finely pointed layers of spines surrounding the sucker (bar = 20 µm). D. Cascades of ciliary microtriches on the tegument of the proglottid (bar = 2 µm).

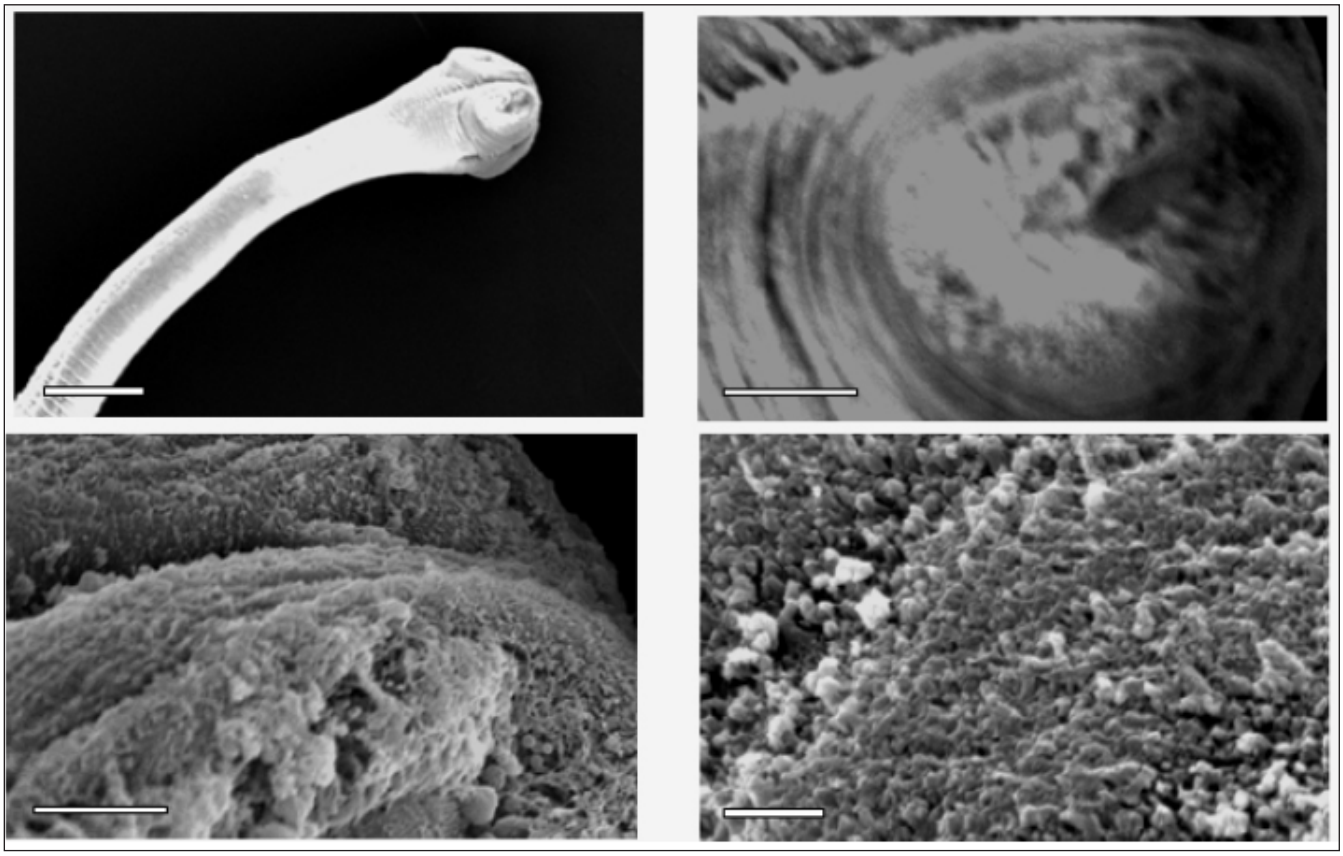

Figure 2: Scanning electron micrographs of R. echinobothrida treated with 20 mg ml^{-1} of the methanol extract of A. caesia stem bark. A. The scolex greatly distorted and the tegument contracted (bar = 15 µm). B. A sucker deformed with some vesicular secretion inside (bar = 2 µm). C. The horny spines completely sloughed off from the rim of the sucker (bar = 20 µm). D. Microtriches reduced to mere adhering dishevelled clumps of tissue on the tegument (bar = 2 µm).

microvillar filaments called microtriches, giving the overall surface a velvety appearance (Fig. 1D).

The cestode treated with 20 mg ml^{-1} of the plant extract showed extensive alterations throughout the fine topography of the body. The scolex appeared greatly contracted forming layers of tegumental folds, with the suckers extensively shrunken (Fig. 2A). Some vesicular secretions obviously due to extreme stress were evident within the sucker (Fig. 2B). Regular spines demarcating the sucker were completely obliterated (Fig. 2C). The fine filaments of the microtriches were entirely degenerated and were reduced to irregularly distributed, clustered protuberances on the tegument (Fig. 2D).

DISCUSSION

The present study provides the evidence for the anthelmintic activity of the extract of *A. caesia* stem bark on the cestode, *R. echinobothrida*. The dose-dependent efficacy of the plant extract on *R. echinobothrida* was quite similar to that of albendazole. However, the plant extract showed significant efficacy only at higher concentrations above 5 mg ml^{-1}, while albendazole was effective at all concentrations tested. The root bark extract of *Millettia pachycarpa* also exhibited similar activity on *R. echinobothrida* (11). From the medicinal plants of a nearby Khasi tribe, the root tuber extract of *Flemingia vestita* was also shown to cause similar concentration-dependent cestocidal activity (10).

Recent explorations of members of the genus *Acacia* documented that they are generally rich in saponins and tannins (12,13). Certain saponins are experimentally validated to possess potent anthelmintic activities (14–16). Two triterpenoid saponins, acaciaside A and B, isolated from *A. auriculiformis* were found to exert significant activities against nematodes and cestodes (15,17). These saponins caused destruction of cell membranes by inducing peroxidation, increasing energy metabolism and inhibiting glucose uptake of the parasites (18,19).

A large number of tannin-rich plants such as *Acacia nilotica*, *A. karoo*, *A. mearnsii*, *A. molissima* and *A. cyanophyla*

have been shown to possess effective deworming properties (20–23), with their tannins component confirmed as the principal anthelmintic compounds (24–26). Inference from the present study corroborates to the nature of these anthelmintic effects, which makes it tempting to assert that the cestocidal activity of *A. oxyphylla* stem bark may be attributed to saponins and tannins.

Scanning electron microscopy reveals extensive morphological destructions throughout the body surface of *R. echinobothrida* after exposure to the plant extract. The observations are clearly consistent with the previously reported structural changes characterized by truncation of microtriches and shrinkage of the tegument under light microscopy (7). Similarly, *M. pachycarpa* reportedly induced extensive erosion of the tegument and distortion of the suckers of *R. echinobothrida* (11). *F. vestita* was also demonstrated to bring about severe degenerative effects including clumping of the microtriches, vacuolization of the tegument and shrinkage of the body of *R. echinobothrida* (10).

Albendazole and its related benzimidazoles are known to enter the helminths by passive diffusion through the external surface of helminths, 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 (27). 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, it has been profusely documented that the distinctive effect of anthelmintic drugs is detrimental alterations and destruction of the cestodes's surface (28–33).

Formation of numerous blebs on the tegument which became detached, leaving debris only, rostellar disorganization and loss of the microtriches were described for the effects of pure albendazole and its sulphoxide combination therapy on the human cestode, *Echinococcus granulosus* (28). Albendazole and praziquantel combination treatment of *E. granulosus* and *Mesocestoides corti* also resulted in the loss of sucker concavity, separation and disintegration of the germinal layers, loss of microtriches and destruction of the tegument (29,33). 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 *E. multiloculoris* (30–32).

CONCLUSION

It can, therefore, be concluded that the present study clearly provides the rationale behind the traditional usage of the extract of *A. caesia* stem bark as an anthelmintic agent. The paralytic and mortality effects with the accompanying structural damages on the fine topography *R. echinobothrida* are typical of those of anthelmintic drugs, suggesting that the plant extract acts trans-tegumentally to induce cestocidal 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.

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