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Anticestodal Efficacy of *Lasia spinosa* Extract Against Experimental *Hymenolepis diminuta* Infections in Rats

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Abstract

The use of *Lasia spinosa* (L.) Thwaites (Araceae) leaves in the treatment of intestinal worm infections is a common ethnobotanical practice in the Naga tribes of India. In the current study, the anticestodal efficacy of *L. spinosa* leaf extract was investigated against a tapeworm using *Hymenolepis diminuta*–rat animal model. The anticestodal effects of *L. spinosa* leaf extract was determined by monitoring the eggs per gram of feces (EPG) counts and percentage worm recovery rates after treatment with leaf extract in single and double doses of 200, 400, 800, and 1600 mg/kg that were given orally for 5 days to the rats harboring immature and mature worms. The effect of plant extract was found to be dose-dependent, and double doses showed better efficacy as compared with single doses. In the case of infections with immature worms, 1600 mg/kg double dose of *L. spinosa* leaf extract reduced the fecal egg counts of *H. dimunta* by 80.8% and worm recovery rate by 16.7%, respectively. Praziquantel, the standard anticestodal drug given in 5 mg/kg single dose, reduced the fecal egg count by 83.2% and worm recovery rate by 16.7%. In the case of efficacy against mature worms, 1600 mg/kg double dose of leaf extract reduced the fecal egg counts of *H. diminuta* by 94.9% and worm recovery rate by 8.5%, respectively. Praziquantel (5 mg/kg, single dose) reduced the fecal egg counts by 95.1% and worm recovery rate by 16.7%. The study suggests that the leaf extract of *L. spinosa* possesses significant anticestodal efficacy and supports its use in folk medicine.

Keywords: Anticestodal efficacy, cestoda, *Hymenolepis diminuta*, India, *Lasia spinosa*, traditional medicine.

Introduction

Lasia spinosa (L.) Thwaites (Araceae) is a spinous perennial herb that is mostly distributed in Southeast Asia. The plant usually grows surrounding the ponds, and its shoots are consumed as traditional food in many communities of Southeast Asia. However, in the folk medicine of the Naga tribes of India, the porridge (pudding) of young tender leaves of *L. spinosa* (known locally as ''jurang'') is frequently used to treat intestinal worm infections. Besides our previous reports on *in vitro* anticestodal and antinematodal efficacy of *L. spinosa* leaf extract (Temjenmongla & Yadav, 2003, 2005), no data are available in the literature regarding any medicinal use, biological efficacy, or chemical profile of *L. spinosa*. In order to substantiate the claims made by local people, the current study was undertaken to evaluate the *in vivo* anticestodal efficacy of *L. spinosa* leaf extract against a tapeworm, using the *Hymenolepis diminuta* Rudolphi (Hymenolepididae) rat model.

Materials and Methods

Plant extraction

Young tender leaves of *L. spinosa* were collected in October 2002 by Temjenmongla from Mokokchung district, Nagaland, India. The plant material was identified by Dr. Jamir, Department of Botany, Nagaland University (Nagaland, India), and a voucher specimen (AKY/002) was deposited at the Department of Zoology, NEHU (Shillong, India). The leaves were air-dried in shade and powdered for extraction in methanol at 40°C by a Soxhlet fractional distillation method (Yadav et al., 1992). The final crude extract was recovered using a

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rotatory evaporator and stored at +8°C until use. The yield of the final extract was 19.1%

Experimental animal model

Male and female Swiss Wistar rats (90–120 g) were used for the study. They were kept at standard environmental conditions and fed with standard rodent diet and water *ad libitum*. All the animal experiments were performed according to the guidelines of the university and animal ethics committee. The infection of *H. diminuta* has been maintained in our laboratory since 2002 by alternating the hosts as described by Dixon and Arai (1991). Gravid segments of *H. diminuta* were scratched smoothly onto filter papers in Petri dishes and mixed with flour powder. *Tribolium confusum* Jacquelin du Val (Tenebrionidae), the intermediate host, were allowed to feed on 1.5 g of flour mixed with the eggs of *H. diminuta* for 72 h and later had free access to flour and kept at 25°C for 14 days or until dissected. On dissecting the beetles, cysticercoids (the larval stage) were collected and suspended in normal saline and inoculated to rats. Each rat was inoculated with four cysticercoids using a feeding tube and later maintained in separate cages.

Administration of plant extract/drug

For each experiment, rats were divided into 13 groups (n = 6). Group 1 rats served as infected untreated controls. Groups 2 to 9 rats were treated with 200, 400, 800, and 1600 mg/kg doses of *L. spinosa* leaf extract as single and double doses for 5 days. Groups 10 to 13 animals were given 5 and 10 mg/kg of praziquantel (PZQ), a broad-spectrum anticestodal drug administered in single and double doses for 5 days. Plant extract and PZQ were administered to the infected rats consecutively from days 8 to 12 and days 21 to 25 postinoculation of cysticercoids for the immature and mature stages of *H. diminuta*, respectively.

From day 18 post infection, 1 g of fresh feces was collected from each cage of the treated and control rats for eggs per gram (EPG) counts (Anon., 1977) for 3 days (days 18–20). Follow-up examination of EPG count was done on days 28–30 (for immature stage) and days 26–28 and days 36–38 (for mature stages). On completion of EPG monitoring, an autopsy was performed, and surviving worms in the intestine were recovered. Accordingly, the worm recovery rate (%) was calculated as described by Rim et al. (1980).

Statistical analyses

All results were expressed as the mean ± standard error of the mean (SEM). Significance was evaluated by the Student's *t*-test (Prasad, 2003). p values <0.05 were considered as significant.

Results

The anticestodal effects of *L. spinosa* leaf extract on immature *H. diminuta* infections in rats as monitored by EPG counts and worm recovery rate (%) are shown in Table 1. The EPG values (800–13,311) of the leaf extract–treated group were significantly reduced in a dose-dependent manner when compared with control (17,166). Treatments with double doses of the extract showed higher reductions

Table 1. Anticestodal efficacy of *L. spinosa* extract against immature *H. diminuta* infections in rats as monitored by eggs per gram of feces (EPG) count and worm recovery rate.

Treatment groups (dose × mg/kg × day)	EPG (mean ± SEM)		% Difference in EPG between A & B	No. of worms recovered/rat (mean ± SEM)	% Worm recovery rate
	Days 18–20 (A)	Days 26–28 (B)			
Control	17,166.7 ± 401.9	16,611.1 ± 309.9	3.2	3.8 ± 0.2	96.0
Plant extract					
1 × 200 × 5	13,311.1 ± 177.8	4866.7 ± 492.9[a]	63.4	2.3 ± 0.2	58.5
2 × 200 × 5	11,222.2 ± 297.5	3822.2 ± 173.3[a]	65.9	1.8 ± 0.3	50.0
1 × 400 × 5	11,733.3 ± 296.1	3911.1 ± 360.1[a]	66.7	1.3 ± 0.2	33.5
2 × 400 × 5	8622.2 ± 176.6	2588.9 ± 110.8[a]	70.0	1.2 ± 0.4	33.5
1 × 800 × 5	8388.9 ± 215.7	2600.0 ± 127.7[a]	69.0	1.2 ± 0.2	33.5
2 × 800 × 5	5177.8 ± 131.8	1377.8 ± 116.3[a]	70.4	1.0 ± 0.3	25.0
1 × 1600 × 5	5844.5 ± 210.6	1533.3 ± 91.1[a]	73.8	0.8 ± 0.3	16.7
2 × 1600 × 5	2088.9 ± 58.2	800.0 ± 64.0[a]	80.8	0.7 ± 0.3	16.7
Praziquantel					
1 × 5 × 5	3844.6 ± 76.3	644.5 ± 88.9[a]	83.2	0.7 ± 0.2	16.7
2 × 5 × 5	1822.2 ± 85.4	200.0 ± 100.4[a]	89.0	0.3 ± 0.2	8.5
1 × 10 × 5	1622.2 ± 47.7	155.6 ± 56.2[a]	90.1	0.3 ± 0.3	8.5
2 × 10 × 5	800.0 ± 64.4	0.0 ± 0.0	100.0	0.0 ± 0.0	0.0

[a]$p < 0.001$ significantly different from mean EPG (A) on days 18–20.

Table 2. Anticestodal efficacy of *L. spinosa* extract against mature *H. diminuta* infections in rats as monitored by eggs per gram of feces (EPG) count and worm recovery rate

Treatment groups (dose × mg/kg × day)	EPG (mean ± SEM)			% Difference in EPG		No. of worms recovered/rat (mean ± SEM)	% Worm recovery rate
	Days 18–20 (A)	Days 26–28 (B)	Days 36–38 (C)	A & B	A & C		
Control	16,955.6 ± 669.4	16,911.1 ± 345.8	16,800.0 ± 336.4	0.3	0.9	3.7 ± 0.2	91.8
Plant extract							
1 × 200 × 5	15,933.3 ± 1699.2	11,777.8 ± 954.9	4777.8 ± 150.7[b]	26.1	70.0	2.3 ± 0.3	58.5
2 × 200 × 5	17,388.9 ± 669.4	10,711.1 ± 498.5	4044.5 ± 2932.0[a]	38.4	76.7	1.3 ± 0.3	33.5
1 × 400 × 5	15,444.5 ± 631.7	9644.5 ± 859.1	3688.9 ± 163.9[a]	37.5	76.1	1.7 ± 0.3	41.8
2 × 400 × 5	17,000.0 ± 178.9	7733.3 ± 232.2	2755.6 ± 150.7[a]	54.5	83.8	1.0 ± 0.4	25.0
1 × 800 × 5	16,977.8 ± 791.7	7600.0 ± 205.8	2666.7 ± 157.8[a]	55.2	84.3	1.0 ± 0.3	25.0
2 × 800 × 5	17,466.7 ± 376.7	4622.2 ± 214.1	1200.0 ± 153.0[a]	73.5	93.1	0.7 ± 0.4	16.7
1 × 1600 × 5	17,311.1 ± 442.4	5177.8 ± 237.1	1711.1 ± 156.5[a]	70.1	90.1	0.8 ± 0.3	25.0
2 × 1600 × 5	16,888.9 ± 425.7	3222.2 ± 385.5	866.7 ± 110.2[a]	80.9	94.9	0.5 ± 0.2	8.5
Praziquantel							
1 × 5 × 5	16,333.3 ± 823.7	3088.9 ± 312.5	800.0 ± 112.9[a]	81.1	95.1	0.7 ± 0.2	16.7
2 × 5 × 5	16,822.2 ± 190.6	600.0 ± 92.7	222.2 ± 58.8[a]	96.4	98.7	0.3 ± 8.5	8.5
1 × 10 × 5	16,200.0 ± 795.5	600.0 ± 59.6	00.0 ± 00.0	96.3	100.0	0.0 ± 0.0	0.0
2 × 10 × 5	16,977.8 ± 187.5	200.0 ± 100.4	00.0 ± 00.0	98.8	100.0	0.0 ± 0.0	0.0

[a] $p < 0.01$ significantly different from mean EPG (A) on days 18–20.
[b] $p < 0.001$ significantly different from mean EPG (A) on days 18–20.

in the EPG counts when compared with single doses. The maximum reduction in the EPG count (80.8%) was observed with treatment of 1600 mg/kg double dose of leaf extract. With regard to the percentage worm recovery rate, the results were comparable with that of the standard drug, PZQ, and the maximum reduction in worm recovery rate was recorded with treatment of 1600 mg/kg double dose of plant extract.

The effects of *L. spinosa* leaf extract on mature *H. diminuta* infections are summarized in Table 2. Treatments of leaf extract against the adult stages of *H. diminuta* revealed a gradual reduction in EPG values, as differences were more in EPG values between days 18–20 and days 36–38 than from days 18–20 to days 26–28. Though the EPG counts of different groups at pretreatment (days 18–20) accounted for uniformity (values ranging from 15,444 to 17,466), significant reductions in the EPG counts and worm recovery rates of the extract-treated groups were recorded during post-treatment periods (days 26–28 and days 36–38). Plant extract treatment in double doses had enhanced the effects as revealed by both reductions in EPG counts as well as lower worm recovery rate than that of its treatment in single doses.

Discussion

Various tribes that use several plant-based recipes in their traditional system of medicine for the treatment of their common diseases inhabit the northeastern part of India. During the course of our studies on ethnomedicine of this region for plants that are used as anthelmintics (Temjenmongla & Yadav, 2003, 2005; Tangpu et al., 2004), we noticed that the young tender leaves of *L. spinosa* have a wide reputation among natives of being curative for intestinal worm infections. Initially, we undertook preliminary studies to test the *in vitro* anticestodal and antinematodal efficacies of *L. spinosa* leaf extract employing *Raillietina echinobothrida* and *Setaria cervi* as model test parasites. The antinematodal efficacy of *L. spinosa* was found to be insignificant when compared with the reference drug, diethylcarbamazine (Temjenmongla & Yadav, 2003). The *L. spinosa* leaf extract, however, showed a significant *in vitro* anticestodal efficacy against *R. echinobothrida*. In this case, the exposure of cestode parasites to 40 mg/ml concentration of plant extract resulted in mortality in as early as 4.3 h compared with reference drug, praziquantel (0.8 h) (Temjenmongla & Yadav, 2005). The good *in vitro* anticestodal efficacy of *L. spinosa* warranted its biological study in animal models. We were, therefore, interested to further assess its acclaimed efficacy by employing an experimental *in vivo* screening model. In the current study, the anticestodal efficacy of leaf extract of *L. spinosa* was evaluated using the *H. diminuta*–rat model.

In the current study, *L. spinosa* leaf extract was tested both in single and double doses against immature as well as mature *H. diminuta* infections in rats. The results indicated that the potency of the plant extract was dose-dependent, and the extract showed activity against both the immature and mature worm infections. In earlier studies, the plant extract effects have been reported only against the adult stage of *H. diminuta* infections.

Ghosh et al. (1996), Saha et al. (1999), and Tangpu et al. (2004), in their studies on effects of plant extracts *Acacia auriculiformis*, *Gladolius gandavensis*, and *Trifolium repens* on adult *H. diminuta*, reported a significant reduction both in the EPG counts and number of worms recovered at necropsy. In order to observe the effects of *L. spinosa* leaf extract on immature stages, the treatment was given on days 8–12 after inoculation of cysticercoids. It is known that on inoculation into the host, the cysticercoids undergo excystation in the small intestine and establish in the lumen of the host. During this phase of development, the 1600 mg/kg double dose of plant extract reduced the EPG count by 80.8% and worm recovery rate by 16.7%. These effects were comparable with the 5 mg/kg single dose of the reference drug, praziquantel. Further, it was observed that effects of plant extract were more pronounced in double doses as compared with single dose.

For the study on effects of leaf extract against the adult stage, the treatment was done at days 21–25 post-inoculation of cysticercoids, and the EPG counts were monitored at days 26–28 and days 36–38. The EPG counts showed a significant reduction in different groups as compared with control where more or less a uniform trend was observed during the entire observation period. The efficacy of plant extract was maximum with 1600 mg/kg double dose, which was comparable with 5 mg/kg single dose of reference drug, praziquantel. This finding is in agreement with other similar studies on effects of leaf extract of *A. auriculiformis*, *G. gandavensis*, and *T. repens* on *H. diminuta* infections in rats (Ghosh et al., 1996; Saha et al., 1999; Tangpu et al., 2004). Ghosh et al. (1996) reported a 100% cure rate of parasite burden and no recovery of eggs in the feces of rats after treatment with funicles extract of *A. auriculiformis*. This could be due to expulsion of adult worms or destrobilation by the effects of extract. A reduction in EPG counts after treatment implies that either the worms are being expelled from the intestine and/or undergoing the process of destrobilation. It has been reported that the process of destrobilation in cestodes generally initiates if they are exposed to hostile physiological conditions, including exposure to anthelmintic drugs (Hopkins et al., 1973).

In conclusion, the reduction in EPG counts and worm recovery rates in extract-treated rats provides support that *L. spinosa* leaf extract possesses an anticestodal property, which validates its use in folk medicine.

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