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Anticestodal Activity of *Trifolium repens* Extract

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Abstract

Trifolium repens L. is an herbal plant that is used in the folk medicine of the Naga tribes of India as a deworming remedy. This study deals with evaluating its anticestodal activity using experimental *Hymenolepis diminuta* Rudolphi infections in albino rats. Doses of *T. repens* aerial shoots extract 200 and 500 mg/kg reduced the mean fecal egg counts of *H. diminuta* by 47.72% and 54.59% and worm recovery rate by 60.00% and 40.00%, respectively. Praziquantel, the standard cestocidal drug, reduced the mean fecal egg count by 65.90% and worm recovery rate by 26.67%. The study suggests that the aerial shoots of *T. repens* bear anticestodal properties and supports its use in the traditional medicine system.

Keywords: Anticestodal activity, cestoda, *Hymenolepis diminuta*, India, traditional medicine, *Trifolium repens.*

Introduction

Trifolium repens L. (Fabaceae) is a small perennial herb that is used as a common forage crop in the temperate and subtropical regions of the world (Majumdar et al., 2004). However, in the folk medicine of the Naga tribes of India, the hot water decoction of aerial shoots of the plant *T. repens* (locally known as *anikatum*) is used as a deworming remedy. The antifilarial activity of the plant has been reported (Tangpu & Yadav, 2003), and its effects have also been studied on gastrointestinal nematode parasites of livestock animals (Tetley, 1953; Bernes et al., 2000). In view of its uses in folk medicine, a study was felt necessary to test and validate its efficacy as claimed by people. We report herein the anticestodal efficacy of *T. repens* using experimental *Hymenolepis diminuta* Rudolphi (Hymenolepididae) infections in albino rats.

Materials and Methods

Plant extraction

The aerial shoots of *T. repens* were collected by Vareishang Tangpu from Paoyi village, Manipur, in January 2002. The plant material was identified by Dr. P.B. Gurung, Department of Botany, NEHU (Shillong, India), and a voucher specimen (no. AKY 211) was deposited in the Department of Zoology, NEHU. The aerial shoots were air-dried under shade and powdered for extraction in methanol at 40°C by Soxhlet fractional distillation method (Yadav et al., 1992). The extract was recovered using a rotatory evaporator; the residue was dried over anhydrous calcium chloride (yield 14.86%) and stored at −4°C until use.

Experimental animal model

Male and female albino rats (100–120 g) were used and were maintained under standard environmental conditions and rodent diet. Institutional animal care and use committee rules were followed throughout the experimental period, and proper care was taken to protect the welfare of the experimental animals. Cysticercoids of *H. diminuta* were prepared from flour beetles, *Tribolium confusum* Jacquelin du Val (Tenebrionidae), which ingested gravid segments of *H. diminuta* and were kept at 25°C for 14 days (Dixon & Arai, 1991). Each rat was inoculated with five cysticercoids using a feeding tube and later maintained in separate cages.

Administration of plant extract/drug

On the 18th day postinoculation, fecal examination revealed that all the rats were infected; their egg counts per gram of feces (EPG) were monitored (Anonymous, 1977) for 3 consecutive days. Animals were divided in to 5 groups (n = 3), keeping uniformity in their EPG counts as far as possible. Group I rats were kept as

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untreated controls. Group II–IV rats were treated with 100, 200, and 500 mg/kg p.o. doses of plant extract, administered singly for 5 days (days 21–25 postinoculation). The rats in the last group V were given a 5 mg/kg p.o. dose of praziquantel, a standard cestocidal drug used in human and animals (Dixon & Arai, 1991) for the same duration for comparing the efficacy of plant extract.

Measurement of anticestodal activity

The anticestodal activity was evaluated in terms of differences in the mean eggs per gram of feces (EPG) count (Rim et al., 1980; Saha et al., 1999) undertaken 3 days each, before treatment and after treatment of plant extract and by direct count of surviving worms (% worm recovery) remaining in small intestines after completion of treatment (Rim et al., 1980) and calculated as follows:

$$\text{Difference in EPG Count} = \frac{\text{Mean EPG at pretreatment} - \text{Mean EPG after treatment}}{\text{Mean EPG at pretreatment}} \times 100$$

$$\text{Worm recovery rate} = \frac{\text{No. of worms recovered}}{\text{No. of cysticercoids given}} \times 100$$

Statistical analysis

The experimental results were expressed as the mean ± the standard error of the mean (SEM). Significance was evaluated by the Student's *t*-test (Prasad, 2003). p values less than 0.05 imply significance.

Results

Tables 1 and 2 summarize the effects of plant extract treatment on EPG count and worm recovery of *H. diminuta* in albino rats. At 100, 200, and 500 mg/kg doses of plant extract, the mean EPG count of *H. diminuta* decreased by 6.31%, 47.72%, and 54.59%, respectively, compared to the counts at pretreatment period. The reduction in the EPG count by the reference drug, praziquantel, was observed to be 65.90%. The mean EPG count in the control group did not show any significant change before (7955.55) and after (7622.22) treatment (Table 1).

With regard to the percentage worm recovery rate at necropsy after treatment, the 100, 200, and 500 mg/kg doses of plant extract showed 86.67%, 60.00%, and 40.00% worm recovery rate compared to control (93.33%). The worm recovery rate by the reference drug, praziquantel, was observed to be 26.67% (Table 2).

The plant extract was administered up to 2000 mg/kg dose, and no mortality or any adverse signs in the animals were observed with regard to body weight, body temperature, and food and water intake.

Discussion

People customarily use the plant(s)/plant-derived preparations and consider them to be efficacious against intestinal worm infections without any scientific base to explain the action of such plants. *T. repens* is one such common plant whose aerial shoots have a reputation of being efficacious against tapeworm infections in the Naga tribes of northeast India. The current study was therefore carried out to assess its acclaimed efficacy, employing the *H. diminuta*–albino rat experimental model. The treatment of rats was done by 100, 200, and 500 mg/kg body wt. dose of plant extract that was administered singly for 5 consecutive days. The anticestodal efficacy of plant extract was evaluated in terms of worm recovery rate and difference in the mean eggs per gram of feces (EPG) count after completion of treatment (Dixon & Arai, 1991; Saha et al., 1999). In general, it emerged that the efficacy of plant extract was almost comparable with that of the standard cestocidal drug, praziquantel. However, this was evident only when the extract was tested at 200 and 500 mg/kg concentration, as at 100 mg/kg dose the efficacy was far below the limits

Table 1. Anticestodal efficacy of *T. repens* extract against *H. diminuta* infections in albino rats by eggs per gram of feces (EPG) count.

Group	EPG count (mean ± SEM)		Difference in EPG count (%)
	Pretreatment (Days 18–20)	Post-treatment (Days 26–28)	
Control	7955.55 ± 596.70	7622.22 ± 505.65	4.19
Plant extract			
100 mg/kg	8099.99 ± 1244.09	7588.89 ± 891.18	6.31
200 mg/kg	9033.33 ± 245.70	4722.22 ± 96.86*	47.72
500 mg/kg	8466.67 ± 618.54	3844.44 ± 194.73**	54.59
Praziquantel			
5 mg/kg	8700.00 ± 366.67	2966.67 ± 333.89*	65.90

*$p < 0.01$ significantly different from mean EPG at pretreatment.
**$p < 0.02$ significantly different from mean EPG at pretreatment.

Table 2. Anticestodal efficacy of *T. repens* extract against *H. diminuta* infections in albino rats by worm recovery rate.

Group	No. of cysticercoids/rat (n = 3)	No. of worms/group (Day 9 post-treatment) (Mean ± SEM)	Worm recovery rate (%)
Control	5	4.67 ± 0.33	93.33
Plant extract			
100 mg/kg	5	4.33 ± 0.33	86.67
200 mg/kg	5	3.00 ± 0.00*	60.00
500 mg/kg	5	2.00 ± 0.58**	40.00
Praziquantel			
5 mg/kg	5	1.33 ± 0.67**	26.67

*$p < 0.01$ significantly different from control.
**$p < 0.02$ significantly different from control.

of comparison with the reference drug. Monitoring the egg counts following treatment with anticestodal drug is a commanly used parameter to access and establish its efficacy as anticestodal (Dixon & Arai, 1991). A reduction in EPG counts following treatment implies that either the worms are being lost from the intestine and/or undergoing the process of destrobilation. It is known 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 the current study, the treatment of *H. diminuta*–infected rats by extract of *T. repens* substantiated the above features needed to prove its efficacy as an anticestodal agent. In a related study on the role of *T. repens* as an anthelmintic agent, Tetley (1953) observed an inhibition of populations of *Haemonchus contortus* (Rudolphi) in sheep fed on white clover (*T. repens*). Similarly, Bernes et al. (2000) and Niezen et al. (2003) reported that *T. repens* reduces the populations of nematode parasites deposited through fecal materials on pastures.

In conclusion, the results of the current study indicate that the aerial shoots of *T. repens* possess cestocidal efficacy, which validates its use in folk medicine.

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