ANTHELMINTIC EFFICACY OF *BUTEA MINOR* EXTRACT AGAINST *HYMENOLEPIS DIMINUTA* INFECTIONS IN RATS

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Summary

Butea minor is a herbal plant that is used in the folk medicine of indigenous tribes in the eastern Himalayan region of India to expel the intestinal worm infections. The main aim of the present study was to evaluate the anticestodal activity of *B. minor* seed extract, using experimental *Hymenolepis diminuta* infections in rats. The plant extract was tested against immature and mature *H. diminuta* infections in the single and double doses of 200, 400, and 800 mg kg body wt. dose that were administered to *H. diminuta* infected rats for consecutive 3 days. The efficacy of the seed extract was adjudged in terms of eggs per gram of feces (EPG) counts and worm recovery rate at necropsy. The results showed that the efficacy of seed extract was more pronounced against mature *H. diminuta* than its immature stages. The maximum efficacy of seed extract was observed with 800 mg/kg double dose of extract, which reduced the EPG counts by 87.50% and showed 20.00% worm recovery rate. The standard anticestodal drug, praziquantel at 5 mg/kg single dose revealed 94.66% reduction in EPG counts and resulted in to 16.60% worm recovery rate. The study suggests that the seed extract of *B. minor* possesses significant anticestodal efficacy and supports its use in folk medicine.

KEYWORDS: Anthelmintic Activity, *Butea minor*, Cestoda, Folk Medicine

The rich floral diversity of India has provided traditional practitioners in the country with an impressive pool of 'natural pharmacy' from which plants are selected as ingredients to prepare herbal remedies to treat various diseases. In India, of the 17,000 species of higher plants, 7500 are known for their medicinal uses [1].

This proportion of medicinal plants is the highest proportion of plants known for their medical purposes in any country of the world for the existing flora of that respective country. *Butea minor* Buch.-Ham. ex Wall (Fabaceae), which is a medium small sub-erect shrub is found distributed in the plains from Himalayas to Ceylon, ascending to 4000 ft. in the north-eastern region of India. In the traditional medicine system of indigenous tribes in the eastern Himalayan region of India its seed's (common name "Kamkutei"), fresh as well as the roasted ones, has a legendry name in expelling gastrointestinal worm infections in children. A perusal of literature reveals that there is no information available regarding any potential use, biological efficacy or chemical constituents of the plant. In view of its uses in folk medicine this study was undertaken to investigate the anticestodal efficacy of seed extract of *Butea minor* as claimed by the local people, using the *Hymenolepis diminuta* - rat model.

Methods

Plant material:

The fresh seeds of *B. minor* were collected in the month of June 2002, around Paoyi village (Manipur, India). The plant material was duly identified and a voucher specimen (Number AKY–215) has been deposited in the Department of Zoology, North-Eastern Hill University, Shillong. The seeds were air-dried under shade and pulverized into powder. The powdered material was extracted using methanol as an organic solvent by Soxhlet fractional distillation apparatus [2]. The final crude extract was recovered using a rotatory evaporator and stored at $+8^0$C until use. The total yield of the final extract was 8.11%.

Reference Drug:

Praziquantel (PZQ; Distocide®) was used as the standard reference drug to compare the efficacy of the extract. Plant extract and PZQ solutions were prepared fresh in phosphate buffer saline (PBS) before treatment to animals harbouring test parasites.

Experimental animal model:

Male and female Swiss Wistar rats (100–120 g) were employed in the study. The animals were acclimatized for 15 days in the laboratory prior to use for experiments. During this period the stool samples were continuously examined in order to ensure that they do not have any intestinal helminthic infections. They were maintained under standard environmental conditions and fed with rodent diet (Pranov Agro Industries Ltd., Delhi) and water *ad libitum*. Proper care was taken to protect the welfare of the experimental animals and all the experiments were performed according to the rules laid down by the Institutional Animal Care and Use Committee. The infection of *H. diminuta* was maintained in the laboratory by alternating the hosts. Adults of *H. diminuta* were collected from the intestine of previously infected rats. Gravid segments of *H. diminuta*

were scratched smoothly onto filter papers in Petri dishes. Flour beetles, *Tribolium confusum* as the intermediate host, were starved for 24 h prior to infection. They were allowed to feed on the eggs of *H. diminuta* for 72 h and had free access to flour and kept for 12-14 days at room temperature or until dissected. On dissecting upon these beetles, cysticercoids were collected and suspended in normal saline and a known number of larvae were inoculated to fresh rats using blunt feeding tube. After 18-20 days, eggs of *H. diminuta* could be observed in the feces of the rats, which were mixed with flour powder and fed to the fresh 24 h fasted beetles. By alternating the infections to these host animals, the life cycle of *H. diminuta* continued in the laboratory conditions throughout the study period.

Administration of plant extract/drug:

For each experiment, rats were divided into 9 groups (n = 6). Group I rats served as infected untreated controls, Groups 2 to 7 rats were treated with 200, 400, and 800 mg/kg doses of *B. minor* seed extract as single and double doses for 3 days. Groups 8 and 9 were given 5 mg/kg of praziquantel (PZQ), a broad spectrum anticestodal drug administered in single and double doses for 3 days. Plant extract and PZQ were administered to the infected rats consecutively from days 8 to 12 and days 21 to 25 post inoculation 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 [3] for 3 days (days 18-20). Follow-up examination of EPG count was done on days 28-30 (for immature stages) 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 [4].

Preliminary acute toxicity test:

The seed extract was administered orally in the doses of 100, 200, 400, 800, 1600 and 3200 mg/kg, p.o. to six animals in each group. The general signs and symptoms of toxicity, intake of food and water, and mortality rates were observed for 72 h post-administration of extract [5].

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. *P* values less than 0.05 imply significance.

Results

The anticestodal effects of *B. minor* seed 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 (12,500-22417) of the seed extract treated group were reduced when

compared with control (28,583). Treatment with double doses of the extract showed higher reduction in the EPG counts when compared with single doses. The maximum reduction in the EPG count (-6.02%) was noted with treatment of 800 mg/kg double dose of seed extract. With regard to the percentage worm recovery rate, the effects were more pronounced only at 800 mg/kg dose of extract. The 200 and 400 mg/kg doses of extract failed to reduce the worm burden and EPG count in a significant manner and showed worm recovery rate between 43.40 to 70.00% as compared to control (90.00%).

The effects of *B. minor* seed extract on mature *H. diminuta* are presented in Table 2. Treatment of seed extract against mature stages revealed a gradual reduction in EPG values, as differences were more in EPG values between days 18-20 to days 28-30. Though the EPG counts of different groups at pretreatment (days 18-20) accounted for uniformity (values ranging from 32,211 to 33,794), significant reductions in EPG counts and worm recovery rates of the extract-treated groups were recorded during post-treatment periods (days 28-30 and 36-38). Plant extract treatment in double doses had enhanced the effects as revealed by both the reductions in EPG counts as well as lower worm recovery rate than that of its treatment in single doses. The 800 mg/kg double dose of extract showed the maximum effects in terms of both reduction in EPG counts as well as worm recovery rate and its effects were comparable with that of 5 mg/kg single dose of the standard drug PZQ.

Discussion

The north-eastern region of India which is inhabited by many distinct tribal populations provides an excellent scope for the ethnomedicinal study. In the quest of evaluating ethnomedicinal plants of these tribes, a list of folklore plants has come into our notice claiming in traditional medicine practice to have therapeutic effects against intestinal worm infections. In our earlier studies, we have reported the anticestodal properties of several such folklore plants, namely- *Trifolium repens, Psidium guajava, Strobilanthes discolor, Houttuynia cordata, Lasia spinosa, Centella asiatica, Zanthoxylum rhetsa,* etc. to name a few [6,7,8,9,10]. During our course of our further study on ethnomedicine of these tribes it was revealed that seed extract of *B. minor* is customarily used by indigenous people to eliminate gastro-intestinal worm infections. In literature no report is available regarding any potential use or biological efficacy of this plant. The current study was therefore carried out to assess its acclaimed efficacy, employing the *Hymenolepis diminuta*-rat experimental model.

In the present study, the seed extract of *B. minor* was tested against immature and mature *H. diminuta* infections in rats. The treatment of rats was done by single and double doses of 200, 400, and 800 mg/kg that were administered for 3 consecutive days. The anticestodal efficacy of extract was evaluated in terms of worm recovery rate and difference in mean eggs per gram of feces (EPG) count after completion of treatment [11, 12].

Table 1 Anticestodal effects of *Butea minor* seed extract[a] against immature *Hymenolepis diminuta* infections in rats[b] as monitored by eggs per gram of feces (EPG) count and worm recovery rate.

Group (dose x mg/kg x days)	EPG (mean ± SEM)		Difference in EPG between A & B (%)	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)
	Days 18-20 (A)	Days 28-30 (B)			
Control	28639±2188	28583±1773	-0.20	4.50±0.22	90.00
Plant extract					
1 x 200 x 3	22306±752 [c]	21745±842 [e]	-2.52	3.50±0.22 [e]	70.00
2 x 200 x 3	19878±1479 [e]	19511±1669 [e]	-1.85	3.00±0.37 [e]	60.00
1 x 400 x 3	22417±1545 [c]	21445±1591 [d]	-4.34	3.00±0.26 [e]	60.00
2 x 400 x 3	16545±972 [f]	15772±969 [f]	-4.67	2.17±0.17 [f]	43.40
1 x 800 x 3	17445±1389 [e]	16728±1104 [f]	-4.11	2.50±0.22 [f]	50.00
2 x 800 x 3	13300±223 [f]	12500±265 [f]	-6.02	2.00±0.00 [f]	40.00
Praziquantel					
1 x 5 x 3	6828±2320 [f]	5678±2207 [f]	-16.84	0.83±0.40 [f]	16.60
2 x 5 x 3	3345±1843 [f]	1906±1227 [f]	-43.20	0.67±0.42 [f]	13.40

[a] Administration of extract on days 8-10 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2 Anticestodal effects of *Butea minor* seed extract[a] against mature *Hymenolepis diminuta* infections in rats[b] as monitored by eggs per gram of feces (EPG) count and worm recovery rate.

Group (dose x mg/kg x days)	EPG (mean ± SEM) Pre-treatment Days 18-20 (A)	Post-treatment Days 28-30 (B)	Follow-up Days 36-38 (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)
Control	34667±1888	34978±1291	34639±1601	+0.90	-0.08	5.00±0.00	100.00
Plant extract							
1 x 200 x 3	33794±1312	30856±965	26406±1482[d]	-8.69	-21.86	3.67±0.37 [g]	73.40
2 x 200 x 3	33789±1072	30045±421[c]	24061±812 [f]	-11.08	-28.79	3.50±0.22 [h]	70.00
1 x 400 x 3	33217±887	28706±1651	25522±1699[d]	-13.58	-23.17	2.83±0.31 [h]	56.60
2 x 400 x 3	32900±811	28822±718[d]	22800±1853[e]	-12.40	-30.70	2.67±0.33 [h]	53.40
1 x 800 x 3	33317±877	22683±1237[f]	13856±2881[e]	-31.92	-58.41	1.67±0.33 [h]	33.40
2 x 800 x 3	32211±1684	8711±1708[f]	4028±954[f]	-72.96	-87.50	1.00±0.37 [h]	20.00
Praziquantel							
1 x 5 x 3	34234±1469	4150±1986[f]	1828±916[f]	-87.88	-94.66	0.83±0.40 [h]	16.60
2 x 5 x 3	33639±1005	695±457[f]	478±304[f]	-97.94	-98.58	0.33±0.21 [h]	6.60

[a] Administration of extract on days 21-23 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[g, h] $p < 0.05$ and $p < 0.01$, vs. control value, Student's *t*-test.

The seed extract of *B. minor* showed comparatively better efficacy against mature *H. diminuta* than immature infections. In this case the treatment of rats with 800 mg/kg body wt. double dose of seed extract showed comparable efficacy with that of standard anticestodal drug, Praziquantel. At 800 mg/kg double dose though the EPG counts of different groups at pretreatment (days 18-20) accounted for uniformity, significant reductions in the EPG counts were recorded in the extract-treated groups during post-treatment period. Similarly, the 800 mg/kg double dose of extract showed percentage worm reduction of 20.00% against the mature stages that was comparable with single 5 mg/kg dose of PZQ. Monitoring the egg counts following treatment with anticestodal drug is a commonly used parameter to access and establish its efficacy as anticestodal [11]. A reduction in the EPG counts implies either the worms are being expelled from intestine and/or undergoing the process of destrobilation. It is a well established fact that the process of destrobilation in cestodes may initiate if they are exposed to adverse physiological conditions, including exposure to anthelmintic drugs [13]. In the current study, the treatment of *H. diminuta*-infected rats by *B. minor* seed extract substantiated the above feature needed to prove its efficacy as an anticestodal agent. In the acute toxicity study, a dose of 3200 mg/kg given singly did not cause any mortality or any changes in body temperature, body weight, and food and water intake. This dose is four times that of the highest dose of the extract (800 mg/kg) administered to the rats. The control and plant-extract treated groups showed normal increases in body weight, which were not significantly different from each other. The preliminary observations indicate that the seed extract is non-toxic in nature. However, the toxicity of extract needs to be further investigated with regard to other parameters.

In conclusion, the results of current study indicate that seeds of *B. minor* possess anticestodal efficacy, which validates its use in folk medicine.

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