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Therapeutic efficacy of *Zanthoxylum rhetsa* DC extract against experimental *Hymenolepis diminuta* (Cestoda) infections in rats

Arun K. Yadav • Vareishang Tangpu

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Abstract *Zanthoxylum rhetsa* DC (Rutaceae), commonly called prickly ash, is used in the folk medicine of Naga tribes in India as a deworming remedy. In the present study, the therapeutic efficacy of *Z. rhetsa* leaf extract was investigated against experimental *Hymenolepis diminuta* (Cestoda) infections in albino rats. The efficacy of extract was determined on the basis of reduction in the eggs per gram of feces (EPG) counts and worm load following treatment with 100, 200, 400 and 800 mg/kg, p.o. doses of plant extract. For comparison's sake, animals were also treated with a reference drug, praziquantel at 5 and 25 mg/kg body weight doses. Three treatment regimes were followed to monitor the effects of extract on cestode parasite: (1) Treatment on day 2–4 postinoculation (pi) of cysticercoids against the larval stages; (2) treatment on day 8–10 pi against the immature stages; and (3) treatment on day 21–25 pi against the adult stages of parasite. The extract revealed its maximum efficacy against the larval stage, where its 800 mg/kg dose showed a worm count reduction of 86.60%, compared to 80.00% by the reference drug, praziquantel (5 mg/kg dose). The EPG counts also decreased drastically from 23,389 ± 2,372 to 0 in the same treatment group, compared to 33,161 ± 1,383 recorded in the control group. The efficacy of extract was found to be of moderate level against the immature and adult stages of parasite. The present investigation holds the evidence that the leaves of *Z. rhetsa* possess significant anticestodal property and supports its use in folk medicine.

Keywords Anticestodal efficacy, *Zanthoxylum rhetsa,* Cestoda, *Hymenolepis diminuta,* Naga tribes, Folk medicine

A. K. Yadav✉ • V. Tangpu
Department of Zoology,
North-Eastern Hill University,
Shillong - 793022, India
e-mail: akynehu@hotmail.com

Introduction

Zanthoxylum rhetsa DC, belonging to family Rutaceae (commonly called prickly ash, or Satin wood and locally known as *Mangangteini*), is a moderate sized deciduous tree with pale corky bark commonly found in shaded moist localities of tropical regions of India at an altitude of 1,800 m. During our course of studies on ethnomedicine of Naga tribes in the North-Eastern region of India, we came across about the use of its leaf decoction in the treatment of intestinal-worm infections. The plant is traditionally used for antidiabetes, antispasmodic, diuretic and anti-inflammatory activities in other regions of India (Pai et al. 2009). Rahman et al. (2002) reported that the plant also bears significant antinociceptive and antidiarrheal activities. Chemically the plant contains a terpenoid, xanthyletin and sesamin, alkaloids and flavonoids and an essential oil, sabinene as its key constituents (Mathur et al. 1967; Ahsan et al. 2000; Pai et al. 2009; Joy et al. 2006).

In view of its popular usage as a deworming agent in the traditional medicine system of Naga tribes in North-East India, the present study was undertaken to investigate the therapeutic effects of *Z. rhetsa* extract against a cestode parasite. The study was performed using *Hymenolepis diminuta* (Cestoda) – rat model, which is considered

to be an appropriate model for the human tapeworms (Andreassen 1991).

Materials and methods

Plant material and preparation of the extract

The leaves of *Z. rhetsa* were collected from Paoyi village, Manipur and authenticated by a plant taxonomist. A voucher specimen of the collected material has been retained in the herbarium record of senior author in the Department of Zoology, NEHU (No. AKY-213). The shade-dried leaves were powdered and extracted with methanol at 40°C by Soxhlet extraction method. The extract was concentrated in a rotary evaporator and the residue was dried in a desiccator over calcium chloride. The final yield (w/w) of the extract was 2.56%.

Drugs

The standard drug used in the study was praziquantel (Distocide®), manufactured by Shin Poong Pharm. Co. Ltd., Seoul, Korea. Plant extract and praziquantel (PZQ) solutions were prepared fresh in 0.9% phosphate buffer saline at pH 7.3 before administration to the animals.

Experimental animals

Healthy adult male and female albino rats (100–120 g) of Wistar stock were used. They were maintained under standard environmental conditions and fed with rodent diet (Pranov Agro Industries Ltd., Delhi) and water *ad libitum*. The animals were acclimatized in the laboratory prior to experimentation to make sure that they were free from any intestinal-worm infections. 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 (IACUC) of our university.

Maintenance of *H. diminuta* infection

The life cycle of *H. diminuta* was maintained in the laboratory in Wistar rats, using flour beetle, *Tribolium confusum* as the intermediate host (Dixon and Arai 1991). In brief, the gravid segments of *H. diminuta* were scratched smoothly onto the filter papers in Petridishes. The beetles were allowed to feed on the eggs of *H. diminuta* for 72 hours and had free access to flour. These beetles were maintained at room temperature for at least 12–14 days for cysticercoid larva to develop. On dissecting the beetles, the cysticercoids were collected and suspended in normal saline and inoculated to previously uninfected rats. After 18–20 days, eggs of *H. diminuta* could be observed in the feces of rats, which were mixed with flour powder and fed to the beetles to continue the life cycle in the laboratory.

Acute toxicity tests

Determination of median lethal dose (LD_{50})

An initial test was done to determine the approximate lethal and non-lethal doses of plant extract (Turner 1965). The rats were divided into 7 groups, comprising of 6 animals in each group. Group 1 of animals served as control and received only distilled water. Groups 2–7 of animals were administered orally 100, 200, 400, 800, 1,600 and 3,200 mg/kg p.o. doses of *Z. rhetsa* leaf extract. The animals were observed for 72 hours and the number of dead rats was recorded to calculate the median lethal dose (LD_{50}) using SPSS software (SPSS Inc., Chicago, IL, USA). Animals were also carefully observed for general signs and symptoms of toxicity in terms of body weight, temperature, intake of food and water, and movement for 72-hour postadministration of extract.

Therapeutic efficacy of *Z. rhetsa* leaf extract

Treatment of leaf extract against larval stages

Seven groups of animals (n = 6) were used in this experiment. Each animal was infected by oral inoculation with 5 cysticercoids and maintained in separate cages. The leaf extract was given in 100, 200, 400 and 800 mg/kg doses to groups 2–5 of animals, while PZQ was given in 5 and 25 mg/kg doses to groups 6–7 of animals. Group 1 served as infected-untreated controls. All the treatments were given on day 2–4 postinoculation (pi) of cysticercoids. From day 18 postinfection, 1 g of fresh faeces was collected from each cage of the treated and control rats for eggs per gram (EPG) counts of parasite using the modified McMaster method (Anonymous 1977) for 3 days (days 18–20). Finally, an autopsy was performed by chloroform anesthesia killing of animals on day 31 and the numbers of surviving worms in the host's intestine were counted. The efficacy of extract was determined on the basis of reduction in number of EPG of feces count and percentage worm load reduction as described by (Rim et al. 1980):

$$\text{Worm load reduction (\%)} = \frac{\text{No. of cysticercoid inoculated} - \text{No. of worms recovered}}{\text{No. of cysticercoid inoculated}} \times 100$$

Treatment of *Z. rhetsa* leaf extract against immature stages

Seven groups of animals (n = 6) were used in this experiment and the extract and PZQ were given in the doses as mentioned above on days 8–10 pi of cysticercoids. The EPG counts of animals were undertaken on days 18–20. The animals were autopsied on day 31 and the numbers of remaining worms in the intestine were counted to calculate the reduction in worm load as described in the above experiment.

Treatment of *Z. rhetsa* leaf extract against adult stages

The experimental protocols followed for this experiment were basically the same as followed in case of efficacy of extract against the larval stage. However, there were slight differences in the schedule of extract and PZQ administration, and also in the timings of EPG and worm counts. The extract and PZQ were given on days 21–25 pi of cysticercoids. EPG counts were done on days 18–20 (pretreatment period) and days 26–28 (post-treatment period) and the percentage reduction in EPG counts was calculated using the following formula (Iqbal et al. 2004):

Finally, all the animals were sacrificed on day 39 and the numbers of remaining worms in the intestine were counted to calculate the percentage reduction in worm load as described above.

Statistical analysis

Data are provided as the mean ±SEM for each group. Student's t-test was used to determine the level of significance of results. The values were considered significant when $p < 0.05$. LD_{50} value was calculated using SPSS software (SPSS Inc. Chicago, IL, USA).

Results

Acute toxicity tests

The leaf extract when given orally to the rats at doses from 100, 200, 400, 800, 1600 and 3,200 mg/kg p.o. to 6 animals in each group did not reveal any toxicity symptoms in terms of body weight, temperature, and food and water intake throughout the 72 hours observation period. The lethal dose (LD_{50}) of *Z. rhetsa* leaf extract was determined to be 2737.34 mg/kg p.o.

Therapeutic efficacy of plant extract agaisnt *H. diminuta*

Effect of leaf extract on larval stages

Table 1 shows the therapeutic effects of leaf extract of *Z. rhetsa* on larval stages of *H. diminuta* infections in rats as monitored by EPG count and worm load. The EPG values of leaf extract treated groups of animals significantly reduced in a dose-dependent manner from 23,389 ± 2,372 to 0 as compared to the control (33,161 ± 1,383). In the 5 mg/kg PZQ-treated group the EPG was recorded to be 8,050 ± 5,033, while no eggs were detected in the feces of animals treated with 25 mg/kg dose of PZQ. The plant extract also showed its significant effects on worm load of animals. The 800 mg/kg dose of extract revealed 86.60% reduction in worm load, compared to 80.00 and 100% by 5 and 25 mg/kg doses of PZQ.

Effect of leaf extract on immature stages

Treatments of leaf extract against immature stages of *H. diminuta* infections on days 8–10 postinfection showed moderate reduction of EPG counts as compared to control. The animals treated with 800 mg/kg dose of extract showed 15,628 ± 1,147 EPG counts compared to 30,461 ± 675 recorded in the control group. The extract also showed a moderate reduction in the worm load as compared to reference drug PZQ. The 800 mg/dose of extract showed 60.00% reduction in the worm load as compared to 86.60 and 93.40% by 5 and 25 mg/kg doses of PZQ, respectively (Table 2).

$$\text{Percentage EPG count reduction} = \frac{\text{Pretreatment EPG} - \text{Post-treatment EPG}}{\text{Pretreatment EPG}} \times 100$$

Table 1 Therapeutic efficacy of *Zanthoxylum rhetsa* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in rats[b] as monitored by reduction in EPG count and worm load

Treatment group (mg/kg × day)	EPG (mean ± SEM) (day 18–20)	Worms recovered at necropsy/rat (mean ± SEM)	Worm load reduction (%)
Control	33,161 ± 1,383	4.83 ± 0.17	3.40
Plant extract			
100 × 3	23,389 ± 2,372[d]	3.00 ± 0.45[c]	40.00
200 × 3	19,200 ± 2,329[d]	2.83 ± 0.40[d]	43.40
400 × 3	13,461 ± 1,678[d]	2.17 ± 0.17[d]	56.60
800 × 3	0[d]	0.67 ± 0.33[d]	86.60
Praziquantel			
5 × 3	8,050 ± 5,033[d]	1.00 ± 0.52[d]	80.00
25 × 3	0[d]	0[d]	100.0

[a] Administration of plant extract on days 2-4 postinoculation with five cysticercoids per rat.
[b] Number of animals in each group is six (n = 6).
[c,d] $p < 0.01$ and $p < 0.001$, versus control value, Student's t-test.

Table 2 Therapeutic efficacy of *Zanthoxylum rhetsa* leaf extract[a] on immature stages of *Hymenolepis diminuta* infections in rats[b] as monitored by reduction in EPG count and worm load

Treatment group (mg/kg × day)	EPG (mean ± SEM) day 18–20	Worms recovered at necropsy/rat (mean ± SEM)	Worm load reduction (%)
Control	30,461 ± 675	5.00 ± 0.00	0.00
Plant extract			
100 × 3	23,934 ± 1,863[c]	3.17 ± 0.31[e]	36.60
200 × 3	21,145 ± 1,965[d]	2.83 ± 0.31[e]	43.40
400 × 3	19,517 ± 136[e]	2.67 ± 0.42[e]	46.60
800 × 3	15,628 ± 1,147[e]	2.00 ± 0.26[e]	60.00
Praziquantel			
5 × 3	5350 ± 3148[e]	0.67 ± 0.42[e]	86.60
25 × 3	1789 ± 1193[e]	0.33 ± 0.21[e]	93.40

[a] Administration of plant extract on days 8–10 postinoculation with five cysticercoids per rat.
[b] Number of animals in each group is six (n = 6).
[c,d,e] $p < 0.02$, $p < 0.01$ and $p < 0.001$, versus control value, Student's t-test.

Effect of leaf extract on adult stages

Treatments of leaf extract against the adult stages of *H. diminuta* revealed moderate reductions in EPG counts at post-treatment periods (Table 3). The animals treated with 800 mg/kg dose of extract showed 51.49% reduction in fecal EPG counts between pre and post-treatment periods. However, the animals treated with 25 mg/kg of PZQ showed 96.48% of fecal EPG reduction. The EPG counts in the control group, however maintained an uniform trend throughout the observation period. The reduction in the worm load by plant extract was also found to be of moderate level as compared to the reference drug PZQ. The maximum reduction (60.00%) in worm load was observed in 800 mg/kg dose treated group of animals as compared to 80.00 and 100% by 5 and 25 mg/kg dose of PZQ (Table 3).

Table 3 Therapeutic efficacy of *Zanthoxylum rhetsa* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in rats[b] as monitored by reduction in EPG count and worm load

Treatment group (mg/kg × day)	EPG (mean ± SEM)		EPG count reduction (%) A and B	Worms recovered at necropsy/rat (mean ± SEM)	Worm load reduction (%)
	Pretreatment (A)	Post-treatment (B)			
Control	31,894 ± 2,057	32,056 ± 1,911	0.50	5.00 ± 0.00	0.00
Plant extract					
100 × 3	32,056 ± 763	27,700 ± 1,649[c]	13.59	3.33 ± 0.33[e]	33.40
200 × 3	33,450 ± 695	24,667 ± 1,518[d]	26.26	3.00 ± 0.37[e]	40.00
400 × 3	30,700 ± 527	20,545 ± 1,168[d]	33.09	2.67 ± 0.33[e]	46.60
800 × 3	34,334 ± 305	16,656 ± 582[d]	51.49	2.00 ± 0.26[a]	60.00
Praziquantel					
5 × 3	33,617 ± 566	11,283 ± 712[d]	66.44	1.00 ± 0.26[e]	80.00
25 × 3	35,195 ± 1,872	1,239 ± 326[d]	96.48	0[e]	100.0

[a]Administration of extract on days 21-25 postinoculation with five cysticercoids per rat.
[b]Number of animals in each group is six (n = 6).
[c,d]$p < 0.05$, and $p < 0.001$, versus pretreatment value, Student's t-test.
[e]$p < 0.001$, versus control value, Student's t-test.

Discussion

Intestinal parasitic infections continue to be a cause of major concern to human and animal health, particularly in tropical and under-developed regions of the world including India, causing malabsorbtion, diarrhea, anemia and other states of poor health (Savioli et al. 1992). Globally, over 3.5 billion people are estimated to be infected with intestinal worms of which, children in the age group 5–15 years suffer the highest infection rate of about 400 million cases of worm burden that are mainly attributed to poor sanitation and hygiene (Luong 2003). It is in this context that the World Health Organization in its Tropical Diseases Control Programme has advocated that the use of traditional medicines be promoted to combat the menace of parasitic diseases globally (Savioli et al. 1992). The North-Eastern region of India is inhabited by many tribes who use a number of medicinal plants for the treatment of various ailments in their folk medicine system. During our studies on ethnomedicine of Naga tribes it was revealed that *Z. rhetsa* has a wide reputation among natives as being a curative agent for intestinal-worm infections. The present study was therefore undertaken to scientifically evaluate the therapeutic efficacy of plant employing *Hymenolepis diminuta* (Cestoda) – rat experimental model.

In this study, the efficacy of leaf extract was investigated against the larval, immature, and adult stages of *H. diminuta*. In most previous studies, however, the anthelmintic effects of plant extracts have been investigated only against the adult parasites (Galal et al. 1991; Ghosh et al. 1996; Saha et al. 1999). The study revealed that *Z. rhetsa* leaf extract possesses a pronounced efficacy against the larval stage of parasite. When administered against the larval stage, at 800 mg/kg body weight dose the extract was able to reduce 86.60% worm load and no parasite eggs were detected in the feces of treated rats. The efficacy was almost comparable to PZQ, the reference drug. Soon after inoculation into experimental animals the cysticercoids are expected to undergo excystation and establishment in the lumen of host. With the extract showing its profound efficacy during this phase of infection, it appears that it probably brings out its action by affecting the excystation or establishment processes of parasite. Contrary to previous findings, the plant extract revealed only a moderate degree of efficacy against the immature stage of parasite. The maximum reduction in the EPG counts and worm load in the extract-treated groups in this case were far below the limits of comparison with that of reference drug PZQ. A more or less similar kind of trend was also noticeable regarding efficacy of extract against the adult stage of parasites. In this case, both the maximum reduction in the EPG counts between pre and post-treatment periods and also the worm reduction in the extract treated group was quite low as compared to reference drug PZQ. The findings of present study are in agreement with the results of Saha et al. (1999) and Tangpu

et al. (2004) who also reported a moderate level of efficacy of *Gladiolus gandavensis* and *Trifolium repens* extracts against *H. diminuta* infections in rats. In another study, Tangpu et al. (2006) reported that *Strobilanthes discolor* leaf extract possesses rather a comparatively higher degree of efficacy against the larval stage than the adult or immature stages of *H. diminuta*. In contrast, Ghosh et al. (1996) in their study reported a 100% cure rate of parasite burden and no recovery of eggs in the feces of rats following treatment with funicles extract of *Acacia auriculiformis*. This could be due to expulsion of adult worms or destrobilation by the effects of extract. It has been reported that the process of destrobilation in cestodes may initiate if they are exposed to hostile physiological conditions, including exposure to anthelmintic drugs (Hopkins et al. 1973). The phytochemical studies on *Z. rthetsa* reveals that it contains terpenoids, alkaloids and flavonoids as major chemical constituents (Mathur et al. 1967; Pai et al. 2009). It is likely that one or more of these constituents may be responsible for the efficacy of plant, as there are several published reports which attest anthelmintic actions of plants to the constituents reported in *Z. rthetsa* (Akhtar et al. 2000; Athanasiadou et al. 2007). In the acute toxicity studies the plant leaf extract was found to be safe in the doses tested and there was no general signs and symptoms of toxicity observed during the observation period. In addition, a comparatively high LD_{50} value (2,673.45 mg/kg p.o.) of the extract is also indicative of its safety profile.

In conclusion, this study indicates that *Z. rhetsa* DC leaf extract possesses significant anthelmintic efficacy without showing any acute toxicity to the experimental animals. The experimental evidence obtained in the animal model may provide a rationale for the traditional use of the plant as anthelmintic.

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