Anticestodal efficacy of *Psidium guajava* against experimental *Hymenolepis diminuta* infection in rats

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ABSTRACT

Objective: To investigate the anticestodal efficacy of *Psidium guajava* L. leaf extract.
Materials and Methods: Anticestodal efficacy was evaluated using experimental *Hymenolepis diminuta* infection in rats. The leaf extract was administered orally to different groups of experimentally infected *H. diminuta* infections in rats. The efficacy was adjudged in terms of parasite eggs/g (EPG) of faeces count before and after treatment, direct count of surviving worms remaining in small intestines after completion of treatment and by host clearance of parasite. In all the experiments, the effect of leaf extract was compared with a standard anticestodal drug, praziquantel (PZQ).
Results: The leaf extract showed reduction in parasite EPG of faeces count in a dose-dependent manner. It further showed comparatively low recovery of worms including scolices in the small intestine and host clearance of parasite in a dose dependent manner. In all the experimental models the anticestodal efficacy of leaf extract was significantly comparable with that of PZQ.
Conclusion: The leaf extract of *P. guajava* possesses anticestodal efficacy. Study supports its folk medicinal use in the treatment of intestinal-worm infections in northeastern part of India.

KEY WORDS: EPG count, praziquantel, scolex.

Introduction

Psidium guajava L., belonging to the family Myrtaceae, is a widely distributed plant which has several medicinal uses: antifilarial,[1] antidiarrheal,[2] CNS depressant,[3] anticough,[4] antiamebic, antispasmodic,[5,6] and antimicrobial activity.[7] Its previously isolated constituents include ascorbic acid,[8] fatty acids,[9] tannins, phenols, triterpenes, essential oils, saponins,[10] carotenoids,[11] lectins,[12] and flavonoids.[5] In the northeastern part of India, various Naga tribes use fresh leaves water decoction of *P. guajava* (locally known as "motiram") as a common remedy for intestinal-worm infections. To substantiate the claims made by local people herein we report the *in vivo* anticestodal efficacy of *P. guajava* using experimental *Hymenolepis diminuta* infection in albino rats.

Materials and Methods

Plant material and extraction

P. guajava L. (Myrtaceae) fresh tender leaves were collected from the Mokokchung District of Nagaland (India) during October 2002 in the supervision of Dr. Jamir, Department of Botany, Nagaland University, Nagaland. A voucher specimen (AKY/001) has been deposited in the Department of Zoology, North-Eastern Hill University, Shillong. The plant material was dried in shade at 15–26°C and finely grounded into powdered form with the help of an electric grinder. The powdered material was hot-extracted in methanol at 40°C for approximately 48 h. The solvent was removed by distillation under reduced pressure using rotatory vacuum evaporator; the residue was dried over anhydrous calcium chloride inside a desiccator and stored at -20°C until further use. The yield of prepared extract was 23.68%.

Animals

Albino rats of either sex were originally purchased from M/s Fanindra Nath Chakravorty, Kolkata, and the stock was inbred in the animal house for several years. Animals (100–120 g) of either sex were used in the study. They were kept under the standard environmental conditions with food and water *ad libitum*. All the animal experiments were performed following the approval of study protocols by the Institutional Animal Ethics Committee.

Acute toxicity study

Eighteen rats were divided into three groups (six rats per group). Groups I and II were treated with the leaf extract at 3200 and 6400 mg/kg body weight dissolved in water. Group III served as the control group, which received water (the vehicle for the extract). The animals were kept under observation for 72 h period for mortality; they were further

observed for any changes in their body weight, body temperature, and food and water uptake over a period of 2 weeks.

Experimental design

Anticestodal efficacy was adjudged on the basis of parasite eggs/g (EPG) of faeces count before and after treatment,[13] direct count of surviving worms including scolices (% worm recovery) remaining in small intestines after completion of treatment,[14] and by host clearance of parasite. These were calculated in percentage as follows:

Percentage difference in EPG count = (mean EPG at pretreatment – mean EPG at post-treatment) X 100/(mean EPG at pretreatment)

Percentage worm recovery rate = number of worms recovered at necropsy X 100/number of cysticercoids given

Percentage host clearance = number of hosts found devoid of worms X 100/number of hosts infected with worms

The infection of *H. diminuta* was maintained in rats by alternating the hosts using wheat flour beetles, *Tribolium confusum* (Tenebrionidae), as the intermediate host.[15] Beetles were starved for 3 days and later allowed to feed for 72 h on the sieved flour powder mixed with the faecal matter containing *H. diminuta* eggs obtained from the gravid segments of parasites, which were maintained in rats in our laboratory. Beetles were later maintained at room temperature with free access to flour until dissected. After 12 days of feeding gravid segments, the cysticercoids (larval stage) were obtained by dissecting the infected beetles in phosphate-buffered saline (pH 7.3). Each rat was inoculated with four cysticercoids suspended in 1 ml of saline using a feeding tube and later maintained in separate cages as described by Dixon and Arai.[15] For each experiment rats were divided into six groups, comprising six animals in each group. Group I rats served as infected, untreated controls. Groups II–V were treated with 1600, 800, 400, and 200 mg/kg dose of leaf extract. Group VI was given 5 mg/kg of praziquantel (PZQ) as a standard anticestodal drug. Different doses of leaf extract and PZQ were calculated out as per the body weight of animals that were dissolved in 1 ml of water and later administered to each animal with the help of a feeding tube. Administration of leaf extracts and PZQ to the infected rats was done consecutively from day 26 to 28 and day 36 to 38.

On the 18th-day postinoculation, faecal examination revealed that all the rats were infected; their EPG of faeces was monitored using the modified Mc Master method.[13] EPG count was done for 3-day pretreatment (day 18–20), 3-day post-treatment (day 26–28) and a follow-up examination of the EPG count was done again for another 3 days (day 36–38), i. e., after 1 week of the previous EPG count.

Statistical analysis

Statistical analysis was performed using two-way and one-way ANOVA followed by Dunnett's test. The significance of difference was accepted at $P < 0.01$.

Results

The results are presented in Tables 1–3. The mean EPG count did not vary much in the control group throughout the experimental period (Table 1). Lower doses of leaf extract had fewer effects on mean EPG counts; whereas 800 mg/kg dose reduced the EPG count by 55.73% and 85.37% at days 26–28 and 36–38, respectively. A dose of 1600 mg/kg body weight of leaf extract showed maximum reduction in the EPG count, 71.23% and 92.17% at days 26–28 and 36–38, respectively. The standard anticestodal drug PZQ decreased the mean EPG counts by 82.37% and 96.39% during the similar days' regime. Treatment with 800 and 1600 mg/kg dose of leaf extracts resulted into comparatively low worm recovery rates (25% and 16.75%, respectively); PZQ reduced the worm recovery rate by 12.5%. Further, the 800 and 1600 mg/kg of dose showed a 16.66% and 33.33% host clearance as compared with PZQ, in which it was observed to be 66.66%. The results thus indicate that leaf extract of *P. guajava* is quite effective against *H. diminuta* parasite.

In the acute toxicity study, 3200 and 6400 mg/kg body weight dose of leaf extract administered to two groups of rats did not show any mortality during the 72 h period of observation. The control and plant-extract-treated groups showed normal increases in body weight (6.29%, 3.4%, and

Table 1

Efficacy of *P. guajava* leaf extract on EPG of faeces count of *H. diminuta* infection in rats

Group	EPG count[a]			Difference in EPG count[a]		% Reduction in EPG[a]	
	Pretreatment (Z) (Days 18–20)	Post-treatment (A) (Days 26–28)	Post-treatment (B) (Days 36–38)	Z and A	Z and B	Z and A	Z and B
Control	16866.68±269.44	16822.22±283.72	16822.22±422.22	44.46±16.66	44.46±15.36	0.26±0.10	0.26±0.09
Extract (mg/kg)							
1600	17044.44±270.34	4911.12±160.24	1000.00±115.48	12133.34±148.00	16044.44±226.23	71.23±0.51	92.17±0.52
800	17133.34±901.84	7600.00±443.90	2533.34±501.84	9533.34±151.56	14600.00±241.27	55.73±1.42	85.37±2.40
400	17133.34±133.34	11222.22±1371.98	4200.00±203.66	5911.12±493.24	12933.34±746.57	35.28±3.94	75.42±0.31
200	17200.00±1488.84	12355.56±978.54	5000.00±983.32	4844.44±544.24	12200.00±946.06	28.12±2.06	70.46±4.07
Praziquantel							
5 mg/kg	17022.24±699.56	3000.00±66.68	600.00±155.46	14022.23±343.89	16422.23±306.76	82.37±0.17	96.39±0.58

[a]Values are mean±SEM. n = 6 animals in each group. P<0.01 (two-way ANOVA).

Table 2

Efficacy of *P. guajava* leaf extract on worm recovery rate of *H. diminuta* infections in rats

Group	Number of Cysticercoids / rat	Mean±SEM of recovered worms / rat (range)	Worm recovery (%)	Host clearance (%)
Control	4	4.00±0.00 (0)	100.00	0.00
Extract				
1600 mg/kg	4	0.67±0.21 (0–1)	16.75	33.33
800 mg/kg	4	1.00±0.26 (0–2)	25	16.66
400 mg/kg	4	1.34±0.21 (1–2)	33.5	0
200 mg/kg	4	2.34±1.00 (2–3)	58.5	0
Praziquantel				
5 mg/kg	4	0.50±0.34 (0–2)	12.5	66.66
One-way ANOVA	F	0.2843		
	P	>0.01		
	df	5,30		

n = 6 animals in each group.

Table 3

Changes in body weight in rats after treatment with *P. guajava* leaf extract

Body weight (g)		Initial	Final	% Increase
Control		110.50±3.06	117.34±1.06	6.29±2.19
Extract				
3200 mg/kg		112.50±5.34	116.17±3.67	3.40±1.93
6400 mg/kg		111.50±4.42	114.84±3.64	3.05±1.23
One-way ANOVA	F	13.3624	0.9595	1.0152
	P	>0.01	>0.01	>0.01
	df	1,10	1,10	1,10

n = 6 animals in each group; values are mean±SEM.

3.05%, respectively, which were not significantly different from each other) (Table 3). No significant differences in body temperature, food, and water intake from the controls were found in the animals.

Discussion

The northeast part of India, which is inhabited by various tribes, has unique tradition of using plant materials or plant-based preparations for various remedies. *P. guajava* is among one such commonly used plants whose young, tender leaves have a reputation of being efficacious against intestinal-worm 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 counting of EPG, recovery of worms, and percentage of host clearance at necropsy after the treatment are conventional methods to establish the anticestodal efficacy of an agent[14,15].

In the present study, methanol extract of the leaves of *P. guajava* revealed a significant level of anticestodal activity against experimentally induced *H. diminuta* infections. The results indicate that the efficacy of leaf extract is dose-dependent. Lower worm counts after treatment periods as recorded in the study may be owing to loss of worm and/or process of destrobilation. Dixon and Arai,[15] in their study on effect of anthelmintic on *H. diminuta*, reported that this may be owing to either expulsion of adult worms or destrobilation by leaf extract. It is known that the cestodes usually undergo the process of destrobilation if exposed to any anthelmintic drug or if owing to any physiological stress in the intestine.[15] The recovery of low number of worms on necropsy after treatment and reduction in EPG count further substantiates the claim regarding the anticestodal efficacy of leaf extract. The worm recovery rate decreased subsequently on increasing the dose of leaf extract. Similar trends were observed with regard to percentage host clearance, which increased with an increase in the dose of leaf extract

In the acute toxicity study, a dose of 6400 mg/kg given singly as well as twice orally 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 (1600 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 leaf extract is nontoxic in nature. However, the toxicity of extract needs to be further investigated with regard to other parameters.

In conclusion, the results of the present study indicate that leaf extract of *P. guajava* possesses significant level of anticestodal efficacy, which validates its use in folk medicine. This plant has a potential for exploring its activity profile against other helminth parasites too.

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