

STUDY ON ACTIVITY OF ALCOHOLIC EXTRACT OF GLANDS AND HAIRS OF FRUITS OF *MALLOTUS PHILIPPINENSIS* IN MURINE CESTODAL INFECTION MODEL

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ABSTRACT

Objective: *Mallotus philippinensis* Muell-Arg has been commonly used in Indian medicine for curing intestinal worm infections. In this study the anticestodal efficacy of *Mallotus philippinensis* fruit extract has been evaluated in rat cestodal (*Hymenolepis diminuta*) intestinal infection model.

Materials and Methods: The anticestodal efficacy of fruit extract was determined in the different groups of rats harbouring adult *Hymenolepis diminuta* infections by monitoring the eggs per gram (EPG) of faeces.

Results: *Mallotus philippinensis* fruit extract at 800 mg/kg twice daily for 3 days was observed to have curative efficacy against mature adult worms. The total follow up period of 90 days did not show any further excretion of eggs in the faeces of treated rats. Praziquantel at the dosage of 5mg/kg also produced a similar effect.

Discussion: The treatment of *Hymenolepis diminuta* adult stage of parasite with ethanolic fruit extract showed dose-dependent decline in EPG count. The anticestodal activity emerged from this study may be attributed due to the presence of phloroglucinol derivatives, chalcones derivatives and some glycosides. However, an active constituent needs to be characterized further.

Conclusion: The study showed that the alcoholic extract of fruit parts of *Mallotus philippinensis* possesses significant anticestodal activity and supports its use in the folk medicine.

Keywords: *Hymenolepis diminuta*; *Mallotus philippinensis*; Anticestodal activity; Cestodes; Fruit; Alcoholic extract.

INTRODUCTION

Mallotus philippinensis Muell-Arg. family Euphorbiaceae, (commonly called Kamala, Kampillaka, Kapila and locally known as Shendri) a very common perennial shrub or small tree found throughout the Indian subcontinent, Malaysia and Philippines. It is also found as high as outer Himalayas ascending to 1500 meters^{1,2}. Different part of the plants i.e. stem bark, leaves, roots and dried glandular and non-glandular capsules, hairs covering the fruits of *Mallotus philippinensis* have been reported being used since long in Ayurvedic (Indian), Arabic, Unani and Chinese traditional Medicine systems as an anthelmintics, antifungal, antibacterial, immuno-regulatory^{3,4}, antifilarial⁶, antiparasitic, anti-ulcers and as an aphrodisiac⁷. Despite the mention of the plant as purgative and anti-intestinal parasitic in Charaka Samhita⁸ and Sushruta Samhita⁹, there are scanty reports on anticestodal activity of crude product of different parts of the plants. Although, Jost (1996)¹⁰, have reported no effect of single oral administration dried powdered fruit of the plant against gastrointestinal nematodes in goats. Further, in a study from India on human subjects where single dose of 1gm for children \leq 5 year and 2 gm for children \geq 5 years has reported to eradicate 96 % of the *Hymenolepis nana* infection¹¹. However no report has been made till date to look into the effect of different extracts of the plant product specially hairs and glands of the fruit of *Mallotus philippinensis* on cestodes either *in vitro* or *in vivo* models to delineate the active component of the plant product. Therefore, the present study was planned to see the effect of alcoholic extract (rich in alkaloids and glycosides) of fruit part of *Mallotus philippinensis* on adult *Hymenolepis diminuta* in rats¹².

MATERIALS AND METHODS

Plant material and preparation of the extract

The fruits of *Mallotus philippinensis* were collected from Botanical Garden, Department of Dravyaguna, Institute of Medical Sciences, BHU, Varanasi, India. The plant was identified and authenticated by Prof. R.K. Asthana, Department of Botany, BHU, Varanasi, India. Reference number RKA/BOT/Sept 10-12 was given to plant sample. The shade dried fruit powder was first defatted with petroleum ether and then extracted with ethanol at room temperature by cold extraction method with occasional shaking. The extract was

concentrated in a rotary evaporator and the residue was dried in desiccators over calcium chloride. The final yield (w/w) of the alcoholic extract was 11.6 %.

Drugs

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

Experimental animals

Healthy adult male and female albino rats (100–120 g) of Wistar stock were used. These 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 not suffering from any infection. 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 Banaras Hindu University, Varanasi.

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 host¹³. In brief, the gravid segments of tapeworm were scratched smoothly on to the filter papers inside the petri dishes, and the beetles were allowed to feed on flour for 72 hr. These beetles were then maintained at room temperature for at least 12–14 days for the cysticercoid larva to develop. Cysticercoids were collected by dissecting the beetles and inoculated to uninfected rats for initiation of infection. After 18–20 days, eggs of *H. diminuta* were detected in the faeces of rats, which were mixed with flour powder and fed to the beetles to continue the life cycle in the laboratory.

Acute toxicity tests

The fruit extract was administered orally at the increasing doses of 100, 200, 400, 800, 1600 and 3200 mg/kg, orally to six animals in

each group. The general signs and symptoms of toxicity, intake of food and water, and mortality were observed for 72 hr post extract administration¹⁴.

Effect of *Mallous philippinensis* fruit extract on the adult stages of *Hymenolepis diminuta*.

Nine groups of animals (n = 6) were used in this experiment and all were maintained in separate cages harbouring *H. diminuta* infection proved by passage of the eggs in their faeces. Administration with fruit extract at different single and double doses per day (200, 400, and 800 mg/kg,) orally in groups 2–7, and with PZQ at single and double doses per day (5 mg/kg, orally) in groups 8–9 were administered to the rats. The group 1 was used as the control and given 1.0 ml of saline per day for the same 3 days. From day 18 post treatment, fresh faeces was collected from each cage of the treated and control rats for eggs per gram (EPG) counts using modified Mc Master method (Anonymous, 1977) for 3 days (days 18–20). The percentage reduction in the EPG counts was calculated as per the criterion of¹⁵. Follow-up examination of EPG was done on days 28–30 following a week EPG count. They were again screened for the presence of *H. diminuta* eggs 90 days later.

Measurement of anticestodal activity

The anticestodal activity was evaluated in terms of differences in the mean EPG count^{16,17} undertaken 3 days each, before treatment and after treatment of plant extract as follows:

$$\text{Difference in EPG Count} = \frac{\text{Mean EPG at A} - \text{Mean EPG at B}}{\text{Mean EPG at A}} \times 100$$

Statistical analysis

All results were reported as mean±S.E.M. These results were further analysed by column analysis using Student's t-test to calculate the significance of the results¹⁸. P values ≤0.0001 % were considered significant.

RESULTS

Toxicity study

The fruit extract when orally given to the rats at doubling doses from 100 up to 3200 mg/kg, orally showed no mortality or any adverse signs in the animals with regard to body weight, body temperature, food and water in take up to 72 h post treatment.

Effect of extract on adult worm of *Hymenolepis diminuta*

The effects of fruit extract on adult stages of *H. diminuta* infections in rats as monitored by EPG counts is shown in Table 1. The EPG values (i.e 0-30,366) of fruit extract treated groups significantly reduced in dose-dependent manner when compared to control (58,300) in Figure 1. The percentage difference in the count between days 18-20 and 28-30 is usually showed gradually increasing trend i.e 3.65 in untreated group to 6.46 in the group receiving 200 mg/kg/day for 3 days to highest 83.65 when 800 mg/kg body weight. However, when 800 mg/kg dose was given twice daily the count observed on the days 18-20 was nil which continued on days 28-30. Interestingly, Praziquantel given in the recommended dosage of 5 mg for rats weighing about 200 gm twice daily for 3 days could not achieve complete cure, despite significant reduction in EPG value.

Table 1: Effect of *Mallotus philippinensis* alcoholic fruit extract on adult stage of *Hymenolepis diminuta* infections in rats

Treatment group(mg/kg×dose×day)	EPG count (mean± SEM)		Percentage difference in count between A & B(%)
	Days 18-20(A)	Days 28-30(B)	
Control ^a	58300 ± 152.752	60433.33 ± 240.37	3.65
Extract			
200×1×3 ^a	30366.66 ± 317.97	28403.33 ± 250.355 ^b	6.46
200×2×3 ^a	28048.00 ± 53.078	26407.66 ± 174.362 ^b	5.84
400×1×3 ^a	24676.66 ± 303.003	22620.00 ± 322.33 ^b	8.33
400×2×3 ^a	10467.66 ± 275.920	8476.00 ± 184.511 ^b	19.02
800×1×3 ^a	5243.33 ± 183.356	857.00 ± 23.067 ^b	83.65
800×2×3 ^a	0 ^b	0 ^b	0
Praziquantel			
5×1×3 ^a	4115.00 ± 7.637	3148.00 ± 26.193 ^b	23.49
5×2×3 ^a	2230.00 ± 11.547	1656.00 ± 29.62 ^b	25.73

^a No. of animals in each group, n=6; ^b p < 0.0001 vs. control value, Student's t-test.



Fig. 1: Effect of increasing doses on egg per gram (EPG) count at different day's interval

DISCUSSION

The present study was designed to evaluate the anticestodal efficacy of alcoholic extract of *M. philippinensis* fruit on adult stage of *H. diminuta* (cestoda) rat experimental model. This animal model has widely been used to evaluate the efficacy of several anticestodal agents^{19,20}. The alcoholic extract essentially known to have various alkaloids and glycosides. The nil EPG at the dose of 800mg/kg twice per day consecutive 3 days indicates that this dose is effective in curing the infection.

This is really encouraging to see that the alcoholic extract at this dose had better potential than the known anticestodal agent i.e Praziquantel at recommended dosage. This observation suggest that the active anticestodal molecule must be either an alkaloid and/or glycoside derivatives of the fruit parts^{21,22}.

Many of the alkaloids and glycosides of *Mallotus philippinensis* had been characterized such as phloroglucinol derivatives (rottlerin, isorottlerin and isoallorottlerin) and chalcones derivatives i.e. mallotophilippens A, B, C, D & E^{23,24}. However, the number of the above derivatives must be subjected to the screening for their activity against the cestodes. The new constituent obtained will be an addition to the only few available anticestodal drugs like Niclosamide and Praziquantel. Moreover, resistance against these two existing molecules have already started coming up. The efficacy of Praziquantel of recommended dose has also been seen unsatisfactory in the present study also and the frequent reoccurrence of the infection after treatment with Niclosamide has been seen very often. On the basis of present study, it may further be suggested that the effect of the different derivatives of Kampilla (*Mallotus philippinensis*) fruit parts should also be screened for its potentials against extra intestinal cestodal infection e.g Cystecercus cellulosa and Hydatid disease etc along with their effect on intestinal form.

There are speculations that the extract might have either expelled the adult worms from the host lumen or caused destrobilation and thus the observed cure. However the hypothesis needs to be proved.

CONCLUSION

It may be concluded that the fruit extract of *Mallotus philippinensis* possesses significant anticestodal efficacy, which validates its use in folk medicine and supports the need for a further investigation in to phytochemical analysis of plant products.

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