Anticestodal properties of *Hibiscus rosa-sinensis* L. (Malvaceae): an in vitro and in vivo study against *Hymenolepis diminuta* (Rudolphi, 1819), a zoonotic tapeworm

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Abstract The aim of this study was to investigate the in vitro and in vivo anticestodal effects of methanol extract of *Hibiscus rosa-sinensis* L. (Malvaceae) leaf against *Hymenolepis diminuta* (Rudolphi, 1819), a zoonotic tapeworm. Under the in vitro study, *H. diminuta* worms were exposed to 10, 20 and 40 mg/ml concentrations of methanol leaf extract and the effects were judged on the basis of physical motility/mortality of worms. On the other hand, in the case of in vivo study, *H. diminuta* infected rats were treated individually with 200, 400 and 800 mg/kg doses of leaf extract for 5 days. The effects were judged on the basis of reduction in eggs per gram (EPG) of faeces and worm counts. In case of in vitro test, the treatment with 40 mg/ml concentration of extract revealed prominent anticestodal effect and caused paralysis of worms in 3.00 ± 0.53 h and mortality in 4.08 ± 0.21 h. However, under in vivo study, the 800 mg/kg dose of extract revealed the highest anticestodal effect and caused 66.55 % reduction in EPG count and 75.00 % reduction in worm count in the treated animals. The results of this study indicated that *H. rosa-sinensis* leaf extract possesses concentration-dependent anticestodal effect against *H. diminuta*, indicating that the plant possesses promising active principle for the control of intestinal helminthic infections.

Keywords Anthelmintics · Anticestodal · Helminthic infections · *Hymenolepis diminuta* · *Hibiscus rosa-sinensis*

Introduction

Intestinal helminthic infections are among the most common infections of humans, especially in tropical and subtropical countries (de Silva et al. 2003). The cost of harbouring these parasites in terms of human misery and economic loss is incalculable, as chronic worm infections often lead to severe manifestations, such as malnutrition, anaemia, stunted growth, poor intellectual development, etc., particularly in children (Anonymous 2004). Though there are some safe and effective anthelmintic drugs to treat the intestinal helminths, the greatest challenge is to extend regular anthelmintic drug coverage to reach all the individuals in endemic areas (WHO 2006). In India, where the prevalence rates of intestinal helminths is fairly high, a large number of people still do not have an easy access to anthelmintic drugs, particularly in rural areas (Qadri 2008). On the other hand, there exist many medicinal plants which are easily available and affordable to the people in developing countries and these plants have been widely used for the treatment of helminthic infections, particularly in Asia, Africa and Latin America (WHO 2002; Tandon et al. 2011).

Hibiscus rosa-sinensis L. (Malvaceae) is a shrub of about 4–5 m height and is widely distributed in tropical and subtropical regions of world. In traditional medicine, almost all the parts of this plant are considered to be precious as they are claimed to have some or other medicinal value (Jadhav et al. 2009). The decoction, juice, and extracts, prepared from the leaves, flowers or roots of this plant are used as diuretics, contraceptives, antidiarrheals, aphrodisiacs, cough suppressants, etc. in traditional medicines (Jadhav et al. 2009). In experimental studies, the leaves and flowers of *H. rosa-sinensis* have been demonstrated to aid in healing of ulcers (Kurup et al. 1979). More

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recently, it has also been demonstrated through controlled experimentations that various parts of this plant also possess cardioprotective (Gauthaman et al. 2006), antibacterial (Ruban and Gajalakshmi 2012) and antioxidant (Sarkar et al. 2014) activities.

During a field study in Tripura, India, it was revealed to us that the leaves of *H. rosa-sinensis* are considered as highly efficacious against intestinal-helminthic infections and therefore used as popular anthelmintic by local people. Despite the wide-spread use of this plant against intestinal-worms, there is no scientific evidence regarding its anthelmintic efficacy. Therefore, in this study we investigated the anticestodal effects of *H. rosa-sinensis* leaf extract against *H. diminuta*, a zoonotic tapeworm.

Materials and methods

Plant material

The leaves of *H. rosa-sinensis* were collected from North Tripura district of Tripura, India and identified by a plant taxonomist. A voucher specimen (AKY-11882) of plant material has been deposited in the Department of Zoology, NEHU, Shillong. The leaves were dried under shade, powdered and extracted in methanol, using a Soxhlet extractor. The final yield (w/w) of plant extract was about 18 %.

Phytochemical analysis of extract

The phytochemical analysis of extract was done for the presence of alkaloids, saponins, flavonoids, tannins, anthraquinones, glycosides, terpenoids and steroids, etc., using various standard qualitative tests, as described in the Ph.D. thesis of first author (Nath 2014).

In vitro anticestodal study

The in vitro anticestodal activity of the leaf extract was evaluated on *H. diminuta* and the effects of extract were studied on the basis of physical motility/mortality of adult tapeworms. For physical motility assay, adult *H. diminuta* worms were collected in 0.9 % Hank's solution from the intestines of freshly necropsied Wistar rats, carrying experimentally induced *H. diminuta* infections (Yadav and Tangpu 2012). The test worms ($n = 6$) were then placed in Hank's solution and kept at 37 ± 1 °C inside a laboratory incubator. The extracts were tested at 10, 20 and 40 mg/ml concentrations, while the reference drug praziquantel (PZQ), was tested at 1 mg/ml concentration. One set of test worms ($n = 6$), maintained exactly on similar conditions, but without plant extract or reference drug, were included

as negative controls. The anthelmintic efficacy of extract/praziquantel was judged on the basis of physical motility of test worms, as evident by their paralysis and/or mortality, and was monitored on regular basis. The paralysis and mortality of test worms was checked as per the methods of (Vijaya and Yadav 2014), where two types of PBS solutions, i.e. one warm (37 ± 1 °C) and the other slightly more warm (40 ± 1 °C) were prepared. Worms not showing any physical movements on gentle stimulation by a soft brush were picked up and transferred first to the warm saline solution. Total loss of noticeable movements in the parasite during exposure to warm solution was considered as a sign of paralysis and the same was recorded for each set of experiment. Likewise, when the parasites showed no movements in this warm saline for about 5 min, they were then immediately transferred to the slightly more warm saline solution so as to finally confirm their mortality. The mortality of parasites was assumed to have occurred when all signs of movements had ceased even after exposing to slightly warmer saline solution for 30 min. The paralysis and mortality time of test parasites were recorded for each experiment. The experiments were undertaken for three times.

In vivo anticestodal testing

Male and female albino rats of Wistar strain, weighing 180–200 g, were used. Prior to the experiment, animals were acclimatized for 15 days in the laboratory and had ad libitum access to standard rodent food and water. During the acclimatization period, the faecal samples of all experimental animals were checked daily to ascertain that they do not harbour any intestinal worm infections. All the experimental protocols that involved use of rats in this study were approved by the Institutional Ethics Committee (Animal Models) of North-Eastern Hill University, Shillong. The infection of *H. diminuta* was maintained in the laboratory by cyclical passage through Wistar rats and flour beetle, *Tribolium confusum* (intermediate host), as described previously (Yadav and Tangpu 2012).

The animals were randomly divided into five groups ($n = 6$). Each animal was then orally infected with four cysticercoids by a blunt feeding tube and maintained in a separate cage. The first group of rats served as the untreated control and received only the vehicle. The second, third, and fourth group of animals were treated with 200, 400 and 800 mg/kg p.o., doses of *H. rosa-sinensis* leaf extract, respectively. Whereas, the fifth group of animals received 5 mg/kg, p.o. dose of praziquantel (PZQ), the reference drug.

To assess the anthelmintic efficacy of extract, treatments of rats was done with extract/PZQ for 5 days, i.e. on days 21–25 post inoculation (p.i.) of cysticercoids. The EPG counts of animals were done for 3 consecutive days (days

18–20 p.i.) before treatment (pre-treatment), and for another 3 days (days 26–28 p.i.) after treatment (post-treatment) (Anonymous 1977). Accordingly, the percentage reductions in EPG counts of animals were calculated at post treatment period (Yadav and Tangpu 2012). On day 39 p.i., all animals were sacrificed and the number of worms in their intestine was counted to calculate the percentage reduction in worm counts (Yadav and Temjenmongla 2011).

Statistical analysis

Data are expressed as mean $\pm$ standard errors of mean (SEM). Statistical evaluations were performed using Student's *t* test. Statistical analysis was done using Origin Pro 8® software. $P < 0.05$ was considered statistically significant.

Results

The phytochemical screening of *H. rosa-sinensis* leaves extract confirmed the presence of alkaloids, saponins, flavonoids, glycosides, and terpenoids, however, tannins and anthraquinones were absent. In the in vitro study, after exposure of adult *H. diminuta* to different concentrations of plant extract, a significant ($p < 0.001$) and dose-dependent effect was observed on the physical motility of test worms (Fig. 1). The extract-treated worms first showed paralysis which was followed by mortality. The highest concentration of extract (i.e. 40 mg/ml) revealed paralysis of worms at 3.00 ± 0.53 h and mortality at 4.08 ± 0.21 h. On the other hand, the worms maintained in the control medium showed their survival till 23.67 ± 3.04 h.

In the in vivo study also the extract showed a significant ($p < 0.001$) and dose-dependent anticestodal effect against *H. diminuta* infections in rats (Table 1). Treatment of rats with increasing concentrations of extract revealed a gradual decrease in the eggs per gram (EPG) of faeces count and worm counts of animals. A single 800-mg/kg dose of extract, given for 5 days, revealed 66.55 % reduction in the EPG and 75.00 % reduction in worm counts, as compared to the control. Herein, the reference drug PZQ (5 mg/kg), given for 5 days, showed slightly better effects and resulted in 87.31 and 83.25 % reductions in the EPG and worm counts, respectively. In the control group, the EPG counts and worm counts did not show any noticeable change during the pre-and post-treatment periods and remained stable throughout the observation period (Table 1).

Discussion

In several anthelmintic screening studies on medicinal plants, the in vitro actions of extracts have been detected

Fig. 1 In vitro anticestodal effects of *Hibiscus rosa-sinensis* leaves extract against *Hymenolepis diminuta*. Physical activity of worms in the control group was recorded up to 23.67 ± 3.04 h. * $p < 0.001$ versus control, Student's *t* test

through their effects on motility or mortality of a suitable test parasite (Tandon et al. 2011). In the present study, exposure of test parasites to *H. rosa-sinensis* extract showed a significant and dose-dependent effect in the form of paralysis and mortality of tapeworm. The best anthelmintic effects were observed at 40 mg/ml concentration of extract, where the parasites showed paralysis and mortality in about 3 and 4 h, respectively. On the other hand, the parasites maintained in the control medium showed physical activity for much longer (23.67 h) time period. On the basis of these findings, it may be assumed that *H. rosa-sinensis* leaf extract exerts significant in vitro effects on test worms and possesses good anthelmintic properties. It is worth mentioning here that in many other in vitro studies, the test worms showing their paralysis or mortality within almost the similar time durations, as noticed in the present work, has also been considered to have significant anthelmintic properties (Tandon et al. 2011).

Although, the in vitro screening assays are easy to conduct and are considered less expensive, but the data obtained from in vitro studies alone usually do not provide a complete picture of biological effect of a test agent, until or unless they are linked to a confirmation test in a suitable animal model (Cos et al. 2006). Therefore, in the present study, we further extended our investigations to test the in vivo anticestodal efficacy of *H. rosa-sinensis* leaf extract in *H. diminuta*-rat animal model. Interestingly, under in vivo investigation as well, the plant extract showed significant and dose-dependent anticestodal effects on *H. diminuta* infections in rats. The treatment of animals with 800 mg/kg dose of extract for 5 days revealed 75.00 % reduction in the worm counts of treated animals. Notably, the reductions in the worm counts of extract-treated

Table 1 In vivo anticestodal effects of *H. rosa-sinensis* leaves extract on *H. diminuta* infections in rats as monitored by eggs per gram (EPG) count of faeces and worm count

Groups	EPG (mean $\pm$ SEM)		Percentage difference in EPG counts (A–B)	No. of worms recovered/rat (mean $\pm$ SEM)	Percentage reduction in worm counts
	Pre-treatment (days 18–20) A	Post-treatment (days 26–28) (B)			
Control	15,860 $\pm$ 139	15,613 $\pm$ 172	1.56	3.84 $\pm$ 0.17	4.00
Plant extract (mg/kg)					
200	16,013 $\pm$ 296	10,711 $\pm$ 210*	–33.11	3.00 $\pm$ 0.22	25.00
400	16,186 $\pm$ 116	9386 $\pm$ 176*	–42.01	1.60 $\pm$ 0.25**	60.00
800	15,394 $\pm$ 218	5150 $\pm$ 134*	–66.55	1.00 $\pm$ 0.30**	75.00
Praziquantel (mg/kg)					
5	15,933 $\pm$ 187	2022 $\pm$ 142*	–87.31	0.67 $\pm$ 0.34**	83.25

Number of animals in each group = 6

* $p < 0.001$ versus pretreatment value, Student's t test

** $p < 0.001$ versus control, Student's t test

animals were found to be fairly comparable with that of standard drug, praziquantel. The present finding about the anticestodal effects of plant extract, as evident by reductions in worm counts is further supported by data on fecal egg reductions. Following extract treatment, a dose-dependent reduction was also noted in the eggs per gram (EPG) of faeces counts of animals. Therefore, the present in vivo findings about the anticestodal effects of plant extract also substantiate our in vitro anticestodal efficacy results.

The findings of the present study are in agreement with some other in vivo studies on anthelmintic effects of medicinal plants, established in *H. diminuta*-rat experimental model. Yadav and Tangpu (2008) studied the anticestodal efficacy of the leaf extract of *Adhatoda vasica* using experimentally induced *H. diminuta* infection in albino rats and established the efficacy of plants on the basis of a significant dose-dependent reduction in the eggs per gram of faeces counts and worm counts following extract treatment of animals (Yadav and Tangpu 2008).

At present, no information is available about the active principle of *H. rosa-sinensis*. However, a preliminary phyto-investigation on leaf extract of this plant revealed that it contains alkaloids, saponins, flavonoids, glycosides, and terpenoids. Nevertheless, there are a number of studies which demonstrate the role of above secondary metabolites of plants in providing them the anthelmintic actions. For example, the anthelmintic activity of alkaloids has been demonstrated against rat nematodes, *Strongyloides ratti* and *S. venezuelensis* (Satou et al. 2002). Similarly, Akhtar and Ahmad (1992) have reported that *Mallotus philippinensis* contains glycosides, which is responsible for its cestocidal effects. Likewise, the flavonoids from plants are believed to stimulate intestinal motility similar to that

produced by acetylcholine (Akendengué 1992), thereby causing rapid worm expulsion from the gastrointestinal tract. In addition, Lahlou (2002) has also reported the anthelmintic effects of flavonoids in the plant, *Origanum compactum*.

Taken together, our findings show that the leaf extract of *H. rosa-sinensis* possesses significant anticestodal effects and therefore this plant possesses promising potentials for the treatment and control of intestinal helminthic infections. Further investigations seem desirable to isolate and characterize the active principle of this plant.

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