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RESEARCH PAPER

In vitro and in vivo cestocidal efficacy of kaempferol derivative isolated from *Lysimachia ramosa* (Wall. ex Duby) against *Hymenolepis diminuta* in Wistar rats

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Abstract
Since ancient times, plant based medicines and other phyto-products are commonly used in different parts of the world. In India, people use different traditional medicines to cure various illnesses. Helminth infection is one of the major health issues in under developed and developing nations like India. In Meghalaya, a north-eastern state of India, people consume aqueous leaf extract of *Lysimachia ramosa* to cure intestinal worm infection. A bioactive compound kaempferol derivative isolated from the leaves of the plant has been found to be efficacious against helminth in domestic fowl in vitro. The present study aims to investigate anthelmintic efficacy of the compound against *Hymenolepis diminuta*, an infectious zoonotic cestode both in vitro and in vivo in Wistar rats taking different parameters such as tegumental enzymatic activities of ATPase, AlkPase, AcPase, mean time of paralyze and death, ultrastructure, EPG count and worm burden in the host animals. The present study demonstrated that kaempferol derivative decrease the mean time of death in the adult worms significantly. Scanning electron microscopic study reveal distorted scolex with deteriorated suckers, shrunken proglottids, loss of microtriches from the worm's body surface. Transmission electron microscopic study records the damages in the tegumental syncytium having deteriorated distal cytoplasm, exposed basal lamina, broken muscle bundle and erosion of microtriches. Distortions in the structure of nucleus and mitochondria were also recorded in the study. ATPase, AlkPase and AcPase decreased in the worms and EPG counts as well as worm recovery rates decreased significantly in the treated rats. It can be concluded from the investigation that the kaempferol derivative recovered from the plant *L. ramosa* is highly efficacious against *H. diminuta* both in vitro and in vivo in rats.

Keywords Kaempferol · Scanning electron microscopy · Transmission electron microscopy · *Hymenolepis diminuta* · ATPase · AlkPase · AcPase · EPG · Wistar rats

Introduction

Parasitic infections are major problem throughout the globe particularly of developing countries like India. Among parasites, helminths are major cause of concern, particularly in areas where adequate sanitisation facilities are lacking. Helminths are also responsible for reduced productivity among livestock (Githiori et al. 2006). To control helminthiases a number of anthelmintic drugs (praziquantel, Albendazole, benzimidazoles, niclosamide, ivermectin, suramin, diethylcarbamazin and imidazothiazoles) are available in the market, however, development of anthelmintic resistance is a threat to agricultural incomes and the increase in disease also poses a great threat to animal welfare (McKellar and Jackson 2004; Kaminsky et al. 2008). Resistance developed by helminth parasites against some anthelmintic drugs (Knox et al. 2012) and the limited availability of such commercial drugs in remote areas (Yadav and Singh 2011; Tandon et al. 2011) has created scientific research aspiration for alternative noble and inexpensive drugs based on traditional knowledge especially use of medicinal plants (Vijaya 2016; Tariq and Tantry 2012). It has been estimated that in developing

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countries majority of people rely predominantly on indigenous practice of plants and plant extracts for controlling various diseases affecting both human beings as well as other animals (Mazid et al. 2012). In India, medical practice include both folk traditions as well as codified knowledge systems with reference in the atharva veda, being textual evidence of the traditional use of medicinal plants. Increasing interest in drug of plant origin may be due to several reasons, viz., conventional medicine can be inefficient, abusive and or incorrect use of synthetic drugs results in serious side effects (Rates 2001), still a large percentage of global population have no access to conventional treatment.

Meghalaya has a wide variety of plants used by natives as curative agent against worm infections (Rao 1981). *Lysimachia ramosa* Wall. ex Duby (Family: Primulaceae) is one such plant, the aqueous extract of its leaves being widely used by Jaintia tribes in the region against intestinal helminth infections. In vitro investigations have been carried out with the ethanolic crude extract in helminth parasites such as *Fasciolopsis buski* and *Ascaris suum* from porcine hosts and *Raillietina echinobothrida* from domestic fowl (Challam et al. 2009). Recently, Dey et al. (2021) isolated a bioactive compound kaempferol derivative from the leaves of the plant and found it to be effective against *R. echinobothrida* in the in vitro experiments as it induced alterations in the enzymes such as acetyl cholinesterase and nitric oxide synthase in the helminths. Since the bioactive compound kaempferol derivative was found to be efficacious against cestodes, therefore the present investigation was undertaken to evaluate the in vitro and in vivo efficacy of the phytoproduct against a zoonotic tapeworm *Hymenolepis diminuta*.

Material and methods

Preparation and isolation of kaempferol derivative from leaves of *Lysimachia* ramosa

The plants *L. ramosa* (Wall. ex Duby) were collected from Jowai region of Meghalaya, India. The locals cultivate the plants and consume as vegetables. Taxonomical identity of the plant was confirmed at Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya, India (Accession No. 98577). Leaves were detached and air-dried and later grounded into fine powder and processed for preparation of crude methanolic extract. The kaempferol derivative was isolated from the most active anthelmintic solvent fraction of crude leaf extract with the help of thin layer chromatography and column chromatography (Dey et al. 2021). The kaempferol derivative was later considered for in vitro and in vivo anthelmintic study.

Experimental animals and test parasites

The animal model Wistar rats were procured from Veterinary Science College, Khanapara, Guwahati, Assam. Before starting the experiments, the animals were kept for 15–20 days to acclimatize to the new laboratory environment. During acclimatization period, all the rats were de-wormed by administering praziquantel at 200 mg/kg body weight dose for 3 days. Ethical permission was obtained from Institutional Ethics Committee (Animal models), North-Eastern Hill University, Shillong (NEC/IEC/2018/003). The temperature of the room was maintained at 22–25 °C and the animals were exposed to 12 h light and 12 h dark. For the in vitro and in vivo efficacy study of kaempferol derivative, the infection of cestode *H. diminuta* was maintained in Wistar rats and flour beetles of the species *Tribolium sp* (intermediate host) as described by Dixon and Arai (1991). After 23 days, the stools were examined for the presence of gravid proglottids and eggs per gram (EPG) of feces were counted to check the worm burden in the rats. When sufficient EPG was counted, then some of the rats were dissected to obtain juvenile and adult *H. diminuta* for in vitro efficacy study of the compound and the other rats were orally administered with the active compound for evaluation of in vivo efficacy of the compound. A diagrammatic representation of the proposed study has been provided (Fig. 1).

In vitro efficacy of kaempferol derivative

Adult *H. diminuta* collected from the small intestine of freshly sacrificed infected Wistar rats were maintained in 0.9% phosphate buffer saline (PBS) at 37 ± 1 °C. To conduct in vitro experiment, first of all various concentrations of kaempferol derivative such as 0.05 mg, 0.10 mg and 0.20 mg/ml of PBS were prepared by dissolving the compound in PBS with 0.1% dimethylsulfoxide (DMSO), where tapeworms were incubated at 37 ± 1 °C for 24 h and assessed continuously. Another set of worms was exposed to 0.20 mg/ml of praziquantel. For each set of experiments, five worms with approximately same size, weight and maturity were used.

Biochemical analyses

The AcPase and AlkPase activities were measured by estimating the p-nitrophenol formation following the procedure described by Plummer (1988). The ATPase activity was assayed by estimating the free phosphate release, following the method of Kaplan (1957). Treated parasites were kept at -20 °C just after they were paralyzed, till they were used for estimation. The estimations were completed within

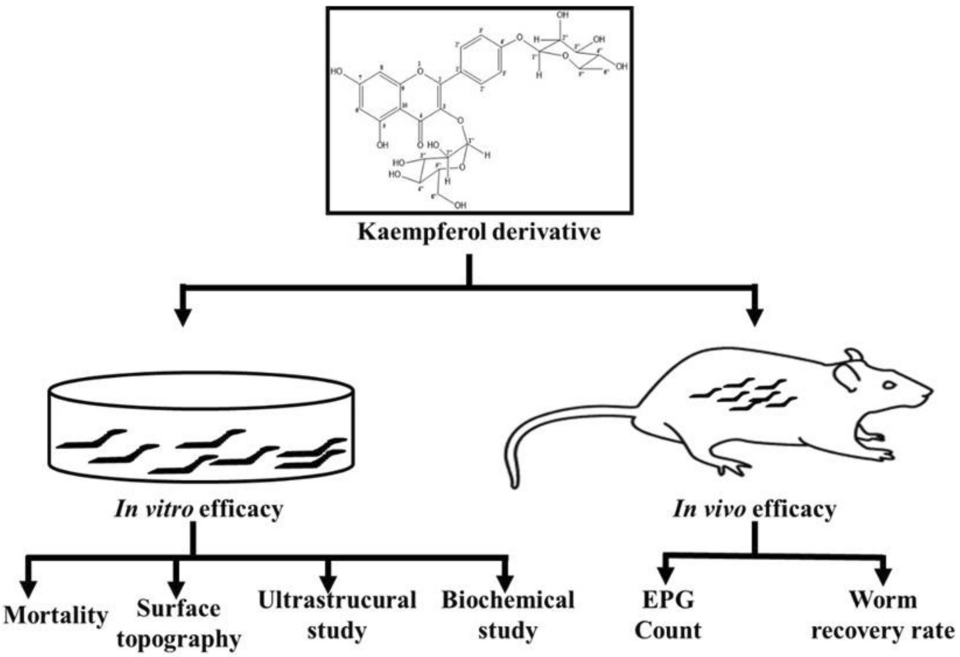

Fig. 1 Diagrammatic representation of the proposed study workflow

2–3 days of sampling during which enzyme activities were supposed to remain unaltered. Protein was estimated following the method of Lowry et al. (1951), using bovine serum albumin as the standard. Specific activity of the enzyme was expressed as the units of enzyme activity per mg protein.

Scanning electron microscopic study

For scanning electron microscopic study, helminths were taken and washed thoroughly in phosphate buffer saline (PBS). Fixations of the tissues were done in 10% neutral buffered formalin (NBF) having pH 7.0. The samples were then passed through ascending grades of acetone for dehydration of the samples. After that, the samples were immersed in tetramethylsilane to dry (Roy and Tandon 1991). The metal-coating of the samples were done with gold in a fine coat Ion Sputter. Finally the samples on stub were viewed under scanning electron microscope (SEM) JSM 6360 (JEOL Ltd, Tokyo, Japan) at electron accelerating voltages ranging between 10 and 20 kV.

Transmission electron microscopic study

The samples were then fixed in the modified Karnovsky's fixative. After that the fixed samples were washed thoroughly in cacodylate buffer. The fixative, 1% osmium tetraoxide (OsO_4) was used for post fixation of the samples. Samples were passed through ascending grades of acetone for dehydration. Tissue embedding was performed in araldite using BEEM capsules. Ultra-thin sections of the samples were prepared with the help of a RMC ultra-microtome (MT-X, USA) having a diamond knife. The sections were then stained with uranyl acetate followed by lead citrate (Reynolds 1963). Finally the sections were examined in a JEM (Jeol) TEM at an accelerating voltage of 120 kilo volt.

In vivo efficacy of kaempferol derivative

The infected Wistar rats were divided into five groups (three rats each) on the 24th day post inoculation. The first group of rats was the control group. 5 mg, 10 mg and 15 mg of kaempferol derivative/kg body weight of rats were administered orally to the rats of second, third and fourth groups respectively. The dose administrations were done daily once for 3 days. The rats of the 5th group received 5 mg of praziquantel/kg body weight of rats. All the rats were observed twice daily for pre-terminal deaths and morbidity. Dead/moribund animals were autopsied immediately at the earliest.

In vivo anticestodal efficacy of the compound was evaluated by analyzing the eggs per gram (EPG) of feces count before and after treatment. Number of surviving worms having scolex (worm recovery percentage), found in small intestine after sacrificing the rats, were calculated using the following formulae.

$$\text{Percentage difference in EPG count} = \frac{\text{Mean EPG count at pretreatment} - \text{Mean EPG count at post treatment}}{\text{Mean EPG count at pretreatment}} \times 100$$

$$\text{Worm recovery rate}\,(\%) = \frac{\text{Number of worms recovered at necropsy}}{\text{Number of cysticercoids given}} \times 100$$

For stool examination, 2 g of feces was collected from each group and average EPG was counted by modified McMaster's method. After treatment was over, all the rats were starved for 24 h and sacrificed by using general anaesthesia and the number of worms in each rat was counted to determine the worm burden.

Statistical analysis

All the data are expressed as mean ± SEM. Results were farther analysed using Students' *t*-test to calculate the significance between the experimental data and respective controls. **p*-value was considered significant at ≤ 0.05.

Results

In vitro anthelmintic efficacy

Adults of *H. diminuta* exposed to different concentrations of the active compound kaempferol derivative isolated from *L. ramosa* showed decrease in motility leading to their deaths. The control helminths showed their physical response up to 72 h following which they became immobilized and dead. At 0.05 mg of kaempferol derivative/ml of PBS, the parasites showed mobility till 12 h of time and then after 24 h, they died. When the adult worms were treated with 0.10 mg/ml of kaempferol derivative, spontaneous movement stopped at 7 h of treatment and then death occurred at an average time of 15 h. Upon exposure to 0.20 mg/ml of kaempferol derivative, it induced loss of motility of the helminths at a mean period of 4 h of treatment and deaths occurred at 7 h. 0.20 mg of praziquantel/ml concentration caused paralysis of the worms at nearly 2 h of treatment and death at an average time of 4 h (Fig. 2).

Scanning electron microscopic study

Control *H. diminuta* showed typical scolex with suckers situated around the scolex. A rostellum was observed on the top of the scolex (Fig. 3a). The body outline was normal with spine like structures covering the whole body surface known as microtriches (Fig. 3c). Proglottids were regular in shape (Fig. 3b). When the cestodes were exposed to kaempferol derivative, the scolex were observed to be distorted. The suckers were shrunken and rostellum surface was noticed to be damaged (Fig. 3d). The proglottids were deteriorated with wrinkled or shrunken surface (Fig. 3e). The microtriches were observed to be eroded from the body surface that lead to the pit formation on the body (Fig. 3f). The praziquantel treated parasites were observed to have completely damaged scolex (Fig. 3g). The normal body outline was distorted. Proglottids were clearly observed to be deteriorated with no proper division between two proglottids (Fig. 3h). Microtriches were completely lost (Fig. 3i).

Transmission electron microscopic study

TEM observations of control *H. diminuta* revealed normal intact structure of tegumental layer and its internal constituents. Regular distal cytoplasm with intact filiform microtriches was observed in the tegumental syncytium, which was followed by basal lamina, circular and longitudinal muscles and various other cellular structures (Fig. 4a). The nucleus was observed to be well intact with perfect nucleolus and continuous nuclear membrane (Fig. 4b). Numerous well-conditioned mitochondria with normal arrangement of cristae were observed in the tegumental cyton (Fig. 4c). Helminths exposed to kaempferol derivative were found to have profound disruption in the tegumental syncytium with complete erosion of microtriches from the tegument surface. Damages were noticed to be in the distal cytoplasm layer and in the form of loose integrity with broken muscle

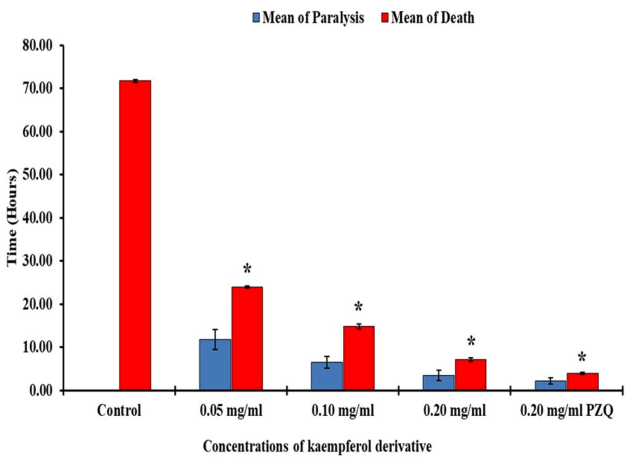

Fig. 2 Graphical representation of effect of different concentrations of kaempferol derivative isolated from leaves of *Lysimachia ramosa* on mean time of death of *Hymenolepis diminuta*. Values are expressed as mean ± SEM, values are significant at **p* ≤ 0.05, when compared to control

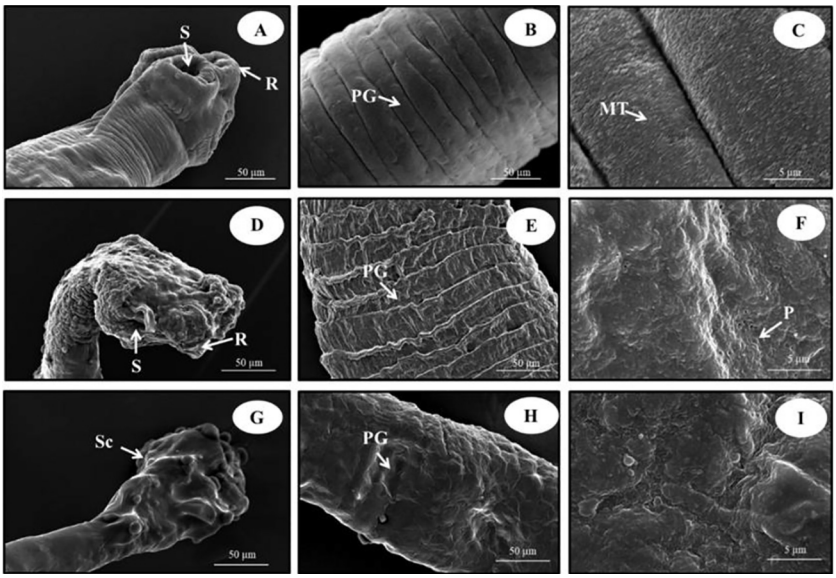

Fig. 3 Scanning electron micrographs of *Hymenolepis diminuta*. **A** Photograph showing typical scolex with suckers (S) situated around the scolex, a rostellum (R) on the top of scolex (Scale bar: 50 µm). **B** showing normal proglottids (PG) (Scale bar: 50 µm); **C** enlarged view of photograph showing spine like structures covering the whole body surface known as microtriches (MT) (Scale bar: 5 µm); **D** photograph showing distorted scolex with shrunken suckers (S) and damaged rostellum (R) on treatment with kaempferol derivative (Scale bar: 50 µm); **E** photograph showing proglottids (PG) with wrinkled and shrunken surface (Scale bar: 50 µm) on treatment with kaempferol derivative; **F** photograph showing erosion of microtriches and pit (P) formation on treatment with kaempferol derivative (Scale bar: 5 µm); **G** photograph showing damaged scolex (SC) (Scale bar: 50 µm) on treatment with praziquantel; **H** photograph showing proglottids with no proper body outline (Scale bar: 50 µm) on treatment with praziquantel; **I** photograph showing enlarged view of body surface that has no microtriches on treatment with praziquantel (Scale bar: 5 µm)

bundles. Disorganizations of cellular structures and inter cellular connections were observed (Fig. 4d). Kaempferol derivative treated helminths showed nuclei with discontinuous nuclear membrane. Nucleolus was observed to be disintegrated (Fig. 4e). Mitochondria of kaempferol derivative treated parasites were observed to have irregular structure of mitochondrial membrane and reduced number of cristae (Fig. 4f). Worms treated with praziquantel revealed total shedding of microtriches with damaged distal cytoplasm, exposed basal lamina and distorted muscle bundles (Fig. 4g). The nuclei of the helminths were observed to have discontinuous nuclear membrane along with disappeared nucleolus (Fig. 4h). Discontinuous membranes of mitochondria and lack of cristae were also noticed in the praziquantel treated worms (Fig. 4i).

Biochemical study

In the control parasites, the activity of the enzyme ATPase was the highest. When the helminths were treated with kaempferol derivative, the average ATPase enzyme activity decreased significantly by 70% from control, whereas, treatment with PZQ caused significant inhibition of the enzyme activity by 65% as observed in Table 1.

The average AlkPase enzyme activity in the worm tissue was calculated to be the greatest in the study. kaempferol derivative inhibited the enzyme activity significantly in the tissue by 54% from control. PZQ worked better than the study compound in inhibition of the AlkPase enzyme activity significantly by 62% as compared to control.

The control helminths were observed to have the highest mean of AcPase enzyme activity, although, the enzyme activity declined significantly by 65% in the kaempferol derivative treated group. PZQ treatment induced the reduction of the enzyme activity in the parasite tissue, significantly by 63%.

In vivo efficacy study

In the control group of rats, there was no significant change in the number of eggs per gram of rat's feces (EPG) (Table 2). When the rats were treated with 5 mg of kaempferol derivative/kg b.w., significant reduction in EPG count (39.33%) was noticed after the treatment period. The number of eggs/gram feces before the treatment was counted to be $21{,}345 \pm 298$, which was reduced to $13{,}020 \pm 385$ after the treatment period was over. Single dose administration of 10 mg kaempferol derivative/kg bw. of rats for 3 days decreased significantly the number of EPG in infected

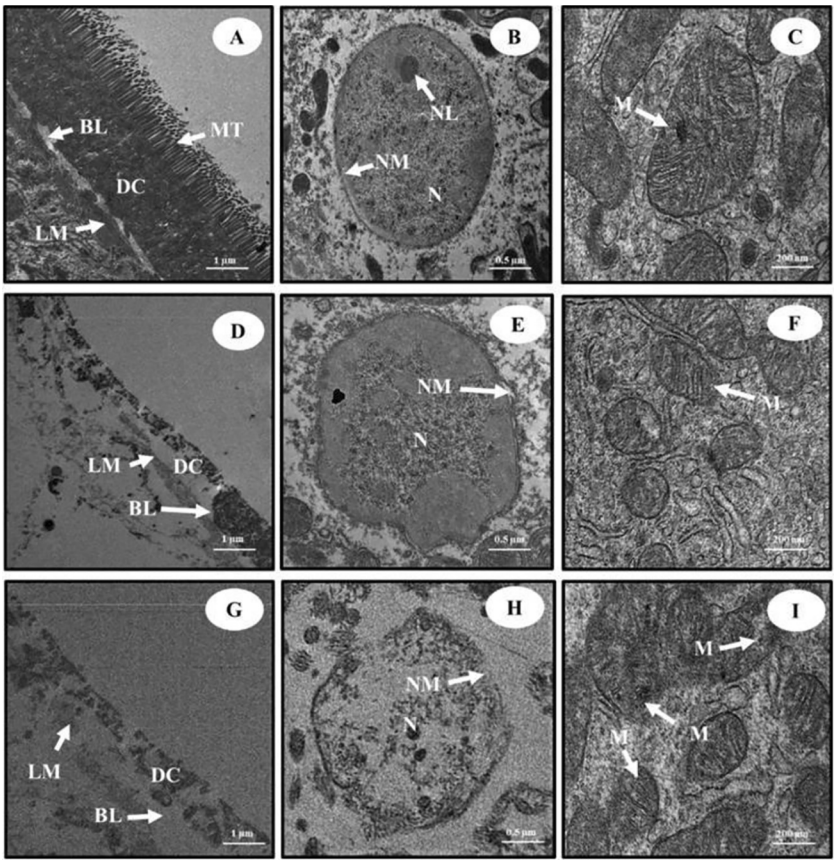

Fig. 4 Transmission electron micrographs of tegument of *Hymenolepis diminuta*. **A** Photograph showing regular distal cytoplasm (DC) with intact filiform microtriches (MT) in the tegumental syncytium, followed by basal lamina (BL) and longitudinal muscles (LM) (Scale bar: 1 µm). **B** Photograph showing normal nucleus (N) with distinct nucleolus (NL) and continuous nuclear membrane (NM) (Scale bar: 0.5 µm). **C** Photograph showing well-conditioned mitochondria with normal arrangement of cristae (Scale bar: 200 nm). **D** Photograph showing damages in distal cytoplasm (DC) layer with broken muscle bundles and complete erosion of microtriches on treatment with kaempferol derivative (Scale bar: 1 µm). **E** Photograph showing nucleus (N) with discontinuous nuclear membrane (NM) and disintegrated nucleolus (NL) on treatment with kaempferol derivative (Scale bar: 0.5 µm). **F** Photograph showing mitochondria with disrupted mitochondrial membrane and reduced number of cristae on treatment with kaempferol derivative (Scale bar: 200 nm). **G** Photograph showing tegument with total shedding of microtriches, damaged distal cytoplasm (DC), exposed basal lamina (BL) and distorted muscle bundles on treatment with praziquantel (Scale bar: 1 µm). **H** Photograph showing nucleus (N) with discontinuous wavy nuclear membrane (NM) and disintegrated nucleolus (NL) on treatment with praziquantel (Scale bar: 0.5 µm). **I** Photograph showing mitochondria with disrupted mitochondrial membrane and reduced number of cristae on treatment with praziquantel (Scale bar: 200 nm)

Table 1 In vitro effect on the activities of Adenosine triphosphatase (ATPase), Alkaline phosphatase (AlkPase) and Acid phosphatase (AcPase) on *H. diminuta* exposed to kaempferol derivative of *L. ramosa*

Control/treatment group	ATPase (tissue/ specific activity)	AlkPase (tissue/ specific activity)	AcPase (tissue/ specific activity)	% decline of ATPase	% decline of AlkPase	% decline of AcPase
Control	412.33 ± 1.45	170.33 ± 0.88	44.67 ± 0.88	_	_	_
	5.11 ± 0.06	4.1 ± 0.06	1.97 ± 0.09			
Kaempferol derivative (0.10 mg/ml)	123 ± 1.52*	78.33 ± 1.2*	15.67 ± 0.33*	70	54	65
	1.54 ± 0.03*	1.7 ± 0.12*	0.70 ± 0.03*			
PZQ (0.10 mg/ml)	144.67 ± 2.6*	63.97 ± 0.04*	16.4 ± 0.26*	65	62	63
	1.67 ± 0.09*	1.53 ± 0.03*	0.62 ± 0.02*			

Values are expressed as mean ± SEM from three replicates (n = 3), values are significant at $*p \leq 0.05$. Tissue activity is expressed as formation of 1 µmole of product/g wet weight/h. Specific activity expressed as unit/mg protein/h. Percentage decrease in tissue activity when compared with control

Table 2 In vivo anticestodal efficacy of kaempferol derivative from *L. ramosa* against *H. diminuta* infections in Wistar rats by number of eggs per gram of feces (EPG)

Groups	EPG count		Percentage reduction
	Pre-treatment (day 24–26)	Post-treatment (day 27–29)	
Control	23,450 ± 1020.3	21,926 ± 849.5	06.50 ± 0.90
5 mg/kg	21,345 ± 298.4	13,020 ± 385.1*	39.33 ± 2.08*
10 mg/kg	23,750 ± 545.7	9500 ± 630.2*	60.33 ± 1.53*
15 mg/kg	20,890 ± 800.2	6058 ± 1289*	70.66 ± 2.08*
5 mg/kg PZQ	22,860 ± 269.6	1989 ± 300.5*	91.33 ± 1.15*

Values are expressed as mean ± SEM, n = 3 rats in each group, values are significant at $*p \leq 0.05$, when compared to control

PZQ praziquental

Table 3 *In vivo* anticestodal efficacy (Worm recovery rate) of kaempferol derivative from *L. ramosa* against *H. diminuta* infections in Wistar rats

Groups	Number of cysticerdoids/rat	Number of worms/group	Worm recovery rate (%)
Control	5	4.70 ± 0.50	93.33 ± 8
5 mg/kg	5	1.70 ± 0.35*	33.33 ± 6*
10 mg/kg	5	1.00 ± 0.10*	20.00 ± 5*
15 mg/kg	5	0.67 ± 0.15*	13.33 ± 3*
PZQ (5 mg/kg)	5	0.33 ± 0.07*	06.67 ± 2*

Values are expressed as mean ± SEM, n = 3 rats in each group, values are significant at $*p \leq 0.05$, when compared to control

PZQ praziquental

treated rats from 23,750 ± 546 to 9500 ± 630. The reduction percentage was recorded to be 60.33%. Treatment with 15 mg kaempferol derivative/kg b.w. reduced the EPG count in the rats significantly by 70.66% from 20,890 ± 800 to 6058 ± 1289, however, in rats exposed to 5 mg of PZQ/kg bw., a significant reduction of 91.33% in EPG count was observed. In the course of treatment, the EPG decreased significantly from 22,860 ± 270 to 1989 ± 301.

In the control rats, after inoculation of 5 cysticercoids, 4.70 ± 0.50 worms were recovered showing the maximum recovery rate of 93%. When the rats were treated with 5 mg of kaempferol derivative/kg bw., there was only 1.70 ± 0.35 worms were obtained on average from 5 cysteceroids. The worm recovery rate significantly dropped to 33%. Oral administration of 10 mg isolated compound/kg bw. of rats, significantly lesser numbers of helminths were found dropping down the recovery rate of worms to 20% in the rats. Treatment with 15 mg kaempferol derivative/kg bw. caused significant decrease in worm number in the rats and the mean value was found to be 0.67 ± 0.15 from 5 inoculated cysticercoids. The decrease in worm recovery rate was significant when compared with the control. However, 5 mg/kg bw. PZQ affected the development of worms and reduced the average number of worms to 0.33 ± 0.07 showing the least worm recovery rate of 6.67% (Table 3).

Discussion

The present investigation showed that the kaempferol derivative isolated from the study plant *L. ramosa* had anthelmintic efficacy against *H. diminuta*, a rat tape worm. It has been observed that when the helminths were treated with the compound, they enter into a state of paralysis soon after incubation. It shows similarity with results from earlier studies carried out involving different plant products (Roy and Giri 2015; Dasgupta et al. 2010). Finally the helminths died maintaining a direct relationship between concentrations of the kaempferol derivative and time taken to attain mortality. Similar to our study, several essential oils have been reported from different plants that showed active anthelmintic activities against *Haemonchus contortus* and *Caenorhabditis elegans* (Pessoa et al. 2002). Like-wise, there are many plant derived phytochemicals that show effective anthelmintic activities against various parasites as reported earlier (Dasgupta et al. 2013).

Resveratrol, an active principle of *Carex baccans*, showed anthelmintic activity when treated against *R. echinobothrida* (Giri and Roy 2014). Similarly, genistein which is an active component of *Flemingia vestita* showed dose dependent anthelmintic activity against *Ancylostoma ceylanicum* (Lyndem et al. 2008).

It is well known that one of the important effects of any anthelmintic is to alter the surface topography of parasitic worm. This is because of the fact that the tegument is the primary host-parasite interface, vital for absorption of nutrients and perception of the surrounding micro-environment provided by the host (Xiao et al. 2004). SRM studies in the present investigation showed extensive deformation and destruction caused by kaempferol derivative on the surface architecture of the helminth *H. diminuta* which was very common to the effects caused by the commercial anthelmintic praziquantel. Like-wise, damages such as surface lesions and loss of spines have also been observed to be induced on body surface of *Fasciola hepatica* by deacetylated (amine) metabolite of diamphenethide (Fairweather et al. 1987). Other broad spectrum anthelmintics such as benzimidazole

derivatives, triclabendazole, oxyclozanide, miltefosine and extract of several plants and their active components have been found to cause destruction of absorptive surface as seen in different parasites (Chetia et al. 2014). Tegumental erosion as observed in the present study caused death of the parasites that may lead to severe nutrient deficiency within their bodies. Apart from this, the role of microtriches present on the helminth surface is to anchor the helminths in the gut so that they do not get removed from the host body. Therefore, damages in the microtriches caused by kaempferol derivative leads to loss of holding capacity of the parasites to the host. Similarly, it has been recorded that the natural compound propolis causes blebbing, swelling of tegument, loss of spines and distortion of suckers in *Fasciola gigantica* exposed in vitro (Hegazi et al. 2007).

The ultrastructural characteristics of a cestode tegument are the presence of the upper glycocalyx layer followed by the tegument, the sub-tegument, distal cytoplasm, the basal lamina, the muscle layers and the sub-tegumental cyton (Smyth 1994). Presence of sub-tegumentalcyton and important cellular elements away from the outer surface of the parasite is an important adaptation to the parasitic lifestyle to avoid immunological attack by the host. In our study, after in vitro incubation with the active compound kaempferol derivative, damages has been observed in the tegument of *H. diminuta* when studied under transmission electron microscope. Deformities in the sub-tegumental cyton in the present investigation indicated the adverse effects of kaempferol derivative on the physiology of the parasite. Generalized stress on the helminths induced by the compound has been supported by the altered nucleus, condensed chromatin, detection of vacuoles, granular cyton and mitochondrial alterations (Ediriweera et al. 2017). However, in the present study, tegumental damage exposing the cellular elements caused high metabolic stress leading to reduction in physical activity of the parasite (Dasgupta et al. 2010). Disruption of tegumental surface as observed in the present study may be correlated with the osmotic imbalances in the parasite that resulted in the impaired ion transfer as reported in *Opisthorchis viverrini* when exposed to amoscanate (Sobhon et al. 1986). The crude extract of *Acacia oxyphylla* and *Securinga virosa* and active compound resveratrol were observed to cause similar type of severe destruction and deformation on cestodes (Dasgupta et al. 2013). The resulted extensive tegumental damage as observed in our present study indicated disturbances in the membrane permeability leading to paralysis and subsequent death of the parasite. Ultrastructural changes, observed in the mitochondrial membranes and cristae may be due to stress released by the plant derived component (Ukil et al. 2022). Under the influence of kaempferol derivative isolated from *L. ramosa*, it can be stated that the altered membrane transport leads to disturbance in ion flux across the membrane together with inhibition of neuromuscular activity resulted in the paralysis and then death of the parasites as observed in the present experiment (William et al. 2001; Ribeiro et al. 2005).

It has been suggested that the enzymes, ATPase, AlkPase and AcPase act in digestion capacity, hydrolyzing phosphate esters to which the cell may be virtually impermeable (Read 1966; Lumsden et al. 1968). Along with membrane transport proteins, phosphatases are important tegument proteins and they are found in parasite's whole body mainly in regions of secretory and absorptive function. Clear dependence of the activities of these enzymes on the degree of maturity of tapeworm segments has been reported with greater activity observed in mature segments. The alkaline phosphatase is believed to participate in the degradation of organic phosphate to free phosphate in the mammalian small intestine. However, there is no evidence for the enzymes to act in substrate transportation (Kaplan 1972). It has been reported that AlkPase supports the absorption and secretory function in *Schistosoma mansoni* (Dusanic 1959). In most of the nematodes the activity of AcPase has been observed to be high and is associated with absorption of glucose through the body wall (Maki and Yanagisawa 1980). According to Skotarczak (1987), both the enzymes play an important role in the metabolic processes of helminth embryos, and their activities depend on the metabolic intensity. They are believed to be responsible for the transport of substances across the walls of excretory canals and also in ionic regulation.

Speculation is that when tissue's ATPase activity decreases, the permeability of the glycocalyx-plasmalemma system are disrupted leading to the damage of plasmalemma parts. This again causes AcPase to diffuse out to digest the damaged membrane parts leading to decrease in the tegumental AcPase level. Damage in membrane also causes increased flow of salts from the external media which gets accumulated in the basal infolds (Fairweather and Boray 1999). To compensate, there is penetration of water from the surrounding tissue leading to swellings in the basal infolds and vacuolisation of tissue. In our study also, it has been observed that when individuals of *H. diminuta* were exposed to kaempferol derivative, the enzymes ATPase, AlkPase and AcPase activities decreased by 70%, 54% and 65% respectively indicating to the cestocidal efficacy of the compound from *L. ramosa*.

The number of eggs excreted in feces has been considered as the endpoint to evaluate the efficacy of the active compound against helminths (Akhtar et al. 1982; Gathuma et al. 2004). An anthelmintic is considered to be efficacious when the level of reduction in fecal egg count of treated animals is in the order of 70%. This is an important criterion that is considered by the researchers to evaluate the anthelmintic efficacy of any drug (Githiori et al. 2002, 2003). In this study, the percentage reduction is nearly 70%

and exactly 70% in the helminth infected rats treated with 10 mg and 15 mg of kaempferol derivative/kg b.w. respectively. Similar to our study, Wabo et al. (2009) found that treatment with 5600 mg/kg b.w. of ethanol extract of *Canthium manni* (Rubiaceae) showed 75% decrease in fecal egg count and 84% reduction in worm burden in rodents during the treatment period of 7 days. Stem bark extract of *Sacoglottis gabonensis* was observed to be effective against helminth in vivo, but its high doses cause toxic effects such as depression, drowsiness, unsteady gait and paralysis of hind limbs etc. (Nwosu et al. 2008). Soren and Yadav (2020) also observed similar kind of effects on *H. diminuta* when treated with extract of *Cyperus compressus*.

It had been observed in the present study that the efficacy of kaempferol derivative was dose dependent. Decrease in worm count after the treatment period as recorded in the present investigation may be due to destrobilation. It is very well known that cestodes undergo the process of destrobilation when exposed to an anthelmitic drug. The worm recovery rate was observed to be decreased as the dose of the compound was increased. Similar kind of results were observed in the study of Tangpu et al. (2004) and Temjenmongla et al. (2006) where they exposed *H. diminuta* to the in vivo treatment with crude extracts of *Trifolium repens* and *Psidium guajava*.

Conclusion

The results from the present study reveal that the active bioactive compound kaempferol derivative isolated from the plant *L. ramosa* has a promising anthelmintic activity against the rodents/human infecting tapeworm *H. diminuta* in dose dependent manner. The compound exerts its anthelmintic efficacy in the helminths by causing damages and deformities in the body covering of the parasite and alterations in the parasite's normal physiological function by targeting some key tegumental enzymes. Therefore it can be concluded that the active compound kaempferol derivative is very effective against cestodes that infect higher animals both in vitro and in vivo.

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Data Availability Statement: The data that supports the finding of this study are available from corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

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