Journal of Helminthology

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Quercetin: An anthelmintic potential against zoonotic tapeworm *Hymenolepis diminuta* (Rudolphi, 1819)

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Research Paper

Cite this article: Ray MS, Mondal C, Saha S, Mandal S and Lyndem LM (2025). Quercetin: An anthelmintic potential against zoonotic tapeworm *Hymenolepis diminuta* (Rudolphi, 1819). *Journal of Helminthology*, **99**, e9, 1–11 https://doi.org/10.1017/S0022149X24000877.

Received: 09 September 2024
Revised: 12 November 2024
Accepted: 25 November 2024

Keywords:
Tapeworm; drug resistant; microtriches; tegument; paralysis

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Abstract

Quercetin, a vital flavonoid found in many medicinal plants, has shown anti-inflammatory, anti-cancerous, anti-aging, anti-tumour, anti-viral, anti-fungal, anti-bacterial, anti-obesity, anti-diabetic, and anti-protozoal activity. However, very little is known of its anthelmintic activity; there is no literature against tapeworm infection so far. The present study was performed to expose its cestocidal role by using the zoonotic tapeworm *Hymenolepis diminuta* as a parasite model. The parasite was exposed to different concentrations of 0.125, 0.25, 0.5, 1, 2.5, 5, 10, 20, and 40 mg/mL Quercetin prepared in RPMI 1640, with 1% Tween 20. Another set of parasites was treated with a standard dose of Praziquantel (0.001 mg/ml), and another set of parasites was kept as control. All experiments were maintained at 37°C ± 1°C in the incubator. Quercetin activity was assessed through viability test, and time of motility was observed through paralysis. After the experiment, worms were processed for light and electron microscopic analysis to observe the post-treatment effect on their tegument. Dose-dependent efficacy was observed in all the treatments. Time of paralysis and time of mortality for 20 mg/mL Quercetin dose was 1.40±0.03h and 2.35±0.03h, respectively, which is at par with the drug Praziquantel. Histological study showed constrictions in the tegument, while extensive damage in suckers and neck region with deformed and shrunken proglottids, sloughed-off microtriches and undistinguished nucleus with loss of envelope architecture were observed in treated parasites under electron microscopic studies, which indicates the negative activity of Quercetin on the parasite thus suggesting its cestocidal activity.

Introduction

Parasitic helminth infection continues to be a neglected disease in developing countries, especially in regions with inadequate sanitation, personal hygiene, and health education, affecting humans and livestock significantly with malnutrition, anorexia, vomiting, and irritability (Hotez *et al.* 2014; Jeza *et al.* 2022). While long-term chronic diseases rarely lead to death, their morbid manifestation in humans can exacerbate conditions like HIV/AIDS, tuberculosis, malaria, and allergies (Gashaw *et al.* 2019; Magalhães *et al.* 2021; Njua-Yafi *et al.* 2016; Rubaihayo *et al.* 2016). Anthelmintic drug development relies on finding an effective method to expel or eliminate helminth parasites without causing significant harm to the host. However, demonstrating anthelmintic efficacy *in vitro* is the primary challenge for a new synthetic compound. Currently, there are anthelmintic drugs available to combat these parasites, but issues such as reduced efficacy, drug resistance, and toxicity underline the need for the discovery of new drug candidates. Nature has played a crucial role in anthelmintic discovery by providing various precursor drug molecules. A diverse array of plants and animals produce defence molecules against predators and parasites, including helminths, offering a vast repository of potential biopharmaceuticals for anthelmintic research (Jayawardene *et al.* 2021). One of the dietary flavonoids that has been investigated the most is Quercetin (QUE) (molecular formula: $C_{15}H_{10}O_7$, IUPAC name: 3,3′,4′,5,7-Pentahydroxyflavone), which is present in a large variety of fruits, vegetables, tea, red wine, nuts, and fruit seeds (Nishimuro *et al.* 2015). It has a wide spectrum of therapeutic potentials, such as antioxidant, anti-viral, anti-cancer, anti-microbial, anti-protozoal, anti-fungal, anthelmintic, anti-inflammatory, anti-tumour, anti-diabetic, and many more, that can effectively protect plants through different physio-biochemical stress responses (Aghababaei and Hadidi 2023). This compound also disrupts bacterial cell walls, cell membrane, and fungal plasma membrane, inhibits nucleic acid synthesis and biofilm formation, modulates quorum sensing, and reduces virulence factor expression as well as triggers apoptosis induction through mitochondrial dysfunction and blocks essential viral enzymes such as polymerases, integrase, reverse transcriptase, proteases, along with suppression of DNA gyrase, and binding viral capsid proteins as evidenced through reports from various workers (Agrawal *et al.* 2020; Kwun and Lee 2020; Qayyum *et al.* 2019; Singh *et al.* 2015; Yang *et al.* 2020). QUE is also reported to

exhibit anti-cancer mechanism of action by triggering both intrinsic (mitochondrial) as well as extrinsic (Fas/FasL) apoptotic cell deaths in MDA-MB-231 and MCF-7 human breast cancer cells, arresting cell cycle by regulating the expression of cyclin-dependent kinases (CDKs), inhibiting the activity of cytochrome P450 (CYP) enzymes, inhibiting metastasis by downregulating the expression of metastatic proteins including matrix metalloproteases (MMPs), angiogenesis suppression and inhibiting neovascularization in tumour microenvironment, disrupting cancer cell crosstalk by reducing extracellular vesicle-mediated VEGFR2 mRNA transfer (Ramos *et al.* 2024; Xiong *et al.* 2024). Plants such as *Allium cepa*, *A. fistulosum*, *Camellia sinensis*, *Capparis spinosa*, *Clerodendrum viscosum*, *Prunus domestica*, *Solanum lycopersicum*, *Coriandrum sativum*, *Hypericum hircinum*, *H. perforatum*, *Apium graveolens*, *Moringa oleifera*, *Nasturtium officinale*, *Asparagus officinalis*, *Centella asiatica*, *Brassica oleracea*, *Calamus scipionum*, *Morus alba*, *Malus domestica*, *Prunus avium*, and *Vaccinium oxycoccos* are rich in QUE (Lakhanpal and Rai 2007). In the prolonged history of QUE consumption as part of the daily diet, no harmful effect is seen (Harwood *et al.* 2007). Many plant-crude extracts containing QUE have been shown to possess anthelmintic efficacy against *Haemonchus contortus*, *Chabertia* sp., *Teladorsagia* sp., *Trichostrongylus* sp., *Ostertagia* sp., *Strongyloides* sp., *Fasciola hepatica*, and *Opisthorchis felineus* (Giovanelli *et al.* 2018; Mordvinov *et al.* 2021; Pereira *et al.* 2016). However, very few literatures reported the anthelmintic activity of the pure compound QUE against helminth infection. It is thus important to explore its role in tapeworm infections, which pose a significant health challenge, primarily attributable to unsanitary conditions and the consumption of contaminated, uncooked food, creating a conducive environment for a high prevalence of infection in developing countries. While these parasites may not be directly fatal, acute infections often lead to complications such as anaemia and malnourishment, which contributes to elevated morbidity and mortality rates (El-Ashram *et al.* 2024). The majority of symptoms are linked to the gastrointestinal tract, including abdominal pain, diarrhoea, anorexia, irritability, and vomiting, accompanied by additional manifestations such as fever, breathlessness, eosinophilia, and anaemia (Hotez *et al.* 2017). Advanced stages of intestinal parasitic infestations can lead to surgical emergencies (Chowdri *et al.* 2021).

Among helminth infections, tapeworm is a totally neglected parasite infection since human taeniasis is mostly asymptomatic, although pains in the abdomen and loss of weight have been observed (El-Ashram *et al.* 2024). However, perforation in the gall bladder, swelling of the appendix, and bowel blockage are infrequent complications of intestinal taeniasis (Yulfi *et al.* 2024). The study report of Global Burden of Disease 2021 revealed the 95% Uncertainty Interval (UI) for the amount of disability-adjusted life years (DALYs) caused by foodborne trematode infections, cystic echinococcosis, schistosomiasis, and cysticercosis (IHME 2024). An estimated 2.8 million disability-adjusted life years have been lost worldwide due to neurocysticercosis, caused by *Taenia solium* larvae (Butala *et al.* 2021).

Hymenolepis diminuta, a zoonotic tapeworm, is considered to be the most suitable parasite model as it can be maintained in the laboratory in rats. The majority of other cestodes share similar basic developmental attributes, and research using advanced molecular techniques has been made possible by studying this parasite (Rozario and Newmark 2015). *H. diminuta* infection in humans is rare, even in developing countries, typically occurring in isolated cases, such as case reports describing a single affected individual of infection rates ranging from 0.001 to 5.5%, especially in children (Rahman *et al.* 2021). Symptoms include abdominal pain, diarrhoea, eosinophilia, due to mucosal damage of intestinal villi, along with rare fever (Panti-May *et al.* 2020; Singh *et al.* 2020). Extraintestinal symptoms, including pruritus, cutaneous itching, and arthromyalgia (Patamia *et al.* 2010), were also reported in some cases, along with eosinophilia and anaemia in children and adolescents (Panti-May *et al.* 2020; Singh *et al.* 2020; Tiwari *et al.* 2014). The present study is thus aimed to evaluate the *in vitro* cestocidal efficacy of QUE against the common zoonotic tapeworm *Hymenolepis diminuta*, maintained in our laboratory.

Materials and methods

Maintenance of parasite model H. diminuta in the laboratory

H. diminuta was reared in our laboratory between a definitive host (Swiss albino rat) and an intermediate host (*Tribolium* sp.) following the methods adopted by Kundu *et al.* (2012). All experiments involving the rats were approved by Institutional Animal Ethics Committee (IAEC), Visva-Bharati with approved number is IAEC/VB/2023-I/01.

Chemicals and drugs

QUE was purchased from (Sisco Research Laboratories Pvt. Ltd.) in the form of Quercetin Dihydrate extrapure, 99% (molecular formula $C_{15}H_{10}O_7.2H_2O$, molecular weight 338.27). The culture media RPMI-1640 was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India, and the drug Praziquantel (PZQ) IP 600 mg, with trade name Zeroquan, was obtained from Aprazer Healthcare Pvt. Ltd. Product, Uttarakhand, India. MTT powder, for molecular biology (Thiazolyl blue; IUPAC name: 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. All other used chemicals of analytical grade were purchased from Merck, USA.

In vitro assay

Fresh adult parasites procured from the intestines of infected rats were washed in phosphate buffered saline (PBS) pH 7.4 and treated with 0.125, 0.25, 0.5, 1, 2.5, 5, 10, 20, and 40 mg/mL of QUE in the RPMI-1640 media with 1% Tween 20. Another set of parasites was treated with the standard dose of PZQ (i.e., 0.001 mg/mL) standardized from our laboratory (Kundu *et al.* 2012). Additionally, a separate batch of parasites was kept in RPMI-1640 media with 1% Tween 20 as a negative control. All experiments were conducted in RPMI-1640 media supplemented with streptomycin and incubated at 37 ± 1°C in the humidity cabinet. The anthelmintic efficacy of QUE was determined through observing the time period of paralysis and morphological alterations.

Parasite motility, paralysis, and mortality assessment

Treated parasites were observed under the stereomicroscope within 5 minutes of start time and thereafter every 30-minute interval to notice the changes in the scolex region. The viability of the parasite in the control and treated worms' group was determined by evaluating the movability index (MI) and the relative

https://doi.org/10.1017/S0022149X24000877 Published online by Cambridge University Press

motility (RM) following the formula below adopted from Zaridah *et al.* (2001).

$$MI = \sum(n\,Nn)/\sum N,$$

where 'N' denotes the motility score and 'Nn' denotes the parasite number with the score n. N denotes 3 when uniform parasitic movement is observed all over the body, N denotes 2 when partial parasitic movement (scolex region or the neck region of the anterior part of the body became immobile) is observed, and N denotes as 1 when parasites became unable to move on their own, but after slight shake, slight mobility could be observed. Further, following the MI value obtained, relative movability (RM) was determined by following the formula below.

$$RM = \left(MI_{sample}/MI_{control}\right) x100$$

When the RM value denotes 100, it designates zero efficacy of the compound; gradually, when the RM value decreases and approaches 0, it denotes stronger compound activity. When the RM value becomes 0, it expresses the most potent activity of the compound.

Viability assessment by MTT assay

The viability of parasites was quantitatively evaluated by MTT assay following the method of Comley *et al.* (1989). Parasites were suspended in MTT solution (2 mg/mL MTT powder in PBS) and further incubated in the dark for 2 hours at 37°C. During the incubation period, a dark formazan crystal formed, which was further dissolved in 500 µL of DMSO. A UV-Visible Spectrophotometer (Beckman Coulter, DU 730) was used in order to measure the absorbance at 595 nm.

Microscopic study of the parasite

Light microscopic study

Another set of paralyzed worms was immediately removed from the medium, stretched with hot PBS, processed for permanent mount with alcoholic eosin stain, and photographed under Upright bright-field microscope (Leica DM 3000) at 10X and 20X magnification.

Histology

Furthermore, using the techniques from Mayer (1896), some paralyzed worms were processed for histological analysis. Mature proglottid tissue sections of 5 µm thickness from control, QUE-, and PZQ-parasites were stained using hematoxylin–eosin counter-staining technique and observed under an upright brightfield microscope (Leica DM 3000) at 40X magnification.

Scanning electron microscopy (SEM)

The treated parasites' ultra-structural changes were observed by SEM analysis following the methodologies of Roy and Tandon (1991). After giving the paralyzed worms a thorough wash in PBS, they were fixed for about 10–12 hours at 4°C in 3% (v/v) glutaraldehyde (prepared with cacodylate buffer), after which the parasites were placed on metal stubs and coated with gold in Quorum SC7620 sputter coater and photographed under Gemini-SEM 450–8216010130 microscope at an electron high tension value ranging from 3.0 kV to 5.0 kV.

Transmission electron microscopy (TEM)

Ultra-structural changes of the treated parasites were further observed by TEM analysis following the method of Dykstra and Reuss (1992). Worms were washed thoroughly in PBS, and 2-mm wide transverse sections of the proglottids were fixed in the mixture of 4% (v/v) Paraformaldehyde and 1% (v/v) Glutaraldehyde, prepared in 0.1 M phosphate buffer (PB) (pH 7.4), for overnight at 4°C. After the primary fixation, further steps of secondary fixation, dehydration in an ethanol series (50%, 70%, 80%, 90%, and 100%), infiltration, and finally embedding in Araldite CY212 resin was done for ultrathin section cutting (60–70 nm thick) using UC7 ultramicrotome (Leica), mounted on copper grids and then stained with 5% uranyl acetate and 5% lead citrate and photographed under Talos F200 Transmission Electron Microscope (Thermo Fisher Scientific) using a FEG filament operated at 200kV

Data analysis

All data are expressed as mean ± standard error of the mean (SEM). One-way ANOVA was used to test the significance among different sets of data, and $p \leq 0.05$ was considered statistically significant.

Results

Motility, paralysis, and mortality

Mean time of paralysis and mean time of mortality in treated parasites gradually decreases with gradual increase of QUE concentrations depicted in Figure 1. Mean time of paralysis and mean time of mortality at 20 mg/mL concentration of QUE-treated parasites were 1.40±0.03 h and 2.35±0.03 h, respectively (Figure 1a–b). This dose was then taken as a standard dose since the time period of paralysis was similar with that of PZQ-treated parasites (1.18±0.04 h and 22.21±0.12 h), reported from our laboratory in earlier studies observed by Kundu *et al.* (2012). Thus, further study was carried out with 20 mg/mL QUE. With the increase in QUE concentrations, RM value decreases, along with the high mortality rate (Figure 2). However, control worms survived up to 69.22±0.23 h.

Dose response curve, EC50 determination

The potency of the drug Quercetin is hereby quantified as EC_{50} (half maximal effective concentration). In the dose response curve, EC_{50} value is determined to be 1.08 log concentration [with 95% confidence intervals, where the coefficient of determination (R^2) is 0.9091], which is 12.03 mg/mL QUE concentration, after 2 hours of exposure time (Figure 3).

Viability assessment by MTT assay

QUE-treated parasites showed only 23.18% of alive parasites, compared to 100% of alive parasites in control (Figure 4), indicating that the viability of treated parasites reduced significantly from the control.

Light microscopic study

QUE-treated parasites showed swollen scolex with bulging out suckers and constriction in the anterior region, compared to the typical scolex with defined suckers and smooth anterior region of the control parasite. PZQ-treated parasites showed evaginated rostellum and constriction in the suckers and the anterior region (Figure 5a–c). QUE- and PZQ-treated parasites showed distorted

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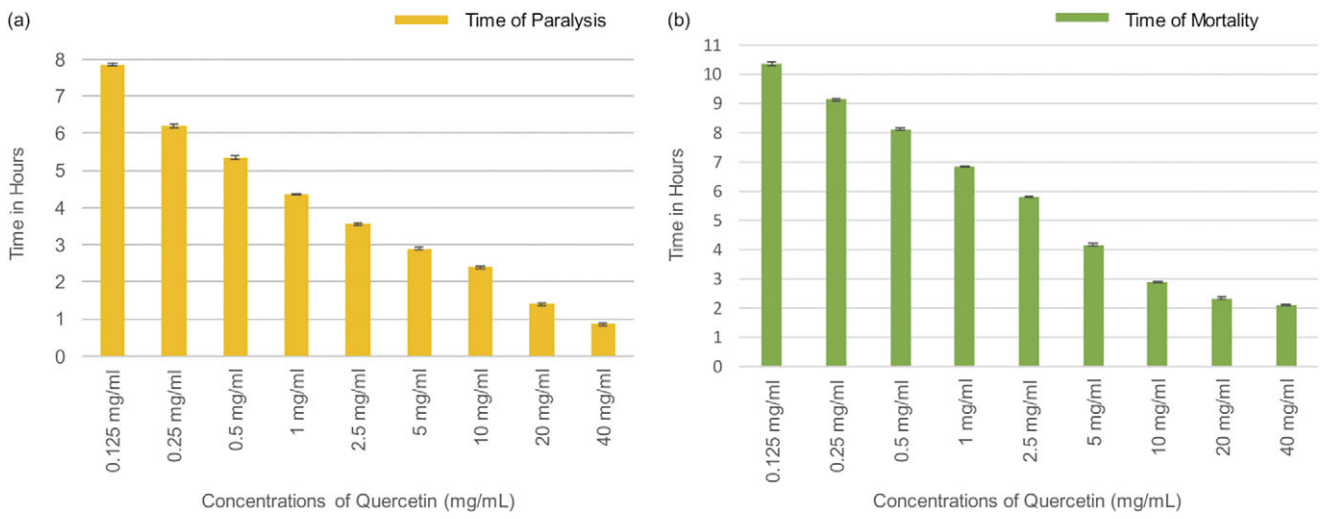

Figure 1. *In vitro* anthelmintic effects of Quercetin (QUE) against *H. diminuta* (a) showing mean time of paralysis (TP) and (b) showing mean time of mortality (TM). Data are expressed as Mean ± SEM (n=6).

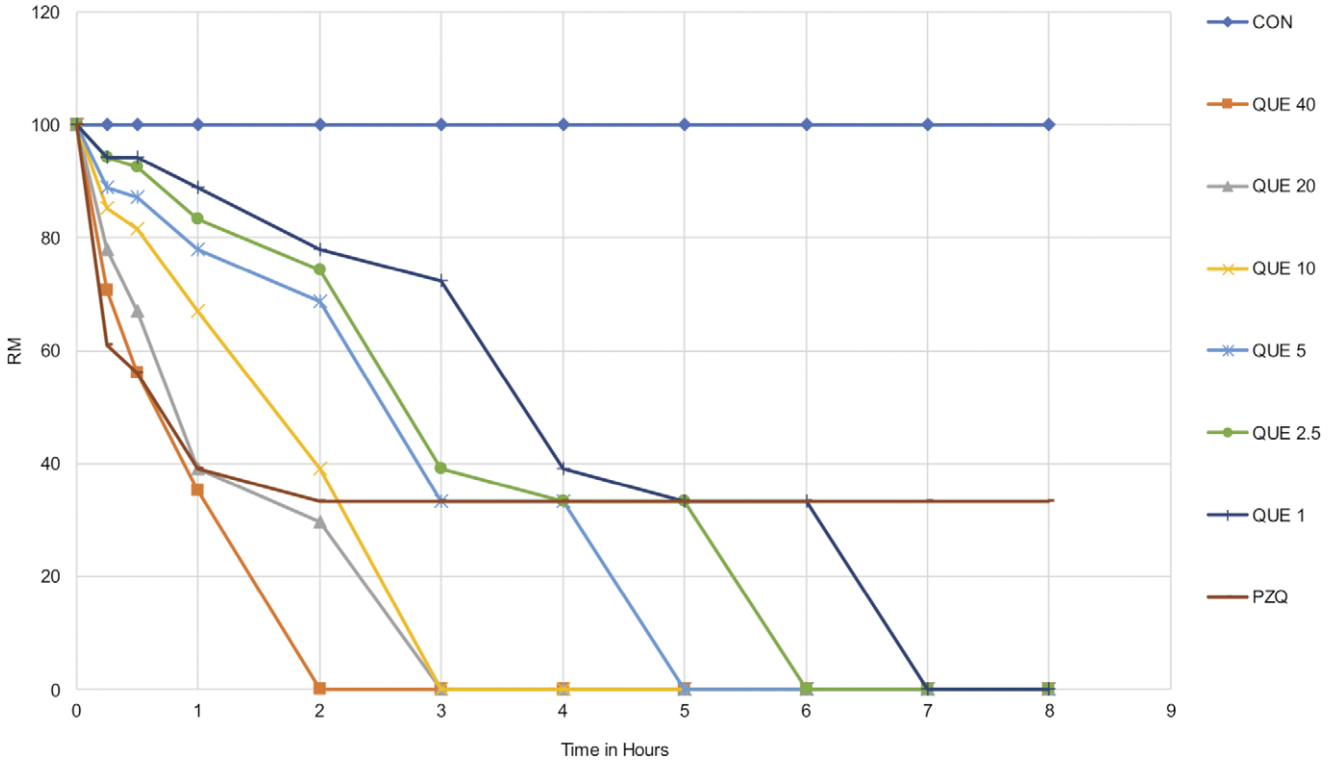

Figure 2. *In vitro* anthelmintic effects of QUE against *H. diminuta* expressed in mean relative movability (RM) value of parasites at different concentrations. Each point in graph is the mean value of triplicate study.

proglottids, compared to the distinctly uniform trapezoid shape in control (Figure 5d–f).

Histology

QUE-treated parasites showed damaged outer tegument with folds and grooves; clumped syncytial and muscle layer was also seen. This contrasts with the distinctly uniform outer tegument and sub-tegument with a clear syncytial layer and defined muscle layer of control. PZQ-treated parasites showed damaged, rough outer tegument and dispersed sub-tegument layer along with indistinct syncytial and muscle layer (Figure 6a–c).

Scanning electron microscopy (SEM)

SEM of control worms showed standard body architecture with four open suckers and an intact tegument (Figure 7a). However, the parasites exposed to QUE showed closed bulging suckers with indistinguishable rostellum and shrinkage in the neck region (Figure 7c). Similarly, PZQ-treated worms showed damaged scolex

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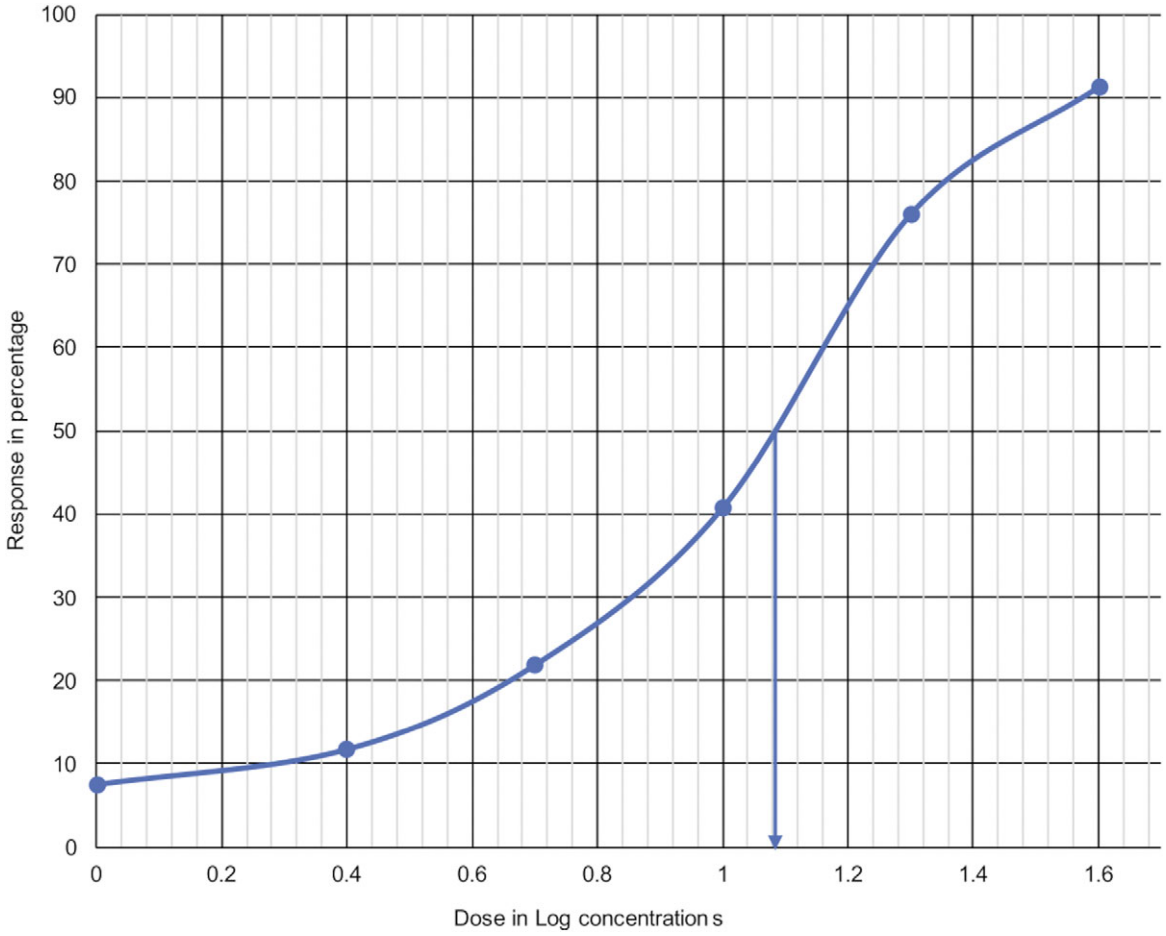

Figure 3. *In vitro* anthelmintic efficacy of QUE against *H. diminuta* showing concentration-dependent dose-response curve with 95% confidence interval. The EC_{50} value is determined as 1.08 log concentration (12.03 mg/mL QUE concentration), and the coefficient of determination (R^2) is 0.9091.

with constricted suckers and constricted neck region with blisters (Figure 7b).

Control parasites showed distinct smooth trapezoid-shaped mature proglottid with definite juncture (Figure 7d), whereas QUE-treated parasites showed wrinkled tegument with folds and grooves in mature proglottid and the loss of trapezoid shape (Figure 7f). PZQ-treated parasites showed inward-folded mature proglottids, along with some blisters (Figure 7e).

Gravid proglottids in control parasites showed standard architecture of thick smooth gravid proglottids filled with eggs (Figure 7g), whereas QUE-treated parasites showed bulging and thin gravid proglottids with exposed eggs (Figure 7i). PZQ-treated parasites also showed folding and tearing of gravid proglottid (Figure 7h).

The tegument of control parasites was covered with microtriches, giving a velvety appearance (Figure 8a), but QUE-treated parasites lost the velvety appearance and replaced with clumped microtriches (Figure 8c), and folding microtriches with blisters were observed in PZQ-treated parasites (Figure 8b).

Transmission electron microscopy (TEM)

The typical tegument architecture of cestode is visible in the control group, with regular-shaped defined layer of microtriches, followed by densely packed syncytial layer, and muscle layer, composed of circular and longitudinal musculature (Figure 9a), while in the QUE-treated parasites, microtriches layer is totally sloughed off, and syncytial and muscle layer are indistinguishable and vacuolization or vesicle formation was visible (Figure 9c). However, PZQ-treated parasites showed short folded microtriches, constricted syncytial layer, and dispersed muscle layer. There was evidence of blebs in muscle layer and syncytial tegument layer (Figure 9b)

A distinguishable, typical eukaryotic nucleus with a nuclear envelope and distinct nucleolus and dense cytoplasm was evident in the control worms (Figure 9d), while QUE-treated parasites showed a nucleus with an indistinct nuclear envelope and nucleolus (Figure 9f). However, PZQ-treated parasites showed nucleus with a distinct nuclear envelope with reduced nucleolus (Figure 9e)

Discussion

The current investigation indicated dose-dependent anthelmintic potential of QUE; other studies using phenolic compounds, including gallic acid, catechin, ferulic acid, and sinapic acid, have reported similar findings (Mondal *et al.* 2023; Saha *et al.* 2024). Other studies using plant extract, containing phytochemicals of anthelmintic potential, also showed anthelmintic efficacy in a dose-dependent manner (Ahmed *et al.* 2013; Buza *et al.* 2020; Carvalho *et al.* 2020; Hajaji *et al.* 2018; Khunkitti *et al.* 2000; Mohammed *et al.* 2024).

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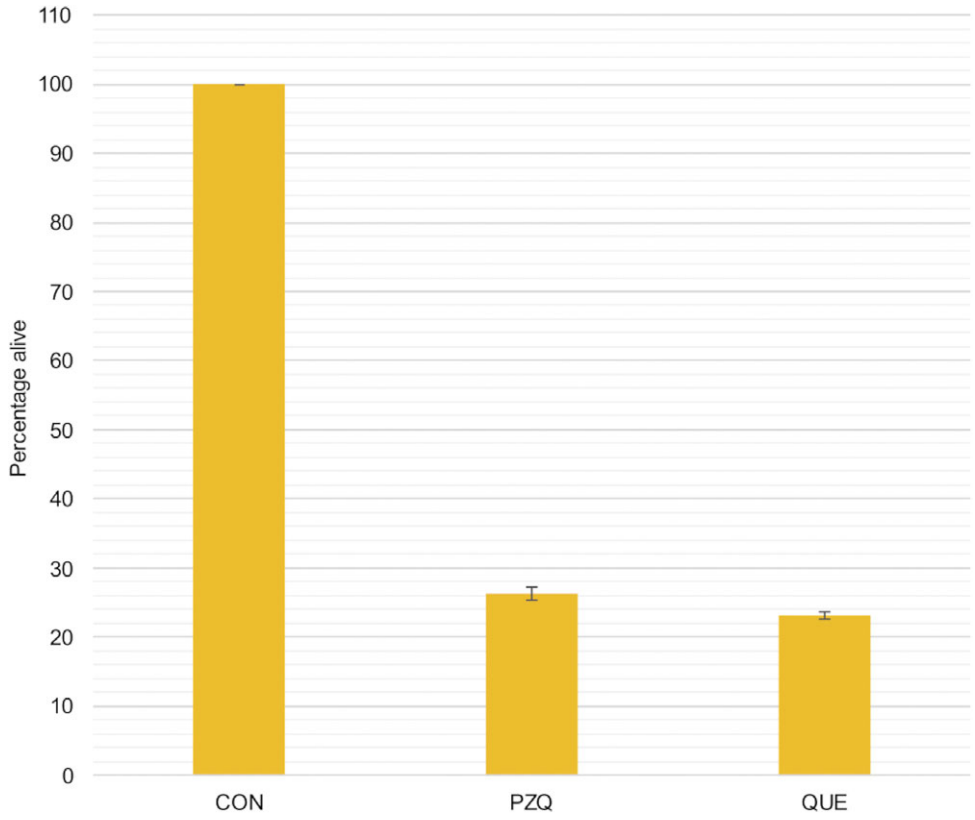

Figure 4. *In vitro* assessment of QUE on viability of *H. diminuta* by MTT assays showing percentage inhibition from control. Data are expressed as Mean ± SEM (n=6). significant p values = $p \leq 0.05$.

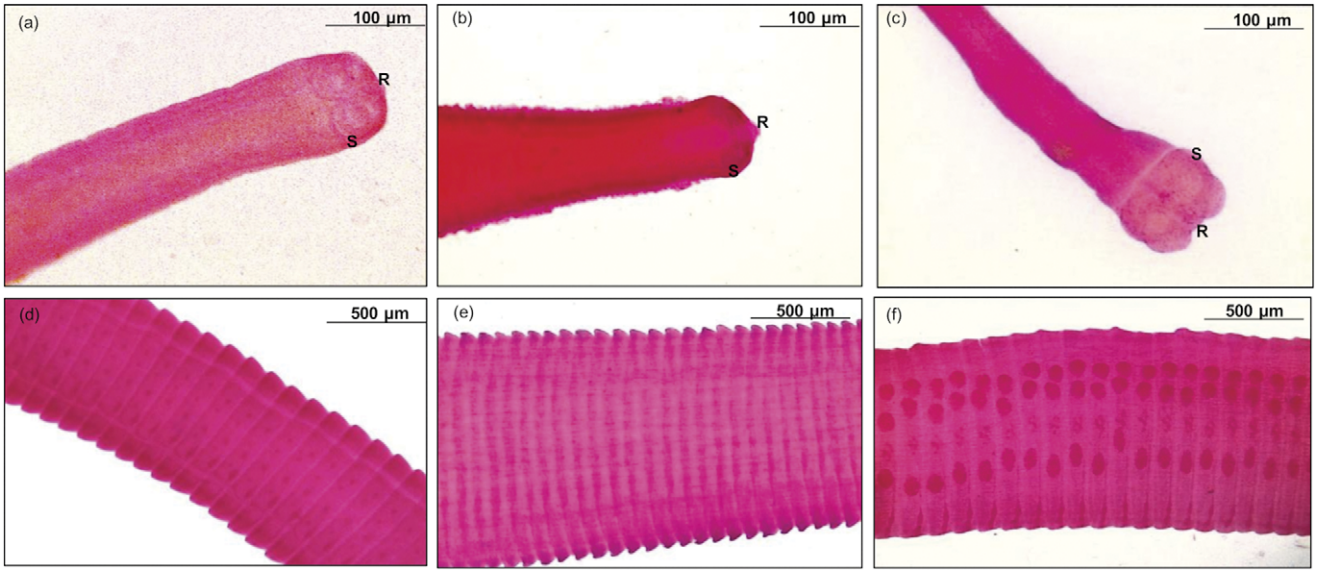

Figure 5. Light microscopic study of *H. diminuta* exposed to Quercetin: (a) Control showing typical scolex with defined four round suckers (s) and smooth body surface; (b) PZQ showing constriction in the suckers as well as the body surface; (c) QUE showing swollen scolex with bulging out suckers (s) while shrinkage over the body surface; (d) Control showing normal trapezoid shaped proglottids; (e) PZQ showing constriction in proglottids; (f) QUE showing flattened and thinned proglottids. Figure (a–c), scale bars are 100 µm, and Figure (d–e) scale bars are 500 µm.

Kundu and Lyndem (2013) reported an *in vitro* dose-dependent cestocidal efficacy of *Cassia* plants against the tapeworm *Raillietina tetragona*, along with the post treatment irrevocable changes in the scolex and proglottids of the parasite.

Shebeko *et al.* (2020) have showed the dose-dependent effectiveness of the combination of N-acetylglucosamine and QUE in treating renal insufficiency in rats. Chen *et al.* (2013) revealed that QUE therapy inhibited the proliferation and migration of oral

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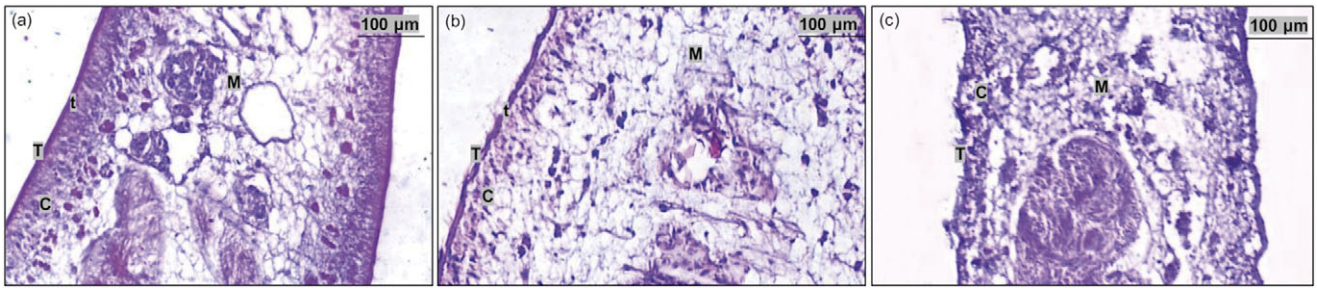

Figure 6. Histological study of *H. diminuta* exposed to Quercetin: (a) Control depicting normal uniform outer tegument (T) and sub-tegument (t) with clear syncytial layer (C) and defined muscle layer (M); (b) PZQ depicting rough outer tegument, dispersed sub-tegument layer and indistinct syncytial and muscle layer; (c) QUE depicting deep grooves in outer tegument, indistinct sub-tegument and clumped syncytial and muscle layer. All scale bars are 100 µm.

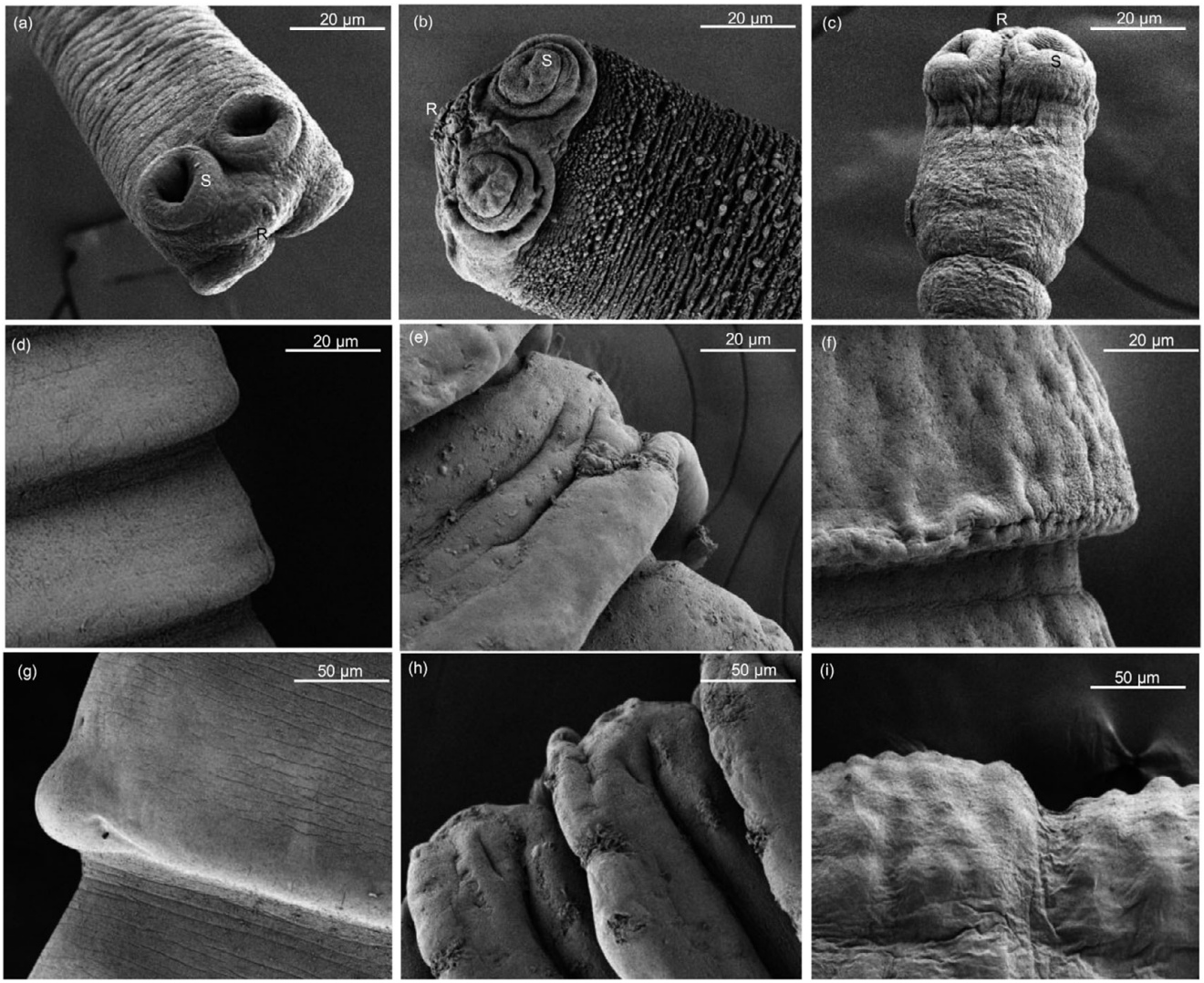

Figure 7. SEM of *H. diminuta* exposed to Quercetin: (a) Control showing scolex with distinct four open round suckers (S) and rostellum (R), and smooth neck region; (b) PZQ showing damaged scolex with constricted suckers and wrinkled rostellum as well as constricted neck region with blisters; (c) QUE showing bulging suckers and undistinguished rostellum and shrinkage in neck region; (d) Control showing distinct smooth trapezoid shaped of proglottids with defined juncture; (e) PZQ showing deep folding with blisters proglottids; (f) QUE showing folds and grooves and wrinkled proglottids; (g) Control showing thick smooth gravid; (h) PZQ showing folding and tearing of gravid proglottid; (i) QUE showing bulged and thin gravid proglottids with exposed eggs. Figure (a–f) scale bars are 20 µm, and Figure (g–i) scale bars are 50 µm.

squamous cell carcinoma in a dose-dependent manner, by the means of reduction of cell viability and colony-forming capacity. Dose-dependent anthelmintic efficacy was also reported in tapeworm *Raillietina tetragona*, treated with *Clerodendrum viscosum* (Nandi *et al.* 2017), which has a high content of QUE (Gupta and Gupta 2012). Wang *et al.* (2016) showed that QUE can dose-

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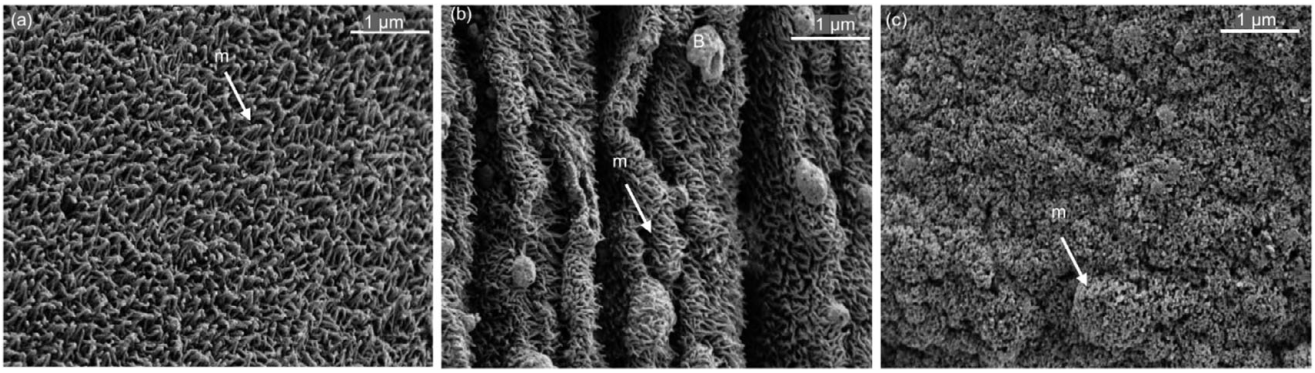

Figure 8. SEM of the tegument of *H. diminuta* exposed to Quercetin at high magnification: (a) Control showing uniform and sharply arranged microtriches (m); (b) PZQ showing folding microtriches with blisters (B); (c) QUE showing clumping microtriches. All scale bars are 1 µm.

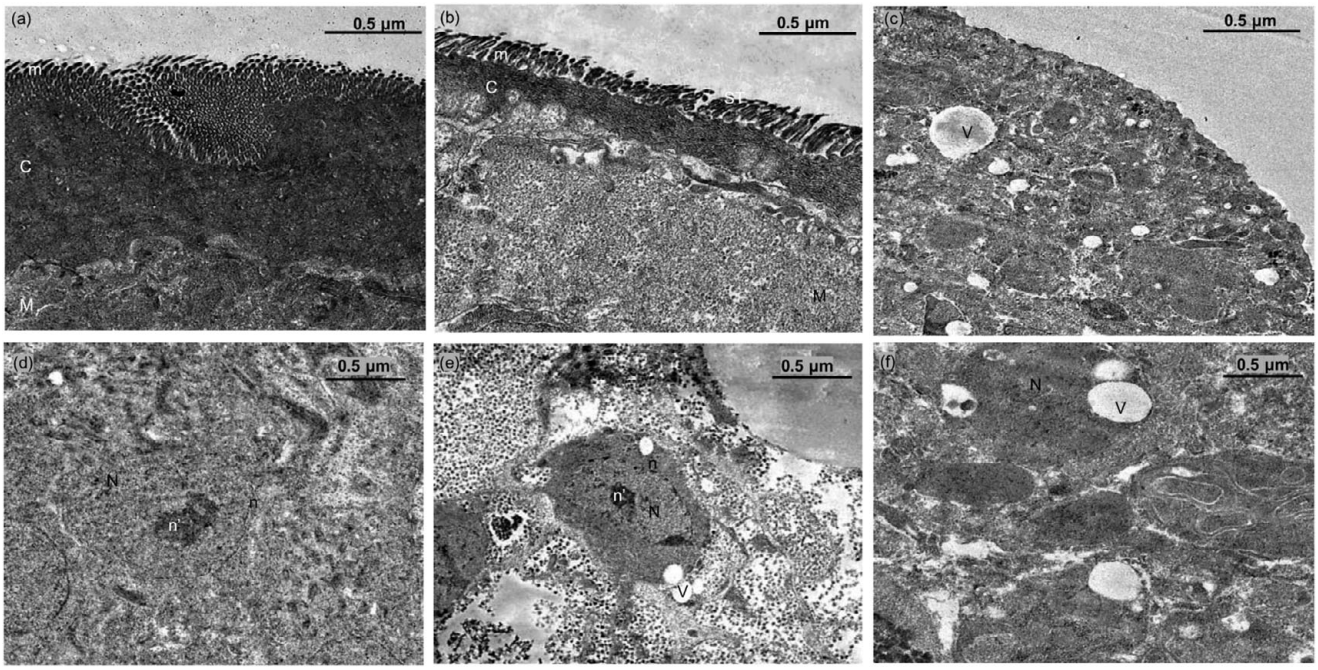

Figure 9. TEM of the tegument and nucleus of *H. diminuta* exposed to Quercetin: (a) Control showing tegument with outer layer of dense microtriches (m), followed by thick syncytial layer (C), muscle layer (M); (b) PZQ showing short folded microtriches (m), constricted syncytial layer and dispersed muscle layer; (c) QUE showing sloughed off microtriches and indistinct syncytial and muscle layer with intermittent vacuolization (V); (d) Control showing eukaryotic type of nucleus (N) with nuclear envelope (n) and distinct dense nucleolus (n'); (e) PZQ showing nucleus with distinct nuclear envelope and reduced nucleolus; (f) QUE showing nucleus with indistinct nuclear envelope and dense nucleoplasm and nucleolus. All scale bars are 0.5 µm.

dependently inhibit the growth of HeLa cells and revealed the number of autophagic vacuoles at higher concentrations of QUE. All these studies support the dose-dependent efficacy of QUE in this present study.

The percentage of alive parasites treated with QUE was significantly less than the control, suggesting the highest percentage of viability reduction from the control. Comparably, research by Baruah *et al.* (2016) revealed that prostate cancer (PC-3) cell viability reduction was reported by QUE treatment, by modulating Wnt pathway components and limiting cancer metastasis. According to Zhao *et al.* (2019), QUE reduced cell viability in oral cancer cells by modulating the miR-16/HOXA10 axis. These studies thus corroborate our present study.

Ultrastructural deformations revealed in the scolex and suckers in the present study might suggest the possible vermifugal action of the compound, as scolex, being the most critical organ for adhesion to the gastrointestinal tract of the host, and damage to it will cause loss of attachment and adhesion of the parasite to the host; *H. diminuta* rostellum cells revealed its major neurosecretory role in inducing strobilization process (Davey and Breckenridge 1967). The damage in the tegument observed in the present study suggests an interference in food intake and metabolism as the tegument serves as a primary interface with the host as well as has a variety of vital enzymes that are crucial to the parasite's survival (Dalton *et al.* 2004; Mondal *et al.* 2023; Pappas 1980; Saha *et al.* 2024). The anthelmintic activity of *Clerodendrum viscosum* that contained a high concentration of QUE (Gupta and Gupta 2012) showed extensive cracks and coarse tegument surface of fowl tapeworm *Raillietina tetragona*, with vacuolization, undistinguishable segments, and sloughed-off microtriches (Nandi *et al.* 2017); von

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Son-de Fernex *et al.* (2023) also reported ultrastructural damage in (L3) of a gastrointestinal nematode *Cooperia punctata* treated with compound combinations of Coumarin and Quercetin, thus supporting our study. Less nutritional absorption and the resulting physiological imbalance are the speculated effect of the clumped microtriches on the outer surface of the tegument, which might cause early paralysis in this present study, as suggested by other researchers (Giri and Roy 2014; Kundu *et al.* 2015). This was also reported by Goel *et al.* (2023) on anthelmintic activity of QUE in *Haemonchus contortus.* Another study by von Son-de Fernex (2015) reported the anthelmintic potential of *Leucaena leucocephala*, a tropical forage legume (of which phytochemical extract is mainly composed of QUE), against *Cooperia* spp eggs by forming little projections and lateral eggshell wall rupture, supporting the present study.

Mean time of paralysis was seen early in all parasites treated to the concentration of QUE. Nevertheless, the post-paralytic period transpired after some time, indicating that if the parasite becomes paralyzed within a host, it may be eliminated from the host's body as a result of the lack of adherence and the host's peristaltic movement (Martin *et al.* 1997). These degenerative alterations in the current study suggest that the worms were stressed, which may have contributed to the parasite's early paralysis and demise. More research is required to fully comprehend QUE's precise and accurate mode of action against *H. diminuta.*

Conclusion

This study reports the anthelmintic potential of QUE. However, further investigation of its mechanism needs to be conducted *in vivo* to understand its mode of action in the future.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0022149X24000877.

Acknowledgements. The authors gratefully acknowledge the Council of Scientific and Industrial Research (CSIR), Pusa, New Delhi, India, for providing research fellowship to Mou Singha Ray (grant no. 09/0202(13912)/2022-EMR-I). Acknowledgement is also extended to Department of Zoology, Visva-Bharati for providing infrastructure; the DST-PURSE (Ref. No. SR/PURSE Phase2/42 (G and C)) for the Field Emission Scanning Electron Microscope facility, Visva-Bharati, Santiniketan; and Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Delhi-110 029, Delhi, India, for the Talos F200 Transmission Electron Microscope facility.

Author contribution. Larisha M. Lyndem: conceptualization, designing, supervision, and final draft editing; Mou Singha Ray: experimentation, result analysis, draft writing, and editing; Chandrani Mondal and Samiparna Saha: experimenting; Sudeshna Mandal: experiment standardization. All authors reviewed the manuscript.

Financial support. The research work was funded by the Council of Scientific and Industrial Research (CSIR), Pusa, New Delhi, India, as a research fellowship to Mou Singha Ray under the grant no. 09/0202(13912)/2022-EMR-I.

Competing interest. The authors declare none.

Ethical standard. The authors assert that all experiments with rats were performed following the standard of practice according to Institutional Animal Ethics Committee (IAEC), Visva-Bharati, and the approved number is IAEC/VB/2023-I/01.

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https://doi.org/10.1017/S0022149X24000877 Published online by Cambridge University Press