Anthelmintic efficacy of *Clerodendrum viscosum* on fowl tapeworm *Raillietina tetragona*

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**ABSTRACT**

**Context:** *Clerodendrum viscosum* Vent. (Verbenaceae) is a shrub, widely used amongst the natives of India against various diseases.

**Objective:** Crude extract of the plant was tested *in vitro* on a tapeworm *Raillietina tetragona* Molin (Davaineidae) to evaluate its potential anthelmintic efficacy and ultrastructural changes in the parasite.

**Materials and methods:** Parasites were exposed to different concentrations of ethanolic leaf extract (10–80 mg/mL) and praziquantel (0.0005–0.005 mg/mL) and incubated in phosphate-buffered saline (PBS). The pH was 7.4 at 37°C, while one set of worms was incubated only with PBS as a control. Permanent immobilization of worms was determined visually when no motility occurred on physically disturbing them. The parasites exposed to high concentrations of leaf extract and praziquantel treatments were processed for histological and electron microscopic studies, as these concentrations took the least time for paralysis and death to occur.

**Result:** With an increase in the concentration of the leaf extract from 10 to 80 mg/mL and praziquantel from 0.0005 to 0.005 mg/mL, the time for the onset of paralysis and death was shortened. The treated parasites lost their spontaneous movement rapidly followed by death. Electron microscopic observations revealed disruptions in the tegument and parenchymal layer, accompanied by deformities in cell organelles.

**Discussion and conclusion:** Extensive structural alterations in the tegument indicate that the plant-derived components cause permeability changes in the parasite leading to paralysis and subsequent death. These observations suggest that phytochemicals present in *C. viscosum* have vermifugal or vermicidal activity, and thus may be exploited as alternative chemotherapeutic agents.

**Introduction**

Helminth infections are one of the major health problems affecting billions of people worldwide (WHO 2010), especially in tropical and sub-tropical countries with low per capita income and poor hygiene conditions (Hotze et al. 2007). Such infections lead to eosinophilia, anemia, pneumonia and malnutrition (Triterraprapab & Nuchprayoon 1998; Stephenson et al. 2000). Traditional medicine practices have relied upon the treatment using several plants against intestinal parasites of human and animals. Recent studies have focused on plants having significant efficacy against helminth parasites (Lasisi & Kareem 2011; Ahmed et al. 2013; Kozan et al. 2013; Rajeshwar & Lalitha 2013; Saowakon et al. 2013; Kundu et al. 2014). *Clerodendrum viscosum* Vent. (Verbenaceae), a shrub found throughout India, is widely used in traditional medicine systems for the treatment of leprosy, diarrhea, post-natal complications, fresh wounds and pain (Warrier et al. 1996; Hamilton 1997; Nadkarni & Nadkarni 2002). It has been used by the natives of India as an antiseptic, anti-inflammatory and antipyretic, vermifuge as well (Warrier et al. 1996). Das et al. (2011) reported its use in the treatment against nematode parasites. However, no literature is available on its anthelmintic activity on cestode parasites. Thus, in order to explore its anthelmintic efficacy, we took up the present study using the cestode parasite *Raillietina tetragona* Molin (Davaineidae), a common parasite of domestic fowl, as a model.

**Materials and methods**

**Preparation of plant extract**

Fresh leaves of *C. viscosum* were collected during the months of March–June in the year 2014 from the surrounding areas of the University campus of Visva Bharati, Santiniketan, West Bengal, India. The flowering plant was identified with the help of Central National Herbarium, Botanical Survey of India, Kolkata, and its voucher specimen (VBNAN 1) was deposited at this Center. The dried leaves (250 g) were powdered, soaked in 90% ethanol and then refluxed in a Soxhlet apparatus for 7–8 h. The product was further processed in rotary evaporator and the final crude extract obtained (18 g) was stored at 4°C until further use.

**Chemicals**

All the chemicals used were obtained from Merck Pvt. Ltd. (Kolkata, India) and these were analytical based. Ethanol was supplied from Bengal Chemicals Kolkata. Praziquantel (trade
name: Distocide), which is a product of Chandrabhagat Pharma Pvt. Ltd (Mumbai, India).

**Experiment design**

Live parasites were collected from freshly slaughtered intestine of domestic fowl and immediately washed with 0.1 M phosphate-buffered saline (PBS). The worms were then incubated in 10, 20, 40 and 80 mg/mL concentrations of the crude leaf extracts and 0.0005, 0.001, 0.0025, 0.005 mg/mL concentrations of praziquantel (PZQ) prepared with PBS. The paralysis time was confirmed when no movement of the parasite was observed, unless placed in a slightly warmer PBS. The time of mortality was recorded when no movement was observed even in warmer PBS or after shaking it vigorously. Worms paralyzed in 80 and 0.005 mg/mL concentrations of plant extract and PZQ, respectively, were used for further analytical studies as paralysis in these two concentrations occurred at a short time span.

**Histology**

Both control and treated parasites were first fixed in Bouin’s fluid and dehydration was done gradually followed by infiltration and embedding techniques. The worms were cut into thin sections (6 microns), stained and mounted with DPX.

**Electron microscopy**

Both control and treated worms were fixed separately in 3% glutaraldehyde prepared in cacodylate buffer at 4°C for 4 h and processed for Scanning Electron Microscopic (SEM) studies following the method described by Roy and Tandon (1991). The surface architecture of the metal-coated parasite was viewed under EVO18 (CARL-ZEISS) Scanning electron microscope (SEM).

Similarly, another set of control and treated worms were processed for transmission electron microscopy (TEM) following the standard procedure of Dykstra and Reuss (1992) and observed under JEM 2100 (JEOL Ltd., Tokyo, Japan) TEM.

**Statistical analysis**

Data were presented as mean ± SD and analyzed for statistical significance following one-way analysis of variance (ANOVA), where ‘F’ values indicated significance, and means were compared by Duncan’s post hoc test, while ‘p’ value less than .05 was considered threshold of statistical significance.

**Result**

Significant (p < .001) dose-dependent efficacy was observed in all treatments with leaf extract and PZQ (Table 1). At 10 mg/mL concentration of leaf extract, paralysis occurred at 9.305 ± 0.36 h, whereas at 80 mg/mL, the worms became paralyzed at a shorter time (1.305 ± 0.35 h). Similarly, PZQ-treated worms took very short time (0.17 ± 0.01 h) to paralyze at 0.005 mg/mL compared to 3.22 ± 0.07 h at 0.0005 mg/mL. The post paralytic time was much longer in both treatments. However, the control parasites survived up to 81.93 ± 4.71 h.

**Histology**

Histological sections from control worms showed a smooth outer tegument followed by a defined muscular layer situated below the sub-tegument (Figure 1(A)), whereas the leaf extract-treated parasites showed a sloughed off tegument, which lost its architecture and the muscle layers disintegrated (Figure 1(B)). These observations were comparable with those of PZQ-treated worms (Figure 1(C)).

**SEM studies**

Control worms revealed a scolex with suckers (Figure 2(A)) and a smooth surface contour (Figure 3(A)). The scolex region of the leaf extract and PZQ-treated parasites showed distortion and shrinkage (Figure 2(B,C)). Similarly, the body surface of the parasite after treatment with leaf extracts (Figure 3(B)) revealed roughness and deep cracks, which were more noticeable than that of PZQ-treated worms (Figure 3(C)).
The entire body of the control parasite was covered with uniform microtriches producing a velvety appearance (Figure 4(A)). This uniformity disappeared and instead, clumping of microtriches was observed after treatment with leaf extract and PZQ (Figure 4(B,C)).

**TEM studies**

Typical architecture of the tegument composed of numerous electron dense microtriches and fuzzy glycocalyx layer followed by numerous secretory bodies in the syncytial layer were observed in control worms (Figure 5(A)). The muscle layer was
also prominent along with numerous cellular organelles present in the parenchymal region of the control worms. However, parasites treated with *C. viscosum* showed displacement of microtriches and distortion in the distal cytoplasmic layer; the basal lamina got degraded, while the muscular and parenchymal layers became distorted (Figure 5(B)), as also observed in PZQ-treated worms (Figure 5(C)). Nuclei, with a dense granular nucleolus and prominent electron dense mitochondria with distinct cristae were found in control worms (Figures 6(A) and 7(A)). In plant-treated worms, though the nucleus was prominent with its nuclear membrane, dense matrix was not found in these worms (Figure 6(B)), but mitrochondria with no distinct cristae and fuzzy appearance was observed (Figure 7(B)). These alterations were also adjudged in PZQ treatment (Figures 6(C) and 7(C)).

**Discussion**

In all concentrations dose-dependent efficacy was observed in the present study. With increase in the concentrations of treatments,
the time taken for paralysis and mortality was shortened. These observations were also reported by many authors on helminths treated with plant crude extracts or plant compounds (Lasisi & Kareem 2011; Bazh & El-Bahy 2013; Giri & Roy 2014; El-Bahy & Bazh 2015; Kundu et al. 2015).

Though early paralysis occurred in all treatments, the death post-paralytic time occurred after a while. It may be suggested that once the parasite gets paralyzed in a host, it would be washed out from the host’s body due to gradual loss of binding and peristaltic movement of the host (Martin et al. 1997).

Shrinkage of the body and distortion of the tegumental architecture as revealed in the treated worms may cause a physiological imbalance. Our study showed clumping of microtriches on the outer surface of the tegument, which may be attributed to less absorption of nutrients and consequent physiological imbalance that may lead to early paralysis (Giri & Roy 2014; Kundu et al. 2015).

Our TEM studies showed electron lucency in the tegument, parenchymal cells and nucleus. Similar results were also observed by Buchanan et al. (2003) and O’Neill et al. (2009) when helminths were exposed to albendazole and artemether drugs. Such observations may suggest a loss of total glycogen content and suppression of glycolysis in the cytoplasm (Wastling & Chappell 1994). The fuzzy appearance with no distinct cristae in mitochondria observed in the treated worms may be related to mitochondrial alteration and could be attributed to depression in energy production of the parasite, which is followed by disruption of intracellular and intercellular transport system as suggested by several workers (Ingold et al. 1999; McConville et al. 2008; Roy et al. 2012). Such degenerative changes indicate that the worms were under a stress condition, which may be the cause responsible for an early paralysis and death of the parasite.

**Conclusions**

Results of our study showed that *C. viscosum* has vermifugal activity on the parasite and some of its chemical constituents may be responsible for anthelmintic activity. Further investigation on the pharmacological properties of the active components of this plant needs to be done.

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**Disclosure statement**

The authors declare no conflict of interest regarding publishing of the manuscript.

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