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Original Article

IN VITRO ANTHELMINTIC EFFECTS OF *SENNA OCCIDENTALIS* (L.) LINK (LEGUMINOSAE) ON RAT TAPEWORM *HYMENOLEPIS DIMINUTA*

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ABSTRACT

Objective: This study was conducted to evaluate the anthelmintic activity of ethanolic leaf extract of *Senna occidentalis on* rat tapeworm *Hymenolepis diminuta*.

Methods: Adult *H. diminuta* worms were obtained from laboratory maintained infection in rats. Different concentrations of crude ethanolic leaf extracts of *S. occidentalis* were tested *in vitro* on *H. diminuta* and praziquantel (PZQ) was used as reference drug. The efficacy of plant was assessed on the basis of motility of the worms. The anthelmintic effects were also evaluated using scanning and transmission electron microscopy.

Results: A dose dependent efficacy was observed in all the experiments. At the highest concentration (80 mg/ml), the mortality of worms was recorded in 12.82±0.24 hrs. The scanning electron microscope observations of extract treated worms showed irrevocable destruction all over the body tegument,which was accompanied with sloughing of microthriches and shrinkage of scolex. Whereas, the transmission electron micrography of treated worms revealed destruction of the syncytial layer along with sparse cytoplasmic cytons and depletion of parenchymatous layer. Exposure of basal lamina, decrease in an electron lucency in the nucleus and intense vacuolization was also observed in our study.

Conclusion: In view of these observations, *S. occidentalis* may be regarded as potential anthelmintic plant and it could be exploited further for the development of a novel anthelmintic.

Keywords: *Senna occidentalis*, Anthelmintic *Hymenolepis diminuta,* Micrography, Tegument.

INTRODUCTION

Drug development towards helminthiasis is poor and due to the asymptomatic nature and less severity of the disease, helminths became overlooked and neglected. Moreover, control of these parasites relies almost exclusively on a limited number of anthelmintic drugs which were manufactured decades ago and have also shown resistant in some parasites [1]. Helminthiasis still remains the most prevalent disease in the world [2] and majority of infections are generally limited to tropical regions which contribute to malnutrition, anemia, eosinophilia and pneumonia [3]. In India, the rural population does not have easy access to drugs [4]and thus depend on traditional medicine to combat the infections. However, a majority of the ethno-veterinary medicine surveys and validation studies also indicate much wider and effective use of plants as anthelmintics compared to other diseases [5-9].

Senna occidentalis (L) Link. Popularly known as 'Coffee Senna' is an abundant species in India. It has been used for different medicinal purposes and already showed some anthelmintic properties against helminths like *Ascaris suum* and *Raillietina tetragona* [10, 11]. Besides, it has also been well established for wound healing [12], hepatoprotective and antioxidant effects [13, 14]. Its roots, flowers, seeds and leaves have been employed in herbal medicine around the world for a variety of purposes such as laxative, expectorant, anti-malarial [15], relaxant [16] and anti-inflammatory [17]. This plant became popular to medical practitioners for vast medicinal properties which may lie within the huge compound contents of glycosides, like anthraquinones. Therefore, it became more important to investigate the anthelmintic potentiality of this plant against rat tapeworm *Hymenolepis diminuta,* as the latter can be raised in animal model rather easily.

MATERIALS AND METHODS

Plant material

Fresh leaves of *S. occidentalis* (L.) Link. were collected from Santiniketan (West Bengal). The plant has been identified and a voucher specimen (VBSL-2) has been deposited at Botanical Survey of India, Kolkata. Leaves were washed thoroughly with distilled water and allowed to dry in an oven at 50 °C, and crushed to powder. The latter was stored in an airtight container and used for further successive extraction.

Preparation of plant extract

About 250 g of the powder were extracted with 1 l of ethanol (90 %) in Soxhlet apparatus for 7-8 h, and the final crude extract (17.25g) was recovered by using rotary evaporator and stored at 4 °C until further use.

Drugs and chemicals

All the chemicals used were of analytical grade and procured from Merck, USA. Ethanol was supplied by Bengal Chemicals, Kolkata, India and reference drug PZQ with trade name Distocide (composed of 600 mg PZQ) is a product of Chandrabhaghat Pharma Pvt Ltd., Mumbai, India.

Parasites

The parasite *H. diminuta* was maintained by routine passage through Sprague-Dawley rats and the beetle *Tribolium confusum* (intermediate host). Each host was infected with 8-10 cysticercoids. At 20-22 days post-infection, rats' intestine was dissected out and worms were recovered, All experimental protocols with rat were approved by the Institutional Animal Ethics Committee (IAEC) of Visva-Bharati University.

In vitro treatment

Worms were washed several times in 0.9 % Phosphate buffer saline (PBS). They were kept in petridishes and exposed to 10, 20, 40 and 80 mg/ml concentrations of crude ethanolic leaf extracts of *S. occidentalis* and PZQ (0.0010, 0.0025 and 0.0050 mg/ml) prepared in 0.9 % PBS at pH 7.4. Another set of worms with only PBS and 1 % dimethylsulfoxide (DMSO), used as solvent for extract, was taken as control. All the experiments were carried out at 37±1 °C inside an incubator. Time for paralysis (PT) of worms was noted at different time intervals when no movement of any sort could be observed, except when shaken

vigorously or kept in slightly warmer PBS. Time of mortality (TM) was recorded when no movements of any sort were observed.

Assessment of plant treatment by electron microscope studies

Scanning electron microscopy (SEM)

Paralysis at 40 mg/ml was observed within a time span that was comparable with worms treated with PZQ at 0,001 mg/ml. Immediately after paralysis, the extract-treated worms of above concentrations and control were fixed immediately for SEM following a standard method [18]. Specimens were later viewed in a Jeol JSM 6360 at an electron accelerating voltage of 20 kV.

Transmission electron microscopy (TEM)

Another group of paralyzed worms was washed gently with warm PBS and immediately fixed for TEM studies according to standard protocol of Sophisticated Analytical Instrumentation Facility (SAIF), North Eastern Hill University (NEHU). JEOL JEM-100 CX-II electron microscope was used for the observation of the ultrathin sections at a magnification range of 2000-27, 000 X.

Statistical analysis

Data are presented as mean values±standard deviations for each group (n=6). For determining statistical significance, standard deviation and analysis of variance (ANOVA) at the 5% level of significance were employed; P<0.001 was considered significant.

RESULTS

Dose responsive studies

Dose dependent efficacy was observed on exposure to various concentrations of leaf extracts of *S. occidentalis* (table. 1). At 40 mg/ml, mortality occurred at 20.42±0.21 which showed almost comparable results with PZQ (22.21±0.12h) at 0.001 mg/ml. The post-paralytic time was comparatively shorter in all concentrations of *S. occidentalis* compared to PZQ. However, control parasite survived up to 69.22±0.23 h.

Table 1: Effects of crude leaf extract of *S. occidentalis* and PZQ on *H. diminuta*

Concentration	PT (hr)	TM (hr)
(mg/ml) *S. occidentalis*		
10	15.04±0.42	28.74±0.6
20	9.90±0.39	27.5±2.91
40	3.86±0.10	20.42±0.21
80	3.19±0.16	12.82±0.24
PZQ		
0.0010	1.18±0.04	22.21±0.12
0.0025	0.67±0.03	18.80±0.05
0.0050	0.31±0.01	17.31±0.13

Survivability of the control parasite is 69.22±0.23 hrs. Each value was represented as mean±SD (n= 6). PT= time of paralysis; TM= time of mortality.

SEM studies

SEM studies of control worm revealed a normal body contour with scolex at its anterior end (fig. 1A). Tegument revealed a uniform, trapezoid shape with densely covered microtriches giving worm surface a velvety appearance (fig. 1D). Plant treated worms possessed irrevocable destruction all over the general topography of the body. Scolex shrinks (fig. 1B) and tegument was extensively damaged (fig. 1E). At higher magnification microtriches were observed to completely loss their velvety appearance with intense sloughing and perforations in plant and PZQ treated worms(fig. 1H and 1I) than that of control (fig. 1G). Overall body shrinkage in the PZQ treated worms was observed (fig. 1C and 1F).

TEM studies

TEM observations on control worms revealed abundant microtriches serially localized followed by a compact syncytial layer in the outer tegument. Distal cytoplasm was electron dense and had abundance of tegumentary cytons. Basal lamina and subtegumental muscles were well organized and compact (fig. 2A). Nucleus bearing well defined nucleolus with condensed chromatin region and electron dense nucleoplasm (fig. 2D) while treated parasites with leaf extract showed noticeable degenerative changes in the tegument. Serially arranged row of microtriches was totally damaged and clumped and syncytial layer was totally diminished.

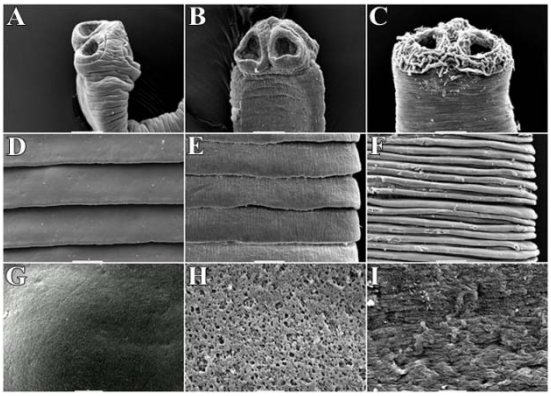

Fig. 1: SEMs of *H. diminuta*: A-control: scolex with suckers; B-*S. occidentalis*: shrunken scolex; C-PZQ: sloughed off tegument near suckers. All bars, 50 µm. D-control: typical trapezoid proglottid velvety appearance, E-*S. occientalis*: infoldings at each proglottid terminal; F-PZQ: intense folding at the edge. G-microthrix with velvety appearance, H-microtriches sloughed off, I-deep pits and ridges are very prominent

Tegument appeared to be completely sloughed off leaving an exposed basal lamina that was only present in remnants and there was release of underlying structures to the outside at the basal lamina. Parenchymatous muscle layer showed intense degradation and was loosely stacked up with loss in continuity (fig. 2B). Worms treated with PZQ showed damage in microthrix layer accompanied with degeneration of muscle stacks (fig. 2C).

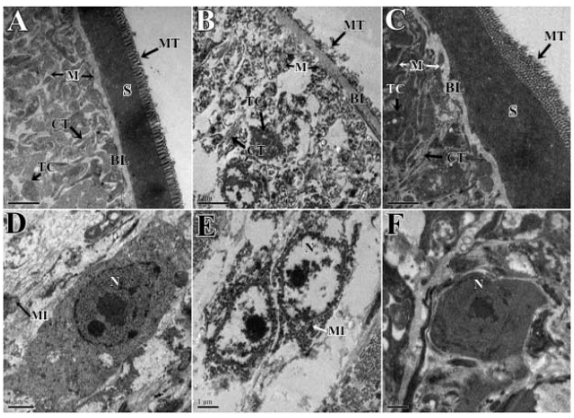

Fig. 2: TEMs of tegument of *H. diminuta*: 2A-Control: with intact microthrix (MT) above the syncytium (S), connective tissue (CT), superficial musculature (M) and basal lamina (BL). 2B-*S. occidentalis*: MT are totally damaged and clumped, exposing BL and CT, sparse syncytium with few tegumentary cytons (TC) intense vacuolization; degradation of longitudinal and circular muscle layers. 2C-PZQ: MT disorganized and stripped off. All bars, 2 µm. 2D control: distinct nucleus (N) with nucleolus, condensed chromatin, electron dense nucleoplasm and mitochondria (MI) with cristae. 2E-*S. occidentalis*: vacuolized nucleus with electron lucency inside, mitochondrial damage observed; 2F-PZQ Nuclear membrane irregular. All bars, 1 µm

Parenchymal cytons of treated parasite showed complete loss of connections with surrounding parenchyma and chromatin in nucleus appeared clumped into large areas of electron-dense heterochromatin in plant treated parasite (fig. 2E) which is similar to that of PZQ (Fig.2F). Overall damage caused by the plant is more pronounced than that of the drug in terms of structural alteration.

DISCUSSION

The present investigation revealed an insignificant difference between time of motility and time of mortality in control, which validates that there was no precession of paralysis to death in treated *H. diminuta* worms. Similar studies were also observed with many ethno medicinal plants [11,19-22]. Dose-dependent efficacy was observed in treated worms which tallies with the observations of many previous workers[23-25]. Although significant mortality was not observed in lowest concentration (10 mg/ml), still a considerable significant paralysis occurred. It may therefore be suggested that plant extracts possibly exert a reversible action on the neuromuscular system of worms and though it did not cause mortality for some time to follow, but once paralyzed, it took very short time for mortality to commence. Thus, it may be suggested that it possesses a vermifugal activity in nature and the inactiveness caused would last long enough for the parasites to be swept out of host's body [26].

The first and most obvious pathological effects of plant treatment observed under SEM were blebs on the worm's surface. Many of these blebs were partially covered with perforated membrane. Surface blebbing is a common feature observed in Schistosomes, *Fasciola hepatica, Moniezia expansa*, *Raillietina sp., Paramphistomum explanatum* after treatment with either drugs or plant extracts [25, 27-32]. This can be regarded as a stress response resulting from emergency repair to a damaged tegument and may be induced by many harmful events [33, 34].

Disorganization of proglottids and microtriches observed ,may be attributed to tegumental enzymes since they are the primary target to such action [35, 36]. Further, present study also revealed infoldings in the edge of proglottid which may be due to an increase in chloride ion conductance of worm muscle membrane producing hyperpolarization and reducing excitability that could lead to muscle relaxation and flaccid paralysis [37, 38].

Progressive erosion of basal lamina was consequent of exposure to the test materials as observed in the present study. Depletion of parenchyma cell in treated worms was evidenced through TEM. This may correspond to the decrease in total glycogen content as reported [39]. Glycogen depletion and mitochondrial dysfunction would, in combination, impair the tegument through reduced glycolysis. In the PZQ treated parasites, most of the damages induced were in the tegument, but degeneration was on a much lesser scale than the plant extract-treated ones. Some of the responses seen, like stripping of tegument, increase in electron lucency of the nucleus and surrounding parenchyma, are typical of a generalized stress response and have been described in other helminthes under drug insult as well [36, 40, 41]. The ultra structural changes such as, clumping of chromatin into heterochromatin are indicative of protein synthesis inhibition [42]. Botanicals like artemisinins were known to cause a collapse of the membrane potential of mitochondria, leading to swelling and inhibition of electron transfer and oxidative phosphorylation [43, 44].

Our investigations revealed swelling of basal lamina and intense vacuolization of the tegument which could have an osmotic basis, due to impairment of energy-dependent ion pumps as observed [29]. Further, vacuolization of the tegument was known to be induced by triggering of calcium ion flux with PZQ [45].

From the present study, it can be concluded that the plant extracts have significant structural effects on the parasites. Similar observations have been described for many plants [31, 46]. *S. occidentalis* being a rich source of anthraquinones and flavones have demonstrated to exhibit antioxidant and hepatoprotective properties [13, 14] and antimalarial activities [15]. Therefore, it may be speculated that they may be the active ingredients responsible for the anthelmintic activity. However, the specific compound (s) and the precise mode of action behind such anthelmintic activity are not determined from the present study and remain to be investigated.

CONCLUSION

Our findings showed that *S. occidentalis* possess anthelmintic effects and therefore this plant show promising potentials for treating intestinal helminth infection and can be a good alternative of chemical drugs.

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CONFLICT OF INTERESTS

Declared None

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