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HISTOPATHOLOGICAL ASSAY OF INDUCED HYMENOLEPIASIS IN *MUS MUSCULUS* AND RESTORATION OF NORMALCY WITH PRAZIQUANTEL

J. PARVATHI* AND K. ARUNA
P. G. Department of Zoology, Vivek Vardhini College,
Jambagh, Hyderabad - 500 017
E-mail: saip_jayanthi@yahoo.co.in

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***Corresponding author**

ABSTRACT
Helminth parasites produce their deleterious effects in their hosts essentially by mechanical trauma, obstruction of hollow viscera and circulatory channels, utilization of substances normally destined for body nutrition, depletion of the blood, chemical intoxication and occupation of space and displacement of tissues. The triumviate interaction of *Hymenolepis nana* infection in the laboratory mouse *Mus musculus* and treatment with praziquantel are depicted in the present work, through histopathological assay of host intestine using heamatoxylin and eosin staining and scanning electron microscopy. The extent of mechanical trauma inflicted in the traumatized host at the morphological level is thus visualized. The research further emphasizes the reasons for the development of many more such broad-spectrum anthelmintics to combat the ever-evolving parasites.

INTRODUCTION

The diversified modes of survival are the outcome of the struggle for existence which necessitates the exploration of various habitats resulting in the exploitation of the available resources. Parasitism is one such mode of survival and Cestode helminths are one such class of parasites which have adapted to virtually every tissue, organ and space in the body of both vertebrates and invertebrates, some of which cause diseases of extreme importance to humans, domestic and wild animals.

Hymenolepis nana (Von Siebold, 1852), the "dwarf tapeworm", is an intestinal cestode helminth of mouse and man, causing Hymenolepiasis. It has a high incidence in the tropics and subtropics, with heavy auto-infections. This is the only known cestode which can be transmitted directly. Proper choice of anthelmintic is important, as most drugs are more effective against some species than others and virtually all antiparasitic drugs induce some adverse effects. The drug selected should offer the best combination of effectiveness and relative safety. The real importance of mice in human disease is found in their role as experimental hosts to infections.

Although much is known about the parasites of laboratory animals, information is often lacking and what is available is scattered. Parasite-induced gastrointestinal pathology has also been intensively studied with murine models (Larsh's (1963) review). The invasion of the mouse bile duct by *Hymenolepis microstoma* (Pappas, 1976) results in hypertrophy of the duct and liver tissues along with enhanced furrowing and erosion of the duct mucosa. However, a more fundamental approach to the actual functioning of these laboratory animals is necessary if experiments are to be correctly evaluated in terms of any human or more general biological application.

Hence to understand these basic host-parasite interactions at the histomorphological level, the intestine of the host *Mus musculus* induced with *Hymenolepis nana* infection and subsequently treated with Praziquantel has been studied.

MATERIALS AND METHODS

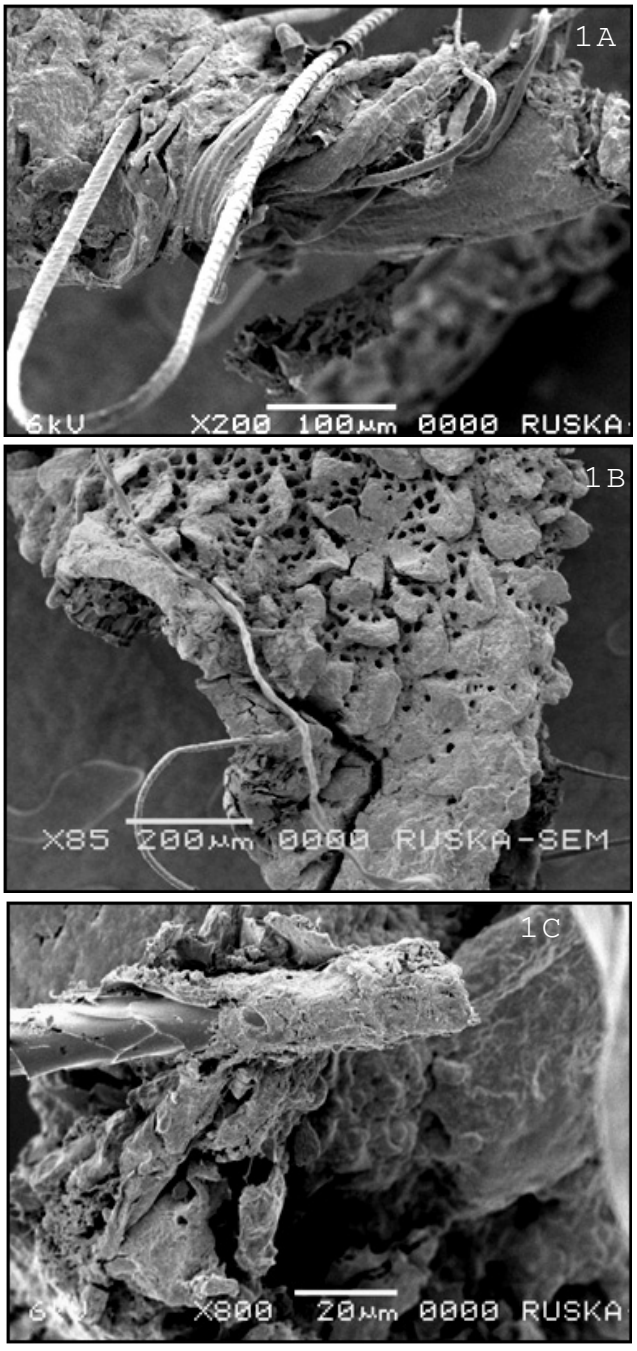
Adult healthy male Swiss albino mice, *Mus musculus* about 4-5 weeks old, weighing 20-25g, maintained under good laboratory practices (GLP) conditions(Lissau, 1994), were divided into three batches. One-third of the mice were the uninfected control batch. Two thirds of total mice were orally infected with about 100 viable eggs of *Hymenolepis nana* per mouse, and maintained as the infected batch. These infected mice were thus maintained until the 16th day of infection to complete the life cycle of the parasite. On the 16th day, half of the infected mice were given a single oral dose of Praziquantel, a 0.2mL suspension at 25mg per kg bodyweight. This batch was maintained for 3 days as the treated batch. The control, infected and treated batches of mice were sacrificed at appropriate times, by cervical dislocation. Some formalin-fixed host tissues were embedded in paraffin blocks in an automatic tissue processor, and 6μ thick formalin-fixed paraffin sections of the host intestine tissues were stained with haematoxylin

and eosin stain(Dawkins and Rees, 1959) and microphotographed for histomorphology. Some part of the intestine was processed for scanning electron microscopy (Bozzola and Russell, 1999).

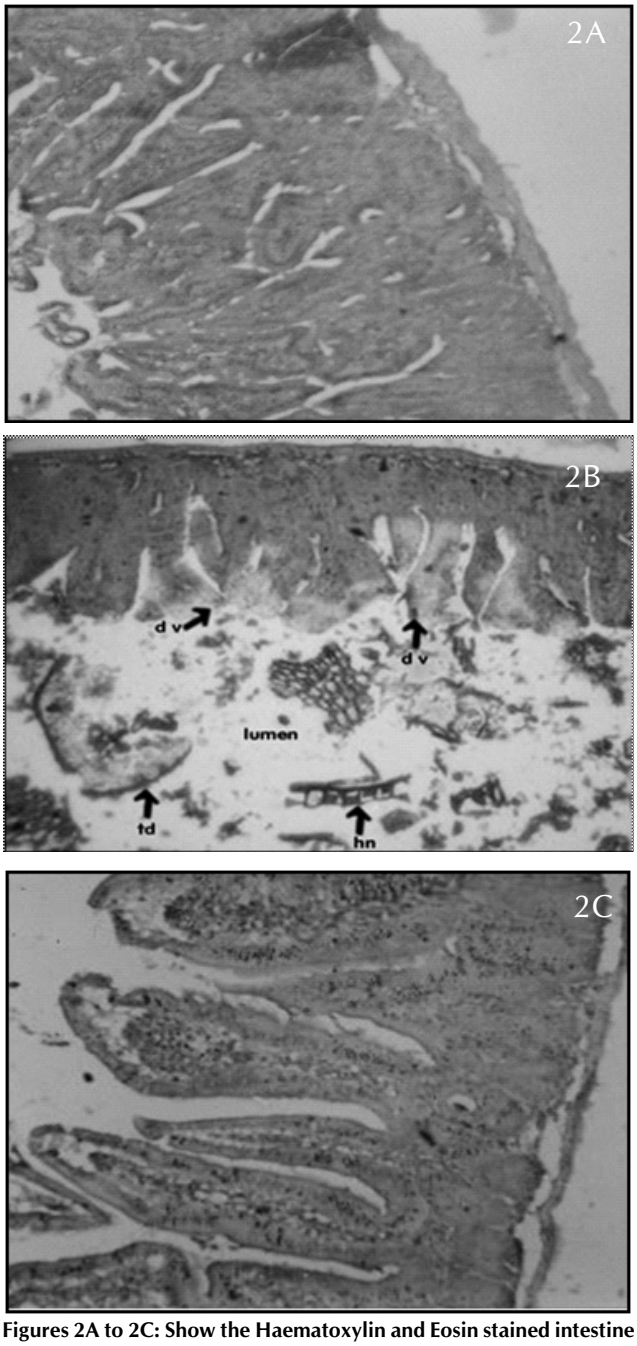
RESULTS

The infected intestine showed a large number of worms in the intestinal wall disrupting more than one site of the host (Fig.1A to 1C). A magnified image of the worm penetrating the host villus has been shown in Fig. 1A and 1B. Many perforations in the intestinal wall are seen in Fig. 1C.

Fig. 2A to 2C is the Haematoxylin and Eosin stained intestine sections of host *Mus musculus*. Fig.2A shows normal



Figures 1A to 1C show Scanning Electron Micrographs of *Hymenolepis nana* infecting the intestine of *Mus musculus;* (1A) shows many worms penetrating the host intestine; (1B) shows *Hymenolepis nana* penetrating a single villus of the host; (1C) shows the highly perforated host intestine due to hymenolepiasis



Figures 2A to 2C: Show the Haematoxylin and Eosin stained intestine sections of host *Mus musculus*; (2A) Control host intestine showing normal histology with intact tissue and villi; (2B) *Hymenolepis nana* infected host intestine showing disrupted tissue, villi, and the worms in the lumen. dv: disrupted villi; td: tissue debris; hn: *Hymenolepis nana* proglottides; (2C) Praziquantel treated host intestine proving the drug's efficacy in restoring normal histology in *Hymenolepis nana* traumatized host

histological features of uninfected control host intestine with intact villi and other tissues. Fig.2B is the section of *Hymenolepis nana* infected host reveals the extent of damage the parasite inflicts at the site of its habitat. The worm disrupts the host's intestinal wall which is visible as the tissue debris in the lumen. Fig. 2C is that of the Praziquantel treated intestine section of *Hymenolepis nana* infected *Mus musculus*. The results reveal the morphology comparable to the uninfected control host intestine, thereby proving the drug's efficacy. The positive healing effect of the disrupted villi, by Praziquantel against hymenolepiasis is further seen.

DISCUSSION

Hymenolepis nana inhabits the ileum part of host's small intestine and it is in this region that the actual extent of damage that the worm can inflict mechanically (Sumner, 1988) is visualized clearly. The infected host tissue investigated, reveals the mechanical injury at the site of the habitat of this cestode. The host's tissue is disrupted, the worm bores into the intestine leading to the leakage of various host's substrates, enzymes and blood and also a lot of tissue debris into the lumen of the intestine (Innes, 1967). The crowding effect(Heyneman,1953) due to recurrent auto-infections in the immuno-compromised hosts leads to many more complications in the host like pronounced intestinal disturbances and other toxic symptoms(Dawkins and Rees, 1959), retarded growth and weight loss in the host(Cheng,1964) and chronic inflammation (Simmons *et al*., 1967). In the warmer countries in populations with poor sanitation, this crowding effect could prove to be a major health problem and even hazardous, especially among children. (El Gholmy *et al*., 1968; Busher and Haley, 1972; Chero *et al*., 2007).

The intricate host-parasite interactions in this triumviate investigation emphasize the gross histopathological alterations during hymenolepiasis in the host intestine which were qualitatively analyzed and compared with the uninfected control counterparts. The perforated host intestine clearly visualizes the devastating extent of the mechanical trauma inflicted in the host traumatized by *Hymenolepis nana*. Moreover, the migration of the parasite toxins to various organs of the host (Simpson and Gleason, 1975; Przyjalkowski, 1977; Wilson *et al.,*1978;) will further induce non-specific damages in turn affecting the metabolism of the host substrates like proteins, carbohydrates, lipids, their enzymes, as well as the haematological parameters.

This study further analyzes the efficacy of the broad-spectrum anthelmintic Praziquantel in reprimanding hymenolepiasis, thus restoring normalcy in *Hymenolepis nana* infected host (Thomas and Gonnert, 1977; Meltem *et al.,* 2009). The histology in the treated intestine proves the healing effect of this drug in bringing back normalcy in the traumatized host. This may serve as the preamble for further research in combating this worm infection for the development of new prophylactic anthelmintics. This is needed because, in the tropics and subtropics, multiple worm infections are common (Khakhavandi *et al*., 2007). If such periodical measures are observed, the mental and physical growth of the children especially in such colonies can be boosted. Moreover, in these countries, there is a need to develop many such cost-effective, safe, single, low-dosage regimen drugs, which can be administered orally without specific diet requirements. The research can still further be extended for the development of vaccines (Leuker *et al*., 1968; Murrell, 1982; Ahmed *et al*., 1986).

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