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Histological Study on the Effect of *Piper longum* (Linn.) against *Haemonchus contortus*

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ABSTRACT

Haemonchosis is the infection caused by the gastro intestinal parasite, *Haemonchus contortus* in the small ruminants which leads to great economic loss to the livestock breeding industry. To overcome this problem various combination of anthelmintic drugs was practised to control the parasitic infection. In recent years, however, use of chemical anthelmintic has led to the development of resistance against *Haemonchus contortus*. Therefore, researchers have focused on the alternate effective sources to treat the parasitic infection. The medicinal plants having wide range of bioactive compounds can be used as anthelmintic agent and to improve the performance of the livestock. In this present study, the effect of *Piper longum* root extract at three different sub-lethal concentrations (0.25%, 0.5% and 1%) was used for structural analysis of the *H. Contortus* based on the dose and time dependent. The parasite incubated in the highest concentration (1%) exposed to 8 hours showed severe damages in the various organ systems; whereas minor structural changes were observed in the parasites incubated at least concentration of the plant extract. The light microscopic study revealed the anthelmintic property of the *P. longum* root extract by destructing the organ system such as cuticle, intestine and ovary of the parasites. Hence, present investigation suggests that *P. longum* could be successfully in the control of haemonchosis.

KEYWORDS: Haemonchosis, *Haemonchus contortus*, Anthelmintic, *Piper longum*, Necrosis

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1. INTRODUCTION

Gastro-intestinal parasitic infection brings great economic loss to both small and large-scale livestock farmers. Among GI parasites the *Haemonchus contortus*, which is most predominant parasite possessing highly pathogenic in small ruminants¹. Haemonchosis mainly controlled by synthetic anthelmintic compounds and frequent use of anthelmintics drugs against parasitic infection which develops drug resistance in the animal². In addition to high prevalence of drug resistance similarly, it results in toxicity and side effects in the animals³. Hence, there is huge necessity to find alternative source to expel the helminthic parasites from the gastro-intestinal tract⁴. The plants possessing various phytochemical constituents which contain anthelmintic property can be used to treat haemonchosis.

Piper longum Linn. belong to family piperaceae and commonly known as long pepper⁵. The root contains active compounds like alkaloids, piper longuminine, piper longumine, piperine has promising biological effects. Pharmacological profile shows that the plant exhibits immunomodulatory activity, antiasthmatic, hepatoprotective, hypocholesterolamic, anti-inflammatory, antibacterial⁶. The root of *Piper longum* possessing a high therapeutic value includes carminative, hepatoprotective, stomachic, abortifacient, haematinic, diuretic, digestive and as a general tonic. It also cures inflammation of the liver, pains in the joint, lumbago, snakebite, scorpion-sting and night-blindness⁷.

2. MATERIALS AND METHODS

2.1 Collection of the parasites

The abomasum of infected sheep was collected from the slaughter house located in Chennai and brought to the laboratory. The Adult female *Haemonchus contortus* was isolated and washed thoroughly in physiological saline and used for subsequent studies

2.2 Preparation of plant extract

The aqueous extract of *Piper longum* root (PIRE) was prepared. A stock solution of 20 % concentrations of each plant extract was prepared using standard procedure and these stock solutions were then serially diluted to 3 %, 5 % and 10 % concentrations with the *in vitro* maintenance medium Hedon-fleig solution⁸. Further sub-lethal concentrations (0.25%, 0.5% and 1%) were used in the experimental studies.

2.3 In vitro study

2.3.1 Gross visual observation on the motility of the parasites incubated in the plant extract

The live and active parasites (n=10) were incubated in various sub-lethal concentrations such as 0.25%, 0.5% and 1% of the extract in airtight sterile container. The activity of the incubated parasites was checked at various time intervals such as 5, 15, 30 min, 1, 2, 4, 6 and 8 hours. Simultaneously, control was also maintained. Based on the visual observation the motility of the parasites were categorized as very active (++++), moderately active (+++), slightly active (++) , sluggish (+) and dead (-).

2.4 Structural Analysis

2.4.1 Light microscopic studies

Alive specimens from control and treated groups were washed in phosphate buffer (pH 7.4) and fixed in 10 % neutral buffered formalin. After fixation the parasites were washed in distilled water and dehydrated through graded alcohol series, cleared in xylene and embedded in paraffin wax. Sections were cut at 5 µm thickness and stained in haematoxylin and eosin⁹ and were observed under binocular compound microscope for various cellular details. Using Nikon Triangular Photomicroscope photomicrographs were taken at various magnifications.

3. RESULTS AND DISCUSSION

3.1 In vitro effects of PIRE on H.contortus

3.1.1 Gross visual observation on the motility of the parasites incubated in PIRE

The result of the effect of PIRE extract on motility of *H. contortus* as evaluated by visual observation is presented in Table 1.

Table No. 1: “Chronological observations on the motility of *H. contortus* exposed to PIRE”

Conc. %	5m	15m	30m	1h	2h	4h	6h	8h
0.25	++++	++++	++++	+++	++	++	++	++
0.5	++++	++++	+++	++	++	++	+	+
1	++++	++++	+++	++	+	+	+	+
untreated	++++	++++	++++	+++	+++	+++	+++	+++

++++ Very active ; +++ Active ; ++ Moderately active ; + sluggish ; - Dead

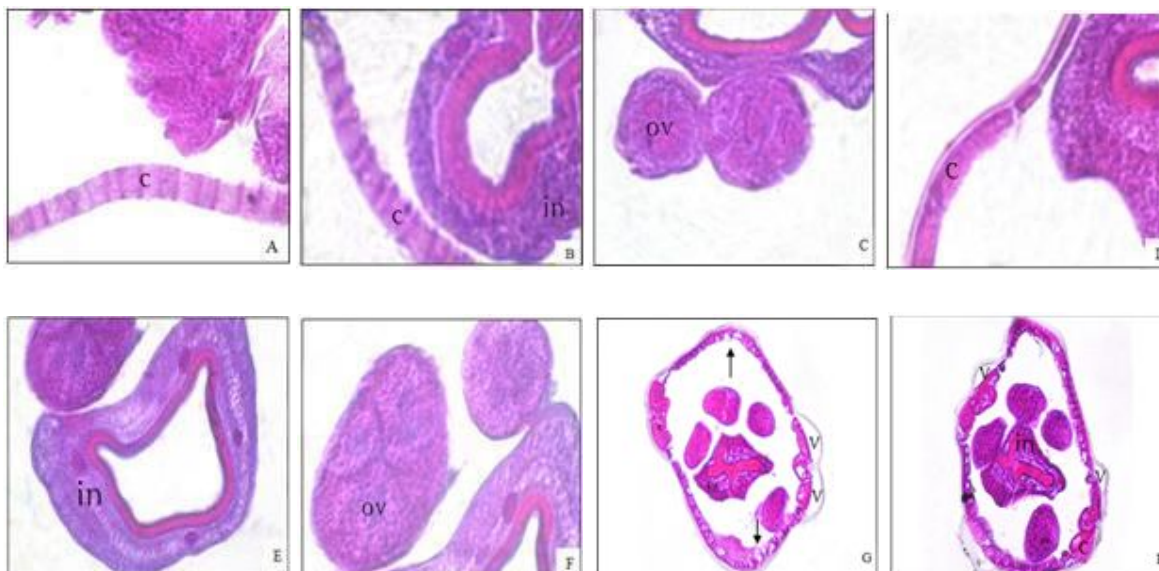
The results showed that the *PIRE* significantly inhibited the motility of *H. contortus*, *in vitro*. The inhibitory effect was dependent on both duration of exposure and concentration of the extracts; longer the duration and higher the concentration, the greater the inhibition of motility.

3.2 Light microscope studies

A thick intact cuticle (c) was observed in untreated *H. contortus* (Fig. A). The intestine (in) showed a complete tube composed of a single layer of epithelial cells resting on a basement membrane (Fig. B). Intact ovary (ov) with a single layer of cuboidal cells (germinal epithelium) resting on a basement membrane was observed in untreated *H. contortus* (Fig. C).

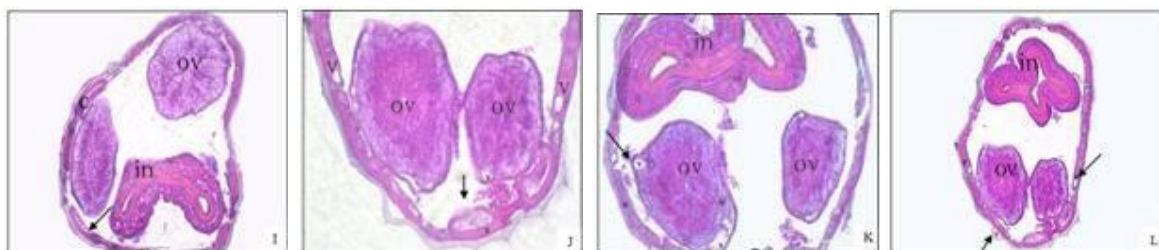
The parasites incubated in *PIRE* at various concentrations showed pathological changes, these changes being dose and time dependant. Severe damages in the various organ systems were observed in the parasites incubated in the highest concentration (1 %) for 8 h; whereas, parasites incubated in the least concentration (0.25 %) of the plant extract showed minor changes.

After 2h exposure, the parasites at 0.25 % concentration showed minor changes only in the cuticle (c) (Fig. D); not many damages were observed in the intestine (in) and ovary (ov) (Figs. E& F). After both 4 h and 8 h exposure at a similar concentration, marked damages were observed in the cuticle (c), with development of vacuoles (v) and severe necrotic lesions (arrows) (Figs. G & H). Further, accumulation of fluid was also observed in the intestinal (in) lumen after 4 h and 8 h exposure (Fig. G & H). Not many changes were observed in the ovary (ov) of *PIRE*-treated parasites at 0.25 % concentration. (Figs. F, G& H).



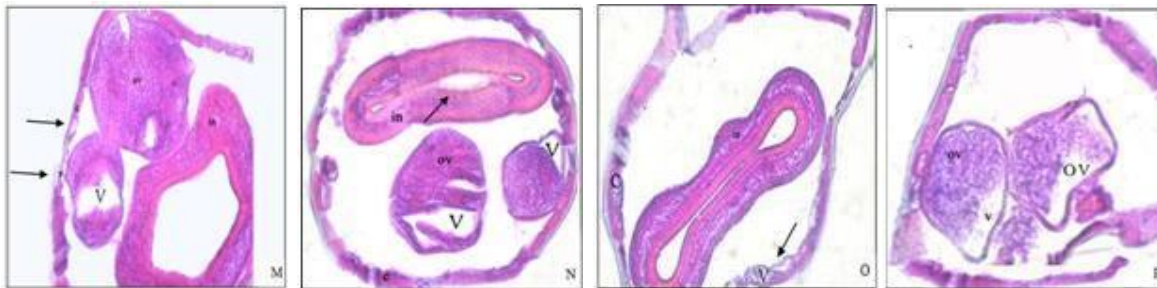
Photomicrographs showing untreated parasite intact cuticle (c) $\times 40$ (A), untreated parasite showing intestine (in) $\times 40$ (B), untreated parasite showing intact ovary (ov) $\times 40$ (C), *PIRE*-treated parasite showing minor damages in the cuticle (c) (0.25 % after 2 h exposure) $\times 40$ (D), *PIRE*-treated parasite showing minor damages in the intestine (in) (0.25 % after 2 h exposure) $\times 40$ (E), *PIRE*-treated parasite showing minor damages in the ovary (ov) (0.25 % after 2 h exposure) $\times 40$ (F), *PIRE*-treated parasite showing vacuole (v) formation in the cuticle (c). (0.25 % after 4 h exposure) $\times 10$ (G), *PIRE*-treated parasite showing development of vacuole (v) in the cuticle (c) accumulation of the fluid in the intestine (0.25 % after 8 h exposure) $\times 10$ (H).

The parasite incubated in 0.5 % concentration of *PIRE* displayed severe damages in the cuticle (c) after 2 h, 4 h and 8 h exposure (Figs. I, K and L). Partial fusion of the absorptive surface of the intestine (in) was also observed in parasites treated with the plant extract after 2 h, 4 h and 8 h (arrows) (Figs. I, K and L). However, development of vacuoles (v) in the ovary (ov) was seen only after 4h of incubation at 0.5 % concentration of *PIRE* (Fig. J). Similar vacuulations were in the ovary also observed in the plant extract treated parasites after 8 h incubation (Fig. L).



Photomicrographs showing *PIRE*-treated parasite showing damages in the cuticle (c) and intestine (in) (0.5 % after 2 h exposure) $\times 10$ (I), *PIRE*-treated parasite showing vacuole (v) formation in the cuticle (c) and ovary (ov) (0.5 % after 4 h exposure) $\times 40$ (J), *PIRE*-treated parasite showing partial fusion of the absorptive surface of the intestine (in) (0.5 % after 4 h exposure) $\times 10$ (K), *PIRE*-treated parasite showing partial fusion of the absorptive surface of the intestine (in) and development of vacuoles in the cuticle (arrows) and the ovary (ov) (0.5 % after 8 h exposure) $\times 10$ (L).

PIRE at the highest concentrations used viz., 1 % caused profound damages in the various organ systems. Marked deformity of the cuticle (c) characterized with lesions (arrows) and vacuoles (v) were observed (Figs. M, N & O). Similarly, the gastrodermis and the absorptive surface of the intestine (in) showed damages and necrotic lesions (arrows) after 2 h, 4 h and 8 h of incubation in the *PIRE*-treated parasites. Vacuolation of the ovary (ov) was observed after 2 h and 4 h exposure to the plant extract (Figs. M & N). Complete destruction of the ovary (ov) with the appearance of vacuoles (v) was seen in the parasites after 8 h exposure to 1 % concentration of *PIRE* (Fig. P).



Photomicrographs showing *PIRE*-treated parasite showing vacuole (v) formation in the ovary (ov) (1 % after 2 h exposure) $\times 40$ (M), *PIRE*-treated parasite showing development of vacuoles (v) in the ovary (ov) and necrotic lesions (arrows) in the intestine (in) (1 % after 4 h exposure) $\times 10$ (N), *PIRE*-treated parasite showing vacuoles in the cuticle (c) (1 % after 8 h exposure) $\times 10$ (O), *PIRE*-treated parasite showing complete destruction of the ovary (ov) (1 % after 8 h exposure) $\times 40$ (P).

PIRE significantly inhibited the motility of *H. contortus*, the inhibitory effect being concentration and time dependent. An inhibitory effect on the motility of larval and adult helminth parasites treated with plant extract has been reported by various authors^{10, 11,12,13}. In GI adult parasites the motility, which is most important characteristic feature helps in survival of the parasite in the host's gut. Similarly, it also helps in their feeding and reproduction of the helminths. Inhibition of motility might be largely due to muscular paralysis, which could also affect the structure and physiological functions of the parasite.

The light microscopic observations on *PIRE*-treated parasites indicate that the plant extract caused conspicuous morphological and structural damages in the parasite. In nematodes, the cuticle performs various functions such as locomotion, selective absorption, excretion, osmoregulation and immunoprotection¹⁴. In the present study *H. contortus* treated with *PIRE*, revealed prominent structural damages to the cuticle. Loose and elevated cuticle with conspicuous fissures is the notable pathological changes observed in *PIRE*-treated worms (0.5 % and 1 %). The degeneration of the cuticle could result in lowered helminth resistance to the host, which lead to the death of the parasite and its subsequent expulsion.

PIRE induced lesions in the gastrodermis of *H. contortus* and notable changes observed in the ovary. Any damages in the absorptive surfaces like cuticle and gastrodermis may remove the essential nutrients of the parasites. Such disruption of the intestinal epithelium could contribute to the

death of the treated worms¹⁵. The drug-induced regression of the female reproductive system would result in reduced egg production and irregular embryonation.

The present study revealed that *Piper longum* induced degeneration of the cuticle, intestine and reproductive tissues. Degeneration of the cuticle exposes the parasites to the GI digestive enzymes of the host. Further, degeneration of the gastrodermis affect the membrane bound phosphatase which is involved in the transport of glucose. Consequently, glucose uptake gets impaired and therefore, results in energy deprivation which leads to death of the parasites.

The results of the present study suggest that *Piper longum* could be successfully used in the control of haemonchosis. The use of cost-effective and locally available plant products, coupled with improved farm management and grazing practices should prove useful to smallholder farmers and pastoralist for the effective control of haemonchosis. Evaluation of acute, chronic and sub-chronic toxicity study of *Piper longum* in mammalian host system could be beneficial in using this extract as an alternative therapy. Plant extracts with multiple modes of action prevent the emergence of resistance. With the rapid escalation of cost, drug resistance to chemical anthelmintics, exploitation of phytotherapy in future would constitute a major part of veterinary medicine. However, coordinated approaches to validate the use of the plant products are mandatory before its field application.

4. CONCLUSION

The present study suggests that *P. longum* could be successfully used in the control of haemonchosis. Plant extract offers an economical alternative to synthetic drugs for the control of haemonchosis. In addition, plant products are eco-friendly and do not leave residues in the animal products. Thus use of *P. longum* coupled with improved farm management and grazing practices could be recommended for the future control of haemonchosis.

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