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Anthelmintic activity of *Acacia nilotica* against *Haemonchus contortus* (Nematoda)

Jatinderpal Singh

Department of Zoology, Baring Union Christian College, Batala, Punjab, India

The anthelmintic activity of leaf and bark extracts of *Acacia nilotica* was tested against *Haemonchus contortus*, a gastrointestinal nematode parasitic in ruminants. The crude aqueous extract of the plant leaves and bark was tested *in vitro* conditions. The adult worms were exposed to varying concentrations of plant extracts in artificial media, Tyrode's solution. The changes in motility, mortality and histology were observed. The motility of the adult worms exposed to varying concentrations of the extract was strongly inhibited after 5 to 10 hours. The 100% mortality was seen after 8 hours (in 50% concentration), 10 hours (in 40% concentration). Microscopic observations on the paralyzed and dead worms revealed wide-scale destruction of the cuticle and muscle cells. The *in vitro* effects of this plant extract showed excellent anthelmintic activity against *Haemonchus contortus*. However, further *in vivo* studies are necessary to validate the anthelmintic properties of this plant.

Keyword: Nematoda, leaf extract, *Haemonchus contortus, Acacia nilotica*, histology

1. Introduction

Parasitic diseases are the major public health problems of tropical countries including India, resulting in great economic losses. Parasites not only infect man but also domestic animals and wild life. Nematodes are the most important parasitic organisms affecting all domestic animals. They parasitize man, livestock, and crops and have a deleterious effect on all.

Infections by gastrointestinal nematode parasites of livestock are among the common and economically important diseases of grazing livestock. Control of internal parasites of sheep and goat in the country and abroad is mainly anthelmintic dependent. The haphazard use and total reliance on anthelmintics have led to emergence of anthelmintic resistant strains of parasites. This problem has threatened anthelmintic based parasite control programme. Recently, a large number of reports are coming from various places reporting the resistance against the commonly used anthelmintics. In India, many cases of anthelmintic resistance in *Haemonchus contortus* to various drugs have been reported from time to time. The resistance of phenothiazine and thiabendazole in *H. contortus* of sheep was reported by various workers [1, 2, 3]. In most cases, the resistance seems clearly to be associated with heavy reliance on chemical control, applied frequently and sometimes haphazardly. This is the most important problem acting against sustainable parasite control. Parasite resistance increases cost of treatment, reduces the efficacy of production, depletes the stock of effective control tools and increase the risk of environment contamination, as frequency of use and dose increase with declining effectiveness of anthelmintics. As resistance to newer anthelmintics is emerging, there is a dire need in India for sustainable integrated parasite management which is

multidisciplinary in nature and is under resourced because of its long-term nature [4].

The growing awareness of environmental conditions have directed research for bio safe, ecofriendly and economically viable approaches. The anthelmintic activities of various plant species have been tested by various workers in different parts of the world. *Acacia* spp. are known to have anthelmintic, anti-fungal and anti-bacterial properties. The active components of *Acacia* are supposed to be condensed tannins. The genus also contains saponins like Acacia side A and B which have been proven to have anthelmintic activity [5, 6]. The alterations in structural and functional integrity of cestode tegument on treatment with *Acacia oxyphylla* was reported by Dasgupta and Roy [7].

The aim of the present study was to evaluate the anthelmintic activites of *Acacia nilotica* against *Haemonchus contortus.* The gastro-intestinal nematode *H. contortus* is considered the most important endo-parasite because of the extensive damage it has caused to livestock. It causes heavy economic losses in small ruminant industry due to high mortality in addition to reduction in productivity.

2. Materials and Methods

2.1 Source of Plant extract

Fresh leaves and bark of *Acacia nilotica* were collected from the different areas of Malwa belt of Punjab and identified botanically before use. The shade dried leaves and bark were grinded to form a powder separately. The powdered leaves were soaked in water in the ratio of 1:5 for overnight. The aqueous leaf extract thus prepared was strained through a fine muslin cloth. This was taken as stock solution (S). Similarly the bark extract was also prepared.

2.2 Collection of parasites

Adult male and female *Haemonchus contortus* worms were collected from abomasum of freshly slaughtered sheep (*Ovis Aries*) from various abattoirs of Batala. (Punjab) India (Fig. 1).

2.3 *In vitro* study experimental design

The efficacy of the leaf extract was evaluated against the adult male and female worms. The worms were exposed to each of the following treatment in petri dish at room temperature at a concentration of 10, 20, 30, 40 and 50% concentrations of the *Acacia* leaf extract plus Tyrode's solution. The *Acacia* leaf extract and Tyrode's solution were taken in the ratio of 1:1 at each dose. Simultaneously, one group of nematodes kept in Tyrode's solution alone served as control group.

The efficacy of the bark extract was evaluated against the adult male and female worms. Four worms were exposed to each of the following treatment in petri dish at room temperature at a concentration of 10, 20, 30, 40 and 50% concentrations of the *Acacia* bark extract plus Tyrode's solution. The *Acacia* bark extract and Tyrode's solution were taken in the ratio of 1:1 at each dose. Simultaneously one group of nematodes kept in Tyrode's solution alone served as control group.

The inhibition of motility along with mortality rate of *H. contortus* was observed after 4, 6, 8, 10, 12 and 14 hours. A score index was made for each observation. The motility was recorded using the following criterion.

Score 5	-	Vigorous motility
Score 4	-	Good motility
Score 3	-	Moderate motility
Score 2	-	Poor motility
Score 1	-	Immobile but alive
Score 0	-	Died

After death of nematodes, two nematodes from each group were processed for observation of histomorphological alterations.

2.4 To study the external morphological changes

For studying the external characters of the parasites, they were stained in 1% methylene blue at 60^0C for 18 hours. Then these were differentiated in 80% alcohol, cleared in lectophenol and mounted in the same. The slides were observed under the research microscope to record the morphological changes induced by neem extract.

2.5 To study the histological alterations

The nematodes were fixed in Bouin's fixative, washed in 70% alcohol, dehydrated in a graded series of alcohol, cleared in xylene and embedded in paraffin wax. The sections were cut in 7 µm in transverse and longitudinal planes. The serial sections arranged on sides were stained with haematoxylin and eosin.

3. Results and Discussion

Nematodes of the control group remained active, with motility score 4, from 0 to 16 hours in Tyrode's solution. The nematodes placed in different concentrations of the *Acacia nilotica* leaf extract became sluggish and paralyzed and died between 8 to 14 hours. The complete mortality in higher concentrations i.e. 40% and 50% was seen after 10 hours and 8 hours respectively. In case of 10% and 20% concentrations the nematodes became sluggish after 8 hours and died after 14 hours. The nematodes have undergone changes in their position in petri dishes and paralyzed before death. The nematodes exposed to medium concentration of the extract i.e. 30% became sluggish and showed feeble movements after 8 hours. The complete mortality was seen after 12 hours in 30% concentration of the leaf extract (Fig. 2 & Table 1).

The motility of the worms placed in *Acacia nilotica* bark extract was also regularly monitored. The worms placed in 10% and 20% concentration showed moderate motility after 6 hours and feeble motility after 14 hours and died after 14 hours. The worms placed in 30% concentration of the bark extract showed regular decline in motility and died after 12 hours. The higher concentrations of the bark extract i.e. 40% and 50% were very effective and killed the worms in 10 and 8 hours respectively (Fig. 3 & Table 2).

The effect of the *Acacia* leaf and bark extract was mainly on the cuticle and muscle layers of the body wall. The histological alterations include deep etching, peeling and wrinkling of the cuticle. These alterations were confined to the epicuticle and cortical layers of the body wall. From this observation it can be concluded that the *Acacia* leaf and bark extract has been able to penetrate the acid mucopolysaccharide barrier present on the surface of the body wall of *Haemonchus contortus* (Fig. 4, 5 & 6).

The anthelmintic activity of *Acacia oxyphylla* stem bark against *Ascaridia galli* caused devasting structural alterations on the fine topography. Severe shrinkage of the cuticle, loosening and collapse of the lips, extensive irregular wrinkles all over the body surface, small swellings or blebs on the cuticle. The extract of *Acacia oxyphylla* stem bark also indicated concentration-dependent efficacy on the nematode [7]. In present study, all the treatments of extracts of *Acacia nilotica* bark and leaves exhibited anthelmintic activity. Leaves of *Acacia nilotica* were found to have higher effects *in vitro* against adult worms of *Haemonchus contortus*. Fruits of *Acacia nilotica* have already been reported for their anthelmintic activity [8]. The efficacy of different anthelmintic drugs was tested on various nematode species by a number of workers. The micro-morphological changes induced by anthelmintic piperazine were assessed *in vitro* on *Ascaris lumbricoides* [9]. Similarily, the histomorphological changes in the body wall of *Ascaridia galli* induced by hertazan, piperazine and santonin were assessed. The effects of these anthelmintics were mainly on the cuticle, hypodermis and muscular layers of the body wall [10].

The anthelmintic levamisole causes serious alterations in the tissues of *Ascaris lumbricoides*. The histological alterations reported by them include thining of muscle cells and their detachment from each other. The atrophy of excretory ducts and nerve cords was also reported [11]. The effect of tribendimidin on *Necator americanus* included histological changes like cuticular swelling, fusion of transverse striations, peeling and erosion of female worms [12]. The *in vivo* effects of benzothiazole and amoscanate derivatives include alterations in the somatic musculature of adult *Brugia* and *Litomosoides*. The electron density of the cytoplasm surrounding the myofilaments in the fibrillar portion of the muscle cells is increased. The appearance of light zone in between the fibrils was also reported. In *Litomosoides carini*, the muscle cells do not increase in size but their

mitochondria swell up and disintegrate. The disintegration of the myofilaments and vacuolation of the cytoplasm was also reported [13]. The disintegration of the cuticle of *Trichinella spiralis* after treatment with cyclosporine was found. In a study on adults and larvae of *T. spiralis* it was found that the cuticle disintegration takes place between the hypodermal pores which appeared somewhat thickened and irregular. Wrinkling of the outer layer of cuticle and bullae formation was also reported [14]. The neuromuscular blocking actions of levamisole and pyrantel type anthelmintics on the gastrointestinal muscles were also reported. Both these anthelmintics have been reported to block the contractility of nematode axial muscle by causing sustained depolarization of the muscle membrane and also by neuromuscular transmission [15].

In the present study on *in vitro* study of *Acacia nilotica* leaf and bark extract, the effect on *Haemonchus contortus* was found mainly on the cuticle and muscle layer of the body wall. The histological alterations included etching, peeling and wrinkling of the cuticle. The second affected area was muscle cells. The muscle cells of the body wall have undergone flattening and detachment from the cuticle. These defects can be due to the loss of fundamental activity of the muscle layer as a result of interruption of glycolytic pathway of metabolism.

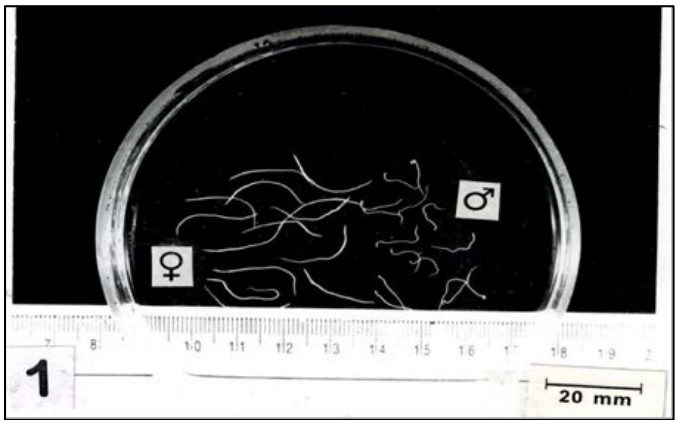

Fig 1: Adult male and female *Haemonchus contortus.*
[Motility Score: Score 5=Vigorous motility; Score 4=Good motility; Score 3=Moderate motility; Score 2=Poor motility; Score 1=Immobile but alive; Score 0=Died]

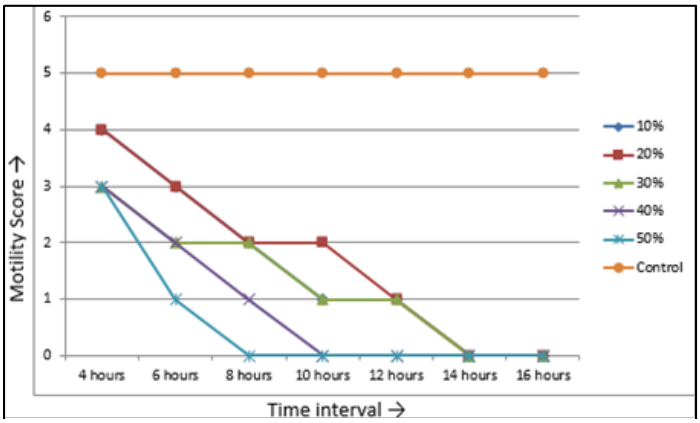

Fig 2: Motility of *Haemonchus contortus* in different concentrations of the leaf extract of *Acacia nilotica* along with control at various time intervals. [Motility Score: Score 5=Vigorous motility; Score 4=Good motility; Score 3=Moderate motility; Score 2=Poor motility; Score 1=Immobile but alive; Score 0=Died]

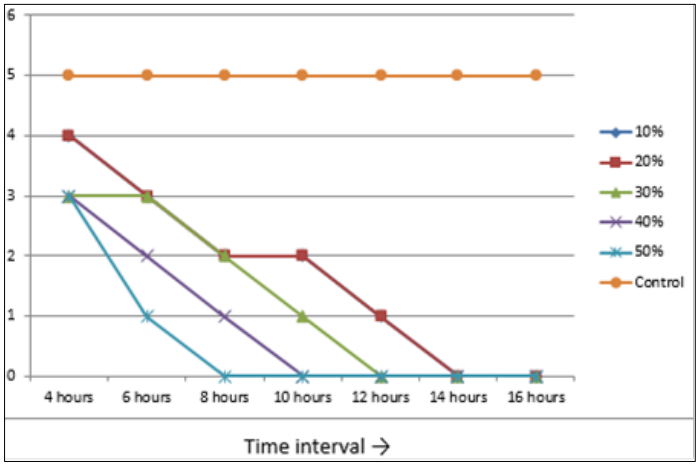

Fig 3: Motility of *Haemonchus contortus in* different concentrations of the bark extract of *Acacia nilotica* along with control at various time intervals.

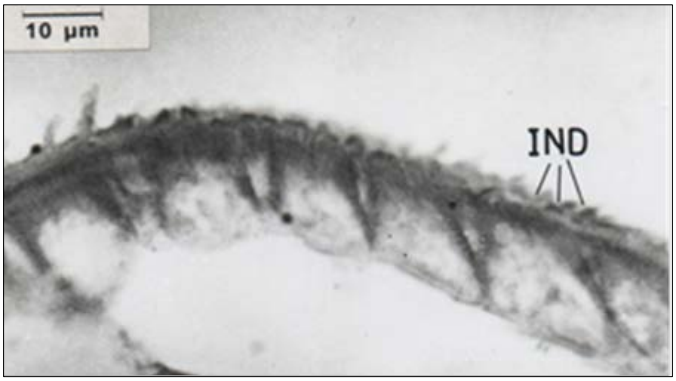

Fig 4: A portion of T.S. of female *Haemonchus contortus* places in 40% concentration of *Acacia* leaf extract showing indentations (IND) on the cuticle.

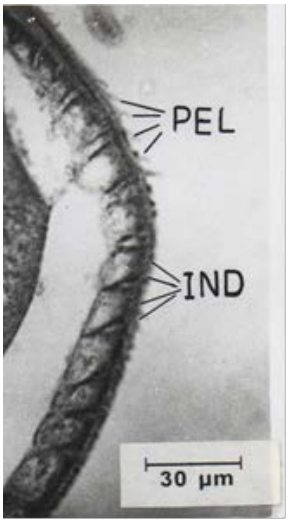

Fig 5: A portion of T.S. of *H. contortus* placed in 50% concentration of *Acacia* leaf extract showing peeling (PEL) and indentations (IND) on the cuticle.

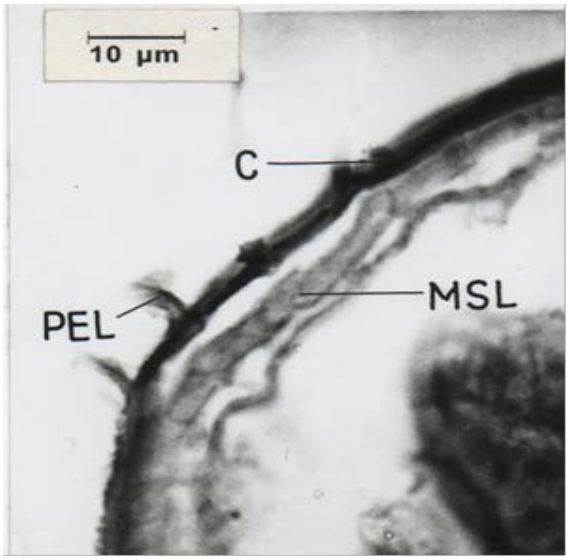

Fig 6: A portion of T.S. of *H. contortus* placed in 50% concentration of *Acacia* bark extract showing peeling (PEL) of the cuticle (C) and detachment of the muscle layer (MSL).

Table 1: Observations on motility and mortality of control and *Acacia nilotica* leaf extract treated *Haemonchus contortus.*

S. No	Concentration of the Neem leaf Extract	Number of worms	Observations on motility score* after the interval of						Total mortality worms/hrs
			4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	14 hrs	
1	10%	6	4	3	2	2	1	0	6/14
2	20%	6	4	3	2	2	1	0	6/14
3	30%	6	3	2	1	1	0	0	6/12
4	40%	6	3	2	1	0	0	0	6/10
5	50%	6	3	1	0	0	0	0	6/8
6	Control	6	5	5	5	5	5	5	-

*Motility Score: Score 5=Vigorous motility; Score 4=Good motility; Score 3=Moderate motility; Score 2=Poor motility; Score 1=Immobile but alive Score 0=Died

Table 2: Observations on motility and mortality of control and *Acacia nilotica* bark extract treated *Haemonchus contortus.*

S. No	Concentration of the Neem leaf Extract	Number of worms	Observations on motility score* after the interval of						Total mortality worms/hrs
			4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	14 hrs	
1	10%	6	4	3	2	2	1	0	6/14
2	20%	6	4	3	2	2	1	0	6/14
3	30%	6	3	3	2	1	0	0	6/12
4	40%	6	3	2	1	0	0	0	6/10
5	50%	6	3	1	0	0	0	0	6/8
6	Control	6	5	5	5	5	5	5	-

*Motility Score: Score 5=Vigorous motility; Score 4=Good motility; Score 3=Moderate motility; Score 2=Poor motility; Score 1=Immobile but alive Score 0=Died

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