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Evaluation of Anthelmintic Effect of Neem (*Azadirachta indica*) Leaves on *Haemonchus contortus* in Goats

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Abstract: A series of tests to examine the utility of Neem leaves as anthelmintic effect on *Haemonchus contortus* in goats was conducted. *In vitro* test revealed that the pure azadirachtin tested at graded level on motility of *Haemonchus contortus* L3 larvae had the maximum anti larval activity at 1000 microgram concentration. The motility of H*aemonchus contortus* L3 larvae was not reduced up to 400 µg. The concentration of azadirachtin content in the Neem leaves was determined to be 0.0244%. In yet another experiment involving twelve weaned nondescript male kids for a duration of 63 days, the efficacy of feeding Neem leaves as an anthelmintic source was studied by infecting with 5000 larvae of *Haemonchus contortus* (L3) on animals fed with or without Neem leaves. It was inferred that feeding Neem leaves reduced the Egg per Gram (EPG) count from 42nd day and worm count was also significantly reduced compared to infected groups fed with complete diet that did not contain Neem leaves. Hence, it is hypothecated that a continuous feeding of Neem leaves for more than 6-9 weeks would result in reducing the EPG count as well as the worm count. It is suggested that at least during the epizootics of worm infection, Neem leaves could play a role as an alternate non-chemical organic source of anthelmintic. Thus Neem can be considered as a potential candidate for organic farming as apart from delivering nutrient and organic source of anthelmintic.

Key words: Neem, *Azadirachta indica*, anthelmintic, *Haemonchus contortus*

INTRODUCTION

Neem (*Azadirachta indica*), a tree in the mahogany family Meliaceae, is native to India, Pakistan and Burma, growing in tropical and semi-tropical regions. The roundworm, *Haemonchus contortus*, is responsible for 80% of worm infections in small ruminants causing heavy economic losses especially to poor farmers in India. Presently, this infection is treated by giving synthetic chemical compounds (anthelmintics), which are costly, causing drug residues in food products. Thus they indirectly affect the health of the human beings. Further, overdependence on these anthelmintics has resulted in drug resistance in animals. To overcome these situations, several plants have been investigated for their anthelmintic properties. Assis *et al.* (2003) observed that maximum inhibition of *Haemonchus contortus* larvae was observed with hexane and methanol extracts of *Spigelia anthelmia*. Significant decrease in faecal egg counts of *Haemonchus contortus* was recorded in Boer goats fed with *Acacia karoo* diets (Kahiya *et al*., 2003). Literature is very scanty about feeding Neem leaves to goats and its effect in reducing the worm load especially *Haemonchus contortus*. Though Costa *et al*. (2006) reported no anthelmintic activity at lower level of feeding Neem leaves to sheep, the neutraceutical

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properties of neem and the level of feeding to derive beneficial effects are yet to be documented. It is in this context, a study was planned to evaluate the anthelmintic efficacy of Neem leaves in goats.

MATERIALS AND METHODS

The experiment involved a series of tests carried out at the Department of Animal Nutrition, Madras Veterinary College, Tamilnadu, India during 2006, to examine the utility of Neem leaves as anthelmintic which include screening azadirachtin for its efficacy against intestinal worms, assessing the concentration of azadirachtin in Neem leaves and evaluating the anthelmintic effect of Neem leaves in complete diet on H*aemonchus contortus* in goats.

Efficacy of Azadirachtin

The efficacy of azadirachtin as anthelmintic was tested against intestinal worms. The abomasal worms, *Haemonchus contortus,* were collected from goats slaughtered at perambur slaughterhouse Chennai, Tamilnadu. The coprocultures of *Haemonchus contortus* were prepared as per Gordon-Whitlock jar method. The culture jars were incubated at 28°C in a dark place for a week (Gathuma *et al*., 2004). The larvae of *Haemonchus contortus* were harvested and aliquoted in 1.5 mL Eppendorf tubes. The larval counts were adjusted to have 100 larvae per 100 µL of distilled water. These larvae were bioassayed in 24 well plates with pure azadirachtin (Sigma, USA A7430) at different concentrations viz., 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg in triplicate. The mortality was observed once in six hours for a period of 24 h. The activity of the larvae was graded as per Boisvenue *et al*. (1983).

Azadirachtin Analysis

The Neem leaves samples collected from six trees were shade dried and ground to pass through 1 mm sieve and the samples were stored for analysis. The azadirachtin content in the Neem leaf samples were analysed as per BIS (1995) and the equipment operating manual for HPLC system used for analyzing the azadirachtin content was EOM/PSP/ACY/-001-00.

Experiment to Evaluate the Anthelmintic Effect of Neem Leaves in Complete Diet on H*aemonchus Contortus* in Goats

A trial was conducted for 63 days in non descript kids to assess the efficacy of feeding Neem leaves as an anthelmintic source. Twelve weaned nondescript male kids (approximately 8-9 months old) were used in the study. The animals were dewormed and dipped for ecto parasites. Four kids were randomly allotted to each of the following three treatments that were fed with complete diet having iso nitrogenous and iso calorific value. The animals were experimentally infected on day one of the experiment with 5000 larvae of *Haemonchus contortus* (L3) cultured as mentioned earlier in the following manner.

Control group (T1)	-	Complete rations without Neem leaves and without infection with *Haemonchus contortus* (L3)
Infected group (T2)	-	Complete rations without Neem leaves and infected with Haemonchus *contortus* (L3)
Infected and Neem leaves fed group (T3)	-	Complete rations with Neem leaves and challenged with *Haemonchus contortus* (L3)

Egg per Gram (EPG) Counts in the Dung

The animals were weighed at fortnightly intervals in an electronic balance to monitor their growth pattern. The EPG was assessed by modified McMaster procedure (Hanson and Perry, 1994) every 7 days from 21st day post infection in dung samples collected per rectum from experimental animals. In this technique, 3 g of dung material was mixed thoroughly with 42 mL of floatation solution

(saturated salt solution in water). This suspension was sieved over a beaker with a 250 micron mesh sieve and the filtrate was agitated and decanted into a 15 mL test tube. The decanted solution was centrifuged at 2000 rpm for 2 min and 0.15 mL of the fluid was removed from the top layer of the test tube and transferred to both chambers of McMaster slide. Number of eggs in etched area of both chambers of the slides was counted and multiplied by 100 to arrive at EPG.

Haemonchus contortus Worms Count in the Abomasum

At the end of ten weeks, the animals were sacrificed to assess the establishment of infection in treated and control group of animals. The worm count was done following the procedure mentioned in the Anonymous (1977). As per this procedure, the abomasums were ligated and removed from the animals. The abomasums was washed thoroughly with stream of water from tap. The mucous membrane being carefully rubbed to remove dry worms adhering to it. The contents of the bowel were passed through a wire mesh screen of aperture of 0.15 mm and washed with stream of water. When all the materials have been washed in this way, the screen was inverted and the worms collected on the screen were washed into a trough which had 4 L of water with 10% formalin. Forty milliliter of this solution were placed in a petridish with worms counted under a dissecting microscope. Total no of worms counted in this sample was multiplied by 100 to get the no of worms present in the abomasums. The worms were also counted manually which were adhering to the contents.

Statistical Analysis

The statistical analysis of the data collected during *in vivo* trial was carried out as per the procedure of Snedecor and Cochran (1989).

RESULTS

Efficacy of Azadirachtin

The efficacy of azadirachtin at different concentrations is given in Table 1. The azadirachtin concentration from 25 to 400 μg had no effect on the motility of L3 larvae of *Haemonchus contortus* even after 24 h. The activity at 500, 600 and 700 μg was also nil though there was some reduction in motility of L3 larvae after 24 h. The azadirachtin concentration at 800 μg had a borderline anti larval activity of L3. At 1000 μg concentration of azadirachtin, there was a reduction in motility up to

Table 1: Efficacy of azadirachtin on the motility of *Haemonchus contortus* L3 larvae in an *in vitro* study

Concentrations (mg)	Motility (%)	Compound activity
25	100.0	0
50	100.0	0
100	100.0	0
200	100.0	0
300	100.0	0
400	100.0	0
500	97.5	0
600	070.0	0
700	055.0	0
800	035.0	1
900	022.0	2
1000	07.5	3

Activity 0 = 50% larvae affected;
Activity 1 = 50-75% larvae affected;
Activity 2 = 76-90% larvae affected;
Activity 3 = 91-97% larvae affected;
Activity 4 = 100% larvae affected

Table 2: Evaluation of anthelmintic efficacy of Neem leaves in goats (Mean±SE)

Particulars	Control	Control challenged	Neem challenged
Initial body weight (kg)	14.47±1.39	14.53±0.88	14.37±1.16
Final body weight (kg)	17.20±1.35	15.97±0.89	16.00±1.23
Average daily Gain (g)	43.33^{a}±1.32	22.86^{b}±.41	25.87^{b}±1.35
DMI as% body weight	3.06±0.18	2.99±0.06	3.08±0.07
Azadirachtin intake (mg)	-	-	57.61±2.89
Average egg per gram count of *Haemonchus contortus* in the dung of goats			
21st day	0^{a}	375^{b}±85.39	275^{b}±47.87
28th day	0^{a}	500^{b}±81.65	450^{b}±86.60
35th day	0^{a}	750^{b}±64.55	600^{b}±70.71
42nd day	0^{a}	1150^{b}±119.02	775^{c}±62.92
49th day	0^{a}	1425^{b}±75.00	975^{c}±110.87
56th day	0^{a}	1975^{b}±149.30	1400^{c}±147.20
63rd day	0^{a}	3200^{b}±91.29	2700^{c}±135.40
Average *Haemonchus contortus* worm count in goats on 63rd day (after slaughter)			
63rd day (after slaughter)	0^{a}	262.25^{b}±15.46	139.25^{c}±12.16

Each value is a mean of four observations, Values in rows with different superscripts vary significantly ($p<0.01$)

92.5% at 24 h suggesting that azadirachtin was able to reduce motility at increased concentrations and it had an activity level 3 while hundred percent of the larvae in the non-treated well (control) were motile.

Azadirachtin Analysis

The concentration of azadirachtin content in the Neem leaves were 0.0244%.

Experiment to evaluate the anthelmintic effect of Neem leaves in complete diet on *Haemonchus contortus* in goats

The performance of kids in T1, T2 and T3 are given in Table 2. The Average Daily Gain (ADG) of the three groups was 43.33, 22.86 and 25.87 g, respectively. The ADG of kids in T1 was significantly higher ($p<0.01$) compared to T2 and T3. Though the Neem leaves fed groups (T3) had a higher ADG of 3 g compared to control challenged group, it was not statistically significant.

Egg per Gram Counts in the Dung

Average EPG count of *Haemonchus contortus* in the dung of goats experimentally infected with *Haemonchus contortus* L3 larvae or with out infection in a trial conducted to evaluate the anthelmintic effect of Neem leaves in complete diet for ten weeks is presented in Table 2. The EPG count between T2 and T3 were not statistically significant at 21st, 28th and 35th day post infection where as it was highly significant ($p<0.01$) from 42nd day onwards.

***Haemonchus contortus* Worms Count in the Abomasum**

Neem leaves feeding was effective in lowering the worm count in the abomasums from 262.25 to 139.25 numbers (Table 2).

DISCUSSION

Efficacy of Azadirachtin

Similar to 1000 μg concentration of azadirachtin, inhibition of motility of *Haemonchus contortus* larvae was observed by Assis *et al*. (2003) at higher concentrations of plant extracts of hexane and methanol indicating the relevance of this screening test in judging anthelmintic property.

Azadirachtin Analysis

At the rate of 0.0244% of azadirachtin, about 4 g of Neem leaves is required to provide 1000 μg of azadirachtin.

Experiment to Evaluate the Anthelmintic Effect of Neem Leaves in Complete Diet on *Haemonchus contortus* in Goats

The ADG was significantly reduced in T2 and T3 compared to T1. Contrary to the results of the current study, Niezen *et al*. (1998) observed no such reduction in daily gain in lambs grazing *Lotus pedunculatus* and *Hedysarum coronarium* plants though there was a slight gain of 3 g in T3 compared to T2. Kukde *et al*. (1999) recorded an increase in total feed intake when Neem leaves were fed to calves in contrast to the present study where there was similar feed intake among the three treatment groups.

Egg per Gram Counts in the Dung

Similar results of highly significant ($p<0.01$) reduction in the EPG count in lambs fed Neem leaves were reported by Arunachal *et al*. (2002). However, Costa *et al*. (2006) reported no anthelmintic activity while feeding the Neem leaves for three months to sheep. While Niezen *et al*. (1998) observed reduction in EPG count of *Trichostrongylus* species by Sulla feeding in ewe lambs, Hordegen *et al*. (2003) observed cent percent reduction in egg counts of *Haemonchus contortus* with the seeds of Neem. Kahiya *et al*. (2003) also observed similar decrease in EPG counts feeding *Acacia karoo* diets. However, Pietrosemoli *et al*. (1999) did not find any difference in EPG count by feeding Neem leaves up to 40% level as blocks to calves.

***Haemonchus contortus* Worms Count in the Abomasum**

Similar to the effectiveness of Neem leaves in lowering the worm count Niezen *et al*. (1998) and Hordegen *et al*. (2003) also reported reductions in worm burden while feeding Sulla and seeds of Neem respectively. Kahiya *et al*. (2003) also reported worm count reduction more with *Acacia karoo* diets (34%) than with *Acacia nilotica* diets (10%). Though the results are promising, the exact mechanism of action as to how the larval activity and worm count is reduced has to be explored. Further studies have to be performed to ascertain the exact mode of action of the inhibitory effect of azadirachtin on L3 larvae of *Haemonchus contortus*.

The anthelmintic resistance in *Haemonchus contortus* is an emerging problem throughout the world. Hence the need for alternative medicines is ever increasing. An effort was made to study the anthelmintic efficacy of Neem leaves in addition to being used as an organic source of feed to goats so that the farmers can derive the double benefit of Neem leaves being used as a feed source as well as an organic anthelmintic.

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