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In vitro Anthelmintic Activity of Neem Plant (*Azadirachta indica*) Extract Against Third-Stage *Haemonchus contortus* Larvae from Goats

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Abstract: The study was conducted to evaluate the *in-vitro* anthelmintic activity of Neem plant (*Azadirachta indica*) extract against third-stage *Haemonchus contortus* larvae from goats. Larvae were tested with different concentrations of methanolic extract. The experiment was repeated twice and results indicated that Neem extract was effective in killing larvae. It was recorded that 4 mg/ml extract gave 40% mortality, the highest percentage of mortalities among all used concentrations after 24 hr treatment.

Key words: Anthelmintic activity · Neem plant · Haemonchus contortus · Goats

INTRODUCTION

Gastrointestinal parasites can cause devastating effects on sheep and goats. There are several important trichostrongylid nematodes which include *Haemonchus contortus, Ostertagia* and *Trichostrongylus spp. Haemonchus contortus* is considered the most prevalent and economically devastating species thriving in warm and humid areas [1]. Several thousand adult females can be found in abomasa of small ruminants and each female can lay up to 10,000 eggs per day [2]. Heavy burdens of trichostrongylid nematodes can lead to anaemia, edema, haemorrhage, traumatic injury to the intestinal epithelium and even death. In addition to losses through mortality, major losses are attributed to reduce feed efficiency, lowered production of meat and labor and drugs associated with control [3]. Due to the economic effects of nematode parasites, it is necessary to have adequate methods of control.

Helminthes control in domestic animals is widely based on the use of anthelminthic drugs [4]. However, the current efficacy of these drugs had been reduced because of resistant nematode strains [5]. The high costs of drugs have awakened an interest in medicinal plants as an alternatives source of anthelminthic drugs [6].

The alternatives to chemotherapy that are used in the control of helminthosis of sheep and goats, that are appropriate to Malaysian conditions, include pasture rotation [7], breeding of selected sheep and goats with natural resistance to nematodes [8], biological control [9, 10] and use of medicated blocks [11].

Exploitation of the possible anthelmintic potentials of medicinal plants such as Neem (*Azadirachta indica*) is also an area of active research [12]. *Azadirachta indica* or Neem plant is a member of the Meliaceae family [13]. The plant is believed to be originated in Assam and Myanmar of South Asia, but other reports suggested various areas of Pakistan, Sri Lanka, Thailand, Malaysia and Indonesia [14]. It is purported to have multiple medicinal applications, including its use as an anti-inflammatory, antipyretic, analgesic, immuno-stimulant, hypoglycemic, anti-fungal and anti bacterial [15, 16]. The Neem had been reported to contain several biologically active constituents such as azadirachtin [17], meliantriol [18] and salanin [19]. Azadirachtin has been demonstrated to inhibit 68% of *H. contortus* eggs from hatching [20].

Goats showed a reduction in faecal egg counts and larval recoveries after daily feeding of fresh Neem leaves [10, 12, 21]. The present paper describes some *in-vitro* anthelmintic activity of *A. indica* against *Haemonchus contortus* third stage larvae from goats.

MATERIALS AND METHODS

Preparation of Plant Extract: Fresh leaf samples of *A. indica* were obtained from Neem trees at the grounds of Universiti Sains Malaysia, Penang, Malaysia. The fresh leaves were crushed manually to fine particles. Extraction was carried out in a 1000ml 1L flask where the crushed leaves were stirred with 500ml of methanol for 1 hour at 60°C. After leaving the methanolic extract to cool, it was filtered through Whatman No.40 filter paper. The filtrate was then freeze-dried overnight.

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Culturing of Third-Stage Worm Larvae: Faecal samples of goats were collected from a goat farm in Penang, Malaysia. Fresh droppings were collected from the rectum and cultured in vermiculites. After being incubated at room temperature (26°C) for 10 days, third stage infective larvae were recovered from the cultures. One hundred larvae taken at random from the culture tubes were identified according to the descriptions of Dikmans and Andrews [22] and Gordon [23].

Anthelmintic Activity Against *H.contortus* Larvae: Neem extract concentrations of 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml and 5 mg/ml were reconstituted in distilled water and its anthelmintic activity was determined in a 96-well micro-titre plate in 3 replicates for each concentration. Five *H. contortus* larvae of similar size were placed in each well and the prepared concentrations were then poured into each well.

Mortality of the worms was recorded at 24, 48 and 72 hr after exposure to the extract. Two trials were carried out for each observation and mean values were recorded. The trials had been carried out in 3 replicates with 5 different concentrations and controls.

Statistical Analysis: Statistical analyses were performed with the Statistical Programs for the Social Sciences (SPSS) version 11.5. Data satisfied the assumptions of the general linear model and were not transformed. Statistical significance of data was assessed by analysis of variance (ANOVA). When ANOVA indicated significant effects ($p<0.05$), the Tukey test was used to compare means. Pearson correlation coefficient was also done to describe the relationship between the percentages of larval mortality with extract concentrations.

RESULTS

Tables 1 to 3 showed the percentages of mortality of larvae after being treated with different concentrations of Neem extract and observed within the period of 24, 48 and 72 hr. The percentage of mortality was significant with extract concentrations as well as the time of observation. Larvae treated with different quantities of extract showed that there was a substantial mortality in the larvae after 24 hr (Table 1). It was recorded that 4 mg/ml extract gave 40% mortality, the highest percentage of mortality among all concentrations after 24 hr treatment.

Table 1: Mortality rate of larvae treated with different concentrations of *Azadirachta indica* extract after 24 hr

	Mortality of larvae after 24 hr						
	Replicates						
Extract concentration (mg/ml)	1	2	3	Total	(%)	Mean	SE (±)
0	0	0	0	0	0	0.00	0.00
1	2	0	2	4	27	1.33	0.67
2	2	0	2	4	27	1.33	0.67
3	2	0	1	3	20	1.00	0.58
4	2	1	3	6	40	2.00	0.58
5	1	0	2	3	20	1.00	0.58

Table 2: Mortality rate of larvae treated with different concentrations of *Azadirachta indica* extract after 48 hr

	Mortality of larvae after 48 hr						
	Replicates						
Extract concentration (mg/ml)	1	2	3	Total	(%)	Mean	SE (±)
0	0	0	0	0	0	0.00	0
1	3	0	2	5	33	1.67	0.88
2	2	0	3	5	33	1.67	0.88
3	2	0	2	4	27	1.33	0.67
4	3	2	4	9	60	3.00	0.58
5	4	2	2	8	53	2.67	0.67

Table 3: Mortality rate of larvae treated with different concentrations of *Azadirachta indica* extract after 72 hr

	Mortality of larvae after 72 hr						
	Replicate						
Extract concentration (mg/ml)	1	2	3	Total	(%)	Mean	SE (±)
0	0	0	0	0	0	0.00	0
1	4	1	2	7	47	2.33	0.88
2	2	1	4	7	47	2.33	0.88
3	2	3	4	9	60	3.00	0.57
4	4	4	5	13	87	4.33	0.33
5	4	4	2	10	67	3.33	0.67

Table 4: Effect of exposure to extracts of *Azadirachta indica* on the mortality of larvae during the 24, 48 and 72 hr of treatment

	Mortality of *H.contortus* larvae (Mean±SD)		
Extract concentrations (mg/ml)	24 hr	48 hr	72 hr
0	0a	0a	0a
1	1.33±0.67ab	1.67±0.88ab	2.33±0.88ab
2	1.33±0.67ab	1.67±0.88ab	2.33±0.88ab
3	1.00±0.58ab	1.33±0.67ab	3.00±0.57ab
4	2.00±0.58b	3.00±0.58b	4.33±0.33b
5	1.00±0.58ab	2.67±0.67ab	3.33±0.67ab

Means followed by a common letter do not differ significantly (Tukey's test, $p<0.05$).

Table 5: Pearson product-moment correlation coefficient describing the relationship between 2 continuous variables

		Concentration (mg/ml)	% of mortality
Concentration (mg/ml)	Pearson Correlation	1	.651(**)
	Sig. (1-tailed)	.	.002
	N	18	18
% of mortality	Pearson Correlation	.651(**)	1
	Sig. (1-tailed)	.002	.
	N	18	18

** Correlation is significant at the 0.01 level (1-tailed).

After 48 hr treatment, 4 mg/ml still recorded the highest mortality of 60%, whereas a concentration of 5 mg/ml recorded 53% mortality. Concentrations of 1 mg/ml, 2 mg/ml and 3 mg/ml, recorded mortalities of 33%, 33% and 27% respectively (Table 2).

An extract concentration of 4 mg/ml again recorded the highest percentage of mortality which is 87% after 72 hr treatment (Table 3). No larval mortality was observed for the control.

Mortality of larvae treated with Neem extract of 4mg/ml, was significantly more than that in the control. Other concentrations also had more larval mortality but not significantly different from control (Table 4).

There was a significant positive relationship exists between the concentrations and percentage of larval mortality ($r = 0.651$, $p<0.05$)(Table 5).

DISCUSSION

The results of the present study indicated that plant extract of *A. indica* could result in the mortality of the L_3 *H. contortus* of goats. The percentage of mortality gave an indication of extract effectiveness in killing *H.contortus* larvae although none of the concentrations caused 100% mortality. The same holds true for other trichostrongylid species as well.

The trials have been carried out in 3 replicates with 5 different concentrations and a control. The higher the concentration was, the darker the aqueous solution would be, making it difficult sometimes to count larvae. Therefore, the choice of the highest concentration in this research was based on whether the larvae could be seen or not after the extract concentrations were poured into the 96-wells plate. The mortality of larvae treated with Neem extract of 4 mg/ml, was significantly more than that in the control. Other concentrations also had more larval mortality but not significantly different from control (Table 4). Table 1 showed that there was a distinct difference in the mortality of larvae treated with Neem extract. The concentrations of 1 mg/ml and 2 mg/ml chalked similar levels of mortality. Both concentrations recorded 27% mortalities. This was similarly observed with 3 mg/ml and 5mg/ml as well after 24 hr of treatment which recorded 20% mortalities.

The current research demonstrated the potential of *A. indica* in the control of *H.contortus* larvae. Previous studies have shown that extracts of some of the plant parts possess insecticidal effects. Studies in the Philippines, India and Malaysia reported the activity of *A. indica* against nematode parasites in ruminants [10, 24, 25]. Similarly, cattle provided with feed blocks containing different levels of dried leaves of *A. indica* had significantly lower EPG than untreated control animals; 90 days post treatment [26].

In other studies, the antihelminthic effect of *Halothamnus somalensis* on gastrointestinal parasites of goats showed a 50% reduction in EPG which was similar to the 52% reduction in EPG count reported for the water extract of *Albizia gummifera* in Ethiopia [27].

Thus further research needs to be conducted before *A. indica* could be safely and effectively used in controlling helminthes in ruminants. Studies to determine the mode of action of *A. indica* on the parasites would be helpful.

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