IN-VITRO ANTHELMINTIC EFFICACY OF CRUDE AQUEOUS EXTRACTS OF NEEM (*Azadirachta indica*) LEAF, STEM AND ROOT ON NEMATODE

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ABSTRACT

The anthelmintic efficacy of the aqueous extracts of neem (Azadirachta indica) leaf and stem and root barks against the hatching of eggs and the survival of larvae of nematode parasites of small ruminants were studied. The results of the in vitro egg hatch assay showed that the aqueous extracts of the leaf and stem bark produced significant anthelmintic effect through reduction in nematode egg hatch. The reduction in egg hatch was concentration dependent being highest (51 % and 50 % for the leaf and stem bark extracts respectively) at the highest concentration (100 mg/ml) of the extracts but inferior to those produced by albendazole (100 % at 40 mg/ml). Aqueous extracts of the leaf and root bark produced significant reduction in larval survival within 60 minutes at ambient temperature (30 – 35 ºC). Larval death was similar in both extracts and concentration dependent, increasing with increasing concentration of the leaf and root bark extracts. The reduction in larval survival due to the extracts was similar to that produced by albendazole. In general, the aqueous extract of neem leaf was more efficacious in limiting nematode larvae survival and in-vitro egg hatch. The results confirm the folkloric claims that neem has anthelmintic effect and thus suggest its possible usefulness as an anthelmintic.

Keywords: Anthelmintic efficacy, Aqueous extract, Leaf, Stem, Root, Bark, *Azadirachta indica*

INTRODUCTION

Gastrointestinal helminthiasis, especially parasitic gastroenteritis (PGE) constitutes a major set back in the productivity of small ruminant livestock in Nigeria and other tropical countries (Schillhorn van Veen, 1973; Akerejola *et al.,* 1979; Chiejina, 1987; Nwosu *et al.,* 1996 ab). The control of PGE is usually achieved through anthelmintic medication and grazing management in cattle (Chiejina and Emehelu, 1986; Chiejina, 1987) and goats (Nwosu *et al.,* 1996b). However, extensive use of anthelmintics has resulted in drug resistance for many nematodes of sheep, goats and cattle (Jackson, 1993; Pritchard, 1994). Since newer anthelmintics are not being brought into the market, there has been increasing search for novel, environmentally friendly and more sustainable drugs for control of helminthosis. Consequently, several plants traditionally said to have medicinal properties are being investigated for their potency. The plant, Azadirachta indica commonly known as neem is a good candidate for such investigation.

All parts of the plant including the leaves, bark, fruits, seed, oil and sap have been shown to have medicinal properties and contain over ten different active components with azadirachtin as the most potent and widely studied (Nwosu, 2001). Neem is very popular in traditional medicine and neem-derived medicinal preparations have been shown to be efficacious against a wide range of animal diseases including bacterial (ITDG and IIRR, 1996), protozoal and other parasitic conditions (Ekanem, 1978; Ivbijaro, 1987; Khalid *et al.,* 1989; Dwivedi, 1999), promote cutaneous wound healing following mange infestation as well as act as fly-repellent against haematophagous insects (ITDG and IIRR, 1996; Nwosu, 2001). However, it is evident that only a small proportion of the possible medicinal usefulness of the plant in veterinary practice has been exploited (Nwosu, 2001). In this paper, we evaluated the anthelmintic efficacy of aqueous extracts of neem leaf, root and stem barks against in-vitro hatching of eggs and the survival of the larvae of nematode parasites of small ruminants.

MATERIALS AND METHODS

Neem: Fresh leaves, stem and root barks of neem were collected from adult plant within the University of Maiduguri, Nigeria. A botanist in the Department of Biological Sciences, University of Maiduguri, Nigeria, where voucher specimen of the plant was deposited, confirmed the identity of the plant. The stem and root barks were pealed off the plant using a sharp knife while the leaves were hand-cut. The samples were collected into polythene bags and transported to the laboratory for processing.

The neem leaves, stem and root barks were sun-fried for 10 days at 8 hours per day. They were separately ground into powder using a pestle and mortar. The active components were then exhaustively soxhlet extracted using the aqueous method (Mittal *et al.,* 1981; WHO, 1992). The extracts were concentrated in a conical flask maintained overnight at 60˚C.

Table 1: *In-vitro* anthelmintic efficacy of crude aqueous stem bark and leaf extracts of neem (*Azadiracta indica*) against strongylid nematodes of small ruminants

Extract/Drug concentration	No. of samples with egg hatch*	No. of larvae hatched Mean ± S.D.	Range	Reduction in egg hatch (%)
		Water control		
Stem bark extract	50	98 ± 47	23 - 283	0**
100 mg/ml	50	49 ± 28	9 - 133	50.0b
50 mg/ml	50	56 ± 30	11 - 146	42.9b
25 mg/ml	50	67 ± 38	17 - 156	31.6c
		Leaf extract		
100 mg/ml	50	48 ± 21	23 - 102	51.0b
50 mg/ml	50	60 ± 32	18 - 167	38.8b
25 mg/ml	50	74 ± 40	36 - 198	24.5c
		Albendazole		
40 mg/ml	0	0	0	100a
20 mg/ml	25	2 ± 1	1 - 4	98.0a
15 mg/ml	43	3 ± 2	1 - 8	96.9a

**Total number of samples tested = 50; **Larval recovery from water control cultures was used as standard (i.e. 0 % reduction in egg hatch); abc Figures in same column with different superscripts are significantly different (P < 0.05).*

Table 2: Survival of infective nematode larvae following incubation in albendazole or neem leaf and root bark extracts for 60 minutes at room temperature

Extract/Drug concentration	Number of surviving larvae Mean ± S.D.	Range	Percent larval death after 60 minutes
Water control	246 ± 101	117 - 608	0*
	Leaf extract		
100 mg/ml	7 ± 7	1 - 34	97.2a
50 mg/ml	12 ± 8	2 - 38	95.1a
25 mg/ml	16 ± 8	4 - 45	93.5a
	Root bark extract		
100 mg/ml	10 ± 7	2 - 30	95.9a
50 mg/ml	14 ± 8	2 - 38	94.8a
25 mg/ml	18 ± 9	2 - 48	92.7a
	Albendazole		
25 mg/ml	10 ± 7	2 – 40	95.9a
12.5 mg/ml	15 ± 7	6 - 47	93.9a
- 6.25 mg/ml	19 ± 8	10 - 54	92.9a

**Larval survival in water (control) was used as standard (i.e. 0% larval death; abc Figures in same column with different superscripts are significantly different (P<0.05)*

Each extract was diluted in three concentrations (25, 50 and 100 mg/ml). The in-vitro anthelmintic efficacy of the various concentrations of the leaf and stem bark extracts was evaluated against the hatching of nematode eggs using the egg hatch assay (Kelly et al., 1981) while the survival of infective larvae in various concentrations of the leaf and root bark was evaluated by culturing a known number of larvae in the solutions for 60 minutes. In all cases, the proportion of unhatched eggs or dead larvae, at each concentration of the extracts was calculated by relating the number of hatched eggs or surviving larvae to the total number of eggs or larvae cultured (Chiejina, 1984).

Faecal Samples: Faecal samples were collected directly from the rectum of trade sheep and goats during slaughter at the Maiduguri Metropolitan Abattoir. Faecal egg counts were determined by the modified McMaster technique using saturated sodium chloride solution as the floating medium (MAFF, 1977). Only samples with counts of at least 500 eggs per gram of faeces were used in the test. Faecal culture and larval recovery were done using the test tube filter paper method described by Harada and Mori (1955). Nematode eggs and larvae were identified using standard parasitological criteria (MAFF, 1977; Soulsby, 1982).

Albendazole: Albendazole, containing 250 mg of Albendazole B. P. (Sam Pharmaceutical, Nigeria Limited) was used for the study. Three dilutions (25, 12.5 and 6.25 mg/ml) of the drug were used for the study based on previous studies (Onyeyili *et al.,* 2001 ab; Nwosu *et al.,* 2001, 2004).

Data Analysis: The results were summarized as means + Standard Error while differences between the means were analysed at the 5 % level of significance using the one way analysis of variance (ANOVA) (GraphPad Instat, 2000).

RESULTS

The results of the egg hatch assay using the aqueous extract of neem leaf and stem bark are presented in Table 1. Compared to the control (water cultures), both the leaf and stem bark extracts showed significant reduction in nematode egg hatch. In both cases, the reduction in egg hatch was concentration dependent with the greatest reduction in egg hatch at the highest concentrations (100 mg/ml) of the

extracts used in the study. The reduction in egg hatch was similar ($P > 0.05$) with the leaf and stem bark extracts at the various concentrations tested but these were significantly less ($P < 0.05$) effective than albendazole in limiting egg hatch.

The survival of strongyle larvae cultured for 60 minutes in water or various concentrations of albendazole and the aqueous extracts of the leaf and root bark of neem are presented in Table 2. The results showed that both the leaf and root bark extracts caused larval death in a similar manner ($P > 0.05$). Larval survival in both extracts was also concentration dependent, decreasing with increasing concentration of the extracts. The reduction in larval survival caused by the leaf and root bark extracts was similar ($P > 0.05$) to that produced by albendazole at the concentrations used in the study.

DISCUSSION

The results of the study revealed that neem leaf, root and stem bark extracts have some anthelmintic properties against strongylid nematodes of sheep and goats since they significantly limited the hatching of nematode eggs and the survival of nematode larvae cultured in them. These findings confirm the folkloric claims regarding the anthelmintic efficacy of neem against intestinal helminths. Previous studies have shown that neem extracts similarly affected the survival of salmonella (ITDG and IIRR, 1996) Plasmodium and *Trypanosoma* species (Ekanem, 1978; Ivbijaro, 1987; Khalid *et al.,* 1989). That the leaf, stem or root bark extract showed similar effects in reducing egg hatch or larval survival suggest that the extracts contain similar active components possibly in similar concentrations and probably possess the same mechanisms of action. These observations suggest the possible usefulness of the aqueous extracts of neem leaf, stem and root barks in the treatment of nematode infections in both man and domesticated livestock.

At the various concentrations used in this study, the efficacy of the extracts was concentration-dependent as they showed graded effect that was highest at the 100 mg/ml concentration. However, at this concentration (100 mg/ml), the effects of the extracts were significantly ($P < 0.05$) inferior to albendazole in preventing nematode egg hatch but similar in preventing nematode larval survival. The efficacy of the extracts or drug in limiting egg hatch has a direct bearing on the effective penetration of their active components through the eggshell to reach the larvae. This may explain the greater efficacy of the extracts and the drug in limiting larval survival than egg hatch at the various concentrations tested during the study.

Previous studies suggested that the anti-trypanosomal activity of neem extracts was associated with the alkaloids or other ingredients and had effect in-vitro but became degraded or metabolised and thus ineffective when introduced into the host animal (Dwivedi, 1999). Consequently, there is need for further studies, especially in-vivo studies, in order to confirm the present observations as well as purify the extract, determine the active components, their lethal dose, appropriate route of administration as well as ascertain which particular parasite species and/or developmental stage are most susceptible to the effect of the extracts so as to further enhance their anthelmintic usefulness. When these studies have been carried out, a new anthelmintic that will be readily available and acceptable to the rural farmers may be produced. Meanwhile, this study highlights the possible anthelmintic usefulness of neem extracts in the control of nematode infections of small ruminants.

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