J. Bangladesh Agril. Univ. 6(1): 87–92, 2008 ISSN 1810-3030

Effects of neem (*Azadirachta indica*) leaves against gastrointestinal nematodes in cattle

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Abstract

The anthelmintic efficacy of neem (*Azadirachta indica*) leaves was evaluated on 12 cross bred cattle (aged between 2-3 years) naturally infected with gastrointestinal nematodes at Bangladesh Agricultural University Dairy farm, Mymensingh during the period of 28 days from 1st to 28th June' 2005. The 12 selected cattle were randomly divided into two groups (A & B), each consisting of six animals, of which each animal of group A was treated with a single orally administered water extract of neem leaves @ 200mg/kg bwt, whereas animals of group B served as untreated control. Egg per gram (EPG) of feces, body weight, certain hemato-biochemical parameters were examined before the treatment and on 3rd, 10th, 17th, and 28th day post treatment. A significant ($P<0.01$) reduction of EPG was recorded in treated group A (48.05-65.7%) but it significantly (P0.01) increased the EPG in untreated cattle. The body weight was significantly increased ($P<0.05$) in treated and decreased in ($P<0.05$) in untreated groups of cattle. The TEC, Hb, PCV and Neutrophil counts increased significantly ($P<0.01$) whereas the TLC and eosinophil count decreased significantly ($P<0.01$) in treated cattle. No significant change was observed in ALT and AST values between treated and untreated groups of animals. The results of this study indicates that a single oral dose of neem extract leaves could reduce 48.05 to 65.77% EPG of gastrointestinal nematode in cattle.

Keywords: Neem, Gastrointestinal nematodes, Hematological, Biochemical, Cattle

Introduction

Parasitism is an important limiting factor that responsible for deteriorating the health and productivity of livestock. The agro-ecological and geo-climatic conditions of Bangladesh are highly favorable for the growth and multiplication of parasites. As a result about 50% apparently healthy cattle population has been demonstrated to be affected with different species of parasites (Garrels, 1975). Among the parasitic diseases, gastrointestinal nematodes such as *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., *Oesophagostomum* spp., *Trichuris* spp. and *Strongyloides* spp. are most common in Bangladesh (Qadir, 1981; Rahman and Mondal, 1983). The greatest losses associated with nematode infections are sub-clinical, and economic assessments have showed that financial costs of internal parasitism are enormous (Preston and Allonby, 1979; McLeod, 1995). Control of parasitic diseases has been mainly based on regular anthelmintic treatment in Bangladesh. However, as these are very expensive and unavailable to farmers in rural areas, livestock producers are not interested to use these anthelmintics. Furthermore, some serious disadvantages of using those anthelmintics, notably the development of resistance to helminth parasites (Waller and Prichard, 1985; Lans and Brown, 1998) against various anthelmintic compounds and classes, as well as their residues and toxicity problems (Kaemmerer and Butenkotter, 1973) poses hazards to livestock development. Medicinal plants are one of the most important natural resources of a country. World Health Organization (WHO, 1993) has recognized the necessity for investigation and mobilization of ancient medicinal practices to fulfill the primary health care systems of the man and animals, and realizes that the traditional system of medicine may play an important role in the development of livestock of the third world countries. Plant remedies were also extensively used as anthelmintics in the developed world before the era of broad spectrum synthetic

drugs. Many currently available therapeutic compounds are plant derived and/or synthetic analogues derived from those compounds (Farnsworth *et al.*, 1985). For these reasons, anthelmintic efficacy of some medicinal plants, especially neem (*Azadirachta indica*) seeds and leaves has been evaluated against gastro-intestinal nematodes of ruminants in Bangladesh (Ahmed *et al.,* 1994, Mostafa *et al.,* 1995, Rob, *et al.,* 2004; Khalid *et al.*, 2005). The medicinal plants for their anthelmintic activity have remained of great scientific interest despite extensive use of synthetic chemicals in modern clinical practices all over the world (Akhtar *et al.*, 2000). *Azadirachta indica* locally known as neem an important multipurpose plant. The various uses of the products of this tree have fascinated agriculturists and chemists and known to have parasiticide activities against different worms (Mostofa *et al.*, 1995). In continuation to the earlier works, this paper describes the the efficacy of neem leaves against gastrointestinal nematodes and its effects on certain hemato-biochemical parameters and clinical parameter (body weight) were also determined in this study.

Materials and Methods

The experiment was performed in the Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh. Bangladesh Agricultural University Dairy Farm was selected as the site for this study. The research was carried out during the period between 1st June, 2005 to 28th June, 2005. Thirty cattle (2-3 years approximately) were selected for this study which were suspected to be suffering from natural gastrointestinal nematodes infection in cattle and they were marked at the ears by the numbered tag. Direct microscopic examination of fecal samples for gastrointestinal nematodes by floatation method (Rahman *et al.*, 1996) were carried out over a week prior to commencement of treatment. On the basis of fecal sample examination results, 12 cattle of both sexes infected with gastrointestinal nematodes were selected for this study and randomly divided into two groups, each group consisting of six (6) cattle.

Group A: Treated with a single oral dose of water extract of neem leaves @100mg/kg body weight

Group B: Used as untreated control group

The fecal sample from both groups were examined by egg counting McMaster method as described by Soulsby (1986) before treatment (day 0) and at 3rd, 10th, 17th and 28th day of post-treatment. Egg per gram (EPG) of feces were recorded. Blood samples were collected from the jugular vein of each cattle at different time intervals mentioned above. Various hematological parameters Total Erythrocyte counts (TEC), hemoglobin (Hb), packed cell volume (PCV), Total Lekocute counts (TLC) and differential leukocyte counts (DLC) were measured following the standard procedures. Biochemical (ALT and AST) parameters were also examined by auto-analyzer (Reflotron® Plus) according to the method described by Deneke and Rittersdorf (1984 and 1985). To determine the body weight gain or loss of neem leaves treated and untreated control groups, the body weight was taken on day 0 (pretreatment) and on 3rd, 10th, 17th and 28th day of experimental period of cattle (Samad, 2001). Collected data were statistically analyzed between normal and treated values by Student's t-test by using the computer statistical package programme of Microsoft Excel.

Results and Discussion

The effects of neem leaves on fecal egg count and body weight in the cattle are shown in Table 1. Neem leaves significantly ($p<0.01$) reduced of EPG count in cattle of group A. The rate of reduction of fecal EPG count on 3rd, 10th, 17th and 28th day post-treatment with neem were 62.33%, 65.77%, 56.70% and 48.05%, respectively of gastrointestinal nematodes in group A. Whereas, the EPG count of untreated control group (B) were significantly ($p<0.01$) increased up to last day of experimental period. The EPG count of untreated control group (B) were significantly ($p<0.01$) increased about 4.76%, 15.33%, 28.57% and 42.86% on 3rd, 10th, 17th and 28th day, respectively. In conformity to the present findings, Khalid *et al.* (2005) reported that neem (10% water extract of leaves) reduced significantly ($p<0.01$) EPG count 47.03%, 46.27%, 41.82% and 37.60% on 7th, 14th, 21st and 28th day, respectively in sheep. Rob *et al.* (2004) observed that water extracts of neem was 53.72% effective against hemonchosis in sheep. Brelin (2002) found that fresh neem leaves significantly reduced *H. contortus* in the abomasum of the treated sheep. Arunachal *et al.* (2002) noted that neem leaves, seeds and bark were 53%, 49% and 38% infective against gastrointestinal helminths in sheep, respectively. Rahman (2002) found the effects of water extract of neem leaves was 62% in goat.

Table 1. Changes in fecal egg count of gastrointestinal nematodes and body weight of cattle at different intervals after treatment with neem leaves

Days after treatment	Eggs per gram feces [EPG increase/decrease (%)]	Body weight (kg)
Day 0		
Neem treated	1411±205.25	124.42 ±4.40
Untreated control	1050±269.81	125.50 ±2.65
Day 3		
Neem treated	533**±86.82 (+62.23)	124.75* ±4.41
Untreated control	1100**±279.91 (-4.76)	125.25* ±2.50
Day 10		
Neem treated	483**±80.86 (+65.77)	125.33* ±4.26
Untreated control	1211**±276.99 (-15.33)	123.83* ±3.24
Day 17		
Neem treated	611**±118.85 (+56.70)	126.00* ±4.37
Untreated control	1350**±236.20 (-28.57)	123.17* ±2.98
Day 28		
Neem treated	733**±119.07 (+48.05)	127.20* ±4.18
Untreated control	1500**±237.67 (-42.86)	122.92* ±2.63

The above values represent the mean ± standard deviation (SD) of 6 cattle ** = Significant at 1 per cent level ($p<0.01$) * = Significant at 5 per cent level ($p<0.05$). '+' = Decrease & '-' = Increase

Neem leaves significantly ($p<0.05$) increased body weight in group A. On the other hand, body weight was decreased significantly ($p<0.05$) in untreated controlled cattle of group B. Similarly, Khalid *et al.* (2005) reported that body weight was increased significantly in neem leaves treated sheep. On the other hand, body weight was decreased in untreated sheep. Hossain *et al.* (1996) also observed neem leaves and neem seed kernels increased body weight of cattle. These results were agreeable with the findings of Ahmed *et al.* (1994) in sheep. The body weight was increased might be due to removal of parasitic load which facilitate the weight regain through proper digestion, absorption and metabolism of feed nutrients in the parasite free gastrointestinal tract.

The results of the effect of neem leaves on hematological and biochemical parameters in cattle are shown in Table 2. After treatment with neem leaves, total erythrocyte count (TEC), hemoglobin (Hb) content and packed cell volume (PCV) were gradually increased significantly ($p<0.01$) at 3rd, 10th, 17th and 28th day post-treatment in cattle. In untreated infected group, TEC and Hb content are decreased significantly ($p<0.01$) from day 3 to day 28. PCV were decreased significantly ($p<0.01$ and $p<0.05$) in untreated control group. Conversely, the total leukocyte count (TLC) were decreased significantly ($p<0.01$) in neem leaves treated cattle. TLC were increased significantly ($p<0.01$) in untreated control group. After treatment with neem leaves, neutrophil count of differential leukocyte count (DLC), were gradually increased significantly ($p<0.01$) in cattle. On the other hand, eosinophil count in differential leukocyte count (DLC) were decreased significantly ($p<0.01$ and $p<0.05$) in treated cattle. Neem leaves caused very little changes in monocyte and lymphocyte count of differential leukocyte count in cattle of group A. Khalid *et al.* (2005) reported that neem (10% water extract of leaves) increased TEC, Hb content, PCV in sheep on 28 day post-treatment. Likewise, Rahman (2002) observed water extract of neem increased TEC, Hb content on 21st day of post-treatment in goat. Similarly, Rob *et al.* (2004) also stated water extracts of neem leaves increased TEC, Hb content, PCV in sheep on 28 day post-treatment. Hossain *et al.* (1996) reported that neem leaves increased Hb content in cattle. Conversely, Rahman (2002) observed water extract of neem decreased TLC on 21st day of post-treatment in goat. The present finding was also agreement with the work of Rob *et al.* (2004) due to water extracts of neem leaves in sheep on 28 day post-treatment. Likewise, Khalid *et al.* (2005) reported that neem (10% water extract of leaves) decreased TLC in sheep.

Table 2. Effects of neem leaves on hematological and biochemical parameters in cattle

Hematological and serum biochemical parameters	Pretreatment values(n=6)		Post-treatment values (n=6)							
	Day 0		3rd day		10th day		17th day		28th day	
	Neem treated	Untreated control	Neem treated	Untreated control	Neem treated	Untreated control	Neem treated	Untreated control	Neem treated	Untreated control
TEC (million/cu.mm.)	8.20 ± 0.53	9.31 ± 0.71	8.26** ± 0.53	9.24** ± 0.67	8.55** ± 0.50	9.06** ± 0.67	8.83** ± 0.48	8.88** ± 0.64	9.06** ± 0.49	8.64 ** ± 0.59
Hb content (gm %)	8.53 ± 0.89	10.43 ± 1.14	8.73** ± 0.99	10.20** ± 1.13	9.73** ± 0.80	9.50** ± 1.01	10.6** ± 0.80	8.60** ± 0.65	11.9** ± 0.77	8.13** ± 0.48
PCV (%)	30.00 ± 2.10	33.17 ± 1.94	30.50** ± 1.64	32.67* ± 1.75	31.83** ± 2.14	32.00* ± 1.67	32.33** ± 1.97	30.83** ± 2.04	32.83** ± 1.94	30.33** ± 1.75
TLC (thousand/cu.mm.)	10.44 ± 0.86	9.11 ± 1.16	10.38** ± 0.84	9.19** ± 1.14	10.19** ± 0.82	9.48** ± 1.12	10.03** ± 0.83	9.79** ± 1.11	9.84** ± 0.87	10.08** ± 1.04
DLC (eosinophil, %)	13.33 ± 1.21	11.50 ± 1.52	13.00 ± 1.09	12.00** ± 1.09	11.67** ± 1.03	12.67** ± 1.37	10.33** ± 1.21	13.17** ± 1.17	9.50** ± 1.05	13.83** ± 1.17
DLC (neutrophil, %)	22.00 ± 2.19	25.67 ± 2.58	22.50* ± 1.87	24.83** ± 2.23	23.83** ± 1.83	24.00** ± 2.10	24.50** ± 1.64	23.33** ± 1.75	25.50** ± 1.64	22.83** ± 2.04
DLC (monocyte, %)	3.50 ± 1.05	4.00 ± 0.89	4.00 ± 1.09	3.67 ± 1.37	4.33 ± 1.63	3.83 ± 1.60	4.50 ± 0.55	4.33 ± 1.03	4.66 ± 1.03	3.50 ± 0.55
DLC (lymphocyte, %)	61.17 ± 2.04	58.83 ± 1.60	60.50 ± 2.07	59.50 ± 2.34	60.17 ± 1.83	59.50 ± 2.17	60.67 ± 0.85	59.17 ± 1.33	60.33 ± 1.63	59.83 ± 1.47
ALT (U/l)	20.53 ± 1.65	17.97 ± 2.21	20.58 ± 2.30	18.22 ± 1.57	19.22 ± 1.69	18.13 ± 2.25	20.50 ± 2.23	17.33 ± 2.54	19.58 ± 2.15	18.65 ± 1.96
AST (U/l)	33.05 ± 2.30	29.68 ± 2.60	31.33 ± 1.80	29.67 ± 2.44	32.58 ± 2.70	30.87 ± 2.02	31.85 ± 3.63	29.27 ± 2.27	31.55 ± 1.98	30.90 ± 2.16

The above values represent the mean ± standard deviation (SD) of 6 cattle
** = Significant at 1 per cent level ($p<0.01$)
* = Significant at 5 per cent level ($p<0.05$)

The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level were not significantly changed in the cattle. These findings cannot be compared due to lack of similar published reports.

It may be concluded that water extracts of neem leaves was moderately effective against gastrointestinal nematode infections in cattle. However, neem leaves may be used as an alternative drugs in field condition of Bangladesh.

Acknowledgement

The financial support of BAURES, Bangladesh Agricultural University, Mymensingh for conducting the experiment is gratefully acknowledged.

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