

*Full Length Research Paper*

## ***In vivo and in vitro* antihelmintic activity of gemmotherapeutically treated *Azadirachta indica* (neem) against gastrointestinal nematodes of sheep and earthworms**

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***In vivo and in vitro* crude powder (CPE), native (NNE) and gemmotherapeutic (GNE) extracts of *Azadirachta indica* (neem) were studied to rationalize its traditional use. Live *Haemonchus contortus* and earthworms were used to access the *in vitro* and anthelmintic effect of crude extracts (NNE and GNE) of neem. The *in vitro* inhibitory effect of both GNE and NNE was evident in the paralysis or mortality of *H. contortus* and earthworms noted at 24 h post exposure. In egg hatch assay, NNE extract demonstrated inhibitory effect on egg hatching of *H. Contortus* after 48 h, with 100% eggs remaining unhatched at 4.0 mg/mL; while in GNE after 48 h, again 100% eggs remained unhatched at 2.0 mg/mL. All these results were also compared with positive control velbazine. For *in vivo* studies, GTNE, NNE and crude powder of neem were administered in increasing doses (1.0-3.0 g/kg) to sheep naturally infected with mixed species of gastrointestinal nematodes. A maximum reduction of 73.6% EPG was recorded in sheep treated with GNE at 3.0 g followed by crude powder of neem at 3.0 g (27%) and NNE at 3.0 g (43.8%). Levamisole, a standard anthelmintic agent, showed 99.23% reduction in faecal eggs per gram (EPG) basis. The data show that GNE and NNE exhibit dose dependent anthelmintic activity both *in vivo* and *in vitro*. The results suggest that utilization of GTNE, NNE and crude powder of neem may be useful in the control of sheep gastrointestinal nematodes.**

**Key words:** Antihelmintic, gemmotherapeutically, *Azadirachta indica*, nematodes, earthworms, Pakistan.

### **INTRODUCTION**

Helminthiasis is among the most important animal diseases inflicting heavy production losses of farm animals. The disease is highly prevalent particularly in third world countries (Dhar et al., 1982) due to poor

management practices. Therefore, multidimensional approaches are in practice for an effective control of helminthes. These include periodical use of anthelmintic and vaccination program (for example, lungworms) coupled with improved management. Development of parasitic resistance to commercially available drugs has become a serious problem (Mascie-Taylor and Karin, 2003) due to misuse of drugs and probably adaptation of

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parasites to commercially available drugs. The drugs are unaffordable, not available or in adequate supply under local conditions (Hammond et al., 1997), hence future animal production is under severe threat. Historically, most pharmaceutical companies started their business by selling plant extracts, and a quarter of all prescription drugs currently sold in the world contains active ingredients derived from plants (Mathias et al., 1996). Use of plants as anthelmintics has also been listed in the British Veterinary Codex until 1965 (Hammond et al., 1997). The potential for new drugs development and discovery depends on documentation and screenings of traditionally used medicinal plants. These naturally produced drugs therefore offer alternatives that can help overcome the problems of Western drugs and are both sustainable and environmentally sound (Dawo et al., 2001; Alawa et al., 2003; Biffa et al., 2004; Waller and Prichard, 1985).

Gemmotherapy was developed in France in the 50s and 60s by a group of medicinal homeopaths who had a wider interest in natural medicine. Gemmotherapy received official recognition in 1965 when it entered French Pharmacopoeia (Churchill, 2003). Gemmotherapeutic remedies are used to stimulate elimination of toxic compounds from the body. Gemmotherapy is believed to work because of the presence of gibberellins, plant growth hormone which acts on the organs to be stimulated. It is a new form of herbal medicine, using remedies specially made from buds and young shoots of trees and shrubs. These are collected in spring, at the peak point of natural cycle of growth and renewal. As a result, gemmotherapy remedies contain many nutrients, vitamins and enzymes released just at this time of the year. From a holistic perspective, they capture the abundant vital energy concentrated in germinating trees and shrubs

Neem is an evergreen tree, cultivated in various parts of the subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity. Neem has been extensively used in Ayurveda, Unani and Homeopathic medicine. The Sanskrit name of the neem tree is Arishlha, meaning reliever of sickness and hence is considered as 'Sarbaroganibarini'. The tree is still regarded as village dispensary in Pakistan. The importance of neem tree has been recognized by US National Academy of Sciences, which published a report in 1992, entitled "Neem, a tree for solving global problems" (Colles et al., 1992).

Chemical investigation on the products of neem tree was extensively undertaken in the middle of the 20<sup>th</sup> century. The isolation of nimbin was reported as first bitter compounds isolated from neem oil. More than 135 compounds have been isolated from different parts of neem. The compounds have been divided into two major classes: isoprenoids and nonisoprenoids. The isoprenoids include diterpenoids and triterpenoids. The non isoprenoids include proteins, carbohydrates (polysaccharides) sulphurous compounds, polyphenolics, such as flavonoids

and their glycosides, dihydrochelone, cumarins and tannins, and aliphatic compounds etc. (Kausik et al., 2002). The present study was therefore carried out to assess: one, anthelmintic activity of gemmotherapeutically treated neem extract and native neem extract *in vitro* using live *Haemonchus contortus* and earth worms; and two, *in vivo* using sheep naturally infected with mixed infection of gastro intestinal worms.

Anthelmintic activity of *Azadirachta indica* has also been reported previously by Akhtar and Riffat (1984), Gowda (1997) and Mostofa et al. (1996) against nematodes. Likewise, Sangwan and Sangwan (1998) have reported extracts of fresh leaves of *Melia azedarach* to kill *H. contortus* larvae *in vitro*. However, Dakshinkar et al. (1997) did not support the anthelmintic effects of *A. indica* against *H. contortus* in Sahiwal x Jersey cow calves compared with moranetl citrate and also reported no *in vitro* anthelmintic activity of *A. indica* against *Ascaridia galli*.

The parts and forms of *A. indica* used by various workers include seeds, leaves, and bark (Gowda, 1997; Jani et al., 1997; Mostofa et al., 1996; Rekha et al., 1997), as a paste (Rekha et al., 1997), decoction (Jani et al., 1997), aqueous extracts (Gowda, 1997; Udeinya, 1993), methanol (Udeinya, 1993; Siddiqui et al., 2000), acetone and dimethylsulfoxoide extracts (Udeinya, 1993) and oils (Rahman, 1975).

## MATERIALS AND METHODS

### Anti-parasitic activity

This experiment was conducted to evaluate the anthelmintic activity of NNE and GNE as follows.

#### *Whole neem extract for anti-parasitic activity*

For this purpose, mature leaves were washed, dried in shade, grinded using an electrical grinder and stored in cellophane bags until use. 500 g powder was soaked in 4 L 70% aqueous ethanol. This mixture was put in cool and shady place for one month. After one month, it was filtered and then concentrated in rotary evaporators at 45°C under reduced pressure. The rest of the alcohol was evaporated in incubator at 65°C until all the alcohol was evaporated. The rest of the extract was stored at 4°C until use and dissolved in distilled water on the day of experiment to prepare stock solution of different dilutions for the purpose of evaluation (Osol, 1975) with some modifications.

#### *Gemmotherapeutic neem extract*

For this purpose, fresh growing shoots and leaves were washed and ground finally in equal amount of glycerine and alcohol (70% aqueous ethanol).

This mixture was put in cool and shady place for one month. After one month, it was filtered and the filtrate was then concentrated in rotary evaporator at 45°C under reduced pressure. Then the rest of the alcohol was evaporated in an incubator at 65°C till all the alcohol was evaporated. The extract was stored at 4°C until use. On the day of experiment the dilutions were prepared in

distilled H<sub>2</sub>O (Churchill, 2002) with some modifications.

## Anthelmintic activity

### *In vitro* anthelmintic activity

*In vitro* anthelmintic activity was assessed through egg hatch test and adult motility assay.

#### 1. Egg hatch test

Adult female *H. contortus* was recovered after necropsy from sheep. This female was crushed and eggs were cultivated *in vitro* to obtain infective larvae. Native lambs were infected with these larvae and faecal samples were collected at day 25 from each lamb. Faecal cultures were realized to obtain the infective stage. These larvae were then used to infest new native lamb to get enough number of eggs for *in vitro* studies. The eggs were isolated as previously described by Hubert and Kerboeuf (1992).

**Test procedure:** Egg hatch test was performed as described by Coles et al. (1992). A suspension of 0.2 mL containing approximately 100 fresh collected eggs per well was distributed in each well of a 24-flat-bottomed micro titre plate and mixed with different concentrations (62.5 to 4000 µg/mL) of NNE and GNE. The negative control plates contained the diluents water and the egg solution and the positive control plates contained Oxfendazole and egg solution. The eggs were incubated in this mixture for 48 h at 25°C. After this time, one drop of Lugol's iodine solution was added. All the eggs and first-stage larvae (L1) in each plate were then counted. There were three replicates for each concentration of NNE and GNE and control.

#### 2. Adult motility assay

Adult motility assay was conducted on mature live *H. contortus* of sheep as described previously by Sharma et al. (1971). Briefly, the mature worms were collected from the abomasums of freshly slaughtered sheep in the local abattoir. The worms were washed and suspended in phosphate buffer saline (PBS). Ten worms were exposed in triplicate to each of the following treatments in separate Petri dishes at room temperature (25-30°C):

1. Levamisole at 550 µg/mL.
2. Gemotherapeutic extract of neem at 125, 250, 500, 1000, 2000, 4000 and 8000 µg/mL.
3. Native neem extract of plants at 125, 250, 500, 1000, 2000, 4000 and 8000 µg/mL.
4. Phosphate buffer saline (PBS).

The mortality of the worms kept in the above treatments was used as the criterion for anthelmintic activity. The mortality was observed on 1, 3, 6, 12, 24 and 48 h post exposure. Finally, the treated worms were kept for 30 min, in the lukewarm fresh PBS to observe the revival of motility.

### *In vivo* anthelmintic activity

Neem selection for *in vitro* trial was based on its *in vitro* anthelmintic activity and its use in the local ethnoveterinary system for *in vivo* trials. The *in vivo* trials were conducted at Livestock Experiment Station, Department of Veterinary Parasitology, University of Agriculture, Faisalabad, Punjab, Pakistan.

For neem, 66 male sheep (young stock of 1 year), weighing 18-

24 kg, and naturally infected with gastrointestinal nematodes were used. These animals were selected after qualitative and quantitative faecal examination using standard parasitological procedures. Experiment was randomly divided into 8 groups of three animals each and assigned to different treatments *Peros* as a single dose as seen below:

- Group 1: Untreated control.
- Group 2: Levamisole HCl at 7.5 mg/kg.
- Group 3: Crude powder (CP) at 1.0 g/kg.
- Group 4: CP at 2.0 g/kg.
- Group 5: CP at 3.0 g/kg.
- Group 6: NNE at the equivalent dose rate of 1.0 g/kg of CP.
- Group 7: NNE at the equivalent dose rate of 2.0 g/kg of CP.
- Group 8: NNE at the equivalent dose rate of 3.0 g/kg of CP.
- Group 9: GNE at the equivalent dose rate of 1.0 g/kg of CP.
- Group 10: GNE at the equivalent dose rate of 2.0 g/kg of CP.
- Group 11: GNE at the equivalent dose rate of 3.0 g/kg of CP.

Faecal samples of each group were collected in the morning, starting from day 0 (pre-treatment) to 3, 5, 7, 9, 11, 13 day treatments and 15 post-treatment. They were evaluated for the presence of worms' eggs using McMaster Egg Counting Technique. Identification of nematode eggs in the faeces was done using standard description of MAFF (1979), Soulsby (1982) and Thienpont et al. (1979).

## RESULTS OF ANTI-PARASITIC ACTIVITY

### *In vitro* anathematic activity

#### *Egg hatch assay activity*

**1) Native neem extract:** It had inhibitory effects on egg hatching of *H. contortus*, after 48 h, with 100% eggs remaining unhatched at 4000 mg/mL in native extract of *A. indica*. The LC<sub>50</sub> was determined graphically from the regression equation as shown in Figure 1. The result of native extract of *A. indica* is also shown in Table 1.

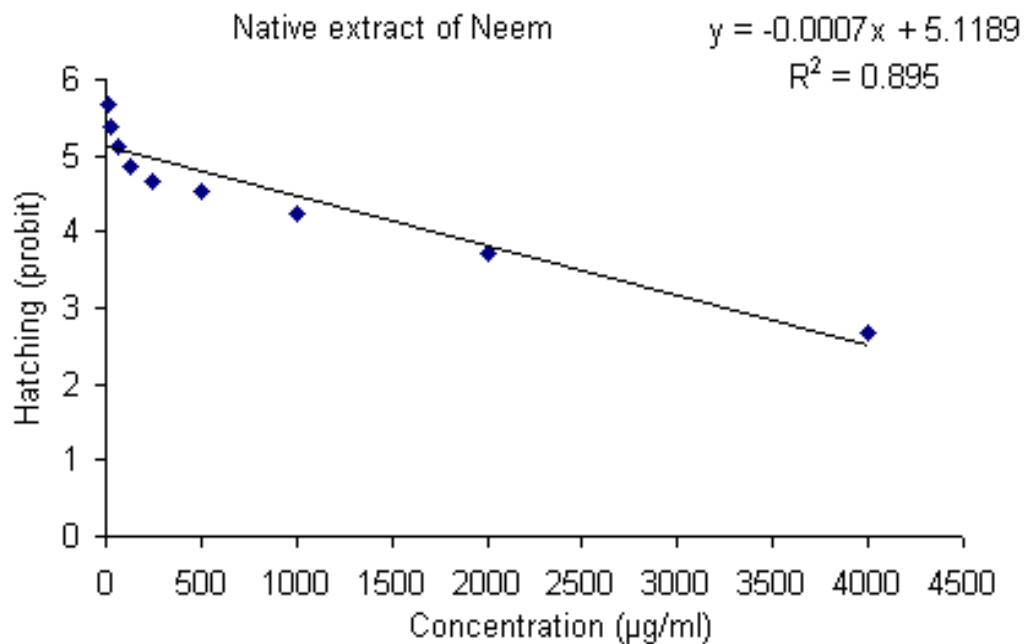
**2) Gemotherapeutic neem extract:** The GT extract of *A. indica* demonstrated inhibitory effects on egg hatching of *H. contortus*. The LC<sub>50</sub> was graphically determined from the regression equation as shown in Figure 2. The calculated LC<sub>50</sub> of GT extract is shown in Table 2.

**3) Effect of velbazine:** This had inhibitory effect on egg hatching of *H. contortus*. The values are shown in Table 3 and LC<sub>50</sub> was determined graphically from regression equation as shown in Figure 3.

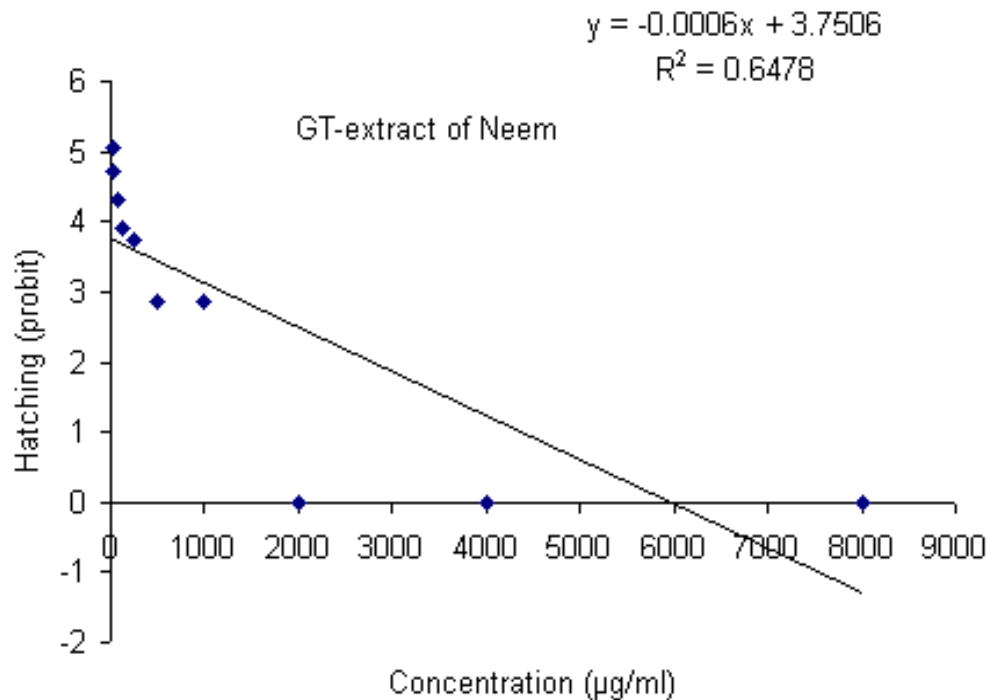
### *Adult motility assay*

#### 1) On *H. contortus*

**i) Native neem extract:** The anthelmintic effect of NNE on the survival of *H. contortus* (Table 4), although lower than levamisole, was time and dose dependent. The higher doses of NNE resulted in an early onset of activity and higher number of dead worms compared to lower



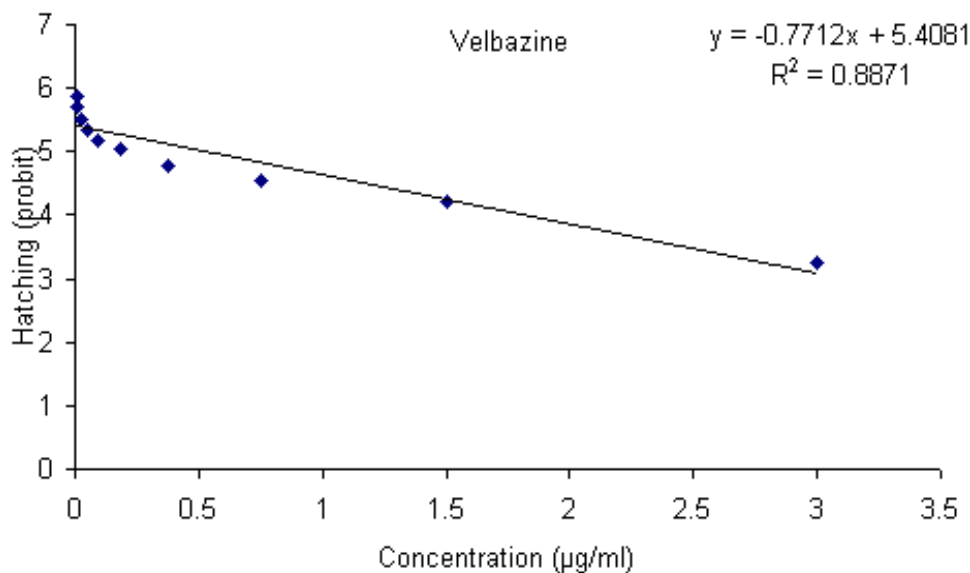
**Figure 1.** Linear relationship between mean eggs hatched and different concentrations of native neem extracts.



**Figure 2.** Linear relationship between mean eggs hatched and different concentrations of gemotherapeutic neem extracts.

doses. The NNE extract at 125 µg/mL exhibited no anthelmintic activity on 12 h post exposure. All the worms exposed to levamisole were found dead at 12 h; whereas, none of the worms was found dead or

paralyzed in PBS at 6 h post exposure. The anthelmintic activity of NNE at 8000 µg/mL was as good as that of levamisole (positive control) from 12 h and onward post exposure.



**Figure 3.** Linear relationship between mean eggs hatched and different concentrations of velbazine.

**Table 1.** Percentage of egg hatched at different concentrations of native extracts of neem (*Azadirachta indica*).

Dose mg/mL	Dose (µg/mL)	Dose (ng/mL)	Hatch (%)	Log dose	Probit	Regression	Slope	Intercept	LC <sub>50</sub>
0.015625	15.625	15625	75.5	4.194	5.69	5.821	-1.061	10.3	4.97
0.03125	31.25	31250	65	4.495	5.385	5.5016			93325.45
0.0625	62.5	62500	55	4.796	5.126	5.1821			93.3 LC <sub>50</sub> µg/mL
0.125	125	125000	45	5.097	4.874	4.8627			
0.25	250	250000	36.5	5.398	4.655	4.5432			
0.5	500	500000	31.5	5.699	4.518	4.2238			
1.0	1000	1000000	22	6	4.228	3.9043			
2.0	2000	2000000	10	6.301	3.718	3.5849			
4.0	4000	4000000	1	6.62	2.676	3.2464			

**Table 2.** Percentage of egg hatched at different concentrations of gemotherapeutic extracts of neem.

Dose (mg/mL)	Dose (µg/mL)	Dose (ng/mL)	Hatch %	Log dose	Probit	Regression	Slope	Intercept	LC <sub>50</sub>
0.015625	15.625	15625	51.67	4.194	5.039	5.58	-2.09	14.3	4.47
0.031250	31.25	31250	39.00	4.495	4.721	4.95			29512.09
0.0625	62.5	62500	24.34	4.796	4.303	4.32			29.5 LC <sub>50</sub> µg/mL
0.125	125	125000	14.00	5.097	3.920	3.69			
0.25	250	250000	10.67	5.398	3.751	3.06			
0.5	500	500000	1.67	5.699	2.852	2.43			
1.0	1000	1000000	1.67	6.000	2.852	1.80			
2.0	2000	2000000	0.00	6.301	0.000	1.17			
4.0	4000	4000000	0.00	6.62	0.000	0.49			
8.0	8000	8000000	0.00	6.903	0.000	-0.08			

**Table 3.** Percentage of egg hatched at different concentrations of velbazine.

Vel ( $\mu\text{g/mL}$ )	Vel ( $\text{ng/mL}$ )	Hatch (%)	log (Vel)	Probit	Regression	Slope	Intercept	LC <sub>50</sub>
0.0058	5.8	81.2	0.763428	5.88	7.323	-0.824	6.6942	2.06
0.0117	11.7	76.2	1.068186	5.71	7.574			log LC <sub>50</sub>
0.0234	23.4	70	1.369216	5.52	7.822			114 LC <sub>50</sub> ng/mL
0.0469	46.9	63.8	1.671173	5.35	8.071			0.11 LC <sub>50</sub> $\mu\text{g/mL}$
0.0937	93.7	57.5	1.97174	5.19	8.319			
0.1875	187.5	51.2	2.273001	5.03	8.567			
0.375	375	41.2	2.574031	4.78	8.815			
0.75	750	32.5	2.875061	4.55	9.063			
1.5	1500	21.2	3.176091	4.2	9.311			
3.0	3000	4	3.477121	3.249	9.559			

**ii) Gemotherapeutic extract:** The anthelmintic effect of GNE on the survival of *H. contortus* (Table 4), although lower than levamisole, was time and dose dependent. The higher doses of GTNE resulted in an early onset of activity and higher number of dead worms compared with lower doses. GNE at 125  $\mu\text{g/mL}$  exhibited no anthelmintic activity observed on 6 h post exposure. All the worms exposed to levamisole were found dead at 12 h; whereas, none of the worms was found dead or paralyzed in PBS at 12 h post exposure. The anthelmintic activity of *A. indica* at 8000  $\mu\text{g/mL}$  was as good as that of levamisole (positive control) from 12 hours and onward post exposure.

## B) On earth worm

**i) Native neem extract:** The anthelmintic effect of NNE on the survival of earthworms (Table 1), although lower than levamisole, was time and dose dependent. The higher doses of NNE resulted in an early onset of activity and higher number of dead earthworms compared with lower doses. The NNE at 125  $\mu\text{g/mL}$  exhibited no anthelmintic activity on 6 h post exposure. All the earthworms exposed to levamisole were found dead at 12 h; whereas, none of the worms was found dead or paralyzed in PBS at 12 h post exposure. The anthelmintic activity of NNE at 8000  $\mu\text{g/mL}$  was as good as that of levamisole (positive control) from 12 hours and onward post exposure.

**ii) Gemotherapeutic extract:** The anthelmintic effect of GNE extract on the survival of earthworms (Table 2), although lower than levamisole, was time and dose dependent. The higher doses of GNE resulted in an early onset of activity and higher number of dead earthworms compared with lower doses. The GNE at 125  $\mu\text{g/mL}$  exhibited no anthelmintic activity on 3 hours post exposure. All the earthworms exposed to levamisole were found dead at 12 h; whereas, none of the earthworms was found dead or paralyzed in PBS at 12 h post

exposure. The anthelmintic activity of GNE at 8000  $\mu\text{g/mL}$  was as good as that of levamisole (positive control) from 12 h and onward post exposure.

## In vivo anthelmintic activity

**i) Levamisole treated (positive control) and untreated sheep:** There was reduction in eggs per gram (EPG) in all the levamisole treated sheep serving as positive control. The range of reduction in EPG 67.3-98.1% in different days is shown in Table 4. In most of the levamisole treated sheep, EPG was dropped; in contrast there was no reduction in all the untreated sheep.

**ii) A. indica treated sheep:** *In vivo* maximum reduction of 73.6% in EPG was recorded in sheep treated with GT extract at 3 g followed by CP at 3.0 g (27%) and MNE at 3.0 g (43.8%). It is evident from the results that GNE extract had higher activity compared with CP and GNE. This may be considered as an indication for the presence of more active ingredients in fresh growing shoots and leaves responsible for anthelmintic activity. All the results are shown in Table 4.

## DISCUSSION

For *in vitro* studies, *Haemonchus contortus* and earth worm proved to be good test worms because of their longer survival in PBS. *H. contour* has previously been used for *in vitro* studies by many workers (Sharma et al., 1971; Prakash et al., 1980; Sangwan and Sanwan, 1998). *In vivo* test requires greater amount of compound, larger number of animals and much more time; while *in vitro* study is simple anthelmintic activity and is economical. *H. contortus* from a few animals and earthworm are sufficient to test many drugs in different concentrations and only little amount of chemicals/ plant extract are required. Moreover, no previous toxicity tests are necessary. Although *in vitro* test does not justify

**Table 4.** Comparison between anthelmintic activity of CP of native neem, gemotherapeutic neem extracts and levamisole.

Day	Crude powder			Native neem extract			Gemotherapeutic extract			Control	
	1.0 g	2.0 g	3.0 g	1.0 g	2.0 g	3.0 g	1 g/kg b.wt	2 g/kg b.wt	3 g/kg b.wt	Untreated	Treated
0	2500.0 ± 577.4 <sup>a</sup>	2666.7 ± 333.3 <sup>a</sup>	3333.3 ± 1202 <sup>a</sup>	2666.7 ± 441.0 <sup>a</sup>	1833.3 ± 375.6 <sup>a</sup>	3233.3 ± 1398 <sup>a</sup>	3333.3 ± 1201.9 <sup>a</sup>	1333.3 ± 600.9 <sup>a</sup>	2333.3 ± 166.7 <sup>a</sup>	2866.7 ± 2518.2 <sup>a</sup>	866.7 ± 185.6 <sup>a</sup>
3	2416.7 ± 591.8 <sup>ab</sup> (3.3)	2400 ± 321.5 <sup>ab</sup> (10)	2933.3 ± 1105.0 <sup>ab</sup> (12)	2600.0 ± 453.7 <sup>ab</sup> (2.5)	1566.7 ± 260.3 <sup>b</sup> (14.6)	2633.3 ± 1452 <sup>ab</sup> (18.6)	2616.7 ± 1160.6 <sup>a</sup> (21.5)	1050.0 ± 436.8 <sup>ab</sup> (21.3)	1633.3 ± 394.1 <sup>b</sup> (30)	2716.7 ± 1433.6 <sup>a</sup> (5.23)	283.3 ± 72.6 <sup>b</sup> (67.3)
5	2366.7 ± 591.8 <sup>b</sup> (5.3)	2116.7 ± 252.2 <sup>bc</sup> (20.6)	2666.7 ± 1047.7 <sup>bc</sup> (20)	2566.7 ± 469.3 <sup>abc</sup> (3.8)	1400.0 ± 312.2 <sup>bc</sup> (23.6)	2383.3 ± 1441 <sup>bc</sup> (26.3)	2116.7 ± 1055.3 <sup>a</sup> (36.5)	900.0 ± 368.6 <sup>abc</sup> (32.5)	1366.7 ± 324.5 <sup>bc</sup> (41.4)	2533.3 ± 1349.8 <sup>a</sup> (11.6)	116.7 ± 16.7 <sup>bc</sup> (86.5)
7	2316.7 ± 548.7 <sup>b</sup> (7.33)	2066.7 ± 233.3 <sup>bc</sup> (22.5)	2350.0 ± 911.5 <sup>c</sup> (29.5)	2516.7 ± 469.3 <sup>bc</sup> (5.6)	1350.0 ± 312.2 <sup>c</sup> (26.4)	2166.7 ± 1424 <sup>bc</sup> (33.0)	2116.7 ± 1012.6 <sup>a</sup> (36.5)	666.7 ± 300.5 <sup>bc</sup> (50)	1116.7 ± 245.5 <sup>cd</sup> (52.1)	2500.0 ± 866.0 <sup>a</sup> (12.8)	16.7 ± 16.7 <sup>c</sup> (98.1)
9	2333.3 ± 548.7 <sup>b</sup> (6.7)	1816.7 ± 142.4 <sup>c</sup> (31.9)	2133.3 ± 841.8 <sup>c</sup> (36)	2466.7 ± 469.3 <sup>c</sup> (7.5)	1300.0 ± 284.3 <sup>c</sup> (29.1)	2016.7 ± 1270 <sup>bc</sup> (37.6)	1783.3 ± 968.0 <sup>a</sup> (46.5)	516.7 ± 164.1 <sup>ac</sup> (61.3)	833.3 ± 261.9 <sup>de</sup> (64.3)	3200.0 ± 1054.0 <sup>a</sup> (-11.6)	50.0 ± 28.9 <sup>bc</sup> (94.23)
11	2366.7 ± 594.7 <sup>b</sup> (5.3)	1733.3 ± 133.3 <sup>c</sup> (35)	2100.0 ± 850.5 <sup>c</sup> (37)	2333.3 ± 352.8 <sup>d</sup> (12.5)	1350.0 ± 288.7 <sup>c</sup> (26.4)	1816.7 ± 1096 <sup>bc</sup> (43.8)	2333.3 ± 1083.3 <sup>a</sup> (9.5)	483.3 ± 261.9 <sup>c</sup> (63.8)	616.7 ± 159.0 <sup>de</sup> (73.6)	3283.3 ± 1293.0 <sup>a</sup> (-14.5)	100.0 ± 50.0 <sup>bc</sup> (88.5)
13	2416.7 ± 594.7 <sup>ab</sup> (3.3)	1850.0 ± 175.6 <sup>c</sup> (30.6)	2133.3 ± 917.1 <sup>c</sup> (36)	2550.0 ± 453.7 <sup>abc</sup> (4.4)	1433.3 ± 318.0 <sup>bc</sup> (21.8)	1650.0 ± 900 <sup>c</sup> (49.0)	3016.7 ± 1307.1 <sup>a</sup> (8)	616.7 ± 324.5 <sup>bc</sup> (53.8)	416.7 ± 136.4 <sup>e</sup> (82.14)	3866.7 ± 1435.9 <sup>a</sup> (-34.9)	50.0 ± 28.9 <sup>bc</sup> (94.23)
15	2516.7 ± 591.8 <sup>a</sup> (-0.7)	2316.7 ± 289.2 <sup>ab</sup> (13.1)	2200.0 ± 1001.7 <sup>c</sup> (34)	2633.3 ± 466.7 <sup>ab</sup> (1.3)	1550.0 ± 332.9 <sup>b</sup> (15.5)	1550.0 ± 826 <sup>c</sup> (52.1)	3066.7 ± 1266.7 <sup>a</sup> (8)	623.3 ± 340.9 <sup>bc</sup> (53.3)	340.0 ± 113.7 <sup>e</sup> (85.3)	3966.7 ± 1560.3 <sup>a</sup> (-38.4)	66.7 ± 33.3 <sup>bc</sup> (92.3)

The worldwide increase in resistance of gastrointestinal trichostrongylids of small domestic ruminants against conventional anthelmintics (Waller, 1985) and the resulting economical damage show the urgent need for alternative methods for reducing the worm burden in the animals and the number of infective larvae. In addition, the worldwide growth of organic agriculture, in which the use of synthetic products is strongly restricted, needs alternatives for helminth control. Research on prophylactic strategies such as grazing management, biological control with nematophagous fungi or food supplementation with leguminous plants accumulating high amounts of condensed tannins is promising but research on the effectiveness of unconventional therapeutic methods such as rational phototherapy is rare (Hammond et al., 1997).

*A. indica* has several diverse chemical compounds and their derivatives include *Azairchtin* (Gowda, 1997; Qadir et al., 1985) and *Nimbicin* (Gowda, 1997). *Azadirachtin* has shown dose and tissue specific inhibition of glutathione S-transferase and has reduced glutathione and UDP-glucuronyl transferase activity in liver, lung, kidney and brain of rats (Gowda, 1997). Rukmini and Raychaudhuri, (1991) have reported that seeds of *A. indica* have oil containing 50% oleic and 15% linoleic acids. Siddiqui et al. (2000) isolated two new triterpenoids, 6 alpha-O-acetyl-7-deacetylnimocinol and meliacinol from the methanolic extract of fresh leaves of *A. indica*.

The anthelmintic activity of *A. indica* may be attributed to the biochemical interference of Azadirachtin, one of the chemical constituents of the plant. Different concentrations of Azadirachtin are available in different parts of the plant. Therefore, the variations in anthelmintic activity of *A. indica* may be attributed to these factors.

In the present study, *A. Indica* exhibited anthelmintic activity against *H. contorts* in naturally parasitized sheep. *In vitro* best anthelmintic activity was exhibited by GNE. The anthelmintic efficacy of *A. indica* NNE and crude power was although lower than the reference drug, levamisole, it is almost similar to that reported earlier by Akhter and Aslam (1988). In case of earthworm, best anthelmintic activity was also exhibited by GNE. *In vivo* maximum reduction in EPG was recorded in sheep treated with GNE at 3.0 g followed by NNE at 3.0 g and then by crude powder at 3.0 g (Table 4).

It is evident from the results that GNE had higher anthelmintic activity compared to NNE and crude powder of neem, which could be an indication for the presence of more parasitic activity in fresh growing shoots and leaves compared to mature leaves. The same trend was also observed in *in vitro* studies.

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