

In vitro* ovicidal and larvicidal activity of *Andrographis paniculata* (Kalmegh) leaves on *Haemonchus contortus

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Gastrointestinal nematodes remain a major constraint to livestock productivity across all production systems and agro-climatic regions throughout the World (Prichard 1994). An epidemiological investigation carried out in both arid and semi-arid region of Rajasthan revealed that *Haemonchus contortus* is predominant (> 80%) parasite in sheep (Anon 2003). The control of this menace is carried out exclusively with synthetic anthelmintics. However, the rapid emergence of resistance to these anthelmintics among nematodes (Singh *et al.* 2002) associated with high cost, food residues and environmental pollution (Hammond *et al.* 1997) have generated need to discover new therapeutics from natural (plant) origin as an alternative source of chemical anthelmintic (Guarrera 1999, Vieira *et al.* 1999). Plants with anthelmintic property are reported mainly from Asia (Akthar *et al.* 2000), Africa (Alawa *et al.* 2003), America (Hansson *et al.* 1986) and Europe (Waller *et al.* 2001).

Andrographis paniculata belonging to family Acanthaceae, commonly known as Kalmegh / Kirayat / Mahatita is widely distributed in tropical and sub-tropical parts of India and is being cultivated in Bengal and Asom. The leaves contain bitter substances and traces of essential oil. The leaves are used as stomachic, tonic, anthelmintic and cholagogue. The aim of present study was to evaluate the *in vitro* effect of aqueous extract of *A. paniculata* leaves on exogenous stages of *H. contortus*.

Plant extract preparation: In a beaker air-dried leaves of *A. paniculata* (40 g) were mixed with water (800 ml) and left in contact for 72 h and shaken vigorously at regular interval. The mixture was then filtered through filter paper (Whatman No. 1) and actual concentration of filtrate was determined after drying it at room temperature. The yield of extract was 7.09%.

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Parasite: Four lambs were experimentally infected with *H. contortus* larvae and used as donors for subsequent *in vitro* assays.

Egg hatch/embryonation assay: The assay was performed as per Coles *et al.* (1992). Faecal pellets were collected from donor lambs and faecal slurry was prepared by soaking them in water. The slurry was filtered through tea strainer and suspension was centrifuged at 3000 rpm for 5 min. The sediment mixed with saturated salt solution and centrifuged at 3000 rpm for 1 min. The tubes were covered with cover glass and allowed to stand for 2–3 min. Clean eggs were collected in a beaker containing water. The number of eggs were counted and adjusted to 20–40 eggs / 100 µl of suspension. Egg suspension (500 µl) was poured in each well of 24-multiwell plate and to this equal volume of extract of varying concentration was added. The final concentration of extract ranged from 0.078 to 1.25 mg ml⁻¹ of water. Control well received water instead of extract.

Eight replicates were employed for each concentration. The plates were incubated for 24 h at 28 °C. A drop of Lugol's iodine was then added to each well. All the eggs (dead / embryonated) and first-stage larvae in each well were counted under microscope (10×) and data were expressed as% hatching (hatched larvae vs. dead + embryonated eggs) and% embryonation (hatched larvae + embryonated eggs vs. dead eggs).

Larval mortality assay: The concentration of freshly collected infective larvae (L₃) of *H. contortus* was adjusted to 40–60 live L₃ / 100 µl in water. To each well of microtitre plate, equal amount (100 µl each) of larval suspension and extract of varying concentrations was added. The final concentration of extract varied from 1.25 to 20.0 mg/ml. In control well only 200 µl of larval suspension was added. The plates were incubated for 24, 48 and 72 h in dessicator containing water at room temperature. The contents of each well were counted for total live and dead L₃ and results were expressed as % L₃ mortality.

Statistical analysis: The data were transformed by arcsine

Table 1. *In vitro* effects (mean±SE) of aqueous extract of *Andrographis paniculata* leaves on eggs of *Haemonchus contortus*

	Concentration of extract (mg/ml)						ED ₅₀ mg/ml(95% frudicial limit)
	1.25	0.625	0.3125	0.156	0.078	Control	
Egg hatching %	0.40 ^A ±0.17	1.67 ^B ±0.30	91.08 ^C ±1.84	95.08 ^C ±0.75	96.51 ^D ±2.58	96.52 ^D ±0.51	0.431(0.283–0.655)
Corrected egg hatching %	0.47±0.17	1.74±0.31	94.38±1.97	99.28±1.13	99.99±1.03	100.00	
Egg embryonation %	21.45 ^A ±3.68	81.18 ^B ±3.62	96.62 ^C ±0.63	96.40 ^C ±0.57	96.85 ^C ±0.57	97.21 ^C ±0.49	0.915(0.894–0.936)
Corrected egg embryonation %	22.06±3.76	83.50±3.65	99.29±1.00	99.20±0.97	99.66±0.99	100.00	

Means with different superscripts differ significantly (P<0.05) from each other in a row

Table 2. *In vitro* effects of aqueous extract of *Andrographis paniculata* leaves on infective larvae of *Haemonchus contortus*

Concentration of extract (mg/ml)	Larval mortality (%)			Corrected Larval Mortality (%)		
	24 h PI	48 h PI	72 h PI	24 h PI	48 h PI	72 h PI
Control	0.54	0.20	1.26	-	-	-
1.25	0.95	1.01	1.27	0.41	0.81	0.01
2.50	2.39	2.55	3.40	1.86	2.35	2.17
5.00	3.02	4.40	14.04	2.49	4.21	12.94
10.00	12.37	10.51	17.32	11.89	10.33	16.26
20.00	12.74	17.71	43.58	12.27	17.5	42.86

transformation and subjected to ANOVA and Duncan's test to compare the mean % egg hatching, egg embryonation and larval mortality among different concentrations of extract and control using SPSS programme ver 10.0. The ED₅₀ values for each assay were calculated by probit analysis.

Aqueous extract *A. paniculata* at the concentration of > 0.625 mg ml⁻¹ caused significant (P<0.05) embryonic death and inhibited hatching in almost all the eggs, indicating potent ovicidal property of the extract. The mean egg hatching was 96.52±0.51% in control well, which reduced significantly (P<0.05) to 1.67±0.30 and 0.40±0.17% at the concentration of 0.625 and 1.25 mg ml⁻¹ respectively (Table 1). The ED₅₀ value of extract required to inhibit hatching in 50% of eggs was 0.431 mg ml⁻¹.

Similarly, the extract also possessed potent embryotoxic activity. The mean% egg embryonation reduced significantly (P<0.05) from 97.21±0.49 (control well) to 21.45±3.68 (@ 1.25 mg ml⁻¹). However, almost double dose was required to cause embryonic death in 50% of the eggs with ED₅₀ value to the tune of 0.915 mg ml⁻¹ (Table 1). On larval mortality assay, it was observed that aqueous extract of *A. paniculata* caused 43.58% mortality of L₃ at the concentration of 20.0 mg ml⁻¹ after 72 h of incubation compared to 1.26% mortality in control well, suggesting presence moderate level of dose dependent larvicidal activity in the extract (Table 2).

In vitro tests using free living stages of parasitic nematodes offers a means of evaluating the anthelmintic activity of the new plant compound (Robinson *et al.* 1990). These tests measure the effect of drugs / compounds directly on the physiological processes such as hatch, development, motility and mortality of parasites (Varady and Corba 1999). The higher rate of egg hatch and embryonation observed in control

well showed that method employed in present study did not interfere with natural development of *H. contortus* eggs. Thus *A. paniculata* offers an opportunity for a new compound. However, more detailed studies are needed to identify and evaluate the active compounds, mechanism of action, *in vivo* efficacy, dose regime and toxicity.

SUMMARY

The aqueous extract from leaves of *Andrographis paniculata* was evaluated for anthelmintic activity against eggs and larvae of *Haemonchus contortus*. The extract was caused marked inhibition of embryonation and hatching of eggs. At the concentration of 1.25 mg ml⁻¹, the corrected mean egg embryonation and egg hatching were 22.06±3.76 and 0.47±0.17%, respectively indicating presence of both ovicidal and embryotoxic activity. However, the extract revealed moderate (43.58%) larvicidal activity at the concentration of 20.0 mg / ml after 72 h post-exposure.

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