INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2003/05–1–86–90
http://www.ijab.org

Anthelmintic Activity of *Adhatoda vesica* Roots

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ABSTRACT

This paper describes the *in vitro* and *in vivo* anthelmintic activity of *Adhatoda vesica* in comparison with Levamisole. *In vitro* studies revealed anthelmintic effects (P≥0.05) of crude aqueous (CAE) and methanol extracts (CME) of *A. vesica* on live *Haemonchus contortus* as evident from their mortality. For *in vivo* studies, roots of *A. vesica* were administered as crude powder (CP), CAE and CME to sheep naturally infected with mixed species of gastrointestinal nematodes. Maximum reduction (37.4%) in EPG was recorded in sheep treated with CAE @ 3 g Kg^{-1} body weight on day 10 post-treatment (PT) followed by CP @ 2 g (33.05%) and CME @ 3 g (25.6%) on day 14 PT. It was found that, although, *A. vesica* roots possess anthelmintic activity against nematodes, yet not comparable with Levamisole (97.8 to 100% reduction in EPG). It is suggested that further research on large scale be carried out on large number of animals on higher doses than those used in the current study, identification of active principles, and standardization of dose and toxicity studies for drug development.

Key Word: *Adhatoda vesica;* Root; Anthelmintic; Nematodes; Sheep

INTRODUCTION

Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent particularly in third world countries (Dhar *et al*., 1982) due to poor management practices. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminths (Geert & Dorny, 1995; Coles, 1997) against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics (Satyavati *et al*., 1976; Lewis & Elvin-Lewis, 1977). A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarni, 1954; Chopra *et al*., 1956; Said, 1969; Akhtar *et al*., 2000).

Adhatoda (A) vesica locally named as Arusa in Pakistan, has been widely used in ethno-veterinary medicine system of Pakistan. However, it lacks enough research based support to be used as anthelmintic in animals. This paper describes anthelmintic activity of *A. vesica* against mixed infection of gastrointestinal nematodes in sheep.

MATERIALS AND METHODS

A. vesica roots were procured from local market (Faisalabad, Pakistan), identified and authenticated by a botanist in the Department of Botany, University of Agriculture, Faisalabad–Pakistan. The material was dried in shade, ground finally in powder in an electric grinder, and stored in cellophane bags at 4°C until use.

Aqueous extract preparation. The crude aqueous extract (CAE) of the ground roots of *A. vesica* was prepared according to the standard methods (Onyeyili *et al*., 2001). One hundred grams of the powdered plant material was mixed with 500 mL of distilled water in a 1 L flask and boiled for 1.5 h. It was allowed to cool to 40°C and then filtered using whatman No.1 filter paper. The filtrate was then concentrated in a rotary evaporator and the extract stored at 4°C until required. The extract yield (% w/w) from the plant material was recorded.

Methanol extract preparation. Powdered plant material was exhaustively extracted with methanol in a Soxhlet apparatus (Asuzu & Onu, 1994). The crude methanol extract (CME) was evaporated to dryness and stored at 4°C until used. The extract yield (% w/w) from the plant material was recorded.

***In vitro* anthelmintic activity.** The *in vitro* trials for anthelmintic activity of CAE and CME were conducted on mature live *Haemonchus contortus* of sheep as described previously (Sharma *et al*., 1971; Lal *et al*., 1976; Singh *et al*., 1985). Briefly, the mature worms were collected from the abomasums of freshly slaughtered sheep in the local abattoir. The worms were washed and finally 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. CAE of *A. vesica* @ 25 mg mL^{-1}
2. CME of *A. vesica* @ 25 mg mL^{-1}
3. Levamisole 0.55 mg mL^{-1}
4. PBS

The inhibition of motility of the worms kept in the above treatments was used as the criterion for anthelmintic activity. The dead worms were easily recognized by their straight flat appearance with no movements at the head and tail regions of the body. The motility was observed on 0, 1, 2, 3 and 4 h intervals. Finally, the treated worms were kept

for 30 min in the lukewarm fresh PBS to observe the revival of motility.

***In vivo* anthelmintic activity** The *in vivo* trials were conducted at Livestock Experiment Station, Rakh Kherewala (Punjab–Pakistan). A total of 44 sheep of both sexes (female and male young stock ≤ 1 year), weighing 18-24 Kg were used for *in vivo* trials. Before the start of experiment, the animals were confirmed to be naturally infected with mixed species of gastrointestinal nematodes by qualitative and quantitative fecal examination using standard parasitological procedures (Soulsby, 1982). Identification of nematode eggs in the faeces was done using standard description of MAFF (1979) and Thienpont *et al*. (1979). The selected animals were suffering from mixed gastrointestinal nematodes species including mainly: *Haemonchus contortus, Trichostrongylus colubriformis, Trichostrongylus axei, Oesophagostomum columbianum, Strongyloides papillosus* and *Trichuris ovis*.

The sheep (n=44) used for experiment were randomly divided into 11 groups of four animals each and assigned to different treatments as given below:

Group 1: Untreated control

Group 2: Treated with Levamisole HCl (Nilverm 1.5% w/v; ICI Pakistan Limited, Animal Health Division) @ 7.5 mg Kg^{-1} body weight (b.w.) as single dose

Group 3: Treated with single dose of CP @ 1 g Kg^{-1} b.w.

Group 4: Treated with single dose of CP @ 2 g Kg^{-1} b.w.

Group 5: Treated with single dose of CP @ 3 g Kg^{-1} b.w.

Group 6: Treated with single dose of CAE at the equivalent dose rate 1 g Kg^{-1} b.w. of CP

Group 7: Treated with single dose of CAE at the equivalent dose rate 2 g Kg^{-1} b.w. of CP

Group 8: Treated with single dose of CAE at the equivalent dose rate 3 g Kg^{-1} b.w. of CP

Group 9: Treated with single dose of CME at the equivalent dose rate 1 g Kg^{-1} b.w. of CP

Group 10: Treated with single dose of CME at the equivalent dose rate 2 g Kg^{-1} b.w. of CP

Group 11: Treated with single dose of CME at the equivalent dose rate 3 g Kg^{-1} b.w. of CP

Fecal samples of each group were collected in the morning, starting from day 0 pre-treatment and at day 3, 5, 7, 10 and 14 post-treatment (PT) and were evaluated for the presence of worms eggs by salt floatation technique (MAFF, 1979). The eggs were counted by the McMaster method (Soulsby, 1982). Egg count (EC) per cent reduction (ECR) was calculated using the following formula:

$$\text{ECR (\%)} = \frac{\text{Pre-treatment egg count per gram} - \text{Post-treatment egg count per gram}}{\text{Pre-treatment egg count per gram}} \times 100$$

The observations were statistically analyzed using SAS software. Test of significance between the mean parameters were performed using analysis of variance.

RESULTS AND DISCUSSION

***In vitro* anthelmintic activity.** The results of *in vitro* anthelmintic activity of CAE and CME of *A. vesica*, PBS and Levamisole on the motility/survival of *Haemonchus contortus* of sheep have been presented in Table I and II. It is evident from these tables that CAE and CME of *A. vesica* demonstrated *in vitro* anthelmintic activity. There was no effect (P≥0.05) of PBS and NS on the motility/survival of worms.

***In vivo* anthelmintic activity.** There was reduction (P≤ 0.05) in EPG from day 3 PT onward compared with that on day 0 PT in Levamisole treated sheep serving as positive control (Table III). There was reduction (P≤ 0.05) in EPG from day 7 PT onward in sheep treated with *A. vesica* CP @ 1 g Kg^{-1} b.w. At 2 and 3 g CP, reduction (P≤ 0.05) in EPG started earlier i.e., day 3 PT onward compared with that with CP @ 1 g. The maximum reduction (33.05%) in EPG was recorded on day 14 PT in sheep treated with *A. vesica* CP @ 2 g. There was no reduction (P≤ 0.05) in EPG in sheep treated with *A. vesica* CAE @ 1 g. At 2 and 3 g CAE, however, there was gradual reduction (P≤ 0.05) in EPG from day 3 PT onward. The maximum reduction (37.4%) in EPG was recorded on day 10 and 14 PT in sheep treated with *A. vesica* CAE @ 3 g. There was reduction (P≤ 0.05) in EPG in sheep treated with *A. vesica* CME @ 1, 2 and 3 g Kg^{-1} body weight from day 5 PT onward. The maximum reduction (25.6%) in EPG was recorded on day 14 PT in sheep treated with *A. vesica* CME @ 3 g.

Table I. *In vitro* effect of aqueous extracts of *A. vesica* in order of decreasing activity on *Haemonchus contortus* of sheep in comparison with positive control (Levamisole)

Treatment/crude aqueous extract	Mean number of worms showing motility at different hours					
	0hrs	1hrs	2hrs	3hrs	6hrs	PBS*
Levamisole @ 0.55 mg/mL	10.0a	3.6b	1.6c	0.3d	0d	0d
A. vesica @ 25 mg/mL	10.0a	7.0b	6.0bc	5.3c	4.6c	4.6c
PBS	10.0a	10.0a	10.0a	10.0a	9.6a	9.6a

Table II. *In vitro* effect of methanol extracts of *A. vesica* in order of decreasing activity on *Haemonchus contortus* of sheep in comparison with positive control (Levamisole)

Treatment/crude methanol extract	Mean number of worms showing motility at different hours					
	0hrs	1hrs	2hrs	3hrs	6hrs	PBS*
Levamisole @ 0.55 mg/mL	10.0a	3.6b	1.6c	0.3d	0d	0d
A. vesica @ 25 mg/mL	10.0a	10.0a	9.6a	7.3b	5.6c	5.6c
PBS	10.0a	10.0a	10.0a	10.0a	9.6a	9.6a

abcd, Means marked with the same letter in a row do not different significantly at P≥0.05; *****indicates that worms were placed in PBS for 30 minutes after exposure of 6 hours to the treatments to confirm their mortality

Table III. Effect of *Adhatoda vesica* administration on Eggs per gram (Mean ± SEM) of feces in sheep naturally infected with nematodes

Day PT	Control		Crude Powder[3]			Crude Aqueous Extract[3]			Crude Methanol Extract[3]		
	UnTreated[1]	Treated[2]	1.0 g	2.0 g	3.0 g	1.0 g	2.0 g	3.0 g	1.0 g	2.0 g	3.0 g
0	645.0±35.7a	705.0±43.3a	742.5±25.6a	817.5±25.6a	660.0±27.3a	742.5±41.3a	742.5±25.6a	742.5±25.6a	712.5±25.6a	705.0±39.6a	735.0±55.4a
3	645.0±31.2a (0)	0b (100)	702.5±17.5abc (5.3)	660.0±17.3b (19.2)	510.0±38.7b (22.8)	705.0±39.6a (4.0)	675.0±25.9b (9.0)	592.5±25.6b (6.8)	660.0±32.4a (7.3)	637.5±44.7ab (10.2)	682.5±37.5ab (7.1)
5	592.5±48.0a (8.1)	0b (100)	687.5±46.9abc (7.4)	687.5±46.9b (15.9)	480.0±32.4b (26.9)	697.5±59.2a (6.1)	615.0±19.3c (17.1)	547.5±33.2b (12.8)	637.5±30.9b (10.5)	585.0±28.7b (17.0)	637.5±53.9bc (13.3)
7	630.0±27.3a (2.3)	15.0±8.6b (97.8)	675.0±19.3bc (9.0)	675.0±19.3b (17.4)	480.0±51.9b (26.9)	682.5±46.4a (8.1)	600.0±27.3c (19.2)	525.0±35.7bc (29.3)	645.0±19.3b (9.4)	577.5±22.5b (18.2)	600.0±47.4bc (18.4)
10	607.5±25.6a (5.8)	7.5±7.5b (98.9)	682.5±7.5abc (8.1)	577.5±33.2c (29.4)	465.0±46.6b (29.5)	720.0±40.6a (2.9)	577.5±33.2c (22.3)	465.0±39.6cd (37.4)	630.0±32.4bc (11.5)	570.0±38.7b (19.1)	570.0±47.4c (22.4)
14	637.5±22.5a (1.2)	15.0±8.6b (97.8)	675.0±19.3bc (9.0)	547.5±30.9c (33.05)	457.5±59.2b (30.8)	720.0±61.2a (2.9)	547.5±33.2c (26.3)	465.0±19.3d (37.4)	600.0±12.2c (15.7)	585.0±28.7b (17.0)	547.5±33.2c (25.6)

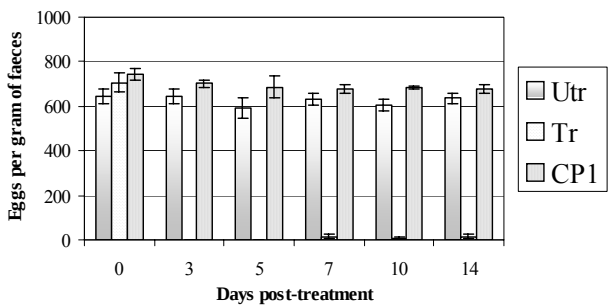
PT= Post-treatment; Means marked with the same letter (abc) in a column do not different significantly at $P \geq 0.05$;[1]Untreated control group;[2] Group treated with Levamisole at the dose rate of 7.5 mg/Kg body weight of animals;[3]*Adhatoda vesica* used as crude powder, aqueous extract and methanol extracts @ 1, 2 and 3 g/Kg body weight of animals

For *in vitro* studies, *Haemonchus contortus* proved to be a good test worm because of its longer survival in PBS. By virtue of its longer survival, more number of observations was recorded on the motility of worms. This worm and some other Strongylids have previously been used for *in vitro* studies by some workers (Sharma *et al*., 1971; Prakash *et al*., 1980; Amorium *et al*., 1998; Asuzu & Njoku, 1996; Sangwan & Sangwan, 1998). *In vivo* tests no doubt give more reliable data, but they require greater amount of compound, large number of animals and much time. The method described above is simple and economical. Worms from few animals are sufficient to test many drugs and their concentrations and only a little amount of chemical compound/plant extract is required. Moreover, no previous toxicity tests are necessary. Although, *in vitro* tests upon parasites in the blood or tissues are not justified, theoretically this method can be used for screening compound/plant extracts against intestinal worms. Since these live in the lumen of gut, the drugs which have been given by mouth reach the parasite in the intestine without much opportunity for chemical modification. It is, however, true that no single chemotherapeutic test can be guaranteed to detect 100% of the compounds/plant extracts. But as a compromise between time, expense and labor the test used in the current study is good.

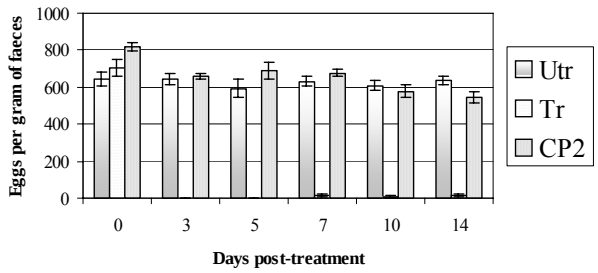
In vivo, maximum reduction (37.4%) in EPG was recorded in sheep treated with *A. vesica* CAE @ 3 g Kg^{-1} b.w. followed by CP @ 2 g (33.05%) and CME @ 3 g (25.6%). It is evident from the results (Table III; Figs. 1, 2 and 3) that CAE had higher activity compared with the CP and CME forms, which may be considered as an indication for the presence of water soluble active principles in *A. vesica* responsible for anthelmintic activity. The higher anthelmintic activity of CAE is consistent with the *in vitro* findings as well. A trend of higher anthelmintic activity was found at higher doses of *A. vesica*. The leaves of *A. vesica* are prescribed in the folk medicine to treat gastrointestinal worm infections and have been listed as an anthelmintic in Pharmacopoeia of Eastern Medicine (Said, 1969). The powdered leaves @ 2 g Kg^{-1} body weight were found to

Fig. 1. Effect of *A. vesica* used as crude powder on gastrointestinal nematodes of sheep compared with levamisole treated and untreated animals

(a) 1 mg Kg^{-1} body weight



(b) 2 mg Kg^{-1} body weight



(c) 3 mg Kg^{-1} body weight

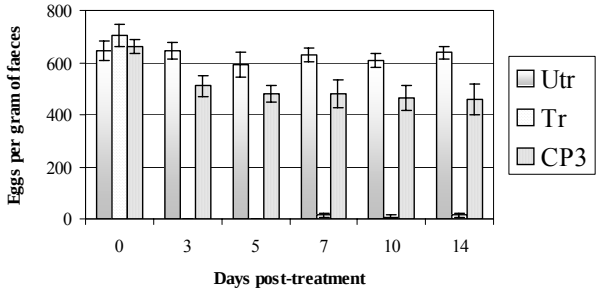


Fig. 2. Effect of *A. vesica* used as crude aqueous extract on gastrointestinal nematodes of sheep compared with levamisole treated and untreated animals

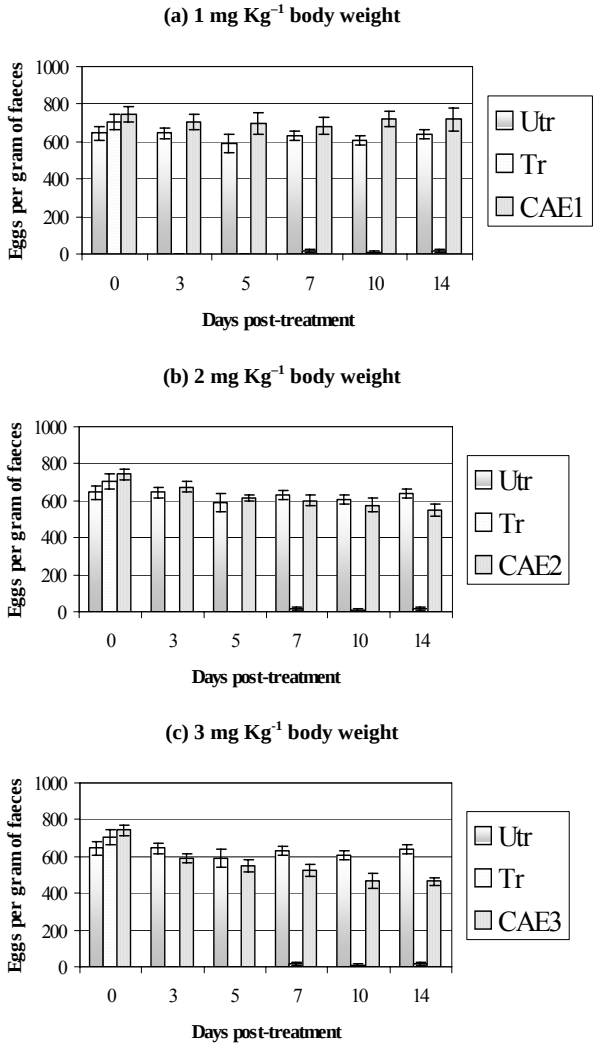
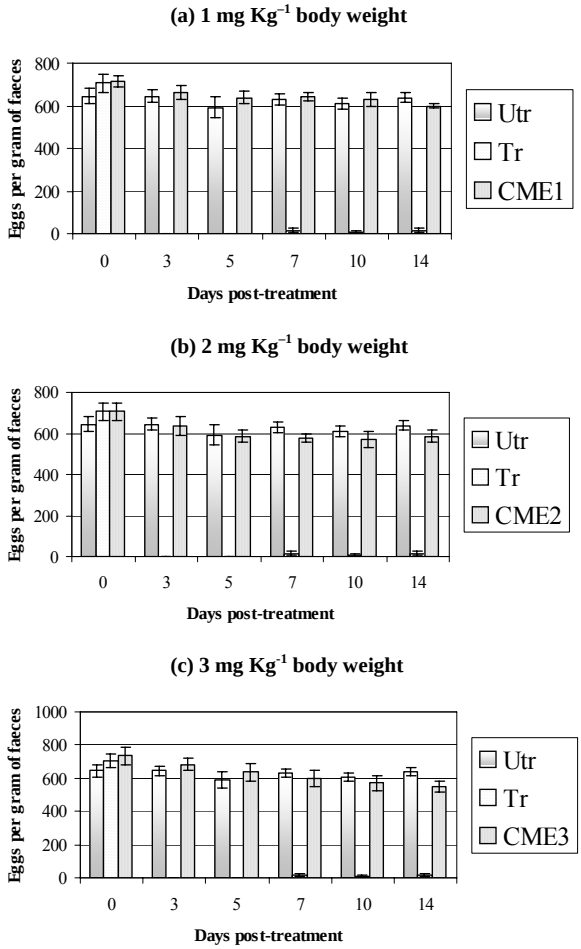


Fig. 3. Effect of *A. vesica* used as crude methanol extract on gastrointestinal nematodes of sheep compared with levamisole treated and untreated animals



result in 62±5.4% reduction in EPG in goats (Akhtar, 1988).

The chemical examination of *A. vesica* revealed to contain alkaloids, glycosides, phenolic components and sterols (Jain, 1968; Akhtar, 1988; Pandey *et al*., 1997). The major constituents identified, however, are vasicine and vasicinone (Brain & Thapa, 1983; Chauhan *et al*., 1999; Shaifali-Srivastava *et al*., 2001) and saponins (Akhtar, 1988). The vasicine content of the plant, responsible for the oxytocic and abortifacient effects of *A. vesica* has, therefore, created doubts about the safe use of this plant in herbal medicine (cited in review by Claeson *et al*., 2000).

The anthelmintic activity of *A. vesica*, as evident from the results of current study and some previous research may be attributed to the tonic and stimulatory effects of the plant. For example, Pandey *et al*. (1997) reported that *A. vesica* had a mild stimulatory effect on the isolated uterus of rat, collected during the metoestrus phase of the oestrous cycle; marked rhythmicitic effect on the uterus collected during the oestrus phase; and on the isolated full-term gravid uterus of rats. *A. vesica* markedly increased the tone of spontaneously motile tissue. Based on its tonic effects, *A. vesica* has also been used as uterotonic in domestic animals (Pandey *et al*., 1997)

In the light of above mentioned pharmacological effects, it may be concluded that the alkaloid content of *A. vesica* may improve tonicity of the gastrointestinal tract and thus expel the worms or may have a direct effect on the nervous system of nematodes. This speculation is supported by the varying rates of effectiveness of *A. vesica*, which may be due to varying contents of total alkaloids in differently aged plants (Rajani & Pundarikakshudu, 1996). This could be the reason that in the current study, the anthelmintic activity of the *A. vesica* was having a dose related trend in anthelmintic effect.

CONCLUSIONS

It is concluded that *A. vesica* roots do possess anthelmintic activity. It is, however, suggested that further research on large scale be carried out on large number of animals on higher doses than those used in the current study, identification of active principles, and standardization of dose and toxicity studies for drug development.

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(Received 10 October 2002; Accepted 20 November 2002)