ANTHELMINTIC ACTIVITY OF *CARUM CAPTICUM* SEEDS AGAINST GASTRO-INTESTINAL NEMATODES OF SHEEP

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ABSTRACT

This paper describes in-vivo anthelmintic activity of *Carum copticum* in comparison with levamisole. For in-vivo studies, the seeds of *Carum copticum* was administered as crude poweder (CP), Crude equeous extract (CAE) and crude methanol extract (CME) at graded doses (1,2 and 3 g kg^{-1}body weight (b.w.) to sheep naturally infected with mixed species of gastrointestinal nematodes. Maximum reduction (79.1%) in eggs per gram (EPG) of faeces was recorded on day 14 post treatment in sheep treated with *Carum copticum* CP at 3g kg^{-1}b.w. It was found that although, *carum copticum* seeds possesses anthelmintic activity against nematodes, it is not comparable with levamisole (99.2% reduction in EPG) at any of the doses tried in this study. However, increase in EPG reduction was noted with an increase in the dose of *Carum copticum* administered as CP, CAE and CME. The graded dose response suggested further studies on a larger number of animals on higher doses than those used in the current study.

Key Words: Anthelmintic, Carum copticum, levamisole crude aqueous extract, crude methanol extract.

INTRODUCTION

Helminths are recognized as a major constraint to livestock production throughout the tropics and elsewhere (Waller, 1987; Ibrahim *et al.,* 1984). Imported manufactured Anthelmintics have long been considered the only effective way of controlling parasitic infection. However, as these are very expensive and often not available to farmers in rural areas, livestock producers have continued to use indigenous plants as dewormers. Furthermore, some serious disadvantages of using manufactured drugs are development of resistance in helminthes (Waller and Prichard, 1985) against anthelmintics and chemical residues and toxicity problems (Kaemmerer and Butenkotter, 1973). This has led to an increased need for research into alternative therapeutic agents for the treatment and control of helminth infections. An estimated 80 percent of the populations of developing countries depend on traditional medicines for primary health care (Plotkin, 1992).

The plants are known to provide a rich source of botanical anthelmintics, antibacterial and insecticides Hammond *et al.,* 1997; Akhtar *et al.,* 2000). A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarni, 1954; Chopra *et al.,* 1956; Said, 1969). The anthelmintic activity of *Carum copticum* seeds against human *Ascaris lumbricoides* is appreciable (Kalesaraj, 1974). This paper reports the results in vivo study on anthelmintic activity of *C. copticum* seeds against gastro-intestinal nematodes of sheep.

MATERIALS AND METHODS

The seeds of *C. copticum* were purchased from local market (Faisalabad, Pakistan), identified and authenticated by Department of Botany, University of Agriculture, Faisalabad, Pakistan. The seeds were dried in shade, finally ground in electric grinder and stored in cellophane bags at 4°C until use.

Aqueous extract preparation: The crude acqueous extract (CAE) of *C. copticum* was prepared according to standard methods (Onyeyili *et al.,* 2001). One hundred grams of the powered plant material was mixed with 500 ml of distilled water in 1 L flask and boiled for 90 minutes. It was allowed to cool to 40°C and then filtered using Whatman # 1 filter paper. The filtrate was then concentrated in rotary evaporator and the extract stored at 4°C until required.

Methanol extract preparation: Powdered plant material was exhaustedly extracted with methanol in Soxhlet apparatus. The crude methanol extract (CME) was evaporated to dryness and stored at 4°C until used.

***In vivo* anthelmintic activity:** A total of 44 sheep of both sexes (female and male young stock $\leq$ 1 year), weighing 18-24 kg were used for *in vivo* trails conducted at the Livestock Experimental Station, Rakh Kherewala (Punjab, Pakistan). Before the start of experiment, the animals were confirmed to be naturally infected with gastrointestinal nematodes by qualitative and quantitative fecal examination using standard parasitological procedures (Soulsby, 1982). Identification of nematode eggs in the feces was done using standard description of

MAFF (1979). The selected animals were suffering from mixed gastrointestinal nematodes species including mainly: *Haemonchus contortus, Trichostrongylus colubriformis, T.axei, Oesophagostomum, Strongyloides papillosus and Trichuris ovis.*

The sheep (n = 44) used for the 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 orally with levamisole HC1 (Nilverm 1.5% (w/v); ICI Pakistan Limited. Animal Health Division) at 7.5 mg kg^{-1} body weight (b.w) as single dose.

Group 3: Treated orally with single dose of CP of *C.copticum* at 1 g kg^{-1} b.w.

Group 4: Treated orally with single dose of CP at 2 g kg^{-1} b.w.

Group 5: Treated orally with single dose of CP at 3 g kg^{-1} b.w.

Group 6: Treated orally with single dose of CAE of *C.copticum* at the equivalent dose rate 1 g kg^{-1} b.w.

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

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

Group 9: Treated orally with single dose of CME of *C.copticum* at the equivalent dose 1 g kg^{-1} b.w.

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

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

Faecal 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-tgreatment (PT) and were examined for the presence of worm eggs by salt floatation technique (MAFF, 1979). The EPG were counted by the McMaster egg counting method (Soulsby, 1982). Egg count (EC) percent reduction (ECR) was calculated using the following formula:

$$\text{E.C. Reduction \%} = \frac{\text{Pre-treatment EPG- Post treatment}}{\text{Pre-treatment EPG}} \times 100$$

The data were statistically analyzed using SAS software (SAS, 1998).

RESULTS AND DISCUSSION

The results of experiment have been presented in *Table 1*. There was a reduction ($P \leq 0.05$) in EPG counts on day 7 PT onward in sheep treated with *carum copticum* CP @ 1 g Kg^{-1} At 2 g CP, there was no reduction ($P \leq 0.05$) in EPG. At 3 g CP, however, EPG reduced ($P \leq 0.05$) from day 3 PT onward. The maximum reduction (78.1%) in EPG was recorded on day 5 PT in sheep treated with *carum copticum* CP @ 3 g.

There was reduction ($P \leq 0.05$) in EPG counts in sheep treated with *Carum copticum* CAE @ 1 g Kg^{-1} on day 3,7,10 and 14 PT but no day 5 PT indicating some fluctuation. Al 2 kg CAE, there was a reduction ($P \leq 0.05$) in EPG on day 7 PT onward. The maximum reduction (53.3%) in EPG was recorded on day 10 PT in sheep treated with *carum copticum* CAE @ 3 g Kg^{-1}. There was no reduction ($P \leq 0.05$) in EPG in sheep treated with *Carum copticum* CME @ 1, 2 and 3 g, rather an increase ($P \leq 0.05$) was recorded in EPG on day 10 and 5 PT @ 1 and 3 g, respectively.

In vivo, the maximum reduction (78.1%) in EPG was recorded in sheep treated with *Carum copticum* CP @ 3 g followed by CAE @ 3 g (53.3%. No anthelmintic effect of *Carum copticum* was observed in its CME, rather an increase was recorded in EPG on higher dose levels. It is evident from the results (Table-1) that CP had higher activity as compared with the CAE form, which may be considered as an indication for a synergistic effect of various chemical constituents in *Carum copticum* responsible for anthelmintic activity. A trend of higher anthelmintic activity was found at higher doses of *Carum copticum.* In case of CME, however, increasing dose levels resulted in an increase in EPG. Although, *Carum copticum* exhibited good anthelmintic activity alongwith fluctuation in EPG counts among different days post-treatment in sheep treated with *Carum copticum* CAE @ 1 and 2 g need further investigation.

The anthelmintic activity of *Carum copticum* has been previously reported (Kranz and Carr, 1967) against human *Ascaris lumbricoides* (Kalesaraj, 1974). Seeds of *Carum copticum* are commonly used for different purposes as alcoholic extracts (Kalesaraj, 1974) and essential oil (Srivastava *et al.,* 1999). The essential oil of the *Carum copticum* seed contains about 50% of the antiseptic thymol (Uma *et al.,* 1993; Krishnamoorthy and Madalageri, 1999; Nagalakshmi *et al.,* 2000), and has relatively high calcium and iron contents (Uma *et al.,* 1993). The essential oil in *Carum copticum* fruit has been found to contain 11 components with carvacrol (45.2%) and delta-cymene (41.98%) as the major constituents (Srivastava *et al.*,1999). Nagalakshmi *et al.* (2000) reported volatile oil of *Carum copticum* to contain 17 constituents of which thymol (39.36%), gamma-terpinene (30.97%), rho-cymene (19.47%) and beta-pinene (5.45%) and alpha-pinene (1.48%) were the major constituents. The yield of the oleoresin was 24.66%, containing 12.15% volatile oil and 87.85% non-volatile material. In another research, the essential oils obtained by steam distillation of the immature and mature seeds of *Trachyspermum roxburghianum* were found to contain 32 compounds accounting for 99.6 and 99.0% of the total yields, respectively. The main component was limonene which made up 40.9-47.2% of the bulk of the respective oils. Other notable constituents in oil from immature and mature seeds were sabinene (20.3 and 8.5%,

respectively), terpinen-4-ol (8.6 and 12.2%, respectively),(Z)-legustilide (5.1 and 8.0%, respectively) and gamma-terpinene (5.6 and 6.7%, respectively).

The anthelmintic activity of *Carum copticum* may be attributed to the antiseptic and immunostimulatory effects of thymol content of seeds (Uma *et al*., 1993; Krishnamoorthy and Madalageri, 1999; Nagalakshmi *et al.*, 2000).

It may be concluded that *C. copticum* seeds considerably possesses anthelmintic activity. Therefore, its use in the ethno-veterinary system of Pakistan as an anthelmintic is justified. It is however, suggested that further research on large scale be carried out on a large number of animals at higher doses.

Table I. Effect of *Carum copticum* administration on Eggs per gram (Mean ± SEM) of faeces in sheep naturally infected with nematodes

Day PT	**Control**		**Crude Powder[3]**			**Crude Aqueous Extract[3]**			**Crude Methanol Extract[3]**		
	Group 1 UnTreated[1]	Group 2 **Treated**[2]	Group 3 **1.0g**	Group 4 **2.0g**	Group 5 **3.0g**	Group 6 **1.0g**	Group 7 **2.0g**	Group 8 **3.0g**	Group 9 **1.0g**	Group 10 **2.0g**	Group 11 **3.0g**
0	1042.5±33.2^{a}	1020.0±32.4^{a}	945.0±43.3^{a}	1215.0±181.8^{a}	1005.0±43.3^{a}	990.0±17.3^{a}	1335.0±77.9^{b}	900.0±121.2^{a}	1035.0±25.9^{b}	1162.5±132.6^{ab}	1125.0±115.2^{b}
3	1012.5±39.4^{a} (2.9)	0^{b}(100)	960.0±34.6^{a} (-1.5)	1200.0±155.8^{a} (1.2)	285.0±8.6^{c} (71.6)	915.0±17.3^{b} (7.5)	1335.0±8.6^{a} (0)	675.0±164.5^{abc} (24.9)	1050.0±34.6^{b} (-1.4)	1087.5±84.2^{ab} (6.4)	1110.0±94.0^{b} (1.3)
5	982.5±28.3^{a} (5.7)	7.5±7.5^{b} (99.2)	885.0±43.3^{ab} (6.3)	1035.0±164.5^{a} (14.8)	210.0±17.3^{d} (78.1)	900.0±86.6^{abcd} (9.1)	1080.0±34.6^{c} (19.1)	705.0±164.5^{ab} (21.7)	1050.0±86.6^{ab} (-1.4)	1290.0±106.7^{a} (-11.0)	1650.0±163.8^{a} (-46.6)
7	1075.0±51.7^{a} (3.1)	0^{b}(100)	750.0±17.3^{c} (20.6)	1020.0±225.1^{a} (16.0)	375.0±8.6^{b} (61.7)	815.0±49.0^{cd} (17.7)	1020.0±17.3^{c} (23.6)	465.0±60.6^{cd} (48.3)	1065.0±25.9^{b} (2.9)	1042.5±111.1^{b} (10.3)	1530.0±271.6^{a} (-35.9)
10	1055.0±37.5^{a} (1.2)	7.5±7.5^{b} (99.2)	810.0±34.6^{bc} (14.3)	1005.0±199.1^{a} (17.3)	360.0±17.3^{b} (63.1)	705.0±60.6^{de} (28.7)	1140.0±138.5^{bc} (5.6)	420.0±51.9^{d} (53.3)	1170.0±51.9^{a} (-13.0)	1110.0±42.4^{b} (4.5)	1365.0±295.0^{ab} (-21.3)
14	1032.5±53.7^{a} (0.9)	7.5±7.5^{b} (99.2)	765.0±25.9^{c} (19.0)	990.0±173.2^{a} (18.5)	285.0±25.9^{c} (71.6)	660.0±51.9^{e} (33.3)	1020.0±51.9^{c} (23.6)	435.0±8.6^{d} (51.7)	1080.0±86.6^{ab} (-4.3)	1095.0±61.8^{b} (5.8)	1350.0±271.6^{ab} (-19.9)

PT= Post-treatment; Means marked with the same letter (abc) in a column do not differ significantly at P ≥ 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]*Carum copticum* used as crude powder, aqueous extract and methanol extracts @ 1, 2 and 3 g/kg body weight of animals respectively.

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