Anti-Nematodal Efficacy of Ivermectin (Oral) and Extracts of Coriandrum sativum in Sheep in Barani Livestock Production Research Institute (BLPRI) Kherimurat, District Attak Punjab Pakistan

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Abstract: Anthelmentic resistance (AR) is one of the major problems of livestock industry in developing countries. Using indigenous medicinal plants may be an alternate way to overcome this problem. Total 60 sheep nematode positive were selected and divided into 6 groups having 10 sheep each. Group A as un-treated control, group B was given Ivermectin at the dose rate of 0.2mg/kg body weight Group C and Group D was given the Chloroformic extract while Group E and F was given the Methalonic extract of Coriandrum sativum whole plant at the dose rate of 50 mg/kg and 100 mg/kg body weight respectively. After treatments, fecal eggs count reduction test (FECRT) (eggs/g) was carried out on day 7, 14 and 28. The EPG in untreated group at day 28 was increased 21.6%. Significant decrease in EPG was only observed at day 28 in Ivermectin treated group (92.6%) and Methanolic extract at a dose rate of 100 mg/kg body weight (60.69%). No significant results were recorded in remaining groups. It was concluded that 100 mg/kg body weight Methalonic extract of Coriandrum sativum would be a best alternative as anti-nematodal drug.

Keywords: Anti-nematodal efficacy, Ivermectin, Coriandrumsativum, sheep, Punjab.

INTRODUCTION

Helminthiasis is one of the most important problems worldwide in reflecting heavy production losses in grazing animals. The disease is especially prevalent in developing countries in association with poor management practices and inadequate control measures [1]. An integrated approach for the control of helminthes includes strategic use of anthelmintics and careful management of the grazing lands are required [2]. The use of the anthelmintics has been done for many decades to obtain optimal performance in ruminant livestock production. The anthelmintic practice typically relies on the use of highly efficacious broad spectrum anthelmintics [3].

A great variety of anthelmintics are available for the treatment of helminthes in sheep belonging to the tetrahydropyrimidine, benzimidazole, probenzimidazole and imidazothiazolegroups [4]. However, some have low activity against the nematodes while others show variable degrees of efficacy depending on the dose rate used and sometimes double or triple dose and repeat treatments are required. Benzimidazoles, imidazothiazoles and ivermectins/milbemycins are the most important anthelmintics groups used for the control of helminthes in ruminant species [5].

The introduction of ivermectin and milbemycin endectocides has resulted in a dramatic reduction in the incidence of clinical parasitic gastroenteritis and bronchitis in first year grazing cattle. It is looked on as one of the dose-limiting species for the current endectocide anthelmintics [6]. Moxidectin was the only drug that was effective on all farms tested (mean reduction in fecal eggs count = 99%) in USA. Moxidectin is a synthetically derived macrocyclic lactone molecule from the milbemycin group that is commercially available in several formulations to treat parasitic infestations in cattle, dogs, horses and sheep. It has a broad spectrum of endectocidal activity and is widely used in many species of animals. The macrocyclic lactones i.e. ivermectin and moxidectin acts as agonists of a family of invertebrate-specific inhibitory chloride channels that are activated by glutamic acid. Moxidectin has been available as a pour on formulation and an aqueous-based injectable formulation for cattle for many years [2].

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Development of anthelmentic resistance to commercially available drugs has become a serious problem. In Pakistan, one of the important factors of high prevalence of nematodes in goats may be the treatment failure with the commonly used anthelmintics. The increasing prevalence of anthelmintic resistant strains of helminthes, drug residues in animal products and high cost of conventional anthelmintics have created an interest in studying medicinal plants as an alternative source of anthelmintics [7]. Coriandrum sativum which belongs to family Umbelliferae is highly reputed ayurvedic medicinal plant commonly known as the Dhanyaka. It contains monoterpenes, α-pinene, limpnene, γ-terpinene, p-cymene, borneol, citronellol, camphor, geraniol, coriandrin, dihydrocoriandrin, coriandronsA-E, flavonoids and essential oils. Various parts of this plant such as seed, leaves, flower and fruit, possess diuretic, antioxidant, antidiabetic, anticonvulsant, sedative, hypnotic, antimicrobial, antimutagenic and anthelmintic activity [8]. Therefore in this study we compare the anthelmentic activity of Ivermectin (oral) and Chloroformic and Methanolic extracts of Coriandrum sativum whole plant.

MATERIALS AND METHODS

Sixty sheep positive for nematodes were randomly selected from the Barani Livestock Production Research Institute (BLPRI) Kherimurat, District Attak (Punjab). Experimental animals were divided into 6 groups (Group A, Group B, Group C, Group D, Group E and Group F) having 10 sheep in each. Group A was un-treated control. Group B was given Ivermectin (orally), Group C and D was treated with Chloroformic extract of Coriandrum sativum whole plant at 50 and 100 mg/kg body weight while Group E and F was treated with methalonic extract of Coriandrum sativum whole plant at 50 and 100 mg/kg body weight respectively.

Preparation of Plant Extract

The Chloroformic extract of the Coriandrum sativum whole plant was prepared by taking the 50g powder of Coriandrum sativum plant and rinsed it with 500ml of chloroform taken in the flask within the Soxhlet apparatus heated up to 60°C [9]. For methanolic extract Coriandrum sativum plant three grams (3 g) of dried and ground spearmint plant was placed in a soxhlet apparatus. Extraction was performed with 150 ml of methanol solvent for 6 hr. After extraction, arotary vacuum evaporator at 40^{o}C was used in order to remove solvent [10].

Sample Collection

Fecal samples were collected directly from rectum on 0, 7, 14 and 28 day post treatment.

McMaster Egg Counting Technique

Fecal egg count (eggs/g) was done by using the modified McMaster technique by mixing 2gm of feces in 28 ml of saturated salt solution [2]. The McMaster chamber was observed under the 100x magnification. The number of eggs was calculated in the chamber by performing the following calculations.

EPG = Total number of calculated eggs in one of the chamber $\times 100$.

EPG = Eggs per gram in one of the chamber.

Calculations

Percentage efficacy will be calculated by applying the following formula:

$$\text{Percent efficacy} = \frac{a-b}{a} \times 100$$

Where, a = EPG premedication.

and b = EPG Post medication.

RESULTS

Mean EPG $\pm$ Standard Error of the Group A on day 0 was 1740.00 $\pm$ 191.02, on day 7 was 1815 ±153.8, on day 14 was 2025 ±169.8 and on day 28 was 2220 ±133.1. Percent increase in EPG on day 7 was 4.13%, and on day 14 and day 28 was 14.07 % and 21.6 % respectively. Group B was given the standard drug Ivermectin (Ivotek drench) at a dose rate of 0.2mg/kg body weight orally. The Mean EPG $\pm$ Standard Error of the Group B on day 0 of the treatment was 1910.00 $\pm$ 210.0, on day 7 was 355 ± 30.2, on day 14 was 245 ± 15.7 while on day 28 was 140 ± 16.3. The percent efficacy of the Group B was calculated and on day 7 was 81.4 %, on day 14 was 87.17 % and on day 28 was 92.60 % post treatment.

Group C were treated with the Chloroformic extract of the Coriandrum sativum plant at a dose rate of 50 mg/kg body weight by oral route. The Mean EPG $\pm$ Standard Error of the Group C on day 0 was 1655.00 $\pm$ 124.0, on day 7 was 1855 ± 137.5, on day 14 was 1930 ± 170.0 while on day 28 was 2135 ± 192.0. There was increase in EPG on day 7 post treatment 10.7 %, on

Table 1: Effect of Medication on Egg per gram (fecal EPG) on Day 0, Day 7, Day 14 and Day 28

Name of medicine	Dose mg/kg	Average EPG ±S.E				% change in EPG		
		Day 0	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Control	Nil	1740 ±191	1815 ±153.8	2025 ±169.8	2220 ±133.1	4.13↑	14.07↑	21.6↑
Ivermectin (oral)	0.2	1910 ±210	355 ±30.2	245 ±15.7	140 ±16.3	81.4↓	87.17↓	92.6↓
Chloroformic extract of CS	50	1655 ±124	1855 ±137.5	1930 ±170	2135 ±192	10.7↑	14.24↑	22.48↑
Chloroformic extract of CS	100	1625 ±98.6	1820 ±85.7	1975 ±79.6	2150 ±83.6	10.7↑	17.72↑	24.41↑
Methanolic extract of CS	50	2210 ±160	1750 ±112	1610 ±129.7	1470 ±125.2	20.81↓	27.14↓	33.48↓
Methanolic extract of CS	100	2150 ±201	1080 ±76.4	940 ±75.2	845 ±83.8	49.76↓	56.27↓	60.69↓

EPG = egg per gram, S.E Standard error, ↑=increase in EPG, ↓=decrease in EPG.

day 14 and day 28 percent increase in EPG was 14.24 % and 22.48 % respectively. No effect was recorded at a dose rate of 50 mg/kg body weight.

The Group D was treated with Chloroformic extract of the Coriandrum sativum at a dose rate of 100 mg/kg body weight. The Mean EPG ± Standard Error of the Group D on day 0 was 1625.00 ± 98.6, on day 7 was 1820 ± 85.7, on day 14 was 1975 ± 79.6 and on day 28 was 2150 ± 83.6. Percent increase in EPG on day 7 post treatment was 10.7 %, on day 14 and day 28 was 17.72 % and 24.41 % respectively. No effect was recorded at a dose rate of 100 mg/kg body weight.

Group E animals were treated with the Methanolic extract of the Coriandrum sativum plant at a dose rate of 50 mg/kg body weight orally. Mean EPG ± Standard Error of the Group E on day 0 was 2210.00 ± 160.0, on day 7 was 1750 ± 112.0, on day 14 was 1610 ± 129.7 and on day 28 was 1470 ± 125.2. Percent efficacy of 50 mg/kg body weight Methanolic extract of Coriandrum sativum was 20.81 % on day 7, on day 14 was 27.14 %, while on day 28 was 33.48 %. Group F animals were treated with 100 mg/kg body weight Methanolic extract of Coriandrumsativum. Mean EPG ± Standard Error on day 0 was 2150.00 ± 201.0, on day 7 was 1080 ± 76.4, on day 14 was 940 ± 75.2 while on day 28 was 845 ± 83.8. Percent efficacy of 100 mg/kg body weight Methanolic extract of Coriandrum sativum was 49.76 % on day 7, on the day 14 was 56.27 %, while on day 28 was 60.69 % (Table **1**).

By comparing the Groups no significant difference was found between control and group C and D treated with Chloroformic extract of the Coriandrum sativum plant at a dose rate of 50 and 100 mg/kg body weight orally P=0.615 and P=0.679. Significant difference was recorded between control and Ivermectin treated group P=0.000 and group E and F treated with Methanolic extract of Coriandrum sativum plant at a dose rate of 50 and 100 mg/kg body weight orally P=0.000 and P=0.000 respectively (Table **2**).

DISCUSSION

In present study 100 mg/kg body weight Methanolic extract of Coriandrumsativum have good antianthelmintic effect although 50 mg/kg body weight

Table 2: Multiple Comparisons between Control and Different Treatment Group at Day 28

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
Control	Ivermectin 2 mg	2080.000*	168.033	.000*
	Chloroformic extract 50 mg	85.000	168.033	.615
	Chloroformic extract 100 mg	70.000	168.033	.679
	Methanolic extract 50 mg	750.000*	168.033	.000*
	Methanolic extract 100 mg	1375.000*	168.033	.000*

(Significant: p-value < 0.05*).

Methanolic extract of Coriandrumsativum also show some percent decrease in EPG. Similarly the hydro-alcoholic and crude aqueous Coriandrum sativum extract have good anti-nematodal effect along with egg hatching inhibition. In some studies Zanthoxylumzanthoxiloides leaves was given at the dose rate of 2.4mg/ml in the form of alcoholic extract on 30% control egg hatching of Haemonchuscontortus up to 40% [11]. Similarly the Ocimumgratissimum essential oil was used at 0.5% concentration and controls the egg hatching up to 100%. In one study the Coriandrumsativum did not show any significant results against nematodes. We know that helminth take substance the non-nutrient or the non electrolyte through cuticular diffusion method. Due to this the oral ingestion is mainly opposed by many parasites as all the broad spectrum anthelmintics are these cuticular diffusion methods [12]. In this study we compare the chloroformic and methanolic extract of Coriandrumsativum to the standard Ivermectin drug and methanolic extract show better results at both dose rates. This may be due to better trans-cuticular transport of methanolic extract than the chloroformic extract. Similarly the aqueous and hydro alcoholic extract of Coriandrum sativum seed antianthelmintic activity was checked and found good results of hydro alcoholic extract than aqueous due to the presence of non-polar organic molecules [13], which give it high lipid solubility and best anthelmintic activity as compare to aqueous extract. Lipid soluble anthelmintics have high capability of cuticular absorption as compare to water soluble [12].

Similarly the Artemisia brevifoliamethanolic extract was used *in vitro* against adult H. contortus at 25mg/ml concentration and gives best results as compare to aqueous extract which show no significant results [14]. In these studies for aqueous and hydro alcoholic extract the dose effect was not checked but such type studies have also been reported earlier [11]. The aqueous extract of Coriandrum sativum efficacy was checked against Haemonchus contortus. After two days of treatment its effect was not so stronger which may be due to high concentration of extract in early days and its effect get looses in later days [15]. Higher efficacy was observed if extract was given at higher dose or giver at repeated doses [16]. Similar was the case in this study, when animals were treated with higher dose of Coriandrum sativum methanolic extract gives significant results. It was concluded that methanolic extract of Coriandrum sativum would be the best alternative as antinematodal drug.

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