



In vivo comparative efficacy of indigenous plants against *Trichostrongylus* of Sheep in District Zhob, Balochistan

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Abstract

The crude aqueous methanolic extract of the medicinal plants, collected from different areas of district Zhob, the north-east region of Balochistan, Pakistan, were used for *in-vivo* studies against Trichostrongylids in sheep. For this purpose, eighty sheep of either sex, aged between three to six months and naturally infested with Trichostrongylids nematodes (*Trichostrongylus* spp. *Haemonchus contortus*, *cooperia* spp. etc) were selected and managed separately for the experiment. These sheep were divided into 4 groups. Group A contained 10 sheep and was kept as untreated control. Thirty (30) sheep kept in group B, were further divided into three equal sub groups i.e. B1, B2 and B3 and treated with three different dose levels of *Chenopodium album* @ 1, 2 and 3 g/kg body weight respectively. Group C having thirty (30) sheep was also divided into three equal sub groups i.e. C1, C2 and C3 and treated with three different levels of *Artemisia brevifolia* @ 1, 2 and 3g/kg body weight respectively. Group D, contained 10 sheep, was treated with Levamisole™ (ICI) @ 7.5mg/kg body weight. Faecal egg count reduction was criterion for evaluation. Faecal samples were collected before treatment on day 0 and on day 3, 5, 7, 10 and 14 post treatments. Best faecal egg count reduction results (87.08%) were observed in Levamisole™ (ICI) treated group as compared to *Artemisia brevifolia* treated group (66.88%) at the dose level of 3gm/kg body weight followed by *Chenopodium album* treated group (51.03%) at the dose level of 3gm/kg body weight. It was concluded that the crude aqueous methanolic extract of the medicinal indigenous plants of Zhob region are not sufficient for any anthelmintic effect as compared to synthetic medicines.

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Introduction

Balochistan is the largest province of Pakistan with a total land mass of 347190sq. km. Out of the total land area almost 92% consists of arid grazing lands, barren Rocky Mountains and deserts. Balochistan has diverse topography in elevation ranging from sea level to 3520 meters. Physiographic units are mountain ranges (52.6%), alluvial fans (21.9), piedmont plains (11.8%), sand plains (7.6%), valley bottom (3.3%), tidal plains (0.8%) and 2.0% plains (Saleem and Ashiq, 2000). The average annual rain fall varies from less than 50 mm in the southwest to above 400 mm in northeast. Cold winters and mild summers characterize the north region including District Zhob. Most winters receive snow, frost and rainfall ranging between 250 to 350 mm. In the southwestern desert region, the annual rainfall ranges between 50 to 125 mm and the region experiences the hottest summer with temperatures rising occasionally to over 51°C. Animals are mostly dependant on open grazing.

The economy of Balochistan is dominated by agriculture, including livestock and fisheries. It accounts for 52% of the province's GDP and employs 65% of the labour force. Of the total agriculture GDP, livestock contributes 40%, fruit 30%, field crops 17%, vegetables 12% and fisheries 1% (Anonymous, 2004-2005). The nomadic people solely depend on livestock whereas; the transhumants and sedentary people derive up to 40% income from livestock. Sheep population estimated at 27.4 million heads in Pakistan (Anonymous, 2008-09) plays an important role in the national economy. 12.8 million (48%) of these, respectively are raised in Balochistan (Anonymous, 2006). In addition to food of high protein value, products such as skin, wool, hair, pellets and goods made from them are a big source of earning foreign exchange.

In developing countries like Pakistan, heavy economic losses in livestock sector have been reported due to parasitic diseases. These parasitic diseases are one of the principal problems in the development of livestock industry. Factors like constant exposure to parasitic infection, variable climatic conditions lack of knowledge on the part of livestock owners regarding parasitic infection play an important role in the development of ecto and endo parasites (Durani, 1991).

Throughout the world, internal parasites pose one of the major health limitations for grazing animals. Sheep and goats are more susceptible to internal parasites than other livestock, due to their grazing behavior and poor immunity (Martin 1983). The impact of parasitic diseases varies greatly between countries and between regions, depending on climate and the intensification of farming in the area (Radostits *et al.*, 1994). Parasites exert adverse effects on the health and productivity of animals in Pakistan (Javed *et al.*, 1992). Khan *et al.* (1988) reported 100% sheep in upland Balochistan get infected with internal parasites.

Trichostrongylid nematodes of sheep, because of their adverse effect lead to lowered productivity, retarded growth rate and even death of lambs (FAO, 1974; Barger, 1982; Steel and Symons, 1982). The prevalence of gastrointestinal nematodes of sheep has also been reported very high (25.1 to 92%) by many workers in Pakistan (Durrani *et al.*, 1981; Mohiuddin *et al.*, 1984; Khan 1985; Iqbal *et al.*, 1993; Qayyum, 1996). Haemonchosis, caused by *Haemonchus* (H.) *contortus*, is among the top 10 most important conditions having an impact on sheep and goat production (Githiori *et al.*, 2004).

Conventionally, trichostrongylids has been tackled with use of synthetic chemicals but owing to development of anthelmintic resistance against major groups of anthelmintics viz., benzimidazole, levamisole and avermectins (Jabbar *et al.*, 2006), people are looking for alternatives to synthetic chemicals like vaccination coupled with improved management and ethnomedicinal practices. Ethnomedicine is an integral part of traditional medical practices in many countries of the developing world. A large proportion of the population uses this form of treatment for primary health care and for the treatment of ailments in their livestock (Waller *et al.*, 2001). The plants are known to provide a rich source of botanical anthelmintic, antibacterial and insecticides (Satyavati *et al.*, 1976). A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarni, 1954; Said, 1969; Akhtar *et al.*, 2000).

Development of herbal products depended upon local botanical flora with the result that different remedies tended to develop in different parts of the world. Recent surveys in the developing countries have identified many plants that have the potential to be used as anthelmintics (Jabbar *et al.*, 2006). However, majority of the evidences reported in ethnoveterinary sources are in the form of observations, rather than from controlled studies (Hammond *et al.*, 1997). There are many plants which have been validated scientifically for their anthelmintic properties based on their traditional uses (Hammond *et al.*, 1997; Iqbal *et al.*, 2001; Iqbal *et al.*, 2003; Iqbal *et al.*, 2004; Iqbal *et al.*, 2005; Iqbal *et al.*, 2006; Githiori *et al.*, 2006).

The present study was planned to evaluate the anthelmintic activity of some locally available indigenous plants against Trichostrongylid in sheep in District Zhob, Balochistan.

Materials and methods

A total of eighty naturally infected sheep age between 3-6 months were selected randomly from the study area. The gastrointestinal nematodes found in sheep were *Haemonchus*, *Trichostrongylus*, *Nematodirus* and *Cooperia* species. *Chenopodium Album*, *Artemisia brevifolia* and Levamisole™ were selected for trails on the infected sheep (Table No. 3.1). Faecal egg count reduction was criterion for evaluation. Faecal samples were collected before treatment on day 0 and on day 3, 5, 7, 10 and 14 post treatments.

Preparation of plants for medication.

The *Chenopodium album* and *Artemisia brevifolia* plants were dried under shade at ambient temperature of ground and whole plants were grinded to powder form by electrical blender and packed in air tight, dark bottles. The powdered plant material was then processed for extraction. The plant material was soaked in methanol for 2 weeks. It was filtered through fine cloth and then Whatman™ filter paper No1. Crude methanol extract of plants was prepared by rotatory evaporator. The solvent (methanol) was evaporated and the extract was obtained (Iqbal *et al.*, 2006). The powder material of plants was placed in a

cellulose thimble and extracted with 90% methanol in a Soxhlets apparatus for 8-12 hours. Solvents were removed at temperature below 50 °C in an oven. The residue (extract) of respective plant material was stored at 4 degree celcius until further used.

Experimental Design

Eighty animals were randomly divided in to eight groups (each group having 10 animals) i.e. A, B1, B2, B3, C1, C2, C3 and D. Animals in group A served as control. Animals in groups B1, B2 and B3 were treated with *Chenopodium album* at the dose rates of 1, 2 and 3 g/kg body weight respectively. Animals in groups C1, C2 and C3 were treated with *Artemisia brevifolia* at the dose rates of 1, 2, and 3 g/kg body weight, respectively. Whereas animals in group D were given Levamisole™ at the dose rate of 7.5mg/ kg body weight.

Results & discussion

Comparison of pretreatment & Post-Treatment EPG in *Chenopodium* & *Artemisia* treated groups with that of control & Levamisole treated groups

To evaluate the efficacy and anti-parasitic activity, two plants of medicinal value, *Chenopodium album* and *Artemisia brevifolia*, and one allopathic medicine Levamisole were selected. Total 80 animals were used for this experiment. Which were infested with either single or multiple parasites, which was subdivided into four groups. A Control group, *Chenopodium* treated group, *Artemisia* treated group, and Levamisole treated group.

These groups which were treated with plants were further sub-divided into three categories; one being treated with 1gm/kg body weight, 2gm/kg body weight, and 3gm/kg body weight respectively. Levamisole was given as per recommended dosage i.e. 7.5mg/kg body weight. Faecal samples were then collected and sent to laboratory, Zhob for examination for EPG, and the effect of dosage was observed. They were observed for 14 days post medication, and the effect of different dosage was observed against parasite (Table 2).

Starting with *Chenopodium* 1gm/kg body weight, EPG for the parasite was found to be at 1325 at day 0. This rate was reduced to 1250 at day 3 and at day 7 this rate was further reduced to 1125. At day 10, eggs per gm of faeces were 995 and finally at day 14 the EPG rate was observed to be at 702. Calculating% EPG Reduction from the results obtained it was found that at day 3% age was 5.66% followed by 15.09% at day 7 & 24.90% at day 10 and 47.01% at day 14. Similarly, introducing 2gm/kg *Chenopodium* into the sheep, EPG was 1280 at day 0 at day 3, 1250; at day 7, 1202; at day 10, 1001; and finally at day 14, this rate was 690. Calculations of% EPG Reduction were: 2.34% at day 3; 6.09% at day 7; 21.79% at day 10; and 46.09% at day 14 respectively.

Increasing the dosage of the plant (*Chenopodium*) at dose rate of 3gm/kg bw the results were also changed as EPG at day 0 was 1250; the rate became 1145 at day 3; 1145 at day 7; 907 at day 10; and 590 at day 14 showing that%age reduction in EPG was 2.15% at day 3; 4.97% at day 7; 24.73% at day 10; and 51.03% at day 14. From above results, it is clear that introducing 3gm of *Chenopodium* gives the best results against the nematodes. Same strategy was adapted for *Artemisia* to evaluate its effectiveness of plants against nematodes. Three groups of sheep were made and were tested for gastrointestinal nematode by

treating them with 1gm/kg body weight, 2gm, and 3gm/ kg body weight. The group treated with 1gm *Artemisia* contained 1203 EPG faeces at day 0.

Treating with 1gm *Artemisia* EPG was reduced to 1173 at day 3; 1115 at day 7 and 700 at day 10th. EPG at day 14 was recorded as 528. % age EPG Reduction was found to be as 2.49%, 7.31%, 41.81%, 56.10% at days 3, 7, 10, and 14 respectively. Introducing 2gm of *Artemisia*, at day 0, EPG was found to be at 1202, after 3 days it was found to be 1020; at day 7 it was declined to 631; at day 10 observed EPG was 546; and, at day 14 EPG was 459.

Table 1. Indigenous Medicinal Plants and allopathic anthelmintic used against *Trichostrongylid* in sheep.

SLName	Family	Part to be used	Local Name
1 <i>Chenopodium album</i>	<i>Chenopodiaceae</i>	Whole plant	Bathu
2 <i>Artemisia brevifolia</i>	<i>Asteraceae</i>	Whole plant	Afsenteen, Turkhy
3 Levamisole _{TM}	N/A	N/A	Levamisole

The percent reduction of eggs per gram faeces of sheep's sample was observed to be 15.14% at day 3; 47.50% at 7th day at day 10% EPG reduction was 54.57% and at day 14 it was found to be 61.81%.

Table 2. Mean \pm S.E for Comparison of pretreatment & Post-Treatment EPG in *Chenopodium* & *Artemisia* treated groups with that of control & Levamisole treated groups.

Treatment	Dosage	EPG Pre. Trt	Post-Treatment EPG					% EPG Reduction			
		0	3	7	10	14	3	7	10	14	
Untreated Control		1350 ± 20	1308 ± 20.6	1250 ± 17	1300 ± 14.8	1290 ± 15.5	-	-	-	-	
<i>Chenopodium</i>	(1gm/kg)	1325 ± 25.5	1250 ± 16.8	1125 ± 23	995 ± 30.1	702 ± 8.3	5.66	15.09	24.90	47.01	
	(2gm/kg)	1280 ± 22.2	1250 ± 13	1202 ± 13	1001 ± 19.7	690 ± 20.1	2.34	6.09	21.79	46.09	
	(3gm/kg)	1250 ± 17.5	1231 ± 18.9	1145 ± 25.9	907 ± 21.8	590 ± 17.2	2.33	4.97	24.73	51.03	
<i>Artemisia</i>	(1gm/kg)	1203 ± 18.9	1173 ± 26.9	1115 ± 19.9	700 ± 22.7	528 ± 10.2	2.49	7.31	41.81	56.10	
	(2gm/kg)	1202 ± 19.5	1020 ± 19.8	631 ± 23.3	546 ± 14.1	459 ± 16.7	15.14	47.50	54.57	61.81	
	(3gm/kg)	1196 ± 18.9	1079 ± 24.2	590 ± 17.2	528 ± 10.2	396 ± 25.4	9.78	24.33	57.38	66.88	
Levamisole	(7.5mg/kg)	1138 ± 25.9	681 ± 61.0	536 ± 34.5	357 ± 35.2	147 ± 25.1	40.15	52.89	68.62	87.08	

*Significant ($P < 0.05$) less than the pre-treatment values. Highly significant ($P < 0.001$) less than the pre-treatment value. Non-significant ($P > 0.05$).

Similarly *Artemisia* was tested for nematode parasite with changed dosage i.e. 3gm/kg body weight. At day 0, EPG was tested to be as 1196; on day 3, it was reduced to 1079; at day 7, it came to 590; after 10 days, EPG rate was declined to 528; and after 14th day eggs per gram faeces was reduced to 396. The % EPG Reduction was found to be 9.78% at day 3; 24.33% at day 7; 57.38% at day 10; and 66.88% at day 14. The results showed that using *Artemisia* 3gm/kg body weight is most effective than 1gm and 2gm.

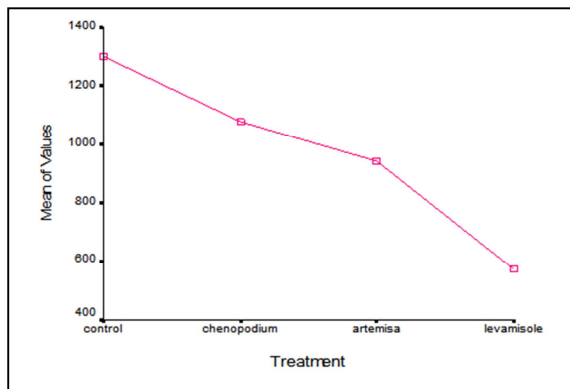


Fig. 1. Comparative efficacy of medicinal plant at low dose rate (1 g/kg) and Levamisole against nematodes.

The above line chart shows comparison of control group and the effect of *Chenopodium*, *Artemisia* and Livamisole, when 1gm of both *Chenopodium* and *Artemisia*, and 7.5mg of Livamisole was introduced into the Sheep infected from mix nematodes infestation. It is clear from the above line chart that Livamisole showed maximum results while *Artemisia* gave better results than *Chenopodium*.

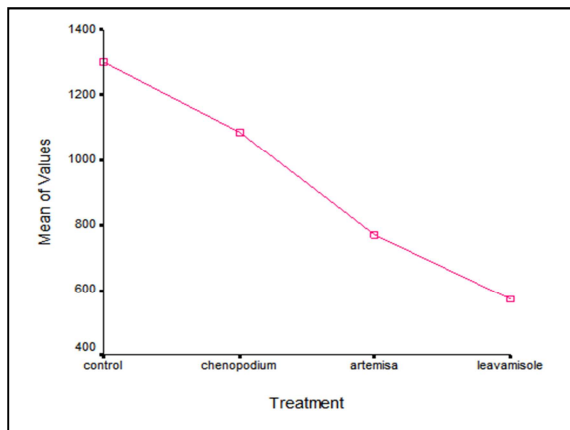


Fig. 2. Comparative efficacy of medicinal plant at the dose rate of 2g/kg and Levamisole against nematodes.

It is clear from above line chart that comparing control group with the plants and medicine, when 2gm of both *Chenopodium* and *Artemisia* was given to the sheep infested with mix nematodes infestation, *Artemisia* showed better results than *Chenopodium*. While Livamisole showed the best results at the dose rate of 7.5mg/kg body weight.

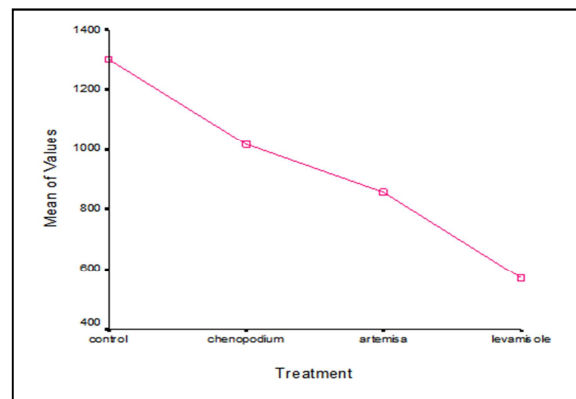


Fig. 3. Comparative efficacy of medicinal plant at the dose rate of 3g/kg and Levamisole against nematodes.

The above line chart shows that while introducing 3gm of both *Chenopodium* and *Artemisia*, and 7.5mg/kg of Livamisole, to sheep infested with mix nematodes infestation, Livamisole showed better results than *Chenopodium* and *Artemisia*, while *Artemisia* showed better results than *Chenopodium*. One group of sheep was tested with recommended Levamisole dosage i.e. 7.5mg/kg body weight against parasite 1. It was noticed that Levamisole gave best results i.e. eggs per gm faeces prior to medicament were 1138, which was reduced remarkably to 681 at day 3rd (40.15% EPG Reduction), 536 on day 7 (52.89% EPG Reduction), 357 on day 10 (68.62% EPG Reduction); and, 147 at day 14th i.e. 87.08%. Levamisole was found to be most effective 87.08% EPG Reduction against GIT Nematodes as compared to *Chenopodium* 51.03% EPG Reduction, using 3gm/kgbw and *Artemisia* 66.88% EPG Reduction using 3gm/kgbw.

Comparative efficacy of medicinal plants and Livamisole against nematodes infestation in sheep

With 1gm/kg body weight CME of *Chenopodium*, % EPG reductions were 5.66%, 15.09%, 24.90% and 47.01% at day 3rd, 7th, 10th and 14th post treatment.

Similarly, introducing 2gm/kg CME of *Chenopodium* EPG reductions were 2.34%, 6.09%, 21.79% and 46.09% at day 3rd, 7th, 10th and 14th post treatment respectively. Increasing the dosage of CME of *Chenopodium* at dose rate of 3gm/kg body weight% age reduction in EPG was 2.15%, 7.97%, 24.73% and 51.03% at day 3rd, 7th, 10th, and 14th post treatment respectively.

From above results, it is clear that introducing 3gm of *Chenopodium* gives the best results against the nematodes. In vivo, maximum reduction in eggs per gram (EPG) of faeces was recorded as 93.9% and 82.2% with *Caesalpinia crista* and *Chenopodium album* AME at 3.0g/kg, respectively (Jabbar *et al.*, 2007). Same strategy was adapted for *Artemisia* to evaluate its effectiveness against trichostrongylids nematodes. The group treated with 1gm CME of *Artemisia* % age EPG reduction was 2.49%, 7.31%, 41.81%, 56.10% at day 3rd, 7th, 10th, and 14th respectively. Introducing 2gm CME of *Artemisia*, the percent reduction of eggs per gram faeces was 15.14%, 47.50%, 54.57% and 61.81% at day 3rd, 7th, 10th and 14th post treatment, respectively. Similarly, CME of *Artemisia* was tested for nematode parasite with further increased dosage i.e. 3gm/kg body weight. The % EPG reduction was 9.78%, 24.33%, 57.38% and 66.88% at day 3rd, 7th, 10th and 14th post treatment, respectively. The results showed that using *Artemisia* at dose rate of 3gm/kg body weight is most effective than 1gm/kg body weight and 2gm/kg body weight.

One group of sheep was treated with recommended Levamisole dosage i.e. 7.5mg/kg body weight against GIT Trichostrongylid nematode. It was noted that Levamisole gave best results i.e. eggs per gm faeces prior to medicament was 1138, which was reduced to 681 on day 3rd (40.15% EPG reduction), 536 on day 7th (52.89% EPG reduction), 357 on day 10th (68.62% EPG reduction) and 147 at day 14th i.e. 87.08% EPG reduction) remarkably.

Tariq (2008) published almost similar results as per present study. They administered extracts of *Artemisia* in sheep and found significant reduction in faecal egg output by the GI nematodes. The CEE was

as effective as the reference drug-albendazole and demonstrated faecal egg count reduction (FECR) of 90.46% in sheep at 2.0g/kg body weight on day 15 PT followed by 82.85% FECR at 1.0 g/kg body weight on day 15 PT. The CAE showed less activity and resulted in maximum of 80.49% FECR at 2.0g/kg body weight. Dosage had a significant ($P < 0.05$) influence on the anthelmintic efficacy of *A. absinthium*.

The anthelmintic efficacy of *Artemisia* and *Chenopodium* recorded in the present study was although lower than the reference drug i.e. Levamisole, yet is having almost similar trends as reported earlier (Iqbal *et al.* (2004). They tested *Artemisia brevifolia* at graded doses (1, 2 and 3g/kg body weight) to sheep naturally infected with mixed species of gastrointestinal nematodes in which maximum reduction (67.2%) in eggs per gram (EPG) of faeces was recorded on day 14th post treatment in sheep. It is not comparable with levamisole (99.2% reduction in EPG) at any of the doses tried in this study. These results correlate with the present study.

Levamisole was found to be most effective (87.08% EPG Reduction) against GIT nematodes at recommended dose level as compared to *Chenopodium* (51.03% EPG reduction) using 3gm/kg body weight and *Artemisia* 66.88% EPG reduction using 3gm/kg.bw. Jabbar *et al.* (2007b) pointed out that in-vivo maximum reduction in eggs per gram (EPG) of faeces was recorded as 82.2% with *Chenopodium album* AME at 3.0g/kg.bw on day 13th post-treatment. Levamisole (7.5mg/kg), a standard anthelmintic agent, showed 95.1-95.6% reduction in EPG.

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Author contributions

N.K (Conceptualization, design, investigation, writing), M.L (Conceptualization, design and funding acquisition and supervision), S.J (Review, Conceptualization, design and supervision), S.M.T & D.K, A.R, A, K & M, S contributed equally in

reviewing, analyzing & sampling. All authors critically reviewed the manuscript and approved the final version of the manuscript.

Conflicts of interest

All other authors declare no conflicts of interest

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