RESEARCH ARTICLE

Anthelmintic Efficacy of Polyherbal Formulation and Fenbendazole in Strongyle Infected Calves

Akanksha Mishra[1], Rajendra K. Bagherwal[2]*, Hemant K. Mehta[3], Pawan Maheshwari[4]

Abstract

The present study was conducted on strongyle infected calves from organized and unorganized cattle farms located in and around Mhow (Indore, India) to assess the efficacy of polyherbal formulation (A mixture of *Azadirchta indica, Momordica charantia*, *Allium cepa, Allium sativum*, *Brassica nigra, Musa paradisiaca*, *Cuminum cyminum*, *Curcuma longa* and *Piper nigrum*) and fenbendazole. Total 12 infected calves (>500 EPG of strongyle) were randomly categorized in 2 groups of 6 calves in each. Group A was administered with polyherbal formulation, and Group B with fenbendazole. The anthelmintic efficacy of polyherbal formulation and fenbendazole was evaluated by faecal egg per gram (EPG) of strongyle and haemato-biochemical parameters. The pre-treatment mean EPG in both treated group were reduced significantly ($p<0.05$) on day 7, 14 and 21 post-treatment. Polyherbal formulation was found more effective against strongyle infection in comparison to fenbendazole. The values of Hb, PCV, TEC were increased significantly and TLC were decreased non-significantly in both polyherbal formulation and fenbendazole treated groups on day 21 post-treatment. Polyherbal formulation treated group showed significant decrease ($p<0.05$) in neutrophil, eosinophil count, whereas increased lymphocyte count.

Keywords: Cow calves, Fenbendazole, Polyherbal formulation, Strongyle infection.

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Introduction

The infection of ruminant livestock with helminths, such as gastrointestinal nematodes (primarily Trichostrongylids) and liver fluke (primarily *Fasciola hepatica*) threatens the profitability and sustainability of livestock health and production (Terefe *et al*., 2012). Helminthes control is heavily reliant on the use of anthelmintics such as benzimidazoles (BZ; e.g. albendazole, triclabendazole), levamisoles and macrocyclic lactones (ML; e.g. ivermectin, eprinomectin, moxidectin). Anthelmintic resistance (AR) in gastrointestinal nematodes has been reported worldwide (Kaplan and Vidyashankar, 2012) and now poses a significant threat to animal health and productivity (Dongre, 2014). There is a need of the hour for judicious use of anthelmintics and also to search for alternative therapies to combat the problem of gastrointestinal nematodasis. For these reasons, interest in the screening of medicinal plants for their anthelmintic activity remains of great scientific interest (Akhtar *et al*., 2000). A variety of plants have been scientifically validated for their anthelmintic properties *in-vitro* and *in-vivo* (Iqbal *et al*., 2010). Looking for safety, effectiveness, and economy of herbal preparation, the present work was planned to assess the comparative anthelmintic efficacy of polyherbal formulation and fenbendazole in strongyle infected cow-calves.

Materials and Methods

Organized and unorganized cattle farms located in and around Mhow were selected to collect the samples. Research work was done in the Department of Veterinary Medicine, College of Veterinary Science & A.H., Mhow (M.P., India). Twelve (12) infected calves (>500 EPG of strongyle) were used in the study. Calves were divided in two groups of six calves in each. Group A was administered with a Polyherbal formulation orally at a dose of 250 g per 50 kg body weight once a day for three consecutive days (NDDB, 2018), whereas group B was administered Fenbendazole at a dose of 10 mg/kg body weight as single oral dose (Singh *et al*., 2012

Polyherbal mixture (250 g) was prepared by combination of neem leaves (*Azadirchta indica*) (25 g), bitter gourd fruit (*Momordica charantia*) (50 g), onion (*Allium cepa*) (40 g), garlic (*Allium sativum*) (5 g), mustard seeds (*Brassica nigra*) (10 g), banana stems (*Musa paradisiaca*) (100 g), cumin seeds (*Cuminum cyminum*) (10 g), turmeric powder (*Curcuma longa*) (5 g) and dry black pepper (*Piper nigrum*) (5 g), collected from

[1-4]Department of Veterinary Medicine, Nanaji Deshmukh Veterinary Science University, College of Veterinary Science and AH, Mhow- 453446, Madhya Pradesh, India

Corresponding Author: Rajendra K. Bagherwal, Department of Veterinary Medicine, Nanaji Deshmukh Veterinary Science University, College of Veterinary Science and AH, Mhow- 453446, Madhya Pradesh, India, e-mail: rkb_vet@yahoo.com

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the local area. After 30 minutes of soaking cumin seeds, mustard seeds and dried black pepper in water, all of the herbs were grinded in a grinder (NDDB, 2018).

Drug Efficacy %

Fresh 8 g faecal sample was collected from the rectum of the calf on day 0 (pre-treatment), 7, 14 and 21 (post-treatment). During the pre- and post-treatment period EPG and clinical performance were monitored. Faeces were examined on day 0 (pre-treatment), 7, 14 and 21 (post-treatment). Efficacy of the drug was calculated as per formula:

$$\text{Efficacy \%} = \frac{\text{EPG pre-treatment} - \text{EPG post-treatment}}{\text{EPG pre-treatment}} \times 100$$

Five mL of blood was collected from the jugular vein of each calf on day 0 (pre-treatment) and day 21 (post-treatment) and was divided in two parts; one containing Ethylene Diamine Tetra-Acetic acid (EDTA) for haematological study and other part without anticoagulant was used for separating the serum. Separated serum was collected in a sterile vial. Haematological parameters were estimated by using Automatic blood cell counter (Diatron, Model Abacus 380) and biochemical parameters were estimated by commercially available kits using Semi-automatic biochemical analyzer (Erba Mannheim EM-200 made in Transasia) following the manufacturer's instructions.

Results and Discussion

Faecal Egg Per Gram (EPG)

The mean values of EPG observed in both the groups A and B, pre (day 0) and post-treatment are presented in Table 1. There was significant reduction in strongyle EPG after treatment in polyherbal group (group A). Nawaz *et al*. (2014) reported that aqueous extract of neem leaves reduce 89% EPG count of GI parasitic infested sheep. Similarly, Kanojiya *et al*. (2015) detected significant reduction of 57% faecal egg count in sheep treated with *Allium sativum*. Worku et al. (2009), Curry and Whitaker (2010), Ahmed *et al*. (2015) reported garlic (*Allium sativum)* as herbal anthelmintic in small ruminants with significant decrease ($p<0.05$) in the faecal egg counts of nematodes. Similar to treatment group A, in treatment group B there was significant reduction in strongyle EPG post treatment

These findings supported the observations of Singh *et al*. (2012) for 99.52% reduction in faecal egg count of infected goats on 13th day post-treatment with fenbendazole @ 10 mg/kg b.wt orally. Efficacy of polyherbal formulation on day 21 was 100%, while, in fenbendazole treated group the efficacy was recorded 98.73%. This suggested that the efficacy of polyherbal formulation was higher than or almost similar to fenbendazole. Jamara *et al*. (2015) reported crude neem leaf powder exhibited 78, 98 and 100 % efficacy on day 7, day 14 and day 28, respectively, in cattle. The total EPG count reduced in fenbendazole treated group might be due to its action on tubulin of the parasite and thus reducing the glucose uptake (Rang *et al*., 2012) thereby paralyzing and resulting in death of parasites.

Haematological Alternations

The mean haematological values observed in both the groups (pre and post treatment) are depicted in Table 2. Haemoglobin, PCV and TEC values improved and returned to normal range in both the groups post-treatment (day 21) due to the elimination of strongyle worms. There was reduction in worm load (strongyle) in the host after treatment thus further reducing the loss of blood from the body. Similar kind of improvement in these blood parameters after *A. indica* treatment has been reported in sheep (Jan *et al*. (2015; Biswas *et al*., 2017).

Table 2: Mean (±SE) values of haematological parameters pre (day 0) and post treatment (day 21) of strongyle infected cow-calves with polyherbal formulation and fenbendazole

Parameters	Day	Group A	Group B
Hb (g/dL)	0	8.66±0.57	8.08±0.23
	21	9.33±0.40	9.08±0.50
PCV (%)	0	26.00 ±1.71	24.25 ±0.71
	21	28.00 ±1.20	27.25 ±1.52
TEC (x10^6/µL)	0	4.33 ±0.28	4.04 ±0.11
	21	4.66 ±0.20	4.54 ±0.25
TLC (x10$^{3.}$/µL)	0	15583.33 ±1248.57	14033.33 ±870.50
	21	13400.00 ±1100.30	12466.67 ±1314.19
Neutrophil %	0	29.16^a±0.98	30.16 ±1.40
	21	26.50^b±0.99	27.33 ±1.60
Lymphocyte %	0	61.33^b±1.54	57.00^b±2.56
	21	67.33^a±1.38	64.66^a±3.32
Monocyte %	0	1.50 ±0.40	1.66^b±0.61
	21	0.83 ±0.40	6.66^a±1.70
Eosinophil %	0	8.00^a±0.68	10.00 ±0.73
	21	5.33^b±0.88	6.66 ±1.70

Day 0= pre-treatment, day 21= post-treatment. Values with different superscripts differ significantly ($p\leq0.05$) within group.

The TLC values also came towards normal level on day 21 (post treatment,). Rowe *et al*. (2008) suggested that

Table 1: Mean (±SE) values of EPG in polyherbal formulation and fenbendazole treated calves

Groups	EPG on Days			
	0	7	14	21
A	633.33^a±23.88	191.66^b±30.04	75.00^c±21.40	0.00^d±0.00
B	791.66^a±32.99	160.00^b±29.15	90.00^{ac}±48.47	10.00^c±9.50

Day 0= pre-treatment, day 7, 14, 21= post-treatment. Values with different superscripts differ significantly ($p\leq0.05$) within group.

generalized inflammation and response to prolonged antigenic stimulus in the form of helminth infection may be responsible for leukocytosis. Decrease in TLC count is indicative of decrease in infection which may be correlated with the decreased strongyle EPG in both treated groups. There is initially (before treatment) nuetrophilia and lymphopenia, however, after therapy in both the groups the neutrophils decrease significantly followed with compensatory increase in lymphocyte count might be due to reduced inflammation of intestinal lumen. Worm fecundity is inversely proportional with lymphocyte percent. Nematode infection increased eosinophil and monocyte counts which on treatment returned to normal level in the both cases. This result was supported by Jamra *et al*. (2015) in strongyle infected cattle treated with neem and fenbendazole.

Biochemical Alternations

The mean values of serum biochemical parameters observed in treatment groups pre and post treatment are presented in Table 3. There was a decrease in ALT and AST values in both the groups , however the decrease was significant in AST whereas it was non significant in ALT. Biswas *et al*. (2017) reported decreased ALT value in neem treated sheep, indicative of the removal of parasites. The elevation of serum ALT level indicates some disruptive activities due to gastrointestinal nematode infection in ruminants (Sharma *et al*., 2014; Bordoloi *et al*., 2012). In both treatment groups AST values were higher on day 0 (before treatment), however, after initiation of therapy the values decreased significantly and came within normal physiological limit after treatment at 21 day.

Table 3: Mean (±SE) values of biochemical parameters pre and post treatment of strongyle infected cow- calves with polyherbal formulation and fenbendazole

Parameters	Day	Group A	Group B
ALT (IU/L)	0	42.81±5.50	37.51±4.27
	21	32.25 ±1.05	30.07±2.59
AST (IU/L)	0	137.56^{a}±20.59	133.36^{a}±15.81
	21	93.79^{b}±4.00	91.57^{b}±4.41
Creatinine (mg/dL)	0	0.97 ±0.13	1.17 ±0.19
	21	0.95 ±0.13	1.10 ±0.19
Albumin (g/ dL)	0	2.37^{b}±0.11	2.30^{b}±0.12
	21	2.96^{a}±0.08	2.95^{a}±0.21
Globulin (g/ dL)	0	2.65±0.16	2.62±0.23
	21	2.91±0.15	2.62 ±0.26
Albumin/Globulin	0	0.89±0.02	0.91±0.10
	21	1.03 ±0.08	1.19 ±0.16
Total Protein (g/ dL)	0	5.03^{b}±0.26	4.92^{b}±0.19
	21	5.87^{a}±0.14	5.57^{a}±0.23

Day 0= pre-treatment, day 21= post-treatment. Values with different superscripts differ significantly ($p \leq 0.05$) within group.

In both the treatment groups A and B as compared to 0 day the mean values of protein, albumin were increased significantly on day 21 post-treatment, but there was no change in globulin value in gr B. A lower value of protein and albumin was also reported in cattle and buffaloes infected with gastrointestinal nematode (Bordoloi *et al*., 2012; Dongre, 2014). This might be because strongyle causes disturbances to the haemopoietic system with anaemia and severe damage to abomasal mucosa resulting in lower serum albumin. The finding was also supported by Jamra *et al*. (2015) in strongyle infected cattle treated with neem and fenbendazole.

Conclusion

Strongyle infection in cow-calves significantly altered the haemato-biochemical parameters which resulted in a significant decrease in neutrophil, eosinophil, lymphocyte in polyherbal formulation treated group, whereas increase in monocyte among fenbendazole treated group. Although lymphocyte, albumin, A/G ratio increased significantly. Polyherbal formulation showed better efficacy as anthelmentic agent than fenbendazole.

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