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Vinod Sinha

Department of Veterinary
Parasitology, College of Veterinary
Science and Animal Husbandry,
Acharya Narendra Dev University of
Agriculture and Technology,
Kumarganj, Ayodhya,
Uttar Pradesh, India

Amit Singh

Department of Veterinary
Parasitology, College of Veterinary
Science and Animal Husbandry,
Acharya Narendra Dev University of
Agriculture and Technology,
Kumarganj, Ayodhya,
Uttar Pradesh, India

VK Pal

Department of Veterinary
Parasitology, College of Veterinary
Science and Animal Husbandry,
Acharya Narendra Dev University of
Agriculture and Technology,
Kumarganj, Ayodhya,
Uttar Pradesh, India

SK Maurya

Department of Veterinary
Biochemistry, College of Veterinary
Science and Animal Husbandry,
Acharya Narendra Dev University of
Agriculture and Technology,
Kumarganj, Ayodhya,
Uttar Pradesh, India

Jaswant Singh

Animal Genetics and Breeding,
College of Veterinary Science and
Animal Husbandry, Acharya
Narendra Dev University of
Agriculture and Technology,
Kumarganj, Ayodhya,
Uttar Pradesh, India

Chandra Shekhar

Department of Veterinary Public
Health and Epidemiology, College of
Veterinary Science and Animal
Husbandry, Acharya Narendra Dev
University of Agriculture and
Technology, Kumarganj, Ayodhya,
Uttar Pradesh, India

Corresponding Author:**Amit Singh**

Department of Veterinary
Parasitology, College of Veterinary
Science and Animal Husbandry,
Acharya Narendra Dev University of
Agriculture and Technology,
Kumarganj, Ayodhya,
Uttar Pradesh, India

In-vivo anthelmintic efficacy of powder formulation of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds on ovine gastrointestinal nematodes

Vinod Sinha, Amit Singh, VK Pal, SK Maurya, Jaswant Singh and Chandra Shekhar

Abstract

Powder formulation of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds was evaluated *in-vivo* for anthelmintic efficacy against gastrointestinal nematodes in sheep. In this study, twelve naturally infected female sheep were selected based on egg per gram (EPG) and then randomly divided into two groups, group I (G I) and group II (G II) having six animals each. G I animals were treated with powder formulations of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds (1:1) at the dose rate of 600 mg/kg.b.wt. orally whereas G II animals were left as untreated infected control. Faecal and blood samples were collected on days 0,3,7 and 10 PT to record reduction in faecal egg count and improvement in haematological and biochemical parameters post treatment. On day 10 PT, 62.07% reduction in faecal egg count was observed. Post treatment, there was improvement in haematological, total protein and albumin level indicating reduction in worm burden. AST, ALT and serum creatinine values post treatment suggested no side effects. Coproculture before and after treatment revealed *Haemonchus contortus* to be a predominant strongyle. The study revealed promising activity of the herbal formulations which can be further exploited to improve the efficacy against gastrointestinal nematodes of sheep.

Keywords: gastrointestinal nematodes, sheep, *Embelia ribes*, *Vernonia anthelmintica*, anthelmintic evaluation

Introduction

The problem of anthelmintic resistance poses a great challenge to the successful control programme for the effective management of gastrointestinal nematodes [1]. This eventually results in huge economic losses to the small and marginal farmers whose livelihood depends upon the rearing of sheep and goat. Emergence of resistance problem has been widely reported from various regions of India [2, 3, 4, 5, 6] which led to the search for alternative means of worm control which is not only effective but economical and eco-friendly. Now a day, efforts being made for the development, formulations and evaluation of anthelmintics of herbal origin as an alternative tool for the effective control of gastrointestinal nematodes.

Keeping the above facts in view, the present study was planned to evaluate the efficacy of powder formulations of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds in sheep naturally infected with gastrointestinal nematodes.

Materials and Methods

Powder preparation- *Embelia ribes* fruits and *Vernonia anthelmintica* seeds after being procured from the local market were put in a hot air oven at 40 °C for 8hrs to remove moisture. They were then pulverized to a fine powder using an electric grinder, sieved and kept in air tight container at 4 °C. Both powders were then mixed in the equal ratio (1:1) to be used for *in-vivo* study.

***In -vivo* experiment**

For *in-vivo* study, twelve (12) adult female sheep, weighing 16-30 kg and naturally infected with gastrointestinal nematodes and having egg per gram of faeces (EPG) more than 500 were selected. They were randomly divided into two groups (Group I and Group II) having six animals each. Group I sheep was treated with *Embelia ribes* + *Vernonia anthelmintica* (1:1)

powder at dose rate of 600mg /kg. b.wt. orally whereas group II was maintained as untreated infected control.

Assessment of efficacy of the drug

Faecal samples from each sheep were collected per-rectum in a sterile container on day 0, 3, 7 and 10 post- treatment, processed as per standard procedure and EPG was calculated by modified McMaster technique [7]

Faecal egg count reduction was calculated on each day using the formula:

$$\% \text{ FECR} = 100 \left\{ 1 - \frac{\text{Mean FEC in treated group on day } n}{\text{Mean FEC in control group on day } n} \right\}$$

FEC - Fecal egg count, FECR - Faecal egg count reduction)

Larvae composition before and after treatment was also studied by carrying out coproculture on collected faecal samples.

Haematological and biochemical parameters

Blood samples were also collected on day 0, 3, 7, and 10 post-treatment to record haematological (Haemoglobin, packed cell volume, total erythrocyte count) and biochemical values (total protein, Albumin, Serum creatinine, ALT (Alanine amino transferase), AST (Aspartate amino transferase) using a commercial kit (SPAN diagnostics).

Statistical analysis

One way ANOVA was employed to analyze haematological and biochemical values [8]

Results and Discussion

Faecal egg count reduction test

Within the treated group i.e. in group I, a significant reduction in faecal egg count was noticed from day 3 post-treatment and on day 10 PT, FECR% of 62.07% (Table 1) was observed. This shows that the combined powder formulations of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds (1:1) given at the rate of 600 mg/kg.b.wt orally was found to induce a significant reduction in gastrointestinal nematodes infestations. Coproculture examination of the pooled faecal samples of the treated group showed 96% larvae population of *Haemonchus contortus*. This suggests the potent anthelmintic activity of formulations against the most pathogenic strongyle of small ruminants.

Haematological and biochemical values

Haematological and biochemical values were also recorded before and after treatment with a powder mixture of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds against gastrointestinal nematodes in ovine and have been depicted in Table 1.

Table 1: Faecal egg count, haematological and biochemical values in sheep naturally infected with *Haemonchus contortus* and treated with powder formulation of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds.

		0 day	3 day PT	7day PT	10 day PT
EPG	GI	1500.0± 99.16	975±77.19 ^{a *}	595.00±46.81 ^{a *}	591.6±30.04 ^{a *}
	GII	1866.66±158.99	2000±146.62 ^b	1966.667±141.81 ^b	1940.667±25.15 ^b
FECR%		-	39.33	62.34	62.07
Hb(g%)	GI	8.1 ± 0.06	8.2± 0.076	8.7 ± 0.054 ^{*a}	9.3 ± .089 ^{*a}
	GII	8.6 ± 0.29	8.6± 0.21	8.3 ± 0.182 ^b	8.31± 0.15 ^b
PCV (%)	GI	24.4 ± 0.18	24.33 ± 0.15	28.5 ± 0.27 ^{*a}	29.9 ± 0.65 ^{*a}
	GII	25.9 ± 0.92	26.3 ± 0.90	25.1± 0.13 ^b	24.24± 0.23 ^b
TEC(x10 ⁶ /μl)	GI	4.7 ± 0.20	4.8± 0.15	6.2± 0.26 ^{*a}	7.3± 0.24 ^{*a}
	GII	5.4 ± 0.55	5.7 ± 0.32	4.9± 0.37 ^b	5.09± 0.27 ^b
Total plasma protein (g/dl)	GI	6.4± 0.10	6.5± 0.16	6.6± 0.08	6.5± 0.16 ^a
	GII	6.4± 0.21	6.46± 0.11	6.46± 0.11	6.45± 0.28 ^b
Serum albumin(g/dl)	GI	2.9± 0.09	2.93± 0.04	2.95 ± 0.02	3.01 ± 0.07
	GII	3.09 ± 0.03	2.98 ± 0.09	2.93± 0.07	2.86± 0.034
AST(IU/L)	GI	61.9± 4.24	64.7± 4.4	64.1 ± 4.25	62.51± 4.27
	GII	57.06± 2.07	61.15± 0.54	60.81± 2.30	59.45± 2.13
ALT(IU/L)	GI	17.8± 0.64	22.86± 0.62 ^{*a}	20.73 ± 0.81	19.31± 0.82
	GII	17.26± 0.67	19.06 ± 0.53 ^b	19.67± 0.72	18.78± 0.66
Serum creatinine (IU/L)	GI	1.08± 0.07	1.03± 0.073	1.10± 0.071	1.13± 0.084
	GII	1.06± 0.06	1.11± 0.031	1.08± 0.07	1.17 ± 0.078

GI: Infect and treated, G II: infected untreated control

Values marked *differ significantly within group with value at day zero ($P < 0.050$), values with dissimilar superscript ^{a, b} differ significantly ($P < 0.05$) between the groups.

After treatment, haemoglobin level (Hb g%) improved significantly on day 7PT in the treated group and when compared with infected control group i.e. group II, values of Group I was significantly higher from day 7 PT onwards. Like haemoglobin, other haematological parameters viz. packed cell volume (PCV%) and total erythrocytic count (TEC x10⁶/μl) too showed similar trends of improvement from day 7 PT onwards. Earlier, in goats [9] and in sheep [10], significant improvements in hematological values were recorded after successful treatment of animals infected with haemonchosis. *Haemonchus contortus* is potent blood sucker as each parasite sucks around 0.05ml of blood per day and

due to this blood sucking activity of parasite, anaemia and death results in small ruminants [11]. Improvement in hematological status of animals after treatment showed that powder formulations was able to induce anthelmintic activity on gastrointestinal nematodes.

Within the treated group, total plasma protein (TPP) levels and serum albumin were found to be significantly increased ($p < 0.05$) on 10 days PT as compared to that of day zero values. Within control group, a significant ($p < 0.05$) decrease in total serum albumin (TA) level was recorded from day 7 PT onwards. Further comparison of treated group with infected control group revealed that albumin level in group I

was significantly ($p < 0.05$) higher than the control group from day 7 PT onwards. Increase in TPP and TA values following successful therapy have also reported earlier [12]. Presence of gastrointestinal nematodes causes protein losing enteropathy resulting in hypoproteinaemia [11]. So increase in TPP and TA values indicates reduction in worm number eventually substantiating the effectiveness of herbal preparation used in the present study.

Among enzymes, AST values were found non-significantly different within and between the groups during the entire study period. However, ALT values were found significantly different on day 3 PT within and between the groups but from day 7 PT onwards, ALT values declined to a non-significant ($p < 0.05$) level. Non significant variation in AST and slight increase in ALT values but in normal range indicates no adverse effects on the liver. Creatinine level remains at normal level within the treated group suggesting that powder formulations did not affect kidney function.

In the present study, powder formulations of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds exerted moderate but promising anthelmintic activity with no side effects. The bioactive molecules embolic acid or embelin present in *Embelia ribes* [13, 14] and vernolin, vernolic acid, vernodalol in *Vernonia anthelmintica* [15] are said to possess anthelmintic activity.

Despite exhibiting moderate anthelmintic action, powder formulations of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds provides an alternative therapeutic hope to combat the menace of anthelmintic resistance. The formulations may be exploited further to increase its efficacy by dose standardization or it may be recommended to be used in conjunction with modern anthelmintics to get rid of resistant worms.

Conclusion

Powder formulation of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds reduced faecal egg count by 62%. Haematological and total protein and albumin values improvement post treatment corroborates the reduction in worm load which was primarily of *Haemonchus contortus*. Hence, promising efficacy was shown by formulation against *Haemonchus contortus* which is widely reported to have developed resistance against commercial allopathic anthelmintics.

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