

*Original Research***Anthelmintic Efficacy of Aqueous-Alcoholic Extracts Formulations of *Embelia ribes* Fruits and *Vernonia anthelmintica* Seeds against Ovine Gastrointestinal Nematodes**Vinod Sinha¹, Amit Singh^{1*}, V. K. Pal¹ and S. K. Maurya²Acharya Narendra Dev University of Agriculture and Technology, Kumarganj, Ayodhya
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Abstract

Aqueous alcoholic (70% ethanol) extracts mixture of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds was evaluated for anthelmintic efficacy in sheep having natural infection of gastrointestinal nematodes. Twelve infected sheep were randomly divided into two groups, Group I and Group II comprising six animals each. Group I sheep were treated with *Embelia ribes* fruits and *Vernonia anthelmintica* seeds extract (1:1) at dose rate of 100mg /kg. b.wt. orally whereas G II was kept as untreated infected control. Faecal and blood samples from each sheep were collected on 0,3,7 and 10-day post-treatment to record egg per gram of faeces (EPG) and haematological (Hb, PCV and TEC) and biochemical indices (Total plasma protein, serum albumin, ALP, ALT, AST & serum creatinine). On day 10 PT, decrease in egg per gram, improvement in haematological values and increase in serum protein level suggests promising anthelmintic values of aqueous alcoholic extract mixture of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds.

Key words: Aqueous-alcoholic *Embelia ribes*, Gastrointestinal Nematodes, Sheep, *Vernonia anthelmintica***How to cite:** Sinha, V., Singh, A., Pal, V., & Maurya, S. (2020). Anthelmintic Efficacy of Aqueous-Alcoholic Extracts Formulations of *Embelia ribes* Fruits and *Vernonia anthelmintica* Seeds against Ovine Gastrointestinal Nematodes. International Journal of Livestock Research, 10(3),60-66. doi: 10.5455/ijlr.20200104064820**Introduction**

Gastrointestinal nematodes pose a major challenge to the health and productivity of small ruminants causing unaccountable economic losses to small and marginal farmers. Control of gastrointestinal nematodes is largely based on the use of chemotherapeutic drugs but improper and injudicious use of anthelmintics has led to the emergence of resistant strain of the gastrointestinal parasites particularly, *Haemonchus contortus* (Coles,1997). The parasite has been documented to be resistant to all three broad spectrum families of

anthelmintics viz. benzimidazole, imidazothiazole and ivermectin (Yadav *et al.*, 1995; Yadav and Garg, 2004; Singh *et al.*, 2010 ; Rathod *et al.*, 2019; Vohra *et al.*, 2019) and against drugs with narrow spectrum of activity such as salicylanilides (Swarnkar *et al.*, 1999).

The emergence of resistance to anthelmintic drugs which is now a worldwide phenomenon (Jackson and Coup, 1997) and the increased awareness of consumers about drug residues that potentially enter the food chain have stimulated investigation into other alternative options. Therefore, as an alternative to the allopathic drugs, there are efforts for the development of eco-friendly, economical and efficacious anthelmintics of herbal origin. Keeping above information in mind, present study was planned to evaluate the efficacy of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds in sheep naturally infected with gastrointestinal nematodes.

Extract Preparation

Embelia ribes fruits and *Vernonia anthelmintica* seeds were procured from local market. Moisture was evaporated by keeping them in hot air oven at 40°C for 8hrs and then pulverized to fine powder using electric grinder, sieved and kept in air tight container at 4 °C. Extract of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds powder were prepared using 70% aqueous ethanol in a Soxhlet apparatus. Powder of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds were placed in thimble and the Soxhlet apparatus was run for 9-12 hours (22-28 cycles). The extracts so obtained were evaporated at room temperature, weighed and then stored in air tight sterile container at 4°C.

In -vivo Experiments

Aqueous alcoholic (70% ethanol) extract of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds was used for *in- vivo* study. For the trial, twelve (12) naturally infected adult female sheep, weighing around 16-30kg and having EPG>500, were selected and randomly divided into two different groups (G I and G II), comprising six animals each. Group I animals were treated with *Embelia ribes* + *Vernonia anthelmintica* (1:1) extract at dose rate of 100mg /kg. b.wt. orally whereas G II was kept as untreated infected control.

Interpretation of Efficacy of Drug

Per-rectal faecal samples from individual sheep from each group was collected in sterile container using rubber glove on day 0, 3, 7 and 10 post- treatment. Egg per gram was calculated by modified McMaster technique (MAFF, 1971). Efficacy was calculated using following 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)

Coproculture was also carried out on collected faecal samples for the larvae composition before and after treatment.

Study of Haematological and Biochemical Parameters

Blood samples were also collected on day 0, 3, 7, and 10 post-treatment to record any haematological and biochemical changes. Haemoglobin (Hbg/dl), packed cell volume (PCV %), total erythrocyte count (TEC $\times 10^6/\mu\text{l}$) were estimated by cyanomethaemoglobin, Wintrobe's haematocrit and haemocytometer method, respectively. Estimation of total protein (g/dl), Albumin (g/dl), Serum creatinine (mg/dl), ALP (Alkaline phosphatase), ALT (Alanine amino transferase), AST (Aspartate amino transferase) and serum creatinine was done using commercial kit (SPAN diagnostics).

Statistical Analysis

All the haematological and biochemical values were analyzed using SPSS software programmed one-way ANOVA.

Results and Discussions

Physical Characteristics of Extracts

Extract of *Embelia ribes* obtained was dark tan in colour, oily and molten mass like consistency. In case of *Vernonia anthelmintica*, the extract was dark brown and semi-solid in consistency. Yield obtained in *Embelia ribes* and *Vernonia anthelmintica* extract was 15.5% w/w and 27.9% w/w, respectively.

Reduction in Faecal Egg Count

In group I, treated with mixture of aqueous ethanolic extract of 1:1 mixture (g/g) of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds, significant reduction in faecal egg count (72.97%) was noticed on day 10 post treatment (PT) (Table 1). Earlier, using similar combination ratio, 93% reduction in faecal egg count with methanolic extract was reported (Javed and Akhtar ,1990). High level of efficacy observed in that study may be due to the high dose (2g/kg. b.wt) of mixture used. Coproculture examination showed the presence of 99% larvae of *Haemonchus contortus* before and after treatment. Reduction in EPG and coproculture reports suggests that the herbal preparations used in the current study were able to reduce worm burden of *Haemonchus contortus*.

Haematological and Biochemical Responses

Haemato-biochemical values (Mean \pm S.E) before and after treatment with ethanol extract mixture of *Embelia ribes* and *Vernonia anthelmintica* against *Haemonchus contortus* in sheep have been depicted in Table 1. Within infected treated group , Hb (g%) values improved significantly on day 7PT. When infected treated group were compared with infected control, Hb (g%) values were observed significantly different

from day 7 PT onwards. Similarly, the PCV and total erythrocyte count values values (TEC x10⁶/μl) improved significantly (p<0.05) from day 7 PT onwards within G. I. and comparison with control groups revealed values of treated groups significantly higher (p<0.05) from day 7 PT onwards. Significant improvement in these parameters following successful treatment of haemonchosis has been recorded earlier in goats (Jayraw and Raote, 2004) and in sheep (Qamar and Maqbool, 2012) The blood sucking habit of *Haemonchus contortus* causes continuous blood loss which might be responsible for decreased value of Hb, PCV, and TEC. The post- treatment increases in these values indicated elimination of the parasite and thereby improved health status of animals.

Table 1: Faecal egg count and haemato-biochemical values (Mean ± S. E.) before and after treatment with ethanol extract mixture of *Embelia ribes* and *Vernonia anthelmintica* against *Haemonchus contortus* in sheep

		0 day	3-day PT	7-day PT	10-day PT
EPG	GI	1723.33±30.84	943.33±31.26*	501.667±5.42*	525±28.137*
	GII	1866.66±158.99	2000±146.62	1966.667±141.81	1941.667±25.15
FECR%	GI	-	52.83	74.49	72.97
	GII	-	-	-	-
Hb(g%)	GI	8.15±0.10	8.23±0.10	9.11±0.07* ^a	9.90±0.13* ^a
	GII	8.60±0.29	8.61±0.21	8.35±0.18 ^b	8.35±0.14 ^b
PCV(%)	GI	24.45 ±0.32	24.33 ±0.33	28.58 ±0.27* ^a	31.28 ±0.51* ^a
	GII	25.90 ±0.92	26.36 ±0.90	25.10 ±0.13* ^b	24.21 ±0.22* ^b
TEC(x10 ⁶ /μl)	GI	4.88 ±0.19	5.39 ±0.18	6.90 ±0.17* ^a	7.83 ±0.14* ^a
	GII	5.48 ±0.55	5.78 ±0.32	4.98 ±0.37 ^b	5.08 ±0.28 ^b
Total plasma protein	GI	6.5 ± 0.21	6.63±0.19	6.78±0.16	6.90±0.17* ^a
	GII	6.4±0.10	6.467±0.10	6.45±0.11	6.25±0.12 ^b
Serum albumin(g/dl)	GI	3.01±0.06	3.05±0.05	3.10±0.03 ^a	3.21±0.031 ^a *
	GII	3.10±0.03	2.933±0.04	2.933±0.04 ^b *	2.86±0.033 ^b *
AST(IU/L)	GI	58.38±1.98	65.76±2.17*	63.01±2.36	61.26±2.09
	GII	57.06±2.07	61.15±0.54	60.81±2.30	59.45±2.11
ALT(IU/L)	GI	18.43±0.74	23.60±0.86* ^a	21.23±1.10	19.88±1.11
	GII	17.26±0.67	19.06±0.53 ^b	19.66±0.72	18.78±0.67
ALP(IU/L)	GI	105.71 ± 5.44	114.61±4.84	97.18±4.71 ^a	86.133±4.55* ^a
	GII	107.26±3.88	105.88±2.50	111.45±3.40 ^b	108.05±3.53 ^b
Serum creatinine (IU/L)	GI	1.20 ± 0.10	1.08 ±0.09	1.15 ±0.12	1.06 ±0.92
	GII	1.06 ±0.06	1.11 ±0.03	1.08 ±0.07	1.18 ±0.07

EPG- Egg per gram ; FECR% - Faecal egg count reduction percentage ; Hb- haemoglobin; PCV-Packed cell volume; TEC- Total erythrocytic count ; ALP -Alkaline phosphatase, ALT-Alanine amino transferase , AST - Aspartate amino transferase; Values marked * differ significantly within group with value at day zero (P< 0.05) , Values with dissimilar superscript a,b differ significantly between groups (P< 0.05).

Within the treated group, total plasma protein (TPP) levels and serum albumin were found to be significantly increased (p<0.05) on 10-day PT as compared to that of day zero values. Within control group,

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 (Patil *et al.*, 1993). So, increase in TPP and TA values indicates the effectiveness of herbal preparation used in the present study.

Within group I, AST values were found significantly ($p < 0.05$) increased on day 3 PT but later on day 7 and 10 PT, the values decreased to non-significant level. Upon comparison with control groups, values in group-I were found non significantly different. ALT level values within GI got significantly elevated ($p < 0.05$) on day 3 PT but later on values declined and eventually became non-significant on day 10 PT. Within control group, there was no significant change in ALT level. When compared with the control group, ALT values on day 3 PT was significantly ($p < 0.05$) higher in GI only to be non-significantly different on day 10 PT. Rise in AST and ALT values in the treated group could be attributed to the consumption of f herbal extract and subsequent hyperactivity of liver, although rise were in ranged with standard upper limit values as mentioned in the standard book (Radiostites *et al.*, 2007). This suggests no significant damage to the liver. Alkaline phosphatase (ALP) levels within the treated group was found significantly decreased on day 10 PT as compared to the values on day zero. However, within infected control group there were no significant changes. When compared with infected control group, the ALP levels in the treated group were significantly lower on day 10 PT. Decreasing trends from day zero indicated the reduction in the number of worms since presence of worms and subsequent damaged to abomasal mucosa induce production of enzyme alkaline phosphatase (ALP) in gastric mucosa which eventually pushes the serum ALP level up. Following treatment, no significant changes were noticed in the creatinine level in all treated groups and were in normal limit. This suggests that extract did not cause any harm to the kidney function.

In the present study, both *Embelia ribes* and *Vernonia anthelmintica* in combination induced moderate anthelmintic activity. The possible mechanism of *Embelia ribes* may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis. It may also have activated nicotinic cholinergic receptor in the worms resulting in either persistent depolarization and hyperpolarisation (Tripathi, 2006; Swarnkar *et al.*, 2009). The molecule which is said to be responsible for anthelmintic acitivity in *Embelia ribes* is embolic acid or embelin. This embelin is of small molecular weight and cell permeable and which alone or synergistic with other bioactive molecules exerts its anthelmintic activity. In *Vernonia anthelmintica*, the anthelmintic property may be attributed to the molecule vernolin, vernolic acid, vernodalol in addition to flavonoids and fatty acids (Javed and Akhtar, 1990). However, the mechanism of action of these compounds is not fully understood. Despite having moderate efficacy, it is clear from the present investigation that *Embelia ribes* and *Vernonia anthelmintica* can very well be the alternative therapeutic approach and can provides an answer to the problem caused by widespread development of

anthelmintic resistance. However, more research work needed to be done regarding standardization of dose in different species of animals for an effective *in- vivo* bioavailability of bioactive molecules, detection of new bioactive substances, mechanism of action of bioactive molecules, toxicity in animals before it can be put to large scale commercial use in animals.

Conclusion

Aqueous alcoholic extract of *Embelia ribes* fruits and *Vernonia anthelmintica* seeds reduced faecal egg count by 72%. Improvement in haematological and biochemical values suggested the reduction in number of worm loads. Coprological examination showed *Haemonchus contortus* as predominant strongyles. Hence the formulation showed promising efficacy against *Haemonchus contortus* which is the most pathogenic worm of small ruminants and has exhibited resistance to number of anthelmintics. However, further study is needed regarding dose standardization so as to further increase the efficacy of the formulation.

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