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In-vivo anthelmintic efficacy of polyherbal formulation and vidangadi churna on gastrointestinal nematodes of sheep

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ABSTRACT

Background: The use of synthetic anthelmintic alone is not effective for the long-term management of gastrointestinal parasites in livestock as it may lead to development of anthelmintic resistance, residue risk and affordability, noting all these problems use of herbal anthelmintics is gaining more importance. **Objective:** The anthelmintic property of polyherbal formulation and vidangadi churna were evaluated against gastrointestinal nematodes of sheep. **Materials and Methods:** Eighty clinically suspected sheep were randomly divided into 8 groups of 10 animals each. Group I and II were dosed with fenbendazole at the rate of 5 mg/kg body weight [Recommended dose] and 15 mg/kg body weight [dose which farmers are using at field level] orally, single dose. While group III, IV and V were dosed with polyherbal formulation in equal proportion according to the dose @30g, 60g and 120g per animal orally, single dose and group VI, VII and VIII were administered different doses of vidangadi churna viz., 12.5g, 25g and 37.5g per animal, orally, single dose. Faecal samples from each individual in all groups were collected and quantitative estimation of faecal sample was done on day 0 (pre -treatment) and 14 days after the treatment. **Results:** Using faecal egg count reduction test (FECRT), on 14th day post treatment polyherbal formulation showed an efficacy of 54.80 per cent, 70.19 per cent, and 87.03 percent at a dose rate of 30, 60 and 120 gram per animal respectively, while vidangadi churna showed an efficacy of 90.78 per cent, 82.44 per cent and 70.20 per cent at a dose rate of 37.5g, 25g and 12.5g per animal respectively. As per World association for advancement of veterinary Parasitology (WAAVP), fenbendazole was found to be resistant in both the doses administered in the present study. **Conclusion:** Thus, the herbal product used in the current study can be employed as an alternate to synthetic anthelmintics.

Keywords: Polyherbal Formulation, Vidangadi Churna, Gastrointestinal Nematode, Sheep, Fecrt.

INTRODUCTION

India is the third-largest country in the world in terms of sheep population, contributing 74.26 million of the 1.176 billion total sheep population worldwide. In India, there are 74.26 million sheep according to the 20th livestock census of 2019, with Karnataka accounting for 11.1 million. However, the sustainability in sheep production can be impeded by various factors including parasitic infections. In developing countries like India, several commercially available synthetic anthelmintics have been employed to control gastrointestinal nematode infections in livestock. In the past 5 decades, chemical control of parasites in animals has been incredibly successful because of its exceptional effectiveness, broad spectrum nature, and good margin of safety. However, due to their extensive use and abuse, anthelmintic resistance has been reported particularly in gastrointestinal nematodes of cattle, sheep, goats, and equines [1]. Currently, anthelmintics most frequently employed in small ruminants are benzimidazoles, levamisole and macrocyclic lactones. However, resistance has emerged to all kinds of anthelmintics [1-6]. Among several approaches to control gastro intestinal nematode infection, herbal preparations are popular for the sustainable and cost-effective management of gastro intestinal nematodes [7]. Herbal anthelmintics are needed primarily to combat the rise of drug-resistant parasites and to provide accessible, affordable deworming alternatives with fewer side effects. In Indian subcontinent, where medicinal herbs are utilized to cure both domesticated animals and human problems, the ayurvedic medical system is becoming more and more popular [8]. The ingredients used in vidangadi churna and polyherbal formulations have shown good anthelmintic efficacy in the previous

studies [9-12]. Therefore, the current study was undertaken to evaluate the *in vivo* anthelmintic efficacy of ayurvedic formulations which may be employed as an alternative to synthetic anthelmintic.

MATERIAL AND METHODS

Chemicals and reagents

The chemicals used in the present study were analytical and molecular grade. Fenbendazole (FBZ) in pure form was procured from Sigma-Aldrich. Both the herbal products under study were procured from Transdisciplinary University, Yelahanka, Bangalore.

Preparation of ingredients

The different doses for the trial were calculated based on traditional usage and available previous studies of different ingredients used in preparation of polyherbal formulation and vidnagadi churna. Ingredients of polyherbal formulation (*Musa paradisiaca*, *Momordica charantia*, *Allium cepa*, *Leucas aspera*, *Azadirachta indica*, *Brassica juncea*, *Curcuma longa*, *Allium sativum*, *Cuminum cyminum* and *Piper nigrum*) of different doses viz., 30g, 60g and 120g were weighed in equal ratio according to the dose required (Figure 1) After weighing, the ingredients were chopped in a fine manner and were grounded. Then jaggery was added to it and a bolus was prepared.

Ingredients of vidangadi churna (*Embelia ribes*, *Acorus calamus*, *Terminalia chebula*, *Hordeum vulgare* and rock salt) of different doses viz., 37.5g, 25g and 12.5g were weighed in equal ratio (Figure 2) and a bolus was prepared by mixing all the ingredients with water and jaggery.



Figure 1: Ingredients of polyherbal formulation



Figure 2: Ingredients of vidangadi churna

Experimental Animals and *In vivo* study

Eighty clinically suspected sheep with more than 600 EPG of faeces without prior treatment with dewormer for two last months and weighing 20-25 kg irrespective of sex were selected for study. The institutional animal ethics committee (IAEC) approval (Ref. No. IAEC/LA-99/2023-24) has been taken for the trial before starting work. The animals were randomly divided into 8 groups of 10 animals each. Group I and II were dosed with fenbendazole at the rate of 5 mg/kg body weight [Recommended dose] orally, and 15 mg/kg body weight [dose which farmers are using at field level] orally, single dose. While group III, IV and V were dosed with polyherbal formulations in equal proportion according to the dose @ 30g, 60g and 120g per animal orally, single dose respectively. While Group VI, VII and VIII were treated orally with single dose of different concentrations of vidangadi churna viz., 12.5g, 25g and 37.5g per animal, respectively. Faecal samples from each individual in all groups were collected on day 0 (pre -treatment) and 14 days after the treatment. The egg per gram were then counted by modified Mc Master technique. Per cent faecal egg count reduction (FECR) was estimated using the formula given by Mc Kenna [13]. $FECRT\% = 100 \times (1 - T2/T1)$, Where, T1 = Pre- treatment FEC, T2 = Post- treatment FEC.

Statistical Analysis

The data obtained in the present study were analysed using SPSS 16.0 version software. The significant effect of *in vivo* test between groups was determined by using two-way ANOVA where P value <0.05 was considered statistically significant.

RESULTS

Fenbendazole which was used as a standard reference drug reduced the faecal egg count (FEC) to 66.95% at 5 mg/kg body weight (Group I) and 89.76% at 15 mg/kg (Group II), respectively. The lower 95% confidence value at the dose 5 mg/kg and 15 mg/kg body weight were 32.64% and 48.63% which is less than 90%. The faecal egg count reduction (FECR) % less than 95% and 95% confidence value less than 90% indicate resistance to fenbendazole as per W.A.A.V.P (Coles *et al.*, 1992).

On 14th day post- treatment, using FECRT polyherbal formulation showed anthelmintic efficacy of 54.80%, 70.19% and 87.03% at a dose rate of 30, 60 and 120g per animal, respectively. On 14th day post-treatment, significant ($p < 0.05$) decrease in mean EPG \pm SE viz., 470 \pm 71.56, 310 \pm 31.44, and 140 \pm 37.11 was observed in groups III, IV, V respectively (Table. 1).

Vidangadi churna showed maximum efficacy of 90.78% at dose rate of 37.5g per animal followed by 82.44% at 25g and 70.20% efficacy at 12.5g per animal, respectively on 14th day post-treatment. The mean EPG \pm SE in all the groups (VI, VII, VIII) on 14th day post treatment was observed to be decreased significantly ($p < 0.05$) as 730 \pm 152.05, 460 \pm 129.27 and 280 \pm 89.19 (Table. 1).

DISCUSSION

The highest faecal egg count reduction (FECR%) of 51.50% was recorded in sheep dosed with neem leaves extract @100 mg/kg body weight [14]. Similarly, 53.72% efficacy of aqueous extracts of neem against *Haemonchus contortus* and 53% efficacy of neem leaves, against gastrointestinal helminths was recorded in sheep [12,15]. The present finding showed efficacy of 87.03% at the rate of 120 g/animal and 70.19% at the rate of 60 g/animal polyherbal formulation which also contain neem leaves. The lower efficacy may be due to the lower dose administered and the source of the leaves.

Table 1: *In vivo* anthelmintic efficacy of fenbendazole, polyherbal formulation and vidangadi churna using Faecal Egg Count Reduction Test (FECRT)

Groups	Anthelmintic	Dose	Mean EPG±SE		FECR %
			0 days	14 th days	
I	Fenbendazole	5mg/kg	1390±203.55	460±89.69	66.95
II	Fenbendazole	15mg/kg	1350±286.45	140±33.99	89.76
III	Polyherbal formulation	30 g/animal	1040±203.96	470±71.56	54.80
IV	Polyherbal formulation	60 g/animal	1040±158.60	310±31.44	70.19
V	Polyherbal formulation	120 g/animal	1080±241.66	140±37.11	87.03
VI	Vidangadi churna	12.5 g/animal	2450±438.49	730±152.05	70.20
VII	Vidangadi churna	25 g/animal	2620±495.93	460±129.27	82.44
VIII	Vidangadi churna	37.5 g/animal	3040±301.18	280±89.19	90.78

The present finding is in accordance with [16] who reported 86% FECR using crude aqueous extract of combined herbal product (Bitter gourd, Neem and Clove) at the rate of 1 mL/kg b. wt. on gastro intestinal nematodes of sheep. Feeding neem leaf meal at 5% concentrate ration to naturally infected sheep reduced the FEC to 88% after 12 weeks, indicating its anthelmintic efficacy [17]. Both methanol extract of *Azadirachta indica* @ 0.5 mL/kg body weight and *Momordica charantia* @ 1 mL/kg body weight results in significant (p <0.01) reduction on 4, 8 and 20 days post treatment indicating its anthelmintic activity [18]. The active principle in *Azadirachta indica* (neem) is the azadirachtin which is water soluble and present in 0.22% concentration in its leaves [19]. The anthelmintic properties of *A. indica* are attributed to azadirachtin [20]. The azadirachtin disrupt the vital functions of the target parasites metamorphosis, growth and fertility [21]. Furthermore, neem leaves contain condensed tannin which exert anthelmintic activity thus disrupting the cuticle of *H. contortus* by binding to the worm cuticle glycoprotein [22].

The anthelmintic efficacy of Poly Herbal Mixtures (PHM) containing *Azadirachta indica* (leaves), *Allium sativum* (cloves), and *Chenopodium album* (leaves) on common gastrointestinal nematodes of goats resulted in 82.85% efficacy at 900 mg/kg [23] which is in agreement with the current study. Aqueous extract of *Allium sativum* bulb given @ 5g/animal resulted in 83% efficacy against naturally occurring gastrointestinal nematode infection in sheep [9]. While Masamha *et al.* [24] observed significant reduction in faecal egg count (FEC) of 97.33% of Strongyles and Trichostrongyles species using 10 mL (80% concentration) of raw garlic juice. This suggest that *A. sativum* is effective in controlling Trichostrongyles and Strongyles, known as the most resistant strains due to their mode of reproduction and thick proteinaceous cuticles. The higher efficacy could be due variation in the dose, moreover in the present study the herbal formulation dosed was crude herbs and seeds. Allicin is the principal compound of *A. sativum* which further gets metabolised, producing active metabolites namely allyl sulphides, ajoene and vinylidithins that attributes to its anthelmintic properties [25]. Like others tanniferous plants, *A. sativum* increases the amount of digestible protein in the rumen by combining with protein to produce a non-biodegradable complex which in turn enhance the host immunity and resistant to nematode infection. High tannin content in *A. sativum* may directly inhibit the growth of resident worm populations by interfering with their ability to absorb nutrients, mobility, and reproduce [24]. The anthelmintic efficacy of bittergourd is attributed to glycosides which is saponin like substance and alkaloids (momordicine) as the secondary metabolites namely glycosides, tannins and saponins of the herbal remedies have shown dose-dependent anthelmintic properties [26].

Maximum FEC reduction of 98.7% was obtained on 18th day of post treatment with combined herbal products (aqueous-methanolic extracts of fruit of *Citrullus colocynthis*, rhizome of *Curcuma longa* and seed of *Peganum harmala*) given at the rate of 4 gm/kg b.wt. [27]. The high efficacy when compared to the present study may be due to the extract methodology. The anthelmintic properties of *C. longa* is attributed to cucurbitacin, its secondary metabolite. Besides, its constituent exhibit protective effects on GI tract like inhibition of intestinal spasm by sodium curcumin, secretion of pancreatic enzyme, biocarbonate, gastrin and secretin by p- tolymethylcarbinol [28]. Also *C. longa* could inhibit formation of ulcer in animals caused by helminths which results in release of alcohol and indomethacin, stress, pyloric ligation resulting in significant increase of gastric wall mucus [29]. Dried ground banana leaves (150g mixed with 15g concentrate per animal per day for 10 days) did not reduced the FEC significantly against gastrointestinal nematodes in sheep [30]. Nogueira *et al.* [31] reported moderate efficacy with aqueous extract of banana leaves. The lower efficacy may be due to the extraction process, dose and frequency of dosing. The extract of Piper nigrum (ethanolic) showed anthelmintic activity against earthworm which is attributed to its phytochemical constituents (piperine, saponins, steroids and alkaloids) [11]. Polyphenolic compounds such as flavonoids and phenolic acids present in the jaggery prepared was found to contribute in enhancing its anthelmintic activity [10]. Jaggery was added in the present formulation to enhance the palability besides its anthelmintic potency.

Kumar *et al.* [32] reported 80% efficacy of *Embelia ribes* leaf powder in Rambouillet sheep naturally infected with GI nematode at a dose rate of 10g orally for three days consecutively. *Embelia ribes* was not effective when used individually against GI nematodes of Goats [33]. Also, a mixture of *E. ribes* fruit and *Vernonia anthelmintica* in equal proportion @ 2g/kg reduced the FEC to 93% that shows the herbal formulation was safe and efficacious in treating caprine gastrointestinal nematodiosis. The higher efficacy may be due to the herbal combination; it may also be due to the fact that the active components of two or more medicinal plants enhance one another or create a new active component [33]. Aqueous-ethanolic extract of *E. ribes* and *V. anthelmintica* caused 72.97% reduction in faecal egg count on 10th day of post treatment [34]. The lower efficacy in their study may be due to low dose (100 mg/kg) of the formulation. The mechanism of *E. ribes* may involves inhibiting the parasites intake of glucose and depleting their glucose synthesis ability. It is believed that embelin is the chemical that causes anthelmintic activity in *E. ribes*. Embelin, a cell permeable and having small molecular weight, alone or in combination with other bioactive components exhibited its anthelmintic activity [33]. The other possible mechanism is that, it may

activate the worm nicotinic cholinergic receptor which result in hyperpolarisation or persistent depolarisation [27].

Medicinal plants used in the present study were evaluated for its anthelmintic property on different species. Some authors have reported the anthelmintic activity of *A. calamus* against *Oesophagostomum* spp in pig [35] and tapeworm in rat [36]. The *A. calamus* plant of Indian variety is reported to contain high β -asarone content, which is the active principle. The active principle (asarone) of *A. calamus* rhizome resulted in rapid inhibition of the mobility, followed by mortality of the larvae of *Toxocara canis*, dog roundworm [37]. The fresh leaves juice of *Azadirachta indica* and *Momordica charantia* at 10% concentration resulted in 72.73% efficacy and 57.89% efficacy against *Ascaridia galli* eggs [38].

CONCLUSION

As per World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.), Polyherbal formulation (P.F) was moderately effective giving 87.03% efficacy at highest dose 120g per animal while vidangadi churna (V.C) was effective at highest dose 37.5g showing efficacy of 90.78% and moderately effective at 25g per animal with 82.44% efficacy, respectively. Statistically, overall *in vivo* study showed significant difference in all groups when dosed with polyherbal formulation and vidangadi churna with respect to post-treatment EPG. As herbal products are easily available especially in rural areas, non-toxic and cost effective when compared to synthetic anthelmintics. Thus, the present finding suggested that polyherbal formulation and vidangadi churna may be employed as an alternative to synthetic anthelmintic treatment in controlling the GI nematode infection in Sheep. However, further studies on its pharmacokinetic and toxic effects are required before employing for extensive use in field condition.

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Author Contributions

PBS, AKJ, DH and KSK were involved in concept design, literature review and execution of research work: SHB carried out research work: SR and RMS conceptualized the manuscript: YHM was involved in statistical analysis. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available and will be made available upon request.

Use of AI in Drafting of Manuscript

The authors declare that they have not used any generative AI/AI-assisted technologies in the writing of this manuscript.

Conflict of interest

The authors declared no conflict of interest.

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