

Anthelmintic Activity of *Withania somnifera*, L. Dunal Water Extract in Sheep

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The anthelmintic activity of *Withania somnifera*, L. Dunal a Maasai traditional anthelmintic was studied in sheep. The McMaster egg count technique and coproculture was used to evaluate worm infestation and the effect of the water extract of the medicinal plant. The water extract was prepared in accordance with the Maasai traditional method. The results showed that the reduction in the egg per gram count in sheep treated with *Withania somnifera* water extract is comparable to that of Lepoxy[®] (a levamisole-based anthelmintic) and was significant ($p < 0.05$) when compared to the control non-treated animals. It is concluded that *Withania somnifera* has anthelmintic activity and that the effect against tapeworms (*Moniezia benedeni* and *M. expansa*) is more predominant.

Key words: *Withania somnifera*, anthelmintic, sheep.

INTRODUCTION

In order to maximize livestock production, new drugs to combat a plethora of livestock diseases, increased drug resistant organisms and new emerging livestock maladies must be discovered. In the recent years, drug resistant organisms appear to be on the increase and anthelmintic resistance in livestock is of particular interest [1]. There is therefore a need to identify new anthelmintics. An important avenue to use in the search for new anthelmintics of veterinary significance is the Maasai traditional medical practice [2]. Traditional medical practices have been used in the control of livestock diseases and especially parasitic diseases, even though the active agents are not known [3]. It has also been observed that plants extracts used to control worm infestation of veterinary importance show promising results [4-6]. An ethnoveterinary bioprospecting field survey conducted in Kenya showed that the Maasai use 18 medicinal plants to combat livestock diseases [2] among them being *Clausena anisata* and *Withania somnifera* used as traditional anthelmintics [7]. Despite the widespread use of these traditional anthelmintics and in particular *Withania somnifera*, no scientific data is available to support their use and their efficacy has never been evaluated. This study was designed to evaluate the efficacy of the water

extract of *Withania somnifera* on endoparasites in sheep.

MATERIALS AND METHODS

Herbal extract preparation

Fresh roots of *Withania somnifera* were collected in the field. The roots were washed with cold water (to remove the excess soil) and sun dried. The roots were weighed and to 0.5 kg of roots, 500 ml of water was added and boiled for 30 minutes in accordance with the Maasai extraction method. The mixture was cooled and after removal of the roots, the extract was filtered and used *ex tempore* on-farm.

Experiment Location

The investigation was carried out at a field station near Kitengela in Kajiado district, 50 km from Nairobi City Centre. The field station had 80 dorper sheep. Cattle and goats grazed on the same pasture. The sheep were housed separately in 8 newly constructed pens holding 10 sheep each.

Animals

Adult dorper sheep were used for the experiment. Thirty-five sheep were randomly screened for worms (helminths) and thirty sheep were used in

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the study. The sheep that had over 300 nematode eggs per gram of faeces were selected for the study. In addition the selected sheep showed clinical signs of poor hair coat, bottle jaw oedema and nasal discharges. The sheep were divided into three groups and given identification numbers using ear-tags before the administration of the various treatments. The sheep in group I were the control and received no treatment; Group II received 10 mls of Lepoxy[®], a commercial levamisole-based anthelmintic at the manufacturers recommended dosage; Group III received 20 mls *Withania somnifera* extract (WSE). After dosing, the animals were allowed to graze naturally in the pasture.

Sample Collection Detection

Fecal samples were collected before treatment (day 0) and on days 1, 3, 5, 7, 10 and 15. The samples were routinely examined for the presence of worm eggs using the McMaster technique.

Technique for nematode detection

The Modified McMaster Egg Count Technique

Briefly, the presence of nematodes eggs in faeces was determined based on 3 g of faeces using the modified McMaster methods of study [8]. A saturated magnesium sulphate solution was poured into glass vials to which 3 g of faeces were added. The contents were mixed thoroughly and passed through a coffee strainer. While stirring the filtrate, a drop was transferred to the Counting Chamber of the McMaster slide. The slide was left for 3 min to allow the eggs to rise to the top of the slide. The slide was examined under low power (x 100 objective) and eggs were counted and identified under the microscope. The count obtained was multiplied by 100 to get the total number of eggs per gram of faeces (EPG).

Efficacy Test

The overall anthelmintic efficacy was calculated from egg count depression. This was the difference between the group mean eggs/g pre- and post-treatment value. The percentage reduction (FECR) was corrected for changes that

may have occurred in the control groups by the equation

$$FECR\% = (1 - [T_2/T_1 \times C_1/C_2]) \times 100$$

Where, T and C are the geometric means for the treated and control groups and subscripts 1 and 2 designate the counts before and after treatment respectively.

Coproculture of infective nematode larvae

Fresh samples from animals that showed high EPG (>1000) and anaemia were cultured on day 0 and 15. Positive samples of EPG >1000 were pooled together, mixed and cultured in a jar with a tightly fitting lid. The incubation period was 10 days at 27 °C. On the 10th day the larvae were recovered using a pasture pipette onto a slide and a drop of Lugol's iodine added and identified under the microscope.

Data Analysis

The data was analyzed using one-way analysis of variance (ANOVA). The level of significance was set at 5 % (p<0.05). LSD was performed to compare means. Results are presented as means and standard error of the mean.

RESULTS AND DISCUSSION

Nematode and tapeworm eggs were present in the faecal samples of the sheep. Compared to the control, the EPG counts decrease (p<0.05) in response to treatment with WSE and Lepoxy[®]. The decrease occurred on day 1 post dosing for both WSE and Lepoxy[®] and progressively decreased to day 15 (Figure 1).

On day zero, coproculture of pooled faecal samples revealed larvae of four main nematodes namely: *Haemonchus spp.*, *Trichostrongylus spp.*, *Oesophagostomum spp* and *Cooperia spp.* On day 15, only *Haemonchus spp.* and *Trichostrongylus spp.* L₃ larvae were identified. In addition, tapeworms *Moniezia expansa* and *Moniezia benedeni* were visually identified on day 0 and on day 15 in the control group. However, no

tapeworms were visually identified in groups receiving Lepoxy[®] and the WSE.

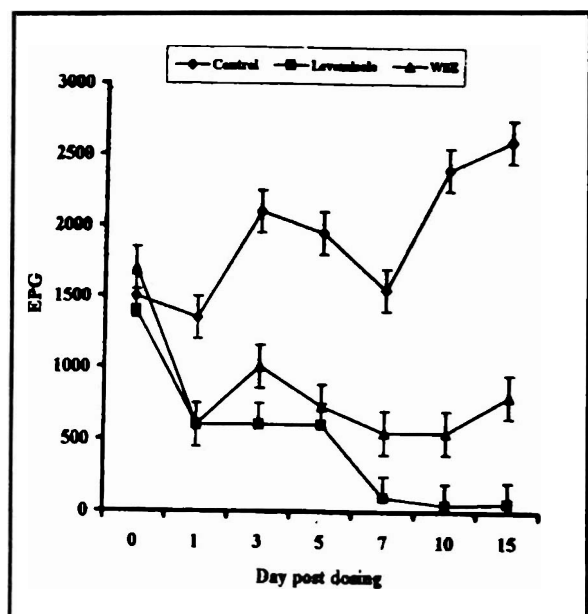


Figure 1: Effect of *Withania somnifera* water extract and Lepoxy[®] (a levamisole-based anthelmintic) on Egg per gram (EPG) counts in sheep

The clinical signs of bottle jaw oedema disappeared in the sheep receiving 20 mL WSE after day 3 of administration and by day 5, all the animals were healthy. Nasal discharges disappeared on day 3. On day 7 all animals with rough hair coat had started to improve and the two treatment groups were not distinguishable. The reversal of clinical signs in the two treatment groups was very comparable. However, in the control group clinical signs of helminthosis persisted.

The antitumor and radiosensitizing properties [9], GABA-mimetic activity [10] and antioxidant activity [11] of *Withania somnifera* have been reported. This study is the first to give laboratory data on the anthelmintic activity of *Withania somnifera*. However, Miaron [7] reported that the Maasai have traditionally used the plant as an anthelmintic and against ecto-parasites. In this study, no attempt was made to identify the active principles. Nevertheless, the herbal preparation appears to have been effective against *Oesophagostomum* and *Cooperia spp.* in addition

to tapeworms. It is also most likely that the reduction in EPGs does suggest some effects on the *Haemonchus* and *Trichostrongylus spp.* This is also supported by the reversal of clinical signs such as disappearance of bottle jaw oedema and nasal discharges. There is definitely a need to determine the pharmacological doses of the anthelmintic active principle in *Withania somnifera*. The tapeworms *M. benedeni* and *M. expansa* visually observed on day 0 of the experiment appear to be more susceptible to the WSE. The overall anthelmintic efficacy calculated from egg count depression was 91 ± 3 for the levamisole-based preparation and 88 ± 5 % for WSE. These value were within the accepted value of >90 %. This study confirms that the Maasai traditional anthelmintic *Withania somnifera* shows activity that is comparable to levamisole (Lepoxy[®]). However, further research to identify the active principle is indicated.

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