Ahmed M, et al., J Anim Res Vet Sci 2017, 1: 003
DOI: 10.24966/ARVS-3751/100003

HSOA Journal of Animal Research and Veterinary Science

Research Article

In vitro and *In vivo* Effects of *Aloe ferox* Extracts on Gastrointestinal Nematodes Control and Live Weight Gain of Young Sheep

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Abstract

Gastrointestinal nematodes reduce production of small ruminants globally. Producing cheap and safe anthelmintic drugs with novel modes of action is the aspiration of many involved in this field. This study evaluated the *in vitro* and *in vivo* effects of a medicinal plant, *Aloe ferox* Mill, on gastrointestinal nematodes in sheep. Ethanolic extracts of different *A. ferox* fractions (dried leaves, pulp and cuticle, and gel of fresh leaves) were tested *in vitro* after dilution with water to concentrations of 5, 10 and 20% of the concentrate. Dried leaves had the greatest effect (P<0.001) on nematode larvae. A further *in vivo* investigation was done using twenty four lambs, aged 3-4 months (initial body weights of 22.1±4.3kg) were used. Gender, initial Eggs Per Gram (EPG) of faeces and initial body weight were used to place lambs into four groups of 6 lambs. Groups were then assigned to 4 treatments randomly. Dried, powdered leaves of *A. ferox* at 0, 50g, 100g and 250g per lamb were given daily for 10 weeks after it was mixed with a standard feed. Lambs were weighed weekly. Rectal faecal samples were taken every 7 days up to Day 70, and EPG were counted in individual samples. Average Daily Gain (ADG) increased with *A. ferox* treatments, whereas EPG decreased (P<0.001) with time. Feeding of 250g of dried, powdered *A. ferox* leaves resulted in the highest ADG and maximum reduction of EPG. These findings suggest that *A. ferox* has the potential to improve animal weight gain and to suppress the production of eggs by gastrointestinal nematodes.

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Citation: Ahmed M, Basha NA, Laing MD, Nsahlai IV (2017) *In vitro* and *In vivo* Effects of *Aloe ferox* Extracts on Gastrointestinal Nematodes Control and Live Weight Gain of Young Sheep. J Anim Res Vet Sci 1: 003.

Received: September 26, 2017; **Accepted:** November 09, 2017; **Published:** November 22, 2017

Keywords: *Aloe ferox*; Biological control; Ethanolic plant extracts; Nematodes; Sheep

Introduction

Gastrointestinal Nematode (GIN) infections are a serious veterinary health challenge and the existing means are in adequate for addressing the problem [1]. In general, GINs reduce productivity of small ruminants by means of lowering fertility, reducing in milk production and depressing weight gain when feed intake is reduced [2].

In addition, loss of endogenous protein, anemia and impact on animal health. Treatment costs are high. In critical situations, death may result from severe infections [3]. Currently, the conventional control strategy for GIN infections is the use of synthetic anthelmintic drugs [4]. However, development of resistance by the parasites to the available drugs is widespread, and alternative control measures are needed. Consumers of animal by-products have also become cautious of possible contamination of meat and milk products as a result of drug residues. Small-scale farmers are leaving the livestock industry because of the unaffordable cost of drugs. Thus, alternative methods for controlling GINs are being studied. These include supplementing feed with medicinal plant products with anthelmintic properties.

The effect of supplementing animal feed with anthelmintic plant products depends upon the availability of that plant, its palatability and selective behaviour of animals. However, many plants have shown potential to control GINs in animals, such as *Sericea lespedeza* Dum. Cours. [5], *Ficus* spp. [6] and *Aloe ferox* Mill [7]. Their anthelmintic properties are attached to various active ingredients and their concentrations in the plants. However, some of these active ingredients have been associated with adverse reactions when fed to livestock. For example, tannins, the recognised active substance in *Sericea lespedeza* and *Acacia* spp., when fed to livestock in large quantities, reduce voluntary feed intake and digestibility [5].

The objectives of the current study were to determine the *in vitro* effects of variousfractions of *A. ferox* plants on nematodes larvae. Further, to examine *in vivo* their anthelmintic activity and their effect on body weight gain of lambs when fed *A. Ferox* plant in feed.

Material and Methods

Plant collection and preparation

Aloe ferox plant material was collected from a private garden in Pietermaritzburg. A voucher specimen of plant was deposited at the UKZN Herbarium, Pietermaritzburg. Ethanolic extracts of three plant fractions (dried leaves, skins of fresh leaves and the gel of fresh leaves) were prepared as per [8]. Ten gram samples of the dried leaves and skin of fresh leaves and 10ml of the gel of fresh leaves were boiled for 24 hours in 100ml of ethanol in a Soxhlet's apparatus. The resulting extracts were transferred to 50ml test tubes and placed in a waterbath (LABOTEC, Model 101, South Africa) at 50°C. Extracts were condensed by evaporation of the solvent to a final volume of 30ml. Condensed extracts were then preserved in airtight glass bottles and stored at 10°C until required for screening.

In vitro anti-nematodal activity of *Aloe ferox* products

Faecal samples were collected from 24 randomly selected Merino sheep grazing on a contaminated kikuyu pasture (*Pennisetum*

clandestinum Hochst. ex Chiov.) at ukulinga research farm. Rectal faecal samples were taken from sheep by hand and stored in plastic bags. Faeces were pooled and mixed. Sub-samples (5g) were placed in trays and incubated for 12 days at 27°C. Samples were kept damp by daily watering at 09: 00h during the period of incubation. On day 10, ethanol extracts of the three plant fractions (dried leaves, skins of fresh leaves and the gel of fresh leaves) were applied in 5, 10 and 20% concentration (v/v water). Each treatment was used to treat 4 trays and there were 4 controls where only an ethanol/water solution at 20% was used. Both control and treated trays were incubated for a further 48 hrs. Baermann Technique was used to count nematode larvae as described by [9]. Samples were examined and larvae were counted using 100x magnification, after adding 0.2ml of iodine stain to the slide and covered with a cover-slip.

In vivo screening of different dosages of *A. ferox* leaves

In a second experiment, dry powdered leaves of *A. ferox* were testedin an *in vivo* trail due to great efficacy in experiment (1). Twenty four lambs (12 females and 12 males), aged 3-4 months, with initial body weights of 22.1±4.3kg, were used. The initial faecal egg counts were determined in rectal faecal samples. Gender, initial Eggs Per Gram (EPG) of faeces and initial body weight were used to place the sheep into four groups of six lambs each and then each group was randomly assigned to a treatment. Sheep were naturally infected with mixed cultures of gastrointestinal nematodes. *Haemonchuscontortus* (Rudolphi, 1803) at 27% predominant species, others like *Trichostrongylus, Strongyloides, Coopera* and *Nematodirus Spp* were also found [4]. Sheep were housed in pens (0.90*1.50m) in sheep facility at ukulinga research farm. Each pen was provided with an individual feeder and drinker on wooden slatted flooring. The facility was temperature regulated with large fans.

Four level of dry powdered leaves of *A. ferox* (0, 50g, 100g and 250g) were added to standard lambs feed which consisted of the following ingredients: Cottonseed cake (37.5kg), hominy chop (33.3kg), molasses liquid (8.6kg) and Vit. Premix (1.1 kg). Lambs were fed the standard diet daily between 07: 00-09: 00h and then given veld hayand water *ad libitum*.

During a 14-days pre-treatment period, sheep were randomly allocated to individual sheep feeding stalls to acclimatize them to handling facilities. At the end of this period, faecal samples were collected and nematode Eggs Per Gram of feaces (EPG) were determined. During this trial, sheep were assigned to one of four treatments: Standard feed without treatment (control), and 50g, 100g and 250g of dry powdered *A. ferox* leaves per sheep daily. Treatments were mixed with the feed formulation given daily for 10 weeks.

Lambs were weighted every week up to Day 70. Rectal grab samples were collected weekly during the experiment for nematode egg counts using the McMaster Technique, according to [9].

Statistical Analysis

Nematode mortality from faecal cultures was calculated using Abbott's Formula [10], as follows:

$$Corrected\% = \left(1 - \frac{n\,in\,T\,after\,treatment}{n\,in\,Co\,after\,treatment}\right) \times 100$$

Where;

n=number of larvae; T=Treated; Co=Control

Nematode larvae counts were analysed using the General Linear Model procedure of SAS (2000). In trial 1, the following statistical model was used to analyse larval mortality at specific concentrations:

$$Y_{ij} = \mu + S_i + e_{ij};$$

Where, Y_{ij}=individual observation; μ=overall mean; S_i=effect of plant parts; e_{ij}=the error term.

Data on faecal egg counts wereanalyzed by using the General Linear Model (GLM) procedure of SAS (2000), according to the following model:

$$Y_{ijkl} = \mu + W_i + T_j + (W*T)_{ij} + G_k + L_l + e_{ijkl;}$$

where: Y_{ijkl}=individual daily observation; μ=overall mean; W_i=weekly effect; T_j=effect of treatment; $(W*T)_{ij}$=interaction between week and treatment; G_k=co-variate effect of initial egg count, L_l=co-variate effect of initial body weight and e_{ijkl}= the error term.

Log transformations were applied to EPG to normalize variance. These data were presented in Table 3 together with the untransformed means, which are easier to interpret. The transformed data were analyzed using the same statistical model.

Results

In vitro screening of *Aloe ferox* extracts for anthelmintic activity

Ethanol extracts from different fractions of *A.ferox* plant had varied (P<0.001) effects on nematode larvae from sheep at the three concentrations (5, 10 and 20%) (Table1). Increasing of the concentration caused stronger larvicidal effects. Dry powdered leaves extract at various concentrations (5, 10 and 20%) had the lowest larvaecounts (75.7-86.9%), followed by skin of fresh leaves (68.3-75.4%), and the gel from fresh leaves (48.7-61.4%). The control had the highest larvae counts (30.5-39.3%) (Table 1).

Aloe Ferox Parts Used	Ethanolic Extract Concentrations% (v/v with water)		
	5	10	20
Dried powdered leaves	75.7±4.2	84.2±3.7	86.9±2.9
Gel from leaves	48.7±2.7	58.3±2.7	61.4±3.3
Skin of fresh leaves	68.3±3.4	72.9±2.1	75.4±1.0
Control	30.5±2.1	33.5±3.4	39.3±2.1
F value	5.74	4.67	4.23
CV%	12.55	10.43	11.67
P<	*0.001*	0.001	0.001

Table 1: *In vitro* efficacy of ethanol extracts of different fractions of a medicinal plant (*Aloe ferox*) against a mixed culture of gastrointestinal nematode larvae of sheep.

CV%=Coefficient of Variance

Live-weight gain

The aloe treatments had significant effects on the live weight of lambs by day 70 (Table 2). All treatments resulted in increased sheep live weight. However, sheep given 250g of A.ferox treatment had the greatest average daily gain of 57.74g, while the Control treatment had the least average daily gain of 14.88g.

Treatments	Wt0 (kg)	Wt70 (kg)	ADG (g)	Rank
50g	25.96	29.92	56.55	3
100g	26.04	29.71	52.38	2
250g	25.71	29.75	57.74	1
Control	25.58	26.63	14.88	4
F value		1.67	15.19	
P<		0.1874	0.001	
LSD		NA	3.51	
CV%		14.66	13.08	

Table 2: Effect of treatments on sheep weight.

W_0=weight on Day 0, W_{70}=weight on Day 70, ADG=Average Daily Gain; LSD=Least Significant Difference; CV% = Coefficient of Variance.

Effect of Aloe ferox plant extracts on nematode eggs count

The nematode egg counts varied (P<0.001) with treatment (Table 3). Pre-treatment EPG counts averaged 1633, 1683, 1758 and 1667 for the Control and sheep fed daily with 50, 100 and 250g of A.ferox /sheep, respectively. However, by week 10, the EPG counts averaged 1542, 900, 833 and 492 for the four treatments, respectively.

Discussion

Ethanolic extracts of all fractions of *A. ferox* used in this study showed larvicidal effects against nematodes larvae *in vitro*. However, dry powdered leaves extracts provided the best larval inhibition at all concentrations. This finding confirms similar work in which an ethanolic extract of *A. ferox* had been used to control *H. contortus* of sheep [7-8] reported *in vitro* larvicidal effects of aqueous extracts of *A. ferox* leaves on *H. contortus* from goats. Gel from *A. ferox* leaves exhibited weak anthelminthic activity compared to other fractions. [11] reported that the gel of *A. ferox* leaves has less secondary compounds with biological activity, as these are found in the sap and outer leaf, and not in the inner gel of the aloe plant. This would explain the efficacy of theskin of fresh leaves. The gel was removed before extracting the rest of the Aloe leaves, thus different results were obtained especially since the polysaccharine gel components would be more water soluble than the rest of the aloe leaves [7]. However, the active compounds are yet to be clearly identified.

In vivo data showed a reduction in the Faecal Egg Counts (FEC) resulting all doses fromaloe treatment compared with the control group, indicating strong anthelminthic activity. Study on goats using *A. Ferox* extracts have reported reductions (P<0.05) in strongyle eggs, administration of two doses of 250 and 500 $mgkg^{-1}$ at a concentration of 100 $mgml^{-1}$ per animal for 9 days decreased faecal egg count dramatically [12,13] also found a significant (P<0.001) decrease of nematode egg counts in naturally infected sheep treated with an *A. ferox* extract. Weekly dosing of 100mg of *A. ferox* kg^{-1} body weight per sheep for 42 days reduced egg counts and numbers of larvae recovered from faecal cultures. [14] found *A. ferox* has a negative effect on nematodes due to the glucoside aloin content, which may cause expulsion of worms from the gastrointestinal tract. *Aloe ferox* extract could affect the egg laying ability of the female adult worms, resulting in reducing of FEC [12]. However, the *in vitro* trial here showed the aloe extract was directly nematicidal.

In this study, there was an increased in live weight gain of treated animals. This confirmed the study of [13], where sheep weight increased when the animals were dosed with an *A. ferox* extract. [15] reported that the means of live weight of lambs treated with *Khayasenegalensis* Desr. extract also improved.

Conclusion

Aloeferox plant possesses anthelminthic properties against nematode parasites of sheep *in vitro* and *in vivo*. Consumption of *Aloe ferox* also resulting in apositive effect on body weight gain. Supplementing sheep feed with powdered *A. Ferox* leaves is a viable treatment to enhance weight gain and control of GI nematodes.

Acknowledgement

The authors are grateful for research funding that was provided by the National Research Foundation, South Africa.

Treatment	Mean Values of Eggs Per Gram of Faeces										
(g of *A.ferox*/sheep)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
50	1683±414	1692±459	1642±486	1617±470	1508±457	1233±459	1158±440	1117±430	1067±433	983±415	900±404
100	1758±257	1750±251	1700±243	1550±225	1450±268	1058±281	1017±248	1000±227	983±199	867±210	833±183
250	1667±311	1675±316	1550±317	1350±304	1242±294	983±287	958±286	900±283	792±295	700±282	592±214
0	1633±447	1667±442	1700±435	1758±432	1758±408	1758±407	1608±403	1558±411	1550±415	1550±422	1542±437
F value	0. 01	0.01	0.03	0.15	0.25	0.95	1.12	1.15	1.47	2.23	3.52
CV%	67.94	64.43	66.13	67.73	70.36	69.69	57.45	58.01	59.44	59.01	60.74
P<	*0.998*	0.999	0.994	0.926	0.863	0.434	0.364	0.354	0.253	0.001	0.001
Treatment	**Transformed egg per gram of faeces (Log transformed)**										
(g of *A.ferox*/sheep)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
50	3.13±0.05	3.13±0.06	3.12±0.06	3.11±0.06	3.09±0.06	3.03±0.06	3.01±0.06	2.99±0.06	2.98±0.06	2.95±0.06	2.92±0.06
100	3.19±0.04	3.18±0.04	3.17±0.04	3.14±0.04	3.11±0.05	2.98±0.06	2.97±0.05	2.95±0.05	2.93±0.04	2.88±0.05	2.85±0.04
250	3.18±0.04	3.19±0.04	3.15±0.04	3.09±0.04	3.04±0.04	2.96±0.04	2.95±0.04	2.93±0.04	2.87±0.05	2.83±0.04	2.68±0.04
0	3.17±0.06	3.13±0.06	3.14±0.06	3.15±0.06	3.15±0.06	3.15±0.06	3.14±0.07	3.13±0.07	3.13±0.07	3.13±0.07	3.13±0.08
F value	0.11	0.1	0.04	0.08	0.21	0.84	0.96	0.98	1.41	2.34	4.79
CV%	8.34	8.31	8.27	8.27	8.58	7.47	6.95	7.19	7.37	7.05	7.23
P<	0.953	0.961	0.988	0.968	0.888	0.487	0.43	0.4238	0.27	0.001	0.001

Table 3: Effect of different concentrations of Aloe ferox plants on the mean number of nematode eggs per gram of faeces in lambs.

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