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Effectiveness of *elephantorhiza elephantina* as traditional plant used as the alternative for controlling coccidia infections in goats.

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ABSTRACT

Elephantorrhiza elephantina Bruch Skeels is used by farmers in the Eastern Cape Province of South Africa to control Gastro-intestinal parasites in goats. The objective of the study was to determine the effects of *E. elephantina* on coccidia oocysts and determine the effectiveness of the dose levels on goats. Thirty six goats (18 males and 18 females) between ages of 8-12 months were used; randomly assigned to six treatments (A-F). Treatments A and B received Prodose orange® at 2ml/10kg and distilled water at 0.5 ml/kg per goat respectively, whereas treatments C (12.5mg/ml), D (25mg/ml), E (50mg/ml) and F (75mg/ml) received aqueous extract of *E. elephantina* at different concentrations dosed at 2ml/10kg. Faecal samples were collected on first month, second month and third month for faecal egg counts (FEC) and body-weights recorded to assess weight changes. Animals receiving the extract had weight increases of between 3 to 4 kg by end of experiment, but those drenched with Prodose orange ® gained by less than 2 kg. Findings are indicative that *E. elephantina* possess some anti-coccidian properties against coccidia oocysts in goats.

Keywords: *Elephantorrhiza elephantina*; faecal egg counts; coccidia oocysts, Ethno-veterinary medicine.

1. Introduction

All adult goats have coccidia in their guts, even healthy ones unless they have been treated against (Gabrielle *et al*., 2011). High numbers of coccidia cause pathological damage to goat resulting in the occurrence of the disease (Muhammad *et al*., 2011). However, goats are easily affected by many diseases because of poor hygienic conditions and inadequate management, and coccidiosis is one of the most serious diseases that are predisposed by these factors (Mahmoud *et al.,* 2003). Therefore, research is needed on how to treat and control internal parasites in goats (Madibela and Kelemogile, 2008). Some resource-limited farmers reported that they use commercial drugs to reduce coccidia oocysts (Mwale and Masika, 2009) but since the commercial drugs are expensive on communal farmers, they favour the use of traditional plants. The research that was conducted by Madibela and Kelemogile, (2008) concluded that exposure of *Melia azedarach* fruits to coccidia infected Tswana goats resulted in reduced oocysts counts. The main objective of this study was to investigate the *in vivo* effects of *E. elephantina* on coccidia oocysts and to determine effective doses on goats.

2. Materials and methods

2.1 *Description of study area*

The study was conducted at Honeydale Farm, University of Fort Hare. The farm is located 5 km east of the town of Alice, Eastern Cape, South Africa and is 520 m above sea level. The average rainfall was approximately 480 mm per year, and most of the rainfall was from November to March. Mean temperature of the farm was about 18.7° C per year (Muchenje *et al*., 2008).

2.2 *Plant material collection and extract preparation*

The roots of *E. elephantina* were collected from a natural population in Matatiele district in the Eastern Cape Province of South Africa. Immediately after collection, the roots were cleaned and air dried under shade for about two weeks. The dried roots were then be crushed into powder using a Waring commercial laboratory blender (South Africa), placed in clean containers and labeled for easy identification. Extraction of the powder was carried out according to the method of Wagner *et al*. (1993), using methanol as the solvent. Briefly, 500 g of

the powder was packed in 2 l flasks, 1 litre 80% methanol added, and extraction allowed to take place for 3 days. The resultant extract was then filtered through a 70 mm diameter filter paper under pressure, and the filtrate evaporated to dryness *in vacuo* at 50^{o}C, using a rotary evaporator (Buchi Labor technik AG),coupled to a thermo regulator, and finally dried in a fume cupboard, was yield about 155 g extract powder. Part of the extract (150 g) was partitioned between *n*-hexane (1200 ml) and water (600 ml), and the aqueous phase was further partitioned using ethyl acetate (EtOAc) (1100 ml). The fractions obtained were then concentrated using a rotary evaporator, and later dried in a fume cupboard. The dried fractions were weighed yielding 8 g for hexane, 42 g for EtOAc and 90.5 g for the aqueous fractions. These were then stored in labeled bottles at 4˚C until required for use, but in this study, the aqueous fraction was used.

2.3 *Solution preparation*

To prepare the stock solution, the aqueous fraction was dissolved in distilled water at concentrations of 12.5, 25.0, 50 and 75mg/ml. Distilled water was used as negative control while Prodose orange ® (Albendazole 1,92% m/v, closantel 3,94% mv), Virbac Animal Health, South Africa) was used as positive control.

2.4 *Animal preparation*

Thirty six naturally infected goats (18 males and 18 females) of 8-12 months old, weighing with the average of 16 kg, which had never been dosed before were used in the experiment. Goats were herded during day for 7 hours, allowed free access to water and penned overnight, and ear tags were used for their identification. The experimental protocol was approved specifically for the experiment and was in compliance with the University of Fort Hare Ethics Committee on research in animals, and internationally accepted principles for animal use and care (Doerfler and Peters, 2006). A local state veterinarian inspected the goats weekly throughout the study period to ensure that compliance with the welfare standards was done.

2.5 *Experimental design and treatment procedures*

Stratified random method was used to allocate goats based on body weight to each of the 6 treatments with six animals each and where mixed as males and females. Treatments A and B received Prodose orange® (Albendazole 1.92% m/v, closantel 3.94% mv) at 2ml/10kg, and water at 0.5ml/kg respectively, whereas treatments C (12.5mg/kg), D (25mg/kg), E (50mg/kg) and F (75mg/kg) received different concentrations of *E. elephantina* aqueous fraction at 2ml/10kg orally using a syringe.

2.6 *Body weight measurement*

Goats were weighed at the beginning of the experiment (day 1) and twice at 28 day intervals post treatment over two months using a commercial scale (Ruddscale, Durbanville, South Africa). The goats were weighed in the morning (08:00) before feeding and watering.

2.7 *Oocyst counting*

The goats to be used in the study were naturally infested coccidial parasites. The coccidia oocysts count were determined before the beginning of the experiment, to assist in the grouping of goats according to coccidian load burden. After grouping the goats, samples were collected per rectum on day 1 of the experiment before dosing, and subsequently twice at 28 day intervals post-treatment to confirm the presence of coccidia. The faecal samples were put in the labeled screw cap bottles that were filled to capacity to ensure exclusion of air from the containers. To preserve the coccidia oocysts, 3 % formalin was added into the faecal samples and the bottles closed tightly. The samples were packed and dispatched in a cooler box.

On arrival at the laboratory, the samples were immediately stored in a refrigerator at 4 ˚C for preservation before laboratory analyses. Faecal oocysts counts per gram of faeces were determined using the Modified McMaster method according to Hansen and Perry (1994). The obtained values were expressed as oocysts per gram (OPG) of fresh faecal samples, with lower limit detection of 50 OPG. Reduction in oocysts counts was based on mean coccidia oocysts count on day 1, which was the first day of the experiment but before treatment and was calculated using the following formula:

$$\text{TFECR } (\%) = \frac{(\text{TEPGpret} - \text{TEPGpostt})}{\text{TEPGpret}} \times 100$$

Where TEPG is the total eggs per gram faeces; pret the pre-treatment; post the post-treatment; and TFECR is the total coccidia oocysts count reduction according to the method of Chapman *et al* (1991), with slight modifications.

2.8 *Statistical analyses*

The differences in coccidia-oocysts count, body condition score and body weights between the treatment groups were analysed using mixed model procedures for repeated measures of SAS (2003). Coccidia-oocysts count data were log transformed [ln (χ+10)] and the resulting transformed variables were tested for normality using probability plots, skewness and kurtosis.

The model was used is:

$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + E_{ijk}$

Where: Y_{ijk}= observation (body weight and coccidia-oocysts count) of each goat; μ= population mean constant common to all observations; T_i=fixed effect of treatment,; P_j=fixed effect of 28 days; $(T \times P)_{ij}$= treatment and infection interaction and E_{ijk}=random error term, assumed to be normally and independently distributed with mean 0 and variance equal to δ^2.

3. Results and Discussion

All the goats in this study were naturally infected with coccidia. The *E. elephantina*'s effectiveness was dose dependant. It differed at different concentration levels for example treatment F which was 75 mg/kg dose level performed better than other dose levels (Table.1), which was highly significant ($P < 0.05$). However, it showed that the higher the extract concentration the more effectiveness it becomes, which means that the *E. elephantina* could be used be at higher concentration for more effectiveness. Since treatment F was most effective than the other treatments so the resource-limited farmers must be advised to use this dose level for controlling and treating of coccidiosis on goats. Then there is a need to find the best concentration that can be used without affecting the meat quality and without involving more expenses.

This high percentage of positive specimens was consistent with the findings of Alyousif et al. (1992) who reported about 90% of the goats in Saudi Arabia were positive for coccidia and Balicka-Ramisz (1999) who reported that of 110 Polish goats about 81% of adults and about 100% of kids were naturally infected with coccidia. Moreover, Harper and Penzhorn (1999) found that 89-100% of adult goats in South Africa were naturally infected with coccidia.

However, all of goats that are naturally infected with coccidia are mostly treated by coccidiostats which are sulfa based drugs but because of their expensiveness and many of commercial drugs are faced with the problems of drug resistance and toxicity in goats (Nweze and Obiwulu, 2009). Drug resistance in a given population develops due to very long usage of the same drugs with no alternative therapy especially in the case of coccidiosis. Medicinal plants are inexpensive, available and naturally alternatives to commercial drugs. *Elephantorrhiza elephantina* has been reported to possess activities against other internal parasites like *Haemonchus cortortus* (Maphosa *et al*., 2010).

However, Jang et al (2007) studied the anti-coccidian effects of green tea-based diet in chickens following oral infection with *Elephantorrhiza maxima* and found that the green tea-fed chickens produced significantly reduced faecal oocysts. Recently, Nweze and Obiwulu (2009) studied the anticoccidial effects of *Ageratum conyzoides* in broilers and found that the extract had anticoccidial potential. The data reported here indicated that *E. elephantina* reduce the oocysts in goats (Table. 2) and also Prodose reduce the oocysts as *E. elephantina* but the plant is cheaper than the Prodose which is the commercial drug making *E. elephantina* to be preferable than the commercial drugs by resource limited farmers. Nweze and Obiwulu (2009) similarly reported that coccidia species were reduced by *Ageratum conyzoides* which is also the medicinal plant.

4. Conclusion

The study showed that *E. elephantina* have some coccidiostatic properties and thus giving it evidence in its ethno-veterinary use against coccidia species in goats. Prodose in this particular appeared to be effective in controlling coccidiosis while *E. elephantina* performance was also effective exactly like Prodose drug and 75 mg/kg is the effective dose level on goats. There is also a need to investigate the presence or absence of side effects of *E. elephantina* in goats.

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Table 1. Mean FEC and Standard error (SE) of the extracts of *E. elephantina* on coccidia in goats

Worm Species	Treatment	Dose mg/kg	1 Month mean±se	2 Month mean±se	3Month mean±se
Coccidia	*A* Prodose		166.7±0.98	108.3±0.88[b]	56±0.70[c]
	B Water		161.8±0.96	182.8±1.30[a]	240.9±1.40 [a]
	C	12.5	164.7±0.97	160.1±0.87 [a]	40±0.69[b]
	D	25	162.7±0.95	156.2±0.85 [a]	20.2±0.60[c]
	E	50	160.7±0.90	154.3±0.80[a]	10.3 ±0.50[c]
	F	75	158.7±0.89	149.2 ±0.79[a]	5.0±0.40[c]

[abcd]Means with different superscripts in a row differ significantly (P<0.05).

In table showed the mean of the fecal egg count (FEC) in all of the treatments including the water which was the negative control of the experiment, the mean was for all the coccidia found in the goats. However, all the treatments showed the (P<0.05) significant differences. Prodose showed a decrease of coccidian-oocysts from first month to third month. Water showed the increase from the first month to third month. The table below showed that the higher the *E. elephantina* concentration the more the coccidian-oocysts were reduced from the goats.

Table 2. Faecal egg count (coccidian oocysts count) reduction percentage over three months

Parasite	Treatment	FECR%1	FECR%2	FECR%3	Significant
Coccidia	*A* Prodose	0	65.97	33.59	*
	B Water	0	112.98	148.89	NS
	C	0	97.21	124.29	*
	D	0	96	12.29	*
	E	0	96.02	64.09	*
	F	0	94.01	3.15	**

** = Highly significant (P < 0.05); * = Significant (P < 0.05); NS = Non-significant.

In table showed that the faecal egg count reduction as percentage per month was zero percent on the first month of data collection then increased in second month (64.97%) to third month (33.59%). All goat treated by the treatment B (water) showed no reduction in first month but second month (112.98%) and third month (148.89%) showed the increase but water was non-significant (P < 0.05). In all the goats treated with extract treatments no faecal egg count reduction at the start of the experiment, however, in second month showed faecal egg counts reduction increase until third month where it showed more reduction which, means less oocysts found, but in goats treated by treatment F only showed 94.01% reduction in second month and also on third month (3.15%) and it showed highly significant (P < 0.05) than other treatments.

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