Research Note

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Evaluation of anthelmintic activity of liquid waste of *Agave sisalana* (sisal) in goats

Avaliação da atividade anti-helmíntica do resíduo líquido de *Agave sisalana* (sisal) em caprinos

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Abstract

It was evaluated the anthelmintic activity of *Agave sisalana* (sisal) juice against gastrointestinal nematodes and its potential toxic effects in goats. *In vitro* tests showed more than 95% reduction in larval counts of the genus *Haemonchus* spp. at concentrations between 86.5 and 146.3 mg.mL^{-1}. *In vivo* the percent reduction of larvae of the fourth (L4) and fifth (L5) stages of *Haemonchus*, *Oesophagostomum* and *Trichostrongylus* was less than 95% in groups GI and GII, and between 80 and 90% in group GIII. *A. sisalana* juice at the concentrations tested *in vitro* was effective against gastrointestinal nematodes in goats; however, its anthelmintic efficacy was reduced when administered to animals.

Keywords: *Agave sisalana*, anthelmintic, goats, nematodes, sisal.

Resumo

Foi avaliada a atividade anti-helmíntica do suco de *Agave sisalana* (sisal) contra nematódeos gastrintestinais e possíveis efeitos tóxicos em caprinos. Nos testes *in vitro,* encontrou-se redução superior a 95% na contagem de larvas do gênero *Haemonchus* spp. nas concentrações entre 86,5 e 146,3 mg.mL^{-1}. *In vivo*, o percentual de redução de larvas de quarto (L4) e quinto (L5) estágios de *Haemonchus*, *Oesophagostomum* e *Trichostrongylus* foi inferior a 95% para o GI e GII, e entre 80 e 90% para o GIII. O suco de *A. sisalana* nas concentrações testadas *in vitro* foi efetivo contra nematódeos gastrintestinais de caprinos, apresentando, no entanto, reduzida eficácia anti-helmíntica quando administrado nos animais.

Palavras-chave: *Agave sisalana*, anti-helmíntico, caprinos, nematódeos, sisal.

The goat industry is a livestock activity of great socioeconomic importance in Northeastern Brazil, but its operation has been limited by frequent gastrointestinal nematode infections (GNI) and inappropriate drug use, which has led to resistance of these parasites to common anthelmintics.

Given the interest in the use of waste liquid of *A. sisalana* and lack of scientific evidence regarding its potential anthelmintic activity, the objective of the present study was to evaluate the effect of *A. sisalana* juice against gastrointestinal nematodes and its potential toxic effects in goats.

Agave sisalana plants were collected in Valente, Bahia. They were identified by botanists and voucher specimens (No. 838) were deposited in the herbarium of the Universidade Estadual de Feira de Santana.

A. sisalana pulp and juice were collected through mechanical defibration of the leaves. The juice was obtained after filtration three times in filter paper and stored at 4 °C for use in *in vivo* tests. For *in vitro* tests, the juice was dried and frozen. Five concentrations were used in this study: 146.3, 112.5, 86.5, 66.5, 51.1 and 39.3 mg.mL^{-1}, which were tested in triplicate. To validate the results, the same experiment was repeated three times.

Larval cultures (UENO; GONÇALVES, 1998) consisted of 2 g of feces, 2 g of sawdust, and 2 mL of juice. Positive and negative controls were treated with doramectin (0.0625 mg.mL^{-1}) and distilled water, respectively.

Twenty-four racially undefined goats between six and 24 months of age were used. According to eggs per gram (EPG) count, the animals were divided into four groups: Group I (GI) was treated with the juice (0.92 g.kg^{-1}) for four days; Group II (GII)

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Table 1. Mean (±S.E.) and percent reduction (%) in the number of eggs of gastrointestinal nematodes of goats treated with the juice of *Agave sisalana*.

Groups	Days of treatment				
	0	8		15	
	Mean ± S.E.	Mean ± S.E.	%	Mean ± S.E.	%
GI	1675 ± 877[a]	1958 ± 1527[a]	0	1250 ± 346[a]	0
GII	1566 ± 1106[a]	1975 ± 1787[a]	0	2162 ± 1400[a]	0
GIII	1658 ± 1454[a]	117 ± 250[b]	97	50 ± 45[b]	95
GIV	2150 ± 1384[a]	1675 ± 1281[a]	-	1063 ± 553[a]	-

Letters differing in the same column signify statistical differences ($p < 0.05$).
GI – 0.92 g.kg^{-1} BW/4 days; GII – 0.92 g.kg^{-1} BW/8 days; GIII - Doramectin (200 µg.kg^{-1} BW); GIV – No treatment.

was treated with the same dose for eight days; Group III (GIII) was treated with a single dose of doramectin (200 g.kg^{-1}); and Group IV (GIV) (negative control) was not treated.

The material was collected on Days 0, 8, and 15; and necropsies were performed on Days 8 and 15. At necropsy, immature and adult parasites in the gastrointestinal tract were identified and counted, and they were histopathologically evaluated.

The effectiveness of juice on eggs and larvae of gastrointestinal nematodes in goats was evaluated at different concentrations by testing percent reduction (PR) of eggs or larvae (VIZARD; WALLACE, 1987). The number of larvae was transformed into a decimal logarithm to standardize the data (BOX; COX, 1964). Differences between the results of egg and larval counts (L4 and L5)

Table 2. Mean (±S.E.) and percent reduction (%) of the number of gastrointestinal nematode larvae (L_4 e L_5) recovered from goats after treatment with the juice of *Agave sisalana*.

Species	GI		GII		GIII		GIV
	Mean ± S.E.	%	Mean ± S.E.	%	Mean ± S.E.	%	Mean ± S.E.
Haemonchus contortus	213 ± 204[a]	40	305 ± 348[a]	14	48 ± 71[b]	87	322 ± 180[a]
Oesophagostomum columbianum	34 ± 23[a]	29	28 ± 14[a]	32	0,2 ± 0,4[b]	80	46 ± 22[a]
Trichostrongylus colubriformis	118 ± 74[a]	0	78 ± 55[a]	16	6 ± 12[b]	83	130 ± 135[a]
Total	366 ± 216[a]	18	412 ± 389[a]	19	54 ± 68[b]	90	498 ± 291[a]

Letters differing in the same line signify statistical differences ($p < 0.05$).
GI – 0.92 g.kg^{-1} BW/4 days; GII – 0.92 g.kg^{-1} BW/8 days; GIII - Doramectin (200 µg.kg^{-1} BW); GIV – No treatment.

Table 3. Mean (±S.E.) of hematological parameters of goats treated with the juice of *Agave sisalana*.

Parameters	Groups and days of treatment											
	GI			GII			GIII			GIV		
	0	8	15	0	8	15	0	8	15	0	8	15
Erythrocytes (×10^6.µL^{-1}) (RR: 8 - 18)	13 ± 3.4[ab]	10 ± 3[a]	9 ± 1.5[a]	13 ± 2.9[ab]	11 ± 2.2[ab]	12 ± 2.1[ab]	15 ± 3.6[b]	14 ± 3.3[b]	13 ± 1.6[b]	12 ± 1.2[a]	10 ± 1.1[a]	10 ± 1.7[ab]
Hematocrit (%) (RR: 22 - 38)	22 ± 5.1[a]	21 ± 5.7[a]	21 ± 6.3[a]	22 ± 3.3[a]	20 ± 5[a]	23 ± 2.7[a]	26 ± 6.1[a]	26 ± 4.9[a]	26 ± 4.6[a]	22 ± 2.7[a]	22 ± 3.3[a]	21 ± 3.9[a]
Mean cell volume (fL) (RR: 16 - 25)	17 ± 1.9[a]	20 ± 2.8[a]	24 ± 3[a]	17 ± 1.4[a]	19 ± 4[a]	19 ± 2.7[b]	17 ± 1[a]	19 ± 3.4[a]	20 ± 1.7[ab]	19 ± 1.4[a]	21 ± 2.4[a]	21 ± 0.7[ab]
Total protein (g.dL^{-1}) (RR: 5.3 - 8.3)	7.2 ± 1.2[a]	6.9 ± 1.4[a]	7.5 ± 1.7[ab]	6.9 ± 0.7[a]	6.6 ± 0.9[a]	7.1 ± 0.3[ab]	7.7 ± 0.6[a]	7.8 ± 0.6[a]	8.1 ± 0.6[a]	7.1 ± 0.9[a]	7 ± 1.1[a]	6,1 ± 0.8[b]
Leukocytes (×10^3.µL^{-1}) (RR: 4 -13 × 10^3)	10.3 ± 3.3[a]	9.1 ± 2.3[ab]	6.6 ± 0.7[a]	9.9 ± 2.9[a]	8.6 ± 1.9[ab]	7.6 ± 1.0[a]	11.1 ± 1.7[a]	11.9 ± 3.7[b]	6.4 ± 1.1[a]	9.7 ± 3.4[a]	8 ± 2.4[a]	7.6 ± 1.4[a]
Segmented Neutrophils (×10^3.µL^{-1}) (RR: 1.2 -7.2 × 10^3)	6.2 ± 2.6[a]	4.2 ± 1.5[a]	1.3 ± 0.2[a]	4.2 ± 1.5[a]	3.5 ± 1.6[a]	2.4 ± 0.4[b]	4.9 ± 0.9[a]	3.7 ± 1.6[a]	2.1 ± 0.1[b]	4.9 ± 2.1[a]	2.9 ± 1.2[a]	3.2 ± 0.6[b]
Lymphocytes (×10^3.µL^{-1}) (RR: 2 - 9 × 10^3)	4 ± 1.2[a]	4.6 ± 1.1[ab]	5.3 ± 0.9[a]	5.3 ± 2.2[a]	4.7 ± 1.9[ab]	4.9 ± 1.3[a]	5.8 ± 1.6[a]	7.6 ± 2.9[b]	3.9 ± 1.1[a]	4.6 ± 1.9[a]	4.7 ± 2.2[a]	3.9 ± 1[a]
Monocytes (×10^3.µL^{-1}) (RR: 0 - 0.55 × 10^3)	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.2 ± 0.2[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.2 ± 0.1[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]
Eosinophils (×10^3.µL^{-1}) (RR: 0.05 - 0.65 × 10^3)	0.1 ± 0.1[a]	0.2 ± 0.1[a]	0 ± 0[a]	0.2 ± 0.1[a]	0.4 ± 0.3[a]	0.2 ± 0.1[b]	0.2 ± 0.2[a]	0.5 ± 0.4[a]	0.2 ± 0.1[b]	0.4 ± 0.7[a]	0.2 ± 0.1[a]	0.4 ± 0.2[b]

Letters differing in the same line signify statistical differences ($p < 0.05$) between the groups.
GI – 0.92 g.kg^{-1} BW/4 days; GII – 0.92 g.kg^{-1} BW/8 days; GIII - Doramectin (200 µg.kg^{-1} BW); GIV – No treatment.

and hematological parameters were evaluated by the univariate test of variance using the statistical program SPSS (version 15.0).

In vitro tests showed more than 95% reduction larval counts at concentrations between 86.5 and 146.3 $mg.mL^{-1}$.

In vivo tests showed no reduction in EPG counts in the animals in GI and GII. GIII, EPG counts were greatly reduced at Day 8 and continued to decrease gradually until the end of the study (Table 1). The reduction in the number of larvae of fourth (L4) and fifth (L5) stage was less than 95% in GI and GII, and between 80 and 90% in GIII. Moreover, there was a significant reduction ($p < 0.05$) of these larvae in GIII compared to other groups (Table 2).

The discrepancy between the results of anthelmintic efficacy *in vitro* and *in vivo* has been attributed to several factors, including availability and/or concentration of active compounds in the preparation of plant material (HOUNZANGBE-ADOTE et al., 2005) and bioavailability and biotransformation of these compounds in animal body (VANDAMME; ELLIS, 2004). In polygastric animals, ruminal microorganisms can affect active chemical constituents of plants and reduce their bioavailability, and this may have contributed to low anthelmintic efficacy of *A. sisalana* juice found in the present study.

The results presented in Table 3 fall within normal physiological range of each parameter, with the exception of hematocrit, which was below normal on Day 8 in GI and GII and on Day 15 in GI and GIV.

Serum GGT (30-58 $IU.L^{-1}$), ALT (25-77 $IU.L^{-1}$), ALP (10-14 $IU.L^{-1}$), urea (36-55 $mg.dL^{-1}$) and creatinine (0,9-1,2 $mg.dL^{-1}$) remained within the normal range throughout the experiment. Body temperature, heart and respiratory rates, and rumen motility remained within the normal range.

The macroscopic changes observed in necropsied animals were consistent with common findings often reported in parasitized animals. These changes included pale mucous membranes, edematous and pale superficial lymph nodes, abomasal edema and hyperemia with mucosal hypertrophy and erosion areas, hemorrhagic enteritis across the small intestine with calcified *Oesophagostomum* nodules in the small and large intestines. The histological changes in liver biopsies were degenerative in nature with discrete inflammation, cellular edema and steatosis with focal hepatitis in all groups. Acute cellular edema and focal discrete tubular necrosis were found in isolated areas of kidney biopsies from two animals in GI and from one animal in GII.

Oral treatment of goats *A. sisalana* juice did not appear to produce clinically toxic effects on animals. Saponins in general have reduced toxicity when administered orally due to their poor absorption in the gastrointestinal tract (PRICE et al., 1987).

In conclusion, *A. sisalana* juice at the concentrations tested was effective against GNI in goats in *in vitro* tests; however, the anthelmintic efficacy was markedly reduced *in vivo*. Studies using higher juice concentrations, or other specific active saponin fractions extracted from the plant are needed to better assess the anthelmintic potential of *A. sisalana*.

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