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In vivo anthelmintic activity of an aqueous extract from sisal waste (*Agave sisalana* Perr.) against gastrointestinal nematodes in goats

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ABSTRACT

The resistance of gastrointestinal nematodes (GINs) of small ruminants to anthelmintics has required the investigation of new alternatives. The aim of the present study was to evaluate the in vivo anthelmintic activity of an aqueous extract from sisal waste (*Agave sisalana*) (AESW) against GINs in goats and to observe the animals for toxic effects. Thirty animals that were naturally infected with GINs were distributed into three groups: group I, was treated with daily doses of AESW (1.7 g/kg) for eight days; Group II, the positive control, was treated with a single dose of levamisole phosphate (6.3 mg/kg); and group III, the negative control, was left untreated. Faecal eggs counts (FECs), coprocultures and post-mortem worm counts were performed to assess the efficacy of the treatments. Clinical and laboratory analyses were performed to evaluate any toxic effects associated with the treatment. In the goats in groups I and II, a significant reduction ($p < 0.05$) of the number of eggs and infective larvae (L_3) was observed. The maximum reductions of the FECs were 50.3% and 93.6% for groups I and II, respectively, whereas the percent reductions of the total number of L_3 larvae were 80% (group I) and 85.6% (group II). There was no difference between groups I and III with respect to worm burden, and the percent reductions were 28.8% and 63.4% for *Oesophagostomum columbianum* and *Trichostrongylus colubriformis*, respectively. No reduction was detected for the *Haemonchus contortus*. The positive control group demonstrated a 74% reduction of the parasites that were recovered from the digestive tract. There were no changes in clinical and haematological parameters. The levels of serum urea and creatinine were higher in group I, but remained within the normal range. At necropsy, pale mucous membranes, abomasitis and enteritis were associated with parasitism. In addition, a histological analysis of the liver and kidney did not reveal any changes suggestive of toxicity. A chemical analysis of the AESW demonstrated the presence of saponins, which after acid-hydrolyses reaction, gave the sapogenins hecogenin and tigogenin. The AESW had a low efficacy for the parasitic stages and was moderately effective against eggs and free-living stages. Furthermore, the treatment was not toxic to the goats.

1. Introduction

Parasitism by gastrointestinal nematodes (GINs) is a major constraint in the production of goats. These infections in goats result in weight loss, diarrhoea, dehydration,

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anaemia, reduced milk production and reproductive changes (Vieira et al., 2009). Synthetic anthelmintics are the primary means of controlling parasitic infections. However, the use of these treatments has led to the development of parasite resistance (Melo et al., 2003).

The flora of Brazil remains promising in the search for active compounds that possess anthelmintic activity (Almeida et al., 2007). For example, *Melia azedarach* (Maciel et al., 2006) and *Chenopodium ambrosioides* (Ketzis et al., 2002) possess anthelmintic activity against GINs of small ruminants. The effects of these anthelmintic plants are related to the activity of secondary metabolites, such as tannins, alkaloids, saponins and glycosides (Athanasiadou and Kyriazakis, 2004).

A. sisalana Perr. (sisal) is a monocotyledonous plant of great economic interest in regions with semi-arid climates, such as northeast Brazil, because the plant is useful for the production of hard fibre. Brazil is the world's largest producer and exporter of sisal, and the state of Bahia is responsible for 95% of the national production (Santos, 2006). However, only 4% of the sisal leaves are used to create fibre. This excessive waste of material, has led to focus on developing other technologies aimed at determining applications for the remaining plant materials, especially the residue (Bandeira and Silva, 2006). Some potential innovations include the use of the material as an organic fertilizer, a supplement in ruminant feed (Bandeira and Silva, 2006) and a raw material in the production of medicine (Debnath et al., 2010).

A. sisalana also exhibits antibacterial (Santos et al., 2009) and insecticide properties (Keriko and Mutua, 2008). The anthelmintic efficacy of the liquid waste from sisal against eggs and larvae of GINs of goats has been demonstrated in vitro (Domingues, 2008; Silveira, 2009). An oral application of this residue in goats decreased the number of GINs adults and L_3 larvae in coprocultures by 18% and 36%, respectively, and it did not influence the evaluated clinical and haematological parameters (Domingues, 2008).

The aim of the present study was to evaluate the anthelmintic efficacy of an aqueous extract from sisal waste (*A. sisalana*) against GINs of goat and to characterise potential toxic effects.

2. Materials and methods

The procedures used in the present study were approved by the Ethics Committee for the Use of Animals at Feira de Santana State University (protocol no. 017/2008).

2.1. Plant material and extract preparation

The sisal waste utilised in the present study was collected directly from a decortication machine on a sisal farm in the city of Valente, Bahia State, Brazil, in July 2009. *A. sisalana* plants that were approximately six-years-old were harvested. Voucher specimens were deposited at the herbarium of the Department of Biology, Feira de Santana State University, Bahia, Brazil (number 838).

The sisal waste (60 kg) was mixed with 60 L of distilled water and boiled for 3 h. After cooling at room temperature, it was filtered using filter paper, resulting in 60 L of the extract, and was stored at −20 °C until needed. Actual concentration of the extract (57.7 mg/mL) was determined by drying three sets of 1 mL sample in a forced air incubator (60 °C) until obtaining constant weight and taking the mean weight of the residue.

2.2. Animals

Thirty goats of both sexes and mixed breed were used in the present study. Goats, between 6 and 18 months of age, weighing 11–27 kg, were infected naturally with GINs. The animals were from the same herd with semi-intensive rearing system in the municipality of Senhor do Bonfim (BA). The animals received no anthelmintic treatment for a period of 60 days prior to the study.

To perform the experiment, the goats were transferred to the Centre for Development of Livestock in Oliveira dos Campinhos (BA). The duration of the study was 22 days, which included an initial one-week period of acclimatisation. The animals were maintained in an indoor area on a concrete floor. Grass hay, water and mineral salt were provided ad libitum.

2.3. Treatment

The goats were divided into three homogeneous groups ($n = 10$). The animals were distributed into each group alternately in descending order of the number of eggs per gram of feces. The mean weight in groups I, II and III were 18.6 ± 4.3, 19.3 ± 3.6 and 19.8 ± 5.9, respectively. Group I was treated with daily doses (1.7 g/kg) of the aqueous extract from sisal waste (AESW) for eight days, group II (positive control) was treated with a single dose of levamisole phosphate (6.3 mg/kg), and group III (negative control) was not subjected to any treatment. The AESW was administered orally by gavage. During the experiment, one animal in group II died due to GINs parasitism on day 11. The FEC of this animal was 6850 and the *Haemonchus* was the most prevalent genera in faecal culture (81%).

2.4. Clinical evaluation

A clinical examination of the animals (Rosenberger et al., 1993) was conducted daily. The assessed parameters were the general condition, lymph nodes, colour of the ocular mucosa, body temperature, heart and respiratory rates, and rumen motility. The body weights of each animal were recorded on days 0 and 9.

2.5. Faecal egg counts (FECs) and coproculture

Faecal samples were collected directly from the rectum of each animal on days 0, 5 and 9 to perform FECs (Gordon and Whitlock, 1939). The generic identification of the nematode population was determined by coproculture (Ueno and Gonçalves, 1998) of individual faecal samples that were collected prior to the start and at end of the treatment.

Table 1
Mean (±S.D.) and percent reduction (%) of the number of eggs of gastrointestinal nematodes of goats treated with AESW.

Groups	Days of treatment				
	0	5		9	
	Mean ± S.D	Mean ± S.D.	%	Mean ± S.D.	%
GI	2480 ± 1665.2^{Aa}	1600 ± 1012.1^{Aa}	25.8	1610 ± 964.9^{Aa}	50.3
GII	2078 ± 1118.1^{Aa}	138.9 ± 167.3^{Bb}	93.6	416.7 ± 612.9^{Bb}	87.1
GIII	2650 ± 1885.3^{Aa}	2155 ± 1394.7^{Ca}	–	3240 ± 2137.7^{Ca}	–

Capital letters compare means in the columns and small letters in the lines ($p < 0.05$). GI: 1.7 g de AESW/kg BW/8 days; GII: levamisole phosphate (6.3 mg/kgPV); GIII: no tretament.

2.6. Haematology and serum biochemistry analyses

Blood samples were collected from each animal by puncturing the jugular vein on days 0 and 9 of the experiment. The blood samples, collected in vacuum tube containing EDTA, were used to perform haemograms and to determine total plasma protein by refractometry (Jain, 1993).

The serum activities of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT) and alkaline phosphatase, as well as the concentrations of creatinine and urea were measured using commercial kits (DOLES®) and spectrophotometry.

2.7. Necropsy procedure

One week after the end of the treatment, six animals from each group were separated randomly and euthanized. The euthanisation procedure followed the recommendations of the Federal Council of Veterinary Medicine (Brasil, 2002). Subsequently, the animals were necropsied. For histopathological examination, fragments of the liver, kidney, abomasum and intestine were collected and fixed in formalin (10%), and then paraffin-embedded sections were prepared (Prophet et al., 1992). Five-millimetre histological sections were stained with haematoxylin–eosin (Luna, 1968).

Aliquots (10%) of the contents of the abomasum and the small intestine from each animal were analysed. The number of nematodes, which were categorised according to the genus, was multiplied by ten. The contents of the large intestine were examined completely (Ueno and Gonçalves, 1998). The identification of GINs species were determined according to Soulsby (1982).

2.8. Statistical analysis

The anthelmintic efficacy was estimated by calculating the percent egg or larvae reduction, using the following formula: $PR = 100\ (1 - T/C)$, where PR is the percent reduction, T and C are the arithmetic means of the eggs or larvae in the treated and negative control animals, respectively (Coles et al., 1992).

The results for the body weight, haematological and biochemical analyses, which demonstrated a normal distribution, were compared by ANOVA followed by Tukey's test (5%). For parameters that did not show a normal distribution (egg, L_3, L_4 and adult worms, basophils, leukocytes and segmented rods), non-parametric analysis was performed: the Kruskal–Wallis test followed by Dunn's multiple comparison test (5%). All analyses were performed using SAS, version 9.1 (SAS, 2004).

2.9. Chemical analysis

An aliquot of the aqueous extract was extracted with isobutanol to remove small water-soluble molecules such as sugar. The iso-butanolic extract (BE) was analysed by ^{1}H NMR (400 MHz, DMSO-d6 as the deutered solvent). The BE was also submitted to hydrolysis and the sapogenin fraction was analysed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) (Blunden et al., 1978).

3. Results

3.1. Effects of treatment on faecal egg and larval counts

In the goats treated with AESW (group I) and levamisole (group II), a decrease in FECs was observed from the fifth day, and this finding was statistically significant compared to the negative control (group III). The largest reductions in FECs corresponded to 50.3% and 93.6% for groups I and II, respectively (Table 1).

The number of L_3 larvae obtained from the faecal cultures of goats treated with AESW or levamisole was significantly lower ($p < 0.05$) compared to the negative control group, excluding the number determined for *Trichostrongylus*. In group I, the percent reduction of L_3 of the genera *Haemonchus*, *Oesophagostomum*, *Trichostrongylus* and the total larvae, corresponded to 82.6%, 79.6%, 53% and 80%, respectively. In contrast, in group II, the percent reduction determined for each genus was 93.8%, 74.1%, 58.6% and 85.6%, respectively (Table 2).

3.2. Effect of the treatment on worm burden

In group I, a reduction of the worm burden for *T. colubriformis* (63.4%) and *O. columbianum* (28.9%) was observed. There was no reduction of the *H. contortus*. In group II, the percent reduction of these parasites varied between 62.4% and 88%. A significant difference ($p < 0.05$) was detected only in group II for *H. contortus* and *O. columbianum*, as compared to the other groups (Table 3).

Table 2
Mean (±S.D.) and percent reduction (%) of the number of third stage larvae of gastrointestinal nematodes observed in faecal cultures from goats treated with AESW.

Genera	Groups and days of treatment							
	GI			GII			GIII	
	Mean ± S.D.			Mean ± S.D.			Mean ± S.D.	
	0	9	%	0	9	%	0	9
Haemonchus spp.	1055.5 ± 825.7^{Aa}	281 ± 174.5^{Ab}	82.6	794.4 ± 393.5^{Aa}	100 ± 111.8^{Bb}	93.8	1255.5 ± 1300^{Aa}	1620 ± 1773^{Ca}
Oesophagostomum spp.	680.5 ± 747.5^{Aa}	166 ± 128.4^{Aa}	79.6	381.1 ± 489.9^{Aa}	210.6 ± 421.3^{Ba}	74.1	488 ± 532.5^{Aa}	812 ± 851^{Ca}
Trichostrongylus spp.	96 ± 75.8^{Aa}	67.5 ± 38.7^{Aa}	53	62.78 ± 40.63^{Aa}	59.4 ± 45.7^{Aa}	58.6	70 ± 67.9^{Aa}	143.5 ± 103.9^{Aa}
Total	1832 ± 1459.4^{Aa}	514.5 ± 234.7^{Ab}	80	1238.4 ± 745.3^{Aa}	370 ± 413.7^{Bb}	85.6	1783.5 ± 1346.3^{Aa}	2575 ± 1708.6^{Ca}

Different capital letters in the line indicate significant difference between groups and different small letters in the line indicate significant difference intragroup ($p < 0.05$). GI: 1.7 g de AESW/kg BW/8 days; GII: levamisole phosphate (6.3 mg/kgPV); GIII: no tretament.

Table 3
Mean (±S.D.) and percent reduction (%) of the number of gastrointestinal nematode larvae (L_4 and adult worms) recovered from goats after AESW treatment.

Species	GI		GII		GIII
	Mean ± S.D.	%	Mean ± S.D.	%	Mean ± S.D.
Haemonchus contortus	295 ± 236.3^{a}	0	30 ± 44.7^{b}	88	250 ± 224.8^{a}
Oesophagostomum columbianum	61.17 ± 33.8^{a}	28.9	17.7 ± 19.8^{b}	79.5	86 ± 63.7^{a}
Trichostrongylus colubriformis	125 ± 154.8^{a}	63.4	128 ± 83.3^{a}	62.4	342 ± 278.5^{a}
Total	481.17 ± 241.9^{a}	29	176 ± 112.1^{b}	74	677.7 ± 354.9^{a}

Different letters in the same line indicate significant difference ($p < 0.05$). GI: 1.7 g de AESW/kg BW/ 8 days; GII: levamisole phosphate (6.3 mg/kg PV); GIII: no tretament.

3.3. Clinical findings

In the clinical evaluation of the animals, the following parameters were recorded: rectal temperature (37.3–38.2 °C), cardiac frequency (60–86 beats per minute), respiratory frequency (15–23 movements per minute), and ruminal movements (1–3 movements per two minute). A slight increase in weight was observed in all of the groups, but no statistically significant differences were observed among the groups ($p > 0.05$).

3.4. Haematological and biochemical findings

The concentration of haemoglobin in group II was significantly increased compared to the other groups on day 9 of the experiment. A comparison of the groups revealed a significant reduction ($p < 0.05$) of the haemoglobin concentration for group I, a significant increase in the erythrocyte count in group II and an increase in the monocyte count in group III (Table 4).

No differences were observed between the groups ($p > 0.05$) with respect to the enzymes GGT, AST, ALT and alkaline phosphatase after treatment ($p > 0.05$). A significant reduction of AST levels (group I) and GGT (group III) was observed on day 9 compared to day 0. Urea and creatinine increase significantly ($p < 0.05$) after the administration of AESW in group I compared to the other groups (Table 5).

3.5. Post-mortem changes and histopathological findings

The main macroscopic findings of the necropsies in all groups were pale mucous membranes, oedematous superficial lymph nodes, acute and subacute

Table 4
Mean (±S.D.) of haematological parameters determined in goats treated with AESW.

Parameters	Groups and days of treatment					
	GI		GII		GIII	
	0	9	0	9	0	9
Erythrocytes ($\times 10^6/\mu L$)	14.4 ± 1.3^{Aa}	15.2 ± 1.5^{Aa}	13.5 ± 1.7^{Aa}	15.9 ± 2.4^{Ab}	13.9 ± 2.4^{Aa}	14.1 ± 2.7^{Aa}
Hematocrit (%)	29.1 ± 4.3^{Aa}	28.4 ± 4.1^{Aa}	28 ± 3.8^{Aa}	29.3 ± 3.9^{Aa}	27 ± 4.5^{Aa}	27.6 ± 4.5^{Aa}
Hemoglobina (g/dL)	11.8 ± 3.3^{Aa}	9.3 ± 1.5^{Ab}	10.8 ± 1.9^{Aa}	12.2 ± 1.3^{Ba}	10.7 ± 2.8^{Aa}	10.4 ± 1.6^{Aa}
Leukocytes ($\times 10^3/\mu L$)	10.4 ± 2.4^{Aa}	14.1 ± 4.1^{Aa}	11.0 ± 2.9^{Aa}	11.7 ± 3.7^{Aa}	12.2 ± 58^{Aa}	12.9 ± 2.0^{Aa}
Segmented Neutrophils ($\times 10^3/\mu L$)	4.4 ± 1.5^{Aa}	8 ± 3.6^{Aa}	5 ± 1.9^{Aa}	6.7 ± 2.4^{Aa}	5.3 ± 2.9^{Aa}	7.8 ± 1.8^{Aa}
Band neutrophils ($\times 10^3/\mu L$)	0.1 ± 0.1^{Aa}	0.1 ± 0.2^{a}	0.1 ± 0.1^{Aa}	0.3 ± 0.4^{Aa}	0.1 ± 0.1^{Aa}	0.1 ± 0.2^{Aa}
Basophils ($/\mu L$)	0 ± 0^{Aa}	61 ± 105.1^{Aa}	51 ± 152.7^{Aa}	20.1 ± 60.3^{Aa}	40 ± 81.4^{Aa}	0 ± 0^{Aa}
Lymphocytes ($\times 10^3/\mu L$)	5.3 ± 2.3^{Aa}	5.2 ± 2.3^{Aa}	4.8 ± 1.5^{Aa}	3.6 ± 1.8^{Aa}	6.1 ± 2.7^{Aa}	4.1 ± 0.2^{Aa}
Monocytes ($\times 10^3/\mu L$)	0.4 ± 0.1^{Aa}	0.5 ± 0.4^{Aa}	0.5 ± 0.3^{Aa}	0.8 ± 0.6^{Aa}	0.4 ± 0.4^{Aa}	0.6 ± 0.1^{Ab}
Eosinophils ($\times 10^3/\mu L$)	0.2 ± 0.3^{Aa}	0.2 ± 0.2^{Aa}	0.6 ± 0.7^{Aa}	0.3 ± 0.4^{Aa}	0.3 ± 0.3^{Aa}	0.4 ± 0.3^{Aa}

Different capital letters in the line indicate significant difference between groups and different small letters in the line indicate significant difference intragroup ($p < 0.05$). GI: 1.7 g de AESW/kg BW/8 days; GII: levamisole phosphate (6.3 mg/kg PV); GIII: no tretament.

Table 5
Mean (±S.D.) of the serum biochemical markers and total plasma protein in goats treated with AESW.

Parameters	Groups and days of treatment					
	GI		GII		GIII	
	0	9	0	9	0	9
GGT (UI/L)	56.6 ± 27.2^{Aa}	44.2 ± 14.2^{Aa}	48.3 ± 27.5^{Aa}	41.2 ± 10.6^{Aa}	89.6 ± 45.3^{Ba}	56.7 ± 28.2 ^{Ab}
AST (UI/L)	39.6 ± 6.7^{Aa}	31.7 ± 4.9^{Ab}	37.3 ± 6.5^{Aa}	33.8 ± 10.6^{Aa}	35.1 ± 7.6^{Aa}	31.7 ± 7.9^{Aa}
ALT (UI/L)	12 ± 1.4^{Aa}	12.3 ± 1.5^{Aa}	11.8 ± 1.7^{Aa}	11.3 ± 2.6^{Aa}	12.1 ± 1.4^{Aa}	11.2 ± 1.6^{Aa}
Alkaline phosphatase(UI/L)	67.8 ± 30.3^{Aa}	44.1 ± 20^{Aa}	89.3 ± 80.1^{Aa}	47.1 ± 25.9^{Aa}	76.7 ± 58.7^{Aa}	45 ± 25.6^{Aa}
Urea (mg/dL)	51.5 ± 8.8^{Aa}	56.9 ± 14.6^{Aa}	50.2 ± 11.7^{Aa}	35.5 ± 15.9^{Bb}	47.7 ± 8.2^{Aa}	40.8 ± 9.5^{bA}
Creatinine (mg/dL)	1 ± 0.1^{Aa}	1.2 ± 0.2^{Ab}	1 ± 0.1^{Aa}	1 ± 0.2^{Ba}	1. ± 0.1^{Aa}	1 ± 0.1^{ABa}
Total plasma protein (g/dL)	6.7 ± 0.6^{Aa}	6.5 ± 0.5^{Aa}	7.2 ± 0.5^{Aa}	6.8 ± 0.5^{Aa}	6.7 ± 0.8^{Aa}	6.2 ± 0.6^{Aa}

Different capital letters in the line indicate significant difference between groups and different small letters in the line indicate significant difference intragroup ($p < 0.05$). GI: 1.7 g de AESW/kg BW/8 days; GII: levamisole phosphate (6.3 mg/kg PV); GIII: no tretament.

abomasitis, haemorrhagic and acute ulcerative enteritis, and calcified nodules of *Oesophagostomum* in the intestines.

The histological analysis revealed a focal to multifocal lymphocytic reaction in the abomasal submucosa in three animals (one from each group). Swollen and vacuolated hepatocytes were reported in all of the animals. In 13 animals, cellular changes were accompanied by a randomly distributed focal to multifocal lymphocytic infiltration. Microscopic changes in the kidneys were characterised by slight cytoplasmic vacuolation in the tubular epithelium and focal lymphocytic inflammatory infiltration in the cortical interstitium. These changes were observed in only one animal in the group treated with sisal, whereas two goats in the negative control group demonstrated tubular changes characterised by a discrete cellular vacuolation and cystic dilatation of some cortical tubular structures.

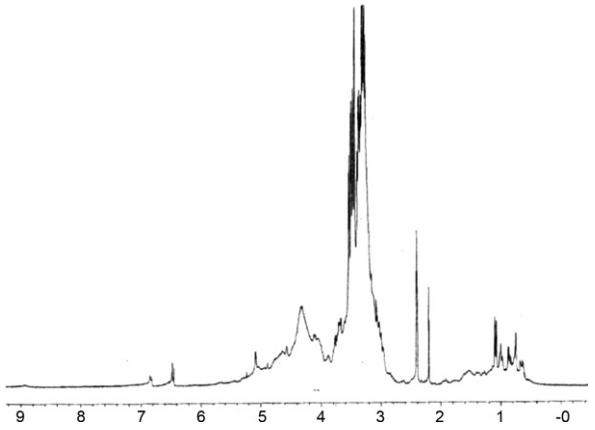

Fig. 1. ^{1}H NMR (300 MHz, DMSO-d6) of the isobutanolic extract obtained of the aqueous extract from sisal waste. The region between 0.5 and 1.5 and 4–5 ppm were characteristics of the sterols and sugars, respectively.

4. Discussion

The treatment of goats with AESW had a partial effect on the number of eggs and L_3 larvae, but there was no difference ($p > 0.05$) in the number of adult worms between the group I and negative control. These results indicate that AESW was not effective in controlling gastrointestinal nematodes, considering that the effectiveness of an anthelmintic is defined a reduction of greater than 90% (Vercruysse et al., 2001). However, fewer parasites were detected in the group treated with sisal compared to the negative control group, which may suggest the presence of compounds with activity against nematodes in the aqueous extract.

The activity of AESW was more effective against the development of L_3. This result is likely due to the residual effects of the extract in the faeces of treated animals, which may contribute to reduce the contamination of pastures with infective-stage parasites (L_3).

Domingues (2008) treated goats with 0.9 g/kg BW/day of liquid waste from *A. sisalana* during a period of eight days, and discovered a 36% reduction in total number of L_3 larvae in coprocultures, but no decrease in FECs. The daily dose of the extract used in that study was 95% higher than the amount used in the current study, which may explain the higher percentage reduction observed for L_3 larvae and FECs. These results suggest an association between the dose and the anthelmintic effect.

The antiparasitic activity of various plants that contain saponins has been described by Chapagain et al. (2008). The associated mechanism of action may be due to the destabilisation of membranes and increased cell permeability (Francis et al., 2002), because the saponins consist of a sugar moiety linked to a hydrophobic aglycone (triterpenoid or steroid), and are characterised based on their ability to reduce the surface tension of water in addition to their, detergent and emulsifying properties.

The steroidal saponins from the Agavaceae family have been described (Simmons-Boyce and Tinto, 2007). Thus, the aqueous extract from sisal waste (AESW) was partitioned using iso-butanol to remove any small water-soluble molecules such as sugar. The iso-butanol was subjected to ^{1}H NMR (Fig. 1) to analyse the major component. In this spectra, two distinct regions were observed: sterols (0.5–1.5) and sugars (4–5 ppm). This observation confirmed the presence of saponins in the original extracts (AESW). The extract was hydrolysed to analyse the aglycones by gas chromatography (Fig. 2), which verified two compounds comprised the majority of the fraction. The GC–MS analysis enabled characterise the compounds as tigogenin and hecogenin, which demonstrated retention times at 6.3 min (Peak a) and 8.1 min (Peak b), respectively.

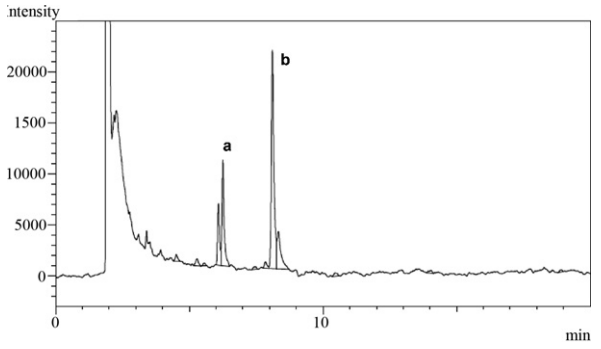

Fig. 2. GC chromatogram of sisal sapogenin. Peaks a and b were characterised as tigogenin and hecogenin, respectively.

The liquid waste of *A. sisalana* was effective against eggs, larvae and adults worms of the gastrointestinal nematodes in vitro (Domingues, 2008; Silveira, 2009). The lack of similarity between the in vitro and in vivo tests can be attributed to several factors. In the in vitro tests, the extracts were in direct contact with the parasites. Furthermore, the concentrations of the potentially active substances in the extract do not always correspond to the bioavailability in vivo (Githiori et al., 2006). Another possibility is the biotransformation of these compounds within the gastrointestinal tract of the animal, which may lead to a loss of biological activity (Athanasiadou and Kyriazakis, 2004).

The bioavailability of plants constituents can be modified by rumen microorganism, which may explain to the low efficacy of AESW against NGIs. In lambs that received an intra-ruminal administration of saponins from *Yucca schidigera* was observed a rapid hydrolysis in the rumen and a large amount of free sapogenins in the contents of omasum and abomasums (Flaoyen et al., 2002).

The effectiveness of levamisole against nematodes in the positive control group was less than 90%. Furthmore, the FEC of one goat of this group remained high eleven day after the treatment, which led to death of this animal. These findings may indicate the resistance of these parasites. Such resistance has been previously reported in goats and sheep in Brazil (Melo et al., 2003).

The oral treatment of goats with AESW did not cause clinical changes that suggested toxicity. The low intestinal absorption of orally administered saponins may be responsible for their reduced toxicity (Price et al., 1987). In addition, the sisal extract did not influence (0.9 g/kg) the clinical, haematological, biochemical and histopathological parameters of the goats (Domingues, 2008). However, saponins may interfere with rumen fermentation, cause an increase in pH, lead to a decrease in the protozoa population and promote the concentration of volatile fatty acids (Santoso et al., 2007).

The haemoglobin level after treatment was higher in group II (positive control) compared to the other groups, which may have been due to a lower level of parasite infection. It is possible that there was an inverse correlation between FECs and haemoglobin (Farias et al., 2002). However, saponins usually cause haemolysis due to their ability to form complexes with sterols in cell membranes and promote cell lysis. in vitro studies investigating the effect of steroidal saponins from *A. sisalana* on human erythrocytes have demonstrated a high level of haemolytic activity (Santos et al., 1997).

Statistical differences in the analysis of erythrocytes, monocytes, AST and GGT were observed between days 0 and 9. Physiological variations were influenced by breed, age, sex, management system, stress, and/or nutritional and environmental conditions may have also contributed to this result (Tucci et al., 1989; Silva et al., 2004).

The activities of AST, ALT, alkaline phosphatase (Tucci et al., 1989) and GGT (Silva et al., 2004) remained within the normal range. In addition, the concentrations of urea and creatinine were normal (Silva et al., 2003), despite a significant increase in the both levels in the group treated with AESW. However, based on histopathology, hepatic lesions were observed in both treated animals and controls. These lesions may be the result of factors that were not explored in the present study. Infection with gastrointestinal nematodes, especially *H. contortus*, can cause anaemia (Vieira et al., 2009), which results in hypoxia and changes in hepatocytes (McGavin and Zachary, 2009). However, the animals did not present symptoms of anaemia at the time of the study. Only one animal in group I displayed slight renal alterations, which were likely not associated with activity of the sisal extract.

In the work reported by Wisløff et al. (2008), the administration of intraruminal *Y. schidigera* juice (63 and 126 mg/kg of sapogenin) in lambs for 21 days resulted in diarrhoea, dehydration, increased levels of creatinine and urea, and acute tubular necrosis and interstitial haemorrhage in the kidneys. No changes were observed in the levels of AST, GGT and bilirubin, but glycogen accumulation and lipid droplets were detected in the cytoplasm of the liver cells.

The lesions observed in the gastrointestinal tract were consistent with a gastrointestinal nematode infection (Vieira et al., 2009), and they were similar to those described by Domingues (2008) in goats treated with the liquid waste from sisal.

In conclusion, the aqueous extract from sisal waste demonstrated a low efficacy against parasitic-stage parasites. However, the extract was moderately effective against eggs and free-living stages of the parasite and did not cause any toxicity in the goats. Future studies employing higher doses or active fractions extracted from the plant are being planned to better assess the potential anthelmintic activity.

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