Veterinary Parasitology 186 (2012) 312–318

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Evaluation of *Cymbopogon schoenanthus* essential oil in lambs experimentally infected with *Haemonchus contortus*

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ARTICLE INFO

Article history:
Received 3 June 2011
Received in revised form
30 November 2011
Accepted 2 December 2011

Keywords:
Cymbopogon schoenanthus
Essential oil
Haemonchus contortus
Small ruminant
Anthelmintic
Nematicide

ABSTRACT

Hematophagous gastrointestinal parasites cause significant economic losses in small ruminant grazing systems. The growing reports of multi-drug resistant parasites call for intensive research on alternative treatments for anthelmintics to help small ruminants cope with these parasites. Two-month-old lambs with mean body weight (BW) of 22.5 kg were experimentally infected with a multidrug-resistant *Haemonchus contortus* strain. Infected animals were dosed orally with *Cymbopogon schoenanthus* essential oil to evaluate its anthelmintic potential. Eighteen animals were allocated into three groups of six animals, and each received one of the following treatments: Group 1 – control (10 mL of water), Group 2 – *C. schoenanthus* essential oil (180 mg/kg BW); and Group 3 – *C. schoenanthus* essential oil (360 mg/kg BW). Animals received the oil once a day for 3 consecutive days. Lambs were evaluated clinically for blood biochemistry before, at 1, 5, 10, 15 and 20 days after treatment, and then were euthanized to assess the total worm burden. No statistically significant reduction in fecal egg count, packed cell volume or total worm count was observed after treatments. Also, no statistical difference among group means for blood levels of urea, creatinine, albumin, alkaline phosphatase, aspartate aminotransferase and gamma glutamyl transferase was found. Larval development assay (LDA) and egg hatch assay (EHA) were performed from feces of treated animals at 1, 5, 10 and 15 days after essential oil administration. An inhibition in LDA was observed 1 day after the 3-day treatment in larvae from feces of animals treated with 360 mg/kg essential oil. In conclusion, the essential oil at the doses of 180 mg/kg and 360 mg/kg was safe to sheep, but failed as an anthelmintic treatment when applied to young sheep artificially infected with a multidrug-resistant *H. contortus* strain.

1. Introduction

Haemonchus contortus is the most important hematophagous gastrointestinal nematode (GIN), representing a major health and economic obstacle for the feasibility of grazing sheep production systems in the tropics. Hot and humid conditions favor larval development, increasing the reinfection rates of animals grazing in contaminated pastures. The severe blood loss caused

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0304-4017/
doi:10.1016/j.vetpar.2011.12.003

by this parasite can result in anemia, anorexia, reduction in body weight, depression, and death. This scenario is aggravated by the multi-drug resistance of *H. contortus* to several commercial anthelmintics (Rowe et al., 2008).

Alternative methods and anthelmintic products to control *H. contortus* are urgently needed for the feasibility of small ruminant production systems. Regarding alternative anthelmintic products, several researchers have tested plant-derived products, including essential oils. For instance, Valencia orange essential oil (Squires et al., 2010) and *Eucalyptus staigeriana* essential oil (Macedo et al., 2010) lead to successful reduction in fecal egg count and parasite burden in sheep and goats, respectively, infected with *H. contortus*.

Cymbopogon schoenanthus belongs to the family Poaceae (Gramineae) and is reported to have sedative, digestive and aromatic properties, with a strong and characteristic aroma, with some reports on its insecticidal activity (Ketoh et al., 2002; Koba et al., 2007). Although we found no studies on the toxicity of *C. schoenanthus* essential oil, it is known that geraniol, the main constituent (59%) from *C. schoenanthus* has an oral LD_{50} in rats of 3600 mg/kg, and geranial (13%), the second main constituent has an oral LD_{50} in rats of 4960 mg/kg (Material Safety Data Sheet, Sigma–Aldrich, 2011).

A previous study (Katiki et al., 2011) demosntrated the *in vitro* anthelmintic activity of *Cymbopogon schoenanthus* essential oil against ovine trichostrongylids through the egg hatch, larval development, larval feeding, and larval exsheathment assays.

The objectives of this work were to evaluate the *in vivo* and *in vitro* anthelmintic activities of *C. schoenanthus* essential oil in lambs infected with a drug-resistant *H. contortus* strain in lambs. *In vivo* parameters included the fecal egg count (FEC), total worm count (TWC), packed cell volume (PCV), and possible toxicity evaluated by changes in the profile of liver and kidney enzymes of lambs treated with the essential oil. *In vitro* parameters included inhibition of both egg hatch and the larval development of nematodes collected from lambs experimentally infected and treated orally with 180 and 360 mg of essential oil per kg of live body weight.

2. Materials and methods

2.1. Animals

Eighteen Santa Ines male lambs, approximately 2 months old, and with mean live body weight (BW) of 22.5 kg were kept indoors in collective stalls, where they were fed Coastcross hay (*Cynodon dactylon*), mineral salt, and water "*ad libitum*". All experimental protocols were approved by the FMVZ-UNESP-Botucatu Animal Care and Use Committee.

2.2. Parasite cleansing pre-experiment

Animals received levamisole phosphate (Ripercol F®, Fort Dodge, Brazil) and albendazole (Valbazen®, Pfizer, Brazil) at double prescription dose every 24 h during 3 consecutive days to treat natural infections by nematodes (Amarante et al., 2004). After treatment, fecal samples were collected directly from the rectum of each animal to confirm their worm-free status.

2.3. Artificial infection

One donor sheep was maintained with a single strain of *H. contortus* that was multidrug-resistant (levamizole, albendazole, ivermectin, moxidectin, closantel and triclorfon), as described by Almeida and collaborators (2010). Fecal cultures were done to obtain infective larvae (L_3). Each lamb was infected orally with 4000 L_3 of this *H. contortus* strain.

2.4. Groups

Twenty-six days after infection, sheep were weighed and nematode fecal egg counts (FEC) were recorded for each animal. Lambs were allocated into experimental groups based on their FEC (highest to lowest). Animals were allocated to groups with six animals each. The groups were: Group 1 – control (10 ml of water); Group 2 – treated with *C. schoenanthus* essential oil (180 mg/kg body weight (BW)); and Group 3 – treated with *C. schoenanthus* essential oil (360 mg/kg BW). These doses were chosen after a pilot experiment where some efficacy, and no toxicity, was observed. Animals were fasted for 8 h pre-treatment and 4 h post-treatment. To make sure lambs received correct volume of essential oil, the volume of oil was pumped first into a graduated syringe, and water was added to a total volume of 10 ml. This water volume was sufficient to carry the oil into the rumen of the animal without losses. This dosing procedure was done three times per treated animal in intervals of 24 h.

2.5. Essential oil chromatographic analysis

C. schoenanthus oil was purchased from WNF Ind. & Com. Ltda (R. Dr. Mario Pinto Serva, 64, Sao Paulo-SP, Brazil), Lot no. 10608, $d = 0.911$.

The oil was analyzed by gas chromatography (GC) coupled to a mass spectrometry (MSD) and flame ionization (FID) detectors (Agilent GC System 6890 Series for the Mass Selective Detector, and Agilent 5973 Network for the FID detector). Each sample was analyzed in two separate columns (HP-5, 30 m, 0.25 mm ID, 0.25 μm film), attached to either the MSD or the FID. The conditions for both inlets and columns were the same with helium as a gas carrier, at a constant flow of 1 mL/min. Inlet temperature was 220 °C, with a temperature program of 60 °C for 1 min, increasing 4 °C/min to reach 200 °C in 15 min. The temperature for the FID was set at 220 °C and for MSD at 280 °C. Qualitative analysis was based on a comparison of retention times and indexes on both columns and mass spectra with corresponding data in the literature and mass spectral libraries (Wiley 275).

2.6. Pre- and post-treatment clinical evaluation

Feces were collected directly from the rectum to determine the FEC before, 1, 5, 10, 15, and 20 days after

treatment. Blood was collected from jugular vein into vacuum tubes containing EDTA sodium to determine PCV, and into vacuum tubes without anticoagulant to evaluate albumin, the liver enzymes alkaline phosphatase (AP), gamma glutamyltransferase (GGT), aspartate aminotransferase (AST), and to monitor urea and creatinine from kidneys before, 5, 10, and 20 days after treatment.

2.7. In vitro assays

Fecal samples were collected directly from the rectum of lambs and mixed. Then, a five-gram sample from each experimental group was washed to recover eggs (Coles et al., 1992) and prepare *in vitro* assays before, 1, 5, 10, and 15 days after treatment.

2.7.1. Egg hatch assay (EHA)

Approximately 100 eggs were added per wells, which were filled with distilled water to a final volume of 500 μL. Tests were performed in 24-well plates, with six replicates per treatment, incubated for 24 h at 27 °C, and read in an inverted microscope to evaluate treatment effect on egg hatching inhibition (Bizimenyera et al., 2006).

2.7.2. Larval development assay (LDA)

Approximately 100 eggs, plus nutritive medium (*Escherichia coli*, yeast extract, amphotericin B), were added per well, according to Hubert and Kerboeuf (1992). Tests were performed in 24-well plates, with six replicates, incubated at 27 °C for 5 days, and read in an inverted microscope to evaluate treatment effect on larval development.

2.8. Total worm count

Twenty days after the last treatment lambs were fasted for 24 h and euthanized in an abattoir. The abomasa were separated; their apertures were closed with a string, identified, packed individually in plastic bags, and sent to laboratory in polystyrene box with ice for total worm count.

An incision was made in the major curvature of each abomasum and the content was deposited in a graduated bucket (20 l). The mucosa was washed with tap water to a total volume of two liters. After mixing this solution, samples were taken from the bucket and placed in flasks to complete a 200-mL aliquot, which corresponds to 10% of total volume of the abomasal content. Ten milliliters of formaldehyde was added to the 200-mL aliquot for preservation and posterior recounting.

Each abomasum was immersed with the mucosa turned down in 200 mL of 0.9% saline solution pre-heated to 40 °C and kept in an incubator at 40 °C during 4 h to release nematodes trapped in the mucosa. After incubation, the solution was placed in flasks and added of 10-mL of formaldehyde for preservation and posterior recounting (Ueno and Gonçalves, 1998).

2.9. Statistics

Significant differences between groups for FEC, total worm count, PCV, plasma protein, albumin, EHA, LDA, AP, GGT, AST, urea, and creatinin were assessed by analysis of repeated measures using the General Linear Models (GLM procedure) of the Statistical Analysis Systems Institute (SAS 9.2, 2008). FEC data were transformed as $\log_{10}(x+1)$ to stabilize the variance. Significant interactions of group × time were reported in the results.

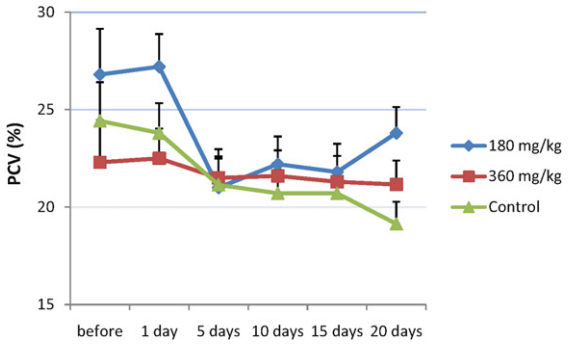

Fig. 1. Mean packed cell volume (PCV) from groups of sheep experimentally infected with 4000 L_3 *Haemonchus contortus* and dosed with water (control) or with *Cymbopogon schoenanthus* essential oil at 180 mg/kg or 360 mg/kg before, at 1, 5, 10, 15 and 20 days after treatment (DAT). Error bars represent standard error of the mean.

3. Results

Treatments with 180 mg/kg or 360 mg/kg provided no anthelmintic activity against *H. contortus*. The differences in dynamics of egg output and PCV resulted in significant interaction between time × group ($P<0.05$). However, there was no significant difference in FEC, TWC or PCV among group means (Table 1, Fig. 1).

The elimination of essential oil in feces and its activity on *H. contortus* egg hatching and larval development at 27 °C were evaluated by EHA and LDA. Regarding these two *in vitro* tests, there was significant interaction between time × group ($P<0.05$). A significant decrease in larval development was observed 1 day after treatment with 360 mg/kg of *C. schoenanthus* ($P<0.05$). Conversely, significantly higher values ($P<0.05$) for EHA were obtained in the same group, 5 days after treatment (Table 2).

No significant differences among group means for the hepatic or kidney parameters evaluated after treatment with essential oil (Tables 3 and 4) were found. However, one animal from the group that received 180 mg/kg was removed from the experiment due to signs of hepatic disease (increasing of liver enzymes, jaundice and weight loss). These clinical signs were noticed 10 days after essential oil administration.

Gas chromatographic analysis established that geraniol (59.42%), geranial (13.49%) and neral (8.98%) were the major constituents of the *C. schoenanthus* essential oil used in this work (Table 5).

4. Discussion

The objectives of this study were to evaluate both *in vitro* and *in vivo* anthelmintic activities of *C. schoenanthus* essential oil in lambs experimentally infected with *H. contortus*, and possible toxic effects of the essential oil.

Table 1
Mean ± standard error of values of $\log_{10}(x+1)$ fecal egg count (log FEC) at before, 1 day, 5 days, 10 days, 15 days and 20 days after treatment (DAT) and $\log_{10}(x+1)$ total worm count (logTWC) from lambs experimentally infected with *Haemonchus contortus* and treated with *Cymbopogon schoenanthus* essential oil at doses of 180 mg/kg or 360 mg/kg.

Groups	logFEC (eggs/g) before	logFEC (eggs/g) 1 DAT	logFEC (eggs/g) 5 DAT	logFEC (eggs/g) 10 DAT	logFEC (eggs/g) 15 DAT	logFEC (eggs/g) 20 DAT	logTWC
180 mg/kg	3.79 ± 0.11	4.18 ± 0.08	4.18 ± 0.10	4.15 ± 0.08	4.08 ± 0.12	4.07 ± 0.09	3.09 ± 0.06
360 mg/kg	3.76 ± 0.10	4.35 ± 0.08	4.05 ± 0.09	4.10 ± 0.07	3.93 ± 0.11	3.87 ± 0.08	3.12 ± 0.06
Control[a]	3.73 ± 0.09	4.26 ± 0.07	4.04 ± 0.08	4.32 ± 0.07	4.27 ± 0.10	4.15 ± 0.08	3.15 ± 0.05

There was no significant difference among group means by Tukey test at 5%.
[a] Control group received 10 ml of water.

Table 2
Mean ± standard error percentage of *in vitro* test egg hatch assay (EHA) and larval development assay (LDA) of *Haemonchus contortus* in lambs at 1 day, 5 days, 10 days and 15 days after treatment (DAT) with 180 mg/kg or 360 mg/kg of *Cymbopogon schoenanthus* essential oil.

	1 DAT	5 DAT	10 DAT	15 DAT
EHA (%)				
180 mg/kg	97.50 ± 0.66a	95.00 ± 0.60a	97.16 ± 0.79a	98.66 ± 0.51a
360 mg/kg	95.00 ± 0.72a	98.80 ± 0.65b	99.60 ± 0.86a	98.40 ± 0.56a
Control	97.33 ± 0.66a	95.50 ± 0.60a	99.16 ± 0.79a	98.00 ± 0.54a
LDA (%)				
180 mg/kg	93.33 ± 1.99a	83.80 ± 1.78a	84.33 ± 1.45a	96.83 ± 0.47a
360 mg/kg	71.80 ± 2.18b	84.80 ± 1.95a	87.20 ± 1.59a	96.80 ± 0.52a
Control	86.16 ± 1.99a	88.00 ± 1.78a	87.16 ± 1.45a	97.33 ± 0.47a

For each *in vitro* test, values with different letters in the columns are significantly different at 5%.

Table 3
Mean ± standard error of serum urea and creatinine evaluated before, at 5 days, 10 days and 20 days after treatment (DAT) in lambs experimentally infected with *Haemonchus contortus* and treated with *Cymbopogon schoenanthus* essential oil at doses of 180 mg/kg or 360 mg/kg.

	Before	5 DAT	10 DAT	20 DAT
Urea (mg/dL)				
180 mg/kg	40.80 ± 1.56	20.00 ± 2.61	21.40 ± 2.98	26.20 ± 3.49
360 mg/kg	40.00 ± 1.56	19.40 ± 2.61	23.00 ± 2.98	30.00 ± 3.49
Control	40.57 ± 1.32	22.14 ± 2.21	27.71 ± 2.52	30.57 ± 2.95
Creatinine (mg/dL)				
180 mg/kg	0.86 ± 0.03	0.92 ± 0.05	1.07 ± 0.05	0.94 ± 0.04
360 mg/kg	0.82 ± 0.03	0.86 ± 0.05	0.97 ± 0.05	0.89 ± 0.04
Control	0.84 ± 0.02	0.92 ± 0.04	1.00 ± 0.04	0.92 ± 0.03

There was no significant difference among group means according to Tukey's test at 5%.

Table 4
Mean ± standard error of albumin, liver biochemical enzymes alkaline phosphatase (AP), aspartate amino transferase (AST) and gamma glutamyl transferase (GGT) evaluated before, at 5, 10 and 20 days after treatment (DAT) in lambs experimentally infected with *Haemonchus contortus* and treated with *Cymbopogon schoenanthus* essential oil at doses of 180 mg/kg or 360 mg/kg.

	Before	5 DAT	10 DAT	20 DAT
Albumin (g/dL)				
180 mg/kg	2.86 ± 0.10	2.76 ± 0.10	2.9 ± 0.14	2.96 ± 0.10
360 mg/kg	2.75 ± 0.13	2.81 ± 0.10	2.75 ± 0.13	2.81 ± 0.10
Control	2.77 ± 0.08	2.81 ± 0.08	2.85 ± 0.12	2.70 ± 0.09
AP (UI/L)				
180 mg/kg	113.60 ± 25.57	79.4 ± 19.17	87.20 ± 21.74	73.80 ± 18.21
360 mg/kg	144.60 ± 25.57	88.00 ± 19.17	81.60 ± 21.74	88.00 ± 18.21
Control	116.3 ± 21.61	107.00 ± 16.20	93.6 ± 18.37	84.57 ± 15.39
AST (UI/L)				
180 mg/kg	54.16 ± 3.05	55.20 ± 5.06	58.20 ± 5.34	57 ± 4.7
360 mg/kg	50 ± 2.43	56.6 ± 5.06	68.20 ± 5.34	58.8 ± 4.7
Control	52.42 ± 2.84	57.14 ± 4.27	59.57 ± 4.51	60 ± 3.97
GGT (UI/L)				
180 mg/kg	44.66 ± 1.46	38.6 ± 3.66	41.6 ± 4.02	42.6 ± 3.33
360 mg/kg	51.33 ± 3.79	41.2 ± 3.66	39.6 ± 4.02	44.2 ± 3.33
Control	49.29 ± 2.06	41.71 ± 3.09	42.85 ± 3.4	43.85 ± 2.81

There was no significant difference among group means according to Tukey's test at 5%.

Table 5
Retention index (RI), retention time (RT), components and their relative percentage in *Cymbopogon schoenanthus* essential oil according to Wiley 275 spectra library.

RI	RT	Component	Relative percentage
858	4.68	(E)-2-hexenal	0.74
988	7.67	6-Methyl-5-hepten-2-one	0.40
1100	11.14	Linalool	0.99
1156	12.88	Citronellal	0.84
1206	14.61	N-decanal	0.42
1232	15.39	Citronellol	3.18
1245	15.8	Neral	8.98
1259	16.28	Geraniol	59.42
1274	16.77	Geranial	13.49
1303	17.78	Geranyl formate	0.17
1356	19.41	Citronellyl acetate	0.33
1360	19.54	Eugenol	0.34
1385	20.37	Geranyl acetate	4.80
1425	21.61	(E) Caryphyllene	2.19
1460	22.65	α-Humulene	0.28
1504	24.01	α-Muurolene	0.13
1528	24.69	γ-Cadinene	0.48
1554	25.43	Cadina 1,4 diene	0.25
1589	26.46	Caryophyllene oxide	0.43
		Total analyzed	94.09

C. schoenanthus essential oil is a complex mixture of terpenes. The oil used in our study had 19 identified components, from which geraniol, geranial and neral were the main ones. Studies with *Caenorhabiditis elegans* (Kumaran et al., 2003) concluded that geraniol had anthelmintic effects against this nematode. A previous study with this essential oil (Katiki et al., 2011) resulted in high *in vitro* anthelmintic activity against sheep trichostrongylids, evaluated by the EHA, LDA, larval feeding inhibition assay (LFIA), and the larval exsheathment assay (LEA). Thus, to follow up on the *in vitro* anthelmintic effects of *C. schoenanthus,* this *in vivo* test was carried out to confirm or refute its anthelmintic activity, and to evaluate the potential oil toxicity to sheep.

In the present experiment, neither 180 mg/kg nor 360 mg/kg of *C. schoenanthus* essential oil resulted in significant reduction in FEC or TWC, refuting the anthelmintic effects observed in the previous *in vitro* study (Katiki et al., 2011). The two doses of essential oil tested were chosen after a pilot test in naturally infected lambs. The doses lead to decreasing values of EPG 15 days after animals were drenched, with no signs of toxicity. In this manuscript we repeated the experiment with the same successful doses. However, lambs from all groups maintained a similarly high FEC throughout the experiment, as recorded at the beginning of the experiment. Although it is possible that oral doses higher than the ones tested, and provided as a single oral dose, could result in better anthelmintic effects, it also could lead to toxicity in the host. A study with Valencia orange oil, containing 95% of the terpene limonene, was very effective in reducing FEC of *H. contortus*-infected sheep. A single dose of 600 mg/kg reduced FEC by 97.4% compared to the control (Squires et al., 2010). These authors reported better results using a single high dose than when dosing animals for 3 consecutive days. FEC reduction of 76.57% also occurred in goats 15 days after treatment with *Eucalyptus staigeriana* essential oil (500 mg/kg, single dose), which also had limonene (28%) as the major oil component (Macedo et al., 2010). In contrast, *C. schoenanthus* oil used in the present study did not have limonene (Table 5).

Besides the nematocidal effect, plant extracts/compounds are tested for their ability to impair egg hatching and larval development from feces of infected animals treated with those plant extracts/compounds. Desired effects can result in reduced re-infection and reduced worm loads leading to decreased pasture contamination levels (Ketzis et al., 2002; Max, 2010). The essential oil of *C. schoenanthus* was very effective in *in vitro* tests with a LC_{50} of 0.045 mg/ml for the EHA and a LC_{50} of 0.063 mg/ml for the LDA (Katiki et al., 2011). Although the elimination of this oil in feces was found to partially inhibit larval development 1 day after the three daily treatments of 360 mg/kg, egg hatching was not inhibited.

When a plant extract or compound is tested *in vivo*, questions are raised about its bioavailability. How much is being absorbed and metabolized versus how much was not metabolized and was eliminated with the gastrointestinal matter. According to Fandohan and collaborators (2008), essential oils are generally absorbed by the gastro intestinal tract, metabolized in the liver and excreted by the kidneys. In addition, a review on the bioavailability and pharmacokinetics of volatile terpenes (essential oil components) in human and animals reported that ^{14}C-labeled terpenes used in three studies with rats found 91–95% of the radioactivity in feces and urine with seven metabolites identified by HPLC (Kohlert et al., 2000). Urine metabolites point to absorption and metabolism of the terpenic compounds, while compounds that are not absorbed and metabolized are found in feces, such as the sesquiterpene artemisinin found in the feces of goats given oral artemisinin (Ferreira and Gonzalez, 2008). However, without an encouraging anthelmintic effect *in vivo*, it is counterproductive to investigate bioavailability and metabolism.

Lambs used in this study had no significant changes in either hepatic (albumin, AST, AP, GGT) or renal (urea, creatinine) parameters, indicating that there was no toxicity of the essential oils at the doses tested. However, lambs from all groups presented marked reduction in serum urea values 5 days after treatment (Table 3). This reduction occurred as a response to their fast pre (8 h) and post treatment (4 h). Urea is synthesized in the liver, resulting from protein that is catabolized into ammonia and eliminated through kidney. The possibility of a change in kidney activity was refuted because no abnormality was found in creatinine. Serum creatinine is a non-proteic nitrogen substance resulting from muscular metabolism, eliminated through the kidneys, and not influenced by protein catabolism or diet (Kaneko, 1997). Also, reduction alkaline phosphatase was observed after 5 days post treatment in all groups (Table 4). According to Bain (2011), a possible reason for this decrease is that young animals have a higher concentration of this enzyme (about three times more) than adult animals. As we used 2-month-old animals in our experiment for a period of 20 days, this reduction (not significant) occurred due to the decrease of osteoblastic activity from those animals

that were undergoing a decrease in growth rate. In small ruminants, alkaline phosphatase has two isoenzymes: L-alkaline phosphatase (hepatic source – this enzyme would predict toxic effects of essential oils), and B-alkaline phosphatase (bones source – high in young animals), and both enzymes were detected by the biochemical tests done.

Although general safety of the oil was observed, one animal that received 180 mg/kg was removed from the experiment, possibly due to toxicity of the essential oil (increasing of AST and GGT at the 10th day after treatment. Toxicity symptoms caused by the essential oil were observed at 360 mg/kg, with signs of discomfort, apathy, lethargy, and drowsiness in treated animals. Those symptoms were noticeable for at least 4 h post-treatment with the oil, but disappeared after that. Although no information about the LD_{50} of *C. schoenanthus* oil was found in literature, geraniol, the main constituent (59%) from *C. schoenanthus* had a high oral LD_{50} (3600 mg/kg) in rats (MSDS Sigma, 2011). For instance, Ketzis et al. (2002) tested 0.1, 0.2 and 0.4 ml/kg of *Chenopodium ambrosioides* essential oil in mixed nematode infection in goats without anthelmintic effect and with high toxicity due to ascaridol (major oil component), which has a LD_{50} of 200 mg/kg (MSDS, Chemcas.com, 2011).

5. Conclusion

There were no statistically significant reductions in FEC or TWC after treatment of lambs artificially infected with *H. contortus* and treated with *C. schoenanthus* essential oil at either 180 mg/kg or 360 mg/kg. No statistical difference among group means regarding blood levels of urea, creatinine, albumin, AP, AST and GGT were found. Feces from animals that received the essential oil were evaluated *in vitro* and larval development was slightly inhibited 1 day after treatment at dose of 360 mg/kg. Lambs that received 360 mg/kg showed signs of apathy, lethargy and anorexia. Thus, although previous *in vitro* studies using four anthelmintic assays with *C. schoenanthus* essential oil were encouraging, these results could not be confirmed in lambs artificially infected with multidrug-resistant H. contortus treated with 180 or 360 mg/kg. Other doses need to be evaluated as well as formulations and adjuvant that could improve efficacy. Further tests with different doses regimen, different hosts (such as goats), and a different strain of *H. contortus* should be considered before discarding the oil of *C. schoenanthus* as a possible anthelmintic against trichostrongylids of small ruminants.

Conflict of interest

The authors declare no conflict of interest. The mention of proprietary names is solely for the convenience of the reader and does not imply endorsement by the authors of the products cited here over similar products.

Acknowledgements

The authors are grateful for the financial support from Embrapa Pecuária Sudeste (CPPSE), and for the technical support of Dr. Sergio Novita Esteves, Dr. Simone Fernandes, Dr. Helder Louvandini, Barbara Rubert, and Camila Olivo de Carvalho.

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