In vitro and in vivo anthelmintic activity of Caryocar brasiliense Cambess. (Caryocaraceae) leaves against Haemonchus contortus in sheep

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Abstract

Effective control of *Haemonchus contortus* showing anthelmintic resistance remains a major challenge in sheep production systems. Plant-based treatments may represent a sustainable alternative for parasite control. This study evaluated the *in vitro* and *in vivo* anthelmintic effects of the leaves of *Caryocar brasiliense*, an important tree species from the Cerrado biome. High-performance liquid chromatography analyses detected tannins and flavonoids in the aqueous (AE) and ethanolic (EE) extracts. In the egg hatching inhibition test, the LC_{90} values of AE and EE were 15.88 and 9.48 mg/mL, respectively. However, both extract showed increased efficacy after tannin extraction. The maximum tolerated dose in both male and female mice was 100 mg/kg body weight. No clinical signs of toxicity, mucosal tissue alterations, changes in animal behavior, or mortality were observed during the four days of extract administration. In the larval development inhibition assay, the LC_{90} values for the dried powder and AE were 64.80 and 38.55 mg/g of fecal culture, respectively. The inclusion of *C. brasiliense* leaves in the diet showed high *in vivo* efficacy in reducing fecal egg counts in lambs infected with the nematode, while blood parameters remained within the physiological reference ranges for sheep. These results highlight the potential of *C. brasiliense*, a Cerrado plant species, as an alternative strategy for the control of *H contortus* in sheep.

1. Introduction

The occurrence of gastrointestinal nematodes (GN) has been a major limitation to the development of sheep production across different continents (Hadi et al., 2021; Hassan et al., 2021; Morais–Costa et al., 2016; Kimeli et al, 2025). *Haemonchus contortus* is the most prevalent nematode infecting sheep, and animals affected by haemonchosis commonly exhibit anemia and submandibular swelling, with high mortality rates particularly observed in young lambs and ewes during the peripartum period. This nematode causes significant economic losses due to reduced growth and weight gain, decreased milk production, and low fertility (Bizimenyera et al., 2006; Bastos et al., 2017; Naeem and Roohi, 2021)

Misuse and indiscriminate use of anthelmintics have promoted the rapid selection of resistant nematode populations (Silva et al., 2018). Resistance to the major classes of anthelmintics has been observed in sheep populations across different continents (Hadi et al., 2021; Hassan et al., 2021; Kimeli et al., 2025).

Thus, it is crucial to explore alternative strategies to overcome anthelmintic resistance and maintain effective control of these nematodes in sheep herds (Molento and Prichard, 2001). The anthelmintic activity of plant extracts has gained increasing attention as an important alternative (Zabré et al., 2017; Hassan et al., 2021).

In particular, plants from the Brazilian Cerrado have shown promising anthelmintic effects against GN (Gaspar et al., 2010; Morais–Costa et al., 2015; Morais–Costa et al., 2016). *Caryocar brasiliense* Cambess. (Caryocaraceae), commonly known as pequi, is a tree species native to the Brazilian Cerrado biome and is widely distributed throughout the Southeast and Midwest regions of Brazil (Maia et al.,

2008). This plant species possess several scientifically proven pharmacological properties (Pinto et al., 2019).

Several studies have reported potential molluscicidal, antifungal, antibacterial, and antileishmanial properties of *C. brasiliense* (Bezerra et al., 2002; Passos et al., 2002; Martins et al., 2009; Paula-Junior et al., 2006; Lopes et al., 2011; Moreira et al., 2019; Filho et al., 2021). A previous study has shown that oral administration of aqueous extract of *C. brasiliense* peel (2 g/kg body weight [bw]) to infected lambs for seven days has shown significant anthelmintic efficacy (33%) (Nogueira et al., 2012), However, the potential of the leaves of this tree, which remain green and leafy throughout the year, to control haemonchosis in small ruminants has not yet been investigated.

In this study, we aimed to determine the *in vitro* anthelmintic effects of the dried powder and aqueous extract (AE) of the leaves of *C. brasiliense*, with or without tannins, as well as the *in vivo* anthelmintic efficacy and effects on blood parameters in lambs infected with *H. contortus* and treated with the plant leaves.

2. Material and methods

2.1. Study area and preparation of *C. brasiliense leaf extracts*

Fresh leaves of *C. brasiliense* were collected from a rural area in the northern region of Montes Claros, Minas Gerais State, Brazil (W 44º 04' 54"; S 16º 30' 93"). The climate of the Montes Claros region is classified as tropical wet with dry summers (As), with an annual rainfall of < 25 mm. Voucher specimens (No. 338) were deposited in the Montes Claros Herbarium (HMCMG) of the Universidade Estadual of Montes Claros (UNIMONTES).

Healthy leaves of *C. brasiliense* were selected and dried to a constant weight in a forced air circulating dryer (TE 394/4, Tecnal Equipamentos CientíficosTecnal, Piracicaba, SP, Brazil) at 40 ºC for 72 h. The dried leaves were ground in a Wiley mill, stored in paper bags, and protected from incident light (Morais–Costa et al., 2015). The aqueous extract (AE) was prepared by infusion with distilled water (30 g/300 mL) at 40°C for 60 min under static extraction conditions. The ethanolic extract (EE) was obtained by static maceration of dried leaves (20 g) with absolute ethanol (1000 mL) in amber glass containers, which were kept in the dark for seven days. The extracts were then filtered through a gauze funnel. Solvents were removed by evaporation at 40°C for 48 h in a forced air circulating dryer to obtain the aqueous (3.5 g) and ethanolic (4.0 g) extracts.

For biological assays, portions of these extracts were subjected to tannin extraction using the method proposed by Nyman et al. (1998), with slight modifications. In summary, a portions of the extract (1 g) was dissolved in 20 mL of water at 90°C. After cooling the extract at room temperature, 0.2 µL of NaCl solution (10% w/v) was added, and an aliquot (1 mL) of this solution was mixed vigorously with 4 mL of

gelatin solution (1% w/v). The solution was centrifuged at 1.800 × *g* for 6 min, and the supernatant was collected and used for the egg hatching inhibition (EHI) assay.

2.2. Characterization of the prepared extracts

Total proanthocyanidin content (condensed tannins) in the extracts was determined according to the method described by Hiermann et al. (1986) by measuring the absorbance of cyanidin chloride formed after acid-catalyzed solvolysis with *n*-butanol/HCl (37%, 95:5). All samples were analyzed in triplicate, and the results are expressed as cyanidin chloride (mean ± standard deviation).

The analyses were performed using high-performance liquid chromatography (HPLC) and gas chromatography (GC). The AE and EE were analyzed using a Waters Alliance 2695 HPLC system (Waters Corporation, Milford, USA) equipped with a quaternary pump, an auto-sampler, a photodiode array detector (DAD 2996), and a Waters Empower Pro data-handling system. The chromatograms were obtained on a LiChrospher 100 RP-18 column (250 × 4 mm i.d., 5 µm; Merck, Darmstadt, Germany) coupled to a LiChrospher 100 RP-18 guard column (4 × 4 mm i.d., 5 µm; Merck) at 40 ºC. The mobile phase consisted of water (A) and acetonitrile (B) acidified with 0.1% (v/v) H_3PO_4 delivered at a flow rate of 1.0 mL min^{-1} through gradient elution as follows: 0 min, 95% A and 5% B; 60 min, 5% A and 95% B, followed by 10 min of isocratic elution. The solvents used were of HPLC grade (Merck, Germany) and degassed by sonication prior to use. Chromatograms were obtained at 210 nm, and UV spectra were recorded online from 190 to 400 nm. For analyses, AE and EE were dissolved in ultrapure water and methanol (HPLC-grade), respectively, at 10 mg/mL. The solutions were centrifuged at 8.400 × *g* for 10 min, and the supernatants (10 µL) were automatically injected into the apparatus.

Chromatographic analysis of AE was performed using a 7890A Gas Chromatography (GC) coupled to 5975C mass spectrometer (Agilent Technologies) on a DB5-MS capillary column (Agilent Technologies), with stationary phase composed of 5% phenyl and 95% methylsiloxane (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness), helium (99.9999% purity) as carrier gas at a flow rate of 1.5 mL/min. The split/splitless injector temperature was maintained at 240°C. The chromatographic column was initially heated at 160°Cand held isothermally for 2 min, then increased at a rate of 2°C/min to 200°C and then up to 240°C at a rate of 10°C/min. After separation of the compounds, the temperature was raised to 300°C and maintained for 3 min (post-run). The interface temperature was maintained at 240°C, and ionization was performed with 70 eV impact. Mass spectra were recorded over a scanning range of m/z 30–600 Da. The sample was injected using CombiPAL autosampler; the injection volume was 1 µL using a split ratio of 1:10.

2.3. Egg hatching inhibition assay

All procedures were performed in accordance with the principles of animal experimentation approved by the 275/2013 protocol of the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Minas Gerais, Brazil.

Three Santa Inês lambs were infected with 2000 third-stage larvae (L3) of albendazole-resistant *H. contortus*. This strain was obtained from a female *H. contortus* collected from the abomasum of a lamb in northern Minas Gerais, Brazil, and multiplied by our sheep, as previously described (Duarte et al., 2012).

Nematode eggs were obtained from fecal samples of two Santa Inês lambs infected with *H. contortus* by flotation, sedimentation, and filtration in saturated NaCl solution (Coles et al., 1992). The average fecal egg count (FEC) was > 1500/g, as determined using the modified McMaster technique (Gordon and Whitlock, 1939).

Immediately after dilution, AE extracts (15.0, 7.5, 3.75, and 1.88 mg/mL) and EE (53.9, 26.9, 13.5, and 6.8 mg/mL) with tannins, and AE (8.13, 4.07, 2.03, and 1.02 mg/mL) and EE (2.54, 1.27, 0.64, and 0.16 mg/mL) without tannin were used for the EHI assay. Positive controls were exposed to levamisole phosphate (0.3 mg/mL), whereas the negative control consisted of sterile purified water. Six experimental groups were used, each with five replicates.

Experimental mixtures consisted of 100 µL of fecal suspension containing average fresh eggs and 100 µL of the plant extracts. The samples were homogenized and incubated in a BOD incubator at 28°C for 48 h. Subsequently, 15 µL of Lugol's solution was added to each tube, and samples were stored at 4°C for counting of unembryonated and embryonated eggs and first stage larvae (L1) (Coles et al., 1992). The number of L1 larvae relative to the initial number of eggs (remaining eggs plus L1) was determined for each replicate and subjected to analysis of variance.

The means were compared using Duncan's test ($P < 0.05$). *Probit* regression was employed to determine the concentrations required to inhibit 50% (LC_{50}) and 90% (LC_{90}) of egg hatching using SAEG software (SAEG, 2007). EHI was calculated using the formula described by Coles et al. (1992) EHI (%) = 100 × (1 − L1/initial number of eggs).

2.4. Larval development inhibition test

To evaluate the effectiveness of dried powder and AE of *C. brasiliense* leaves, a larval development inhibition (LDI) test was performed by the quantitative coproculture method (Borges, 2003; Morais-Costa et al., 2016) performed using feces from two lambs infected with *H. contortus* (mono-infection). Seven treatments were conducted, each with five replicates, for dried powder leaves at concentrations of 133.37, 100.0, 66.67, and 33.37 mg/g dry weight (dw), whereas the AE was tested at concentrations of 66.67, 50.0, 33.3, 25.0, and 16.67 mg/g dw.

For both experiments, a positive control consisting of 2 mL of ivermectin solution (16 µg/mL) added to 2 g of feces was included, and a negative control consisted of 2 mL of sterile distilled water. The cultures were incubated in a BOD incubator at 28°C for three and seven days and subsequently evaluated for the presence of infective larvae (L3). The % LDI was calculated using the formula adapted from Borges (2003):

% LDI = 100 × (1 − LPGF of the treated group/LPGF of the untreated group),

where LPGF represents the number of larvae (L3) per gram of feces.

The data were log-transformed [log(x + 1)] and subjected to analysis of variance. The means were compared using Duncan's test ($P < 0.05$). The LC_{90} was determined by *Probit* analysis using SAEG software (SAEG, 2007).

2.5. Assessment of toxicity of *C. brasiliense extracts in mice*

Toxicity testing in mice was performed according to Walum (1998) and adapted from Morais-Costa et al. (2016) to determine the maximum tolerated dose (MTD). AE and EE of *C. brasiliense* and 1 × phosphate-buffered saline (PBS; control) (20 µL each) were administered to six Swiss mice (3 males and 3 females), aged 6–8 weeks and with a mean bw of 20 g for four consecutive days.

On days 1 and 2, the extracts were administrated at doses of 25.0 mg/kg bw and 50.0 mg/kg bw, respectively. On days 3 and 4, the extracts were administered at a dose of 100.0 mg/kg bw. On day 5, mice were euthanized by cervical dislocation following the administration of AE, EE, and 1 × PBS. The internal organs and mucosa were clinically examined during necropsy to assess possible toxic effects.

2.6. In vivo anthelmintic activity

Fecal egg count reduction was assessed in twenty-four Santa Inês lambs (4–8 months old) of both sexes, with an average bw of 26.5 kg. Prior to the start of the trial, all lambs were administered albendazole (10 mg/kg bw) and levamisole phosphate (0.6 mg/kg bw) to ensure that they were worm-free (FEC = zero in four consecutive tests).

During the 10-day adaptation period, the animals were individually confined and fed a balanced diet consisting of sorghum silage, concentrate, and mineral premix, with water provided ad libitum.

Treatments were conducted in the morning, and lambs were monitored for clinical signs and weighed in the morning before feeding on the day of treatment and on days 7, 14, 21, and 28 post-treatment.

The *in vivo* anthelmintic test was performed as described by Coles et al. (1992). The 24 lambs with zero FEC were infected with 800 (L3) of *H. contortus* per 10 kg bw. Twenty-eight days post-infection, the lambs were assigned to one of three homogeneous groups based on FEC, bw, and sex, and the treatments were administered in the morning after a 12-h fasting period. The first group of untreated animals served as the negative control, the second group was orally administered levamisole phosphate (0.6 mg kg^{-1} bw) and served as the positive control, and the third group was administered crude powder of the leaves of *C. brasiliense* at a dose of 1.35 g dry matter (dm)/kg bw for three consecutive days. This dose was based on the LC_{90} estimated from the LDI test for the dried leaf powder.

The initial FEC values were recorded on days 1 and 2 before and on the day of treatment, and the mean value was used to standardize the groups. Subsequently, the mean FEC values were evaluated on days 7,

8, and 9 (second period); days 14, 15, and 16 (third period); days 21, 22, and 23 (fourth period); and days 28, 29, and 30 (fifth period) (Morais-Costa et al., 2016). The mean weight gain of the groups was compared using Tukey's test at a 5% significance level. The mean of two FEC counts was obtained for each collection day per animal, and the McMaster technique was performed in duplicate with the addition of saturated NaCl solution (Gordon and Whitlock, 1939). Treatment efficacy was determined using a formula adapted from Coles et al. (1992):

FEC reduction (%) = 100 × [1 – (mean FEC of treated group/mean FEC of untreated group)].

2.7. Blood parameters of lambs

Blood samples were collected from the jugular vein on days 0, 7, 14, 21, and 28 into tubes containing ethylenediaminetetraacetic acid (EDTA) and stored at 4°C. The erythrogram and leukogram were evaluated using an electronic automatic analyzer (2.800 BC Vet®; Mindray Medical International Ltd. Shenzhen, China). Total protein and albumin concentrations were analyzed using Bioclin® (Quibasa Basic Chemicals Ltd., Belo Horizonte, MG, Brazil) by colorimetric spectrophotometer using an automatic biochemical analyzer (BIOPLUS BIO-2000, Shenzhen, China).

2.8. Statistical analysis of in vivo tests

The data obtained were transformed to $\log_{10}(x + 10)$ and subjected to analysis of variance in a split-plot design with respect to the four evaluated periods. The means were compared using Duncan's or Scott-Knott's tests at a 5% significance level. Additionally, regression analysis was applied to evaluate the response of fecal egg count (FEC) over time and among treatments using SAEG software (SAEG, 2007). The percentage reduction in FEC was calculated using the formula adapted from Coles et al. (1992):

FEC reduction (%) = 100 × [1 – (mean FEC of treated group/mean FEC of control group)].

3. Results

3.1. Characterization of extracts

The proanthocyanidin contents in the AE and EE were 2.66% ± 0.14% and 2.20% ± 0.03%, respectively. The chromatographic profiles obtained by HPLC-DAD showed a high degree of similarity between the two extracts, with a predominance of peaks corresponding to polar compounds and UV spectra characteristic of polyphenols. The presence of tannins and flavonoids were detected in the AE, with maximum absorbance at λ 212.0–261.5 and λ 376.8–376.1, respectively (Fig. 1). In the EE, flavonoids were detected with absorbance in the region of λ 274.5–376.8 nm (Fig. 2).

Gas Chromatography analyses revealed that the major peak (n^{o} 35) detected in the chromatogram of the AE (Fig. 3), representing 29.49% of the total chromatographic area, was gallic acid (3,4,5-trihydroxybenzoic acid). In addition, 13 other acids and 12 carbohydrates were detected (Supplementary Material).

3.2. In vitro anthelmintic activity

In the EHI test, efficacy increased with increasing concentrations of the extracts (Table 1). The LC_{90} values of AE was 9.95 mg/mL (95% confidence interval [CI]: 9.34–11.61), and that of EE was 9.48 mg/mL (95% CI: 8.89–10.23). After tannin extraction, the AE at 8.13 mg/mL and EE at 2.54 mg/mL were 78% and 93% efficacy, respectively (Table 2). The LC_{90} values for AE and EE after tannin removal were 12.16 mg/mL (95% CI: 10.78–13.98) and 1.15 mg/mL (95% CI: 1.05–1.28), respectively. At all tested concentrations, embryonated eggs presented degraded embryos with structural malformations (Fig. 4).

In the LDI test, the mean numbers of infective larvae were significantly lower than those observed in the negative control with sterile distilled water ($P < 0.05$), with the highest efficacies of 100% at concentrations > 133.37 mg/g for the dried leaf powder and 97.2% at 66.67 mg/g for the AE (Table 3). The LC_{90} values for the dried leaf powder and AE were 64.80 mg/g and 38.55 mg/g, respectively.

3.3. Toxicity test in mice

The maximum tolerated dose in both male and female mice was 100 mg/kg bw. No clinical signs of toxicity, mucosal tissue alterations, changes in animal behavior, or mortality were observed during the four days of extract administration or at necropsy, and macroscopic examination revealed no alterations in the liver, kidney, spleen, lung, or other viscera.

3.4. In vivo test

FEC analysis showed a significant interaction between treatments and the periods of evaluation ($P < 0.0001$; Table 4). The leaves of *C. brasiliense* showed high efficacy in reducing FED, differing significantly from the untreated group during the four weeks post-treatment, with the maximum reduction of 92.7% observed one week after treatment. Treatment with levamisole phosphate remained highly effective throughout all evaluated periods (> 97%). The polynomial regression analysis showed significant effect ($P < 0.01$), indicating that fecal egg count (FEC) reduced non-linearly over time in response to treatments (Fig. 5).

The mean daily weight gain among the three groups of lambs were similar (1.33 ± 0.16 kg). Animals treated with *C. brasiliense* did not exhibit behavioral changes, submandibular edema, weakness, or loss of appetite during the experiment.

3.5. Blood parameters of lambs

According to Kahn and Line (2010), erythrocyte counts and hematocrit (Hct) values were within the reference ranges. However, lambs treated with levamisole phosphate showed lower erythrocyte and Hct values than those in the other lambs groups ($P < 0.05$).

The mean total serum protein was lower ($P < 0.05$) in lambs treated with *C. brasiliense* leaves than in untreated lambs. However, after 28 days, no significant differences were detected, and the values remained within the reference range for sheep. For albumin concentrations, differences were observed

among the lamb groups; however, at 28 days post-treatment, the mean values were significantly higher than those observed during the other evaluated periods (Table 5).

No significant differences were detected in the total leukocyte, neutrophil, eosinophil, lymphocyte, and monocyte counts among the lamb groups. However, lymphocyte counts were significantly higher during the initial periods and at 14 days after treatment (Table 6).

Discussion

High-performance liquid chromatography analyses revealed flavonoids as the major components of *C. brasiliense* extracts, which showed high *in vitro* EHI after the removal of tannins, suggesting that flavonoids are responsible for the anthelmintic effect. Plant secondary metabolites have been recognized are effective alternatives for the control of *H. contortus*. *Caryocar brasiliense* leaves, which are rich in tannins, saponins, and other secondary metabolites, warrant further studies to confirm their efficacy in controlling gastrointestinal parasites. The anthelmintic activity may also results from synergism among these metabolites (Morais-Costa et al, 2014) or from tannin-rich extracts that interfere with the biology of gastrointestinal nematodes (Minho et al., 2008; Lima et al., 2019).

The scientific validation of new alternative anthelmintic compounds requires proper characterization of their effects on ruminant gastrointestinal parasites, as well as evaluation of their toxicity through *in vivo* experiments involving administration of plant materials to animals (Morais-Costa et al., 2014).

Several studies have reported that secondary metabolites of plants are responsible for their effects on the eggs, larvae, or adult stages of gastrointestinal nematodes. In particular, condensed tannins have been widely reported to exhibit anthelmintic activity (Githiori et al., 2006; Minho et al., 2008; Manolaraki et al., 2010; Lima et al., 2021).

Other studies have reported the anthelmintic action of secondary compounds such as saponins, flavonoids, alkaloids, terpenoids, and coumarins (Silveira et al., 2012; Ademola et al., 2009; Barrau et al., 2005; García et al., 2005). *C. brasiliense* leaves have been reported to contain condensed tannins, hydrolyzable tannins, flavonoids, terpenoids (Bezerra et al., 2002; Paula-Junior et al., 2006), and saponins (Paula-Junior et al., 2006). The degeneration of L1 larvae and embryonated eggs observed in this study may have resulted from the synergism among these secondary compounds present in *C. brasiliense*.

Studies have shown that exposure of infective larvae of gastrointestinal nematodes in goats to extracts *of Onobrychis viciifolia*, a plant rich in tannins, results in alterations in the subcutaneous vesicles of the cytoplasm and degeneration and/or death of muscle and intestinal cells (Brunet et al., 2011). The anthelmintic activity of flavonoids has been attributed to changes in enzymatic activities and metabolic processes in parasites (Kerboeuf et al., 2008). Nandi et al. (2004) reported that the nematicidal action of saponins from *Acacia auriculiformis* was related to membrane damage caused by the formation of free radicals that induce lipid peroxidation in the cell membranes. In the present study, EE was more effective than AE in the EHI test. After tannin removal, both extracts showed increased efficacy and a substantial

reduction in LC_{90}, suggesting the presence of other metabolites capable of inhibiting embryogenesis and egg hatching. These results suggest that tannins are not the metabolites primarily responsible for the observed anthelmintic effect.

In another study analyzing Cerrado plant species, HPLC analyses revealed flavonoids as the major components of leaf extracts of *Piptadenia viridiflora*, which showed high *in vitro* EHI after the removal of tannins (Morais-Costa et al., 2016). The authors suggested that flavonoids were responsible for the observed anthelmintic effect, as higher EHI values were observed after tannin extraction. In another study involving Cerrado plant species such as *Turnera ulmifolia*, *Parkia platycephala*, and *Dimorphandra gardneriana*, phytochemical analyses detected phenols and condensed tannins, which were associated with high anthelmintic efficacy in LDI assays (Oliveira et al., 2017) and other helminths (Ahmad et al., 2023).

However, synergistic effects between condensed tannins and flavonoids such as quercetin and luteolin have been reported in LDI tests (Klongsiriwet et al., 2015). In contrast, the leaf extracts of *C. brasiliense* evaluated in this study were more effective after tannin removal than in their crude forms, suggesting the absence of synergism with flavonoids.

In this study, a modified method of quantitative coproculture allowed eggs and subsequently larvae to come into contact with the extract metabolites present in host feces. This test simulates the natural environment in which larval development occurs during outbreak, thereby providing a natural condition for evaluating larval development inhibition (Nogueira et al., 2012).

According to Athanasiadou et al. (2007), the efficacy of plant extracts may vary depending on the bioavailability of their bioactive compounds across different compartments of the gastrointestinal tract, as well as on the parasite species infecting the host. This variability may lead to discrepancies between in vitro and *in vivo* results for the same plant species.

The presente study provides the first evidence of high *in vivo* efficacy of ingesting leaves from a Cerrado plant for the control of *H.* contortus. The ingestion of dried leaves of *C. brasiliense* resulted in a substantial reduction in FEC after one week of treatment in lambs experimentally infected with this nematode. These findings indicated that the plant metabolites remain biologically active under abomasal conditions and after ruminal fermentation, demonstrating in vivo stability.

Anthelmintic efficacy was most pronounced during the first week post-treatment (92.7%), suggesting that the plant metabolites may either reduce the adult nematode population in the abomasum or inhibit egg production during oral administration. Future studies should evaluate the continuous inclusion of *C. brasiliense* leaves in the diet at higher doses to achieve more effective and sustained reductions in pasture contamination.

Regression analysis revealed a consistent, treatment-dependent reduction pattern, with distinct response dynamics among experimental groups. A polynomial model provided the best fit to the data, as

a linear model did not adequately describe the FEC reduction. This pattern may be associated with the intrinsic variability of FEC data, as well as the short duration of leaf ingestion (three days). During this limited exposure period, the concentration of plant metabolites may not have been sufficient to fully eliminate the nematode population; however, it may have been adequate to inhibit oviposition, thereby contributing to the marked reduction in FEC observed during the first weeks post-treatment.

In the present study, administration of dried leaf powder of *Caryocar brasiliense* significantly reduced mean fecal egg counts (FEC), demonstrating high anthelmintic efficacy at a dose of 1.3 mg (DM)/kg body weight (BW). In comparison, a previous study evaluating another Cerrado plant species reported that the aqueous extract of *Piptadenia viridiflora*, administered in vivo at 230 mg/kg BW, achieved an efficacy of 47.20% during the first week post-treatment (Morais-Costa et al., 2016). Notably, *C. brasiliense* exhibited greater efficacy at a substantially lower administered dose.

The potential anthelmintic effects of *C. brasiliense* have also been reported by Nogueira et al. (2012), who evaluated the AE from fruit peels. In the LDI test using the quantitative coproculture method, the lowest tested concentration (40 mg/mL) showed an efficacy of 99%, with an LC_{90} value of 53.19 mg/mL. Ribeiro et al. (2012) also reported anthelmintic activity of both the AE and powder obtained from the fruit peels of *C. brasiliense* against the root-knot nematode *Meloidogyne javanica*. The authors reported that this extract reduced egg hatching and increased mortality of the second-stage nematode young larvae. In addition, Gaspar et al. (2010) evaluated the *in vivo* anthelmintic activity of the leaf powder of *C. brasiliense* mixed with the leaves of *Eugenia dysenterica* in Santa Inês sheep at a dose of 1.2 g/kg bw and observed a reduction of 81% in FEC by the 14th day of evaluation. Moreover, no significant weight loss was observed in the treated animals.

The maximum intraperitoneally tolerated dose of AE in mice was 100 mg/kg bw, indicating low toxicity (Walum, 1998). The mean total serum protein level was lower ($P < 0.05$) in lambs treated with *C. brasiliense* leaves than in untreated lambs. However, after 28 days, no significant differences were observed, and the values remained within the reference range for sheep. For albumin concentrations, differences were detected among the lamb groups; however, at 28 days post-treatment, the mean values were significantly higher than those observed during the other evaluated periods (Table 5).

No significant differences were detected in blood parameters among the lamb groups. In this study, the lambs did not develop anemia, as the diet provided during the experiment contributed to clinical resistance against blood loss caused by the parasite. Lymphocyte counts were significantly higher during the initial periods in both lamb groups; however, on day 28, these values returned to normal levels for sheep, suggesting resolution of the acute phase of haemonchosis (Kahn and Line, 2010). Ingestion of *C. brasiliense* leaves promoted a slight reduction in serum protein levels during the first week post-treatment, which could be attributed to its tannin content, capable of forming complex with dietary proteins. However, by 28 days post-treatment, serum protein levels returned to normal values in sheep. Another study on sheep infected with *H. contortus* showed that the total serum protein levels were lower than those of reference values (Kahn and Line, 2010) in all lambs during the initial infection period.

Hematophagy of this nematode and abomasitis, which may impair protein digestion during the acute phase of infection, may explain these low protein concentrations. After day 14, protein levels were significantly elevated compared with the initial period, suggesting recovery of animals treated with the AE of *Piptadenia viridiflora* (Morais-Costa et al., 2016).

Conclusion

The leaves of *Caryocar brasiliense* showed high efficacy in inhibiting larval development and egg hatching of *Haemonchus contortus*. Phytochemical analyses of the extracts revealed the presence of tannins such as gallic acid and flavonoids; however, both the aqueous and ethanolic extracts showed greater efficacy in inhibiting egg and larvae development after tannin removal. In mice, AE exhibited no signs of toxicity, and the ingestion of dried leaves resulted in high in vivo efficacy after one week of treatment, indicating that *C. brasiliense* leaves may represent a promising complementary and alternative measure for the control of *H. contortus*.

Declarations

Conflict of interest

All procedures were performed in accordance with the principles of animal experimentation approved by the 275/2013 protocol of the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Minas Gerais, Brazil.

Author Contribution

Franciellen Morais-Costa: Investigation, Resources, Writing – Review and Editing, Supervision, Project administration. Ana Cláudia Maia Soares: Formal analysis, Resources, Methodology. Gabriela Almeida Bastos: Resources, Methodology, Formal analysis. Leydiana Duarte Fonseca: Validation, Investigation, Resources, Writing – Review and Editing, Methodology, Formal analysis. Otávio Cardoso Filho: Supervision, Project administration. Eduardo Robson Duarte: Supervision, Project administration. Kaike Magno de Macêdo: Validation, Investigation, Resources, Writing – Review and Editing, Methodology, Formal analysis. Fernão Castro Braga: Supervision, Project administration. Eduardo Robson Duarte: Supervision, Project administration. Neide Judith Faria de Oliveira: Formal analysis, Resources, Methodology. Walter dos Santos Lima: Supervision, Project administration.

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Tables

Tables are available in the Supplementary Files section.

Figures

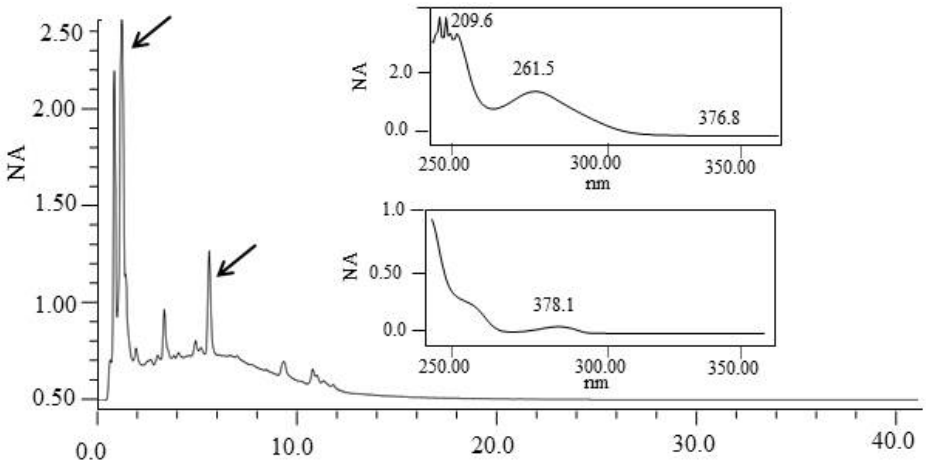

Figure 1

HPLC-DAD chromatogram of the aqueous extract of *C. brasiliense* leaves, showing spectrum characteristics of tannins and flavonoids (shown in panels). Retention times: first RT = 0.972 min (UV λ 209.6–376.8 nm) and second RT = 5.872 min (UV λ 378.1 nm).

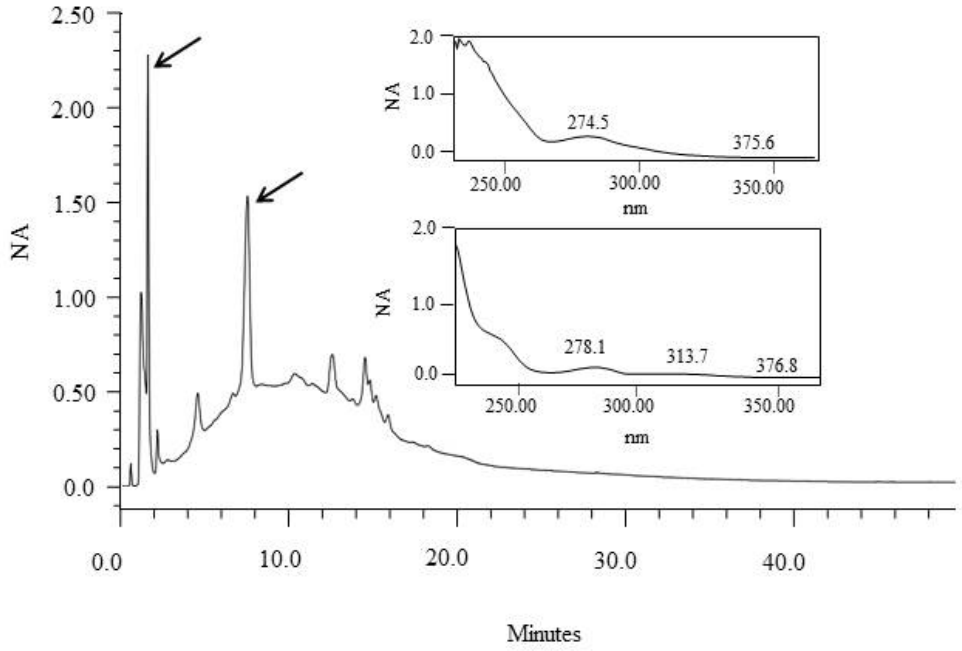

Figure 2

HPLC-DAD chromatogram of the ethanolic leaf extract of *C. brasiliense*, showing spectrum characteristics of flavonoids (shown in panels). Retention times: first RT = 0.989 min (UV λ 274.5–375.6 nm) and second RT = 1.378 min (UV λ 278.1–376.8 nm).

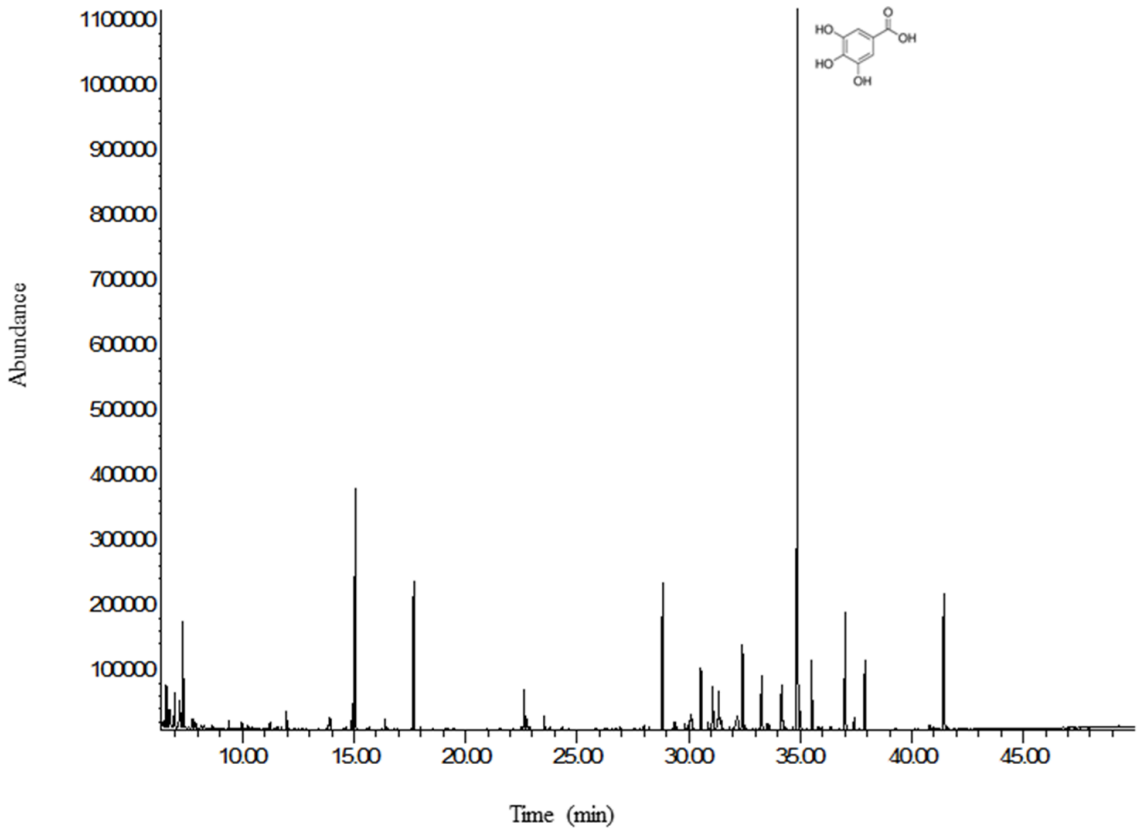

Figure 3

GC-MS chromatogram of the aqueous leaf extract of *C. brasiliense* used in the anthelmintic assay. Peak 35 corresponds to gallic acid. Chromatographic conditions are described in the Experimental section. Retention time = 34.872 min; area = 29.49%.

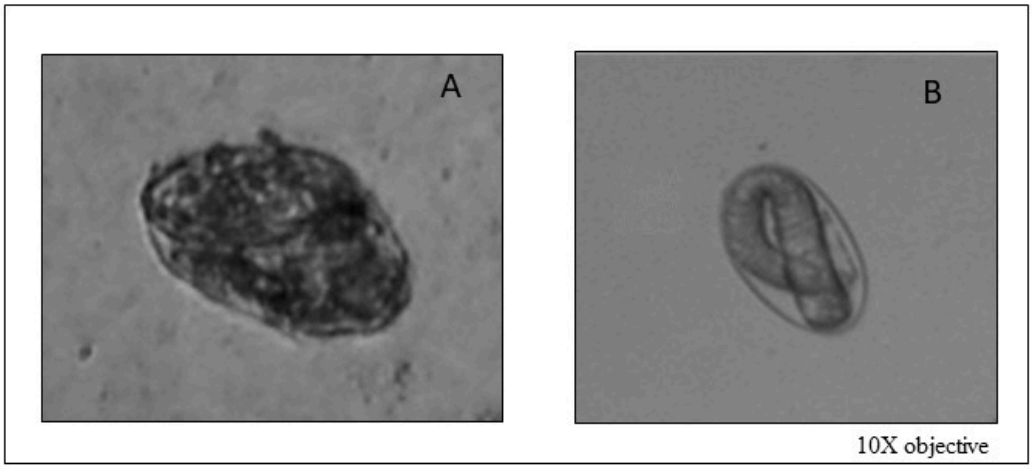

Figure 4

Eggs with structural malformations. A) Treated with *Caryocar brasiliense* leaves. B) Untreated.

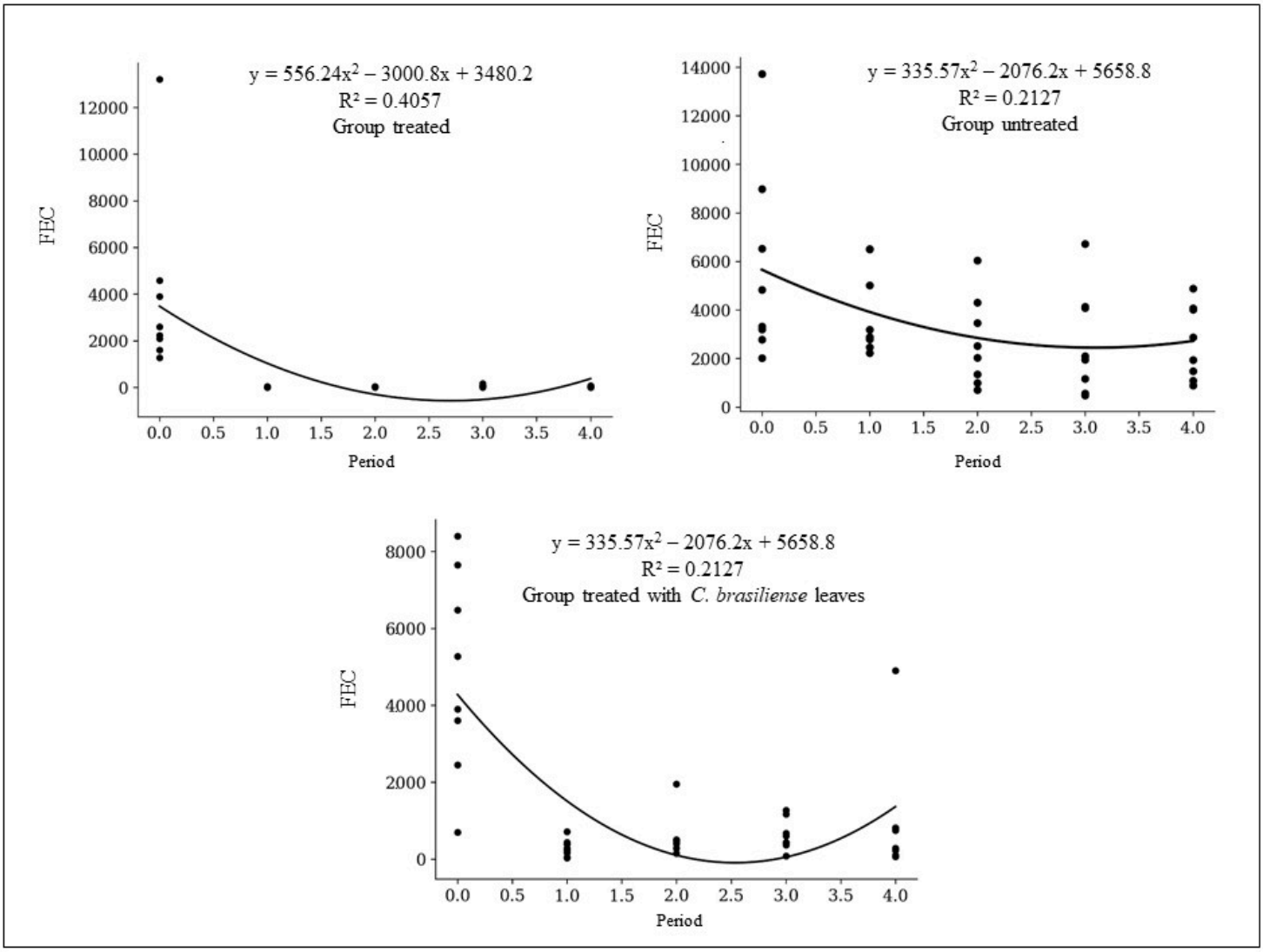

Figure 5

Polynomial regression analysis showing fecal egg count dynamics in three sheep groups: treated, untreated, and animals treated with *Caryocar brasiliense* leaves

Supplementary Files

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- Table2.docx
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