Evaluation of the anthelmintic activity and toxicity of an aqueous extract of *Chenopodium ambrosioides* in goats*

Gisele Dias da Silva[1], Mariana Borges Botura[2], Hélimar Gonçalves de Lima[3], João Victor Alvaia de Oliveira[4], Eduardo Luiz Trindade Moreira[5], Francianne Oliveira Santos[6], Thiago Sampaio de Souza[7], Maria Angela Ornelas de Almeida[8] and Maria José Moreira Batatinha[9+]

ABSTRACT. da Silva G.D., Botura M.B., de Lima H.G., de Oliveira J.V.A., Moreira E.L.T., Santos F.O., de Souza T.S., de Almeida M.A.O. & Batatinha M.J.M. **Evaluation of the anthelmintic activity and toxicity of an aqueous extract of *Chenopodium ambrosioides* in goats.** [Avaliação da atividade anti-helmíntica e toxicidade do extrato aquoso de *Chenopodium ambrosioides* em caprinos.] *Revista Brasileira de Medicina Veterinária, 38(Supl.1):156-162, 2016*. Programa de Pós-Graduação em Ciência Animal nos Trópicos, Universidade Federal da Bahia, Av. Ademar de Barros, 500, Ondina, Salvador, BA 40170-110, Brasil. E-mail: mjmb@ufba.br

The objective of this study was to evaluate the anthelmintic activity of an aqueous extract (AE) from *Chenopodium ambrosioides* on goat gastrointestinal nematodes (GINs) and its toxic effects. The anthelmintic activity *in vitro* was investigated using the inhibition of egg hatching assay (EHA), while cytotoxicity on Vero cells was evaluated using the MTT test. *In vivo*, thirty goats that were naturally infected with GINs were divided into three groups: group I, treated with a daily dose of AE *C. ambrosioides* (700mg/kg) for eight days; group II (positive control), treated with a single dose of levamisole phosphate (6.3mg/kg); and Group III, untreated (negative control). Treatment efficacy was assessed on the basis of egg counts (FEC), faecal cultures and post-mortem worm burden counts. Clinical and laboratory evaluations were performed to detect toxic effects associated with treatment. In the EHA, the EC_{50} and EC_{90} corresponded to 1.6 and 1.9mg/mL, respectively. The AE promoted a slight reduction in cell viability in the cytotoxicity test. The AE reduced ($p < 0.05$) the number of infective larvae of the genera *Haemonchus* and *Oesophagostomum*. The anthelmintic treatment of goats with AE *C.ambrosioides* resulted in moderate efficacy against infective larvae, but revealed neither ovicidal nor toxic activity towards adult nematodes. No toxic effects of this plant-derived treatment were observed in the animals.

KEY WORDS. Anthelmintic, *Chenopodium ambrosioides*, herbal, ruminants, toxicity.

*Received on March 21, 2016.
Accepted for publication on May 9, 2016.

[1] Médica-veterinária, MSc, Departamento de Anatomia, Patologia e Clínicas (DAPC), Escola de Medicina Veterinária e Zootecnia (EMVZ), Universidade Federal da Bahia (UFBA), Av. Adhemar de Barros, 500, Salvador, BA 40170-110. E-mail: gdsilva05@hotmail.com

[2] Médica-veterinária, DSc, Departamento de Saúde, Universidade Estadual de Feira de Santana, Av. Transnordestina, s/n, Novo Horizonte, Feira de Santana, BA 44036-900. E-mail: mbbotura@hotmail.com

[3] Médica-veterinária, MSc. Programa de Pós-Graduação em Ciência Animal, EMVZ, UFBA, Av. Adhemar de Barros, 500, Salvador, BA 40170-110. E-mail: helima_vet@hotmail.com - bolsista CAPES.

[4] Médico-veterinário, DAPC, EMVZ, UFBA, Av. Adhemar de Barros, 500, Salvador, BA 40170-110. E-mail: vitoralvaia@hotmail.com

[5] Médico-veterinário, DSc, DAPC, EMVZ, UFBA, Av. Adhemar de Barros, 500, Salvador, BA 40170-110. E-mail: eltm@ufba.br

[6] Médica-veterinária, MSc. Programa de Pós-Graduação em Ciência Animal, EMVZ, UFBA, Av. Adhemar de Barros, 500, Salvador, BA 40170-110. E-mail: francy_medvet@yahoo.com.br - bolsista FAPESB.

[7] Médico-veterinário, DSc, Hospital Universitário de Medicina Veterinária, Universidade Federal do Recôncavo da Bahia, Av. Rui Barbosa, 710, Cruz das Almas, BA 44380-000. E-mail: tss123@gmail.com

[8] Médica-veterinária, DSc, DAPC, EMVZ, UFBA, Av. Adhemar de Barros, 500, Salvador, BA 40170-110. E-mail: aornelas@gmail.com

[9] Médica-veterinária, DSc, DAPC, EMVZ, UFBA, Av. Adhemar de Barros, 500, Salvador, BA 40170-110. +Author for correspondence, E-mail: mjmb@ufba.br

RESUMO. O objetivo deste estudo foi avaliar a atividade anti-helmíntica do extrato aquoso (EA) de *Chenopodium ambrosioides* sobre nematoides gastrintestinais de caprinos (NGIs) e seus efeitos tóxicos. A atividade anti-helmíntica *in vitro* foi investigada utilizando o teste de inibição da eclosão dos ovos (EHA), ao passo que a citotoxicidade em células Vero foi avaliada utilizando o teste de MTT. *In vivo*, trinta caprinos, naturalmente infectados com NGIs foram divididos em três grupos: grupo I, tratado com uma dose diária do EA de *C. ambrosioides* (700mg/kg) durante oito dias; o grupo II (controle positivo), tratados com uma única dose de fosfato de levamisol (6,3mg/kg); e grupo III, não tratados (controle negativo). A eficácia do tratamento foi avaliada com base na contagem de ovos (OPG), coprocultura e contagem da carga parasitária post-mortem. As avaliações clínicas e laboratoriais foram realizadas para detectar efeitos tóxicos associados com o tratamento. No EHA, a CE_{50} e CE_{90} correspondeu a 1,6 e 1,9mg/mL, respectivamente. O EA promoveu uma pequena redução da viabilidade celular no teste de citotoxicidade. O EA reduziu ($p < 0,05$) o número de larvas infectantes dos gêneros *Haemonchus* e *Oesophagostomum*. O tratamento anti-helmíntico de caprinos com o EA de *C. ambrosioides* resultou em moderada eficácia contra larvas infectantes, mas não revelou atividade ovicida nem tóxica para nematoides adultos. Nenhum efeito tóxico foi observado nos animais tratados com esta planta.

PALAVRAS-CHAVE. Anti-helmíntico, ruminantes, *Chenopodium ambrosioides*, plantas, toxidez.

INTRODUCTION

Gastrointestinal parasites restrict the production of small ruminants, resulting in low productivity due to reduced feed consumption, weight loss, decreased fertility, decreased milk production and higher mortality rates (Lima et al. 2010).

Alternative control measures are being investigated in an effort to decrease the development of nematodes that are resistant to anthelmintics, and these measures include the use of medicinal plants (Camurça-Vasconcelos et al. 2005).

Chenopodium ambrosioides, commonly known as mastruz, belongs to the family Chenopodiaceae. Its medicinal properties include leishmanicidal (Patrício et al. 2008) and anthelmintic activities (Almeida et al. 2007). *In vitro,* the anthelmintic efficacy of *C. ambrosioides* on GINs in goats has been demonstrated by using the essential oil (Ketzis et al. 2002) and the aqueous extract (Almeida et al. 2007) of the plant. Ascaridol is the main component of the essential oil, and many of the medicinal properties of the plant are attributed to this component. However, MacDonald et al. (2004) described the nematicidal action of the leaf infusion of *C. ambrosioides* against *Caenorhabditis elegans* and suggested that the use of the infusion as an anthelmintic for mammals is safer than the essential oil, because toxic effects were observed in mammals exposed to ascaridol. The goal of the present study was therefore to assess both the *in vitro* and *in vivo* efficacy of the aqueous extract of *C. ambrosioides* on GINs in goats and the possible toxic effects in Vero cell culture.

MATERIALS AND METHODS

The experimental protocol was approved by the Ethics Committee for the Use of Animals at the School of Veterinary Medicine, Federal University of Bahia (protocol # 05/2009).

The leaves of *C. ambrosioides* were collected in the municipality of Senhor do Bonfim in the State of Bahia, Brazil. Plant identification was performed at the Laboratory of Botany of the Bahia Agricultural Development Company, Brazil (number 548). For the preparation of the aqueous extract (AE), 8kg of dry leaves were obtained from plants grown at room temperature and were triturated in a mixer apparatus. Sixty-four litres of distilled water (the quantity required to achieve saturation) ware added, and the mixture was subjected to mechanical mixing for a period of 24h, followed by filtration. A total of 40L of the filtrate was obtained, which was lyophilised aliquot of 200mL for use in *in vitro* assays. The concentration of the extract was 24.5mg/mL, which was determined by taking the average of the dry weight of three 1mL aliquots.

For the evaluation of *in vitro* ovicidal activity of AE of *C. ambrosioides,* the hatching eggs test (EHA) was employed (Coles et al. 1992). Fecal samples were collected from goats naturally infected with GINs, with 81% of *Haemonchus* spp., 14% *Oesophagostomum* and 5% of *Trichostrogylus* spp. in their faecal culture. Recovery of eggs was performed as described by Hubert & Kerbeoeuf (1992), using 10g of feces homogenised with distilled water and filtered through 1mm, 100, 55 and 25μm sieves. The egg suspension was distributed in 96-multiwell plates (100eggs/100μL/well), and equal volumes of extract, diluted in distilled water, were added at concentrations of 4.0, 2.0, 1.0, 0.5 and 0.25mg/mL. In each plate, negative and positive controls were included, containing distilled water and albendazole (0.025mg/mL), respectively. After incubating the plates in an oven B.O.D. at 24ºC for 48 hours, Lugol's iodine solution was added to stop the reaction, and all eggs and larvae (L1) in each well were counted.

In the cytotoxicity assay, Vero cells culture was maintained in RPMI (Roswell Park Memorial Institute) buffer, supplemented with 10% fetal equine serum and antibiotics (100IU/mL penicillin G, 100mg/mL strepto-

mycin) at 37°C in a CO_2 incubator. The cells were distributed into 96-well plates (3.5 x 10^4cells/well). After 24 hours of incubation at 37°C and 5% CO_2, the culture medium was removed and replaced with AE of *C. ambrosioides* diluted in RPMI medium at the following concentrations: 4.0, 2.0, 1.0, 05 and 0.25mg/mL. In control wells, only untreated cells were used. After 24 hours of treatment, the cell viability was assessed by testing the solution of 3- (4,5-dimethylthiazol-2yl)-2,5-difenitetrzólio (MTT) according to Hansen et al. (1989). The results are presented as the percentage of cell viability (mean and standard deviation) compared to the control, which was considered to be 100%. Three replications with five replicates for each concentration and controls were performed in the *in vitro* assays.

In vivo, a total of thirty goats of both sexes, mixed breed, aged between 6 and 18 months and weighing 11-27 kg, who had become naturally infected with GINs were used for the experiments outlined below. These animals were obtained from the Municipality of Senhor do Bonfim, Bahia, Brazil, where they were kept in a semi-intensive system and received no anthelmintic treatment during the period of 60 days prior to the beginning of the experiment. The experiment was conducted at the Centro de Desenvolvimento da Pecuária, Bahia, Brazil, and the animals were held for 22 consecutive days in an area with a concrete floor. Grass hay, water and mineral salt were provided *ad libitum.*

The goats were divided into three homogeneous groups (n = 10) according to the number of eggs per gram of feces (EPG). The mean weights of the animals in groups I, II and III were 18 ± 4.2, 19.3 ± 3.4 and 18.1 ± 5.0, respectively. Group I was treated once daily for eight days with doses of the AE of *C. ambrosioides* (700mg/kg/day). Group II (the positive control group) was treated with a single dose of levamisole phosphate (6.3mg/kg). This active principle showed better results among chemical groups tested. Group III (the negative control group) was not subjected to any treatment. The AE was administered orally by gavage.

The animals were subjected to clinical examination daily, and the following parameters were recorded: general condition, lymph nodes, color of the ocular mucosa, body temperature, heart and respiratory rates, and rumeinal motility. Weight recordings were performed on days 0 and 9 of the experimental period.

Fecal samples were collected directly from the rectum of each animal on days 0, 5 and 9 of the trial period to determine the fecal egg count (FEC) (Gordon & Whitlock 1939). The different genera of nematodes were identified by the larvae that were present in the fecal cultures (Roberts & O'Sullivan 1950). Fecal cultures were conducted individually on days 0 and 9 of the experimental period.

Blood samples were collected from the jugular vein of each animal on days 0 and 9 of the experimental period and stored in vacuum tubes containing ethylenediamine tetra-acetic acid (EDTA) in order to generate haemograms and determine total plasma protein using refractometry (JAIN, 1993). The enzymatic activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), alkaline phosphatase, urea and creatinine were measured in the serum samples using commercial kits (Doles ®) and spectrophotometry.

One week after the end of the treatment, six animals from each group were randomly selected and euthanized. After a macroscopic examination of the organs, fragments of the liver, kidney, abomasum and intestine were analysed histopathologically. These fragments were fixed in formalin (10%) and processed using a paraffin-embedding technique (PROPHET et al., 1992). Five-millimetre histological sections were stained with haematoxylin-eosin (Luna 1968). Aliquots containing 10% of the contents of the abomasum and small intestine were collected from each animal. The number of the nematodes larvae (L4 and L5), which were categorised according to the genus, was multiplied by ten. The contents of the large intestine were examined completely (Ueno & Gonçalves 1998). The identification of GIN species were performed according to the method proposed by Soulsby (1982).

The results obtained from the hatching of eggs and cytotoxicity tests were analysed by ANOVA followed by Tukey's test with a significance level of 5%. The EC_{50} and EC_{90} were calculated by nonlinear regression analysis using the GraphPrism program (version 5.0).

The anthelmintic efficacy *in vivo* of each treatment was determined using the formula: PR = 100 (1 - T / C), where PR represents the percentage of reduction in the number of eggs or larvae and T and C represent the arithmetic means of the number of eggs or larvae in treated animals and animals in the negative control group, respectively (Coles et al. 1992). Body weight and the results of the haematological and biochemical analyses were compared using an ANOVA, followed by a post-hoc analysis using Tukey's test. Parameters that were not normally distributed, including eggs, larvae, basophils, eosinophils, band neutrophils and monocytes were analysed using the non-parametric Kruskal-Wallis test, followed by Dunn's multiple comparison test. The results were processed using SAS statistical software (version 9.1), and the significance level was 5%.

RESULTS

The AE of *C. ambrosioides* inhibited the hatching of GIN eggs in goats in a concentration-dependent manner. The mean percentage of inhibition varied between 8.7 and 100%. Greater than 90% efficacy was observed at the concentration of 4mg/mL and the positive control did not differ (albendazole, 0.025mg/mL) (Figure 1). The EC_{50} and EC_{90} for this extract corresponded to 1.6 and 1.9mg/mL, respectively.

The AE of *C. ambrosioides* exhibited moderate toxicity to cultures of Vero cells in the MTT assay, with percentages of cell viability between 60.84 and 80.91%, which differed significantly ($p < 0.05$) from the control group (96.24%) (Figure 2).

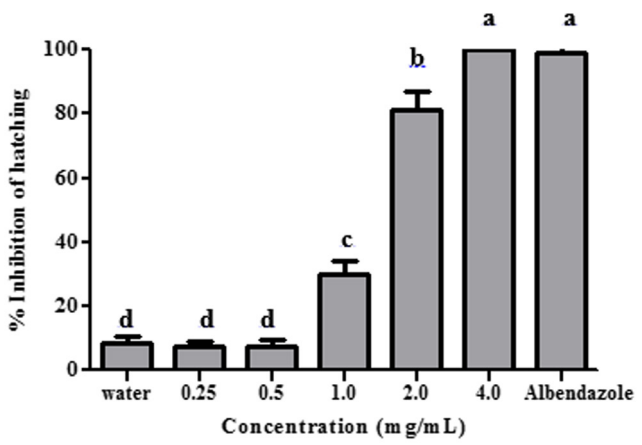

Figure 1. Inhibition of gastrointestinal nematode egg hatching following treatment with AE from *Chenopodium ambrosioides* and albendazole.

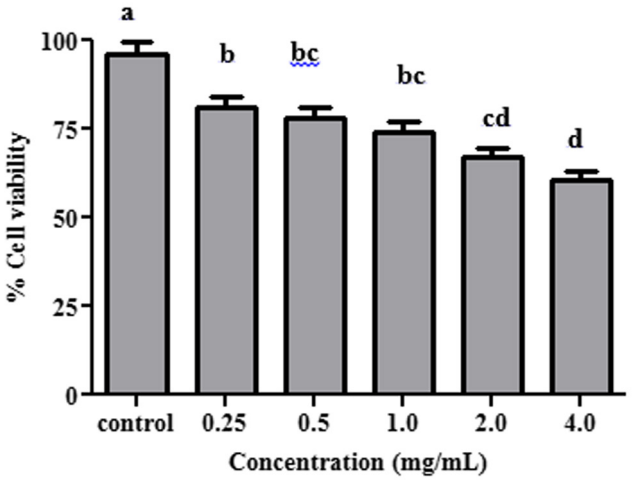

Figure 2. Percent cell viability following treatment with AE *Chenopodium ambrosioides.*

No reductions in EPG were observed in the goats treated with the AE of *C. ambrosioides* (group I), while a significant decrease in the EPG was observed on the fifth and ninth days of treatment ($p < 0.05$) in the animals treated with levamisole (GII) when compared to the negative control group (G III), as shown in Table 1.

The number of infective larvae (L3) obtained from the faecal cultures of goats treated with either the AE (83,89%) or levamisole (85,63%) was significantly decreased ($p < 0.05$) when compared with the control group (GIII), with the exception of the genera *Trichostrongylus* (59,23 and 58,58%, respectively). In group I, the percentage reduction of the number of L3 larvae was greater for genus *Oesophagostomum* (90.15).

The number of *T. colubriformis* in goats treated with the AE was reduced (42.44%), although the reduction was not statistically significant when compared with the results of the negative control group. The group treated with levamisole differed from the other groups, however, in the percentage of reduction of number of *H. contortus, O. columbianum* and *T. colubriformis*. The percentage of reduction of each genus was less than 90% (Table 2).

We did not observe any clinical changes in the animals. All clinical parameters were within normal ranges: the rectal temperature ranged from 37 to 38.2° C, the cardiac frequency was between 60 and 92 beats per minute, the respiratory frequency ranged 14 to 19 movements per minute, and the ruminal movements were measured at 1-3 per two minutes.

The erythrocyte count increased significantly in group II ($p < 0.05$) after treatment ($13{,}51 \times 10^6/\mu L \pm 1{,}73$ to $15{,}89 \times 10^6/\mu L \pm 2{,}40$). In group I, there was a significant increase ($p < 0.05$) in leukocyte count after nine days ($10{,}73 \times 10^3/\mu L \pm 1{,}81$ to $15{,}35 \times 10^3/\mu L \pm 4{,}13$). The counts of monocytes ($0{,}37 \times 10^3/\mu L \pm 0{,}37$ to $0{,}6 \times 10^3/\mu L \pm 0{,}16$) and segmented neutrophils ($5{,}33 \times 10^3/\mu L \pm 2{,}94$ to $7{,}8 \times 10^3/\mu L \pm 1{,}85$) were increased significantly in the control group (GIII) after the treatment period. Significant differences ($p < 0.05$) were observed in haemoglobin levels and lymphocyte counts among the groups after nine days.

In groups I (91,29UI/L±33,56 to 61,16UI/L±22,33) and III (48,32UI/L±16,68 to 41,23UI/L±10,63), a significant decrease ($p < 0.05$) in the levels of the enzyme GGT was observed after treatment. In addition, Group I also displayed significant differences in AST (51,96UI/L±13,02 to 34,22UI/L±7,09) and creatinine (0,93mg/dL±0,11 to 1,06mg/dL±0,11) on day nine of the experimental period. The urea content (50,25mg/dL±11,77 to 35,46mg/dL±15,91) decreased significantly ($p < 0.05$) after treatment with levamisole (GII). On day zero, the values of AST in group I were significantly ($p < 0.05$) higher than those in the other groups. Differences in the levels of the GGT enzy-

Table 1. Number of eggs of gastrintestinal nematodes of goats treated with aqueous extract of *C. ambrosioides* and levamisole.

Groups	Days of treatment				
	0	5		9	
	EPG	EPG	%[k]	EPG	%
GI	2700±2186,96[Aa]	3605±4141,49[Aa]	0	3310±2829,59[Aa]	0
GII	2077,78±1118,07[Aa]	138,89±167,29[Bb]	93,56	416,67±612,88[Bb]	87,14
GIII	2650±1885,32[Aa]	2155±1394,72[Aa]	-	3240±2137,73 [Aa]	-

Capital letters compare values between groups and small letters compare values (Means and standard desviation) between days by group ($p < 0.05$), GI: 700mg of aqueous extract of *C. ambrosioides*/kg BW/8dias; GII: levamisole phosphate (6.3 mg /kg BW single dose); GIII no treatment; kpercent reduction

Table 2. Number of gastrintestinal nematode larvae (L4 and L5) recovered from goats after aqueous extract of *Chenopodium ambrosioides* and levamisole.

Species	GI		GII		GIII
	Larvae L_4 and L_5	%k	Larvae L_4 and L_5	%	Larvae L_4 and L_5
Haemonchus contortus	278±137,46[a]	0	30±44,72[b]	88	250±224,77[a]
Oesophagostomum columbianum	87,5±62,55[a]	0	17,67±19,84[b]	79,46	86±63,75[a]
Trichostrongylus colubriformis	197±94,16[a]	42,44	128±83,29[a]	62,44	342±278,53[a]
Total	562,5±183,49[a]	16,99	176±112,13[b]	74,03	677,67±354,90[a]

Capital letters compare values between groups and small letters compare values (Means and standard deviation) between days by group ($p < 0.05$), GI: 700mg of aqueous extract of *C. ambrosioides*/kg BW/8dias; GII: levamisole phosphate (6.3 mg /kg BW single dose); GIII no treatment; [k] percent reduction

me among the groups were observed on days zero and nine of the experimental period.

The macroscopic findings observed in all groups consisted of pale mucous membranes, edematous superficial lymph nodes, acute and subacute abomasitis, haemorrhagic and ulcerative enteritis, and calcified nodules of *Oesophagostomum* in the intestines.

During the microscopic analysis, kidney changes, which were characterised by lymphocytic interstitial nephritis, were observed in two animals treated with the AE of *C. ambrosioides* (Group I). Two of the animals in group III (the negative control group) had mild, diffuse steatosis, and one animal in this group had cystic dilatation of the cortical tubular structures. Animals treated with levamisole (group II) showed no kidney changes. In the liver, mild to moderate diffuse steatosis and lymphocytic hepatitis was observed in all groups. Haemorrhagic foci or focal haemorrhage was observed in two animals in groups II and III and one animal in group I. We also found mild hepatic septal fibrosis in one animal from group I, telangiectasia and atrophy of the liver epithelial cords in two animals from group II, and apoptotic hepatocytes and focal cholangitis in two animals from group III. In the intestine, acute ulcerative enteritis was observed in animals from group II (two animals) and group I (one animal), and granulomatous enteritis was observed in one animal from group I. In each group, at least one animal had multifocal lymphocytic abomasite, with four animals from group I suffering from this condition.

DISCUSSION

The AE of *C. ambrosioides* (EC_{90} 1.9mg/mL) proved to be efficacious with GIN eggs, but not with the larval stage (L3) of GINs in goats. At the highest concentration used (4mg/mL), this extract produced a 100% inhibition of eggs hatching; identical results were also found by Almeida et al. (2007) when using a concentration 27 times higher (110.6mg/mL). The variation in these percentages may be due to the difference between the methodologies. In the present study, the extract was applied directly to the suspension of eggs, explaining the higher sensitivity and precision of this technique, while Almeida et al. (2007) applied the aqueous extract in fecal culture.

In previous studies, the aqueous/methanol extract (70:30) of *C. album* showed an inhibitory effect on the hatching of eggs with an EC_{50} of 0.45mg/mL, and this action was likely due to a hydrophilic component or to a low polar extract (Jabbar et al. 2007). However, the anthelmintic activity of *C. ambrosioides* has been attributed to a lipophilic compound (ascaridol). MacDonald et al. (2004) suggested that there are hydrophilic components present in this plant, which also may be related to this activity.

Nematode hatching is initiated by a signal from the environment, which stimulates the embryo to secrete enzymes such as proteases, lipases, and chitinases that degrade the egg membrane (Young et al. 1999). In this sense, it is important to investigate whether constituents present in the AE of *C. ambrosioides* could be acting as inhibitors of these enzymes, which would explain the ovicidal activity.

The AE of *C. ambrosioides* exhibited low toxicity to Vero cells. This is important because it increases the chances that this product can be used safely in animals. However, other studies of cytotoxicity with extracts of this plant have shown different results. The methanol extract of *C. ambrosioides* did not induced a toxic effect on a human hepatocellular carcinoma line at concentrations from 15.5 to 1000g/mL (Ruffa et al. 2002). However, toxicity of AE of *C. ambrosioides* was demonstrated on cultured human lymphocytes (Gadano et al. 2007). The essential oil of *C. ambrosioides* and some components of this oil (ascaridol, carvacrol and caryophyllene

oxide) exhibited toxicity a culture of mouse macrophages, and the mechanism of cytotoxic action was discerned to be an inhibition of respiratory function in the mitochondria within the cells (Monzote et al. 2009).

Treatment of goats with the AE of *C. ambrosioides* had no effect on the number of eggs, and only resulted in a partial effect (83.89%) on L3 larvae and a reduced effect (16.99%) on (L4 e L5) adult nematodes. These results demonstrate the low efficacy of the AE of *C. ambrosioides* on the control of GINs because a product having anthelmintic action is considered effective when the rate of reduction is greater than or equal to 90% (Vercruysse et al. 2001). However, the extract reduced the number of L3 of the genus *Oesophagostomum* by more than 90% (90.15%) and reduced the number of *Haemonchus* by approximately 80%, which can help to reduce the number of infective larvae (L3) in pastures.

The reduced anthelmintic efficacy of the AE of *C. ambrosioides in vivo* may be attributed to the action of ruminal microorganisms, which act to decrease the availability of active chemical constituents (Vandamme & Ellis 2004).

The differences observed between the *in vitro* and *in vivo* studies can be explained by the fact that in *in vitro* assays, the extracts are in direct contact with the parasites. In addition, the concentrations of potentially active substances contained in the extract do not always correspond to the *in vivo* bioavailability of these substances (Githiori et al. 2006). Reports of the degradation of chemical compounds from plants by bacteria in the bovine rumen (Krumholz et al. 1986) have also been described.

The low *in vivo* efficacy of *C. ambrosioides* was also verified by Vieira et al. (1999). *H. contortus* infected goats were treated with a single dose of 0.75g/kg of the leaves and seeds of the plant in the form of a juice. Reductions of only 33.6% in the egg counts in the faeces, and 9.6% of the number of nematodes recovered in the digestive tract of these animals were observed. Goats treated with fresh leaves of the plant over the course of five days, did not show a reduction in the EPG or of the number of *H. contortus* (Ketzis et al. 2002).

The oral treatment of goats with the AE of *C. ambrosioides* resulted in no clinical changes. Some of the haematological parameters evaluated in this study showed variations after treatment. However, these were within the reference values for the species (Bezerra et al. 2008).

The lowest hemoglobin concentration evident in groups I and III after treatment may also be attributed to the parasitic infection, mainly as a result of blood loss caused by the action of *H. contortus*. According to Mir et al. (2007), lambs infected with *H. contortus* have reduced hemoglobin concentrations, haematocrit and numbers of erythrocytes.

The activities of AST, ALT, alkaline phosphatase, and GGT, and the concentrations of urea and creatinine remained within the reference values ranges for the species (Tucci et al. 1989, Silva et al. 2003, 2004). For these parameters, factors such as climate, management system, breed, sex and age can contribute to serum the variations, as observed in this study (Silva et al. 2004).

The significant differences observed in GGT values in groups I and III before and after treatment, as well as those observed between groups, can be attributed to the increased activity of this enzyme at the beginning of the experiment. Similar changes in the values of the AST enzyme were observed in group I, which were significantly increased in relation to other groups before treatment, as well as in the same group on the ninth day of treatment. Although the concentration of creatinine was slightly higher in group I post-treatment, this concentration remained within the reference range and was not significantly different from that of the other groups. Liver and kidney lesions observed during histopathologic analysis in all groups suggested that previously existing factors, which were not explored in this study, may have interfered with the biochemical parameters.

The lesions observed in the gastrointestinal tract of animals during necropsy are consistent with GINs infection and do not suggest signs of plant extract induced toxicity (Vieira et al. 2009).

CONCLUSION

The AE of *C. ambrosioides* has high ovicidal effect on GINs from goats *in vitro*, and has low toxicity to Vero cells. *In vivo* the aqueous extract of *C. ambrosioides* was not effective against the egg and adult stages of the genera *Haemonchus, Oesophagostomum* and *Trichostrongylus*. However, moderate efficacy was found for third stage larvae of the genera *Oesophagostomum* and *Haemonchus*. Signs of toxicity were not observed in goats treated with the aqueous extract of *C. ambrosioides*.

Acknowledgements. The authors would like to thank the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and the Programa de Pós-graduação em Ciência Animal nos Trópicos da Universidade Federal da Bahia for financial support.

REFERENCES

Almeida M.A.O., Domingues L.F., Almeida G.N., Simas M.M., Botura M.B., Cruz A.C.F.G., Silva A.V.A.F., Menezes T.P. & Batatinha M.J.M. Efeitos dos extratos aquosos de folhas de *Mentha piperita* L. e de *Chenopodium ambrosiodes* L. sobre cultivos de larvas infectantes de nematódeos gastrintestinais de caprinos. *Revista Brasileira de Parasitologia Veterinária,* 16:57-59, 2007.

Bezerra L.R., Ferreira A.F., Camboim E.K.A., Justiniano S.V., Machado P.C.R. & Gomes B.B. Perfil hematológico de cabras clinicamente sadias criadas no Cariri Paraibano. *Ciência Agrotécnica,* 32:955-960, 2008.

Camurça-Vasconcelos A.L.F., Morais S.M., Santos L.F.L., Rocha M.F. G. & Bevilaqua C.M.L. Validação de plantas medicinais com atividade anti-helmíntica. *Revista Brasileira de Plantas Medicinais,* 7:97-106, 2005.

Coles G.C., Bauer F.H.M., Borgsteede S., Greetrs S., Klei M.A. & Waller P.J. World association for the advancement of veterinary parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology,* 44:35-44, 1992.

Gadano A., Gurni A. & Carballo M.A. Herbal medicines: Cytotoxic Effects of Chenopodiaceae species used in Argentine folk medicine. *Pharmaceutical Biology,* 45:217-222, 2007.

Githiori J.B., Athanasiadou S. & Thamsborg S.M. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Veterinary Parasitology* 139:308-320, 2006.

Gordon H. & Whitlock H.V. A new technique for counting nematode eggs in sheep faeces. *Journal of the Council for Scientific and Industrial Research,* 2:50-52, 1939.

Hansen M.B., Nielsen S.E. & Berg K. Re-examination, and further development of a precise and rapid dye method for measuring cell growth/cell kill. *Journal of Immunological Methods,* 119:203-210, 1989.

Hurbert J. & Kerboeuf D. A microlarval development assay for the detection of anthelmintic resistance in sheep nematodes. *Veterinary Record,* 130:442-446, 1992.

Jabbar A., Zaman M.A., Iqbal Z., Yaseen M. & Shamim A. Anthelmintic activity of *Chenopodium album* (L.) and *Caesalpinia crista* (L.) against *Trichostrongylid* nematodes of sheep. *Journal of Ethnopharmacology,* 114:86-91, 2007.

Jain N.C. *Essentials of Veterinary Hematology*. Lea and Febiger, Philadelphia, 1993.

Ketzis J.K., Taylor A., Bowman D.D., Brown D.L., Warnick L.D. & Erb H.N. *Chenopodium ambrosioides* and its essential oil as treatments for *Haemonchus contortus* and mixed adult-nematode infections in goats. *Small Ruminant Research*, 44:193-200, 2002.

Krumholz L.R., Crawford R.L., Hemling M.E. & Bryant M.P. A rumen bacteium degrading quercetin and trihydroxybenzenoids with concurrent use to formate or H_2. *Progress in Clinical and Biological Research,* 213:211-214, 1986.

Lima W.C., Athayde A.C.R., Medeiros G.R., Lima D.A.S.D., Borburema J.B., Santos E.M., Vilela V.L.R. & Azevedo S.S. Nematoides resistentes a alguns anti-helmínticos em rebanhos caprinos no Cariri Paraibano. *Pesquisa Veterinária Brasileira,* 30:1003-1009, 2010.

Luna L.G. (Ed.), *Manual of the Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. McGraw Hill, New York, 1968.

MacDonald D., Vancrey K., Harrison P., Rangachari P.K., Rosenfeld J., Warren C. & Sorger G. Asacaridole-less infusions of *Chenopodium ambrosioides* contain a nematocide(s) that is(are) not toxic to mammalian smooth muscle. *Journal of Ethnopharmacology,* 92:215-221, 2004.

Mir R.A., Chishti M.Z., Zarger M.A., Tak H. & Ganie S.A. Clinicopathological changes in sheep experimentally infected with *Haemonchus contortus*. *World Journal of Agricultural Sciences,* 3:562-566, 2007.

Monzote L., Stamberg W., Staniek K. & Gille L. Toxic effects of carvacrol, caryophyllene oxide, and ascaridole from essential oil of *Chenopodium ambrosioides* on mitochondria. *Toxicology and Applied Pharmacology,* 240:337-347, 2009.

Patrício F.J., Costa G.C., Pereira P.V.S., Aragão-Filho W.C., Sousa S.M., Frazão J.B., Pereira W.S., Maciel M.C.G., Silva L.A., Amaral F.M.M., Rebêlo J.M.M., Guerra R.N.M., Ribeiro M.N.S. & Nascimento F.R.R. Efficacy of the intralesional treatment with *Chenopodium ambrosioides* in the murine infection by *Leishmania amazonensis*. *Journal of Ethnopharmacology,* 115:313-319, 2008.

Prophet E.B., Mills B., Arrington J.B. & Sobin L.H. *AFIP Laboratory Methods in Histoteccnology*. *American Registry of Pathology*, Washington, 1992.

Roberts F.H.S. & O'Sullivan J.P. Methods for egg counts and larval cultures for strongyles infesting the gastrointestinal tract of cattle. *Australian Journal of Agriculture Research,* 1:99-102, 1950.

Ruffa M.J., Ferraro G., Wagner M.L., Calcagno M.L., Campos R.H. & Cavallaro L. Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. *Journal of Ethnopharmacology,* 79:335-339, 2002.

Silva S.L., Fagliari J.J. & Cesco F.T.R.S. Atividade sérica das enzimas AST, ALP e GGT de caprinos das raças Anglo-Nubiana e Saanen criados nos Estados de São Paulo e Paraíba. *Ars Veterinária,* 20:22-27, 2004.

Silva S.L., Fagliari J.J. & Cesco F.T.R.S. Concentrações séricas de cálcio, fósforo, magnésio, bilirrubinas, uréria e creatinina de caprinos das raças Anglo-Nubiana e Saanen criados nos Estados de São Paulo e Paraíba. *Ars Veterinária,* 19:87-95, 2003.

Soulsby E.J.L. *Helminths, arthropods and protozoa of domesticated animals*. 7th ed. Bailliere Tindall, London, 1982.

Tucci T.V., D'Angelino J.L., Ishizuka M.M., Birgel E.H. & Ribeiro L. Estudo comparativo dos valores normais das provas de função hepática em caprinos das raças Saanen, Parda Alpina, e mestiços do Estado de São Paulo. *Revista da Faculdade de Medicina Veterinaria e Zootecnia da USP,* 26:241-247, 1989.

Ueno H. & Gonçalves P.C. *Manual para diagnóstico das helmintoses de ruminantes*. 4th ed. JICA, Tokyo, 1998.

Vandamme T.F. & Ellis K.J. Issues and challenges in developing ruminal drug delivery systms. *Advanced Drug Delivery Reviews,* 56:1415-1436, 2004.

Vercruysse J., Holdsworth P., Letonja T., Barth D., Conder G., Hamamoto K. & Okano K. International harmonisation of anthelmintic efficacy guidelines. *Veterinary Parasitology,* 96:171-193, 2001.

Vieira L.S., Cavalcante A.C.R., Pereira M.F., Dantas L.B. & Ximenes L.J.F. Evaluation of anthelmintic efficacy of plants available in Ceará State, North-east Brazil, for the control of goat gastrointestinal nematodes. *Revue Médecine Vétérinaire,* 150:447-452, 1999.

Vieira L.S., Chagas A.C.S. & Molento M.B. Nematoides Gastrintestinais e Pulmonares de Caprinos, p.65-94. In: Cavalcante A.C.R., Vieira L.S., Chagas A.C.S. & Molento M.B. (Eds), *Doenças Parasitárias de Caprinos e Ovinos-Epidemiologia e Controle*. Embrapa Informação Tecnológica, Brasília, 2009.

Young A.R., Mancuso N. & Bowles V.M. Biochimical aspects of egg hatch in endo- and ectoparasites: potential for retional drug desing. *International Journal for Parasitology,* 29:861-867, 1999.