ORIGINAL ARTICLE

Efficiency of bitter melon (*Momordica charantia*) extract as anthelmintic in ovine from Bom Jesus, Piaui, Brazil.

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ABSTRACT: The objective of this research was to evaluate the efficiency of bitter melon (*Momordica charantia* L.) extracts as anthelmintic in naturally infected ovines in the region of Bom Jesus, Piaui, Brazil. The experiment was performed at the Federal University of Piaui, from August 2012 to July 2013. A single already lambed female ovine was used in the experiment. Leaves and branches of bitter melon were collected to obtain the extract used in the experiment. The *in vitro* effect of vegetal extracts on the development of helminthes' eggs was analyzed using a hatching test. The number of eggs *per* gram of feces observed in the experiment was of 5.200. Eggs showed typical morphological characteristics of the *Trichostrongylidea* Family. Only in samples within the control group, with distilled water, eggs with larvae and free living larvae were observed. In *in vitro* tests, the ethanolic extract from leaves and branches of bitter melon inhibited the development of eggs of gastrointestinal nematodes in the ovine, with the percentage of viable eggs decreasing as the concentration of the extract increased.
Keywords: infection, sheep, parasites, vermifuge.

RESUMO: Eficiência do extrato de melão de São Caetano (*Momordica charantia* L.) como anti-helmíntico em ovinos de from Bom Jesus, Piauí, Brasil. Objetivou-se com a pesquisa avaliar a eficácia do uso do extrato de Melão de São Caetano (*Momordica charantia* L.) como anti-helmíntico em ovinos naturalmente infectados da região de Bom Jesus, Piauí, Brasil. O experimento foi conduzido na Universidade Federal do Piauí, no período de agosto de 2012 a julho de 2013. Foi utilizada uma fêmea ovina parida. Foram coletadas folhas e ramas de Melão de São Caetano para produção do extrato utilizado no teste. O efeito *in vitro* do extrato vegetal sobre o desenvolvimento dos ovos foi analisado utilizando-se o teste de eclodibilidade. A contagem de ovos por grama de fezes do ovino utilizado no experimento foi de 5.200. Os ovos apresentavam características morfológicas típicas da Família *Trichostrongylidea*. Apenas nas amostras do grupo controle com água destilada é que foram observados ovos larvados e larvas de vida livre. O extrato etanólico de folhas e ramas do melão de São Caetano apresentou efeito na inibição de desenvolvimento de ovos de nematódeos gastrintestinais de ovinos, em testes *in vitro*, constatando-se que o percentual de ovos viáveis decresce com o aumento da concentração do extrato.
Palavras-chave: infecção, ovelha, parasitas, vermífugo.

INTRODUCTION

Sheep are used to produce high biological value nutriments such as meat and milk, in addition to enlarge farmers' incomes by trading live animals and animal skins (SILVA et al. 2010). Changes in the praxis of sheep farming in the Brazilian Northeastern region resulted in models of intensive production within reduced areas, favoring the incidence of parasites, which have a remarkable importance as limiting factors for sheep production (COSTA et al. 2011).

Due to the high number of animals raised in small areas the environmental contamination with free living stages of parasites increases. This fact results in the increment of the index of parasite

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larvae in the pastures, which consequently becomes a constant infection source (MOLENTO, 2004). In an attempt to minimize the problem with parasites several integrated control strategies are used, including the use of herbal products with anthelmintic effect (NERY et al. 2009).

In Brazil, more than 300 species of medicinal plants are used as anthelmintic, amongst them, bitter melon (*Momordica charantia* L.). This plant originated in Asia is a tropical climbing plant which is well adapted to the Brazilian climate and is very common in different region of the country, growing easily in coastal and/or planted areas and being well known by farmers due to its medicinal properties (ZURLO & MITZI, 1989; LENZI et al. 2005; CORDEIRO et al. 2010).

It is commonly used to cure skin affections as *Pityriasis versicolor*, itches, scabies and *Tinea corporis*. Nowadays new uses and applications for this plant's extracts are being studied, as in the case of pest control and disease control in agricultural crops (ZOCOLER et al. 2006).

This is a tropical vine belonging to the family Cucurbitaceae and is composed by approximately 120 genera and nearly 825 species (COUTINHO et al. 2009). According to the classification system APG II (2003), this family belongs to the order Curcubitales, with their members being found mainly in the tropics and rarely occurring in the temperate regions.

The main parts of bitter melon plant used for medicinal purposes are their leaves, branches and fruits (PEREIRA et al. 2010). Fruits, although edible, are not recommended for consumption once they can induce abortion, diarrhea, hypoglycemia and vomits. There are reports on the use of this plant as febrifuge, anthelmintic, hypotensive, hypoglycemiant, intestinal analgesic, pain reliever for malaria fever and dysentery as well as commonly used against rheumatism (GONZALES et al. 1995).

The objective of this research was to evaluate the efficiency of the bitter melon (*Momordica charantia* L.) extract as anthelmintic in naturally infested ovine in the region of Bom Jesus, Piaui, Brazil.

MATERIAL AND METHODS

The experiment was performed at the Domestic Animal's Parasitic Disease Laboratory (LDPAD), the Organic Chemistry Laboratory (LQO) and the Ovine and Goat Breading Sector from the Federal University of Piaul – UFPI, *Campus* Professor Cinobelina Elvas – CPCE, from August 2012 to July 2013.

Acquisition of eggs and helminthes

In order to obtain the helminthes' eggs to test against *Mormodica charantia* L. extracts, a four-year old lamb female was used. The female was raised in a semi-intensive system and had a parasitological status with a count of 5.200,00 eggs *per* gram of feces, according to the Gordon & Whitlock (1939) method.

Eggs observed showed typical morphological characteristics of eggs produced by nematodes from the superfamily *Strongyloidea*, Family *Trichostrongylidae*.

Approximately 10 g of feces were collected from the rectal ampulla. Samples were conditioned in plastic bags, packed at room temperature and sent to the Parasitology Laboratory at UFPI. The material was macerated in a porcelain grail containing supersaturated saline solution, filtered in sieves with mesh of 250 mn/µm (nº 60) and 180 mn/µm (nº 80). This solution was added to essay tubes (1.4 x 9.8 cm) and centrifuged at 2000 rpm for two minutes.

Then, the supernatant was transferred to another centrifuge tube and submitted to three consecutive washes with distilled water (2000 rpm/2min). In the last washing the sediment was added with a small volume of distilled clay, re-suspended and transferred to essay tubes (1.8 x 10 cm) in aliquots of 1000 µl, in order to compose a suspension with approximately 3.640 eggs per tube.

Extracts of *Momordica charantia* L.

Leaves and branches of *Momordica charantia* L. were used to produce vegetal extracts. Leaves and branches were collected in the county of Bom Jesus – PI, Brazil, at the sides of the BR 135 highway, near to the River Gurguéia. The samples of the plant were obtained following the usual methodology, being registered in the Hdelta herbarium under the number 4593

The extract was obtained at the Laboratory of Organic Chemistry from the Federal University of Piaui (UFPI), *Campus* Professor Cinobelina Elvas (CPCE). Leaves and branches were dried at room temperature for five days, before being milled and then conditioned in capped glass flasks containing ethanol.

In order to concentrate the extracts a rotary evaporator at a constant temperature of 50°C was used, obtaining a crude ethanolic extract from leaves and branches of *M. charantia* L. The crude extract was weighted and separated at concentrations of 100; 50; 25; 12,5; mg/ml (GUERRERO et al. 2012).

Larval development inhibition test

The *in vitro* effect of the vegetal extracts on the development of eggs was evaluated using a hatchability test, in order to determine the

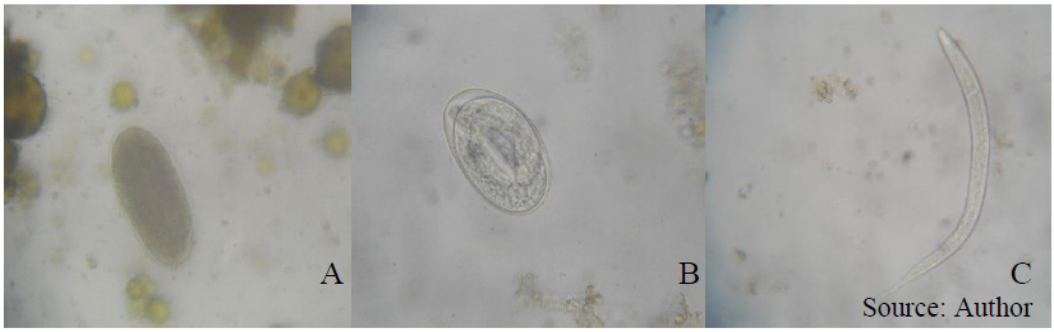

FIGURE 1. Family *Trichostrongylidea*: A- Blastomered egg, B- Larvae in egg and C- Ecloded larvae.

anthelmintic efficiency, as proposed by BATISTA et al. (1999) with modifications.

Essay tubes, in duplicate, containing 1000 µl of suspension with eggs and equal volume of vegetal extract were incubated at room temperature. After incubation periods of 24, 48 and 72 h, the material was placed in Petri dishes and observed under light microscope to classify eggs observed in the sample according to the development stage, namely: blastomered egg, showing a rounded mass formed by a great number of cells; egg with larvae, showing a small coiled moving larvae; Ecloded larvae, larvae outside of the egg, stationary or moving (Fig. 1).

The procedure was repeated with distilled water, constituting the control treatment.

RESULTS

The treatment to control eggs of gastrointestinal helminthes using ethanolic extracts from bitter melon was efficient, showing a significant reduction in the percentage of viable eggs, with an evident decreasing percentage in viability as the extract concentration increased.

In this study, the concentration of 12.5 mg/ml of extract resulted in 58.8% of viable eggs after 24 h of extract exposure and 35.5% of viable eggs 72 h after. At a concentration of 25mg/ml the percentage of viable eggs was of 42.5% after 24 h of extract exposure and 27% viable eggs after 72 h.

At a concentration of 50 mg/ml, a total of 65.0% of non-viable eggs were observed after 24 h of extract exposure and 78.5% of non-viable eggs after 72 h. At a concentration of 100 mg/ml was observed a percentage of 23.5% viable eggs after 24 h and 13.5% of viable eggs after 72 h (Table 1).

Evaluation of egg's development 24 h after incubation showed 95% of viable eggs (OVV) and 5% non-viable eggs (OVI) when treated with distilled water. Evaluation of egg's development 72 h after incubation resulted in 0% of viable eggs, 5% non-viable eggs and 95% non-viable free living larvae (LL), when treated with distilled water (Tabela 1).

Eggs with larvae and free living larvae were observed only in samples from the control group with distilled water, implying that bitter melon's extract was efficient in all samples and concentrations tested (Table 1).

Figures 2 and 3 show the percentage of trichostrongylid eggs development under effect of *M. charantia* L. extracts at different concentrations, 24 and 72 h after incubation, respectively.

The ethanolic extracts from leaves and branches of bitter melon (*Momordica charantia*

TABLE 1. Evaluation of the effect of *Momordica charantia* L. extracts on the development of trichostrongylid nematode eggs.

	24 h			48 h			72 h		
	OVV (%)	OVI (%)	LL (%)	OVV (%)	OVI (%)	LL (%)	OVV (%)	OVI (%)	LL (%)
H2O	95	5	-	50	5	45	0	5	95
12.5 mg/ml	58.8	41.2	0	46.5	53.5	0	35.5	64.5	0
25 mg/ml	42.5	57.5	0	33.5	66.5	0	27	73	0
50 mg/ml	35	65	0	25.5	74.5	0	21.5	78.5	0
100 mg/ml	23.5	76.5	0	18.3	81.7	0	13.5	86.5	0

OVV – viable eggs; OVI – non-viable eggs; LL – Free Living larvae.

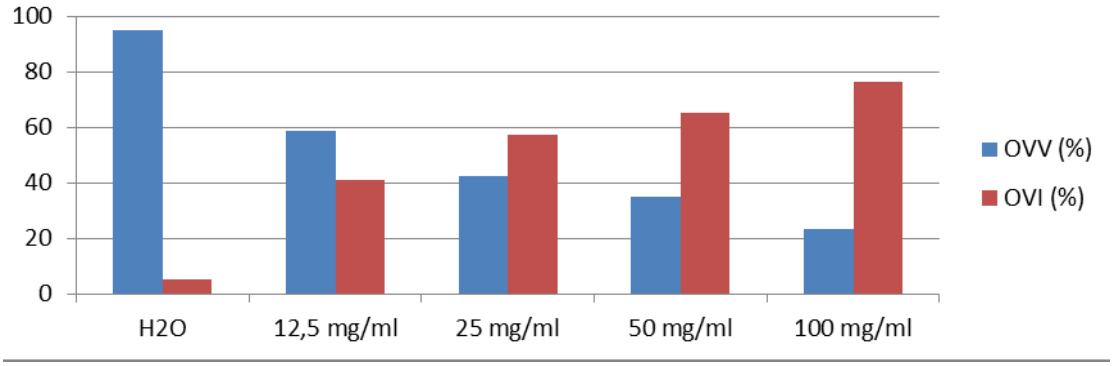

FIGURE 2. Evaluation in percentage of the development of trichostrongylid eggs under effect of *Momordica charantia* extracts at different concentrations 24 h after incubation. OVV – viable eggs; OVI – non-viable eggs.

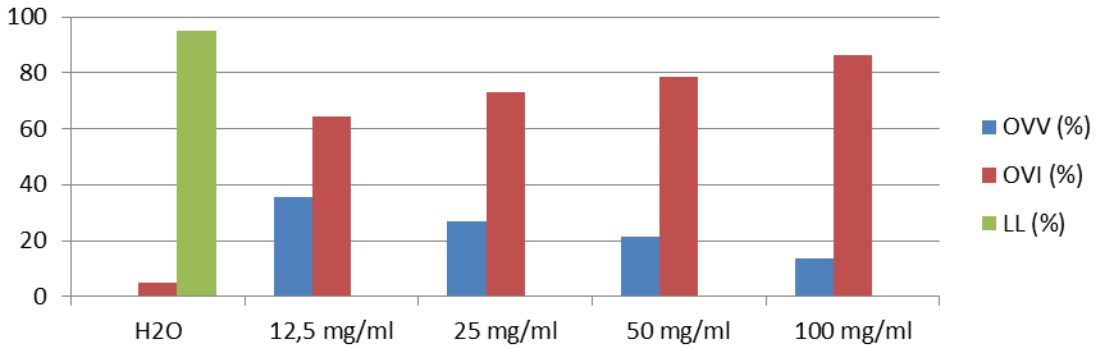

FIGURE 3. Evaluation in percentage of the development of trichostrongylid eggs under the effect of *Momordica charantia* extracts at different concentrations 72 h after incubation. OVV – viable eggs; OVI – non-viable eggs; LL – Free Living larvae.

L.) had an inhibitory effect on the development of gastrointestinal nematodes' eggs in ovine, in *in vitro* tests, verifying the percentage of viable eggs decreases with the increase of the extract concentration.

DISCUSSION

In order to any anthelmintic treatment be considered efficient, it must affect the development of eggs, preventing eclosion of larvae and larvae development, with a product inhibiting eggs at the stage of blastomere being considered as the ideal one.

Similar results were observed by Girão & Carvalho (1999), while testing *Momordica charantia* L. (bitter melon) extracts. These authors observed an inhibition of 43.0% in the eclosion of ruminant nematodes' eggs. Also, Batista et al. (1999), while studying the natural extracts from plants, demonstrated the inhibitory effects of *M. charantia* extracts over the eclosion of eggs of up to 50% for *Haemonchus contortus* obtained from sheep.

The previously mentioned results from different authors and the present study are in agreement with Sousa Gomes et al. (2009) whom, analyzing the effect of bitter melon extracts, verified the percentage of viable eggs decreased with the increase of the extract concentration. These authors used concentrations starting at 6% and verified the percentage of viable eggs decreased to 72.91%. The authors also observed that extracts concentrations of 25% and 50% were the most efficient to reduce viable eggs after 72 h, with percentages reduced in 64.53% and 62.86%, respectively.

Results in this study are supported by the studies performed by Almeida et al. (2007), when these authors evaluated the efficiency of bran and ethanolic extracts of bitter melon (*Momordica charantia* L.) leaves, Indian jalap (*Operculina hamiltoni* L.) leaves and seeds of summer squash (*Cucurbita pepo L.*) as anthelminthics, as well as Brito-Júnior (2006), whom evaluated the *in vivo* anthelmintic effect of alcoholic extracts from Indian jalap and bitter melon and observed these plants may be used alternatively to control gastrointestinal nematodes of small ruminants, reducing the number of eggs per gram of feces (OPG).

In the state of Paraíba, Brazil, Sousa Gomes et al. (2011) concluded that the utilization of

extracts form Indian jalapa and bitter melon to control gastrointestinal nematodes in small ruminants represents a feasible alternative, a recommendation also supported by Athayde et al. (2004), whom using seeds of summer squash (*Cucurbita pepo*), Indian jalapa (*Operculina hamiltoni* L.) and bitter melon (*M. charantia* L.) in rural groups located in Paraiba, verified anthelmintic activity with OPG reduction in naturally infected small ruminants, 30 days after the extracts were applied.

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