In vitro ANTHELMINTIC EFFECTIVENESS OF ETHANOLIC EXTRACTS OF *Operculina hamiltonii (*G. DON) D.F. Austin & Staples (1983) – BATATA DE PURGA

EFICÁCIA ANTI-HELMÍNTICA *IN VITRO* DO EXTRATO ETANÓLICO DE *OPERCULINA HAMILTONII* (G. DON) D.F. AUSTIN & STAPLES (1983) – BATATA DE PURGA

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SHORT COMMUNICATION

SUMMARY

The experiment *in vitro* was realized to evaluate the action of the ethanolic extract of *Operculina hamiltonii* (G. DON) D.F. Austin & Staples (1983), known as batata de purga, on eggs and larvae of gastrointestinal nematodes of goats. The recovery of the eggs was realized in tamises and the larvae were obtained by means of larval culture, from feces of naturally infected goats of the semi-arid central region in Paraiba state, NE of Brazil. The extract was used at concentrations of 50; 25; 12.5; 6.25 and 3.12 $mg.mL^{-1}$ in both tests. Moxidectin (0.2 $mg.kg^{-1}$) was used as positive control, and in the negative control group was used distilled water. The plates were examined by optical microscopy, and the eggs and larvae counted were classified as: in development and movable and immovable larvae, after 24h, 48h and 72h of incubation. The concentrations of the ethanolical extract of *C. erosa* differed as the number of nonviable eggs and on the test of larval motility. The percentage of viable eggs decreased as the concentration of the extract of Batata de purga increased. When extract concentration reached 25%, the percentage of viable eggs decreased to 53.07%, and decreased further to 29.57% at concentration of 50%. The concentration of Batata de purga was responsible for the reduction of the percentage of viable larvae, where it was observed that the concentration of 50% affected negatively 66.87% of the larvae.

KEY-WORDS: Farming goat. Gastrointestinal helminthiasis. Phytotherapy.

RESUMO

O experimento *in vitro* foi realizado para avaliar a ação do extrato etanólico da Batata de purga - *Operculina hamiltonii* (G. DON) D.F. Austin & Staples (1983), sobre ovos e larvas de nematóides gastrintestinais de caprinos. A recuperação dos ovos foi realizada em tamises e as larvas foram obtidas por meio de coproculturas, a partir de fezes de caprinos naturalmente infectados da mesorregião do Sertão Paraibano. O extrato foi utilizado nas concentrações de 50; 25; 12,5; 6,25 e 3,12 mg.mL-1 para ambos os testes e como controle positivo 0,2 $mg.kg^{-1}$ de moxidectina e para testemunha, utilizou-se água destilada estéril. As placas foram examinadas ao microscópio óptico para contagem dos ovos em desenvolvimento e larvas móveis e imóveis, após 24 h, 48 h e 72 h de incubação. As concentrações do extrato etanólico da Batata de purga e os tratamentos testemunha e fármaco diferiram quanto ao número de ovos inviáveis e no teste de motilidade larval. O percentual de ovos viáveis decresceu com o aumento da concentração do extrato de Batata de purga, e que a partir da concentração de 25% do extrato, o percentual de ovos viáveis caiu para 53,07%, chegando a 29,57% na concentração de 50%, valores estes inferiores e significativos quando comparados com o controle positivo e negativo. A concentração do extrato da Batata de purga foi responsável pela redução no percentual de larvas viáveis, onde se observa que o extrato na concentração de 50% foi capaz de afetar negativamente 66,87% das larvas.

PALAVRAS-CHAVE: Caprinocultura. Helmintoses gastrintestinais. Fitoterapia.

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Submitted: 14/07/2010 Accepted: 20/06/2011

Although gastrointestinal nematodes cause losses to the goat culture all over the country, this fact is more evident in the Northeast, where the species is more extensively cultivated. Therefore, anthelmintics are used against gastrointestinal nematodes in small ruminants, especially goats. The majority of goat farmers use several groups of anthelmintic at different dosages per year, which decreases the effectiveness of these products
(VIEIRA & CAVALCANTE, 1998).

Frequent worm outbreaks in goat farms of the semiarid central region of Paraiba state characterize the inadequate control that is being used by the the farmers. The use of sub-doses, the persistent use of anthelmintic based on the same chemical principle, bad timing for deworming and lack of appropriate techniques, as well as their availability to the farmers contribute to the rapid development of anthelmintic resistance (ALMEIDA, 2005).

Several research studies are being conducted to develop products based on natural substances that are capable of interfering on the biological processes of the parasites, such as growth regulators and feeding behavior (CHAGAS 2004).

Phytotherapy becomes an alternative to increase the profits of the farm and to reduce the use of conventional anthelmintics (VIEIRA, 1991). Roeder (1988) refers to the importance of using medicinal plants to treat diseases of livestock in the semiarid regions of northeastern Brazil and suggests to increase their use.

This study aimed to evaluate *in vitro* the action of ethanolic extract obtained from *Operculina hamiltonii* (G. DON) D.F. Austin & Staples (1983), known as batata de purga, on the eggs and larvae of gastrointestinal nematodes of goats.

The experiment was conducted in the Laboratory of Parasitic Diseases of Domestic Animals (LDPAD) of the Health and Rural Technology Center (CSTR), Universidade Federal de Campina Grande (UFCG) and Laboratory of Natural Products Research (LPPN), Universidade Regional do Cariri (URCA).

Samples of *Operculina hamiltonii* (G. DON) D.F. Austin & Staples (1983), known as Batata de purga, were collected at the Health and Rural Technology Center (CSTR/UFCG), Campus de Patos, between August and September, 2008. After identification of the parts suitable for the ethnopharmacological study, the prepared dried specimen were deposited in the Herbario Caririense Dárdano de Andrade – Lima, of Universidade Regional do Cariri (URCA) under the number #3750.

The plants were air dried for 48 hours, subsequently placed in a forced air oven at 60ºC for 24 hours, and soon after that, weighed and milled.

Ethanolic extracts were obtained following methodology described by Matos (1997) at Laboratório de Pesquisa de Produtos Naturais (LPPN), of the Universidade Regional do Cariri (URCA). The ratio used was 500.01 g of batata de purga powder to 1.000 mL of ethanol PA. This mixture was allowed to stand for 72 hours, followed by filtration and concentration using a rota-evaporator to obtain a viscous material. For an effective evaporation of the solvent, the material was placed in weighed glass jars in a water bath.

Helminth eggs and larvae were obtained from one goat of the Moxoto breed of the Centro de Saúde e Tecnologia Rural (CSTR/UFCG). Fecal samples were collected daily from the rectum, placed in plastic bags and sent immediately to the Laboratório de Doenças Parasitárias e Animais Domésticos (CSTR/UFCG), at room temperature.

The eggs were separated using a series of four sieves (UENO & GUTIERREZ, 1983). In the suspension obtained by the technique described by Gordon & Whitlock (1939) 2 mL of ethanolic extract was added at the following concentrations: 50; 25; 12; 6 and 3% mg/mL^{-1} for 200 eggs in 2 mL, according to Hubert & Kerboeurf (1984). Subsequently, the samples were placed in a Petri dish and the following variables were quantified: viable egg (OVV) and non-viable egg (OVI), identified by the genus.

The infecting larvae were obtained from fecal culture by the technique of Roberts & O'Sullivan (1950). The larvae present in the suspension resulting from the fecal culture were counted and identified. Another 2 mL of the ethanolic extract was used at the concentrations of 50; 25; 12; 6 and 3% mg/mL for 200 larvae in 2 mL, according to Hubert & Kerboeurf (1984). The samples were placed in Petri dishes and the following variables were quantified: viable larvae (LVV) and non-viable larvae (LVI). This procedure was repeated using distilled water for the negative control and albendazole 5% (1Farmazole 1.9%® - Fagra Laboratory) for the positive control.

All the trials were carried out in triplicate. The optical microscope readings to determine the number of eggs and developing larvae were conducted after 24 h, 48 h and 72 hour incubation time. The results for five ethanolic extract concentrations, as well as positive and negative controls, all in triplicate, were evaluated at three different incubation times (24, 48 and 72 hours) and compared statistically by Tukey test at 5%. All data were treated as percentage.

The parasitological analysis resulted in a mean count of 4.500 EPG (eggs per gram of feces). The eggs had morphological characteristics typical of *Trichostrongylidea* superfamily.

Larva counts and identification resulted in an average of 2.150 larvae per plate, where 70% of the larvae were identified as belonging to the genus *Haemonchus*, 16% *Trichostrongylus* and 14% *Oesophagostomum*.

With the methodology used in the experiment, it was found that the percent of viable eggs decreased as the concentration of *Operculina hamiltonii* (G. DON) D.F. Austin & Staples (1983), batata de purga extract increased. Further, at the concentration of 25% extract, the percentage of viable eggs decreased to 53,07% and at 50%, the viable eggs were 29,77%; these values were significantly lower compared to the positive and negative controls (Table 1), which shows the effectiveness of the extract as an inhibitor of hatching eggs of gastrointestinal nematodes of small ruminants.

Table 1 - *In vitro* anthelmintic effectiveness of the extract of batata de purga on the eggs of gastrointestinal helminths of goats of the semiarid in Paraiba state.

OVV	24h	48h	72h
Negative control	95,67Aa	96,33Aa	92,33Aa
Positive control	93,63Aa	75,73Aa	52,83Bb
Ext. 3%	88,33Aa	38,33Bb	52,53Bb
Ext. 6%	77,83Aa	57,13ABa	68,07ABa
Ext. 12%	67,57Aa	52,50ABa	59,60ABa
Ext. 25%	21,53Ba	44,57ABa	53,07Ba
Ext. 50%	20,93Ba	38,00Ba	29,77Ba

Same uppercase letters in the columns and lowercase in the rows do not differ statistically by Tukey 5%.
Obs: OVV= viable eggs.

Table 2 - *In vitro* anthelmintic effectiveness of the extract of batata de purga on the larvae of gastrointestinal helminths of goats of the semiarid in Paraiba state.

LVV	24h	48h	72h
Negative control	100,00Aa	100,00Aa	99,27Aa
Positive control	100,00Aa	98,43Aa	95,97Aa
Ext. 3%	100,00Aa	93,00ABa	97,43Aa
Ext. 6%	97,67Aa	90,77ABa	95,60Aa
Ext. 12%	98,77Aa	87,70ABab	74,70Bb
Ext. 25%	91,40Aa	84,10ABa	62,33Bb
Ext. 50%	85,50Aa	70,70Ba	33,13Cb

Same uppercase letters in the columns and lowercase in the rows do not differ statistically by Tukey 5%.
Obs: LVV= viable larvae.

As for the incubation time, it was concluded that the exposure time of gastrointestinal helminth eggs to the extract did not affect significantly the counts; therefore, the extract concentration and not the time is responsible for the anthelmintic action.

The number of studies in the literature about the action of plant extracts on gastrointestinal nematodes is small and inconclusive, thus making difficult a meaningful comparative study. However, the findings by Girão et al. (1998), who conducted a ethno-veterinary survey of plants that show anthelmintic action in goats in the state of Piaui, can be used for this purpose. In this *in vitro* study, the authors determined the ovicidal action of batata de purga on eggs of gastrointestinal nematodes of goats, administered in doses that ranged from 0,4 to 5 g, of crushed dried plant for 10 g of feces, using the fecal culture method. Athayde et al., (2004) used pumpkin seeds (*Cucurbita pepo*), Batata de purga - *Operculina hamiltonii* (G. DON) D.F. Austin & Staples (1983) and São Caetano melon (*M. charantia*), in rural centers located in the municipalities of Patos, São Mamede and Santa Terezinha, PB, verified this anthelmintic activity, due to the decreasing number of EPG in goats naturally infected, 30 days after its administration.

In assessing the action of the batata de purga extract on the viability of larvae, it can be seen that the percentage of viable larvae decreased as the extract concentration increased. This difference became significant from the concentration 12%, and resulted in

33,13% of viable larvae at 50% concentration (Table 2). Despite the significant difference when compared to the control, it can be observed that the batata de purga extract in tests *in vitro* is less efficient to control gastrointestinal helminth larvae in small ruminants when compared to those proposed by the Grupo Mercado Comum for chemical substances (GMC 1996), that recommends the following levels: highly effective > 98%, effective between 90 and 98%, moderately effective about 80-89% and insufficiently effective < 80% (not recordable).

In the state of Piaui, Girão et al. (1998) based on information obtained from goat farmers, listed 14 plants as having anthelmintic activity. The following plants were listed: *Operculina sp*. (Batata de purga), *Cucurbita moschata* (pumpkin), *Luffa operculata* (loofah paulista, Cabacinha), *Heliotropium sp*. (cockscomb), *Mentha sp.* (mint), *Carica papaya* (Mamoeiro), *Chenopodium ambrosioides* (Mastruço), *Momordica charantia* (são caetano melon), Milome (scientific name not identified), *Plumeria sp* (Pau de leite, Janguba), *Jatropha curcas* (White pine, Pinhão de purga), *Scopalaria dulcis* (Vassourinha) and *Croton sp* (canopy).

Almeida et al. (2003), in a study also conducted *in vitro,* observed the action of *Cymbopogon citratus* and *Digitaria insularis* extracts on gastrointestinal nematodes of goats, and reported a reduction above 95,00% of the *Strongyloidea* superfamily. The doses were respectively 224 and 355,2 mg/mL, for both plants.

Exposure of the gastrointestinal helminth larvae to the batata de purga extract over time (24, 48 and 72 hours) resulted in significant reduction of the number of viable larvae. This reduction increased over time, after 72 hours the number was significantly lower compared to 24 and 48 hours. Extract concentration was also responsible for the reduction of viable larvae, at concentration of 50%, 66,7% of the larvae became non-viable. This reduction in the number of viable larvae was attributed to exposure time to the extract, as well as concentration.

The results of this study suggest that batata de purga extract was effective during *in vitro* experiment to treat gastrointestinal nematodes of goats. However, further *in vivo* studies are needed to establish its use as an alternative control of parasitism in animals.

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