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IN VITRO STUDY OF *Vitex gardneriana* SCHAUER TOXICITY ON *Artemia salina* AND ITS EFFECT ON EGGS AND LARVAE OF *Haemonchus contortus*

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ABSTRACT This research aimed to investigate the toxic action of *Vitex gardneriana* on microcrustacean *Artemia salina* by using hydroalcoholic extract from leaves (EHF) and acute toxicity test (CL50) concentrations of 100, 500, 1.000, 10.000, 20.000, and 50.000/mL. Sodium hypochlorite (NaClO) at 1% was used as positive control, and saline and 1% DMSO were used as negative control. *Vitex gardneriana* showed low toxic potential, with 50% mortality in the microcrustacean metanauplii population (concentration: 34.600 mg/mL). *A. salina* is a seawater microcrustacean widely used in bioassays for determining the toxic dose of bioactive substances because of its low costs and its related simple, aseptic procedures. To determine the toxic action of the hydroalcoholic extract of *V. Gardneriana* on eggs and larvae of *Haemonchus contortus*, 2,5mL of EHF with a concentration of 34,600 mg/mL were added into single-species nematode fecal cultures containing approximately 12.000 eggs. Only 26,6% of the eggs hatched, corresponding to an EHF efficiency of 73,3%, whereas the hatchability was 50% in control, with 6.000 larvae recovered. The findings show the potential of this plant as an alternative in parasite control.

Keywords: Bioassay, *Vitex gardneriana*, *Haemonchus contortus*.

INTRODUCTION

The production of small ruminants is currently regarded as an important source of household income in developing

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countries. The Brazilian Northeast region concentrates a large proportion of herds, with 94% of the country's goat and 55% of its sheep (LIMA et al., 2013). However, a major obstacle to the satisfactory growth of these animals is the helminthic diseases caused by gastrointestinal nematodes (COSTA et al., 2011).

The effective technique for controlling these pests has traditionally been the use of synthetic drugs, resulting in anthelmintic resistance and increased concern about residues in animal products (SILVA et al., 2010) and the environment. New forms of parasite control have been tested to improve breeding, including the use of phytotherapy and the promising potential of plants rich in bioactive substances, especially tannins, in the control of nematodes (OLIVEIRA, 2013).

The parasite *Haemonchus contortus* stands out for its high prevalence and its high pathogenic potential and prolificacy (FONTES & MOLENTO, 2013), directly interfering with the economic results of small ruminant production (Cunha et al., 2014). In the tropics, on average 95% of caprine are infected, mainly by *Haemonchus* spp. and *Trichostrogylus* spp., with a mortality rate as high as 40% (GITHIGIA et al., 2001).

The *Vitex gardneriana* species, popularly known as "jaramataia", belongs to the Lamiaceae family and is endemic in Brazil. Distributed in Northeast, it is usually found in the banks of rivers, lakes, streams and floodplain lands. The genus has a vast history of use in popular medicine as either an infusion or a syrup, notably in the control of inflammatory syndromes (MARINHO et al., 2011). Several articles have reported on the therapeutic effects of other species of *Vitex* (BARRETO et al., 2008), such as the *Vitex agnus-castus,* used to control irregularities in premenstrual syndrome, including mammary gland pain, liquid retention, hyperprolactinemia, and infertility caused by decreased progesterone levels (IFTODA et al., 2006).

Considering the alleged properties of the plant, this study aimed to evaluate the toxic effect of the *V. gardneriana* leaves' alcoholic extract on *A. salina* and its ovicidal and larvicidal potential for ovine's *H. contortus*.

MATERIALS AND METHODS

Research Location

This research was carried out at the Multidisciplinary Laboratory for Environmental Research (LAMPA) of Universidade Federal de Campina Grande – UFCG – Departments of Pharmacology and Parasitic Diseases of Domestic Animals (LDPAD).

Collecting Botanical Material

Plant materials were collected and herborized based on the Technical Manual of Brazilian Vegetation (IBGE, 2012). *V. gardneriana* leaves were collected from the banks of the Piranha River in the Municipality of Pombal, State of Paraíba (06 ° 46'55.8 "S / 37 ° 48'30.6" W) in November 2013. Plant preparation, identification, herborization and tipping were performed by the Rural Health and Technology Center herbarium's taxonomy staff at Universidade

Federal de Campina Grande – Patos/PB, and deposited with number #4960.

Obtaining Botanical Extracts

An extract was made from the leaves of *V. gardneriana* by using a method described in Pereira et al. (2009). Grain alcohol 70% was used as organic solvent. After 96 hours of cold extraction, simple filtrations were performed, and organic extract was stored at room temperature (30 ± 2°C) for completely evaporating the solvent and obtaining the botanical extract. That was followed by roto evaporation (32 r.p.m.) at a pressure of 540 mmHg at 45°C for 03 hours for concentrating the extract.

Determining LC50

The LC50 was determined using an assay method described in Araújo et al. (2010). Assays were performed in triplicate with ten *A. salina*'s metanauplii in plates containing NaCl (pH 7 to 8) extract dissolved in a Dimethylsulfoxide's solution (DMSO) at 1% for concentrations of 100, 500, 1.000, 10.000, 20.000, and 50.000 µg /mL in a final volume of 5mL. Sodium hypochlorite (NaClO) at 1% was used as positive control, and saline and 1% DMSO were used as negative control. After 24 hours in contact with these solutions, the surviving metanaupliis were counted -- larvae were considered dead if they remained immobile for as long as 10 seconds.

Data Analysis

The average numbers of dead metanauplii were statistically analyzed. A lethal concentration of 50% (LC50) was estimated for the larvae through Probit Analysis and Spearman-Karber test with 95% of confidence interval using the TRIMMED program version 1.5.

Biological action of *Vitex gardneriana* hydroalcoholic extract on eggs and larvae of *Haemonchus contortus*

To evaluate the ovicidal and larvicidal action, coproculture was performed using a methodology adapted from Roberts O'Sullivan (1950). A volume of 2,5mL of *V. gardneriana* aqueous extract in concentration of 34.600,79 µg/mL was added into monospecific *Haemonchus contortus* fecal cultures containing approximately 12.000 eggs, and for the control group the extract was replaced with distilled water (Table 2). The treatments were performed in triplicates. After seven days at room temperature, recovery and counting of third-stage larvae (L3) were performed using optical microscope.

An adaptation of the formula described by Suede-Vasconcelos et al. (2007) was used to estimate the efficiency of the extract on eggs and larvae in treatments.

ET: Initial L3 - L3-treated group
Initial L3

Where:

ET extract efficiency

L3 corresponds to the initial estimate number of larvae in each coproculture

L3 treated group corresponds to the number of larvae recovered after eight days of incubation with different treatments.

RESULTS

In the toxicity assessment of the hydroalcoholic extract of *V. gardneriana* leaves, the mortality indices for the *A. salina* groups treated at concentrations of 100 to 10.000 µg/mL showed no deaths, while higher concentrations of 20.000 and 50.000 µg/mL showed mortalities above 50% (Table 1), determining a LC50 of 34.600,79 µg/mL for the plant's EHF. This toxicity is considered low by Mayer (1982), which determines that a substance is toxic when its LC50 is equal to or lower than 1000 p.p.m.

Table 1. The mortality of *A. salina* and LC50 values calculated for the alcoholic extract of *Vitex gardneriana* leaves and respective confidence intervals of 95%.

Concentration	Mortality of *A.salina*				LC_{50}	Confidence interval
Of EHF	G1	GC1	GC2	GC3	µg/mL	
100	-	-	-	30		
500	-	-	-	29		
1.000	-	-	-	30	34.600,79	31.504,75 – 38.001,07
10.000	-	-	-	30		
20.000	26	-	-	30		
50.000	29	-	-	30		

G1: (Group treated with Hydroalcoholic Extract of the *V. gardneriana* Leaves); GC1: Negative Control Group 1% DMSO); GC2: (Control Group: Isotonic Saline); GC3: (Control Group: Positive NaClO 1%); (-): Inactive; LC50: (Lethal Concentration at 50%).

The methodology applied to study the efficiency of the *V. gardneriana* hydroalcoholic extract on *Haemonchus contortus* in coprocultures showed that only 26,6% of the total of 12.000 eggs hatched, with a total recovery of 3.200 larvae, which represents an efficiency of 73,3% (Table 2), confirming its efficacy in controlling the parasite.

Table 2. Percentage and efficiency of the concentration of *V. gardneriana* aqueous extract on ovine's *Haemonchus contortus* eggs and larvae.

Treatment	**Concentration (µg/mL)**	**Total L3**	**Hatchability (%)**	**Efficiency (%)**
Vitex gardneriana	34.600,79	3.200	26,6	73,3
Control	Distilled water	6.000	50	50

DISCUSSION

Sá-Barreto et al. (2008) evaluated the acute toxicity of the extract from *V. gardneriana* leaves and stems and found no toxic effect on rats after 72 hours of observation. They found a LD50 above 2.000 mg/kg, considering from the toxicological the use not only from the extract of the leaves as well as the twigs' bark.

The use of genus *Vitex* has often been applied in research with several therapeutic actions (OLIVEIRA et al., 2012). Sá Barreto et al. (2007) performed preliminary tests with dried extracts of leaves, stem barks and aucubin isolated from *V. gardneriana.* They showed significant molluscicidal activity against *Biomphalaria glabrata*'s embryos can be used to control the intermediate

schistosomiasis host, an endemic tropical pathology. Aucubin, an iridoid glycoside, is prevalent in the genus *Vitex sp*. and has extensive pharmacological effects, including anti-inflammatory, antioxidant, and antiprotozoal properties, but few studies have addressed its anthelmintic action.

The *Vitex gardneriana* low toxicity on *A. salina* enables the safe use of this plant in biological activities. Along with the results of the tests applied to ovicidal and larvicidal action on *Haemonchus contortus*, this finding points to the use of this plant as a potential alternative in parasitic control.

In the evaluate the extract in coproculture with an anthelmintic action of the EHF of *Vitex gardneriana,* further phytochemical studies are required to identify and isolate components in the plant, specifically the leaves, with probable antiparasitic effects. It is known that this species has scientific reports of the presence of different chemical groups with possible therapeutic uses.

CONCLUSIONS

Artemia salina is a suitable model for *in vitro* toxicity assessment. The hydroalcoholic extract of *Vitex gardneriana* showd low toxicity on the metanauplii population of this microcrustacean. Tests carried out with eggs and larvae of *Hemonchus contortus* revealed that plant *Vitex gardneriana* can be an excellent therapeutic alternative in the control of this parasite.

ESTUDO *IN VITRO* DA TOXICIDADE DE JARAMATAIA (*Vitex gardneriana* SCHAUER) SOBRE *Artemia salina* E SEU EFEITO EM OVOS E LARVAS DE *Haemonchus contortus*

RESUMO: O objetivo da pesquisa foi investigar a ação tóxica da espécie *Vitex gardneriana* sobre o microcrustáceo *Artemia salina* utilizando extrato hidroalcóolico das folhas (EHF) com ensaios de toxicidade aguda (CL50) nas concentrações: 100, 500, 1.000, 10.000, 20.000 e 50.000 µg/mL. Como controle positivo, utilizou-se Hipoclorito de Sódio (NaClO) a 1%; para controle negativo, utilizaram-se solução salina e DMSO a 1%. Houve letalidade em 50% da população de metanaúplios do microcrustáceo sobre a concentração 34.600 µg/mL, demonstrando seu baixo potencial tóxico. *Artemia salina,* um microcrustáceo de água salgada, tem sido bastante utilizado como bioensaio em laboratórios para determinação de doses tóxicas de substâncias bioativas por ter baixo custo com procedimentos simples de assepsia. Para a determinação da ação tóxica do extrato hidroalcóolico da *V. gardneriana* sobre ovos e larvas de *Haemonchus contortus,* foram adicionados 2,5ml do EHF na concentração de 34.600 µg/ml em culturas fecais monoespecíficas do nematódeo, contendo aproximadamente 12.000 ovos. Os resultados demonstraram que o EHF permitiu apenas 26,6% de

eclosão, correspondendo a uma eficiência de 73,3%, enquanto no controle a eclodibilidade e eficiência foram de 50%, sendo recuperadas 6.000 larvas. Os dados obtidos nesta pesquisa demonstraram o potencial terapêutico desta planta para uma possível utilização alternativa no controle parasitário.

Palavras-chaves: Bioensaio, *Vitex gardneriana*, *Haemonchus contortus*.

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ANIMAL ETHICS

This study was submitted to the Animal Ethics Committee of Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural: Protocol No. **CEUA 197/2014 (Segunda via 48/2019)**. A partitioning of the project was requested because the main project involves other variables, which are not addressed in this paper.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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