Sincurá et al., Vector Biol J 2017, 2:2
DOI: 10.4172/2473-4810.1000120

Vector Biology Journal

Research Article A SCITECHNOL JOURNAL

Effect of Aqueous Extract of *Protium heptaphyllum* (Burseraceae) on *Lutzomyia longipalpis* (Diptera: Psychodidae), a Proven Vector of Visceral *Leishmaniasis* in Brazil

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Abstract

Objective: To evaluate the toxicity of aqueous extract of *Protium heptaphyllum* on *Lutzomyia longipalpis* a proven vector of visceral leishmaniasis (VL).

Methods: Aqueous extract of the leaves of *P. heptaphyllum* were prepared at 0.25, 0.10,0.05 and 0.025 mg/mL concentrations, and 100 µL was applied to adults of *Lu. longipalpis* (n=20). Controls included 20 adults that were treated with 100 µL distilled water (negative control) and 20 adults that were treated with 100 µL alpha-cypermethrin at 196 µg/mL concentration (positive control). The percentage of dead insects in each group was observed at 1, 2, 4, 8, 12, 24, 48 and 72h after application.

Results: The mortality rate of *Lu. longipalpis* after 2h of exposure to the extract at a concentration of 0.1 mg/mL was 52%, behaving similarly to the positive controls (cypermethrin), and the extract at a concentration of 0.25 mg/ml was able to kill 56% of insects within the first hour of exposure. After 72h, the aqueous extract of *P. heptaphyllum* showed a mortality rate of about 81% and 83%, at concentrations of 0.1 and 0.25 mg/mL, respectively.

Conclusion: The extract of *P. heptaphyllum* contains terpenes, a molecule with recognized toxicity in insects, and which may be responsible for killing the phlebotomines. These results demonstrate the potential use of this extract in the control of this vector.

Keywords

Phlebotominae; Sand flies; Insecticidal activity; Bioinsecticide; Vector control

Introduction

Visceral leishmaniasis (VL) is an infectious parasitic disease that mainly affects hematopoietic organs such as liver, spleen and bone marrow causing hepatosplenomegaly, malnutrition, jaundice and can lead the individual to death, if not treated in time [1]. In the Americas, about 90 % of human cases have been recorded in Brazil [2], where the disease is distributed in all regions and is present in 26 of the 27 federative units [3-5].

In the Americas, the protozoan *Leishmania infantum* is transmitted to humans primarily through blood feeding of female *Lutzomyia longipalpis*, a sand fly that has shown high ecological plasticity and adaptability to various environments [6-8], and therefore, it has been incriminated as one of those vectors responsible for the urbanization phenomenon of the disease in Brazil [9,10].

The use of indoor residual insecticides has been an action used by the Ministry of Health to control VL. Currently, synthetic pyrethroids such as cypermethrin and deltamethrin are the most commonly used insecticides in Brazil. However, low efficiency in vector control was observed and some studies have pointed to resistance of *Lu. longipalpis* for these compounds, which increases the interest in new chemical products [11-14].

Popularly known as pitch-black, mastic, wild musk, among others, *Protium hetaphillum* is an evergreen tree species found in areas of forest and savannah ("cerrado"), highly very widespread in Brazil and South America, present from the Guianas to Argentina [15]. Studies show their sedative, anxiolytic, antidepressant, anti-inflammatory, antimicrobial and acaricidale activities [16-19]. Considering so many positive effects, this study aimed to investigate the insecticidal activity of the aqueous extract of *P. heptaphyllum* leaves on *Lutzomyia longipalpis*, a vector of vsceral leishmaniasis (VL) in Brazil.

Methods

Collection, identification, and preparation of plant material

In June 2015, Protium heptaphyllum leaves were collected in rock field area in the city of Diamantina (Minas Gerais, Brazil) (18° 11'S - 43°34'O). The stuff was deposited in the DIAM / UFVJM herbarium in Diamantina under no. DIAM nº 5878 and its identification made based on morphological characters [15].

Obtaining the plant extract

The collected material was subjected to drying in a forced circulation oven and air renewal to 60ºC and milled in a knife mill to obtain a fine powder of uniform granulation. To prepare the aqueous extract was added to the glass beaker, the powder obtained after grinding and distilled water at room temperature, keeping at rest for 48h.

Collection and identification of phlebotomine sand flies

The phlebotomine sand flies were collected in Diamantina / MG in the town of Aroeira (18° 88' S - 43° 38' W), using light traps HP [20] type exposed for two nights. The specimens were transported to the Laboratório de Parasitologia of Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM) and put in cages. From this material, a sample was taken for identification according to the classification proposed by Young & Duncan [21]. The sand flies remained at rest for 24h until perform the test, being offered to them sugar solution of distilled water and honey (1:1).

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Received: September 23, 2017 **Accepted:** October 03, 2017 **Published:** October 10, 2017

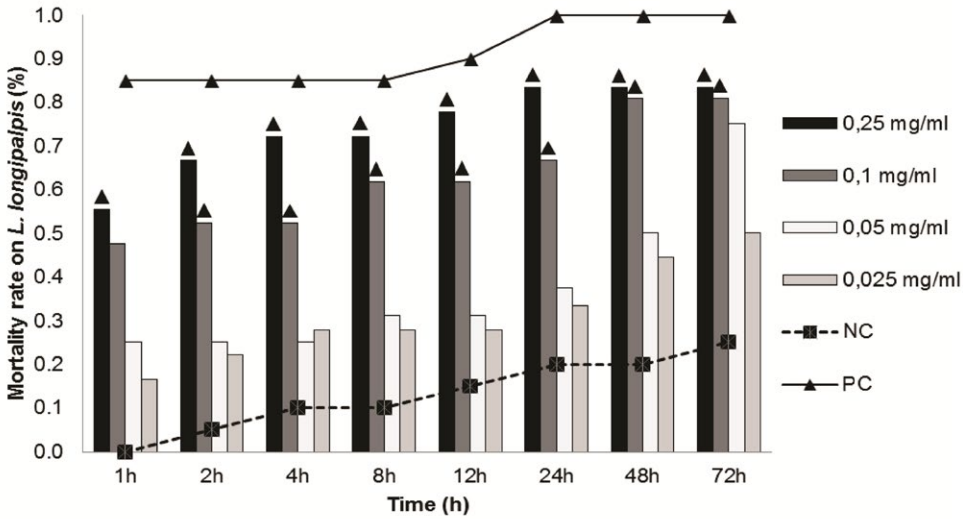

Figure 1: Proportion of *Lutzomyia longipalpis* (%) killed at four concentrations of the aqueous extract of *Protium heptaphyllum*: 0.25 mg/ml; 0.1 mg/ml; 0.05 mg/ml; 0.025 mg/ml; PC (positive control) = 196 µg/ml; NC (negative control) = 300 µl of distilled water; (n = 20). ▲ = values with non-significant difference (p> 0.05) in relation to PC (equality test of proportions test - chi-square), with continuity correction or Fisher's exact test.

Biological tests on *Lutzomyia longipalpis*

The aqueous extract of P. heptaphyllum was applied on the filter paper in the following concentrations: 0.025 mg/mL; 0.05 mg/mL; 0.1 mg/mL; 0.25 mg/mL and introduced inside translucent plastic pots. Controls included 20 adults that were treated with 100 µL distilled water (negative control) and 20 adults that were treated with 100 µL alpha-cypermethrin at 196 µg/mL concentration (positive control). Tests, including controls, were performed in duplicate, with 15 males and 15 females in each pot. After 1h, 2h, 4h, 8h, 12h, 24h, 48h and 72h the dead sand flies were counted. The overall temperature of the laboratory was 28ºC.

Statistical analysis

The statistical test used to compare proportions between groups at each time point was the proportion of equality test (chi-square), with continuity correction or Fisher's exact test. The software used for statistical analysis was the R (version 2015) [22], considering the 0.05 significance level.

Results

The aqueous extract of *Protium heptaphyllum* demonstrated toxic properties in all concentrations tested. The mortality rate of Lu. longipalpis after 2h of exposure to the extract at a concentration of 0.1 mg/mL was 52%, behaving similarly to the positive controls (cypermethrin), and the extract at a concentration of 0.25mg / ml was able to kill 56% of insects within the first hour of exposure. The aqueous extract of *P. heptaphyllum* showed a mortality rate of about 81% and 83% after 72 h, at concentrations of 0.1 and 0.25 mg/mL, respectively (Figure 1).

Discussion

The use of pyrethoids insecticides, mainly deltamethrin and alpha-cypermethrin, to eliminate phlebotomines constitutes a strategy of visceral leishmaniasis control. Deltamethrin has shown low efficacy on *Lu. longipalpis* [23-25]. Similarly, the alpha- cypermethrin had low mortality rates in the wall, which led the authors to assume the possibility of resistance of *Lu. longipalpis* strain used or even the low efficacy of this insecticide [26].

Studies of plants with insecticidal action effects on *Lu. longipalpis* have been performed [14]. It showed the capacity of the neem seed oil (*Azadirachta indica*) on the development of Lu. longipalpis. In the same way, *Antonia ovata* and *Derris amazonica* displayed significant insecticide effect against *Lu. longipalpis* [27]. At the same time, studies have demonstrated the effects of plant extracts on other organisms. Tests with P. heptaphyllum on *Candida krusei*, *Cryptococcus neoformans*, *Staphylococcus aureus* and *Tetranychus urticae* demonstrate a fungicidal, bactericidal, acaricidal action, anti-inflammatory and healing activity [12,16-19,28,29-32]. In these works, the action effect found was associated with the presence of terpenes, components that are found in plants, used in defense against natural enemies [5]. Terpenes have been identified in large numbers in leaves of *P. heptaphyllum*, with β- caryophyllene being the most abundant component [33,34]. In view of the above, the presence of terpenes in extracts of P. heptaphyllum leaves, a molecule with recognized toxicity in insects, has been shown to be responsible for killing the phlebotomines and that the significant mortality rates observed in the present study show that P. heptaphyllum merits further study as to its potential use as an bioinsecticide.

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