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In vitro activity of *Tityus metuendus* and *Brotheas amazonicus* scorpion venoms against *Plasmodium falciparum* FRC3

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ABSTRACT

Introduction: Scorpion venoms contain different classes of molecules with possible pharmacological activities, making them sources of bioactive molecules for the development of new drugs against infections caused by pathogens, such as malaria, a disease caused by protozoa of the genus Plasmodium. Malaria faces challenges in its control due to pathogen resistance to available antimalarials.
Materials and Methods: In this study, we evaluated the venom activity of the Amazonian scorpions *Tityus metuendus* and *Brotheas amazonicus* against *Plasmodium falciparum* FRC3, the analysis was performed by flow cytometry.
Results: At the analyzed concentrations, we found that the crude venom of *B. amazonicus* had an average inhibition of 87% at the concentration of 100 μg/mL, above that obtained with the drug (quinine), which had mean inhibition of 84% against *P. falciparum* FCR3. Regarding the venom of *T. metuendus*, lower activity was observed in comparison with the inhibition potential of the *B. amazonicus* venom and the standard drug, *B. amazonicus* venom showed low toxicity against the human fibroblast MRC5.
Conclusion: Because peptides and toxins from scorpion venom are related to biological functions, they can be used in the design of new therapeutic agents, with *B. amazonicus* venom being a possible source of molecules for the development of antimalarial drugs.

1. Introduction

Malaria is caused by parasites of the genus *Plasmodium*. Five species are the etiological agents of malaria in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae, Plasmodium ovale* and *Plasmodium knowlesi*. During the life cycle of this parasite, two hosts are infected: 1) a vertebrate, in which the parasites reproduce asexually; and 2) an invertebrate, which acts as a vector for disease transmission between vertebrate hosts. In addition to humans, reptiles, birds, rodents and primates can host *Plasmodium* species.[1] These parasites affect various tissues when not neutralized by the immune response, causing a wide range of clinical outcomes, from the absence of symptoms to severe malaria and death. Thousands of cases of malaria occur annually in tropical and subtropical regions, mainly Africa, South and Central America, India, Southeast Asia and Oceania.[2] Globally in 2020, there were an estimated 241 million malaria cases and 627 thousand deaths worldwide.[3] Epidemiological data indicate that cases of malaria are most prevalent in poorer countries and regions, and infection can even harm the socioeconomic development of these regions.[4] One of the main obstacles to malaria control in the world is the emergence of resistance.[5] Studies of malaria parasites report that the

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etiological agent *P. falciparum* has shown resistance to available antimalarials.[4,6] In response, efforts are being made to discover new drugs with antimalarial potential. Animal venom is a source of proteins and cationic peptides, with possibilities for pharmacological applications that can serve as the basis for new drugs.[7] Studies of venoms from scorpions in the Amazon region are scarce.[8] However, they demonstrate therapeutic potential against various microorganisms, such as fungi, bacteria and viruses.[9] The venoms of two scorpion species (*T. metuendus* and *B. amazonicus*) that occur in Central Amazonia (Manaus region) were extracted for experimental evaluation of their antimalarial activity.

2. Materials and Methods

2.1. Extraction and maintenance of venoms

The venoms of the scorpions *B. amazonicus* and *T. metuendus* were extracted according to Batista et al.,[10] freeze-dried and stored at room temperature for later use. For the tests, the venoms were solubilized in sterilized water and evaluated at four concentrations: 100 μg/mL, 50 μg/mL, 10 μg/mL and 1 μg/mL.

2.2. In vitro culture of P falciparum

The *P. falciparum* strain FRC3 was grown in RPMI medium with 10% AB+ human plasma and normal human erythrocytes in a low-oxygen atmosphere. This suspension of infected erythrocytes was incubated at 37 °C in a candle jar.

2.3. Evaluation of antiplasmodial activity by flow cytometry

The antimalarial tests were performed in triplicate at the Carlos Borborema Clinical Research Institute of the Tropical Medicine Foundation (IPCCB-FMT). For the evaluation of the antiplasmodic activity, the cultivation of *P. falciparum* was carried out in 96-well plates with flat bottom with final parasitemia of 1% and hematocrit of 2%.[11] The substance was solubilized in sterilized water at different concentrations (100 μg/mL, 50 μg/mL, 10 μg/mL and 1 μg/mL). After 72h of incubation, the samples were washed with 1X PBS buffer and ethyl bromide. At the end, the substances were resuspended in 200 μl of 1X PBS for analysis in a BD FACSCanto II flow cytometer (BD Biosciences, San Jose, USA). The analysis was performed by flow cytometry using the blue laser (488 nm) in the FL-3 channel (670 LP filter) with the Getting Started with BD FACSDiva^{TM} software. To determine the morphometric characteristics and the percentage of fluorescence of the samples, FlowJo^{TM} version 10 was used. Non-parasitized erythrocytes (healthy red blood cells) were used as a negative control and hematocrit (*P. falciparum* + culture medium) was used as a positive control. As a reference drug control, quinine (Sigma-Aldrich) was used, tested at the same concentrations.

2.4. In vitro cytotoxicity and cell viability assay

The cytotoxicity and cell viability assay were performed at Oswaldo Cruz Foundation – Leônidas e Maria Deane Institute (ILMD). The sample was solubilized in H_2O. Substances were tested at four concentrations: 100 μg/mL, 50 μg/mL, 10 μg/mL and 1 μg/mL. The MRC5 strain was cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco), supplemented with 10% inactivated fetal bovine serum (Gibco), and penicillin (50 μg/mL). All assays were performed in triplicate. The culture conditions of the MRC5 strain were adapted for use of the ILMD RPT11H-Bioassays of Biotechnological Compounds Platform.

The assay results were determined by the Alamar Blue method.[12] Cells were plated at a concentration of 1.0 x 10 cells/well in a 96-well plate, and incubated in an oven under 5% CO_2 at 37 °C for 24h for cell adhesion, then treated with the venoms at concentrations which ranged from 100 μg/mL to 1 μg/mL. The plates were kept in a CO_2 incubator for 72h at 5% CO_2 at 37 °C. After this period, 10 μL of 0.4% resazurin (diluted 1:20) was added to each well and the Alamar Blue (Sigma-Aldrich) was allowed to metabolize for 2h. Fluorescence was monitored in a microplate reader (GloMax® Explorer) at an emission wavelength of 580-640 nm and excitation of 520 nm. Cell growth medium was used as a positive control and 0.1% DMSO was used as a negative control. The percentage of cell viability was calculated according to the formula: %Viability= Ft x 100/Fb, where Ft = (cell fluorescence + medium + substance + resazurin) and Fb = (cell fluorescence + medium + resazurin).

3. Results

3.1. Antiplasmodial activity of scorpion venoms against P falciparum

At the analyzed concentrations, the venom of *B. amazonicus* was active against *P. falciparum* FRC3 with mean inhibition of 87% at the concentration of 100 μg/mL, while the quinine had average inhibition of 84% at the concentration of 100 μg/ml. Regarding *T. metuendus*, we observed that the venoms showed activity at a concentration of 100 μg/mL with inhibition of 58%, but at other concentrations the activity declined, with lower activity in comparison with *B. amazonicus* and the drug (Figure 1). The *B. amazonicus* venom showed values of plasmodium inhibition similar to quinine, as can be seen in Table **??**. For IC_{50}, *B. amazonicus* (0.078 μg/mL) presented a lower concentration than quinine (0.092 μg/mL) against *Plasmodium falciparum* FRC3.

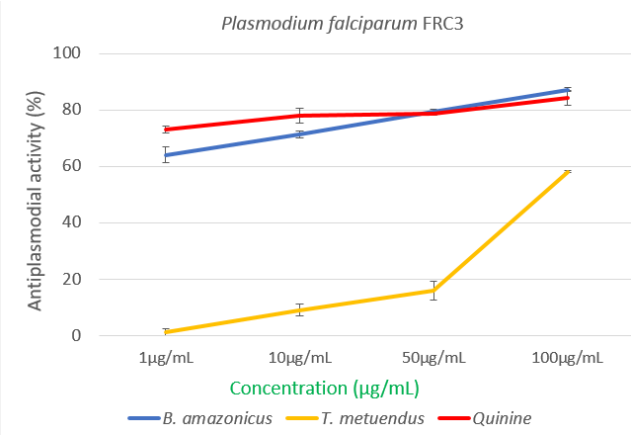

Figure 1: Antiplasmodial activity of *B. amazonicus* and *T. metuendus* venom against *P. falciparum* (FRC3) by flow cytometry.

3.2. In vitro cytotoxicity

The venoms of both species were submitted to the viability test against the MRC5 human fibroblast line at the same concentrations used in the assays against promastigotes. *B. amazonicus* venom showed an average viability above 78% at a concentration of 100 μg/mL, while the *T. metuendus* venom showed viability above 72% at the same concentration, in relation to 100% viability present in the control cells (Figure 2). Thus, the venoms show low cytotoxicity against the cells at the higher tested concentration, as can be seen inTable **??**.

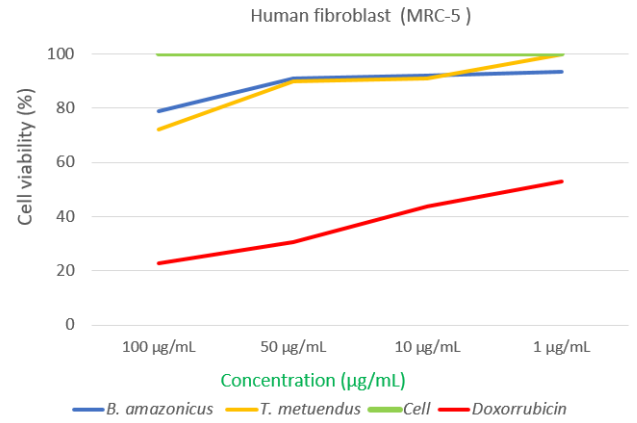

Figure 2: Cytotoxicity and cell viability test of the human fibroblast line (MRC5) using doxorubicin, *B. amazonicus* and *T. metuendus* venoms.

4. Discussion

In addition to neurotoxins, promising sources in the treatment of channelopathies,[13] scorpion venoms contain a

Table 1: Antiplasmodial activity and cytotoxicity of scorpion venoms.

	Inhibition of *P. falciparum* FRC3 (%)			**MCR5 human fibroblast viability (%)**		
Venom concentration	**Quinine**	***B. amazonicus***	***T. metuendus***	**Doxirrubicin**	***B. amazonicus***	***T. metuendus***
1 μg/mL	73,0±1,11	64,0±2,79	1,1±1,17	52,8±0,21	93,4±0,13	99,8±0,05
10 μg/mL	77,9±2,67	71,3±1,33	8,9±2,06	43,7±0,46	91,8±0,01	90,9±0,13
50 μg/mL	78,5±0,37	79,2±0,98	15,8±3,39	30,5±0,27	90,8±0,05	89,6±0,08
100 μg/mL	84,3±2,67	87,1±0,64	58,0±0,36	22,7±0,52	78,7±0,03	72,1±0,46

wide range of other molecules with interesting therapeutic properties, making them a valuable source of bioactive molecules that can serve as the basis for developing new drugs for the treatment of various diseases.[14] The literature reports the anticancer potential, and against pathogen infections, such as antimicrobial, antifungal, antiviral, antimalarial, antitrypanosoma and antileishmanial effects.[15–17] A peptide (scorpine) with 75 amino acid residues from the venom of the scorpion Pandinus imperator (with sequence obtained from a cDNA library) demonstrated antimalarial activity against *P. berghei*.[18] A recombinantly expressed scorpine produced 98% mortality in sexual stages of *P. berghei* at 15 μM and 100% reduction in *P. falciparum* at 5 μM.[19] A synthetic peptide (meucine-24) derived from the cDNA of the venom gland of the scorpion *Mesobuthus eupeus* inhibited the development of *P. berghei* and was effective against *P. falciparum* at micromolar concentrations.[20]

The Brazilian Amazon is home to scorpions with promising potential for pharmaceutical applications, but few have had their venoms studied between 2001 and 2021, and little research covers the species *B. amazonicus* and *T. metuendus*.[8] The *T. metuendus* scorpion has medical relevance in the Amazon region,[9] and its venom has leishmanicidal potential against promastigotes of *L. amazonensis* and *L. guyanensis*.[21]

Venom from the Amazonian scorpion *B. amazonicus*, which lives in the region of Manaus, Amazonas state, Brazil, had *in vitro* activity against *P. falciparum* FRC3. This scorpion in the Manaus region lives in leaf litter and inside fallen tree trunks, and has low toxicity to humans.[9] The venom of *B. amazonicus* demonstrated leishmanicidal potential against promastigote forms of *Leishmania guyanensis*.[21] The proteolytic activity of the *B. amazonicus* venom degraded the Aα and Bβ subunits of fibrinogen, and had low toxicity, making it a candidate for new drugs.[22] The scientific interest in compounds derived from animal venom has given rise to several studies due to the broad spectrum of activity of these molecules.

5. Conclusion

B. amazonicus and *T. metuendus* venoms showed low cytotoxicity against the MRC-5 human fibroblast line. Regarding antiplasmodial activity, both venoms showed good activity at the maximum concentration tested, but the activity of the *T. metuendus* venom decreased at other concentrations in relation to the *B. amazonicus* venom and the standard drug. The urgent need to find new ways to treat diseases such as malaria is spurring investigation of peptides and toxins with therapeutic potential present in scorpion venoms, and *B. amazonicus* venom is a possible source of molecules for the development of antimalarial drugs.

6. Source of Funding

None.

7. Conflicts of interest

The authors declare no conflict of interest.

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