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Original Article

Cytotoxic activity and abdominal writhes promoted by snake venom from *Philodryas nattereri* Steindachner, 1870

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ABSTRACT — *Philodryas nattereri* is distributed in arid and semiarid regions of South America and is most common in northeastern Brazil. The aims of the work were to investigate the effects of the venom from *P. nattereri* in cultured of MDCK and RAW cells and abdominal writhes in mice. Based on oxidative metabolism, it was possible to observe that the venom was capable of significantly reducing cell viability only at higher concentrations of venom at 50 and 100 µg/mL for MDCK cells, while in 200 µg/mL to RAW cells, with an IC_{50} of 169.5 µg/mL. Regarding writhing in mice promoted by the poison and acetic acid, it held a greater number of writhes when compared to promoted by saline. The venom of *P. nattereri* has a cytotoxic effect in MDCK and RAW cells and abdominal writhes, which appears to be similar to those caused by acetic acid.

Key words: Snake venom, Citotoxic activity, Cell culture, Abdominal writhes

INTRODUCTION

Philodryas nattereri Steindachner, 1870 of the family Dipsadidae (Zaher *et al*., 2009), commonly called the brown racer, has an olive green coloration with the final portion of its body colored brown. This snake is 1.20 to 1.60 m long, has large eyes with round pupils, is fast and has an intense daily activity (Vitt and Colli, 1994). The snakes' habitat is related to the environment's physical structure, food availability, the presence of predators and the physiology of these snakes, which are diurnal, arboreal and semi-arboreal. These snakes feed on small mammals, birds and lizards, are oviparous and lay from 6 to 20 eggs. The snakes' dentition is opisthoglyphous and connected to the Duvernoy's gland (Fundação Nacional da Saúde, 2001).

P. nattereri is distributed in arid and semiarid regions of South America and is most common in northeastern Brazil (Ceará and Rio Grande do Norte) (Mesquita, 2010).

During evolution, snakes have specialized in affecting the vital functions of their prey by releasing a large number of toxins (enzymes, proteins and peptides) through venom that destabilize the physiological levels of hormones, alter the activity of enzymes, receptors or ion channels, and promote cardiovascular and nervous system imbalance in their prey. The use of snake toxins as pharmacological tools and prototypes for drug development is increasing (Paioli, 2011).

Snake venoms comprise of different substances whose proportion and characteristics differ among families, genera and species. The composition of snake venoms may also vary due to age, sex, feeding habit, geographical distribution and individual characteristics of the snake, as well as seasonality. Example of this occurs in females of *Bothrops jararaca*, which produce five times more venom than males (Furtado *et al*., 2006).

Snake venoms contain organic and inorganic substanc-

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es. The main organic substances are proteins, which have enzymatic activities. Protein and peptides make 90 to 95 percent of the dry weight of venom. Some snake venoms also contain carbohydrate, lipid, biogenic amines, and free amino acids (Gutiérrez, 2002). In addition to that snake venoms contain inorganic cations such as sodium, calcium, potassium, magnesium and small amounts of zinc, nickel, cobalt, iron, manganese (Santos *et al*., 2008).

A large number of toxins from snake venoms have been purified and characterized and this includes those from various species of *Bothrops* (Theakston and Kamiguti, 2002).

Although there are several studies on the venoms of Proteroglyphs and Solenoglyphs snakes, little information is found with respect to Opisthoglyph. Thus, little is known about the composition of toxic oral secretions of Dipsadidae, which are opisthoglyphous species (Zingali *et al*., 2011).

These toxins are usually involved in defense mechanisms, in addition to possess characteristics of lethality, since are used to capture, killing and digestion of possible enemies or preys. Their possible therapeutic applicability has stimulated studies that contribute in the development of pharmaceutical tools and understating of the mechanism of their actions which can allow for the development of protocols in the treatment of patients involved in accidents with snakes (Lewis and Garcia, 2003).

The study aimed to evaluate the citotoxic activity in MDCK and RAW cells and abdominal writhes in mice promoted by *P. nattereri* venom.

MATERIALS AND METHODS

Venom extraction

Philodryas nattereri snakes were captured on Aroeiras Farm in the municipality of Upanema (5°38'32'' S and 37°15'27''W), state of Rio Grande do Norte and transported to NUROF (Ophiology Regional Nucleus of Ceará).

The animals were maintained in individual cages with free access to water and fed with 15 g mice every 30 days. Venom pools were made from more than 40 individual snakes and collected from the venom gland into capillary tubes to prevent contamination with saliva. After the outflow of the venom into the capillary tube, the venom was frozen and lyophilized.

Cell culture

Epithelial Madin-Darby Canine Kidney (MDCK) and Rat Peritoneal Macrophage (RAW-246.7) cells were cultured in RPMI-1640 and DMEM-High-Glucose medium, respectively and supplemented with 10% fetal bovine serum, 1% penicillin (10,000 IU/mL), and streptomycin (10 mg/mL). For each experiment cells were removed and incubated with trypsin-EDTA (0.25/0.02% v/v) at 37°C about 5 min. After this, the cells were counted in a Neubauer chamber and suspended in culture medium (1×10^5 cells/mL) and 24 hr later used for the experiments.

In vitro Cytotoxicity Assay

Cell viability was assessed by MTT (4,5-dimetilazil-2-il)-2,5 diphenyl tetrazolium) assay as described by Mosmann (1983). The MDCK and RAW cells were seeded at a concentration of 1×10^5 cells/mL on microplates and incubated at 37°C with 5% CO_2 for two hours. After cells were washed with sterile phosphate buffered solution (PBS) at pH 7.4, they were treated with venom from *Philodryas nattereri* (6.25, 12.5, 25, 50, 100 and 200 µg/mL, the last concentration used only for Raw cells). After 24 hr of treatment, the cells were incubated with 0.5 mg of MTT/mL for 4 hr.

The formazan crystals that resulted from MTT reduction were dissolved by adding SDS (10%) to each well followed by incubation for 17 hr. The absorbance was read at 570 nm in a microplate reader, and cell viability was calculated by comparing the resulting absorbances with the mean absorbance of the control wells (without venom, considered to be 100% viable).

Abdominal writhing test

The response to intraperitoneal injection of a 0.6% acetic acid solution induced according to procedures described by Koster *et al*. (1959), was a contraction of the abdominal muscle and stretching of the hind limbs.

Male Swiss mice (25-30 g) were divided into 3 groups, each comprising 6 mice. Animals were treated with *Philodryas nattereri* venom (100 mg/kg diluted in 0.15 M of saline solution) using intraperitoneal (IP) administration. The negative control animals received a similar volume of saline solution (0.9%). Positive control mice received acetic acid solution 0.6% (v/v). After challenge, pairs of mice were placed in separate transparent boxes and the number of abdominal writhes was counted a period of 20 min. The antinociceptive activity was expressed as the reduction of the number of abdominal writhes.

The experiments were conducted with prior approval from the Ethics Committee on Animal Research of the Federal University of Ceará (protocol n. 68/08).

Statistical analysis

Statistical analysis was done using one-way ANOVA followed by the Holm-Sidak post-test, with $p < 0.05$. The

IC_{50} was defined as the venom concentration resulting in 50% cell viability as estimated by interpolation between the mean percentages of dead cells and venom concentrations, and was obtained by non-linear regression analysis.

RESULTS

Cytotoxic effects of *Philodryas nattereri* venom on MDCK and RAW cells

P. nattereri venom cytotoxicity was assessed in the MDCK and RAW cell cultures after 24 hr of exposure to various venom concentrations (6.25, 12.5, 25, 50, 100 and 200 µg/mL). In the MTT assay, it was observed that the venom significantly reduced the viability of MDCK cells at concentrations of 50 and 100 µg/mL tested when compared to control, with an IC_{50} of 169.5 µg/mL. While, in the Raw cells viability was significantly reduced only in the concentration of 200 µg/mL, since this concentration occurred was the formation of formazan compound (Fig. 1).

Abdominal writhing caused by *P. nattereri* venom

The analgesic activity was evaluated using the writhing test in mice. The *P. nattereri* venom and acetic acid promoted 40 ± 1.2 and 45.1 ± 2.1 of abdominal writhes, respectively. In the control group (saline) 20.0 ± 1.2 (Fig. 2).

DISCUSSION

In the present study, we evaluated the cytotoxic potential of venom using MDCK and RAW cells with MTT assay. MDCK cells constitute a very well established cell line with morphological and functional characteristics similar to the cells of the collecting duct and/or mammalian distal tubule and have been extensively employed in the investigation of a variety of cellular processes, including epithelial transport and cellular response to toxic agents (Collares-Buzato *et al*., 2002; de Morais *et al*., 2013).

The results showed that the venom of *Philodryas nattereri* has a citotoxic effects to MDCK and RAW cells, significantly reducing cell viability at high concentrations studied.

The cytotoxic action was also promoted to *Bothrops* venom in various cell types (Gutiérrez and Lomonte, 1995; Damico *et al*., 2007). And, the cytotoxicity induces apoptosis through the production of hydrogen peroxide (H_2O_2) by inducing rupture of membranes, disrupting cell growth and consequently cell death (Naumann *et al.*, 2011).

Intraperitoneal injection of the venom significantly induced inflammatory processes similar to those caused by acetic acid evaluated as the number of writhing. These values suggest a new mode of studying the medical significance of the venom from the family of Dipsadidae.

Thus, the research of biological activity of the toxins from snakes of different species become an important screening tool for further studies about the mechanism of action and toxicity, in search of substances with pharmacological and therapeutic value and diagnostic (Ferreira *et al.*, 2011).

In conclusion, the venom of *P. nattereri* showed cyto-

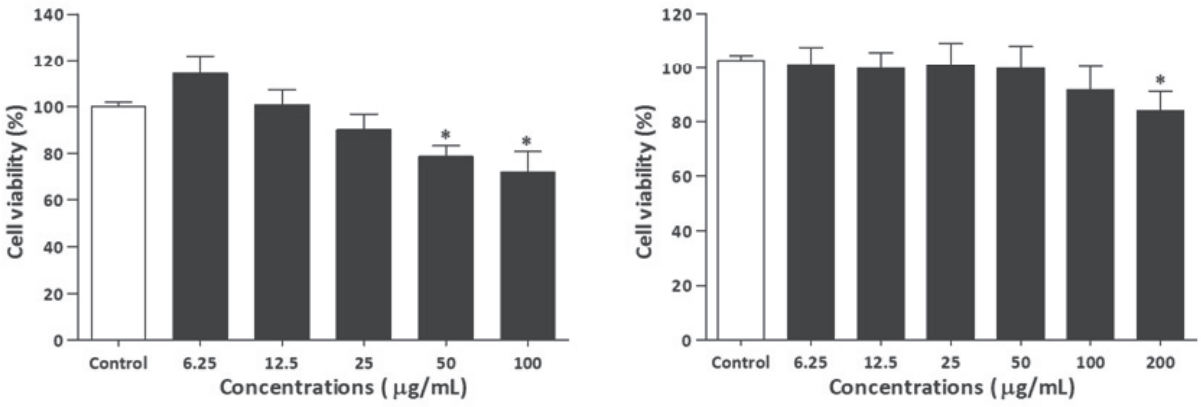

Fig. 1. Cytotoxic effects of *P. nattereri* venom applied in the concentration range of 6.25-200 µg/mL on MDCK and RAW cells by the MTT method. Data are expressed as mean ± S.E.M., ANOVA, Holm-Sidak post-test,* $p < 0.05$, compared to the corresponding control group.

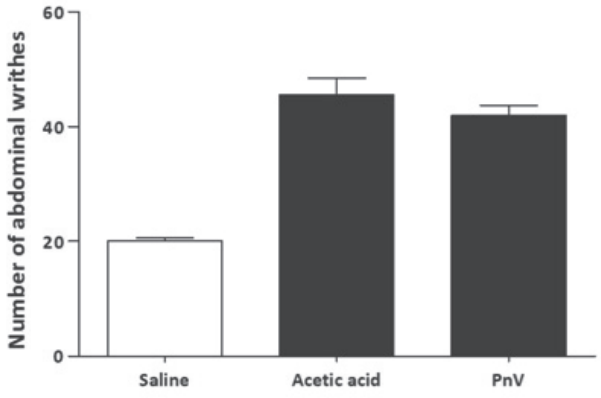

Fig. 2. Effects of intraperitoneal administration (IP) of venom from *P. nattereri* (PnV) (100 mg/kg, p.o.) and acetic acid induced writhing in mice. Saline solution (0.9%) was used as positive control. Data are expressed as mean ± S.E.M., ANOVA, Holm-Sidak post-test, *p < 0.05, compared to the corresponding control group.

toxic effects on MDCK and RAW cells in higher concentrations, regarding the number of abdominal writhes caused by this poison was similar to that found for acetic acid.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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