Biotecnologia

***Scientia Amazonia*, v. 7, n.2, B1-B14, 2018**
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

Antitumor and antibacterial activity of white and yellow venoms of *Crotalus durissus ruruima* tested individually

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Abstract

The aim of this study was to evaluate the antitumor and antibacterial potential of the yellow and white venoms of the Amazonian rattlesnake *Crotalus durissus ruruima* tested individually. The yellow venoms had antibacterial activity against *Staphylococcus aureus*. The pool of yellow venoms was cytotoxic to SK-Mel-103, MCF-7, HCT-116 tumor cell lines and MCR-5 cell lines. The white venoms did not exhibit any cytotoxicity to all the cell lines tested. Analysis of the damage index in HCT-116 cells revealed that yellow venoms cause DNA damage and double-stranded DNA breaks in this cell line. In conclusion, there is intrapopulational variation in *C. durissus ruruima* venoms; unlike the white venoms, the yellow venoms have antimicrobial activity and are cytotoxic and genotoxic to tumor cell lines. These findings indicate that the venoms are potential sources of components for new drugs to combat cancer.

Keywords: Amazonian rattlesnake, venoms, cytotoxicity, anti-*Staphylococcus aureus*, antitumor activity.

Resumo

O objetivo deste estudo foi avaliar o potencial antitumoral e antibacteriano dos venenos amarelos e brancos da serpent amazônica *Crotalus durissus ruruima* testados individualmente. Os venenos amarelos tiveram atividade antibacteriana contra *Staphylococcus aureus*. O *pool* de venenos amarelos foi citotóxico para as linhagens tumorais SK-Mel-103, MCF-7 e HCT-116 e para a linhagem não tumoral MCR-5. Os venenos brancos não exibiram citotoxicidade para todas as linhas celulares testadas. A análise do índice de danos nas células HCT-116 revelou que os venenos amarelos causam danos no DNA e quebra de DNA de fita dupla nesta linhagem celular. Em conclusão, há variação intrapopulacional em venenos de *C. durissus ruruima*; ao contrário dos venenos brancos, os venenos amarelos têm atividade antimicrobiana e são citotóxicos e genotóxicos para as linhas celulares

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tumorais. Esses achados indicam que os venenos são fontes potenciais de componentes para novos medicamentos no combate ao câncer.

Palavras-chave: cascavel amazônica, venenos, citotoxicidade, anti-*Staphylococcus aureus*, atividade antitumoral.

1. Introduction

Cancer is a very serious public health problem and in 2015 alone was responsible for the deaths of 8.8 million people worldwide (WHO, 2017). Various therapies are used to treat the condition, including radiation, surgery, chemotherapy, immunotherapy and hormone therapy, of which the most widely used nowadays is chemotherapy. However, one of the main obstacles associated with chemotherapy is that patients very often do not respond to the treatment or sometimes develop resistance after the initial treatment, as well as serious side effects (KUMAR et al., 2013).

Another major public health concern is bacterial infections, which are among the ten most common causes of death around the world. The resistance of certain clinically important pathogenic agents to antimicrobials is a consequence of the indiscriminate use of these drugs and is considered the main reason for the increase in the morbidity and mortality of infectious diseases (SANTOS, 2004). For example, the Gram-positive bacteria *Staphylococcus aureus*, which is commonly found in the squamous epithelium lining the nasal cavity, is currently resistant to methicillin, an antibiotic widely used to treat infections by antibiotic-resistant bacteria (LOWY, 1998; FOSTER, 2004).

It is therefore imperative to develop new, more potent, less toxic drugs based on natural sources to treat not only infectious diseases but also cancer. Various classes of promising natural molecules that are toxic to pathogens and tumor cells have been identified (KOH et al., 2006; PERUMAL SAMY et al., 2006; KUMAR et al., 2013; PERUMAL SAMY et al., 2017), including proteins and peptides from the venom of scorpions (CONDE et al., 2000), spiders (BENLI; YIGIT, 2008), bees (HEGAZI et al., 2015), wasps (JALAEI et al., 2014) and snakes.

Researchers have concentrated their efforts on characterizing the structure of snake venom proteins with potential biological activities that could be used to produce new medicines (WHITE, 2000).

Brazil, a country with some of the greatest biodiversity on the planet (MITTERMEIER et al., 2005), is home to various genera and species of snakes in the families Elapidae, Viperidae and Colubridae. Notable among these are the subspecies of the rattlesnake *Crotalus durissus*, whose venoms are a source of biologically active agents with antifungal, antileishmanial, antiplasmodial, antiviral, antibacterial and antitumor activities (DIZ FILHO et al., 2009; SOARES et al., 2010; BARROS et al., 2011; MULLER et al., 2012; QUINTANA et al., 2012; VARGAS et al., 2013; BARROS et al., 2015; NEVES et al., 2015).

When the venom and the toxins isolated from *Crotalus durissus terrificus*, the South American rattlesnake, were tested separately they conferred resistance to infection by the dengue and yellow fever viruses on Vero E6 cells (MULLER et al., 2012). Furthermore, one of the toxins isolated from this venom exhibited antiparasitic activity and inhibited development of *Plasmodium falciparum* dose-dependently (MALUF et al., 2016).

The cytotoxic effect of fractions isolated from *C. durissus terrificus* venom on the following cell tumor lines has been evaluated in several studies: MEL (murine erythroleukemia), Hs578T (human mammary duct), SK-LU-1 (lung adenocarcinoma), CHO-K1 (Chinese hamster ovary), RT2 (glioblastoma), GH3 (benign pituitary adenoma), SK-MES-1 (human lung) (CORIN et al., 1993; RUDD et al., 1994; DA SILVA et al., 1997; TAMIETI et al., 2007; SOARES et al., 2010; HAN et al., 2014).

The enzyme L-amino acid oxidase (LAO) was isolated from the venom of *Crotalus durissus cascavella*, the rattlesnake found in Northeastern Brazil, and exhibited activity against the Gram-negative bacteria *Xanthomonas axonopodis* pv *passiflorae*, the Gram-positive bacteria *Streptococcus mutans*

Scientia Amazonia, v. 7, n.2, B1-B14, 2018
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

and promastigote forms of *Leishmania amazonensis in vitro* (TOYAMA et al., 2006).

Snakes of the subspecies *Crotalus durissus ruruima*, the rattlesnake found in Northern Brazil, secret two types of venom: white and yellow. A study of both types of venom found that a mixture of white venoms was lethal and triggered coagulant, myotoxic, edema-forming and myolytic activities. In addition to the activities triggered by the white venoms, a mixture of yellow venoms induced hemorrhage and necrosis and, in a test using casein as the substrate, exhibited proteinolytic activity (DOS-SANTOS et al., 1993). When tested individually, the venoms making up these mixtures had different biological activities and different strengths, demonstrating the existence of intrapopulational variability among *C. durissus ruruima* venoms (DOS-SANTOS et al., 2005).

In an attempt to identify new bioactive agents, this study sought to evaluate the antitumor, antimicrobial and other biological activities of white (Cdr110 and Cdr173) and yellow (Cdr68 and Cdr69) venoms from *C. durissus ruruima* tested individually.

2. Material and methods

2.1. Venoms

Venoms were collected from four adult snakes of the subspecies *C. durissus ruruima* from Boa Vista (RR) kept in the Poisonous Animals Unit at the Amazonas Institute of Tropical Medicine. The snakes were anesthetized with carbon dioxide gas before the venom was extracted. The venoms were collected individually, separated according to color (white or yellow), filtered through a 0.45µm Millipore filter, lyophilized and stored at -20°C. The yellow venoms were identified as Cdr68 and Cdr69, and the white venoms as Cdr110 and Cdr173.

The pool of venoms from *C. durissus terrificus* came from snakes from the state of Minas Gerais and was identified as Cdt.

2.2. Assessment of the *in vitro* cytotoxic potential 2.2.1. Assessment of cytotoxicity by the Alamar Blue method

The Alamar Blue assay was performed following AHMED et al. (1994) in 96-well plates. The cells were cultured in DMEM medium (Gibco®, Life Technologies, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco®, Life Technologies, USA), 50 U/mL of penicillin and 50 µg/mL of streptomycin (Invitrogen) and incubated at 37°C, 5% CO_2. To determine the IC_{50} values (the concentration of venom that causes 50% cell death), cells plated at a density of $5x10^3$ cells/well were treated with the venoms at concentrations of 100 to 1.56 µg/mL. The test was performed in triplicate. Doxorubicin (5 µg/mL) (Sigma) was used as a positive cell death control, and the cell culture medium (cell diluent) as a negative control. After 72 h of treatment, 10 µL of 0.4% Alamar Blue® solution (Sigma) were added to each well, and fluorescence was measured in a microplate reader (DTX800 Beckman Coulter) after 2 h of exposure.

Cytotoxicity was initially assessed with samples of Cdr110, Cdr173 and Cdt individually and a pool of venoms (Cdr68+Cdr69) in three human tumor cell lines (SK-Mel 103, a melanoma line; HCT-116, a colorectal carcinoma line; and MCF-7, a breast adenocarcinoma line) and two human non-tumor cell lines (MRC-5, a human fibroblast line, and THP-1, a monocyte line). The Cdr68 and Cdr69 venoms were then tested individually in the HCT116 and MRC-5 lines at concentrations of 10 to 0.15 µg/mL.

2.2.2. Assessment of cytotoxicity by the MTT method

THP-1 cells were cultured at 37°C in a humidified 5% CO_2 incubator in RPMI 1640 medium (Gibco®, Life Technologies, USA) supplemented with 10% FBS (Gibco®, Life Technologies, USA) and 1% 2mM L-glutamine and penicillin/streptomycin (Gibco®, Life Technologies, USA). To induce monocytes to differentiate into adherent macrophages, the cells were resuspended in fresh medium containing phorbol myristate acetate (PMA) at 200 ng/mL. The cells were distributed between the 96 wells at a density of 100,000 cells/well and incubated for 48 h. The macrophages were washed with fresh medium and left to rest for 2 days with a daily change of medium.

The THP-1 cells were treated with 1, 10, 100 and 1000µg of the Cdr68, Cdr69,

Cdr110, Cdr173 or Cdt venoms for 48 h. The controls were treated with 5 µM staurosporine or medium. After the treatment period, the media containing the venoms and controls were removed and 90 µL of RPMI and 10 µL of MTT (5 mg/mL) were added to each well. The cells were incubated for 3 h, and the medium was then removed. A volume of 100 µL of DMSO was added, and the plate was left for 20 minutes under shaking so that all the formazan crystals dissolved.

Absorbance at 560 nm, which is linearly proportional to the number of live cells, was read in an ELISA reader.

2.3. Assessment of genotoxicity – comet assay

Alkaline and neutral comet assays were performed following SINGH et al. (1988). MRC-5 or HCT-116 (2 x 10^5) cells were plated in 24-well plates. After 24 h, when the cells had adhered to the slides, they were treated with the Cdr68 3 µg/mL, Cdr68 3 µg/mL, DMSO 0.2% (negative control) or doxorubicin 5 µg/mL (positive control) for 3 h. The assay was performed with 10 µL of the cell suspension after the treatment. The suspension was homogenized with 100 µL of 0.5% low melting point agarose (Sigma) and dissolved in PBS (phosphate buffered saline) pH 7.4. Cells suspended in agarose were spread on microscope slides previously prepared with 1.5% normal melting point agarose. A cover glass was placed over the cells, which were kept at 4°C for 5 minutes. The cover glasses were then removed, and the slides were immersed in a lysis solution containing 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100 and 10 mM Tris pH 10 for 24 h at 4ºC. After lysis, the slides were electrophoresed in 300 mM NaOH/1 mM EDTA buffer pH 13 for 20 min (20 V or 300 mA). After the electrophoretic run, the slides were washed in neutralizing buffer (0.4 M Tris-HCl pH 7.5) for 15 minutes. They were then dried at room temperature, immersed in ethanol for 3 minutes and dried again at room temperature.

The procedure for the comet assay in neutral pH was the same as for the alkaline assay except for the electrophoresis solution, which was prepared with sodium acetate and Tris–HCl, pH 8.5, and the electrophoretic run (20 minutes, 20 v or 300 mA) (WOJEWÓDZKA et al., 2002). Each slide was stained with 50 µL of ethidium bromide (20 µg/mL) and analyzed immediately in a LEICA fluorescence microscope. All the steps were performed in the dark.

2.4. Morphological analysis by hematoxylin-eosin staining

Cell morphology was analyzed following Wang et al. (2009). The HC-T116 cells were plated on 24-well plates ($7x10^4$ cells/mL). After 24 h, the cells were treated with Cdr68 or Cdr69 at concentrations of 0.5 and 5 µg/mL. After a 72 h incubation period the cells were trypsinized and 100 µL of each sample was cytocentrifuged at 2000 rpm for 5 minutes. The cells were fixed with methanol for 1 minute, and the slides were stained with hematoxylin and eosin. Changes in cell morphology were assessed using an optical microscope (Eclipse Ni, Nikon) and Nis-Elements 4.30.01 (Nikon).

2.5. Assessment of antimicrobial activity

2.5.1. Assessment of antimicrobial activity by the disk diffusion method

The following bacteria from the American Type Culture Collection (ATCC) were used for the antimicrobial activity assays: Gram-negative –*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Klebsiela pneumoniae* ATCC 700603; Gram-positive –*Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228.

The agar disk diffusion assay was performed with disks containing the crude Cdr68, Cdr69, Cdr110 or Cdr173 venoms following Bauer et al. (1966) and the CLINICAL AND LABORATORY STANDARDS INSTITUTE (2012).

The inocula were prepared in a 0.85 % saline solution by the direct colony suspension method. The turbidity of the inoculum was compared and adjusted to the 0.5 McFarland test standard.

After homogenization, the inoculum was plated on a Mueller-Hinton agar culture medium (DIFCO). The disks containing the

Scientia Amazonia, v. 7, n.2, B1-B14, 2018
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ISSN:2238.1910

venoms and the controls were then applied to the surface of the agar. The plates were incubated at 35°C (±2ºC) for 24 h.

The presence of a clear halo without bacterial growth around the disk was considered to indicate antimicrobial activity. The diameters of the halos (inhibition zones) were measured in mm. All the assays were performed in triplicate, and antimicrobial activity was expressed as the mean of the diameters of the halos for a given concentration.

2.5.2. Microdilution test

The venoms that exhibited antimicrobial activity in the disk diffusion assay were tested by the broth microdilution technique (ELOFF, 1998; CLINICAL AND LABORATORY STANDARDS INSTITUTE, 2003). Serial dilutions of the Cdr68 and Cdr69 venoms (1000 to 0.12 µg/mL) were performed, and 95 µL of each dilution were added to each well. A *Staphyloccus aureus* ATCC 2592 inoculum was prepared by adjusting the turbidity of the suspension to the 0.5 McFarland test standard and diluting it 10 times. The inoculum (5 µL) was added to each well immediately after the venoms were added. The plates were incubated at 37°C for 24 h, and the absorbance at 625 nm was then read in a spectrophotometer.

2.6. Statistical analysis

The results were expressed as mean ± standard deviation. The means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-test. The IC_{50} was determined by nonlinear regression. The analysis was performed with GraphPad Prism 6.0. A significance level of $p<0.05$ was used.

3. Results

3.1. Assessment of cytotoxicity

The pool of yellow venoms (Cdr68+Cdr69) was cytotoxic to all the tumor cell lines tested. The IC_{50} (Table 1) varied from 9.39 µg/mL for MCF-7 cells to <1.56 µg/mL for HCT116 cells.

The IC_{50} of the Cdr68+Cdr69 pool for MRC-5 cells was 4.21 µg/mL, showing that this pool was less toxic than doxorubicin, for which the IC_{50} was 0.14 µg/mL.

When the Cdr68 and Cdr69 were tested individually on the HCT116 tumor cell line at lower concentrations (10 to 0.15 µg/mL), they had similar cytotoxicity; the IC_{50} of Cdr68 was 1.8 µg/mL (1.5-2.2) and the corresponding figure for Crd69 was 1.3 (1.1 - 1.5). The cytotoxic activity was concentration dependent.

Table 1. *In vitro* cytotoxicity of the white and yellow venoms of *Crotalus durissus ruruima* for MRC-5 (normal human fibroblast) cells and the following tumor cell lines: SK-Mel-103 (melanoma), MCF-7 (breast adenocarcinoma), HCT116 (colorectal carcinoma) and THP-1 (monocyte).

Venoms	MRC-5	MCF-7	HCT116	SK-Mel-103	THP-1
	IC_{50}(µg/mL)				
Cdr110	>100	>100	>100	>100	>1000
Cdr173	>100	>100	>100	>100	>1000
Cdt	>100	>100	>100	>100	>1000
Cdr68+Cdr69	4.21 (3.96 - 4.48)	9.39 (8.980 - 9.819)	n.d.	5.43 (4.491 - 6.581)	-
Cdr68	-	-	1.8 (1.5-2.2)	-	508.3 (284.1- 909.3)
Cdr69	-	-	1.3 (1.1 - 1.5)	-	532.8 (316.8-896.0)
Doxorubicin	0.14 (0.10 - 0.17)	0.84 (0.50 - 1.43)	0. 52 (0.27 - 0.97)	0.90 (0.29 – 1.80)	-

Results expressed as IC_{50} with a 95% confidence interval.
n.d. not determined

Scientia Amazonia, v. 7, n.2, B1-B14, 2018
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

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3.2. Assessment of genotoxicity – comet assay

Cdr68 and Cdr69 (3µg/mL) were tested for their capacity to damage DNA in MRC-5 cells and HCT116 tumor cells using the alkaline and neutral versions of the comet assay.

In MRC-5 cells treated with Cdr68 or Cdr69 the damage index (DI) in an alkaline pH was higher than in the negative control (p<0.0001) but lower than in the positive control (p<0.0001). Cdr69 had greater genotoxic potential than Cdr68 (p=0.0185). In neutral pH, treatment with each venom caused DNA damage, and the result was statistically significant compared with the negative control (p<0.0001). The difference between the positive control and Cdr68 was not statistically significant (p>0.05) (Figure 1). In the alkaline comet assay, the most common types of damage were class 1 for venoms and class 2 for doxorubicin, while in the neutral comet assay the most common were classes 2 and 3, respectively (Figure 2).

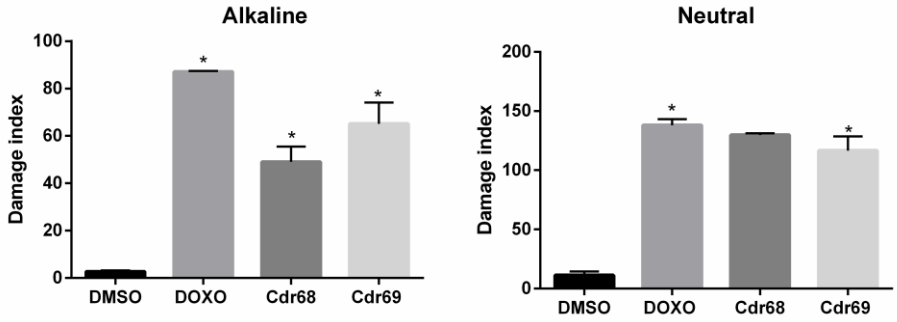

Figure 1. Damage index in MRC-5 cells treated with Cdr68 and Cdr69 in alkaline and neutral comet assays. In the alkaline comet assay both venoms had a lower damage index than the positive control, while in the neutral assay only Cdr69 had a statistically significantly lower damage index than the positive control. Doxo – Doxorubicin (positive control). DMSO- Dimethyl sulfoxide (negative control).

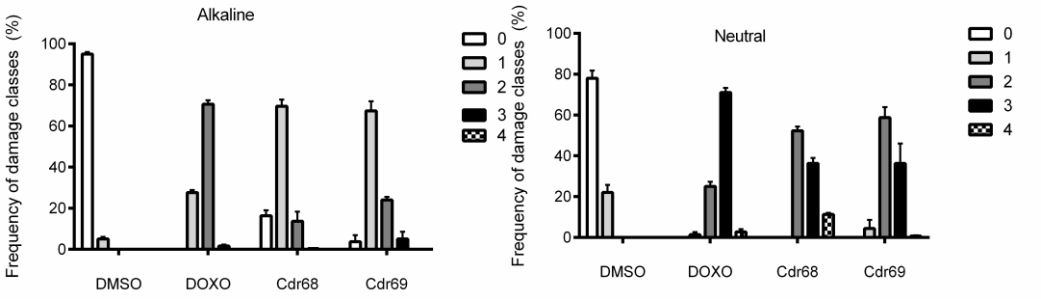

Figure 2. Frequency and distribution of damage classes in MRC-5 cells treated with Cdr68 and Cdr69 in alkaline and neutral comet assays. The most frequent damage class for the venoms was class 1 in the alkaline assay and class 2 in the neutral assay. Doxo – Doxorubicin (positive control). DMSO - Dimethyl sulfoxide (negative control).

A statistically significantly higher DI was observed for HCT-116 cells treated with the venoms in the alkaline and neutral comet assay than for the positive and negative controls (p<0.0001). Cdr68 had a greater genotoxic potential than Cdr69 (p< 0.0001) in the alkaline assay, but in the neutral assay there was no statistically significant difference between the venoms (Figure 3). The most common types of damage in the alkaline assay were classes 2 and 3 for the venoms and class 2 for doxorubicin, while in the neutral comet assay the most common were classes 4 and 3, respectively (Figure 4).

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Scientia Amazonia, v. 7, n.2, B1-B14, 2018
Revista on-line http://www.scientia-amazonia.org
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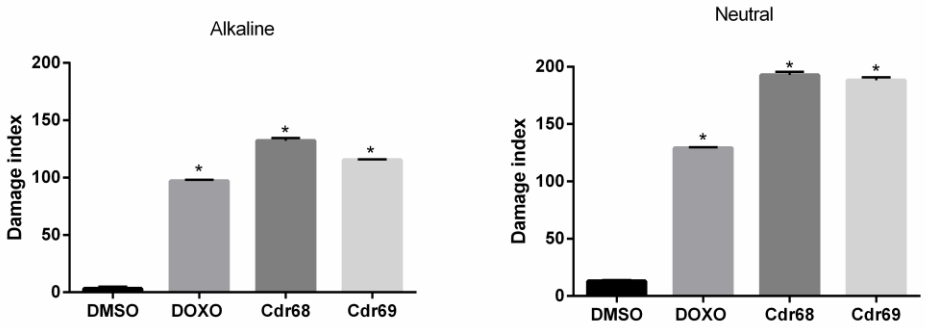

Figure 3. Damage index for HCT-116 cells treated with Cdr68 and Cdr69 in alkaline and neutral comet assays. In both assays the venoms had a higher damage index than the positive control. Doxo – Doxorubicin (positive control). DMSO- Dimethyl sulfoxide (negative control).

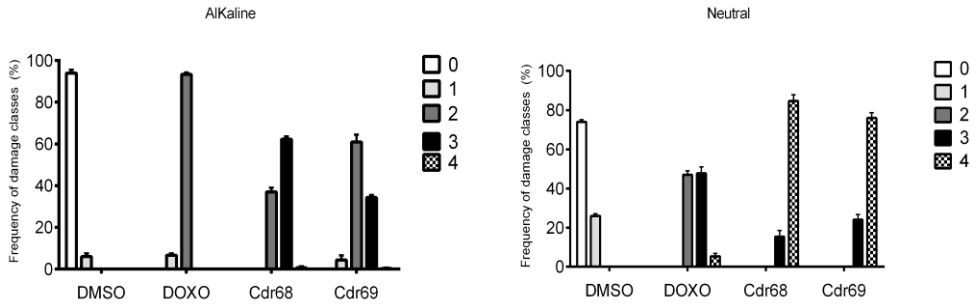

Figure 4. Frequency and distribution of damage classes in HCT116 cells treated with Cdr68 and Cdr69 in alkaline and neutral comet assays. The most frequent damage classes for the venoms were classes 2 and 3 in the alkaline assay and class 4 in the neutral assay. Doxo – Doxorubicin (positive control). DMSO- Dimethyl sulfoxide (negative control).

3.3. Morphological analysis by hematoxylin-eosin staining

Exposure of HCT116 cells to Cdr68 or Cdr69 for 72 h caused morphological changes such as cell shrinkage, pyknotic nuclei, chromatin condensation and cytoplasmic vacuolization, and in some cases cellular remains were observed, showing that the cells had been destroyed (Figure 5).

3.4. Antimicrobial activity The diameters of the halos for the yellow venoms (Cdr68 and Cdr69) measured 9.6 ± 1.52 (SD) mm and 10.6 ± 0.57 (SD) mm, respectively, for the *S. aureus* ATCC 25923 strain. There was no statistically significant difference between the activities of the two venoms for this strain. The microdilution test was performed with the same strain and Cdr68 and Cdr69 venoms at concentrations of 1000, 500, 250, 125 and 62.5 µg/mL. Cdr69 had greater inhibitory potential than Cdr68 ($p \leq 0.05$). However, at a concentration of 0.12 µg/mL the Cdr68 yellow venom had greater inhibitory potential ($p \leq 0.05$), as shown in Figure 6.

4. Discussion

The particular characteristics of each type of cancer, the mechanisms of resistance to cancer therapies and the broad spectrum of side effects of cancer treatment make prevention and treatment of this condition a challenge (VENDRAMINI-COSTA et al., 2016).

In the last three decades, many studies have investigated the anti-cancer properties of venoms, and this has led to the discovery of various molecules with promising activities, some of which are being tested in clinical trials and may in future be the basis for cancer therapy drugs (GOMES et al., 2010).

The present study assessed the cytotoxicity of white (Cdr110 and Cdr173) and yellow (Cdr68 and Cdr69) snake venoms,

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which had been characterized in a previous study (DOS-SANTOS et al., 2005). We found that, in the concentrations tested, the white venoms and the venom of *C. durissus terrificus* were not toxic to the cell lines tested. However, the mixture of yellow venoms was toxic to SK-Mel-103, MCF-7 and HCT116 tumor cells, of which the last were the most sensitive. Although the mixture was toxic to MRC-5 cells, at low concentrations it only showed a high cytotoxic effect for colorectal carcinoma cells.

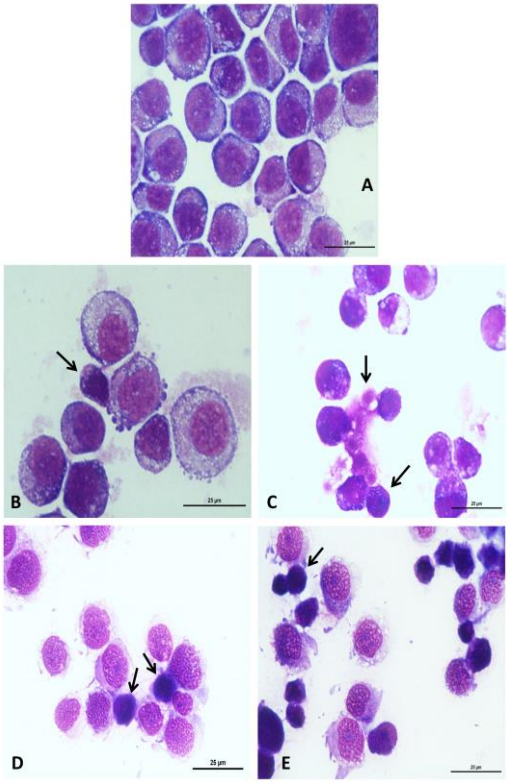

Figure 5. Optical micrographs showing the morphology of HCT116 cells after 72 h of treatment with Cdr68 or Cdr69. H&E staining. (A) negative control; (B and C) cells treated with Cdr68 at concentrations of 0.5 and 5 µg/mL, respectively; (D and E) cells treated with Cdr69 at concentrations of 0.5 and 5 µg/mL, respectively. Changes are indicated by arrows.

Studies carried out with venoms from snakes in the families Elapidae, Crotalidae and Viperidae have shown that these can cause lysis in Yoshida sarcoma cells (BRAGANÇA et al., 1967). Venoms from *Bothrops jararaca* and *C. durissus terrificus*, which belong to the family Viperidae, have been shown to act directly on tumor cells and to induce an inflammatory response mediated by the cytokine TNF-α and chemokine CXCL-8 (DA SILVA et al., 1996).

DA SILVA et al. (1997) assessed *in vivo* the effect of *C. durissus terrificus* venom on the growth of Ehrlich tumor. Although treatment with the venom did not completely eliminate the tumor cells, there was an increase in the animals' survival time and significant macrophage stimulation. The authors suggest that this effect may be due to activation of inflammatory responses.

All possible types of DNA damage can be detected by the alkaline comet assay, while the neutral assay detects mainly double-stranded DNA breaks (OLIVE, 1999).

The alkaline comet assay with MRC-5 cells treated with Cdr68 or Cdr69 showed that these venoms caused less DNA damage than doxorubicin. In the neutral comet assay, there was no statistically significant difference between Cdr68 and doxorubicin, showing that these venoms damage DNA by causing double-strand breaks. Cdr69 was less toxic than Cdr68 and doxorubicin.

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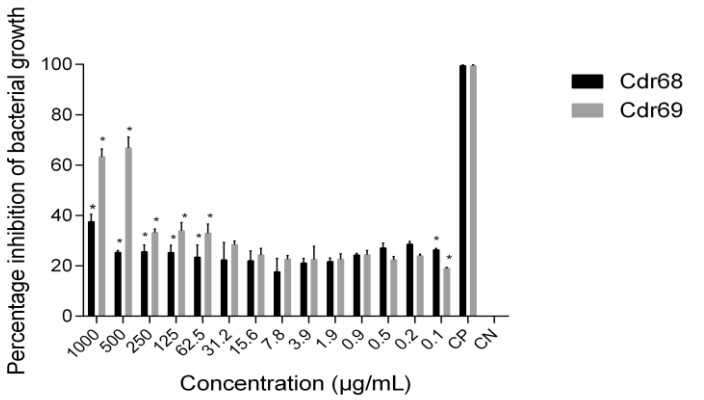

Figure 6. Percentage inhibition by Cdr68 and Cdr69 of growth of the *Staphylococcus aureus* 25923 strain. At concentrations of 1000, 500, 250, 125 and 62.5 µg/mL the Cdr69 yellow venom had greater inhibitory potential than Cdr68 ($p \leq 0.05$). PC - Oxytetracycline (positive control). NC – Mueller Hinton culture medium (negative control).

Analysis of the results of the alkaline and neutral comet assays in the HCT116 cells revealed that Cdr68 and Cdr69 had statistically significantly higher DIs (class 4) than the positive control, showing that they cause DNA damage and double-stranded DNA breaks in in this tumor cell line. Double-stranded DNA breaks represent a threat to genome integrity because they can result in chromosome aberrations that simultaneously affect many genes, leading to cell malfunctioning and, consequently, cell death (VAN GENT et al., 2001). Our results suggest that the cytotoxicity of Cdr68 and Cdr69 is associated with DNA damage. Nevertheless, further studies are required to clarify the cell death mechanisms induced by these venoms.

MARCUSSI et al. (2011) assessed the genotoxicity of *C. durissus terrificus* crude venom and the toxins isolated from this venom to human lymphocytes. In the comet assay, all the toxins tested (crotamine, crotoxin, phospholipase A_2 and crotapotin) and the crude venom exhibited genotoxicity; the toxins, however, exhibited greater genotoxicity than the crude venom. The same authors also evaluated the genotoxicity of the venoms of the snakes *Bothrops jararacussu*, *B. atrox*, *B. moojeni*, *B. alternatus* and *B. brazili* to human lymphocytes. The crude venoms of *B. brazili*, *B. jararacussu* and *B. atrox* all exhibited genotoxic potential, and the latter two induced five times more DNA breaks than the negative control (MARCUSSI et al., 2013).

TAMIETI et al. (2007) reported structural changes in actin filaments, the endoplasmic reticulum and the nucleus as a result of the action of *C. durissus terrificus* venom on the CHO-K1 hamster ovarian cancer cell line. These structural changes and the DNA fragmentation the authors observed suggest that the venom can induce apoptosis.

SOARES et al. (2010) observed morphological changes in RT2 glioma cells and GH3 benign pituitary adenoma cells treated with crude *C. durissus terrificus* venom or crotoxin isolated from this venom. These included irregular cell shapes and cell shrinkage in cells treated with the crude venom. Cells treated with crotoxin exhibited shrinkage, irregular shapes, condensation of the nucleus and the formation of apoptotic bodies, all changes characteristic of apoptosis. OVCAR-8 ovarian carcinoma cells treated with *C. durissus cascavella* venom showed a reduction in cell volume, irregular shapes, nuclear fragmentation, pyknotic nuclei and the formation of apoptotic bodies. Activation of caspases 3 and 7 in these cells confirmed that the venom induces cell death by apoptosis (ARAÚJO et al., 2016).

HCT-116 cells treated with Cdr68 and Cdr69 displayed morphological changes such as cell shrinkage, pyknotic nuclei, condensed chromatin and cytoplasmic vacuolation, and in

some cases cellular remains were observed, indicating apoptosis. However, as this is to our knowledge the first study of morphological changes in cells treated with *C. durissus ruruima* venom, further specific studies are required to clarify the mechanism of death induced by these venoms.

Snake venoms contain a mixture of biologically active substances, each of which may exercise its action separately or jointly with other components, producing a synergic effect (LIPPS, 1995; RANGEL-SANTOS et al., 2004). The use of venoms to treat tumors is therefore not straightforward and can be dangerous as venoms are complex mixtures of proteins and peptides that can affect homeostasis (LU et al., 2005). Some of these difficulties could be overcome, however, if venoms were used with nanoparticles, which have shown great promise for treatment of cancer patients. Nanoparticles bound to anticancer agents can increase the concentration of these agents in specific target tissues and be phagocyted and/or endocyted. Because of this internalization, the drug's efficiency increases and toxicity reduces, increasing its therapeutic index (BARRATT, 2003).

Badr et al. (2014) assessed the effect of *Walterinnesia aegyptia* venom on its own and coupled to nanoparticles on human breast cancer cells isolated from biopsies. In both cases it inhibited proliferation, changed the cell cycle and induced apoptosis, and when bound to nanoparticles it increased the antitumor effect. The IC_{50} for the venom on its own and for the venom bound to the nanoparticles was 50 ng/mL and 20 ng/mL, respectively, indicating the potential of nanoparticle-bound venoms in cancer treatment.

In the present study, Cdr173, Cdr110, Cdr68, Cdr69 and Cdt were also evaluated for antimicrobial activity. While Cdr173, Cdr110 and Cdt did not exhibit any activity against the bacterial strains tested, Cdr69 and Cdr68 both exhibited activity against *S. aureus* 25923, but the latter had the greater activity ($p \leq 0.05$).

The first reports of the antimicrobial activity of snake venoms were in 1948 and 1968, in studies using venoms from snakes in the families Elapidae and Viperidae (GLASER, 1948; ALOOF-HIRSCH et al., 1968). The venoms of *Naja* spp. and *Hemachatus haemachatus* were shown to contain direct lytic factor, indicating that they could break down the phospholipid membranes of *S. aureus* and *E. coli*, respectively (ALOOF-HIRSCH et al., 1968).

Various proteins have been isolated from crotalic venoms, including crotoxin, L-amino acid oxidase and crotamine, and their antimicrobial activity has been investigated and confirmed (OLIVEIRA et al., 2003; TOYAMA et al., 2006; OGUIURA et al., 2011). Further studies of the venoms of snakes in the North of Brazil are therefore needed to gain a better understanding of their action against tumor cells and antibiotic-resistant bacteria. Our research group is already fractionating and isolating proteins from the white and yellow venoms of the Amazonian snake *C. durissus ruruima* and evaluating the cytotoxicity and antibacterial activity of these fractions.

Acknowledgments

The authors would like to express their gratitude to the CNPq (National Council for Scientific and Technological Development) for awarding a productivity grant to Maria Cristina dos Santos (303032/2016-2); to the FAPEAM (State of Amazonas Research Foundation) for providing a doctoral fellowship for Ilia Gilmara Carvalho dos Santos; and to FAPEMIG (State of Minas Gerais Research Foundation) for providing a research and technological development incentive grant for Consuelo Latorre Fortes-Dias.

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