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Genotoxic effect of *Bothrops* snake venoms and isolated toxins on human lymphocyte DNA

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ABSTRACT

In the present study, micronucleus with cytokinesis blocking and comet assays were used to evaluate the genotoxic potential of *Bothrops jararacussu*, *Bothrops atrox*, *Bothrops moojeni*, *Bothrops alternatus* (*Rhinocerophis alternatus*) and *Bothrops brazili* snake venoms, and also of some isolated toxins (MjTX-I, BthTX-I and II myotoxins, BjussuMP-II metalloprotease, and BatxLAAO L-amino acid oxidase) on human lymphocytes. Significant DNA damages were observed, indicating genotoxic potential after exposure of the lymphocytes to the toxins BthTX-I, II and BatxLAAO compared to untreated and Cisplatin-treated controls, which were able to induce greater formation of micronuclei. *B. brazili*, *B. jararacussu* and *B. atrox* crude venoms also presented genotoxic potential, and the latter two induced DNA breakage 5 times more often than in normal environmental conditions (control without treatment). *B. jararacussu* venom and its isolated toxins, as well as an LAAO from *B. atrox*, were able to cause lymphocyte DNA breakage in the comet test with more than 85% damage levels. The DNA damage evaluation allows a widening of the toxic-pharmacological characterization of snake venoms and their toxins and also contributes to the understanding of the mechanisms of action of these molecules in several human pathologies.

1. Introduction

Bothrops snake venoms are mainly composed of enzymes such as phospholipases A_2 (PLA_2s), metalloproteases (SVMPs), and L-amino acid oxidases (LAAOs), that can induce a wide range of toxic effects, such as myotoxicity, hemorrhage, blood coagulation, neurotoxicity, cytotoxicity, edema, cellular apoptosis, genotoxicity, as well as others of medical interest, such as antimicrobial, antiparasitic, antifungal and antiviral activities (Iwanaga and Suzuki, 1979; Kang et al., 2011; Vonk et al., 2011; Marcussi et al., 2011; Soares, 2012).

PLA_2s from *Bothrops* venoms are the main components responsible for cellular damage through the hydrolysis of membrane phospholipids. Those PLA_2s known as myotoxins belong to the IIA group of PLA_2s and may be

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classified into two subgroups: (i) Asp49 myotoxins (for example, *Bothrops jararacussu* BthTX-II), with low to moderate enzymatic activity, and (ii) Lys49 myotoxins (as *B. jararacussu* BthTX-I), which do not show any hydrolytic activity on synthetic substrates (Soares et al., 2004; Lomonte and Gutiérrez, 2011; Lomonte and Rangel, 2012).

BjussuMP-II is a P–I class metalloprotease isolated from *B. jararacussu* venom with molecular mass of 24 kDa, which showed fibrinogenolytic and caseinolytic activities, without presenting hemorrhagic or myotoxic effects (Marcussi et al., 2007). An LAAO from *Bothrops atrox*, named BatxLAAO, is a single-chained glycoprotein with a molecular mass of 67 kDa, pI 4.4 and 12% sugar content. It presents moderate edematogenic activity and does not induce hemorrhage. Moreover, it presents cytotoxic activity on different tumor cells, but not on normal cells (mononuclear cells from peripheral blood) (Alves et al., 2008).

Molecules isolated from venoms show a significant medical-scientific relevance due to their action on cells, and could be used in structural studies in order to improve the understanding of several cell processes and mechanisms. These molecules can also be used as models in to the development of new therapeutic agents that could be used in new therapies for snakebite accidents and pathologies, such as cancer, thrombosis and hypertension (Koh et al., 2006; Lomonte et al., 2010; King, 2011; Koh and Kini, 2012; Soares, 2012).

Considering several papers that describe the therapeutic potential of animal venom toxins for various diseases, the evaluation of their toxicity against human cells is necessary to gauge the difference between non effective, therapeutic or toxic doses for these molecules in order to adjust administration protocols.

Genotoxicity evaluation of venoms and their isolated toxins is a powerful tool for the identification of potential therapeutic agents for the treatment of some cancer types, and shows significant information for the understanding of the mechanisms of action of these toxins. The present work aims to evaluate the genotoxic potential of venoms from *B. jararacussu, Bothrops alternatus* (*Rhinocerophis alternatus*), *B. atrox*, *Bothrops brazili* and *Bothrops moojeni* together with some isolated toxins (BthTX-I, BthTX-II, MjTX-I, BjussuMP-II and BatxLAAO) by micronucleus and comet assays using human lymphocytes.

2. Material and methods

2.1. Chemical agents

Doxorubicin (DXR, Rubidox®, chemical abstract service register number 25316-40-9) was kindly provided by Laboratório Químico Farmacêutico Bergamo Ltda (São Paulo, Brazil). DXR was diluted with distilled water according to manufacturer recommendations. Cisplatin (PLATINIL®) was kindly provided by Quiral Química do Brasil S.A. RPMI 1640 medium, penicillin/streptomycin, phytohemagglutinin and fetal bovine serum were purchased from Cultlab. Cytochalasin B and ethidium bromide were purchased from Sigma Aldrich. All other reagents used were of the highest purity degree.

2.2. Toxins and venoms

Dried crude *Bothrops* venoms were obtained from Bioagents Serpentarium, Batatais-SP, Brazil. Toxins MjTX-I, BthTX-I and II were isolated from *B. moojeni* and *B. jararacussu* snake venom, respectively, as previously described by Andrião-Escarso et al. (2000); BjussuMP-II was isolated from *B. jararacussu* snake venom according to Marcussi et al. (2007); BatxLAAO was isolated from *B. atrox* snake venom as previously described by Alves et al. (2008).

2.3. Micronucleus assay

2.3.1. Cell preparation and materials

Human blood was obtained from 6 healthy volunteers between 18 and 30 years old, women or men, after obtaining their formal consent. Volunteers have not made use of any medication in a minimum period of one month before the blood collection. Briefly, venous blood was collected in heparinized tubes and distributed in fractions of 500 μL per flask for cultivation. Peripheral blood mononuclear cells (PBMCs) were cultivated in total blood RPMI 1640 medium (5 mL) supplemented with 10% fetal bovine serum (FBS, Gibco BRL), 100 U/mL penicillin and streptomycin and 1% phytohemagglutinin (Gibco BRL) in 5% CO_2 at 37 °C. Experiments were approved by the Research Ethics Committee of FCFRP-USP (n° 102).

2.3.2. Cell viability

In order to determine the concentrations of venoms or toxins which would allow the evaluation of the DNA damage without affecting the cell cycles or inducing cell death, cellular viability tests were performed using a concentration response curve before carrying out the micronucleus and comet tests. The toxicity of samples on human lymphocytes, using ficoll®, was assayed using the Trypan blue exclusion method after incubation of cells with samples of *B. jararacussu* snake venom or BthTX-I at the concentrations of 5, 15 and 30 μg/mL for 24 h. Viable cells were determined based on the ability of cells to exclude the dye.

2.3.3. Cytokinesis-block micronucleus (CBMN) test

This test allows the observation of human lymphocytes after one nuclear-division phase in the presence of treatments. Samples were tested at three different concentrations (5, 15 and 30 μg/mL). Three cell culture flasks were used for each concentration/experiment totalizing 6 different volunteers. The mutagenic potential on human cell cultures was analyzed for *B. jararacussu*, *B. alternatus*, *B. atrox*, *B. moojeni* and *B. brazili* crude venoms and isolated toxins (BthTX-I, BthTX-II, BjussuMP-II and BatxLAAO). The samples were added 24 h after the initiation of the cultures. After 44 h, cytochalasin-B (4 μg/mL, Sigma) was added to the cultures. The CBMN test preparations were performed according to Fenech and Morley (1985a,b). The analyses were carried out after 72 h. Scores were taken according to the criteria of Fenech (2000). All slides were coded and scored blindly. Three slides were made for each flask/treatment/experiment, and 1000 binuclear cells were counted considering the presence or absence of micronuclei, this way making it possible to determine the

genotoxic effect of venoms or isolated toxins. Based on the values obtained for the controls that contained only cells and culture media, in which the micronuclei formation mean was of approximately 1.0, mean values higher than 2 micronuclei/1000 binuclear cells (MN/1000 BN cells) were considered significant for the assayed samples. The antineoplastic drug, Cisplatin (PLATINIL®, Quiral Química do Brasil S.A.) (6 μg/mL) was used as positive control.

2.3.4. Cell proliferation index

The cytokinesis-block proliferation index (CBPI) was calculated by counting 500 cells, considering the number of nuclei (mono, bi, tri or tetranucleated). The CBPI defines whether the cultures are multiplying normally after the addition of samples. The following formula was used according to Kirsch-Volders (1997): CBPI = [1 (mono) + 2 (bi) + 3 (tri + tetra)] / 500.

2.4. Comet assay

2.4.1. Nucleoid preparation and comet assay

This test was performed according to the methodology described by Singh et al. (1988). The lymphocytes were cultured in total blood obtained from 6 healthy volunteers and each one corresponded to one experiment. The concentration and incubation times were performed according to Marcussi et al. (2011). Three cell culture flasks were used for each treatment/experiment, and the culture period was of 7 h at 37 °C. The cells were incubated with different treatments for 4 h at 37 °C, and were then utilized to prepare the slides before the first cellular division. A cellular suspension containing approximately 10^5 cells/mL was used to obtain 5–8 million cells per slide. Three slides were made for each flask of each treatment/experiment, although only 100 nucleoids were evaluated per flask/treatment/experiment-volunteer, totalizing 300 nucleoids/treatment/volunteer.

Approximately 60 μL of each cell culture were transferred to microtubes containing 300 μL of LMP (low melting point) agarose, for the slides preparation in triplicate. The mixture was homogenized and dropped on the slides (100 μL/slide) with NMP (normal melting point) agarose and covered with the coverslips. The cells were submitted to 4 °C for 10 min, and after that, the coverslips were removed and the slides immersed in lyses solution (containing 0.25 M NaCl, 100 mM EDTA, 10 mM Trizma base, pH 10 adjusted with 10 M NaOH, 5% DMSO and 1% Triton X-100), remaining there for 2 h, being this procedure responsible for the achievement of the nucleoid. Doxorubicin (Bergamo Ltda) (2 μg/mL) was used as a positive control. All the procedures described above and the electrophoresis were carried out in the dark.

2.4.2. Electrophoresis

Before the electrophoretic run, the slides were kept in electrophoresis solution (300 mM sodium hydroxide and 1 mM EDTA, pH 13) for 20 min at 4 °C. The electrophoretic run was programmed at 25 V and 300 mA, and the run time was fixed as 25 min. After the run, the slides were immersed in neutralization solution (0.4 M Tris–HCl, pH 7.4) for 10 min, dried at room temperature and fixed with 100% ethanol for 3 min.

2.4.3. Staining and analysis

The coloration was performed with ethidium bromide solution at 20 μg/mL. To that end, 100 μL of this solution was placed over each slide, protected from light, covered with a coverslip and immediately analyzed by fluorescence microscopy at 400X. Comet standards were analyzed by visual scores according to Collins et al. (1993), with minimal modifications as previously described (Marcussi et al., 2011). The cells analyzed were classified by DNA injury extent in 5 classes: class 0, without damage (damage <5%); class 1, low level of damage (5–20%); class 2, medium level of damage (20–40%); class 3, high level of damage (40–95%) and class 4, totally damaged (damage> 95%). In order to perform comparative analysis, data were calculated with arbitrary units as described by Collins (2004).

2.5. Statistical analysis

Data are presented as means with standard deviations (mean ± S.D.). A p value of less than 0.05 was deemed to be statistically significant (Kruskal–Wallis).

3. Results

Initially, cell viability tests were performed using a concentration response curve, before carrying out the micronucleus and comet tests, in order to determine the quantities of venoms or toxins which allowed the evaluation of the DNA damage without affecting the cell cycles or inducing cell death. The effective doses chosen were 5, 15 and 30 μg/mL. As positive control the mutagenic and antineoplastic drug Cisplatin was used (6 μg/mL).

The micronucleus test indicated that BthTX-I and BthTX-II from *B. jararacussu* and BatxLAAO from *B. atrox* were potentially genotoxic as there were more than 2 MN/1000 BN cells for a mean of 6 experiments with 30 μg/mL (7.6, 8.7 and 6.6 respectively) (Table 1), suggesting a potential genotoxic effect. Concerning the crude venoms, only *B. jararacussu* and *B. atrox* showed to be potentially genotoxic, yielding (at 30 μg/mL) an average of 6 and 7.3 MN/1000 BN cells in 6 experiments performed with lymphocytes isolated from the blood of the 6 volunteers (Table 2). These results confirm the significance of myotoxins and LAAOs in the composition of *B. jararacussu* and *B. atrox* venom. The mean values obtained for the cytokinesis-blocking proliferating index (CBPI) show that the treated cultures were similar to those of the untreated cultures (negative controls), demonstrating that toxin and venom concentrations used were not cytotoxic to cells and therefore do not interfere with their development and division (Tables 1 and 2), as observed for *Crotalus durissus terrificus* snake venom and its isolated toxins at concentrations of 1, 3 and 6 μg/mL (Marcussi et al., 2011). Data showed no significant differences between male and female regarding the induction of micronuclei, allowing the pooling of results in Tables 1 and 2.

Although it is not possible to determine precisely whether there was an apoptotic or necrotic effect of the toxins on the lymphocytes, significant morphologic differences between cells after the treatments were observed. In the slide related to BthTX-I and BthTX-II (15 and 30 μg/mL),

Table 1
Distribution of micronuclei and cytokinesis-blocking proliferating index (CBPI) in cells treated with different toxins from *Bothrops* venoms or with the antitumor agent Cisplatin (6 μg/mL).

Treatments (μg/mL)		% Cell/500 cells				MN/1000 BN cells, mean ± S.D.	CBPI ± S.D.
		Mono	Bi	Tri	Multi		
Cisplatin	6	38.7	33.8	15.3	12.2	13.8 ± 0.15	1.89 ± 0.01
Negative control	–	35.6	34.6	16.7	13.1	1.0 ± 0.05	1.94 ± 0.06
BthTX-I	5	39.3	36.1	15.8	8.8	3.8 ± 0.03[a,b]	1.85 ± 0.03
	15	32.8	38.6	15.2	13.4	5.4 ± 0.07[a,b]	1.96 ± 0.04
	30	36.8	36.6	13.8	12.8	7.6 ± 0.09[a]	1.89 ± 0.02
BthTX-II	5	34.1	37.9	15.2	12.8	3.9 ± 0.08[a,b]	1.94 ± 0.02
	15	36.8	36.5	15.9	10.8	6.5 ± 0.08[a,b]	1.90 ± 0.05
	30	38.5	37.9	13.6	10.0	8.7 ± 0.2[a]	1.85 ± 0.03
BjussuMP-II	5	34.6	39.7	13.2	12.5	1.0 ± 0.02[b]	1.91 ± 0.02
	15	34.5	39.7	13.0	12.8	1.1 ± 0.02[b]	1.91 ± 0.04
	30	33.9	39.6	13.8	12.7	1.5 ± 0.06[b]	1.92 ± 0.08
BatxLAAO	5	39.9	14.7	11.7	33.7	1.6 ± 0.03[a,b]	1.93 ± 0.03
	15	39.0	16.5	10.5	33.9	4.3 ± 0.18[a,b]	1.93 ± 0.05
	30	43.1	14.1	10.5	32.2	6.6 ± 0.12[a,b]	1.92 ± 0.06

BN: binucleated cells; MN: micronuclei; CBPI: cytokinesis-blocking proliferating index. 1000 binuclear cells were analyzed per volunteer for each treatment, with six volunteers. Data are represented as means ± S.D. The values of CBPI were not statistically different compared to the control ($p < 0.05$).
[a] Significantly different from the negative control ($p < 0.05$).
[b] Significantly different from the positive control, Cisplatin ($p < 0.05$).

approximately 5% of the analyzed cells were deformed, possibly presenting necrotic nuclei (data not shown).

The myotoxin isolated from *B. moojeni* (MjTX-I) did not show high rates of DNA damage when assayed by the comet test, however, its genotoxic potential was revealed when these rates were compared with the results obtained for the negative control. The damages observed in the DNA of lymphocytes were most pronounced after treatment with the crude venoms from *B. jararacussu* and *B. atrox* and the toxins BthTX-I, II and BatxLAAO. The standardization of the comet assay for the evaluation of snake venom toxins was performed according to Marcussi et al. (2011). The concentration chosen (7.5 μg/mL) did not induce cell death but resulted in DNA damage. In this test, the isolated toxins showed similar results to the positive control. However, BjussuMP-II induced more genotoxicity than the control drug, doxorubicin, at the concentration used. In contrast, BatxLAAO induced lower damage than that observed for the positive control, but greater damage than that obtained for the culture without treatment (negative control). The values of arbitrary units calculated according to Collins (2004) clearly show significant differences between controls and treatments.

Crude venoms from *B. jararacussu* and *B. brazili* showed similar genotoxicity to that of isolated toxins, but *B. alternatus*, *B. atrox* and *B. moojeni* crude venoms showed no statistical differences in relation to the negative control (Table 3). The obtained results suggest that venoms from different species belonging to the same genus present different genotoxic properties.

Table 2
Distribution of micronuclei and cytokinesis-blocking proliferating index (CBPI) in cells treated with different *Bothrops* venoms or with the antitumor agent Cisplatin (6 μg/mL).

Treatments (μg/mL)		% Cell/500 cells				MN/1000 BN cells, mean ± S.D.	CBPI ± S.D.
		Mono	Bi	Tri	Multi		
Cisplatin	6	45.4	42.2	9.2	3.2	14.1 ± 0.03	1.67 ± 0.02
Negative control	–	41.5	40.3	10.6	7.6	1.2 ± 0.05	1.77 ± 0.01
B. jararacussu	5	37.6	35.8	13.8	12.8	1.7 ± 0.02[b]	1.89 ± 0.03
	15	39.9	40.4	14.1	5.6	2.8 ± 0.01[a,b]	1.80 ± 0.02
	30	41.4	40.2	12.8	5.6	6.0 ± 0.01[a,b]	1.77 ± 0.07
B. alternatus	5	40.6	37.2	13.2	9.0	1.0 ± 0.05[b]	1.82 ± 0.03
	15	39.0	36.2	14.0	10.8	1.5 ± 0.07[b]	1.86 ± 0.05
	30	41.8	33.4	13.5	11.3	2.0 ± 0.03[a,b]	1.83 ± 0.06
B. moojeni	5	46.4	36.5	10.2	6.9	1.1 ± 0.02[b]	1.71 ± 0.04
	15	45.1	38.4	11.3	5.2	2.3 ± 0.01[a,b]	1.71 ± 0.02
	30	40.1	40.7	8.5	10.7	2.7 ± 0.02[a,b]	1.79 ± 0.01
B. brazili	5	43.1	41.2	7.4	8.3	2.3 ± 0.04[a,b]	1.73 ± 0.03
	15	41.4	38.6	8.6	11.4	3.7 ± 0.02[a,b]	1.79 ± 0.04
	30	38.9	39.5	12.5	9.1	4.1 ± 0.03[a,b]	1.83 ± 0.04
B. atrox	5	38.5	44.6	10.6	6.3	2.5 ± 0.05[a,b]	1.78 ± 0.07
	15	42.3	38.1	11.4	8.2	4.2 ± 0.08[a,b]	1.77 ± 0.05
	30	40.5	43.8	9.8	5.9	7.3 ± 0.04[a,b]	1.75 ± 0.08

[a] Significantly different from the negative control ($p < 0.05$).
[b] Significantly different from the positive control Cisplatin ($p < 0.05$).

Table 3
Frequencies of cells with comet and distribution of comet classes after treatment with *Bothrops* snake venoms, isolated toxins and the antitumor agent doxorubicin (DXR, 2 μg/mL).

Treatments (7.5 μg/mL)	Cells with comet (%)	Arbitrary units (0–400)	Comet classes (%)				
			0	1	2	3	4
Negative control	27.75	28.75	72.25	26.75	1	0	0
Positive control (DXR)	69.25	121.5	30.75	37	18	8.5	5.75
Toxins							
BthTX-I	70.25[a]	108.25	29.75	45.5	14.75	6.75	3.25
BthTX-II	67.25[a]	97.75	32.75	49	9.25	5.75	3.25
MjTX-I	32.5[b]	44	67.5	23.5	6.5	2.5	0
BjussuMP-II	85.25[a,b]	153.75	14.75	37.5	31.5	11.75	4.5
BatxLAAO	57.5[a,b]	80.25	42.5	45.25	5.5	3	3.75
Venoms							
B. jararacussu	67[a]	110	33	40	15	8	4
B. alternatus	36[b]	48	64	30	1	4	1
B. atrox	38[b]	38	62	38	0	0	0
B. moojeni	33[b]	36	67	30	3	0	0
B. brazili	56[a]	66	44	48	6	2	0

Data represent the mean of each treatment ± S.D. for 6 individual experiments, one for each volunteer. Three flasks were prepared for each treatment and for each volunteer's blood, with cells remaining in culture for 7 h. 100 nucleoids were observed by flask, with three flasks per treatment/volunteer resulting in a total of 300 nucleoids.
Arbitrary units (0–400) calculated according to Collins (2004).
[a] Significantly different from the negative control ($p < 0.05$).
[b] Significantly different from the positive control DXR ($p < 0.05$).

4. Discussion

In a previous paper, the micronucleus method was applied in human lymphocytes in order to evaluate the genotoxic potential of *C. durissus terrificus* snake venom and its isolated toxins and the results showed significant DNA damage production (Marcussi et al., 2011). The presence of micronuclei indicated breakage and/or aneuploid induction in DNA produced by the venom and toxins tested, showing that these breakages were not likely to be corrected during the process of checking and cell repair resulting in permanent damage that could produce changes in cell morphology or physiology, and may also be transmitted hereditarily to future generations (Fenech and Morley, 1985a,b; Fenech, 2007, 2008).

The chromosome damage observed in genotoxic assays performed with animal venoms showed that these toxins may possibly be used in the development of new therapeutic strategies for cancer control. There are some interesting examples with venoms from scorpions, bees and snakes (Zargan et al., 2011; Lee et al., 2007; Varanda et al., 1999; Wang et al., 2000; Wang and Groopman, 1999; Lerda et al., 2005; Brugger et al., 2006; Dönmez-Altuntas et al., 2007).

The obtained results suggest that different toxins could induce breakages in DNA by different ways, which is corroborated by the results obtained with BthTX-I, BthTX-II and BatxLAAO, which resulted in permanent breakages likely to be observed in the micronucleus assay. Conversely, the high rate of DNA breakage induced by BjussuMP-II is not maintained after the action of cell repair system, as observed in the micronucleus assay. Interesting, BthTX-I is an enzymatically inactive PLA_2-like enzyme and showed similar mutagenic effect to BthTX-II, which is catalytically active, suggesting that the genotoxicity is not related to the catalytic activity.

The mechanisms of action of snake venom genotoxicity are not yet elucidated. The production of free radicals induced by some toxins is a valuable hypothesis that should be considered, since they participate in inflammatory processes and the mediators are intimately related to the oxidative stress. However, the apoptosis induction cannot be discarded considering the high number of published works describing this effect for different classes of toxins such as LAAOs, metalloproteases and PLA_2s (Iwanaga and Suzuki, 1979; Kang et al., 2011). Corroborating this hypothesis, the induction of oxidative stress has been described for some snake venoms and isolated toxins (Zhang and Cui, 2007; Yamasaki et al., 2008). This effect can also be associated with the DNA damage induced by venom toxins, through the formation of free radicals that could induce genotoxicity and, in high levels, even mutagenicity or cellular apoptosis. The induction of micronuclei and DNA damage of lymphocytes observed after cell exposure to different concentrations of an LAAO from *B. atrox* showed in the present work are an indication that substantiates the hypothesis cited above. Future experiments using anti-oxidant agents, together with the toxins could elucidate the suggested mechanism, as showed for zearalenone (Ouanes et al., 2003).

The venoms from *B. brazili* and *B. atrox* did not induce DNA damage when assayed by the comet test, however, both showed genotoxic potential when assayed by the micronucleus test. It is possible to conclude that the time of incubation with the cells, which was of 4 h, was not enough to induce damage during the comet assay in contrast to the 48 h of incubation used in the micronucleus test.

Although various proteins from animal venoms have been isolated and characterized enzymatically, pharmacologically, toxicologically and/or structurally, the knowledge concerning their biotechnological potential is still very scarce, and each new research developed opens up new possibilities of potential uses for the development of future medications, which could bring fewer collateral effects with major efficiency for the treatment of many degenerative diseases (Koh et al., 2006; Lomonte et al., 2010; King, 2011; Kang et al., 2011; Koh and Kini, 2012).

5. Conclusion

The present work demonstrates the genotoxic potential of *B. jararacussu*, *B. brazili* and *B. atrox* venoms, as well as the isolated toxins BthTX-I, BthTX-II, BjussuMP-II and BatxLAAO. Concentrations up to 5 μg/mL were able to induce breakage in the DNA of human lymphocytes in the tested conditions. The micronucleus test demonstrates the perpetuation of DNA breakage in the first cell generation produced after the treatment, showing that the DNA breaks were maintained even after the action of the cellular repair systems. These results could also be related to other pharmacological and toxic activities induced by venoms and toxins, being useful for the elucidation of their mechanisms of action.

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Conflict of interest statement

The work does not show any conflict of interest.

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