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Cytotoxic, genotoxic/antigenotoxic and mutagenic/antimutagenic effects of the venom of the wasp *Polybia paulista*

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ABSTRACT

Hymenoptera venoms are constituted by a complex mixture of chemically or pharmacologically bioactive agents, such as phospholipases, hyaluronidases and mastoparans. Venoms can also contain substances that are able to inhibit and/or diminish the genotoxic or mutagenic action of other compounds that are capable of promoting damages in the genetic material. Thus, the present study aimed to assess the effect of the venom of *Polybia paulista*, a neotropical wasp, by assays with HepG2 cells maintained in culture. The cytotoxic potential of the wasp venom, assessed by the methyl thiazolyl tetrazolium assay (MTT assay), was tested for the concentrations of 10 μg/mL, 5 μg/mL and 1 μg/mL. As these concentrations were not cytotoxic, they were used to evaluate the genotoxic (comet assay) and mutagenic potential (micronucleus test) of the venom. In this study, it was verified that these concentrations induced damages in the DNA of the exposed cells, and it was necessary to test lower concentrations until it was found those that were not considered genotoxic and mutagenic. The concentrations of 1 ng/mL, 100 pg/mL and 10 pg/mL, which did not induce genotoxicity and mutagenicity, were used in four different treatments (post-treatment, pre-treatment, simultaneous treatment with and without incubation), in order to evaluate if these concentrations were able to inhibit or decrease the genotoxic and mutagenic action of methyl methanesulfonate (MMS). None of the concentrations was able to inhibit and/or decrease the MMS activity. The genotoxic and mutagenic activity of the venom of *P. paulista* could be caused by the action of phospholipase, mastoparan and hyaluronidase, which are able to disrupt the cell membrane and thereby interact with the genetic material of the cells or even facilitate the entrance of other compounds of the venom that can act on the DNA. Another possible explanation for the genotoxicity and mutagenicity of the venom can be the presence of substances able to trigger inflammatory process and, consequently, generate oxygen reactive species that can interact with the DNA of the exposed cells.

1. Introduction

Each year, thousands of new substances are synthesized and released into the environment and avoid the exposure to them is practically impossible. Several studies correlate the exposure of living organisms with the induction of

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damages in their genetic material. For this reason, several studies have been developed aiming to find substances that can protect the DNA from damages caused by xenobiotics.

Hymenoptera venoms, such as bees and wasps, have in their composition substances with antimicrobial action, cytolytic peptides and a complex mixture of enzymes, neurotoxins and low molecular weight compounds (Kuhn-Nentwig, 2003). According to Santos et al. (2007), there is almost 500 species of social wasps in Brazil, of which little is known about the biochemistry, pharmacology and immunology of their venoms. Venoms of the Vespidae family (wasps) contain phospholipases A and B, as well as hyaluronidases, acid phosphatases, proteases and mastoparans (Nakajima et al., 1985; King and Valentine, 1987).

Several studies have described the presence of substances with pharmacological potential in wasp venoms, and among them some with antimicrobial (Čeřovský et al., 2008), anticonvulsant (Cunha et al., 2005) and anticoagulant potentials (Han et al., 2008). These studies have also shown that Hymenoptera venoms can constitute a rich and promising study area for the discovery of new biopharmaceuticals, among them those that have the ability to decrease and/or avoid mutations in the genetic material.

Polybia paulista is a Neotropical wasp that is endemic to south-eastern Brazil, of very aggressive behaviour that, due to its stings, causes many accidents in the region (Santos et al., 2007). Studies made with the venom of this species verified that it has in its composition substances with antimicrobial (Souza et al., 2005, 2009) and antitumour potential (Wang et al., 2008).

This study aimed to evaluate the cytotoxicity (ability to induce the cell death); genotoxicity (ability to induce damages in the DNA, which can be repaired or not) and antigenotoxicity (ability to prevent damages in the DNA); mutagenicity (ability to induce mutations or increase their frequency) and antimutagenicity (ability to prevent mutations) of the venom of the wasp *P. paulista*, by assays with human cells maintained in culture (HepG2).

2. Material and methods

2.1. Material

2.1.1. Substance used

2.1.1.1. Wasp venom (WV). Wasps of the species *P. paulista* were identified and kindly provided by the Centre for the Study of Social Insects (Centro de Estudos de Insetos Sociais – CEIS) of the Institute of Biosciences from the Universidade Estadual Paulista (UNESP), campus of Rio Claro. After the capture, the insects were immediately frozen at −80 °C to be dissected later. To obtain the venom, 1160 venom glands were extracted with the aid of tweezers. The glands were carefully washed, perforated and gently agitated in a solution containing 1 mM of protease inhibitor (PMSF – phenylmethylsulphonyl fluoride) and centrifuged at 8000 rpm, for 10 min at 4 °C. The supernatant was used as crude extract of the venom. These samples were lyophilized in lyophilizer "Centrivap Concentrator" (Labconco), coupled to a condenser solvent "Centrivap Ultra Low Cold Trap" (Labconco).

2.1.2. Experimental model

2.1.2.1. Human cell culture. HepG2 cells were isolated from a human hepatoblastoma and they retained the activity of certain enzymes of phase I and phase II, enzymes that are related to the activation and detoxification of genotoxic carcinogens and, thus, have been widely used in genotoxicity studies (Uhl et al., 1999, 2000).

The cells were obtained from the American Type Culture Collection (ATCC No HB 8065, Rockville, MD) and were grown in culture flasks of 25 cm^2 in 5 mL of MEM (Minimum Essential Medium – Cultilab), supplemented with 10% of foetal bovine serum (FBS) and 0.1% of antibiotic-antimycotic solution (penicillin 10.000 U.I./mL/streptomycin 10 mg/mL, Cultilab) in CO_2 incubator (5%), until they reached confluence.

2.2. Methods

2.2.1. MTT test

The MTT test (Thiazolyl Blue Tetrazolium Bromide – CAS n. 298-93-1, Sigma) with HepG2 cells was performed according to the protocol of Mosmann (1983), with some modifications. In each well of a 96 well plate, 2.34×10^4 cells were seeded. Subsequently, this plate was incubated for 24 h for stabilization of the cells. After this period, the medium was removed from the wells and it was added 200 uL of culture medium (without serum) in the negative control (NC), culture medium without serum plus Triton X-100 at 1% in the positive control (PC) and culture medium without serum plus the treatments (different concentrations of the wasp venom). After 3 h of incubation, the treatments were removed from the wells and it was added 150 uL of a solution of 5 mg/mL of MTT. The plate was incubated for 4 h, in incubator at 37 °C. After this period, the MTT solution was discarded and it was added, in each well, 100 μL of dimethyl sulfoxide (DMSO).

The plates were then read in spectrophotometer with microplate reader (Apparatus Multiskan FC – Thermo Scientific) in filters of 540 nm. The statistical analysis was performed by the ANOVA parametric statistic test (1 way), followed by the Dunnet's comparison test ($p < 0.05$).

2.2.2. Comet assay with HepG2 cells

The comet assay was performed to evaluate the genotoxic and antigenotoxic potential of the wasp venom and it was made according to the protocol described by Singh et al. (1988) and Tice et al. (2000), with some modifications. The assays were conducted in triplicate/treatment.

For the genotoxicity and antigenotoxicity assay, 5×10^5 cells were seeded in culture flasks of 25 cm^2. The flasks were incubated for 24 h in incubator at 37 °C, 5% CO_2 in humid atmosphere, for a stabilization period. After this period, two evaluations were made, one to assess the genotoxicity, where the cells were exposed to different concentrations of the wasp venom for 3 h, and the other to evaluate the antigenotoxicity, where four different types of treatment were performed:

- pre-treatment (PT): the cells were exposed to the different concentrations of the wasp venom for 3 h. After this period, the medium was removed and the cells were exposed to new culture medium containing methyl methanesulfonate (MMS, CAS No 66-27-3), at the concentration of 4×10^{-2} M, for another 3 h;
- post-treatment (PostT): the cells were exposed to MMS (4×10^{-2} M) for 3 h. After this period, the medium was removed and the cells were exposed to new culture medium containing different concentrations of the wasp venom for another 3 h;
- simultaneous treatment (ST): the cells were exposed simultaneously to MMS (4×10^{-2} M) and to the different concentrations of the wasp venom for 3 h;
- simultaneous treatment with incubation (STI): MMS (4×10^{-2} M) was previously incubated, during 1 h at 37° with the wasp venoms and after this incubation period, the cells were exposed to this mixture, for 3 h.

Besides these treatments, it was also performed the negative control (NC, 50 μL of PBS) and positive control (PC, MMS, 4×10^{-2} M).

After the treatment periods, both for the genotoxicity and antigenotoxicity evaluation, the cells were collected and, after obtaining the cell suspension, were subjected to the cell viability test with Trypan Blue (Gibco), according to the methodology described by Salvadori et al. (2003). For this evaluation, 5 μL of the cell suspension was mixed with 5 μL of Trypan Blue, where it was counted 100 cells of each treatment. The cells stained in white were considered live and the ones stained in blue dead.

After counting the cell viability, 20 μL of the cell suspension was mixed to 120 μL of low melting point agarose at 37 °C. Then, this cell suspension was placed on slides previously coated with normal agarose and covered with coverslips. After a brief period of solidification at 4 °C (15 min), the coverslips were removed and the slides incubated in lysis solution (1 mL of Triton X-100, 10 mL of DMSO and 89 mL of lysis stock – NaCl 2.5M, EDTA 100 mM, Tris 10 mM and ~8 g of NaOH, pH = 10), in the dark, at 4 °C, for, at least, 1 h. After lysis, the slides were transferred to an electrophoresis vat and covered with an alkaline buffer (NaOH 300 mM + EDTA 1 mM, pH > 13), where they remained for 20 min for stabilization. After this period, they were subjected to electrophoresis at 39 V, 300 mA (~0.8 V/cm) for 20 min. After the electrophoresis period, the slides were removed and neutralized in Tris buffer (0.4 M Trizma Hydrochloride, pH 7.5), fixed in absolute ethanol for 10 min and stored at 4 °C, until the time of analysis. The slides were stained with 50 μL of GelRed® solution (15 μL of GelRed 10,000× in water, 5 mL of NaCl at 1M, and 45 mL of distilled water) and immediately analysed after staining. It was analysed, in Leica epifluorescence microscopy, magnification of 400×, filter B – 3^4 (excitation: i = 420 nm–490 nm, barrier: I = 520 nm), 100 nucleoids per slide, totalling 600 nucleoids per treatment. The nucleoids were visually classified and allocated in one of the four classes (0, 1, 2, 3) according to the migration of the fragments as follows: class 0, no tail; class 1, small tail with size smaller than the diameter of the head (nucleus); class 2, size of the tail equal to the diameter of the head or even twice the diameter of the head and class 3, tail larger than the diameter of the head (Rigonato et al., 2005).

The total score was obtained by multiplying the number of cells in each class by the class damage, according to the formula: Total score = $(0 \times n_1) + (1 \times n_2) + (2 \times n_3) + (3 \times n_3)$, where n = number of cells in each class analysed. Thus, the total score could vary from 0 to 300. The statistical analysis was performed by the ANOVA parametric statistic test (1 way), followed by the Dunnet's comparison test ($p < 0.05$).

2.2.3. Cytokinesis-blockage MN test

The cytokinesis-blockage micronucleus test (CBMN), used to evaluate the mutagenicity and antimutagenicity of different concentrations of the wasp venom, was performed according to the protocol described by Natarajan and Darroudi (1991), with some modifications.

For the mutagenicity and antimutagenicity test, 5×10^5 cells were seeded in culture flasks of 25 cm^2. The flasks were incubated for 24 h in incubator at 37 °C, 5% CO_2 in humid atmosphere, for a stabilization period. After this period, two evaluations were made, one to assess the mutagenicity, where the cells were exposed to the different concentrations of the wasp venom for 3 h and another to assess the antimutagenicity, performed by 4 different types of treatment, as already described for the comet assay.

After the treatments, both for the evaluation of the mutagenicity and antimutagenicity, the cells were washed with PBS. The medium was changed and it was added to the culture cytochalasin B (final concentration of 3 μg/mL). The cells were then incubated for more 28 h, collected, treated with hypotonic solution (sodium citrate 1%) and fixed with formaldehyde (40%) and ethanol-acetic acid (3:1). The slides were stained with Giemsa 5% for 8 min.

It was counted 2000 binucleated cells per replica, and it was counted micronuclei, nucleoplasmic bridges and nuclear buds (Fenech and Crott, 2002). The analysis of the significance of the results was made by the ANOVA (one way) parametric statistic test, followed by the Dunnet's comparison test ($p < 0.05$).

3. Results

3.1. MTT assay

The MTT test is a cytotoxicity assay in which it is measured the cell viability. This test is based on the ability of the viable cells on converting the MTT salt (Thyazolyl Blue Tetrazolium Bromide). This salt is soluble in water and is converted, by the viable cells, into an insoluble salt of purple colouration. This product cannot cross the cell membranes of viable cells, and, therefore, accumulates in their interior (Fotakis and Timbrell, 2006).

In this study it was tested the concentrations of 160 μg/mL, 80 μg/mL, 40 μg/mL, 20 μg/mL, 10 μg/mL, 5 μg/mL, 1 μg/mL of the venom of the wasp *P. paulista*. The results obtained for the MTT assay are shown in Table 1, Fig. 1.

From the results obtained, it was observed that the concentrations of 10 μg/mL, 5 μg/mL and 1 μg/mL were not statistically different from the negative control. Thus, it could be inferred that these concentrations were not

Table 1
Cell viability by the MTT assay performed with HepG2 cells exposed to different concentrations of the venom of the wasp *P. paulista*.

Treatments	Viability% (mean ± SD)
NC	100 ± 0.020
PC	14.1 ± 0.002
160 μg/mL	34.2 ± 0.075*
80 μg/mL	54.2 ± 0.048*
40 μg/mL	72.7 ± 0.035*
20 μg/mL	77.2 ± 0.028*
10 μg/mL	89.4 ± 0.008
5 μg/mL	98.7 ± 0.031
1 μg/mL	99.4 ± 0.04

SD = standard deviation; NC = negative control; PC = positive control. Values followed by * are statistically significant in relation to the negative control negativo (ANOVA/Dunnet, $p < 0.05$).

cytotoxic for the cells exposed, after the period of 3 h of exposure. The other concentrations were significant in relation to the negative control, showing cytotoxicity and, therefore, were not suitable for use in the genotoxicity and mutagenicity tests, since these tests need viability equal or superior to 80% to be performed.

3.2. Comet assay

By the results obtained with the MTT assay, it was chosen, to carry out the initial evaluation of the genotoxicity of the wasp venom, the concentrations of 1, 5 and 10 μg/mL of this substance. The results are shown in Table 2. Since these concentrations were genotoxic, new concentrations were tested in order to find concentrations that did not induce genotoxic damages (0.5 μg/mL, 0.1 μg/mL, 0.05 μg/mL, 0.01 μg/mL, 1 ng/mL, 100 pg/mL and 10 pg/mL).

From the results obtained, it was observed that the concentrations of 1 ng/mL, 100 pg/mL and 10 pg/mL were not statistically significant in relation to the negative control. Thus, these three concentrations were used in the assessments of the antigenotoxic potential of the wasp venom. The data concerning the antigenotoxic evaluation are shown in Table 3.

By the results observed, none of the concentrations tested (1 ng/mL, 100 pg/mL and 10 pg/mL) was effectively able to decrease and/or inhibit the genotoxic action of MMS.

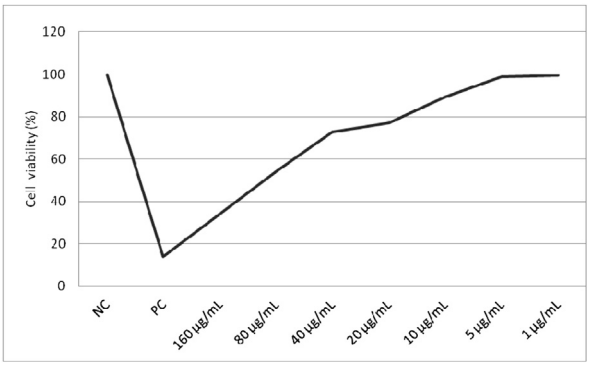

Fig. 1. Viability (%) of the HepG2 cells exposed to different concentrations of the venom of the wasp *P. paulista*. NC = negative control; PC = positive control; 160 μg/mL, 80 μg/mL, 40 μg/mL, 20 μg/mL, 10 μg/mL, 5 μg/mL, 1 μg/mL = concentrations tested of the venom of *P. paulista*.

3.3. Cytokinesis-blockage MN test (CBMN)

The same concentrations used in the comet assay were also used to evaluate the mutagenicity of the wasp venom (10 μg/mL, 5 μg/mL, 1 μg/mL, 0.5 μg/mL, 0.1 μg/mL, 0.05 μg/mL, 0.01 μg/mL, 1 ng/mL, 100 pg/mL and 10 pg/mL). The results are shown in Table 4.

As for the genotoxic damages, the concentrations of 1 ng/mL, 100 pg/mL and 10 pg/mL were not statistically significant in relation to the negative control. Thus, these three concentrations were selected to be used in the evaluations of the antimutagenic potential of the wasp venom (Table 5).

Likewise in the antigenotoxicity assay, none of the concentrations tested was able to inhibit and/or decrease the mutagenicity induced by MMS, therefore, they were not considered good antimutagenic agents.

4. Discussion and conclusion

Venoms of social wasps are rich in biogenic amines, biologically active peptides and proteins (Lorenzi, 2002; Nakajima et al., 1986). Among these substances it can be highlighted the phospholipases, hyaluronidases and mastoparans.

In the present study it was observed that concentrations above 10 μg/mL are able to induce death of the HepG2 cells, and the concentration of 80 μg/mL was capable of inducing the death of approximately 50% of the cells. We highlight, therefore, that it is very difficult to occur exposure to this concentration, since in a single sting of vespids it can be injected into the skin only about 20 μg of the venom. This concentration can, according to Reisman and Livingston (1992), be enough to trigger the sensitization process in human beings. However, from our results the concentration of 20 μg/mL did not induce high cytotoxicity for the exposed cells. Our results also showed that, although concentrations lower than 17 μg (10 μg/mL, 5 μg/mL, 1 μg/mL, 0.5 μg/mL, 0.1 μg/mL, 0.05 μg/mL, 0.01 μg/mL) had not induced cytotoxicity for the HepG2 cells, they present a genotoxic and mutagenic potential for these cells. This capacity may have been triggered as a result of the action of several proteins present in the venom on the cell membrane, which can lead to an alteration in the permeability of these cellular structures. This alteration in the permeability may allow the entrance of several compounds into the cell and their interaction with the DNA, causing mutations.

Among the proteins that are able to modify the cell permeability, are the hyaluronidases. Hyaluronidases are glycosidases (El-Safory et al., 2010) capable of hydrolysing the hyaluronic acid and, thus, digest partially the extracellular matrix (Hoffman, 2006), increasing the infiltration and, possibly, the action of other compounds of the venom on cellular structures. The hyaluronic acid is a polysaccharide of high molecular weight found in the extracellular matrix, especially in connective tissues. This polysaccharide is known as a “lubricant” responsible for the viscoelastic properties of fluid tissues and as a stabilizing

Table 2
Comet assay with HepG2 cells exposed to different concentrations of the venom of the wasp *P. paulista*.

Treatments	Number of cells in each comet class				Damage score (mean ± SD)	Cell viability % (mean ± SD)
	Migration categories (mean ± SD)					
	0	1	2	3		
NC	93.6 ± 1.53	1 ± 1	2.33 ± 0.57	3 ± 0	14 ± 1	96 ± 0.016
PC	0.33 ± 0.58	3.67 ± 1.15	6.33 ± 1.15	89.67 ± 1.53	285.33 ± 2.08*	97 ± 0.015
10 μg/mL	68.67 ± 3.06	13.67 ± 6.11	4 ± 1	13.67 ± 2.08	62.67 ± 2.52*	96.43 ± 0.004
5 μg/mL	70.67 ± 4.16	4.67 ± 0.58	4.33 ± 0.58	20.33 ± 3.51	73.33 ± 10.07*	96 ± 0.017
1 μg/mL	76.67 ± 1.53	7.67 ± 0.58	4.67 ± 2.52	11 ± 1	50 ± 3.46*	96.03 ± 0.02
0.5 μg/mL	80.33 ± 10.07	6.33 ± 2.89	3.67 ± 2.08	9.67 ± 7.37	42.67 ± 24.54*	95 ± 0.018
0.1 μg/mL	89.33 ± 2.52	4.33 ± 1.15	3.33 ± 2.08	3 ± 1	22 ± 8.54*	95.73 ± 0.021
0.05 μg/mL	89.5 ± 1.38	4.5 ± 0.84	2 ± 1.26	4 ± 0.63	20.5 ± 2.58*	95.18 ± 0.068
0.01 μg/mL	92.5 ± 1.38	1.33 ± 1.21	1.5 ± 1.04	4.66 ± 1.03	18.33 ± 2.87*	97.17 ± 0.019
1 ng/mL	92 ± 2	1.33 ± 1.03	2.16 ± 0.75	3.66 ± 0.81	16.6 ± 1.21	98.65 ± 0.005
100 pg/mL	93 ± 1.55	2.33 ± 1.63	1.16 ± 0.41	3.5 ± 1.76	15.16 ± 4.79	98 ± 0.005
10 pg/mL	95.5 ± 1.87	1.83 ± 1.33	0.66 ± 0.51	2.5 ± 1.37	10.66 ± 2.84	99 ± 0.005

NC = negative control, PC = positive control, SD = standard deviation. Damage score: 0 = no damage; 300 = maximum damage; * = statistically significant in relation to the negative control (ANOVA/Dunnet, $p < 0.05$).

and moisturizing agent of connective tissues (El-Safory et al., 2010). According to Wahby et al. (2012), hyaluronidase increases the permeability of the cell membranes and causes a reduction in the viscosity of the fluids injected into the tissues.

Another protein that can be related to the mutagenicity of the wasp venom is phospholipase. Phospholipases are proteins that also have action on the lipid bilayer of the cells, by disrupting the phospholipids of the biological membranes, since they can catalyse the hydrolysis of ester bonds at specific positions of the 1,2-diacyl-3,*sn*-phosphoglyceride, releasing fatty acids (Santos et al., 2007). According to Aoki et al. (2007), some phospholipases A (phospholipase A_1 – PLA_1) can hydrolyse both phospholipids and triacylglycerols, as well as galactolipids. But there are also some PLA_1 that only hydrolyses phosphatidylserine and phosphatidic acid. Denaturation of the phospholipids leads to the formation of pores in the membrane, allowing an easier entrance of other compounds into the cells, leading to cell lysis, inflammation and tissue damages (Dotimas and Hider, 1987). *P. paulista* presents phospholipase A_1 that has direct haemolytic action in erythrocytes (Santos et al., 2007).

Mastoparans, the main components of the vespid venoms (Souza et al., 2009), seem to promote the formation of ionic channels in the lipid membranes, leading to cell lysis (Li et al., 2000). These compounds also increase the permeability of the membrane to ions and small molecules, by forming pores when in high concentrations. According to Gusovsky et al. (1991), this action is due, probably, to the interaction of the mastoparans with the guanine nucleotide binding protein, so that there is a collapse of phosphoinositol. Furthermore, mastoparans can stimulate the activity of phospholipase A2 and C (Perianin and Snyderman, 1989), mobilization of Ca^{2+} from the sarcoplasmic reticulum (Hirata et al., 2000, 2003), induce the mitochondrial permeability transition (Pfeiffer et al., 1995) and cell death by necrosis and apoptosis (Perianin and Snyderman, 1989).

Table 3
Comet assay with HepG2 cells exposed to different treatments with the venom of the wasp *P. paulista*.

Treatments		Number of cells in each comet class				Damage score (mean ± SD)	Cell viability % (mean ± SD)
		Migration categories (mean ± SD)					
		0	1	2	3		
NC		93.6 ± 1.53	1 ± 1	2.33 ± 0.57	3 ± 0	14 ± 1	96 ± 0.016
PC		0.33 ± 0.58	3.67 ± 1.15	6.33 ± 1.15	89.67 ± 1.53	285.33 ± 2.08*	97 ± 0.015
1 ng/mL	PT	0 ± 0	0 ± 0	0 ± 0	100 ± 0	300 ± 0*	9738 ± 0.006
	PosT	0 ± 0	4.5 ± 3.5	6.5 ± 5.85	89 ± 9.25	281.5 ± 18.47*	98.47 ± 0.016
	ST	0 ± 0	0 ± 0	0 ± 0	100 ± 0	300 ± 0*	96.63 ± 0.009
	STI	0 ± 0	0.16 ± 0.4	1 ± 1.09	98.83 ± 1.17	298.66 ± 1.36*	88.93 ± 0.049
100 pg/mL	PT	0 ± 0	0 ± 0	0.83 ± 1.32	99.16 ± 1.33	299.16 ± 1.33*	95.87 ± 0.034
	PosT	0.16 ± 0.4	2 ± 3.34	1.5 ± 1.97	96.33 ± 5.08	294 ± 8.22*	97.17 ± 0.011
	ST	0.33 ± 0.81	0 ± 0	0.16 ± 0.41	99.5 ± 0.83	298.83 ± 2.4*	95.33 ± 0.019
	STI	0 ± 0	0.83 ± 0.4	2 ± 1.09	97.16 ± 1.17	296.33 ± 7.11*	98.5 ± 0.011
10 pg/mL	PT	0.17 ± 0.41	2.33 ± 2.07	6.17 ± 4.54	91.33 ± 5.89	288.67 ± 7.63*	97 ± 0.004
	PosT	0.33 ± 0.82	16.33 ± 17.18	22.83 ± 19.8	60.50 ± 37.03	243.50 ± 54.88*	96.77 ± 0.036
	ST	0.17 ± 0.41	0.17 ± 0.41	1 ± 2.45	98.67 ± 2.8	298.17 ± 3.25*	96.2 ± 0.013
	STI	0 ± 0	0.67 ± 1.21	3.67 ± 5.39	95.67 ± 6.35	295 ± 7.38*	95.10 ± 0.013

PT: pre-treatment, PosT: post-treatment, ST: simultaneous treatment; STI: simultaneous treatment with incubation, SD = standard deviation. Damage score: 0 = no damage; 300 = maximum damage; * = statistically significant in relation to the negative control (ANOVA/Dunnet, $p < 0.05$).

Table 4
Cytokinesis-blockage micronucleus test performed with HepG2 cells exposed to different concentrations of the venom of the wasp *P. paulista*.

Treatments	MN (mean ± SD)	Bud (mean ± SD)	Bridge (mean ± SD)
NC	22 ± 1	9 ± 1.73	0.66 ± 0.57
PC	57.33 ± 2.51*	18.66 ± 1.15*	0 ± 0
10 μg/mL	87 ± 2.64*	41 ± 4.35*	1.33 ± 0.57
5 μg/mL	89.66 ± 6.43*	48 ± 6.08*	2.33 ± 1.52
1 μg/mL	73.33 ± 8.32*	30.66 ± 9.23*	2 ± 1.7
0.5 μg/mL	47.33 ± 3.05*	26 ± 3.46*	0 ± 0
0.1 μg/mL	42.66 ± 6.11*	18.66 ± 2.31*	1.33 ± 1.15
0.05 μg/mL	43.33 ± 4.16*	22.66 ± 2.30*	0.66 ± 1.15
0.01 μg/mL	37.33 ± 1.15*	19.33 ± 1.15*	0.66 ± 1.15
1 ng/mL	24.33 ± 3.78	13 ± 1	3 ± 1.73
100 pg/mL	23.33 ± 1.52	15.66 ± 3.05	3 ± 1.57
10 pg/mL	22 ± 1	14 ± 2	2.66 ± 1.15

NC = negative control; PC = positive control; MN = micronucleus; SD = standard deviation, * = statistically significant in relation to the negative control (ANOVA/Dunnet, $p < 0.05$).

Mastoparans are tetradecapeptide with variable cationic character and hydrophobicity, but are generally hydrosoluble, but when they interact with the lipid bilayers or when in anisotropic environments they assume an amphipathic helical form and have as main target the phospholipidic matrix of the membranes and their preferential interaction is with the anionic phospholipids of the membrane (Cabrera et al., 2011). Mastoparans present several biological activities such as degranulation of mast cells, release of histamine, activation of GTP-binding proteins, bactericidal potential and haemolytic activity (Čeřovský et al., 2008), besides being able to inhibit, *in vitro*, the transport of Golgi vesicles (Weidman and Winter, 1994).

Venoms of Neotropical vespids have polycationic peptides, such as polybines, which seem to be related with the occurrence of inflammation, including the initial process of the cell membrane lysis (Ribeiro et al., 2004). According to a study performed by de Paula et al. (2006), the venom of *P. paulista* causes acute inflammation, but according to Ferguson and Laing (2010), this event can lead to a series of adverse effects, including an increase in the rates of somatic mutation. Furthermore, during the inflammatory process there is the formation of reactive oxygen species (ROS), which are highly reactive molecules and able to interact and cause damages in the genetic material of the cells (Azad et al., 2008).

Table 5
Cytokinesis-blockage micronucleus test performed with HepG2 cells exposed to different treatments of the venom of the wasp *P. paulista*.

Treatments		MN (mean ± SD)	Bud (mean ± SD)	Bridge (mean ± SD)
NC		22 ± 1	9 ± 1.73	0.66 ± 0.57
PC		57.33 ± 2.51*	18.66 ± 1.15*	0 ± 0
1 ng/mL	PT	50.33 ± 5.85*	16 ± 2*	3.66 ± 1.15
	PosT	54.66 ± 2.51*	20 ± 1.73*	4 ± 1.73
	ST	48.66 ± 0.57*	15 ± 2.64*	5 ± 1
	STI	42.66 ± 4.93*	14.33 ± 1.52*	2.33 ± 3.21
100 pg/mL	PT	53.33 ± 0.57*	21 ± 2.64*	3.66 ± 1.52
	PosT	55 ± 3.6*	21.66 ± 0.57*	1.66 ± 1.52
	ST	61 ± 4.58*	26.33 ± 4.04*	8.66 ± 2.3*
	STI	52.33 ± 6.43*	19.66 ± 8.32*	5.66 ± 4.04
10 pg/mL	PT	62 ± 10.53*	26.33 ± 9.23*	2 ± 1.73
	PosT	68 ± 6.24*	26.33 ± 3.78*	1.33 ± 0.57
	ST	56.66 ± 15.63*	25.66 ± 5.03*	0 ± 0
	STI	48.33 ± 11.3*	26 ± 6.92*	2.33 ± 1.52

PT: pre-treatment, PosT: post-treatment, ST: simultaneous treatment; STI: simultaneous treatment with incubation, MN = micronucleus, SD = standard deviation. * = statistically significant in relation to the negative control (ANOVA/Dunnet, $p < 0.05$).

At low concentrations, the venom of *P. paulista* is not able to induce, by itself, damages in the genetic material, but when a substance with genotoxic and mutagenic potential is administered together with the venom, the substances present in the venom (phospholipase, hyaluronidase and mastoparans) seem to help in the entrance of the aggressor agent, since they can disrupt the cell membrane and, consequently, allow the entrance of xenobiotics into the cell. Although several substances, such as Polybia-MPI present anti-tumour activity (Wang et al., 2008, 2009), caution is needed when administering substances derived from the venom of *P. paulista* in the treatment of cancer, since it was observed in this study that very low concentrations of the wasp venom do not present cytotoxic potential but can induce genotoxicity and mutagenicity.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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