UFSM

CIÊNCIA e NATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, v. 45, e19, 2023 • https://doi.org/10.5902/2179460X66067
submitted: 02/06/2021 • approved: 20/07/2022 • Published: 11/10/2023

Biology-Genetics

Plant cytogenetics tests can predict toxic effects on human cells: genotoxic and mutagenic effects of *Tityus serrulatus* scorpion venom on plant and human cells

Testes de citogenética vegetal podem prever efeitos tóxicos em células humanas: efeitos genotóxicos e mutagênicos da peçonha do escorpião *Tityus serrulatus* em células vegetais e humanas

Silvana Marcussi[I], Marcel José Palmieri[I], Larissa Fonseca Andrade-Vieira[I], Amanda Ribeiro Barroso[I], Mariana Aparecida Braga[I], Marcus Vinicius Cardoso Trento[I]

[I] Federal University of Lavras, Lavras, MG, Brazil

ABSTRACT

The effects of *Tityus serrulatus* venom was accessed on the cell cycle and genetic material of *Lactuca sativa* L. and compared with the damages to human leukocytes, in order to evaluate its toxicity on two different cell types. Incubations of venom with human leukocytes were also held with subsequent evaluation of cell proliferation index, micronucleus and fragmented DNA. The *T. serrulatus* venom showed cytogenotoxic, reducing mitotic index and induced alterations in the plant cell cycle and micronuclei formation. The occurrence of cell death was evidenced by the detection of condensed nuclei, positive TUNEL signals, and presence of DNA fragmentation on lettuce cells. The scorpion venom induced DNA fragmentation and micronuclei in leukocytes. Further, the role of peptides and proteases from this venom in inducing the observed damage was discussed.

Keywords: Cytogenotoxicity; Mutagenic action; Tunel; Comet assay; Micronuclei

RESUMO

Os efeitos da peçonha de *Tityus serrulatus* foram avaliados no ciclo celular e material genético da *Lactuca sativa* L. e comparados com os danos a leucócitos humanos, a fim de avaliar a toxidade da mesma em dois tipos celulares diferentes. Também foram realizadas incubações de peçonha com leucócitos humanos com posterior avaliação do índice de proliferação celular, micronúcleo e DNA fragmentado. A peçonha de *T. serrulatus* mostrou-se citogenotóxica, reduzindo o índice mitótico e induzindo alterações no ciclo celular vegetal e formação de micronúcleos. A ocorrência de morte

celular foi evidenciada pela detecção de núcleos condensados, sinais TUNEL positivos e presença de fragmentação de DNA em células de alface. A peçonha do escorpião induziu fragmentação de DNA e micronúcleos em leucócitos. Além disso, o papel dos peptídeos e proteases desta peçonha na indução do dano observado foi discutido.

Palavras-chave: Citogenotoxicidade; Ação mutagênica; Túnel; Ensaio do cometa; Micronúcleos

1 INTRODUCTION

Scorpionism is considered a public health problem all over the world. The species *Tityus serrulatus* (Brazilian yellow scorpion), is considered the most dangerous in South America (Reckziegel; Pinto Jr, 2014), being responsible for the majority of the accidents related to venomous animals (Cupo, 2015).

The *T. serrulatus* venom is composed by a complex mixture of substances. Among these are enzymes (hyaluronidases and proteases), peptides, mucus, amino acids, nucleic acids and salts. Neurotoxins (peptides) are the main components of the venom, and act on different ion channels, being responsible for physiological alterations that may result in death. Moreover, the proteases and hyaluronidases may leverage the effects of the neurotoxins, since they act as spreading factors of the venom (Abdel-Rahman; Quintero-Hernández; Possani, 2016; Oliveira-Mendes *et al*., 2019). Also, antimicrobial components and other peptides with different biological functions have already been described (Pucca *et al*., 2016; Cerni *et al*., 2017).

Considering that the components of animal venoms may act on the hemostatic, immunological and neural systems, in addition to induce cell death, and that the occurrence of the *T. serrulatus* in Brazil is prolific, studies that allow understanding the mechanisms of action of its venom are of great value in the medical-scientific scope.

Active agents found in venoms have been used as model for the development of drugs. The works in this area have intensified over the last decades, resulting in the discovery of new medicines with wide therapeutic uses, like anticoagulants (e.g. Ancrod, Batroxobin and Echistatin), antihypertensive (e.g. Captopril and

Ranatensin) (Koh; Kini, 2012), as well as analgesics and pharmaceuticals that treat Diabetes Mellitus (Koh; Kini, 2012; Harvey, 2014; Zambelll *et al*., 2016).

However, both the characterization of pharmacologically active molecules and the development of pharmaceuticals require tests that use animals. This is a practice which often faces logistic and economic problems related to the maintenance of the animals, in addition to legal and ethical barriers. An alternative for animal tests could thus be the use of plant organisms as models to investigate mechanisms of action, especially for pilot assays and initial screenings with different samples and dosages.

The use of plant models in bioassays that prospect toxicity of environmental pollutants and vegetable compounds has been of great value in ecotoxicology and allelopathy researches (Fiskesjö, 1985; Fiskesjö, 1988; Leme; Marin-Morales, 2009; Andrade-Vieira *et al*., 2011; Andrade-Vieira *et al*., 2017; Santos *et al*., 2018). These models are accepted as efficient methods to evaluate cytological and genetic material damage (Leme; Marin-Morales, 2009; Andrade-Vieira *et al*., 2014; Palmieri *et al*., 2016). The assays using higher plants as models are advantageous and justifiable due to presenting low cost, good correlation with other test models and systems, apart from being highly reliable (Grant; Owens, 2006; Dong; Zhang, 2010). Besides, they are considered adequate test systems to evaluate toxicity by the United Nations Environment Programme, the World Health Organization and the United States Environmental Protection Agency (Grant, 1999).

Therefore, the main objective in this work was to evaluate the validity of plant bioassays for the screening of toxic effects of animal toxins. In order to achieve this tag, the cytotoxic and genotoxic effects of *T. serrulatus* venom on *Lactuca sativa* cells and on the DNA of human lymphocytes were evaluated.

2. MATERIAL AND METHODS

2.1 Obtainment of the biological material

2.1.1 Tityus serrulatus venom

The protocol followed Brazilian Institute of Environment (Ibama) and Brazilian College for Animal Experimentation (COBEA) guidelines. Scorpions maintained at the "Biotério Central" vivarium (University of São Paulo, Ribeirão Preto, Brazil) had their venom extracted by the electrical stimulation technique (Lowe; Farrell, 2011), following this process the venom was then lyophilized and frozen.

2.2 Root tips treatment

Commercial seeds of *L. sativa*, were pre-germinated in Petri dish, covered by filter paper moistened with distilled water, for 24 h. After this period, five seeds with emitted roots of about 0.5 mm length were transferred to a second Petri dish, covered with filter paper, containing 500 µL of *T. serrulatus* venom solution (0.5 mg mL^{-1}), in total the seeds were exposed to 0.25 mg of venom. These concentrations were based on pilot studies. The negative control was achieved with distilled water. The Petri dishes were sealed with *Parafilm*® and remained in germination chamber at 20 ºC for 24 h.

2.3 Human cell preparation

Human blood from 20 volunteers (18-30 years old) was collected in heparinized tubes, after obtaining their formal consent. This study received approval of Research Ethics Committee of FCFRP-USP (nº 102).

The peripheral blood was distributed in flasks for cultivation (500 µL per flask), in RPMI 1640 medium (5 mL) supplemented with 10% fetal bovine serum (FBS, Gibco BRL), 100 U mL^{-1} penicillin and streptomycin and 1% phytohemagglutinin (Gibco BRL) in 5% CO_2 at 37°C. Each treatment/experiment/volunteer was evaluated

in triplicate, and the culture period was of 72 h at 37ºC for the micronucleus and 7 h for the comet assay.

2.4 Bioassays in plant cells

2.4.1 Effects on the cell cycle

Exposed roots (2.1.2) were collected and fixed for 24 h in ethanol:acetic acid solution (3:1). Then, fixed roots were washed in distilled water and hydrolyzed in 5 N HCl at 25ºC for 12 min. The slides were prepared and the material was stained with 2% acetic orcein. For each treatment, 5,000 cells were analyzed.

The evaluated parameters were: (1) mitotic index (MI), given by the ratio between the total of dividing cells and the total of evaluated cells, (2) cell cycle alterations (CCA): the sum of sticky chromosomes, c-metaphases, chromosome fragments, multipolar anaphases, chromosome bridges and non-oriented chromosomes, micronuclei (MCN) and condensed nuclei (CN).

2.5 DNA fragmentation test

The DNA from 1 g of *L. sativa* roots freshly collected, exposed to venom solution, was extracted (Doyle; Doyle, 1987). The roots were homogenized in liquid nitrogen and incubated in buffer containing 2% hexadecyltrimethylammonium bromide (CTAB) at 65 ºC. The DNA was extracted with chloroform-isoamyl alcohol (24:1) and precipitated in cold isopropanol. The DNA molecules were maintained in TE buffer (10 mM Tris-HCl, pH 7.4; 1 mM EDTA). Then, the obtained samples were subjected to 1.2% agarose gel electrophoresis in TBE (Tris/Borate/EDTA) containing 0.5 μg mL^{-1} of ethidium bromide. The presence or not of fragmentation DNA fragmentation was observed under UV light. A standard (100 bp to 1500 bp; Promega) and DNA molecules from non-treated roots, were applied as comparators. The data represent three repetitions performed in two independent experiments.

2.6 TUNEL test

The kit DeadEnd™ Fluorometric TUNEL System (Promega®) was used for testing. For preparation of the slides, freshly exposed root meristems were hydrolysed with HCl 1M at 60°C for 15 min., and next fixed in 4% paraformaldehyde for 25 min. at 4°C. The positive reaction was marked with fluorescein, and the countermarking was accomplished with use of propidium iodide. The slides were evaluated in epifluorescence microscope (Olympus BX60) using the filters with excitation interval of 460-490 nm to evidence the fluorescein, and of 530-550 nm for propidium iodide. The data represent three repetitions and 100 cells were analyzed per slide.

2.7 Bioassays in human cells

2.7.1 Cytokinesis-block micronucleus test

The Cytokinesis-block micronucleus test (CBMCN) was performed according to Fenech and Morley (1985). The samples (*T. serrulatus* venom; 1 to 120 µg mL^{-1}) were added 24 h after the initiation of the cultures. After 44 h, cytochalasin-B (4 µg mL^{-1}, Sigma) was added to the cultures. The analyses were carried out after 72 h. Scores were taken according to the criteria of Fenech (2000). For each treatment/experiment-volunteer 1,000 binuclear cells were counted considering the presence or not of micronuclei. Cisplatin (PLATINIL®) (6µg mL^{-1}) was used as positive control.

2.7.2 Cell proliferation index

The cytokinesis-block proliferation index (CBPI) was calculated, to determine non-cytotoxic doses of the venom, by counting 500 cells, considering the formula described by Kirsch-Volders (1997): CBPI = 1 (nuclei mono) + 2 (bi) + 3 (tri + tetra) / 500.

2.8 Comet assay

Using the methodology described by Singh *et al*. (1988), the cells were incubated with the treatments (*T. serrulatus* venom; 1.0 and 120 µg mL^{-1}) for 4 h at 37ºC, and used to prepare the slides. For each treatment/experiment-volunteer, 900 nucleoids were evaluated. For the slides preparation in triplicate, 60 µL of each cell culture (cellular suspension at 10^5 cells mL^{-1}) were transferred to microtubes containing 300 µL of low melting point agarose. The mixture was dropped on the slides (100 µL/slide) with normal melting point agarose and covered with the coverslips. After gel polymerization, the coverslips were removed and the slides immersed in lyses solution (0.25 M NaCl, 100 mM EDTA, 10 mM Trizma base, pH 10, 5% DMSO and 1% Triton X-100), remaining there for 2 hours. Doxorubicin (Bergamo Ltda) (6 µg mL^{-1}) was used as positive control.

2.8.1 Electrophoresis

The slides were subjected to electrophoretic run (at 25 V, 300 mA, for 35 min.) after being kept for 20 min at 4 ºC in a 300 mM sodium hydroxide solution (pH13). The slides were then submerged for a period of 20 min in a 0.4 M Tris-HCl solution (pH 7.4) for neutralization. Afterwards they were kept at room temperature to dry and finally immersed in a 100% ethanol solution for fixation.

2.8.2 Nucleoids evaluation

The slides were protected from light, covered with a coverslip, stained with a solution of ethidium bromide (2 µg mL^{-1}) and analyzed by fluorescence microscopy at 200 and 400X magnificence. The nucleoids were analyzed by visual scores (Collins *et al*., 1993), and classified by levels of DNA fragmentation: 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 (40-85%) and class 4, totally damaged (damage > 85%). The average frequency of damage was calculated from the sum of the percentages of nucleoids with damage 1, 2, 3 and 4. The data were also calculated with arbitrary

units - AU (0-400; where 0 = no damage and 400 = 100% damage), by the equation (1 x number of nucleoids grouped in class 1) + (2 x number of nucleoids in class 2) + (3 x number of nucleoids in class 3) + (4 x number of nucleoids in class 4), (Collins, 2004).

2.9 Statistical analysis

Statistical analyses were performed using the free Software "R" (R Core Team, 2015). The parameters evaluated in the cell cycle and TUNEL test assessments were subjected to analysis of variance (ANOVA) followed by Tukey's test of means at 5% significance. For the comet and micronucleus assays the data of each treatment were compared by Duncan ($P<0.05$) test.

3 RESULTS

3.1 Bioassays in plant cells

Our results present the utilization of plant and human cells as model systems to evaluate the genotoxic and/or mutagenic action of animal venom.

The data obtained show that *T. serrulatus* venom has a toxic effect on lettuce cells and DNA, affecting the chromosome structure, causing breaks in the DNA molecule, and leading to cell cycle alterations. In the cell cycle analyses, significant differences were noticed in the two evaluated parameters (MI and CCA). The cells exposed to *T. serrulatus* venom presented an increase of about 15 times in the CCAs in relation to the control (Table 1).

Table 1 – Effect of *Tityus serrulatus* venom on the mitotic index (MI), cell cycle alterations (CCA) and TUNEL test after exposure of *Lactuca sativa* roots to 0.25 mg of venom

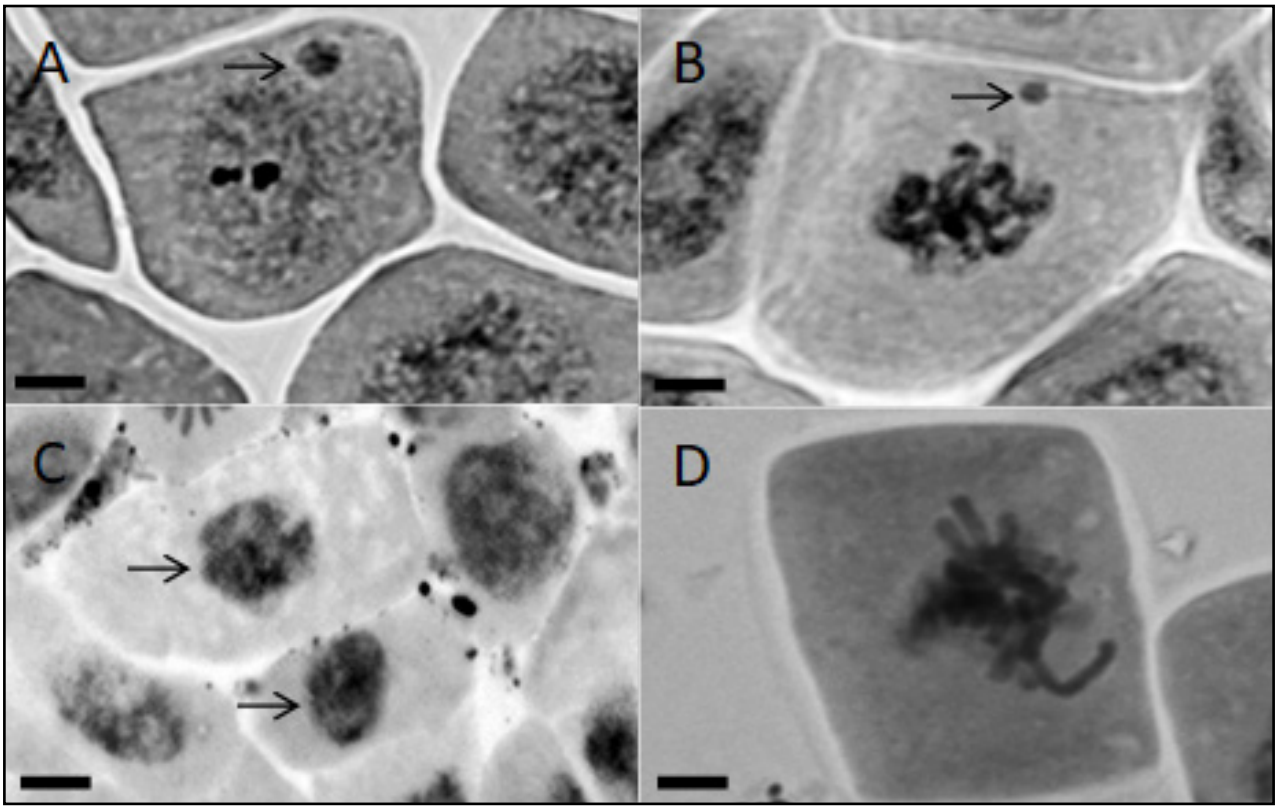
Treatments	MI	CCA	TUNEL
Control	9.29 ± 2.40	0.99 ± 1.03	0.20 ± 1.49
T. serrulatus	6.23 ± 2.19*	14.9 ± 5.80*	23 ± 7.74*

Source: By the author

Observed results followed by standard deviation in the two evaluated parameters and in the TUNEL test. MI is expressed as percentage, and CCA as frequency per thousand cells. The TUNEL result is given by the percentage of cells with the positive mark. Data followed by * differ significantly from the control according to Tukey test (α=5%).

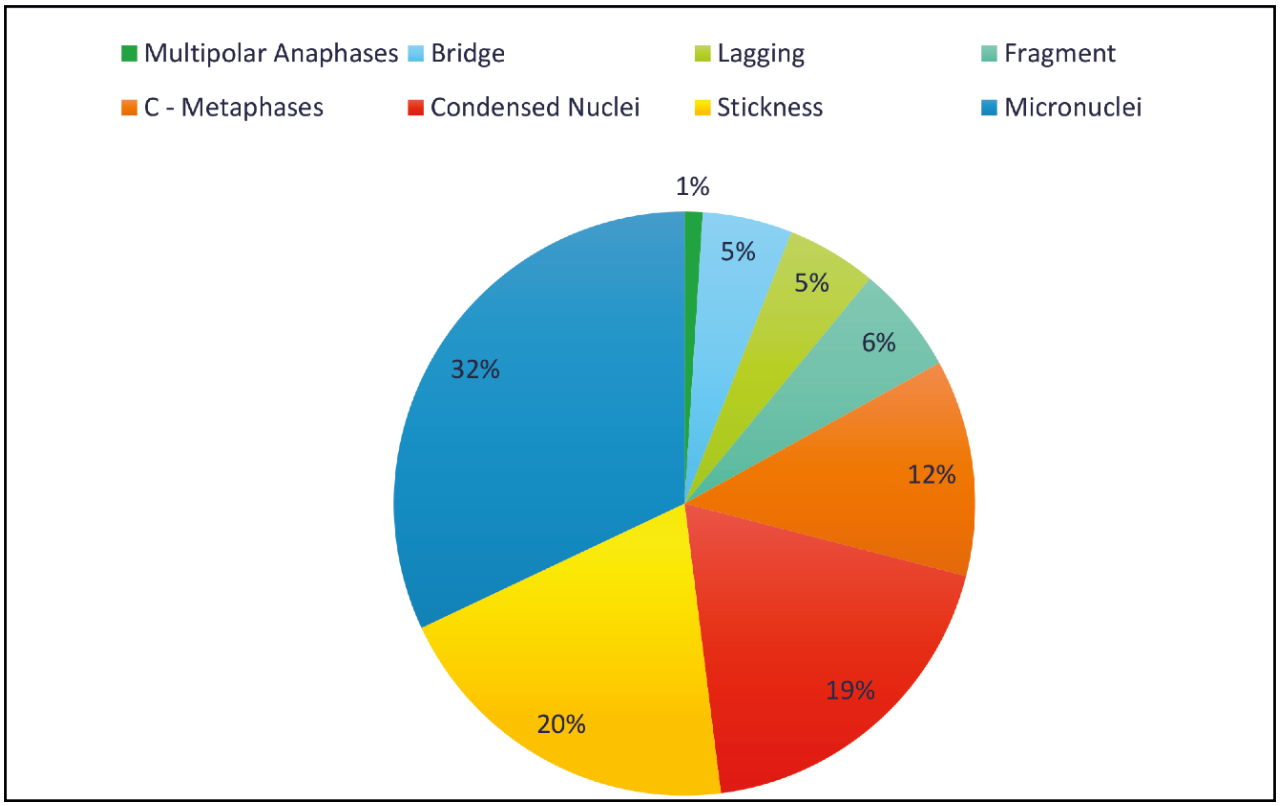
MCN, sticky chromosomes, c-metaphases, bridges, chromosome fragments, non-oriented chromosomes, multipolar anaphases and condensed nuclei were observed. Figure 1 brings examples of the CCA most commonly found in the slides evaluated (Figure 2).

Figure 1 – Alterations in the cell cycle found with higher frequency in root tip cells of *Lactuca sativa* exposed to *Tityus serrulatus* venom. A and B – Micronuclei (arrow); C – Condensed nuclei (arrow); D – Metaphase with sticky chromosomes. Bar = 5 µm



Source: By the author

Figure 2 – Percentage of CCAs observed on root tips of *Lactuca sativa* exposed to *Tityus serrulatus* venom

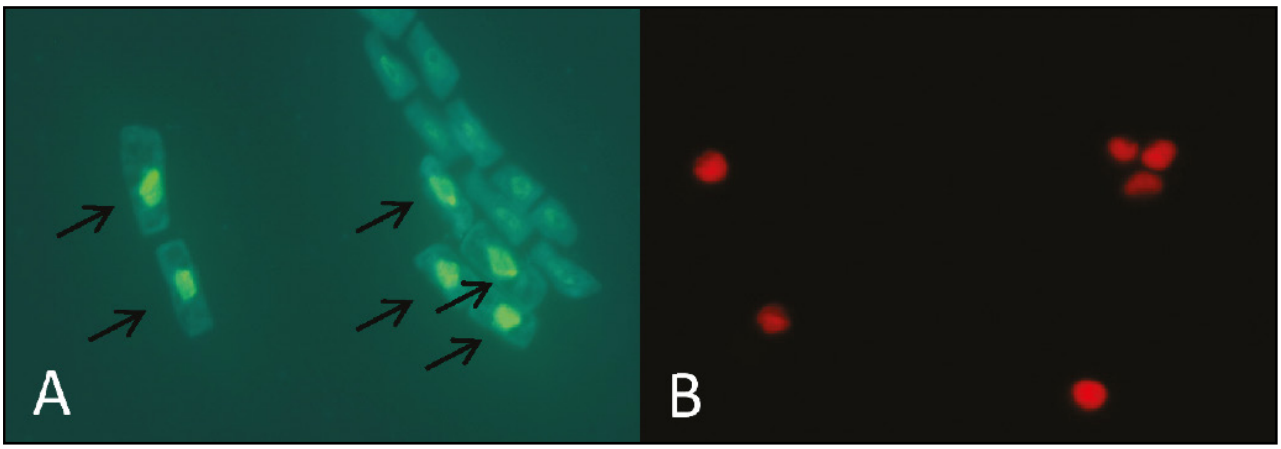


Source: By the author

The most abundant alterations were MCN (Figure 1A, B), representing 32% of the total of observed alterations (Figure 2). The second highest observed alteration was sticky chromosomes (Figure 1C), which represent 20% of the total alterations (Figure 2), and finally metaphase with sticky chromosomes (Figure 1D).

The high index of CN found, 18.67%, suggests that *T. serrulatus* venom presents genotoxic potential. As consequence, plant aborts the damaged cells. The DNA laddering effect becomes evident by the fragmentation of the genetic material, which showed fragments of 100 to 500 bp (data not shown). These data are corroborated by the positive result of the TUNEL assay, which detected damage in the DNA molecules in 23% of the evaluated cells (Table 1 and Figure 3).

Figure 3 – TUNEL test. Results observed after exposure of *Lactuca sativa* roots to 0.25mg of *Tityus serrulatus* venom. A – Positive signal of the TUNEL test on cells exposed to the *Tityus serrulatus* venom (seen on the fluorescein filter). B – Control cells showing no positive markings (image is composed of the juxtaposition of the fields observed on the fluorescein and iodide filters)



Source: By the author

Both the DNA fragmentation and the detected alterations have an impact on the MI, which decreased approximately 33% in cells exposed to venom in relation to the control (Table 1).

3.2 Bioassays in human cells

Thus, the results obtained in the present work demonstrate a genotoxic potential of *T. serrulatus* venom on lettuce cells. However, it is important to point that the effects of this venom were also evaluated over human leukocytes, using comet test, and its genotoxic potential was once again noticeable.

Figure 4 shows the percentage of damage and the AU on human leukocytes exposed to concentrations of 1, 2.5, 5, 7.5, 10, 15, 30, 60 and 120 μg mL^{-1} of *T. serrulatus* venom. Significant differences for the higher comet classes, especially on the higher concentrations, in relation to the negative control were observed (Table 2). In addition, at the highest concentration applied, the total percentage of induced

damage (approximately 40%) and AU (approximately 75) were very similar to those observed for the doxorubicin (6μg mL^{-1}). This data is in accordance with what was detected by the TUNEL test and by the fragmentation on DNA observed in the agarose gel electrophoresis.

Table 2 – Distribution of comet classes observed in nucleoids from leukocytes treated with *Tityus serrulatus* venom in different concentrations

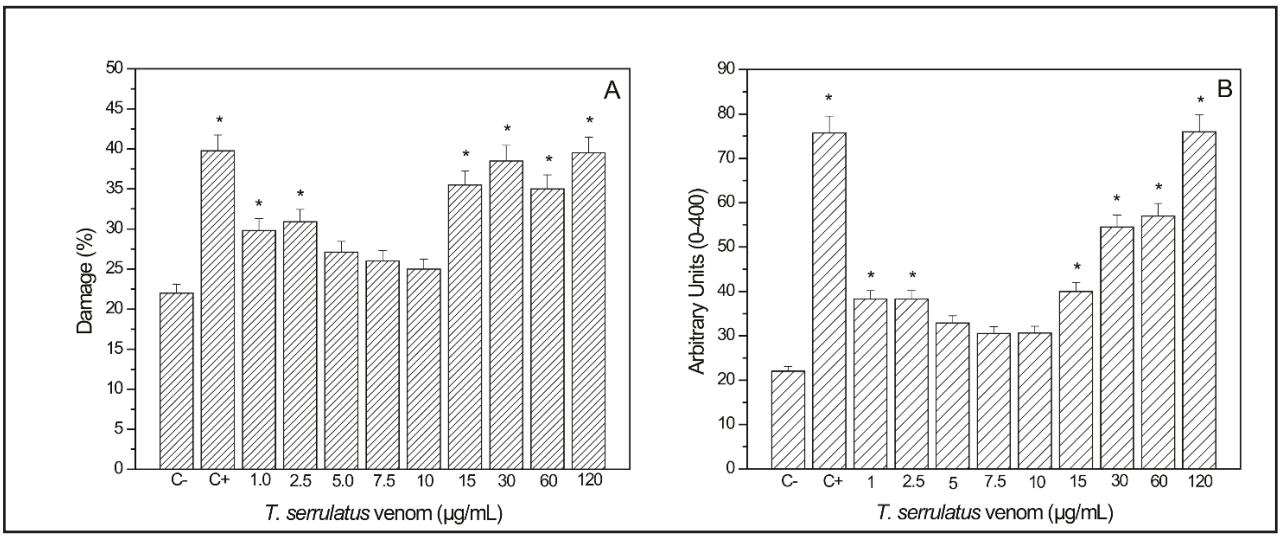
Treatment [μg mL^{-1}]		**Comet classes (%)**				
		0	**1 (5-20%)**	**2 (20-40%)**	**3 (40-85%)**	**4 (damage ≥85%)**
Negative control	-	78±0.02	22.0±0.03	0±0.01	0±0.02	0±0.02
Positive control	6	33.5±0.04a	25.75±0.05	2.5±0.02	1±0.05	10.5±0.04a
T. serrulatus	1	70.2±2.1ab	23.5±1.3	4.1±0.8a	2.2±1.7a	0±0.03
	2.5	69.1±5.4ab	24.6±2.5	5.2±1ab	1.1±0.3	0±0.2
	5	72.9±7.0b	21.8±3.1	4.8±0.5a	0.5±0.1	0±0.1
	7.5	74.0±6.4b	22.5±1.1	2.5±0.6	1.0±0.1	0±0.4
	10	75.0±4.0b	20.4±1.4	3.6±0.8	1.0±0.4	0±0.8
	15	64.5±3.2ab	31.0±3.5ab	4.5±1.2a	0±0.2	0±0.5
	30	61.5±5.4ab	26.0±2.8	9.0±1.3ab	3.5±0.2ab	0±0.5
	60	75.0±3.8b	19.0±1.6	11.5±1.1ab	3.0±0.7ab	1.5±0.2
	120	60.5±2.6ab	15.5±2.2ab	14.5±1.6ab	6.5±1.1ab	3.0±0.3ab

Data represents the mean of each treatment ± S.D. for 10 individuals. DXR = doxorubicin.
a Significantly different from the negative control, PBS ($p<0.05$).
b Significantly different from the positive control, DXR-Doxorubicin ($p<0.05$).
Source: By the author

Nevertheless, for the MCN test in human leukocytes, the venom of *T. serrulatus,* on the concentrations between 1 and 30 μg mL^{-1}, did not induce micronucleus formation significantly different from the negative control (1.1 ± 0.1 MCN) (Table 3). It is suggested that the low frequency of MCN found may be the result of effective action of the cell repair mechanisms. This way, the venom is inducing damage that is liable to correction, and thus not observed by the mentioned test. However, the data obtained here show that at least part of this damage is not repaired effectively, since

the occurrence of CN and MCN was observed in high frequency on the tests using *L. sativa* as model and the two highest concentrations tested (60 and 120 μg mL^{-1}).

Figure 4 – Comet test. (A) Frequency of nucleoids from leukocytes with comet and (B) Values of arbitrary units calculated to nucleoids with damage exposed for 4 hours at *Tityus serrulatus* venom in different concentrations, or doxorubicin



Source: By the author

Data represent the mean of each treatment ± S.D. for 10 individual experiments, one for each volunteer. The percentage of nucleoids with damage was calculated on the sum of nucleoids classified with damage1, 2, 3 and 4. Arbitrary units (0–400) calculated according to Collins (2004). * Significantly different from the negative control ($p<0.05$).

Nevertheless, for the MCN test in human leukocytes, the venom of *T. serrulatus,* on the concentrations between 1 and 30 μg mL^{-1}, did not induce micronucleus formation significantly different from the negative control (1.1 ± 0.1 MCN) (Table 3). It is suggested that the low frequency of MCN found may be the result of effective action of the cell repair mechanisms. This way, the venom is inducing damage that is liable to correction, and thus not observed by the mentioned test. However, the data obtained here show that at least part of this damage is not repaired effectively, since the occurrence of CN and MCN was observed in high frequency on the tests using *L. sativa* as model and the two highest concentrations tested (60 and 120 μg mL^{-1}).

Table 3 – Distribution of micronuclei and cytokinesis-blocking proliferating index (CBPI) in leukocytes treated with *Tityus serrulatus* venom in different concentrations or with the antitumor agent cisplatin

Treatment [µg/mL]		% cells/500cells				MCN/1000BN cells, mean ± S.D.	CBPI ± S.D.
		Mono	Bi	Tri	Multi		
Negative control	--	50.3	39.0	7.3	3.4	1.1±0.1	1.604±0.6
Positive control	6	48.5	38.7	7.6	5.2	12±0.5	1.643±0.5
T. serrulatus	1	48.9	42.5	6.2	2.4	1.0±0.1b	1.686±0.3
	2.5	47.8	36.2	11.0	5.0	0.8±0.2b	1.652±0.4
	5	46.9	41.3	8.9	2.9	1.2±0.2b	1.649±0.7
	7.5	35.3ab	48.6ab	8.6	7.5a	1.2±0.1b	1.808±0.2
	10	46.9	37.6	9.3	6.2	1.4±0.3b	1.686±0.6
	15	52.4	37.9	6.4	3.3	1.5±0.3b	1.572±0.9
	30	51.4	38.3	5.8	4.5	1.8±0.4b	1.598±0.9
	60	37.4ab	41.2	13.3ab	8.1a	5.4±0.2ab	1.840±0.5
	120	45.3	38.2	14.1ab	2.4	9.7±0.5a	1.712±0.4

BN: binucleated cells; MCN: micronuclei; CBPI: cytokinesis-blocking proliferating index. Data are represented as means ± S.D. The values of CBPI were not statistically different compared to the control ($p<0.05$). Negative control = PBS. Positive control = cisplatin.

a Significantly different from the negative control ($p<0.05$).

b Significantly different from the positive control, Cisplatin ($p<0.05$).

Source: By the author

4 DISCUSSION

Proteases of *T. serrulatus* venom can act on the protein scaffold of the chromosome, destabilizing it, and resulting in formation of sticky chromosomes. Another hypothesis is the action of these enzymes on the mitotic spindle proteins, resulting in the occurrence of non-oriented chromosomes, multipolar anaphases and c-metaphases (Fiskesjö, 1985) (detected in the present work, Figure 2).

The proteases can also act on proteins associated to the DNA, favoring the occurrence of breaks, which can be evidenced by the formation of bridges and fragments (Figure 2). The extremities without telomeres tend to fuse, leading the

formation of a bi-centric chromosome, observed as a bridge between the two poles and then segments excised are eliminated by way of MCN (Leme; Marin- Morales, 2009; Andrade-Vieira *et al*., 2011).

This instability and the damage in the DNA structure may also be evidenced by the occurrence of condensed nuclei (CN), which are markers of cell death (CD) (Andrade-Vieira *et al*., 2011), related to DNA fragmentation (Üstün; Hafrén; Hofius, 2017).

The reactive oxygen species (ROS), associated to oxidative stress, are also responsible to the induction of DNA damage followed by CD (Zhoua *et al*., 2018). The exposure of the root cells to the *T. serrulatus* venom induces CD. In addition, the neurotoxins act on ion channels and induce oxidative stress (Cologna *et al*., 2009; Galvani *et al*., 2017). Some of these peptides can act on ion K^+ channels, to activate the CD mechanism, generating CN (Housley *et al*., 2017), observed in the present study.

The presence of DNA damage induced by different toxins is also reported on literature (Kang *et al*., 2011; Marcussi *et al*., 2011; 2013). Specifically, on *T. serrulatus* it is known that Ts2 and Ts6 toxins are capable of affecting the immune system and trigger inflammation in human cells (Zoccal *et al*., 2013), could result in the formation of ROS causing DNA damage. Cells in CD process, or that present inadequate transport of K^+ ions do not divide (Lebaudy; Véry; Sentenac, 2007). *Androctonus crassicauda* venom is able to limit the growth of SH-SY5Y and MCF-7 cancer cell lines by a mechanism of apoptosis induction and DNA synthesis blocking (Zargan *et al*., 2011).

Apis mellifera venom induced DNA fragmentation and formation of micronuclei on HL-60 cancer cells (Lee *et al*., 2007). The micronuclei reflect genomic instabilities that point to degradation of somatic DNA (Hara and Marin-Morales, 2017). Gajski and Garaj-Vrhovac (2008), observed DNA instability in human lymphocytes after exposure to venom from *Apis mellifera*. Gupta *et al*. (2007) observed cytotoxic and pro-apoptotic effects induced by the *Heterometrus bengalensis* venom on human leukemic cells. In addition, Palmieri *et al*. (2019) demonstrated the cytogenotoxic effects of *Polybia* venoms on human and vegetal cells.

The results demonstrate that the toxins were able to cross the cell wall and induce toxicity in plant cells. It is also important to consider that in plant models the exposure occurs in the meristematic tissue, which undergoes many rapid cell divisions (Palmieri *et al*., 2016b), thus increasing its sensitivity to external agents, allowed their use in screening for toxic or protective substances (Andrade-Vieira *et al*., 2017). In addition, bioassays using plant systems are inexpensive, reliable, high reproducibility and results obtained demonstrate a good correlation with human cells (Reis *et al*., 2017; Silva *et al*., 2020).

5 CONCLUSIONS

The *T. serrulatus* venom presented cytotoxicity in the meristematic cells of *L. sativa*, evidenced by the reduction in the mitotic index, and genotoxicity, demonstrated by the high frequency of abnormalities in the cell cycles.

Under the evaluated conditions, the venom induced cell death, manifested by damage to the DNA molecule, positive TUNEL assay, fragmentation of the genomic DNA subjected to agarose gel electrophoresis, and by the presence of micronuclei, condensed nuclei, and chromosome bridges and fragments. Similarly, the *T. serrulatus* venom induced DNA damage in human leukocytes, observed in the comet assay.

Moreover, the employed plant model revealed itself as viable test system to evaluate the toxicity of animal venoms, with perspective of use for investigation of different natural compounds, representing a rapid, low-cost, easy-to-use alternative to complement or even substitute animal models.

ACKNOWLEDGEMENTS

This article has segments of the material and methods and some excerpts from the other topics that were extracted from the doctorate thesis entitled "*Lactuca sativa*: Bioindicador Para Análise do Efeito Genotóxico de Peçonhas" (Palmieri, 2016).

This work was supported by grants from the Brazilian Agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

The authors express their gratitude to Universidade Federal de Lavras for the support structural to the researchers.

REFERENCES

ABDEL-RAHMAN, M. A.; QUINTERO-HERNÁNDEZ, V.; POSSANI, L. D. Scorpion venom gland transcriptomics and proteomics: An Overview. *In*: GOPALAKRISHNAKONE, P.; CALVETE, J. (eds). **Venom Genomics and Proteomics**. Dordrecht: Springer, 2016. p. 1-17.

ANDRADE-VIEIRA, L. F.; BOTELHO, C. M.; LAVIOLA, B. G.; PALMIERI, M. J.; PAÇA-FONTES, M. M. Effects of *Jatropha curcas* oil in *Lactuca sativa* root tip bioassays. **An Acad Bras Cienc.**, [*s.l*]. v. 86, n. 1, p. 373–382, 2014.

ANDRADE-VIEIRA, L. F.; GEDRAITE. L. S.; CAMPOS, J. M. S.; DAVIDE, L. C. Spent Pot Liner (SPL) induced DNA damage and nuclear alterations in root tip cells of *Allium cepa* as a consequence of programmed cell death. **Ecotoxicol Environ Saf.**, [*s.l.*], v. 74, n. 4, 882–888, 2011.

ANDRADE-VIEIRA, L. F.; PALMIERI, M. J.; BOTELHO, C. M.; LUBER, J.; SILVA, M. F. F. Evaluation of the antimutagenic potential of *Psidium guajava* L. extracts via plant bioassays. **South A J Botany**, [*s.l.*], v. 113, p. 443–448, 2017.

CERNI, F. A.; PUCCA, M. B.; ZOCCAL, K. F.; FRANTZ, F. G.; FACCIOLI, L. H.; ARANTES, E. C. Expanding biological activities of Ts19 Frag-II toxin: Insights into IL-17 production. **Toxicon**, [*s.l.*], v. 134, p. 18-25, Aug. 2017.

COLLINS, A. R.; DUTHIE, S. J.; DOBSON, V. L. Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA. **Carcinogenesis**, [*s.l.*], v. 14, n. 9, p. 1733–1735, 1993.

COLLINS, A. R. The comet assay for DNA damage and repair: principles, applications, and limitations. **Mol. Biotechnol.**, [*s.l.*], v. 26, n. 3, p. 249–261, 2004.

COLOGNA, C. T.; MARCUSSI, S.; GIGLIO, J. R.; SOARES, A. M.; ARANTES, E. C. *Tityus serrulatus* scorpion venom and toxins: an overview. **Protein Pept Lett.**, [*s.l.*], v.16, n. 8, p. 920–932, 2009.

CUPO, P. Clinical update on scorpion envenoming. **Rev. Soc. Bras. Med. Trop.**, [*s.l.*], v. 48, n. 6, p. 642-649, 2015.

DONG, Y.; ZHANG, J. Testing the genotoxicity of coking wastewater using *Vicia faba* and *Hordeum vulgare* bioassays. **Ecotoxicol Environ Saf**., [*s.l.*], v. 73, n. 5, p. 944–948, 2010.

DOYLE, J. J.; DOYLE, J. L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. **Phytochem Bull.**, [*s.l.*], v. 19, n. 1, p.11–15, 1987.

FENECH, M.; MORLEY, A. A. Measurement of micronuclei in lymphocytes. **Mutat Res Mutagen Relat Subj.**, [*s.l.*]. v. 147, p. 29–36, 1985.

FENECH, M. The *in vitro* micronucleus technique. **Mutat Res - Fundam Mol Mech Mutagen.**, [*s.l.*], v. 455, n. 1-2, p. 81–95, 2000.

FISKESJÖ, G. The *Allium* test as a standard in environmental monitoring. **Hereditas.**, [*s.l.*], v. 102, n. 1, p. 99–112, 1985.

FISKESJÖ, G. The *Allium* test - an alternative in environmental studies: the relative toxicity of metal ions. **Mutat Res - Fundam Mol Mech Mutagen.**, [*s.l.*], v. 197, n. 2, p. 243–260, 1988.

GAJSKI, G.; GARAJ-VRHOVAC. V. Genotoxic potential of bee venom (*Apis Mellifera*) on human peripheral blood lymphocytes *in vitro* using single cell gel electrophoresis assay. **J Environ Sci Heal Part A.**, [*s.l.*], v. 43, p. 1279–1287, 2008.

GALVANI, N. C.; VILELA, T. C.; DOMINGOS, A. C.; FAGUNDES, M. Í.; BOSA, L. M.; DELLA VECHIA, I. C.; SCUSSEL, R.; PEREIRA, M.; STEINER, B. T.; DAMIANI, A. P.; CHÁVEZ-OLÓRTEGUI, C.; ANDRADE, V. M.; ÁVILA, R. A. M. Genotoxicity evaluation induced by *Tityus serrulatus* scorpion venom in mice. **Toxicon.** [*s.l.*], v. 140, p. 132-138, 2017.

GRANT, W. F. Higher plant assays for the detection of chromosomal aberrations and gene mutations - a brief historical background on their use for screening and monitoring environmental chemicals. **Mutat Res.**, [*s.l.*], v. 426, p. 107–112, 1999.

GRANT, W. F.; OWENS, E. T. *Zea mays* assays of chemical/radiation genotoxicity for the study of environmental mutagens. **Mutat Res.**, [*s.l.*], v. 613, p. 17–64, 2006.

GUPTA, S. DEBNATH, A.; SAHA, A.; GIRI, B.; TRIPATHI, G.; VEDASIROMONI, J. R.; GOMES, A.; GOMES, A. INDIAN black scorpion (*Heterometrus bengalensis* Koch) venom induced antiproliferative and apoptogenic activity against human leukemic cell lines U937 and K562. **Leuk Res.**, [*s.l.*], v. 31, p. 817–825, 2007.

HARA, R. V.; MARIN-MORALES, M. A. *In vitro* and *in vivo* investigation of the genotoxic potential of waters from rivers under the influence of a petroleum refinery (São Paulo State - Brazil). **Chemosph.**, [*s.l.*], v. 174, p. 321-330, 2017.

HARVEY, A. L. Toxins and drug discovery. **Toxicon.**, [*s.l.*], v. 92, p. 193–200, 2014.

HOUSLEY, D. M.; HOUSLEY, G. D.; LIDDELL, M. J.; JENNINGS, E. A. Scorpion toxin peptide action at the ion channel subunit level. **Neuropharm.**, [*s.l.*], v. 127, p. 46-78, 2017.

KANG, T. S.; GEORGIEVA, D.; GENOV, N.; MURAKAMI, M. T.; SINHA, M.; KUMAR, R. P.; DEY, S.; SHARMA, S.; VRIELINK, A.; BETZEL, C.; TAKEDA, S.; ARNI, R. K.; SINGH, T. P.; KINI, R. M. Enzymatic toxins from snake venom: Structural characterization and mechanism of catalysis. **FEBS J.**, [*s.l.*], v. 278, p. 4544–4576, 2011.

KIRSCH-VOLDERS, M. Towards a validation of the micronucleus test. **Mutat Res.**, [*s.l.*], v. 392, p. 1-4, 1997.

KOH, C. Y.; KINI, R. M. From snake venom toxins to therapeutics - Cardiovascular examples. **Toxicon.**, [*s.l.*], v. 59, n° 4, p. 497–506, 2012.

LEBAUDY, A.; VÉRY, A. A.; SENTENAC, H. K^+ channel activity in plants: Genes, regulations and functions. **FEBS L.**, [*s.l.*], v. 581, p. 2357–2366, 2007.

LEE, Y. J.; KANG, S. J.; KIM, B. M.; KIM, Y. J.; WOO, H. D.; CHUNG, H. W. Cytotoxicity of honeybee (*Apis mellifera*) venom in normal human lymphocytes and HL-60 cells. **Chem Biol Interact.**, [*s.l.*], v. 169, p. 189–197, 2007.

LEME, D. M.; MARIN-MORALES, M. A. *Allium cepa* test in environmental monitoring: A review on its application. **Mutat Res - Rev Mutat Res.**, [*s.l.*], v. 682, p. 71–81, 2009.

LOWE, R. M.; FARRELL, P. M. A portable device for the electrical extraction of scorpion venom. **Toxicon.**, [*s.l.*], v. 57, p. 244-247, 2011.

MARCUSSI, S.; SANTOS, P. R. S.; MENALDO, D. L.; SILVEIRA, L. B.; SANTOS-FILHO, N. A.; MAZZI, M. V.; SILVA, S. L.; STÁBELI, R. G.; ANTUNES, L. M. G.; SOARES, A. M. Evaluation of the genotoxicity of *Crotalus durissus terrificus* snake venom and its isolated toxins on human lymphocytes. **Mutat Res - Genet Toxicol Environ Mutagen.**, [*s.l.*], v. 724, p. 59–63, 2011.

MARCUSSI, S.; STÁBELI, R. G.; SANTOS-FILHO, N. A.; MENALDO, D. L.; PEREIRA, L. L. S.; ZULIANI, J. P.; CALDERON, L. A.; SILVA, S. L.; ANTUNES, L. M. G.; SOARES, A. M. Genotoxic effect of *Bothrops* snake venoms and isolated toxins on human lymphocyte DNA. **Toxicon.**, [*s.l.*], v. 65, p. 9–14, 2013.

OLIVEIRA-MENDES, B. B. R.; MIRANDA, S. E. M.; SALES-MEDINA, D. F.; MAGALHÃES, B. F.; KALAPOTHAKIS, Y.; SOUZA, R. P.; CARDOSO, V. N.; BARROS, A. L. B.; GUERRA-DUARTE, C.; KALAPOTHAKIS, E.; HORTA, C. C. R. Inhibition of *Tityus serrulatus* venom hyaluronidase affects venom biodistribution. **PLoS Negl Trop Dis.**, [*s.l.*], v. 13, e0007048, 19 April, 2019.

PALMIERI, M. J.; ANDRADE-VIEIRA, L. F.; CAMPOS, J. M. S.; GEDRAITE, L. S.; DAVIDE, L. C. Cytotoxicity of Spent Pot Lineron *Allium cepa* root tip cells: A comparative analysis in meristematic cell type on toxicity bioassays. **Ecotoxicol Environ Saf.**, [*s.l*], v. 133, p. 442-447, 2016b.

PALMIERI, M. J.; ANDRADE-VIEIRA, L. F.; TRENTO, M. V. C.; ELEUTÉRIO, M. W. F.; LUBER, J.; DAVIDE, L.; MARCUSSI, S. Cytogenotoxic effects of Spent Pot Liner (SPL) and its main components on human leukocytes and meristematic cells of *Allium cepa*. **Water Air Soil Pollut.**, [*s.l.*], v. 227, p. 156-166, 2016a.

PALMIERI, M. J. ***LACTUCA SATIVA*: BIOINDICADOR PARA ANÁLISE DO EFEITO GENOTÓXICO DE PEÇONHAS**. Doctorade—[s.l.] Universidade Federal de Lavras (UFLA), 2016.

PALMIERI, M. J.; BARROSO, A. R.; ANDRADE-VIEIRA, L. F.; MONTEIRO, M. C.; SOARES, A. M.; CESAR, P. H. S.; BRAGA, M. A.; TRENTO, M. V. C.; MARCUSSI, S.; DAVIDE, L. C. *Polybia occidentalis* and *Polybia fastidiosa* venom: a cytogenotoxic approach of effects on human and vegetal cells. **Drug Chem Toxicol.**, [*s.l.*], v. 44, p. 1-9, 2019.

PUCCA, M. B.; CERNI, F. A.; PINHEIRO-JUNIOR, E. L.; ZOCCAL, K. F.; BORDON, K. C. F.; AMORIM, F. G.; PEIGNEUR, S.; VRIENS, K.; THEVISSEN, K.; CAMMUE, B. P. A.; MARTINS JÚNIOR, R. B.; ARRUDA, E.; FACCIOLI, L. H.; TYTGAT, J.; ARANTES, E. C. Non-disulfide-bridged peptides from *Tityus serrulatus* venom: Evidence for proline-free ACE-inhibitors. **Peptides.**, [*s.l.*], v. 82, p. 44-51, 2016.

R DEVELOPMENT CORE TEAM. R: **A Language and Environment for Statistical Computing.** Viena R Found Stat Comput. 2015.

RECKZIEGEL, G. C.; PINTO, J. R. V. L. Scorpionismo in Brazil in the years 2000 to 2012. **J. Venom Anim. Toxins Incl. Trop. Dis.**, [*s.l.*], v. 20, p. 46-54, 2014.

REIS, G. B. D.; ANDRADE-VIEIRA, L. F.; MORAES, I. C.; CESAR, P. H. S.; MARCUSSI, S.; DAVIDE, L. C. Reliability of plant root comet assay in comparison with human leukocyte comet assay for assessment environmental genotoxic agents**. Ecotoxicol Environ Saf.**, [*s.l.*], v. 142, p. 110-116, 2017.

SANTOS, F. E.; CARVALHO, M. S. S.; SILVEIRA, G. L.; CORREA, F. F.; CARDOSO, M. D. G.; ANDRADE-VIEIRA, L. F.; VILELA, L. R. Phytotoxicity and cytogenotoxicity of hydroalcoholic extracts from *Solanum muricatum* Ait. and *Solanum betaceum* Cav. (Solanaceae) in the plant model *Lactuca sativa.* **Environ Sci Pollut Res.**, [*s.l.*], v. 25, p. 1-11, 2018.

SILVA, M. A.; SOUZA, T. G.; MELO, M. E. G.; SILVA, J. M.; LIMA, J. R.; LIRA, A. F. A.; AGUIAR-JÚNIOR, F. C. A.; MARTINS, R. D.; JORGE, R. J. B.; CHAGAS, C. A.; TEIXEIRA, V. W.; TEIXEIRA, A. A. C. *Tityus stigmurus* venom causes genetic damage in blood and testicular cells and affects the number and morphology of gametogenic lineage cells in mice. **Toxicon.**, [*s.l.*], v. 185, p. 114-119, 2020.

SINGH, N. P.; MCCOY, M. T.; TICE, R. R.; SCHNEIDER, E. L. A simple technique for quantitation of low levels of DNA damage in individual cells. **Exp Cell Res.**, [*s.l.*], v. 175, p. 184–191, 1988.

ÜSTÜN, S.; HAFRÉN, A.; HOFIUS, D. Autophagy as a mediator of life and death in plants. **Curr Opin Plant Biol.**, [*s.l.*], v. 40, p. 122-130, 2017.

ZAMBELLI, V. O.; PASQUALOTO, K. F.; PICOLO, G.; CHUDZINSKI-TAVASSI, A. M.; CURY, Y. Harnessin the knowledge of animal toxins to generate drugs. **Pharmacol Res.**, [*s.l.*], v. 112, p. 30-36, 2016.

ZARGAN, J.; SAJAD, M.; UMAR, S.; NAIME, M.; ALI, S.; KHAN, H. A. Scorpion (*Androctonus crassicauda*) venom limits growth of transformed cells (SH-SY5Y and MCF-7) by cytotoxicity and cell cycle arrest. **Exp Mol Pathol.**, [*s.l.*], v. 91, p. 447–454, 2011.

ZHOUA, S.; HONG, Q.; LI, Y.; LI. Q. Wang M. Autophagy contributes to regulate the ROS levels and PCD progress in TMV infected tomatoes. **Plant Sci.**, [*s.l.*], v. 269, p. 12–19, 2018.

ZOCCAL, K. F.; BITENCOURT, C. S.; SORGI, C. A.; BORDON, K. C. F.; SAMPAIO, S. V.; ARANTES, E. C.; FACCIOLI, L. H. Ts6 and Ts2 from Tityus serrulatus venom induce inflammation by mechanisms dependent on lipid mediators and cytokine production. **Toxicon**., [s.l.], v. 61, p. 1–10, 2013.

Authorship contributions

1 – Silvana Marcussi

Biologist, Doctor in Biochemistry
https://orcid.org/0000-0002-4674-6911 • marcussi@ufla.br
Contribuition: Conceptualization; Methodology; Writing – original draft

2 – Marcel José Palmieri

Biologist, Doctor of Genetics and Plant Breeding
https://orcid.org/0000-0001-6726-1369 • marcelpalmieri@yahoo.com.br
Contribuition: Conceptualization; Methodology; Writing – original draft

3 – Larissa Fonseca Andrade-Vieira

Biologist, Doctor of Genetics and Plant Breeding
https://orcid.org/0000-0002-7947-7498 • larissa.vieira@ufla.br
Contribuition: Conceptualization; Writing – original draft

4 – Amanda Ribeiro Barroso

Biologist, Master in Pharmacology
http://lattes.cnpq.br/7510999297252005 • barrosobioufla@gmail.com
Contribuition: Methodology; Writing – original draft

5 – Mariana Aparecida Braga

Chemistry, Doctor in Agrochemistry
https://orcid.org/0000-0003-0758-3993 • marybraga07@yahoo.com.br
Contribuition: Methodology. Writing – original draft. Review and editing

6– Marcus Vinicius Cardoso Trento

Biologist, Doctor in Agrochemistry
https://orcid.org/0000-0001-8463-9052 • marcusvinicius.ct@gmail.com
Contribuition: Writing – original draft. Review and editing

How to quote this article

MARCUSSI, S.; PALMIERI, M. J.; ANDRADE-VIEIRA, L. F.; BARROSO, A. R.; BRAGA, M. A.; TRENTO, M. V. C. Plant cytogenetics tests can predict toxic effects on human cells: genotoxic and mutagenic effects of *Tityus serrulatus* scorpion venom on vegetal and human cells. **Ciência e Natura**, Santa Maria, v.45, e19, 2023. DOI 10.5902/2179460X66067. Available in: https://doi.org/10.5902/2179460X66067.