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Cytotoxic and genotoxic effects of *Solanum lycocarpum* St.-Hil (Solanaceae) on the cell cycle of *Lactuca sativa* and *Allium cepa*

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ABSTRACT. *Solanum lycocarpum* St.-Hil popularly known as 'fruta-de-lobo' or 'lobeira' is native to the Brazilian Cerrado, and used in folk medicine due to its phytotherapic properties. The action of *S. lycocarpum* on the cell cycle and chromosomes in order to demonstrate whether there are aneugenic and/or clastogenic effects is unknown. Thus, this study aimed at investigating the cytotoxic and genotoxic potential of methanol and hexane extracts of *S. lycocarpum* on growth and cell cycle of *Lactuca sativa* and *Allium cepa*. Roots from both species were exposed for 72 hours to methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} of *S. lycocarpum*. Slides were prepared by the squash technique and then analyzed to determine the mitotic index and the total of chromosomal and nuclear abnormalities. The frequencies of chromosomal and nuclear abnormalities were high and significant with a dose-dependent effect, indicating that *S. lycocarpum* has a cytotoxic and genotoxic action depending on the dose used on meristem cells of *A. cepa* and *L. sativa*.

Keywords: aneugenic, Cerrado, clastogenic, lobeira, medicinal plant.

Efeito citotóxicos e genotóxicos de extratos *Solanum lycocarpum* St.-Hil (Solanaceae) no ciclo celular de *Lactuca sativa* e *Allium cepa*

RESUMO. *Solanum lycocarpum* St.-Hil (Solanaceae) conhecida popularmente como fruta-de-lobo ou lobeira é uma planta nativa do Cerrado brasileiro, utilizada na medicina popular nos tratamentos de diabetes, obesidade e redução do colesterol. Ainda não é conhecida a ação de *S. lycocarpum* sobre o ciclo celular e os cromossomos, demonstrando se possuem efeitos aneugênicos e/ou clastogênicos. O objetivo desse estudo foi investigar o potencial citotóxico e genotóxico dos extratos metanólico e hexânico de *S. lycocarpum* sob o crescimento e ciclo celular de *Lactuca sativa* e *Allium cepa*. As raízes de ambas as espécies foram expostas por 72h aos extratos metanólico e hexânico com 50, 100 e 200 μg mL^{-1} de *S. lycocarpum*. As lâminas foram montadas pela técnica de esmagamento e em seguida foram analisadas, afim de determinar o índice mitótico e anormalidades cromossômicas e nucleares. As frequências de anormalidades cromossômicas e nucleares foram altas e significativas, com efeito dose-dependente e confirmando que *S. lycocarpum* tem ação citotóxica e genotóxica de acordo com a dose utilizada sobre as células meristemáticas de *A. cepa* e *L. sativa*.

Palavras-chave: aneugênico, Cerrado, clastogênico, lobeira, planta medicinal.

Introduction

In Brazil, it is a common practice to use teas, infusions, and plasters with raw plants for the treatment of pathologies (Bighetti, Antonio, Foglio, & Posseni, 2005). Plant extracts may be effective in the treatment of several diseases, but some plant compounds have a toxic, carcinogenic, and teratogenic potential (Ferreira & Vargas, 1999, Akinboro & Bakare, 2007). Moreover, there is no knowledge on the cumulative effect of these plants on organisms.

Thus, there is a great concern in studying the genotoxic and mutagenic effects of species with medicinal potential, which may induce genetic damages and cause several health problems. In order to assure the quality of medicines, many bioassays for genotoxicity and mutagenicity have been conducted (Sousa & Viccini, 2011).

Some plants can be used as models in bioassays, such as *Allium cepa*, *Tradescantia,* and *Lactuca sativa* in order to investigate the action of some substances in the cell cycle. With the aid of these studies, it is possible to identify new mutagens by observing abnormalities in the different phases of the cell cycle. The model organisms have great advantages

and, therefore, are used in bioassays because they are easy to store and manipulate at a low cost, presenting a large number of dividing cells, well-known chromosomes, and good correlation with other testing systems (Fiskesjö, 1985, Grant, 1994).

Solanum lycocarpum (Solanaceae) popularly known as 'fruta-de-lobo' or 'lobeira' (Oliveira, Salazar, Duarte, Moreira, & Paula, 2010) is a plant native to the Brazilian Cerrado. Due to its phytotherapeutical potential, *S. lycocarpum* is widely used in folk medicine (Munari et al., 2012) for the treatment of diabetes, obesity, and cholesterol-lowering (Cruz, 1985). However, there are various studies reporting other pharmacological properties of *S. lycocarpum*. The leaves have properties that act as a sedative in the nervous system against epilepsy, spasms, kidney and abdominal pains. The flowers, besides being expectorant, aid in minimizing the symptoms of hemorrhoids whereas the roots are used for the treatment of hepatitis (Munari et al., 2012).

As for the popular use, the fruit pulp is crushed or macerated, which is denominated 'polvilho de lobeira' (lobeira starch), and then consumed (Oliveira et al., 2010). Currently, the lobeira powder, rich in polysaccharides, is marketed in capsules throughout Brazil (Dall'Agnol & Von Poser, 2000).

The toxic effect of *S. lycocarpum* is still debated in the literature (Oliveira et al., 2010). For example, Chang, Felício, Reis, Guerra, and Peters (2002) reported a fetotoxic effect in pregnant rats exposed to *S. lycocarpum*, inducing reduction in placenta, lungs and kidneys.

The action of *S. lycocarpum* extracts has been well reported for germination and growth of other plants, such as the studies by Oliveira et al. (2004a, 2004b) and Aires, Ferreira, and Borghetti (2005), who studied the allelopathic potential of *S. lycocarpum* in sesame seeds. They found that the aqueous fruit and leaf extracts of *S. lycocarpum* may promote changes in the morphology of sesame seeds, besides impairing germination and growth. On the other hand, there were no reports on the effects of *S. lycocarpum* extracts on the cell cycle, demonstrating whether they have aneugenic or clastogenic effects.

Thus, this study aimed at investigating the cytotoxic and genotoxic potential of methanol and hexane extracts of *S. lycocarpum* on the growth and cell cycle of *Lactuca sativa* and *Allium cepa*.

Material and methods

Plant material and extraction

Unripe fruits (498.20 g) of *S. lycocarpum* were collected in São Sebastião do Oeste (20° 14' 38.96" S, 45° 2' 14.38" W), Minas Gerais State, Brazil, in August, 2013. Ph.D. Alexandre Salino identified the plant material and a voucher specimen (BHCB 159397) was deposited in the herbarium belonging to *Instituto de Ciências Biológicas* at the *Universidade Federal de Minas Gerais* in Belo Horizonte, Minas Gerais State, Brazil.

Hexane (ACS reagent ≥ 98.5%, Sigma-Aldrich, USA) and methanol (ACS reagent ≥ 99.8%, Sigma-Aldrich, USA) were used as solvents (700 mL, 6 hours) to obtain the extracts from 74.62 g of dried and powdered unripe fruit, using a Soxhlet extractor. The extracts were then concentrated in a rotary evaporator at 40°C under reduced pressure to produce hexane (HEX, 2.26 g) and methanol (MET, 10.23 g) extracts.

Germination and growth of *Lactuca sativa* and *Allium cepa*

Seven-hundred seeds of *Lactuca sativa* cultivar 'White Boston' and *Allium cepa* cultivar 'Baia Periforme' were germinated in distilled water in bod chamber (BOD) at 24°C. These two model species were used to increase the reliability of the allelopathic tests of *S. lycocarpum* extracts.

After the protrusion of the radicle, for each assay, one-hundred seeds of *L. sativa* and *A. cepa* were transferred to a Petri dish lined with germination paper moistened with 5 mL distilled water (control), 50, 100, and 200 μg mL^{-1} hexane and methanol dried extracts of *Solanum lycocarpum* dissolved in distilled water. The doses chosen were based on the lowest doses used to promote growth inhibition of *A. cepa* and *L. sativa* seeds, according to Araújo et al. (2013).

After 72 hours in contact with the extract, 25 root tips per treatment of the *L. sativa* and *A. cepa*, selected randomly, were measured with the aid of a caliper (Ribeiro et al., 2013) and subsequently fixed in Carnoy (ethanol: acetic acid 3:1) for 24 hours at room temperature. Afterwards, roots were transferred to a new Carnoy solution and stored at -4°C until preparation of the slides.

Cytogenetic analyses

For the analysis of the cell cycle of *Lactuca sativa* and *Allium cepa*, roots were rinsed in distilled water and hydrolyzed in HCl 1 N at 60°C for 10 min. The slides were mounted, with five roots per slides, through the squash technique (Guerra & Souza,

2002), and subsequently stained with 2% acetic orcein. Slides were analyzed in a microscope Axio Lab. A1 (Zeiss) coupled with a camera AxioCam Icc1 at 400X magnification. We evaluated five slides per treatment and 1000 cells on each slide, totalizing 5000 cells per treatment for both species. For each treatment, the mitotic index (MI) and the total chromosomal and nuclear abnormalities were determined at all phases of the cell cycle.

Statistical analyses

A multiple contrast test (Mukerjee, Robertson, & Wright, 1987, Bretz, Genz, & Hothorn, 2001) coupled with the Dunnett procedure was applied to perform simultaneous treatment levels comparisons versus the negative control (Hothorn, Bretz, & Westfall, 2008). In this case, the significance and 95% confidence interval of each concentration level for both extracts (Hexane and Methanol) and biological models (*Allium cepa* and *Lactuca sativa*) was tested against the negative control for each response variable. Cytogenetic variables are non-binomial proportions and hence were logit-transformed prior to analysis for satisfying modelling assumptions as suggested by Warton and Hui (2011).

All the statistical analysis was run using the computing environment R (R Core Team, 2016), using the package multcomp (Hothorn, 2016) for generating the Dunnett procedure statistics and simultaneous confidence intervals.

Results and discussion

Root growth

Lactuca sativa roots responded differently to the exposure to hexane and methanol extracts. The two-sided confidence intervals and Dunnett's t-test for all differences to control suggest a significant effect of every *S. lycocarpum* concentration level on the *Lactuca sativa*-Methanol treatment (Table 1; Figure 1A and B). In the case of *Lactuca sativa* - Hexane treatment, only one concentration level (100 μg mL^{-1} of *S. lycocarpum*) showed significant effect (Table 1; Figure 1A and B).

Allium cepa roots that were not exposed to *S. lycocarpum* showed higher growth, differing statistically from the remaining roots subjected to different extracts and concentrations of *S. lycocarpum*. These treatments, in turn, were not significantly different from each other, except for the concentration of 200 μg mL^{-1} *S. lycocarpum*, which showed smaller root growth (Table 1; Figure 1C and D).

Cell cycle

The analysis of the cell cycle of both species showed similar results for the effects of *S. lycocarpum* extracts. Methanol and hexane extracts of *S. lycocarpum* at all concentrations showed cytotoxic potential on *Lactuca sativa* and *Allium cepa*, because they decreased the mitotic index and induced chromosomal abnormalities statistically significant in relation to the control treatment.

Lactuca sativa roots treated with distilled water showed the highest mitotic indices, differing statistically from roots subjected to methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *S. lycocarpum* (Table 2; Figure 2A and B). The mitotic indices obtained for roots exposed to *S. lycocarpum* extracts were significantly different from each other in a dose-dependent way, in which the increasing concentration of *S. lycocarpum* decreased the mitotic index for the species studied.

For *Allium cepa*, the extracts of *S. lycocarpum* have also significantly decreased the mitotic index as the concentrations of the extracts increased. The lowest mitotic index observed was for the treatment with 200 μg mL^{-1} of methanol extract of *S. lycocarpum* whereas the highest mitotic indices were found with 50 μg mL^{-1} for both extracts (Table 2; Figure 2C and D).

Table 1. Statistical analyses for growth of *Lactuca sativa* and *Allium cepa* roots treated with methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *Solanum lycocarpum* and control treatment with distilled water.

Model/Reagent	Concentration comparison	Estimate	Standard Error	t value	Significance	Pr (>|t|)
	50-0	-0.21	0.1299	-1.617		0.2562
Lactuca sativa -Hexane	100-0	-0.35	0.1299	-2.695	*	0.0236
	200-0	-0.245	0.1299	-1.886		0.1546
	50-0	-0.5	0.1895	-2.638	*	0.0273
Lactuca sativa - Methanol	100-0	-1.175	0.1895	-6.199	***	< 0.001
	200-0	-1.33	0.1895	-7.017	***	< 0.001
	50-0	-0.12	0.2329	-0.515		0.919
Allium cepa - Hexane	100-0	0.195	0.2329	0.837		0.738
	200-0	0.06	0.2329	0.258		0.988
	50-0	-0.255	0.2109	-1.209		0.4841
Allium cepa - Methanol	100-0	-0.395	0.2109	-1.873		0.1586
	200-0	-0.55	0.2109	-2.608	*	0.0296

*Denotes a statistically significant difference to the respective control. Significance codes: 0; ***0.001; **0.01; *0.05; 1. t: Dunnett's test value. Pr: Significance level.

Table 2. Statistical analyses for mitotic index of *Lactuca sativa* and *Allium cepa* roots treated with methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *Solanum lycocarpum* and control treatment with distilled water.

Cytogenetic variable	Model/Reagent	Concentration comparision	Estimate	Standard Error	t value	Significance	Pr (>|t|)
Mitotic Index	*Lactuca sativa* -Hexane	50-0	-0.34	0.02	-16.69	***	< 2e-16
		100-0	-0.42	0.02	-20.53	***	< 2e-16
		200-0	-0.81	0.02	-39.27	***	< 2e-16
	Lactuca sativa - Methanol	50-0	-0.43	0.03	-16.51	***	< 2e-16
		100-0	-0.57	0.03	-22.09	***	< 2e-16
		200-0	-0.72	0.03	-27.64	***	< 2e-16
	Allium cepa - Hexane	50-0	-0.21	0.03	-6.65	***	1.33E-05
		100-0	-0.27	0.03	-8.65	***	< 1e-05
		200-0	-0.56	0.03	-17.71	***	< 1e-05
	Allium cepa - Methanol	50-0	-0.15	0.02	-5.95	***	< 1e-04
		100-0	-0.38	0.02	-15.30	***	< 1e-04
		200-0	-0.98	0.02	-39.63	***	< 1e-04

*Denotes a statistically significant difference to the respective control. Significance codes: 0; ***0.001. t: Dunnett's test value. Pr: Significance level.

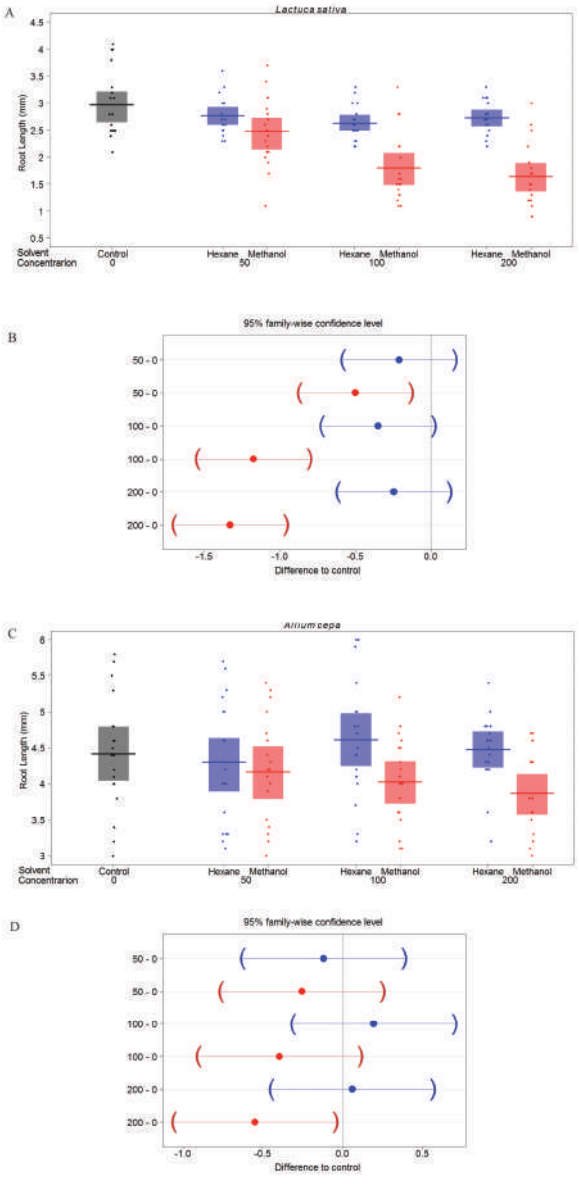

Figure 1. Growth of *Lactuca sativa* (A-B) and *Allium cepa* (C-D) roots treated with methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *Solanum lycocarpum* and control treatment with distilled water. *Two-sided confidence intervals and Dunnett's t-test.

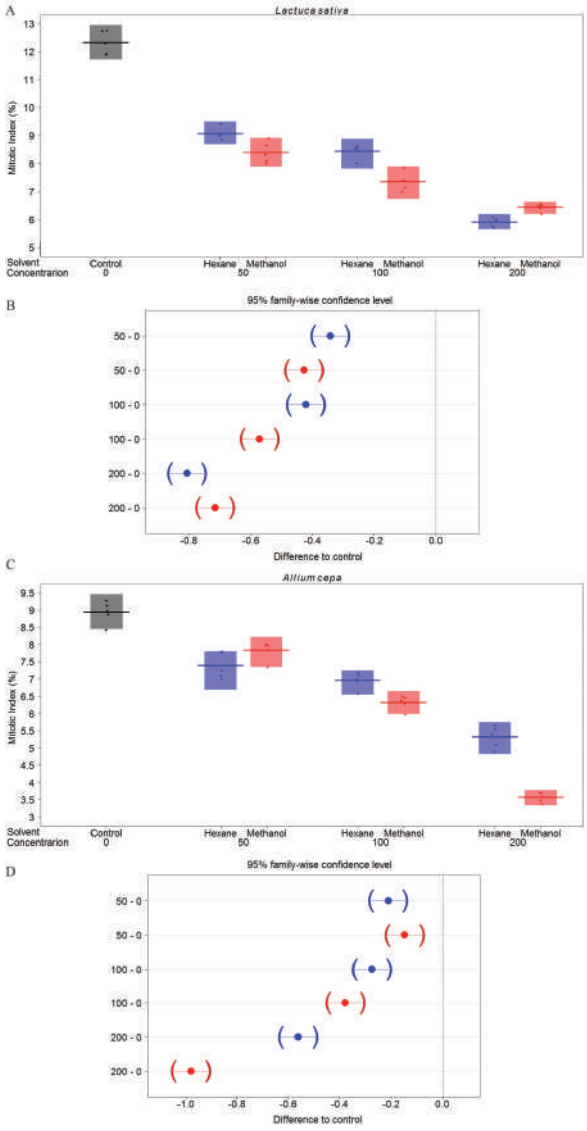

Figure 2. Cytotoxic and genotoxic effects of methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *Solanum lycocarpum* on roots of *Lactuca sativa* (A-B) and *Allium cepa* (C-D) expressed in mitotic index. *Two-sided confidence intervals and Dunnett's t-test.

For the control treatment, we observed no chromosomal abnormalities in roots of *Lactuca sativa*

and *Allium cepa*, differing statistically from the other extracts and concentrations of *S. lycocarpum*. Roots of both species exposed to hexane extracts showed the highest rates of chromosomal abnormalities, statistically significant in comparison to methanol extracts (Table 3; Figure 3). The predominant abnormalities consisted of chromosomes/ nuclei in stickiness, bridges at anaphase and telophase, C-metaphases, unoriented chromosomes at metaphases, and chromosomes/chromatids or even lost fragments at anaphases (Table 4 and 5, Figure 4).

Regarding nuclear abnormalities, *Lactuca sativa* roots were most affected at the concentration of 200 μg mL^{-1} for both extracts, with no statistical differences from each other (Table 6, Figure 2). For *Allium cepa*, a larger number of cells with nuclear abnormalities was observed at the concentration of 200 μg mL^{-1} of methanol and hexane extracts of *S. lycocarpum*, which were statistically different From each other (Table 2; Figure 5). Condensed nuclei feature cell death and it was the only nuclear abnormality found in *Lactuca sativa* and *Allium cepa* roots. Micronuclei have not been observed in any treatment for both species.

Mitotic index is also related to cell proliferation, since it is considered the ability of the cell population to increase (Hao, You, & Deng, 2002). There are cells in intense division in growing tissues, as observed in root apical meristems. Such tissues have greater or lesser susceptibility to several biotic or abiotic stresses, making it possible to test the toxicity of a given substance (Molina, Tillmann, Biccadode, & Viégas, 2006).

Table 3. Statistical analyses for chromosomal abnormality of *Lactuca sativa* and *Allium cepa* roots treated with methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *Solanum lycocarpum* and control treatment with distilled water.

Cytogenetic variable	Model/Reagent	Concentration comparision	Estimate	Standard Error	t value	Significance	Pr (>|t|)
Chromosomal Abnormality	*Lactuca sativa* -Hexane	50-0	0.80	0.06	14.09	***	< 1e-10
		100-0	1.01	0.06	17.79	***	< 1e-10
		200-0	1.27	0.06	22.23	***	< 1e-10
	Lactuca sativa - Methanol	50-0	0.72	0.04	18.79	***	< 1e-10
		100-0	0.79	0.04	20.55	***	< 1e-10
		200-0	0.98	0.04	25.29	***	< 1e-10
	Allium cepa - Hexane	50-0	0.74	0.04	20.84	***	< 2e-16
		100-0	1.11	0.04	31.53	***	< 2e-16
		200-0	1.42	0.04	40.16	***	< 2e-16
	Allium cepa - Methanol	50-0	0.72	0.03	24.55	***	< 2e-16
		100-0	0.81	0.03	27.74	***	< 2e-16
		200-0	1.01	0.03	34.29	***	< 2e-16

*Denotes a statistically significant difference to the respective control. Significance codes: 0; ***0.001. t: Dunnett's test value. Pr: Significance level.

Table 4. Chromosomal abnormalities (%) observed in the cell cycle of meristems of *Lactuca sativa* roots exposed to methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *Solanum lycocarpum.*

	Abnormalities (%)					
Treatment	Stickiness	Bridges	Unoriented chromosomes	C-Metaphase	Chromosome/ chromatids or lost fragments	Mean number of cells with chromosomal abnormalities
Control	-	-	-	-	-	-
Met 50 μg mL^{-1}	25	50	-	12.5	12.5	16±0.71
Met 100 μg mL^{-1}	-	23.08	30.76	23.08	23.08	18.2±1.48
Met 200 μg mL^{-1}	61.52	23.07	-	-	15.38	24.8±4.32
Hex 50 μg mL^{-1}	22.22	44.44	-	22.22	11.11	13.8±2.17
Hex 100 μg mL^{-1}	23.07	42.3	34.62	-	-	19.2±3.27
Hex 200 μg mL^{-1}	-	35.7	-	50	14.28	27.6±4.34

Table 5. Chromosomal abnormalities (%) observed in the cell cycle of meristems of *Allium cepa* roots exposed to methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *Solanum lycocarpum.*

	Abnormalities (%)					
Treatment	Stickiness	Bridges	Unoriented chromosomes	C-Metaphase	Chromosomes/ chromatids or lost fragments	Mean number of cells with chromosomal abnormalities
Control	-	-	-	-	-	-
Met 50 μg mL^{-1}	87.5	12.5	-	-	-	18±0.71
Met 100 μg mL^{-1}	76.9	7.1	15.38	-	-	21.4±1.82
Met 200 μg mL^{-1}	62.5	12.5	12.5	12.5	-	29.4±3.65
Hex 50 μg mL^{-1}	15.38	23.07	34.62	11.54	15.38	12±0.71
Hex 100 μg mL^{-1}	43.09	33.33	-	4.54	19.04	22.4±1.82
Hex 200 μg mL^{-1}	7.69	23.09	69.23	7.69	7.69	34±4

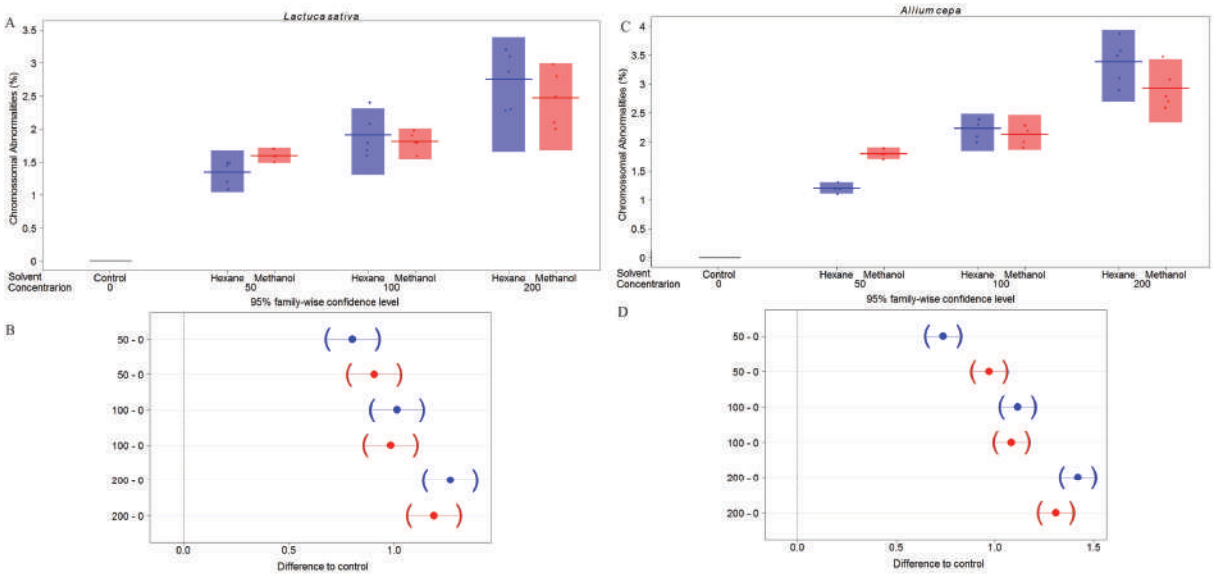

Figure 3. Cytotoxic and genotoxic effects of methanol and hexane extracts with 50, 100, and 200 μg ml^{-1} *Solanum lycocarpum* on roots of *Lactuca sativa* (A-B) and *Allium cepa* (C-D), expressed in chromosomal abnormalities. *Two-sided confidence intervals and Dunnett's t-test.

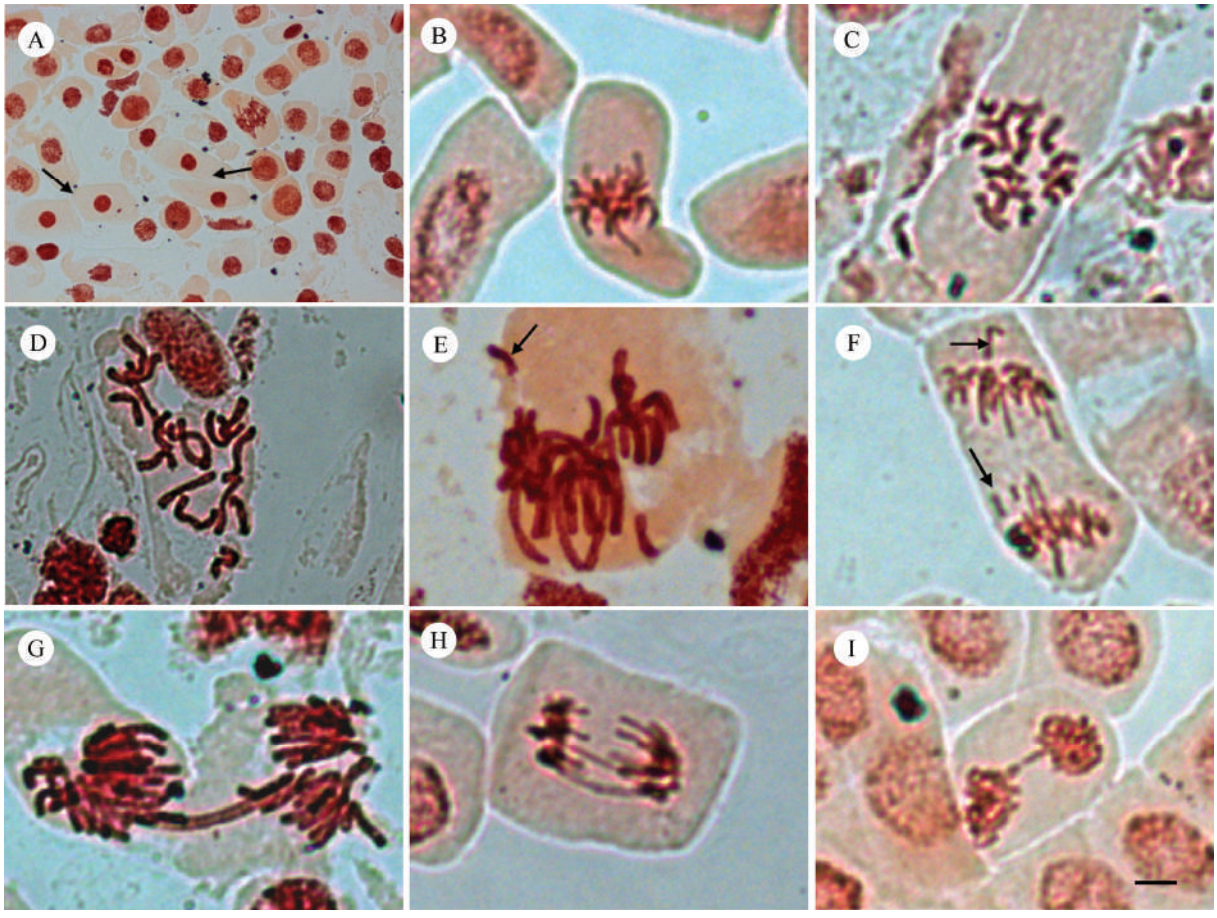

Figure 4. Chromosomal and nuclear abnormalities in *Lactuca sativa* and *Allium cepa* exposed to methanol and hexane extracts of *Solanum lycocarpum*. A. Condensed nuclei (arrow), B. Stickiness, C-D. C-Metaphase, E. Unoriented chromosome (arrow), F. Chromosome/chromatid (arrow) and fragment (arrowhead) lost at anaphase, G. Bridge at anaphase, H. Double bridge at anaphase, I. Bridge at telophase. Bar 5 μm.

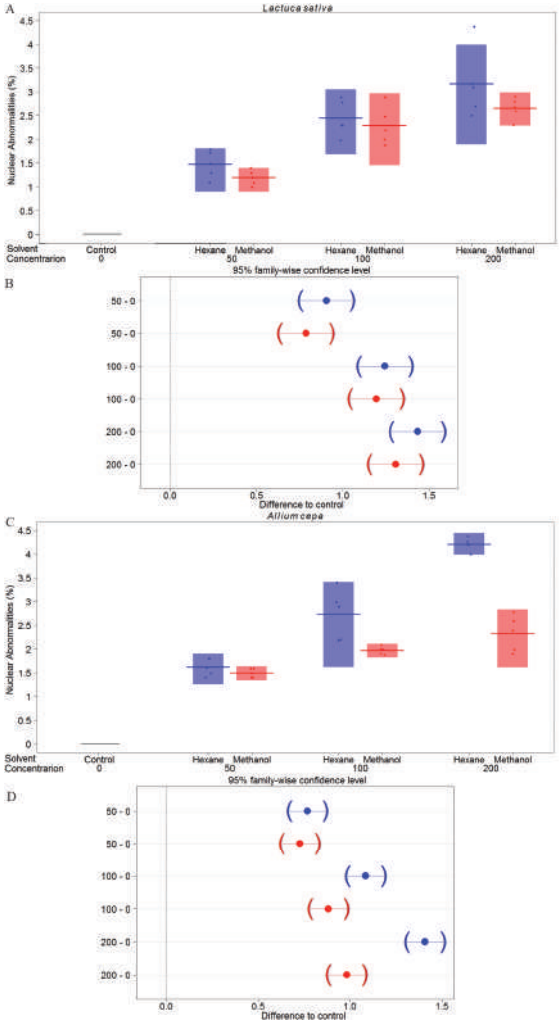

Figure 5. Cytotoxic and genotoxic effects of methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *Solanum lycocarpum* on roots of *Lactuca sativa* (A-B) and *Allium cepa* (C-D) expressed in nuclear abnormalities. *Two-sided confidence intervals and Dunnett's t-test.

According to Çelik and Äslanturk (2006), the mitotic index may be lower when exposed to a cytotoxic substance because it can inhibit the cell cycle. However, the cytotoxic potential may be expressed through a combination of other substances and depending on the concentrations used (Sousa, Silva, Campos, & Viccini, 2009). *Solanum* species have high concentrations of alkaloids such as solasonine and solamargine. These substances are partially responsible for toxicity and also exhibit cytotoxic properties against cancer cells (Vieira, Costa, Silva, & Chen-Chen, 2010, Munari et al., 2014). The decreased rootlet growth observed with the methanol extract can be explained by the reduced dose-dependence of the mitotic index. This is an important trace of the cytotoxic effect of *S. lycocarpum* extracts on meristem cells of *Lactuca sativa* and *Allium cepa*. Similarly, lower concentration of *S. lycocarpum* have also been reported to significantly reduce mitotic index of V79 cells (64 µg mL^{-1} in a 1 to 256 µg mL^{-1} range) (Tavares et al., 2011). Sousa et al. (2009), studying the cytotoxic potential of extracts of two medicinal species, *Lantana camara* L. and *Lippia alba* (Mill.), observed the reduction in root growth with increasing concentrations of extracts of these plants. The same occurred with the mitotic index, which is pointed out as an agent for decreasing root growth.

Usually, the root growth and the mitotic index are correlated parameters, however this has not been observed for hexane extracts. The cell expansion can explain the growth of roots treated with hexane extracts. In the early development of plants, the early cells divide, which is followed by a cell expansion, allowing the growth of organs, and subsequent reentering cell division (Taiz & Zeiger, 2006).

The increase of condensed and fragmented nuclei is the first sign of apoptosis. Such events may be responsible for the decrease in the mitotic index, as observed for cells treated with *S. lycocarpum* extracts. When there is a significant decrease of mitosis due to the action of toxic substances, there is a direct influence on growth and development of the organism exposed, because cytotoxic substances can block the G2 phase of the cell cycle, preventing the cell from entering into mitosis or inhibiting the DNA synthesis, leading to a decrease of the mitotic index in relation to control organisms (Turkoglu, 2008, Leme & Marin-Morales, 2009).

Table 6. Statistical analyses for nuclear abnormality of *Lactuca sativa* and *Allium cepa* roots treated with methanol and hexane extracts with 50, 100, and 200 μg mL^{-1} *Solanum lycocarpum* and control treatment with distilled water.

Cytogenetic variable	Model/Reagent	Concentration comparison	Estimate	Standard Error	t value	Significance	Pr (>|t|)
Nuclear Abnormality	*Lactuca sativa* -Hexane	50-0	0.85	0.07	11.62	***	< 1e-10
		100-0	1.18	0.07	16.08	***	< 1e-10
		200-0	1.37	0.07	18.60	***	< 1e-10
	Lactuca sativa - Methanol	50-0	0.79	0.05	15.70	***	< 2e-16
		100-0	1.19	0.05	23.85	***	< 2e-16
		200-0	1.31	0.05	26.09	***	< 2e-16
	Allium cepa - Hexane	50-0	0.77	0.05	16.30	***	< 1e-10
		100-0	1.08	0.05	23.02	***	< 1e-10
		200-0	1.41	0.05	29.88	***	< 1e-10
	Allium cepa - Methanol	50-0	0.73	0.04	20.30	***	< 2e-16
		100-0	0.88	0.04	24.66	***	< 2e-16
		200-0	0.98	0.04	27.45	***	< 2e-16

*Denotes a statistically significant difference to the respective control. Significance codes: 0; ***0.001. t: Dunnett's test value. Pr: Significance level.

The large number of cells with chromosomal abnormalities observed in roots treated with methanol and hexane extracts with 100 and 200 μg mL^{-1} of *S. lycocarpum* shows that these extracts have also a genotoxic potential in cells of *Lactuca sativa* and *Allium cepa*. The chromosomal abnormalities may arise from the dysfunction of mitotic spindle and breakage along chromosomes, especially near telomeric regions. Such events interfere with the chromosomal segregation, resulting in the formation of daughter cells with changes in chromosome structures and/or the total number of chromosomes. Thus, the presence of chromosomal abnormalities indicates the genotoxic effects of a certain substance (Natarajan, 2002, Russel, 2002, Fernandes, Mazzeo, & Marin-Morales, 2007).

Some events observed in cells, such as unoriented chromosomes and chromosome bridges, indicate that these extracts at the concentrations tested promote aneugenic and clastogenic damages, respectively. The aneugenic event characterized by the loss of whole chromosomes may result in the formation of C-metaphases, lost and unoriented chromosomes, indicating that the cell have components that prevent the polymerization of microtubules, therefore, preventing the formation of mitotic spindle fibers (Fernandes et al. 2007). On the other hand, the clastogenic event causes chromosome breakage, forming chromosome bridges and stickiness. When there is the breakage of chromosome segments, it may fuse (inter- and intra-chromatic fusions) getting sticky and irreversible forms (stickiness) or even leading to cell death (El-Ghamery, El-Kholy, & El-Yousser, 2003). Another consequence of chromosomal segment breakages is the cycle of breakage-fusion-bridge described by McClintok (1941) in a classic study on maize cytogenetics. In this study, the author proposed that the non-repair of the chromosomes that had lost the telomeric region generates cycles of breakage-fusion-bridge, since there is the fusion of sister chromatids with no telomeres, transforming them into a chromosome with two centromeres. As the genetic material move to the polar region of the cell, at anaphase, these centromeres are pulled to opposite directions, creating the chromosome bridge. The chromosome bridge may break at a random region, generating a chromosomal segment without telomeres that can be transferred to the next generation and, therefore, restart the cycle of breakage-fusion-bridge.

The frequencies of chromosomal and nuclear abnormalities were high and significant with a dose-dependent effect, confirming that *S. lycocarpum* has cytotoxic and genotoxic action depending on the dose applied on meristematic cells of *Allium cepa* and *L. sativa*.

Conclusion

Taking into account the doses and the period of exposure, we found that methanol extracts of *S. lycocarpum* have an inhibitory effect on the growth of *L. sativa* roots.

In general *L. sativa* was more affected by the *S. lycocarpum* extracts, for both nuclear abnormality and chromosomal abnormality.

The extracts of *S. lycocarpum* have cytotoxic potential because they decreased the mitotic index and induced chromosomal abnormalities in the cell cycle of both species at all concentrations studied.

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