

Effects cytotoxic and genotoxic of *Psittacanthus acinarius* and *Psittacanthus cordatus* (mistletoe) on *Allium cepa*

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Abstract

This study assessed the cytotoxic and genotoxic potentials of extracts of *Psittacanthus acinarius* (Mart.) Mart. and *Psittacanthus cordatus* (Hoffmanns.) in the root cell cycle of *Allium cepa* L. Aqueous leaf extracts of *P. acinarius* and *P. cordatus* at three concentrations: 0.00, 5 and 20 mg/mL for 24 hours. Histological slides were prepared and mitotic indices (MI %) and chromosomal alteration indices (CAI %) were determined. Inhibitory effects of the aqueous extract of leaves of *P. acinarius* were observed in 46.33 and 46.00% at concentrations of 5 and 20 mg/mL, respectively, in relation to the control (62.83%), in addition to a higher chromosomal alteration index by 0.26% at a concentration of 20 mg/mL. In the aqueous extract of leaves of *P. cordatus*, the greatest inhibitory effects were 33.83 and 35.50% in the concentrations of 5 and 20 mg/mL, respectively, in relation to the control (88.16%) and the highest alteration index chromosomal (3.30%) at 5 mg/mL. The aqueous leaf extracts of *P. acinarius* and *P. cordatus* at concentration of 5 and 20 mg/mL inhibit MI %, reveal an irregular recovery or prevent it, and induce chromosomal alterations, suggesting cytotoxic and genotoxic effects on division of meristematic cells of *A. cepa*.

Keywords: Chromosomal alteration. Medicinal mistletoe. Mitotic index. Bioassays.

Introduction

The family Loranthaceae Juss. (Santalales), with 70 genera and 940 species^[1], has a great diversity of mistletoes distributed mainly in tropical areas, but also temperate regions^[2]. Among the genera of this family, the *Psittacanthus* Mart. has approximately 120 species distributed from California (USA) to Argentina, extending through Jamaica and other Caribbean islands^[3]. In Brazil, it is the most representative genus, being found in the Amazon, Atlantic Forest, Caatinga, Cerrado, and Pantanal^[4]. Its flowers produce nectars enriched with biomolecules attractive to pollinators^[5,6].

Besides presenting an ecological interaction, this genus has therapeutic potential, with description of plants used for diabetes and hypertension^[7], with anti-inflammatory^[8], antibacterial, and antioxidant activity^[9], vasodilator^[10] and anti-hyperglycemic effect, and genotoxicity^[11].

The species *Psittacanthus acinarius* (Mart.) Mart. and *Psittacanthus cordatus* (Hoffmanns.) are among the plants of this genus. These species have been used popularly in the treatment of cancer^[12]. Also, *P. acinarius* has medicinal uses for treating wounds, ulcers, and diabetes and disorders of the urinary, respiratory, cardiovascular, and reproductive systems^[12], while *P. cordatus* is used to treat eye infections^[13]. Despite the medicinal importance of these species, few studies have described the possible toxic compounds synthesized by plants, being required studies that reveal the possible damage to human health.

The cytotoxic and genotoxic effects of plant extracts can be assessed in several cytogenetic assays, such as in the root tips of plant species^[14]. Among these assays is the *Allium cepa* L. test, which allows the contact of the root of the test organism with the tested substance, allowing assessing different doses of the studied plant, showing possible cytotoxic and mutagenic potential^[15]. Inhibition in the cell cycle, interruption in metaphases, induction of numerical and structural chromosomal alterations, and sister chromatid exchanges are among the alterations observed cytologically^[15]. In addition to assessing various genetic parameters, it also allows verifying the mechanism of action of the substances tested in the DNA and stating that the action of a compound is cytotoxic, genotoxic, or carcinogenic^[16]. Therefore, it is an important tool for such assessments^[17-19]. Thus, this study aimed to assess the cytotoxic and genotoxic potentials of concentrations of aqueous leaf extract of *P. acinarius* (EAFPa) and *P. cordatus* (EAFPC) in the cell cycle of *A. cepa*.

Material and methods

Linking

This study is linked to the Mistletoes Project, under the responsibility of the Research Group Unemat/CNPq FLOBIO (Study of the Flora Bearing Bioactive Substances of Mato Grosso).

Plant material

Leaves of *Psittacanthus cordatus* (Hoffmanns.) and *Psittacanthus acinarius* (Mart.) Mart. were collected on plants of aroeira preta [*Astronium urundeuva* (M.Allemão) Engl., Anacardiaceae] and guava (*Psidium guajava* L., Myrtaceae), respectively, at the University Campus Jane Vanini of the Mato Grosso State University "Carlos Alberto Reyes Maldonado" (UNEMAT), in the municipality of Cáceres, Mato Grosso, Brazil, located at 16°03'46.1" N, 57°40'48.82" W, with an altitude of 123 m. The identification was carried out in 2015 with the assistance of Dr. Claudenir Simões Caires from the State University of Southwest Bahia (UESB), who has experience in the botany area, with an emphasis on morphology and taxonomy of Loranthaceae and Viscaceae.

Preparation of extracts

The leaves (500 g of each species) were dehydrated in an air-circulation oven at 45°C for seven days and ground in a mill (IKA®, model M20). The extracts were prepared with 150 g of the crushed material and 750 mL of distilled water at ambient temperature for 60 minutes, being subsequently filtered on ordinary filter

paper. This process was repeated three times. The extract was then taken to an air-circulation oven at 45°C for seven days until the water evaporated, obtaining the concentrated aqueous extract.

Model system

Onions (*Allium cepa* L.; 2n=16) obtained commercially were subjected to rooting in 50 mL of distilled water and maintained in a germination chamber of the biochemical oxygen demand (BOD) type at 25°C and under constant white light for 72 hours. The roots reached an average length of 2 cm and were submitted to concentrations of aqueous extract for each species, that is, D₁=5 mg/mL and D₂=20 mg/mL, including the negative control (NC, D₀=0 mg/mL), which remained exclusively in distilled water. The experimental unit consisted of six replications arranged in a completely randomized design. The average diameter values (mm) of onions within each species varied little (*P. acinarius*: NC or D₀=51.66, D₁=51.16, and D₂=51.66; *P. cordatus*: NC or D₀=39.00, D₁=38.83, and D₂=38.16). The pH values of all doses were determined with a portable Kasvi pH meter (model K39-0014PA) (**TABLE 1**). The roots (still in the onions) were subjected to diluted doses in 50 mL of distilled water for 24 hours, whose environment was qualified as treatment (Ti). After this period, 50% of the roots were collected and the remaining roots were subjected to distilled water without the concentrations for 24 hours to assess the recovery of the mitosis process, which was qualified as the recovery environment (Ri). The roots collected in the process of submission to treatments and the recovery process were fixed in Carnoy solution (95% ethanol + glacial acetic acid, 3:1 v/v), and stored in a refrigerator for further analysis.

TABLE 1: Solution pH values at doses of aqueous leaf extracts of *Psittacanthus acinarius* (Mart.) Mart. and *Psittacanthus cordatus* (Hoffmanns.) applied to the roots of *Allium cepa* L.

Species	Solutions (S) with the used doses (D _i) (mg/mL)		
	D ₀ =0	SD ₁ =5	SD ₂ =20
	pH		
<i>P. acinarius</i>	7.2	5.2	5.0
<i>P. cordatus</i>	7.2	6.5	6.1

Cytogenetic analysis

Root ends were washed in distilled water for four minutes, hydrolyzed in 5N HCL for five minutes, and washed again in distilled water for four minutes. The root ends were transferred to histological slides and sectioned with cutting blades. Subsequently, a drop of Giemsa dye (5%) was superimposed on the coverslip, the tissue was crushed, the excess dye was removed using a filter paper^[20], and photographed under an optical microscope (400x magnification). A total of 3000 cells were identified in each treatment, and normal cells at different division stages and those showing alterations in the cell cycle were counted (**FIGURES 1A-1H**). Mitotic indices (MI %) were calculated with the number of normal cells at each phase of cell division, divided by the total number of cells counted, while the cell alteration index (CAI %) was obtained by dividing the total number of cells with alterations in the cell cycle by the total number of cells counted. Both indices were multiplied by 100.

The data were subjected to analysis of variance and the means compared by the Tukey (for treatments) and Student tests (for submission environment) at the 5% significance level, using the statistical program SISVAR® for analysis^[21]. The compared means were considered different when the conclusive error was

likely to occur in a proportion lower than 1/20 ($P < 0.05$). Equal and different letters were used between similar and different means, respectively.

Results and Discussion

Cytotoxicity effect

An inhibitory effect ($P < 0.05$) of the mitotic index (MI %) was found in roots of *A. cepa* for doses of aqueous leaf extracts of *P. acinarius* (EAFPa) and *P. cordatus* (EAFPC) in the exposure environments (Ti) and with irregular partial recovery in the recovery environment (Ri) (**TABLE 2**).

TABLE 2: Mitotic indices (MI %) observed in meristematic cells of *Allium cepa* L. exposed to aqueous leaf extracts of *Psittacanthus acinarius* (Mart.) Mart. (EAFPa) and *Psittacanthus cordatus* (Hoffmanns.) (EAFPC).

Species: <i>P. acinarius</i>		
Doses of EAFPa (mg/mL)	Environments	
	Submission (Ti)	Recovery (Ri)
	MI % (Mean \pm SD)	
D ₀ = 0	62.83 \pm 4.20 aB	71.33 \pm 3.40 aA
D ₁ = 5	46.33 \pm 3.21 bB	59.00 \pm 2.80 bA
D ₂ = 20	46.00 \pm 6.97 bA	44.50 \pm 2.68 cA
Species: <i>P. cordatus</i>		
Doses of EAFPC (mg/mL)	Environments	
	Submission (Ti)	Recovery (Ri)
	MI % (Mean \pm SD)	
D ₀ = 0	88.16 \pm 6.91 aA	77.83 \pm 6.06 aB
D ₁ = 5	33.83 \pm 5.60 bB	60.83 \pm 1.57 bA
D ₂ = 20	35.50 \pm 3.13 bB	56.66 \pm 4.76 bA

SD – standard deviation. Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other by the Tukey test (for treatments) and Student t-test (for submission environment) at the significance level of 5% probability.

The mitotic indices (MI %) for the species *P. acinarius* in the treatment environment (Ti) (presence of the EAFPa extract) were similar and lower at the tested doses (MI_{D1} = 46.33% and MI_{D2} = 46.00%) compared to the control (MI_{D0} = 62.83 %). Also, the MI % recovered less with an increase in doses (MI_{D0R0} = 71.33, MI_{D1R1} = 59.00 %, and MI_{D2R2} = 44.50%) when submitted to the recovery process (Ri). The recovery of MI% at the highest dose (D₂ = 20 mg/mL) ceased, characterizing an irreversible effect in the cell division process at the highest dose (MI_{D2} = 46.00% and MI_{D2R2} = 44.50%) and a partially reversible effect at the lowest concentrated dose (MI_{D1} = 46.33 % and MI_{D1R1} = 59.00%) (**TABLE 2**).

The mitotic indices (MI %) of the species *P. cordatus* showed a similar trend relative to *P. acinarius*, although with different values. The MI % values were similar in the treatment environment (Ti) (presence of the EAFPC extract) at the two tested doses (MI_{D1} = 33.83% and MI_{D2} = 35.50%) compared to the control (MI_{D0} = 88.16%).

Moreover, the IM % recovered less with increasing doses (MI_{D0R0} = 77.83 %, MI_{D1R1} = 60.83%, and MI_{D2R2} = 56.66%) when submitted to the recovery process (Ri). The recovery of MI % occurred partially at both doses. It evidences the reversible effect on the cell division process for both doses (MI_{D1} = 33.83% and MI_{D1R1} = 60.83%; MI_{D2} = 35.50 % and MI_{D2R2} = 56.66%) (**TABLE 2**).

Genotoxicity effect

The aqueous leaf extracts of *P. acinarius* and *P. cordatus* at the doses D₁ (5 mg/mL) and D₂ (20 mg/mL) under submission (Ti) and recovery (Ri) presented chromosomal alterations (**TABLE 3**). However, no chromosomal alterations were observed in the cell cycle of roots of *A. cepa* exposed only to distilled water, that is, the zero dose (D₀) or control (NC) (**TABLE 3**).

TABLE 3: Types and count (number of cases) of chromosomal alterations observed in meristematic cells of *Allium cepa* L. exposed to aqueous leaf extracts of *Psittacanthus acinarius* (Mart.) Mart. (EAFPa) and *Psittacanthus cordatus* (Hoffmanns.) (EAFPC).

Species: <i>P. acinarius</i>			
Chromosomal alterations	Submission environment (Ti) Doses (mg/mL of EAFPa)		
	D ₀ = 0	D ₁ = 5	D ₂ = 20
	Count (number of cases)		
Metaphase with chromosomal adhesion	–	–	3
Micronucleus	–	–	3
Binucleated prophase	–	–	2
Total	–	–	8
Chromosomal alterations	Recovery environment (Ri)		
	D ₀ = 0	D ₁ = 5	D ₂ = 20
	Count (number of cases)		
Anaphase with chromosomal disorganization	–	15	8
Bud cell	–	2	1
C-metaphase cell	–	5	–
Metaphase with chromosomal adhesion	–	1	1
Polyploid metaphase	–	1	1
Micronucleus	–	1	2
Chromosomal bridge	–	6	1
Achromatic spindle breakage	–	6	–
Chromosomal rupture	–	2	2
Total	–	39	16

Species: <i>P. cordatus</i>			
Chromosomal alterations (Discrimination)	Submission environment (Ti) Doses (mg/mL of EAFPa)		
	D ₀ = 0	D ₁ = 5	D ₂ = 20
	Count (number of cases)		
Anaphase with chromosomal disorganization	–	64	10
Diagonal anaphase	–	5	–
Bud cell	–	1	–
C-metaphase cell	–	15	1
Micronucleus	–	1	–
Chromosomal bridge	–	6	–
Binucleated prophase	–	–	1
Achromatic spindle breakage	–	6	4
Chromosomal rupture	–	1	–
Total	–	99	16
Chromosomal alterations (Discrimination)	Recovery environment (Ri)		
	D ₀ = 0	D ₁ = 5	D ₂ = 20
	Count (number of cases)		
Anaphase with chromosomal disorganization	–	72	96
Diagonal anaphase	–	–	2
Bud cell	–	1	–
C-metaphase cell	–	5	4
Metaphase with chromosomal adhesion	–	6	3
Polyploid metaphase	–	–	5
Micronucleus	–	–	2
Chromosomal bridge	–	32	40
Binucleated prophase	–	–	1
Achromatic spindle breakage	–	1	1
Chromosomal rupture	–	1	1
Total	–	118	155

No significant differentiated effect ($P < 0.05$) was observed under the extract of *P. acinarius* in the chromosomal alteration index (CAI %) during the cell division as a function of the tested doses ($D_0 = 0.00\%$, $D_1 = 0.00\%$, and $D_2 = 0.26\%$) in the environment with exposure (Ti) of roots of *A. cepa*. However, the CAI % in the recovery environment of D_1R_1 (1.30%) was higher than in D_2R_2 (0.53%) and D_0R_0 (0.00%). In turn, the CAI% with extracts of *P. cordatus* under the submission environment was higher at the dose D_1 (3.30%)

relative to the control ($D_0 = 0.00\%$) and the highest dose D_2 (0.53%). However, the CAI% under recovery presented similar values between D_1 (3.93%) and D_2 (6.20%), but higher values than the control ($D_0 = 0.00\%$), in which the CAI % was null or absent (TABLE 4).

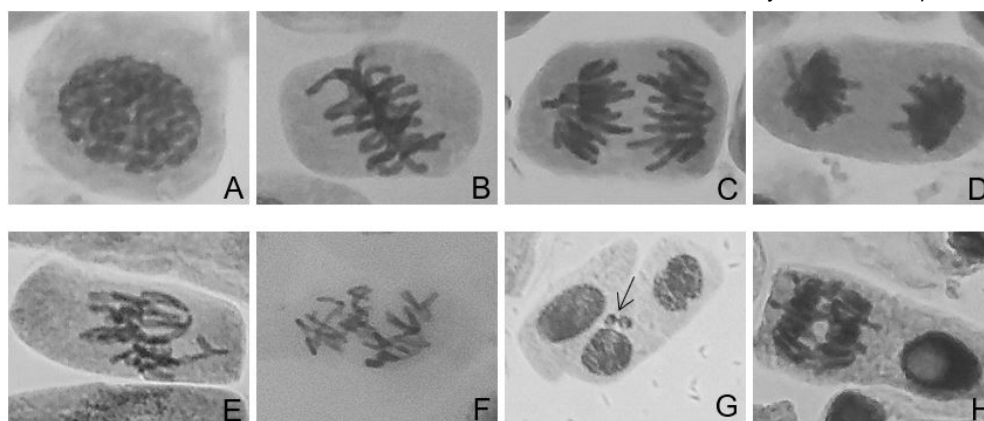
The effects of the aqueous extracts of *P. acinarius* and *P. cordatus* at the tested doses suggest a cytotoxic action, which is evidenced by the inhibition of cell division in meristematic cells of *A. cepa* exposed to the treatments, having as reference the control results. Several studies in the literature have shown inhibitory effects on the cell cycle of *A. cepa* when submitted to the extracts of medicinal plants, such as aqueous, methanolic, and hexanic extracts of bark and inflorescences of *Phoradendron mucronatum* (DC.) Krug & Urb[19], phenolic compounds (rosmarinic acid)[22], the leaf extract of *Parkinsonia aculeata* L.[23], and the aqueous extract of *Grewia lasiocarpa*[24].

TABLE 4: Chromosomal alteration indices (CAI %) observed in meristematic cells of *Allium cepa* L. exposed to aqueous leaf extracts of *Psittacanthus acinarius* (Mart.) Mart. (EAFPa) and *Psittacanthus cordatus* (Hoffmanns.) (EAFPa).

Species: <i>P. acinarius</i>		
Doses of EAFPa (mg/mL)	Environments	
	Submission	Recovery
	CAI % (Mean ± SD)	
$D_0 = 0$	0.00 ± 0.00 aA	0.00 ± 0.00 bA
$D_1 = 5$	0.00 ± 0.00 aB	1.30 ± 0.80 aA
$D_2 = 20$	0.26 ± 0.36 aA	0.53 ± 0.62 bA
Species: <i>P. cordatus</i>		
Doses of EAFPa (mg/mL)	Environments	
	Submission	Recovery
	(Mean ± SD)	
$D_0 = 0$	0.00 ± 0.00 bA	0.00 ± 0.00 bA
$D_1 = 5$	3.30 ± 3.20 aA	3.93 ± 0.89 aA
$D_2 = 20$	0.53 ± 0.53 bB	6.20 ± 1.36 aA

SD – standard deviation. Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other by the Tukey test (for treatments) and Student t-test (for submission environment) at the significance level of 5% probability.

FIGURE 1: Normal cells and with chromosomal alterations observed in the cell cycle of *Allium cepa* L.



A - Prophase; B - Metaphase; C - Anaphase; D - Telophase; E - Anaphase with chromosomal disorganization; F - C-metaphase cell; G - micronucleus; H - Chromosomal bridge. 400x magnification.

The reviewed literature showed no studies revealing the chemical constituents present in the studied plants. However, several chemical constituents of the secondary metabolism were found in plants of the genus *Psittacanthus*, which may be related to the inhibition of the cell cycle division. The presence of flavonoids, coumarins, and hydrolyzable tannins was observed in the phytochemical analysis of aqueous and hydroalcoholic leaf extracts *P. plagiophyllus* Eichl.^[25] Also, found gallic acid, flavonoids, and non-protein amino acid in the soluble fraction of methanol from the aqueous extract of *P. calyculatus* (DC.) G. Don^[26]. Identified tannins, flavonoids, and phenylpropanoids in the methanolic extract of *P. calyculatus* and the absence of anthraquinones, steroids, and alkaloids^[11].

The mitotic index did not return to the level of the lowest dose ($D_1 = 5$ mg/mL) and control ($D_0 = 0$ mg/mL) in the process of recovering from the highest dose of the *P. acinarius* extract (EAFPa) ($D_2 = 20$ mg/mL), showing an irreversible cytotoxic effect.

The alterations found in the tested doses suggest damage that plant extracts may cause to living organisms, including humans. Among the anomalies found, the most frequent for the two studied species were the anaphase with chromosomal disorganization (5.6%), chromosomal bridge (2.4%), C-metaphase cell (0.53%) (**FIGURE 1E, 1F, 1G, 1H and TABLE 3**). The effects caused in meristematic cells of roots of *A. cepa* are related to spindle inhibition, leading to chromosome displacement, viscosity, bridging in anaphase, and several other types of abnormalities as the dose of the tested plant extract increases^[24].

Conclusion

The aqueous leaf extracts of *P. acinarius* and *P. cordatus* at doses of $D_1 = 5$ and $D_2 = 20$ mg/mL inhibit MI%, show irregular recovery or prevent it, and induce chromosomal alterations, suggesting cytotoxic and genotoxic effects on the division of meristematic cells of *A. cepa*.

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