



Antiproliferative action of aqueous extracts of *Hymenaea stigonocarpa* Mart. (Fabaceae) on the cell cycle of *Allium cepa* L.

LOURRAN P. LACERDA, GEIZ MALAQUIAS and ANA PAULA PERON

Universidade Federal do Piauí (UFPI), Núcleo de Pesquisa Aplicada a Saúde e ao Meio-Ambiente (NUPBSAM),
Laboratório de Citogenética Vegetal e Animal, Campus Senador Helvídio Nunes de Barros (CSHNB),
Rua Cícero Duarte, 940, Bairro Junco, 64600-000 Picos, PI, Brasil

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ABSTRACT

In this study we evaluated the action of crude aqueous extracts obtained from rhytidome of *Hymenaea stigonocarpa* (jatobá-do-cerrado) on *Allium cepa* meristematic root cells in three concentrations: 0.082, 0.164, 0.328g/mL, at exposure times of 24 and 48 h. The slides were prepared by the crushing technique, and cells analyzed throughout the cell cycle, totaling 5000 for each control group and concentration. It was found that all three concentrations, including the lowest which is considered ideal for use, in all exposure times, had significant antiproliferative action on the cell cycle of this test system. For cells under division, we observed a high number of cells in prophase. Therefore, under the conditions studied *H. stigonocarpa* indicated to be cytotoxic.

Key words: *Allium cepa*, cellular division, cells in prophase, medicinal plant.

INTRODUCTION

Leguminosae is a plant family of great economic and medical importance to temperate and tropical regions worldwide. Among these plant species of this genus *Hymenaea*, used in Brazil as medicinal plants.

Worldwide, many plant species are used for the treatment and prevention of diseases, but most of them have not yet been evaluated for their cytotoxic, genotoxic and mutagenic potential, which is essential for an effective use of herbal medicines by the population (Bagatini et al. 2007).

The outer surface of the stem or rhytidome of *Hymenaea stigonocarpa* Mart. (jatobá-do-cerrado) is widely used in the form of tea in folk medicine in the states of northern and northeastern Brazil to

reduce cholesterol and glucose levels, ease pain and stomach cramps, bronchitis and asthma, and to cure urinary tract infections (Ramos et al. 2007). The chemical composition of the rhytidome of this plant contains diterpene acids, anthraquinones, high concentration of mineral salts, tannins, flavonoids, oligosaccharides and xyloglucans (Cartaxo et al. 2010). No data was found in scientific literature on the cytotoxic potential of this medicinal plant.

The *Allium cepa* test plant system is an excellent biomarker for the first screening of medicinal plant cytotoxicity due to its kinetic properties of proliferation for having large chromosomes which are few in number ($2n = 16$) (Fachinetto et al. 2007) and its reliability and agreement with other toxicity tests, greatly aiding human health damage prevention studies (Bagatini et al. 2007, Leme and Marin-Morales 2009).

Correspondence to: Ana Paula Peron
E-mail: anapapegenpes@hotmail.com

The present study aimed to evaluate the toxic effects of crude aqueous extracts of the jatobá-do-cerrado rhytidome, on the cell cycle of *Allium cepa* roots at different concentrations and exposure times.

For this work, samples of the *H. stigonocarpa* stem rhytidomes were collected in a medicinal garden in the city of Teresina, in the state of Piauí, in May 2012. The plant identification and collection of rhytidomes were performed by Prof. Maria do Socorro Meireles de Deus, Master in botany and Professor at the Federal University of Piauí.

For this cytotoxicity evaluation, 03 concentrations were established, 0.082; 0.164 and 0.328g/mL, of which of 0.082 g/mL, is considered normal, according to Sousa et al. (2006). For the preparation of concentrations, rhytidome pieces, 80g each, were placed in boiling water for infusion where they remained for 20 minutes. After this time, the crude aqueous extracts were filtered out and placed to cool at room temperature. The teas were prepared in the same way as the population would prepare them.

The *A. cepa* bulbs were placed for rooting in flasks with distilled water at 25°C and aerated constantly, until obtaining roots of about 1.0cm long. For the analysis of each concentration we stipulated an experimental group with five bulbs. Before placing the roots in contact with their respective concentrations, some roots were collected and fixed to serve as control (CO) of the bulb itself. Then the remaining roots were placed on their respective concentrations, for 24 hours, this procedure being called the exposure time of 24 hours (ET 24 h).

After this time some roots were removed and fixed. Subsequently, the remaining roots of each bulb were once again placed in their respective concentrations where they remained for 24 more hours, procedure being called the exposure time of 48 hours (ET 48 h). Fixing occurred in Carnoy 3:1 (ethanol: acetic acid) at room temperature for about 24 hours. For each collection root retired, on average, three roots per bulb onion.

Slides, average of 03 per bulb, were prepared following the protocol proposed by Guerra and Souza (2002) each slide was stained with drops of 2% acetic orcein, and analyzed by light microscopy, in a 40X objective lens. For each bulb we analyzed 1,000 cells, totaling 5,000 for each concentration. Values of the average number of cells in each *A. cepa* cell cycle phase were calculated and the mitotic index (MI) determined. The statistical analysis was performed by the χ^2 test, with a probability level <0.05, using the BioEstat 3.0 statistical software (Ayres 2007).

Table I shows the number of cells in interphase and at different stages of cell division, and mitotic index values obtained for the root cells of *A. cepa* treated with water (CO) and with the concentrations of *H. stigonocarpa* teas for 24 and 48 hours. Significant χ^2 values are also shown.

From the results obtained (Table I), it can be observed that the concentrations tested, including normal, greatly decreased the mitotic index of *A. cepa* meristematic root cells compared to the MI obtained for the respective controls. One could also observe that most of the dividing cells were in prophase. These results suggest a cytotoxic action of the *H. stigonocarpa* aqueous extracts studied at the two exposure times evaluated, and in the test system used. MI values obtained for ET of each concentration were not significant among themselves.

To date, few studies have been conducted to evaluate *Hymenaea* genus cytotoxicity, however the results of these studies corroborate those obtained here for *H. stigonocarpa*. Among the studies is that by Pettit et al. (2003) who found that the flavonoid palstatin, in joint action with diterpenes extracted from the leaves of *Hymenaea palustris*, dramatically inhibited cell division in human stomach cancer cell lines. Likewise, Closa et al. (1997) found that the flavonoid astilbin and diterpenes extracted from leaves of the *Hymenaea martiana* species have cytotoxic action on rodent liver cells treated with

TABLE I
Number of cells in interphase and at different stages of cell division, total cell analyzed in cell cycle root tips of analyzed in cell cycle root tips of *A. cepa* treated with water (control) and three concentrations rhytidome tea of *H. stigonocarpa*, 0.082, 0.164, 0.328g/mL under ET of 24 and 48 h. 5,000 cells were analyzed for each control group and concentration.

Concentration (g/mL)	ET	Intephase Cells	P	M	A	T	Cells under Division	MI (%)
0.082	CO	3566	452	394	378	270	1494	28.7a
	24h	4270	474	71	91	94	730	14.6b
	48h	4320	413	91	90	82	676	13.5b
0.164	CO	3131	500	493	487	389	1869	37.4a
	24h	4201	535	91	80	93	799	16.0b
	48h	4140	587	88	93	92	860	17.2b
0.328	CO	3279	462	455	394	410	1721	34.4a
	24h	4277	469	80	93	81	723	14.5b
	48h	4427	438	48	55	32	573	11.5b

CO – Control; ET – Exposure time, P – Prophase, M – Metaphase A – Anaphase, T – Telophase, MI – Mitotic Index.

Means followed by the same letter do not differ significantly at 5% by the χ^2 test.

a clastogenic drug, significantly inhibiting their cell division rate and indicated as hepatoprotective. Abdel-Kader et al. (2002) found that diterpenes indicated in the rhytidome of the *Hymenaea courbaril* trunk had the potential to reduce the mitotic index of human ovarian cancer cells. These authors suggest that the rhytidome of this species has chemopreventive potential.

Thus, from the results obtained in *A. cepa* for *H. stigonocarpa* as well as those reported in the literature for other species of the *Hymenaea* genus, it becomes relevant to conduct other studies to evaluate toxicity of this plant with other test systems, different exposure times and different treatments to thereby establish what the optimal, efficient and safe concentrations are for the utilization of this plant, and verify, with proprietary, its antiproliferative capacity.

RESUMO

Neste estudo avaliou-se a ação de extratos aquosos brutos obtidos do ritidoma de *Hymenaea stigonocarpa* (jatobá-do-cerrado) sobre as células meristemáticas de raízes de *Allium cepa*, em três concentrações: 0,082;

0,164; 0,328g/mL, nos tempos de exposição de 24 e 48 h. As lâminas foram feitas pela técnica de esmagamento, e analisaram-se células em todo ciclo celular, totalizando 5.000 para cada grupo controle e concentração. Verificou-se que as três concentrações, inclusive a menor e considerada ideal para consumo, em todos os tempos de exposição tiveram ação antiproliferativa significativa sobre o ciclo celular deste sistema teste. Para as células em divisão observou-se um grande número de células em prófase. Portanto, nas condições analisadas a *H. stigonocarpa* mostrou-se citotóxica.

Palavras-chave: *Allium cepa*, divisão celular, células em prófase, planta medicinal.

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