Biotemas, 28 (1): 45-49, março de 2015
ISSNe 2175-7925
http://dx.doi.org/10.5007/2175-7925.2015v28n1p45

Antiproliferative effect of the hydroalcoholic extract of *Hymenaea stigonocarpa* Mart. ex Hayne (Fabaceae, Caesalpinioideae) on the meristematic cells of *Allium cepa* L. roots

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Submetido em 06/06/2014
Aceito para publicação em 20/11/2014

Resumo

Efeito antiproliferativo do extrato hidroalcoólico de *Hymenaea stigonocarpa* Mart. ex Hayne (Fabaceae, Caesalpinioideae) sobre as células meristemáticas de raízes de *Allium cepa* L. Este estudo avalia o extrato hidroalcoólico obtido do ritidoma de *Hymenaea stigonocarpa*, (jatobá-do-cerrado) sobre as células meristemáticas de raízes de *Allium cepa*, em três concentrações, 0,5; 1,0 e 1,5 mg/mL, e dois tempos de exposição, 24 h e 48 h. As lâminas foram preparadas pela técnica de esmagamento. As células foram analisadas em todo ciclo celular, totalizando 5.000 para cada grupo controle e concentração. Verificou-se que as três concentrações diminuíram significativamente o índice de divisão celular no tempo de exposição de 48 h quando comparadas aos seus respectivos controles. O índice mitótico do tempo de exposição de 48 h foi estatisticamente diferente quando comparado ao índice de divisão celular do tempo de exposição de 24 h em todas as concentrações. O número de anormalidades celulares observado não foi estatisticamente significativo pelo teste do qui-quadrado utilizado. Portanto, nas condições analisadas, as três concentrações testadas do ritidoma do jatobá-do-cerrado reduziram de forma estatisticamente significativa o índice de divisão celular das células meristemáticas de raízes de *A.* cepa.

Palavras-chave: Índice mitótico; Inibição da divisão celular; Jatobá-do-cerrado; Sistema teste vegetal

Abstract

This study evaluates the hydroalcoholic extract obtained from the cork cambium of *Hymenaea stigonocarpa* (jatobá-do-cerrado) on the meristematic cells of *Allium cepa* roots, at 3 concentrations, 0.5, 1.0, and 1.5 mg/mL, and 2 exposure times, 24 h and 48 h. The slides were made through the crushing technique. Cells were analyzed throughout the cell cycle, totaling 5,000 for each control and concentration group. We found that the 3 concentrations significantly decreased the cell division rate at the exposure time of 48 h when compared to their respective controls. The mitotic index of the exposure time of 48 h was statistically different when compared to the cell division rate of the exposure time of 24 h for all concentrations. The number of cell abnormalities observed was not statistically significant by means of the chi-square test used. Therefore, under the conditions analyzed, the 3 concentrations tested for the cork cambium of jatobá-do-cerrado significantly decreased, in statistical terms, the cell division rate in the meristematic cells of *A. cepa* roots.

Key words: Cell division inhibition; Jatobá-do-cerrado; Mitotic index; Plant test system

Introduction

Leguminosae is a plant family of great economic and medical importance to temperate and tropical regions worldwide. Among these plants, the species *Hymenaea stigonocarpa* Mart. ex Hayne, known in Brazil as jatobá-do-cerrado, is mainly found in the states of the Central-West, North, and Northeast regions, in open formations of cerrado and caatinga.

The human population in these regions consumes the outer surface of the bark of this legume species in tea form, in order to cure urinary tract infections (DIMECH et al., 2013), alleviate stomach pain, cure intestinal infections (ORSI et al., 2014), fight against helminths (VALENTE et al., 2014), and treat depression (ORSI et al., 2014). According to Cartaxo et al. (2010), this bark contains many groups of metabolites, such as diterpene acids, anthraquinones, high concentrations of minerals, tannins, flavonoids, xyloglucan, and oligosaccharides. So far, there are no phytochemical studies on *Hymenaea stigonocarpa*, thus its bioactive compounds are not known.

Although the medicinal properties of the bark of this species have already been scientifically proven in laboratory experiments and despite its wide use by the population in these three Brazilian regions, no data was found in the literature regarding its toxic potential at the systemic and cellular levels. However, according to Cartaxo et al. (2010), studies have shown that the bark of some *Hymenaea* species exhibited cytotoxic and mutagenic potential in the culture of both normal and tumor cells, as well as by using an *in vivo* test system. Thus, it is worth analyzing the action of the bark of *Hymenaea stigonocarpa* at the cell level.

Bioassays with plants are regarded as highly sensitive, quick, and simple to monitor toxic effects of chemical compounds at the cell level. Among them, the meristematic cells of *Allium cepa* (onion) root are efficient test organisms for the first screening for cytotoxicity of herbs (SABINI et al., 2011; HERRERO et al., 2012), given their kinetic proliferation properties, as they have few and large chromosomes (2n = 16), something which facilitates their analysis (CARITÁ; MARIN-MORALES, 2008), and they allow good visualization of cell abnormalities when present (BAGATINI et al., 2007). Besides, Fachinetto et al. (2007) report that the results obtained by means of this test system are excellent parameters for cytotoxicity and genotoxicity analysis and they have been used as an indicator to warn the population about the consumption of certain foods and synthetic and natural medicines.

In this context, this study aims to assess the activity of the hydroalcoholic extract from the bark of *H. stigonocarpa* on the cell cycle of *A. cepa* roots at different concentrations and exposure times.

Material and Methods

For this study, bark pieces of *H. stigonocarpa* were purchased from a herbal store in Picos, Piauí, Brazil. The study was carried out in December 2013 and January 2014.

Obtaining the hydroalcoholic extract

Before extraction, the bark pieces were dried at room temperature for 5 days and ground in a knife mill. Then, the plant material was weighed and placed in infusion in a 80% ethanol extraction solution at a 1:5 ratio (COUTINHO; HASHIMAMOTO, 1971) and stored at room temperature for 11 days, shaking the macerate once a day. After this procedure, the material was filtered by using filter paper and the filtrate was concentrated in a rotary evaporator, under low pressure.

Cytotoxicity test using Allium cepa L.

To assess the cytotoxicity, we established 3 concentrations of the hydroalcoholic extract, 0.5, 1.0, and 1.5 mg/mL. Bulbs of *Allium cepa* were allowed to root in flasks with distilled water at 25°C, under constant aeration, until obtaining roots about 1.0 cm long. For analyzing each concentration, an experimental group was set with 5 bulbs. Before placing the roots in contact with their respective concentrations, some of them were collected and fixed to serve as control (CO) for the bulb itself. Then, the remaining roots were placed at their respective concentrations for 24 h; this procedure is referred to as the 24 h exposure time (24 ET).

After this period, some roots were removed and fixed. The remaining roots of each bulb were placed in contact with their respective concentrations of extract for further 24 h (48 h exposure time – ET 48). After this, roots were collected and fixed. The material was fixed in 3:1 Carnoy's fixative (ethanol: acetic acid) for about 24 h.

On average, 5 slides were prepared per bulb, according to the protocol proposed by Guerra and Souza (2002) and analyzed through light microscopy at 40× objective. For each bulb, 1,000 cells were analyzed, totaling 5,000 cells for each tested concentration of the extract. During the analysis, we observed cells in interphase, prophase, metaphase, anaphase, telophase, and the presence of aneugenic effects and micronuclei. Mean values were calculated for each phase of the cell cycle of *A. cepa* and the mitotic index (MI) was determined. Statistical analysis of data was performed by means of $\chi 2$ test with a probability level < 0.05, using the software *BioEstat*, version 5.0 (AYRES, 2007).

Results and Discussion

Table 1 lists the number of cells in interphase and at different cell division stages and the mitotic index values obtained for the cells of *A. cepa* roots treated with water (CO) and with the concentrations of the extract obtained from *H. stigonocarpa* for 24 h and 48 h. Significant X^2 values are also presented.

The results (Table 1) indicated no significant difference between MI of CO and MI of ET 24 for any

TABLE 1: Total number of cells analyzed and the cell cycle phases of *A. cepa* roots treated with water (control) and with the hydroalcoholic extract of the bark of *H. stigonocarpa* (0.5; 1.0, and 1.5 mg/mL) at ET 24 h and 48 h. Analysis of 5,000 cells per group.

Concentration (mg/mL)	ET	Interphase cells	P	M	A	T	Total dividing cells	MI (%)
0.5	CO	4,732	95	24	78	71	268	5.4 [a]
	24 h	4,811	148	7	13	21	189	3.9 [a]
	48 h	4,970	16	7	2	5	30	0.6 [b]
1.0	CO	4,714	178	55	40	13	286	5.7 [a]
	24 h	4,821	151	11	17	0	179	3.6 [a]
	48 h	4,979	11	4	4	2	21	0.4 [b]
1.5	CO	4,685	124	66	67	58	315	6.3 [a]
	24 h	4,788	179	14	12	37	212	4.2 [a]
	48 h	4,909	15	4	4	8	31	0.6 [b]

ET – Exposure time; CO – Control; P – Prophase, M – Metaphase, A – Anaphase, T – Telophase, MI – Mitotic Index. Means followed by the same letter are not significantly different at the 5% level by chi-square test.

of the 3 treatments. On the other hand, MI of ET 48 h was significantly different from the cell division index of CO and TE 24 h, in the 3 concentrations.

It is worth mentioning that, at all concentrations tested, despite ET 24 h had no reduction in the mitotic index in relation to their respective controls, it may be seen that the number of cells in prophase, when the mitotic spindle has not been organized, yet, is higher than in other cell division stages (Table 1).

Thus, under the conditions studied, the 3 concentrations promoted antiproliferative effect on the meristematic cells of *A. cepa* root in ET 48. Cell abnormalities were found, such as anaphase and telophase bridges, micronuclei and colchicine-metaphase, in ET 48 for the 3 concentrations, however, the numbers were not significant ($p > 0.05$).

Lacerda et al. (2014) investigated the activity at the cell level of the bark aqueous extract of *H. stigonocarpa* on meristematic cells of *A. cepa* root at concentrations commonly used in traditional medicine by the population and they found that the three concentrations tested caused statistically significant inhibition on the cell cycle of the test system used. Orsi et al. (2014), through classic and gas chromatography, they found that the hydroalcoholic extract of the bark of jatobá-do-cerrado consists of high flavonoid and tannin concentrations. According to Silva et al. (2012), tannins generally have the property of changing the permeability of membranes and causing cell death. Thus, it may be suggested that the results observed here took place on the basis of these chemical compounds, a condition intensified with increasing ET. However, further studies should be carried out to define the action of this plant part and its chemical compounds at the cell level.

Few studies have been conducted to evaluate the cytotoxicity in some species of the genus *Hymenaea*. Among them, Pettit et al. (2003) stands out, they noticed that the flavonoid palstatina combined with diterpenes extracted from the leaves of *Hymenaea palustre* has the potential to strongly inhibit cell division of strains from human stomach cancer cells. Likewise, Closa et al. (1997) reported that the flavonoid astilbin and diterpenes extracted from the leaves of *Hymenaea martiana* significantly inhibited cell division in liver cells of rats treated with a clastogenic drug (ClC_4), showing a hepatoprotective potential. Abdel-Kader et al. (2002) observed that diterpenes observed on the cork cambium of *Hymenaea courbaril* were cytotoxic to human ovarian cancer cells, A2780 strain. Therefore, the results obtained by these studies corroborate those obtained by our research group for *H. stigonocarpa*, i.e. these plants have the ability to inhibit cell proliferation.

Many currently used antitumor drugs were isolated from herbs, such as paclitaxel, vinca alkaloids, and camptothecin (WALL; WANI 1995), something which makes molecular bioprospection a significant issue regarding the search for new therapeutic approaches to cancer. The test with *A. cepa*, despite preliminary, points out new potential antitumor drugs, as observed in this study, thus, there is a need for conducting further studies to evaluate the toxicity of *H. stigonocarpa* at the cell level by using other test systems, such as testing in cancer cell lines under different exposure times and different treatment regimens, in order to accurately determine the antiproliferative potential of this legume species.

References

ABDEL-KADER, M.; BERGER, J. M.; SLEBODNICK, C.; MALONE, S.; WISSE, J. H.; WERKHOVEN, M. C.; MAMBER, S.; KINGSTON, D. G. Isolation and absolute configuration of ent-Halimane diterpenoids from *Hymenaea courbaril* from the Suriname rain forest. **Journal of Natural Products**, Columbus, v. 65, n. 1, p. 11-15, 2002.

AYRES, M. **BioEstat 5.0:** aplicações estatísticas nas áreas das Ciências Biológicas e Médicas. 5. ed. Belém: Sociedade Civil Mamirauá: Brasília CNPq, 2007. 220 p.

BAGATINI, M. D.; SILVA, A. C. F.; TEDESCO, S. B. Uso do sistema-teste *Allium cepa* como bioindicador de genotoxicidade de infusões de plantas medicinais. **Revista Brasileira de Farmacognosia**, Curitiba, v. 18, n. 3, p. 509-516, 2007.

CARITÁ, R.; MARIN-MORALES, M. A. Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. **Chemosphere**, Elmsford, v. 72, n. 1, p. 722-725, 2008.

CARTAXO, S. L.; ALMEIDA, D. E.; SOUZA, M. M.; ALBUQUERQUE, U. P. Medicinal plants with bioprospecting potential used in semi-arid northeastern Brazil. **Journal of Ethnopharmacology**, Shannon, v. 131, n. 2, p. 326-342, 2010.

CLOSA, D.; TORRES, M.; HOTTER, G.; BIOQUE, G.; LÉON, O. S.; GELPÍ, A. N. D.; RÓSELLO-CATAFAU, J. Prostanoids and free radicals in Cl4C- induced hepatotoxicity in rats: effect astilbin.

Prostaglandins, Leukotrienes and Essential Fatty Acids, Shellfied, v. 56, n. 4, p. 331-334, 1997.

COUTINHO, L. M.; HASHIMOTO, F. Sobre o efeito inibitório da germinação de sementes produzido por folhas de *Calea cuneifolia* DC. **Ciência e Cultura**, São Paulo, v. 23, n. 6, p. 759-764, 1971.

DIMECH, G. S.; SOARES, L. A.; FERREIRA, M. A.; OLIVEIRA, A. G.; CARVALHO, M. C.; XIMENES, E. A. Phytochemical and antibacterial investigations of the extracts and fractions from stem bark of *Hymenaea stigonocarpa* Mart. Ex Hayne ande effect on ultrastructure of *Staphylococcus aureus* induced by hydroalcoholic extract. **The Scientific World Journal**, Nars City, v. 14, p. 862-763, 2013.

FACHINETTO, J. M.; BAGATINI, M. D.; DURIGON, A. C. F. S.; TEDESCO, S. B. Efeito anti-proliferativo das infusões de *Achyrocline satureioides* DC (Asteraceae) sobre o ciclo de celular de *Allium cepa*. **Revista Brasileira de Farmacognosia**, Curitiba v. 17, p. 49-54, 2007.

GUERRA, M.; SOUZA, M. **Como observar os cromossomos:** um guia de técnicas em citogenética vegetal, animal e humana. Ribeirão Preto: FUNPEC, 2002. 191 p.

HERRERO, O.; PEREZ, J. M. M.; FERNÁNDEZ, P. F. Toxicological evaluation of three contaminant of emerging concern by use of *Allium cepa* test. **Mutation Research**, Amsterdam, v. 743, p. 24-34, 2012.

LACERDA, L. P.; MALAQUIAS, G.; PERON, A. P. Antiproliferative action of aqueous extracts of *Hymenaea stigonocarpa* Mart. (Fabaceae) on the cell cycle of *Allium cepa* L. **Anais da Academia Brasileira de Ciências**, Rio de Janeiro, v. 86, n. 3, p. 119-122, 2014.

ORSI, P.; BONANIN, F.; SEVERI, J. A.; SANTOS C. R.; VILEGAS, W.; HIRUMA-LIMA, C. A.; STASI, L. C. *Hymenaea stigonocarpa* Mart. Ex Hayne: a Brazilian medicinal plant with gastric and duodenal antiulcer and antidiarrheal effects in experimental rodent models. **Journal Ethnopharmacology**, Shannon, v. 143, n. 1, p. 81-90, 2014.

PETTIT, G. R.; MENG, Y.; STEVENSON, C. A.; DOUBEK, D. L.; KNIGHT, J. C.; CICHACZ, Z.; PETTIT, R. K.; CHAPUIS, J. C.; SCHMIDT, J. M. Isolation and structure of palstatin from the Amazon tree *Hymenaea palustris*. **Journal of Natural Products**, Columbus, v. 66, n. 2, p. 259-162, 2003.

SABINI, M. C.; CARIDDI, L. N.; ESCOBARA, F. M.; BACHETTI, R. A.; SUTIL, S. B.; CONTIGIANI, M. S.; ZANON, S. M., SABINI, L. I. Evaluation of cytogenotoxic effects of col aqueous extract from *Achyrocline satureioides* by *Allium cepa* L test. **Natural Products Communications**, Westerville, v. 6, n. 7, p. 995-998, 2011.

SILVA, M. R.; AMBROSI, A.; RAMOS, G. M.; TESSARO, I. C. Rejuvenating polyamide reverse osmosis membranes by tannic acid. **Treatment Separating and Purification Technology**, Washington, v. 100, p. 1-8, 2012.

VALENTE, P. P.; AMORIN, J. M.; CASTILHO, R. O.; RIBEIRO, M. F. 2014. *In vitro* acaricidal efficacy of plant extracts Brazilian flora and isolated substances against *Rhipicephalus microplus* (Acari: Ixodidae). **Parasitology Research**, Heidelberg, v. 113, n. 1, p. 417-413, 2014.

WALL, M. E.; WANI, M. C. Camptothecin and taxol: discovery to clinic-thirteenth Bruce F. Cain Memorial Award Lecture. **Cancer Research**, Philadelphia, v. 55, n. 4, p. 753-760, 1995.