ARTÍCULO ORIGINAL

Cytotoxic action of the stem aqueous extract of the stem of *Cereus jamacaru* DC. (mandacaru)

Acción citotóxica del extracto acuoso del tallo de *Cereus jamacaru* DC. (mandacaru)

Lic. Darciella Moura da Silva,[I] Lic. Francisco Ronielson de Sousa Carvalho,[I] Lic. Antonio Gabriel Moura,[I] DraC. Lívia Martins,[II] Prof. Paulo Michel Tinheiro Ferreira,[III] DraC. Ana Paula Peron[III]

[I] Campus Senador Helvídio Nunes de Barros, Universidade Federal do Piauí. Brasil.
[II] Campus Ministro Petrônio Portella, Universidade Federal do Piau. Brasil.
[III] Universidade Federal do Piauí. Brasil.

ABSTRACT

Introduction: *cereus jamacaru* DC. (Cactaceae), with popular name mandacaru, is a plant from the Brazilian semiarid region with medicinal properties against various diseases.
Objective: to evaluate the effect of different concentrations of crude aqueous extracts obtained from *C. jamacaru* on the cell cycle of meristematic root cells of *Allium cepa* L.
Methods: the *C. jamacaru* aqueous extracts were prepared by trituration of pieces from inside the cladodes immersed in distilled water. The concentration considered optimal for the treatment of diseases is 500/2 L. From this concentration were more established other two 500/1.5 and 500/1 L. Each concentration was evaluated at times of 24 and 48 hours exposure. A group of five bulbs of *A. cepa* was used. The root tips were collected and fixed in acetic acid (3:1) for 24 hours. The slides were prepared by crushing and stained with 2 % acetic orcein. Data analysis was performed using the Chi-square ($p < 0.05$).
Results: the results showed that the concentrations of 500/1.5 and 500/1 g/L have an antiproliferative effect and induced a statistically significant number of cellular aberrations in the test system cells in question.
Conclusion: under the conditions studied, these two concentrations present as cytotoxic.

Key words: *Cereus jamacaru*, antiproliferative effect, cellular aberrations, plant test system.

RESUMEN

Introducción: c*ereus jamacaru* DC. (Cactaceae), conocido popularmente como mandacaru, es una planta medicinal utilizada contra diversas enfermedades en el semiárido brasileño.
Objetivo: evaluar el efecto de los extractos acuosos de la maceración del tallo de *C. jamacaru* (mandacaru) en las células meristemáticas de la raíz de *Allium cepa* L.
Métodos: extractos acuosos de *C. jamacaru* se prepararon por trituración de piezas del interior de los cladodios sumergidas en agua destilada. La concentración considerada óptima para el tratamiento de enfermedades es 500/2 L. De esta concentración se estableció más otros dos de 500/1,5 y 500/1 L. Cada concentración se evaluó en tiempos de 24 y 48 h de exposición. Para cada concentración fue utilizado un grupo de cinco bulbos de *A. cepa.* Las puntas de las raíces se recogieron y se fijaron en ácido acético (3:1) durante 24 h.
Las láminas fueron preparadas por aplastamiento y teñidas con orceina acética al 2 %. Los datos fueron analizados mediante la prueba de Chi-cuadrado ($p < 0,05$).
Resultados: los resultados mostraron que las concentraciones de 500/1 y 500/1.5 g/L tienen un efecto antiproliferativo e indujeron un número estadísticamente significativo de aberraciones celulares en las células del sistema de prueba en cuestión.
Conclusiones: en las condiciones estudiadas, estas dos concentraciones se muestran citotóxicas.

Palabras clave: *Cereus jamacaru*, efecto antiproliferativo, aberraciones celulares, sistema de ensayo de la planta.

INTRODUCTION

Currently, about 70 % of the world population uses medicinal plants in primary health care.[1] In Brazil, this practice is widespread, but almost always conducted indiscriminately and without medical supervision.[2]

The species *C. jamacaru* DC. (Cactaceae), popularly known as mandacaru, is widely used in traditional medicine in the northeastern region of Brazil, mainly in the states of Maranhão, Piauí, Ceará and Paraíba. It is native to the Caatinga (scrublands) and along with other cactaceae, shape the typical landscape of the semiarid region of this country.[3] Its cladode, which can reach two meters in height, is woody, with erect branches and quite thorny, having in its phytochemical constitution, sodium nitrate; B-sisterol; the amines tyramine, N-methylamide and hordenine; many fibers and unsaturated fatty acids, such as oleic acid and linoleic acid; saturated fatty acids, such as the palmitic, and stearic; betalaine and indicaxanthin.[4]

Much of what is known about the medicinal activity of the mandacaru stem has been described in the scientific literature based on reports made by popular culture

about activities such as the antimicrobial, vasodilation,[5] anti-inflammatory, contraceptive and anti constipating.[6] However, some experimental studies have been conducted and show that the aqueous extract of the cladode of this cactus has action on the control of diabetes and albuminuria, on the treatment of renal disorders, the easing of respiratory problems such as coughs and bronchitis,[7] control of high cholesterol,[8] and anthelmintic activity.[9,10]

Many medicinal plants have shown to have cytotoxic, genotoxic and/or mutagenic action,[11,12] conditions that can contribute significantly to the development of cancer.[13] Thus, toxicity evaluation studies at the cellular level, in various test systems, are needed to assist in the standardization of safe quantities and effective uses of these herbal medicines for the population.[2]

Bioassays with plants are considered highly sensitive, rapid and simple for the monitoring of chemical compound toxic effects at the cellular level, where we can cite the meristematic root cells of *Allium cepa* (onion) as efficient organisms for the first test screening of cytotoxicity of crude aqueous extracts of medicinal plants.[12,14] These cells are efficient because of the function of their kinetic properties of proliferation, for having large chromosomes and few in number (2n = 16), which facilitates their analysis,[15] and for allowing good visualization of cellular aberrations when present.[16] Furthermore, Fachinneto et al.[17] report that the results obtained by this test system are excellent cytotoxic and genotoxic analysis parameters, and it has been widely used as an indication to warn the human population about the consumption of certain foods and natural and synthetic drugs.

In this context, this study was aimed to evaluate the effect of different concentrations of crude aqueous extracts obtained from *C. jamacaru* cladodes on the cell cycle of meristematic root cells of *A. cepa.*

METHODS

This work was developed in the Laboratory of Plant and Animal Cytogenetics at the Senador Helvidío Nunes de Barros Campus (CSHNB), Federal University of Piauí (UFPI), Municipality of Picos, Piauí State, in the months of October and November 2013.

Plant collection

For the experiments, samples of *C. jamacaru* cladodes were collected in a small orchard of CSHNB/UFPI. The *C. jamacaru* plant is identified with the number 008 in the garden registry. Before starting the collections, this Cactaceae was characterized taxonomically as the species *C. jamacaru* DC., by Maria do Socorro Meireles de Deus, master botanist and Biological Sciences professor at CSHNB/UFPI.

Preparation of different concentrations of aqueous extracts

The *C. jamacaru* aqueous extracts were prepared by trituration of pieces from inside the cladodes immersed in distilled water in an industrial blender. Before maceration, the epidermis and the thorns of each piece to be used were removed with a stylet.

After this procedure, the obtained solutions were strained and then placed in contact with the *A. cepa* bulb roots. Three concentrations were established: 500/2; 500/1.5 and 500/1 g/L, the 500/2 g/L being recommended by Portal Educação – Homeopatia e Fitoterapia.[18]

Obtaining meristematic cells for the cytogenetic analysis

A. cepa bulbs were rooted in flasks with distilled water at a temperature of 25 °C and constantly aerated until obtaining roots with about 1.0 cm. For analysis of each concentration, each experimental group was established with five onion bulbs. Before placing the roots in contact with their respective concentrations, some roots were collected and fixed to serve as control (CO) for the bulb itself. Continuing, the remaining roots were placed in their respective concentrations for 24 hours, this procedure being referred to as the 24 hours exposure time (24 hours ET).

After this time some roots were removed and fixed. This done, the rest of the roots from each bulb were returned to their respective concentrations where they remained for 24 hours, denominated the 48 hours exposure time (48 hours ET). After this period, roots were collected and fixed again. Exposure times of 24 and 48 hours were chosen in order to evaluate the effect of the concentrations studied in more than one cell cycle.

The fixation of the roots occurred in Carnoy 3:1 (ethanol: acetic acid) at room temperature for 24 hours. For each root collection, we removed an average of three roots per onion bulb.

Preparation and reading of the slides, and data analysis

An average of 3 bulbs per slide were mounted following the protocol proposed by *Guerra and Souza*.[19] Each slide was stained with two drops of 2 % acetic orcein[20] and examined under an optical microscope at 40X. For each bulb 1,000 cells were analyzed totaling 5,000 cells for each concentration and control. During the analysis we observed cells in interphase, prophase, metaphase, anaphase and telophase. The number of cells in interphase and in division for each control and exposure time was calculated and the mitotic index (MI) determined. The MI was calculated by dividing the total number of dividing cells by the total number of cells analyzed in each exposure time studied.

We also evaluated the presence of cellular aberrations, such as mitotic cycle anomalies (colchicine metaphases, anaphase and telophase bridges) and interphase anomalies (micronucleated cells and binucleated cells). For this evaluation 1,000 cells were analyzed for each control and exposure time.

Statistical analysis of all data was performed by the Chi-square (χ^2) test, with a signification level < 0.05, through the BioEstat 3.0[21] statistical software.

RESULTS

Table 1 presents the number of cells in interphase and during different phases of cell division and the mitotic index values obtained from *A. cepa* meristematic root cells treated with water (control) and different concentrations of *C. jamacaru* at exposure times of 24 and 48 hours.

From the results obtained it can be seen that the concentration of 500/2 g/L did not alter the cell division rate of root meristem cells of *A. cepa* when comparing the MI obtained for its two ETs with the MI of its respective CO. When examining the cell division index values obtained for the two ETs of this concentration it was found that they were not significant among themselves.

As for the 500/1 and 500/1.5 g/L concentrations, it was observed that the MI obtained for both ETs were significantly lower than the MI obtained for their respective controls, demonstrating that these two concentrations had a significant antiproliferative effect on the cell cycle of *A. cepa* root meristem cells. It is important to point out, and as can be seen in Table 1, that for these two concentrations, in the two ETs evaluated, there occurred a greater number of cells in prophase than in other cell division phases.

Table 1. Types and total cells analyzed in the cell cycle of *A. cepa* root tips treated with water (control) and the crude aqueous extracts from the *C. jamacaru* cladodes at concentrations of 500/2, 500/1.5 and 500/1 g/L

Concentration	ET	Total cells analyzed	Cells in interphase	P	M	A	T	Total cells in division	MI (%)
500/2 g/L	CO	5.000	4.405	233	143	108	111	595	11.9a
	24h	5.000	4.628	190	76	82	24	372	7.4a
	48h	5.000	4.882	218	82	54	17	371	7.4a
500/1.5 g/L	CO	5.000	4.435	225	130	122	88	565	11.3a
	24h	5.000	4.857	102	09	04	28	143	2.8b
	48h	5.000	4.833	140	01	01	25	167	3.3b
500/1 g/L	CO	5.000	4.321	200	269	127	135	679	13.6a
	24h	5.000	4.724	244	00	00	32	276	5.5b
	48h	5.000	4.702	257	00	00	41	298	6.0b

ET–Exposure time, C–control, P-prophase, M-metaphase, A-anaphase, T-telophase, MI-mitotic index.
Means followed by the same letter do not differ significantly at the 5 % level by the X^2 test.

Table 2 presents the number of colchicine metaphases, anaphase and telophase bridges, micronuclei, and total cellular aberrations present in meristematic root cells of *A. cepa* treated with water and with different concentrations of *C. jamacaru* in exposure times of 24 and 48 hours. At the two ETs of the four concentrations evaluated the presence of colchicine metaphases, anaphase bridges and telophase bridges, and mainly micronuclei, was verified. All concentrations induced a number of cellular aberrations that differed significantly from their CO, but did not differ among themselves. It is important to report that the number of cellular aberrations at the two ETs, highlighting the number of micronuclei, increased in the 500/1 g/L concentration. Thus, the results obtained in relation to the cell division index and number of cellular aberrations showed that concentration 500/1 and 500/1.5 g/L were cytotoxic to *A. cepa* meristematic roots cells.

Table 2. Types and total cellular aberrations found in each control and concentrations of 500/2, 500/1.5 and 500/1 g/L of aqueous extracts of *C. jamacaru*

Concentration	ET	Total cells analyzed	Colchicine metaphases	Metaphase and telophase bridges	Micronuclei	Total aberrant cells
500/2 g/L	CO	1.000	02	00	00	02a
	24h	1.000	01	01	221	223b
	48h	1.000	01	00	219	220b
500/1.5 g/L	CO	1.000	02	00	00	02a
	24h	1.000	01	02	220	223b
	48h	1.000	04	03	221	228b
500/1 g/L	CO	1.000	01	00	00	01a
	24h	1.000	01	01	243	245b
	48h	1.000	01	00	254	255b

ET– Exposure time, CO– control.
Means followed by the same letter do not differ significantly at the 5 % level by the X^2 test.

DISCUSSION

According to the results obtained in this study, the three concentrations tested we have an antiproliferative effect and induced a statistically significant number of cellular aberrations in the test system cells in question.

Similarly, *Souza*[22] found significant antiproliferative effect of hydroethanolic extract from the cladodes of *C. jamacaru* on tumor cells induced in Wistar rats (Sarcoma 180) at concentrations of 250 and 500 mg/Kg in acute treatment. Schwarz et al.[23] found in the ethanolic extract obtained from *C. jamacaru* in a *Daphnia magna* acute toxicity test (24h) was able to yield in 100 % death and 95 % death at a dilution of 1:100. No death was observed at 1:1000. These authors also reported that an acute toxicity test (48h) with zebra fish eggs showed that the extract had effects on the survival of the embryos and only slight effects on other end points of this test.

The work of these authors was the only one found in the scientific literature, to date, on the action of extracts of the mandacaru stem at the cellular level. Despite the mandacaru being used by people in northeastern Brazil for a long time, and studies having already demonstrated its medicinal activity, this plant has not yet been cataloged by the Brazilian Sanitary Surveillance Agency (ANVISA) as an herbal medicine, due only to the lack of studies on the toxicity of this plant.

According to *Carvalho*,[24] it is known that for a plant to be indicated as a medicine, it is also necessary that it undergoes toxicological testing, such as cytotoxicity, genotoxicity and mutagenicity. Thus, it is expected that the results obtained in this

work, assist in establishing ideal doses for ingestion by the public. It is also hoped that these results will serve as a stimulus for further studies to evaluate cytotoxicity as well as genotoxicity and mutagenicity and thus determine the real action of this property on plant genetic material and cell division.

It is also very useful to highlight that there is a scarcity of studies evaluating toxicity for most species of Cactaceae of the genus *Cereus* and not only for *C. jamacaru*. These species are widely used as food and in traditional medicine by population, especially the poorest, in the periods of intense drought in northeastern Brazil and this fact calls for the conduction of toxicity tests with these plants in various test bodies and concentrations as soon as possible, to comprehensively assess the action of these plants at the systemic and cellular level.

Other mandacaru stem crude aqueous extract toxicity evaluations must be performed varying the test system, the concentrations and exposure times in order to establish, with propriety, the real action of this herbal medicine at the cellular level.

From the results obtained, it was found that the aqueous extract of the stem of *C. jamacaru*, at concentrations of 500/1.5 and 500/1 g/L, presented as cytotoxic to meristematic root cells of *A. cepa*.

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Ana Paula Peron. Rua Cícero Duarte, 940. Bairro Junco. Universidade Federal do Piauí (UFPI), Campus Senador Helvídio Nunes de Barros (CSHNB). Núcleo de Pesquisa Aplicada a Saúde e ao Meio-ambiente (NUPBSAM). Laboratório de Citogenética Vegetal e Animal, Picos, Estado do Piauí, Brasil.
Correo electrónico: anpapegenpes@hotmail.com