Original Article

TOXICITY OF *Jatropha curcas L.* LATEX IN *Allium cepa* TEST

TOXICIDADE DO LÁTEX DE *Jatropha curcas L.* NO MODELO DE *Allium cepa*

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ABSTRACT: The latex obtained from *Jatropha curcas* (physic nut) is used in traditional medicine to treat a variety of disturbs, including burns, hemorrhoids, ringworm and ulcers. Phytochemical analyses have shown that *J. curcas* latex contains natural compounds with therapeutic potential. In this study, the toxicity, cytotoxicity and genotoxicity effects of *J. curcas* latex on the root cells of *Allium cepa* were examined. Onion seeds and bulbs were exposed to seven different concentrations of latex and then the roots were submitted to macro and microscopic analyses. Water and sodium azide were used as negative and positive controls, respectively. The analysis of root growth showed that *J. curcas* crude latex or 50% diluted is highly toxic. Cytogenetic results showed that the mitotic index of the onion roots submitted to latex treatment decreased significantly compared to the negative control, which suggests that the latex is cytotoxic. High incidence of chromosome aberrations in the cells treated with *J. curcas* latex was observed too, indicating that the latex also presents genotoxic effect. The analyses presented in this report suggest the toxic, cytotoxic and genotoxic effects of *J. curcas* latex. Then, the indiscriminate use of *J. curcas* latex in folk medicine could bring risk to human health.

KEYWORDS: Antiproliferative effect. Genotoxicity. Medicinal plants and physic nut.

INTRODUCTION

Medicinal herbs have been used in folk medicine for a long time and they have played a promising role in the treatment and prevention of various human diseases (AL-ASMARI et al., 2014). Despite the therapeutic advantages of medicinal plants, their potential toxicity is not recognized by the general public or by many professional groups of traditional medicine (SOETAN; AIYELAAGBE, 2009). Many plant species commonly considered medicinal can contain potentially dangerous substances (RODRIGUES et al., 2011; MELO-REIS et al., 2011). Recent research studies conducted *in vitro* and *in vivo* revealed that many plants used as food or in traditional medicine have cytotoxic and genotoxic effects (SEHGAL et al., 2006; DALLA NORA et al., 2010; CARDOSO et al., 2014). Cytotoxic and genotoxic studies have great importance in the establishment of standard quantities and extraction methods for safe and effective use of these plants by the population (ASARE et al., 2012).

Jatropha curcas, known as physic nut, is a multipurpose plant from the Euphorbiaceae family (DUTTA et al., 2007). People in almost all parts of the world have been using this plant against alopecia, ascites, burns, convulsions, cough, dermatitis, diarrhea, dropsy, dyspepsia, fever, syphilis, tumors, ulcers, yaws and others (DEBNATH; BISEN, 2008). The latex of this plant is particularly used in the treatment of burns, hemorrhoids, ringworm and ulcers (DEBNATH; BISEN, 2008). Besides the widespread use of *J. curcas* by the population, there is some information about the toxic potential of different parts of this plant (SANTOS et al., 2008). Poisoning with *J. curcas* seeds or fruits have been reported. Gastrointestinal disorders within the first hours following ingestion, such as nausea, vomiting, diarrhea and abdominal pain, were always presented. Neurological or cardiovascular signs, and hepatic or renal disorders were sometimes associated (LANGRAND et al., 2015).

Phytochemical analyses showed that *J. curcas* latex contains natural compounds with biological activities. Peptides present in *J. curcas* have been reported as cytotoxic, such as curcucyclins A and B (INSANU et al., 2012). Moreover, it has been isolated a protease from the *J. curcas* latex, known as curcain, that exhibit wound-healing property when tested on mice (NATH;

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DUTTA, 1991). *J. curcas* latex also contains alkaloids, including jatrophine and jatropham that have been demonstrated as anti-cancer (THOMAS et al., 2008). Despite the knowledge of the phytochemical composition, few studies have reported the toxic potential of *J. curcas* latex.

The *Allium cepa* assay has been widely used for evaluation of cytotoxic and genotoxic activity of various compounds (RIBEIRO et al., 2016; DEL CAMPO et al., 2005; BARBÉRIO et al., 2009; ROA et al., 2012; FRESCURA et al., 2013), including medicinal herbs (BAGATINI et al., 2007). It has been validated by an international collaborative study by the United Nations Environmental Program (UNEP), World Health Organization (WHO) and US Environmental Protection Agency (USEPA) as an efficient test for genetic monitoring (BADMUS et al., 2013). One of the parameters evaluated in this assay is the mitotic index (MI), characterized by the number of cells in division relative to the total number of analyzed cells. The MI is used as a parameter to assess the cytotoxicity of several agents. MIs significantly lower than the negative control can indicate alterations deriving from the chemical action on the growth and development of the exposed organism. On the other hand, MIs higher than the negative control indicate an increase in cell division, which can be harmful to the cells and lead to disorderly cell proliferation and even to the formation of tumor tissues (LEME; MARIN-MORALES, 2009). Other parameters analyzed in the *A. cepa* assay were the presence of chromosome aberrations and micronucleus formation, indicating genotoxic potential of the tested compound, and root appearance and length alterations, which could point to toxicity potential of the tested compound (LEME; MARIN-MORALES, 2009).

Therefore, the *Allium cepa* assay is an efficient bioindicator for the first screening of medicinal plant toxicity, cytotoxicity and genotoxicity for: 1) its kinetic proliferation properties; 2) having large chromosomes which are few in number (2n = 16); and 3) its reliability and agreement with other toxicity tests (LACERDA et al., 2014; NEVES et al., 2014; ALMEIDA et al., 2014; FRESCURA et al., 2013; LEME; MARIN-MORALES, 2009). Thus, this assay has been aiding human health damage prevention studies (BAGATINI et al., 2007). So, the present study was undertaken to evaluate the toxic, cytotoxic and genotoxic potential of different concentrations of *J. curcas* latex on *Allium cepa* meristematic root cells.

MATERIAL AND METHODS

Latex extraction

The latex of *J. curcas* was extracted from Universidade Estadual de Goiás tree collection, in Ipameri (Goiás, Brazil). A voucher specimen (10.042) was deposited at the University Herbarium (Universidade Estadual de Goiás , Anápolis, Goiás, Brazil). The latex was collected into a sterile container through cuts made into the tree trunk. The cuts were made into the bark with a knife and had approximately 10 cm length and 0.5 cm depth.

Germination test

*Allium cepa s*eeds of the commercial cultivar (Sementes Vidasul Ltda) were used in order to evaluate the toxic effects of *J. curcas* latex. The latex was mixed with ultrapure water and quickly applied on germination paper in gerbox. Seven concentration of the latex were tested for toxicity (100%, 50%, 10%, 5%, 1%, 0.5% and 0.1%). The experiments were made in triplicate using 35 seed in each time. Ultrapure water was used as negative control solution.

Onion seeds were pre-soaked for 48 h in gerbox containing moistened germitest paper with distilled water and maintained at 21 ± 1 °C. After the seeds were transferred to gearboxes containing paper soaked in different extracts, except the negative control sample, which remained in water distilled. The count was performed six days after the experiment beginning and the germination test was performed. The seeds that presented radicle with at least 50% of the seed size were considered germinated (FERREIRA; L'AQUILA, 2000). The germinability (G) was calculated using the following formula: G = (N / A) x 100. Where N is total number of germinated seeds; A is the total number of seeds placed to germinate. The length of radical was measure at 6 days after germination induction using scale.

Allium cepa assay

Onion bulbs were grown in water at room temperature for 2–3 days. When the roots were 2–4 cm in length, the bulbs were treated with different concentrations of *J. curcas* latex (10%, 5%, 1%, 0.5% and 0.1%). Another set of plants was placed in sodium azide (2 M) as a positive control, while for negative control, a set of *A. cepa* remained in water. The solutions were changed daily, and after 48 h the root tips from each bulb were harvested and fixed in Carnoy's fixative solution (1:3 acetic acid: ethyl alcohol) for 24 h. Then, they were prepared for microscopic analysis or stored in 70% ethyl alcohol.

Before the slide preparation the roots were rinsed a few times with distilled water. After, they were hydrolyzed with 5 M HCl solution at room temperature for 20 min. After hydrolysis, the roots were dissected in acetic acid (45%) and squashed with cover slip. The cover slips were removed after freezing in liquid nitrogen and stained with Giemsa (5%) for 5–10 min.

The slides were evaluated using a LEICA® Optical Microscope with magnification of 40 or 100 x. In total, 1,000 cells were analyzed per bulb, in 5 bulbs per treatment. The cytotoxic potential was calculated through observation of the mitotic index (MI). The MI was calculated for each treatment, by comparing the number of dividing cells with the total number of cells (SETH et al., 2008). The genotoxic potential was estimated by the frequency of anomalies in the mitotic cycle (AMC).

For the statistical analysis the groups were divided into five treatments, each containing five replications, and the values were measured through variance analysis (ANOVA) and compared through Tukey test (SISVAR, 2010). The value $p < 0.05$ was considered as indicative of significance.

RESULTS

The inhibitory effect of *J. curcas* latex on the germination and growth of *A. cepa* root meristems was evaluated and compared with the positive control (Table 1). The germinability rate was calculated and showed that the *J. curcas* crude latex or 50% diluted decrease and almost prevented seed germination. On the other hand, the others latex solution concentrations (10%, 5%, 1%, 0.5% and 0.1%) do not seem to interfere in the germination process, once the germination frequency was similar to the water. In relation to the radicule length, a progressive increase in root during the 6 days of incubation was observed in the seeds exposed to water and diluted latex solutions (10%, 5%, 1%, 0.5% and 0.1%). On the other hand, high latex concentrations (100 and 50%) cause a significant decrease in root length. This result indicates that *J. curcas* in high concentration are toxic to onion seeds, decreasing the germination rate and root length.

Table1. Germination rate (GR) and root length in *Allium cepa* seeds after *J. curcas* latex exposure.

Treatment	Germination Rate (%)	Root Length Mean ± SD
Control (water)	72,85	21.9 ± 14.5 a
0.1%	64,3	28.3 ±12.7 b
0.5%	75,7	29.2 ±13.5 b
1%	74,28	27,4 ±16.3 b
5%	78,57	23.4 ± 14.4 a
10%	70	22.7 ± 12,0 a
50%	34,2^{+}	5.3 ± 5.1 c*
100%	5^{+}	4.3 ± 1.5 c*

* $p < 0.05$; Same letters represent no significant difference using Tukey test; + Reduction of 50% in relation to the control.

Besides the seed germination, we used the onion bulbs exposed to *J. curcas* latex to evaluate the cytotoxic and genotoxic potential of the physic nut latex. The mitotic index (MI) was taken into consideration to evaluate cytotoxicity, and chromosome aberrations (CA) was recorded to evaluate genotoxicity. The results obtained showed that all three latex concentrations of *J. curcas* significantly inhibited the MI of *A. cepa* meristematic root cells (Table 2), and does not differ significantly from positive control. In addition, the MI decreased progressively as the latex concentration increased (Table 2), showing that latex concentration is negatively correlated with MI. To be considered cytotoxic, a substance should cause MI reduction greater than 50%, when compared with the control substance (GREENWOOD et al., 2004). The results obtained for *J. curcas* latex in the present work were higher than 50% in all concentrations, which suggested that *J. curcas* latex is cytotoxic even at very low concentrations.

To evaluate the genotoxic potential it was observed the chromosome aberrations frequency. The results showed that chromosome aberrations were observed in low incidence at 1% and 0.5% latex concentrations. However the number of aberrations increased drastically when the latex was diluted to 0.1% (Table 2). One possible explanation for these results is that at high latex concentrations, the MI is very low and it is not possible to observe cells in division. But when the MI increased with

latex dilution (0.1% group), it was possible to observe cells in division. Among the cells dividing in the bulbs exposed to 0.1% latex, it was possible to identify high occurrence of chromosome aberrations. Among the different types of abnormalities observed, the most common were: chromosome stickiness, chromosome bridging and lagging chromosomes (Figure 1).

Table 2. Cytogenetic analysis of *A. cepa* roots exposed to different concentrations of *J. curcas* latex.

Treatments	Total number of cells	Interphase cells	Division cells	Cytotoxicity	Genotoxicity
				Mitotic index (MI %)	Chromosome aberrations (CA %)
Latex 1%	5000	4500	50	1.0 A	3 a
Latex 0.5%	5000	4921	79	1.6 A	23 a
Latex 0.1%	5000	4643	357	7.1 A	253 b*
Water	5000	3705	1295	26 B*	0 a
Sodium azide	5000	4579	421	8.4 A	254 b*

* $p < 0.05$; Same letters represent no significant difference using Tukey test.

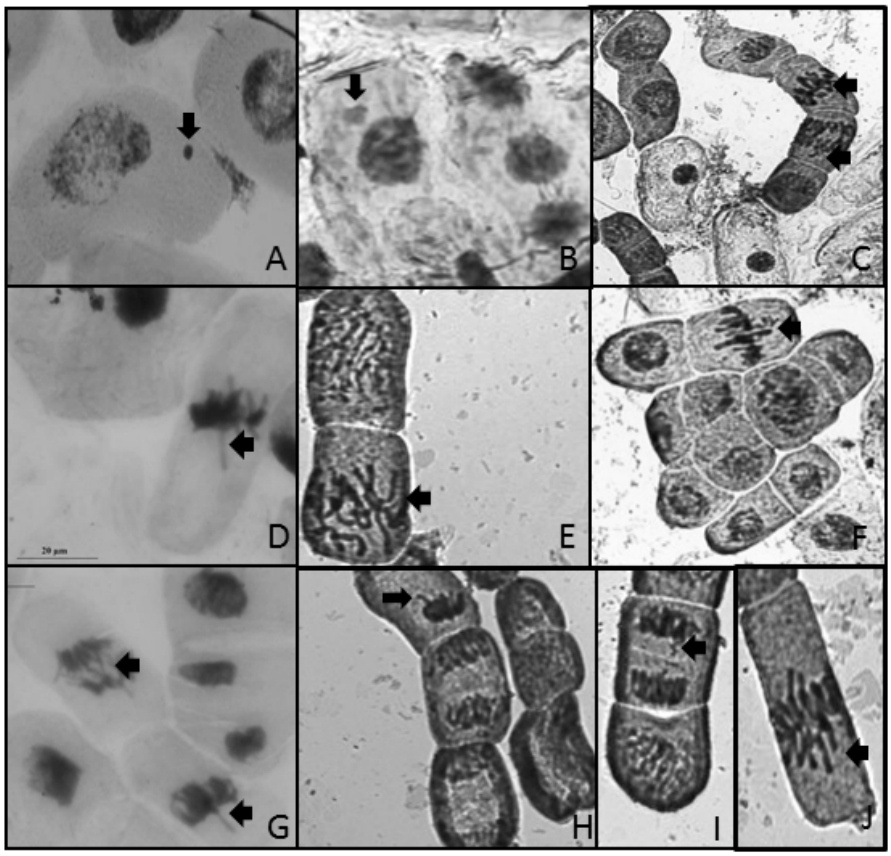

Figure 1. Mitotic and chromosomal aberrations after the *J. curcas* latex treatments in *Allium cepa* root tip meristem cells visualized with light microscopy. **A and B**: interphase cells with micronucleus; **C:** chromosome stickiness and lagging; **D:** chromosome lagging; **E:** chromosome stickiness; **F:** chromosome break; **G.** chromosome bridges; **H and I:** chromosome lagging; **J.** chromosome stickiness. Arrows indicated the abnormalities.

DISCUSSION

Although an expressive number of medicinal plant species are known and used in popular medicine, detailed studies of the pharmacological and biological activity of many of them remain elusive. In the case of *J. curcas,* different parts of this plant, such as roots, bark latex, leaves, seeds and oil, have been employed empirically in popular medicine (THOMAS et al., 2008). However, detailed evaluation of their effectiveness, pharmacological and toxic potential, molecular action, or proper dosage remains elusive.

In the present study, the *A. cepa* test system was used to evaluate the toxic (seed germination rate and root growth), cytotoxic (mitotic index frequency) and genotoxic (chromosome abnormality) effects of different concentrations of *J. curcas* latex. Regarding toxicity, the results obtained showed retarded growth of all the roots submitted to the latex treatment. Crude latex and diluted 50% with water suggesting that *J. curcas* latex are highly toxic. Also, it was possible to infer that toxicity was dose-dependent, since higher latex concentrations showed the highest toxic effects. On the other hand, latex diluted solutions stimulate the root growth comparing with water treatment. In that way, the root growth was dose-dependent and the smallest latex concentration showed the highest root growth.

In addition to medicinal use, *J. curcas* has been considered to be one of the most promising oilseeds for biofuel production (DEVAPPA et al., 2012). In this way, the seeds have been the main economic use of this species. Considering the economic potential of this part of *J. curcas*, some authors have evaluated the toxic potential of the oilseeds using different bioassays. Andrade-Viera (2014) showed the phyto, cyto and genotoxic effects of oil extracted from *J. curcas* seeds in *Lactuca sativa*, and suggested that this assay can be used to identify toxic and nontoxic varieties of physic nut. Devappa et al. (2012) showed that phorbol esters from *J. curcas* seeds exert a toxic effect on snail, brine, shrimp and daphinia bioassays, and also in microorganisms. Rakshit et al. (2008) tested *J. curcas* oil toxicity in rats and observed that all rats showed reduced appetite and low diet intake, accompanied by diarrhea. All authors have been attributed the toxicity of natural compounds present in *J. curcas* to the phorbol esters. The phorbol esters are diterpenes having tigliane skeleton. In *J. curcas*, six types of phorbol esters have been reported (HAAS et al., 2002).

In specific regard to *J. curcas* latex cytotoxicity, there are few reports indicating cytotoxic activity of the crude extract. Ribeiro et al. (2012) tested the leaves cytotoxicity in human tumor cell lines using the thiazolyl blue test (MTT) assay and observed significant cell mortality. Despite the high mortality, the mechanism responsible for the cytotoxic activity has not been investigated yet. The authors suggest that the cytotoxicity could be associated with the presence of substances curcin, curcusone (A, B, C and D), ultidione, jatropholone and acetoxyjatropholone in the *J. curcas* species.

On the other hand, the toxic effect of these isolated compounds has been intensely evaluated. For example, there are different reports showing the toxic activity of curcin (LIN et al., 2003; QIN et al., 2010; HE et al., 2011; PRAHAN et al., 2012; ZAHO et al., 2012; JARAMILLO-QUINTERO et al., 2015). Curcin is a toxalbumin protein extracted from *J. curcas* seeds that present *in vitro* effect inhibiting protein synthesis, which is called ribosome-inactivating protein (RIP). Curcin has shown antitumor effect on different tumors cells (gastric cancer cell line; mouse myeloma cell line; carcinoma cell line), and its mechanism is related to N-glycosidase activity (LIN et al., 2003). The curcusones are other important compounds extracted from *J. curcas* latex. They are diterpenes with strong inhibitory and cytotoxic activity (AIYELAAGBE et al., 2011). Four curcusones have been identified in this species (curcusones A, B, C and D), and all of them showed anticancer activity. Curcusone B, in particular, effectively suppresses the metastatic processes at doses that are non-toxic to cells, which may be of therapeutic benefit for the treatment of metastatic cancers (MUANGMAN et al., 2005). Another diterpene found in the latex is the jatropholone, which also presented antiproliferative activity in human cell culture (THEODULOZ et al., 2009). *J. curcas* latex also contains cyclic peptides, such as octapeptides (curcacycline A and jatrophidin) and cyclic nonapeptides (curcacycline B), that could possess cytotoxic, antimalarial and antimicrobial activity (SABANDAR et al. 2013).

In relation to the genotoxic activity of the latex, to the best of our knowledge to date there are no reports evaluating the genotoxic effect of *J. curcas* latex. Our results showed that at 1% and 0.5% the latex did not present detectable genotoxic effect when compared with the negative control (water). However, at the lowest concentration (0.01%) the latex showed highly genotoxic activity. In fact, the genotoxic effect is not observed in cells submitted to higher concentrations of latex because the latex inhibits cell division (MI), and it is not

possible to visualize the chromosomes in the *A. cepa* assay. At lowest latex concentration it was possible to observe chromosome aberrations, such as chromosome stickiness, chromosome bridges and lagging chromosomes. The sticky chromosome represents changes in the chromosomal structure and can be associated with the action of toxic compounds on DNA and/or protein structure. The sticky chromosome change is usually irreversible and generally leads to cell death (EL-GHAMERY et al., 2003). A chromosome bridge rises when the chromosome fails to separate due to chromosome stickiness (YADAV, 1986). Sticky chromosomes and chromosome bridges are abnormalities that characterize the aneugenic action of the components of the *J. curcas* latex. The genotoxic potential of *Jatropha gossypiifolia* latex was also investigated. Similarly to what was observed to *J. curcas* latex, *J. gossypiifolia* latex showed a significant decrease in root growth and in the mitotic index for the tested concentrations (0.1%, 0.2%, 0.5%, 1% and 2%). The 0.1%, 0.2%, 0.5% concentrations induced significant chromosome adherences, C-metaphases and/or chromosome bridges, suggesting genotoxic effects (ALMEIDA et al., 2015).

The present study demonstrated that the latex extracted from *J. curcas* has natural compounds that exert toxic, cytotoxic and genotoxic effects on *Allium cepa* roots. The knowledge of the latex effect on cells is still preliminary, requiring more studies to determine the mechanisms of action of the latex natural compounds and the suitable dosage for safe and effective use by the population. Studies involving the genotoxicity of medicinal plants are important, because they can alert the population about possible and eventual damages to health (TEDESCO et al., 2015). Our results showed that the empirical utilization of *J. curcas* latex in popular medicine could be harmful to human health and should be avoided.

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RESUMO: O látex obtido de *Jatropha curcas* (pinhão manso) é usado na medicina tradicional para tratamento de diversos distúrbios, como queimaduras, hemorroida, micose e úlcera. Análises fitoquímicas apontaram que o látex de *J. curcas* contém compostos naturais com potencial terapêutico. Este estudo avaliou a toxicidade, citotoxicidade e genotoxicidade do látex de *J. curcas* em células da raiz de *Allium cepa*. Sementes e bulbos de cebola foram expostos á sete diferentes concentrações de látex e, então, as raízes foram submetidas a análises macro e microscópica. Água e azida sódica foram utilizadas como controle negativo e positivo, respectivamente. A análise do comprimento das raízes mostrou que o látex de *J. curcas* puro e diluído a 50% é altamente tóxico. O índice mitótico das raízes de cebola submetidas ao tratamento com o látex diminuiu significativamente comparado com o controle negativo, o que sugere que o látex é citotóxico. Uma alta incidência de aberrações cromossômicas em células tratadas com o látex de *J. curcas* também foi observada, indicando que o látex apresenta efeito genotóxico. Essa análise sugere que o látex de *J. curcas* possui efeitos tóxico, citotóxico e genotóxico, sendo que o uso indiscriminado do látex de *J. curcas* na medicina popular pode trazer risco à saúde humana.

PALAVRAS-CHAVE: Efeito antiproliferativo. Genotoxicidade. Plantas medicinais e pinhão manso.

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