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The Mitigating Effect of *Jatropha curcas* L. Latex against Genotoxicity Induced by Doxorubicin

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PRMR, LMA and PJG initiated the work and wrote the protocol. Author FSM obtain the latex samples. Authors IRP, JGVS, MAMS and LLB conducted the laboratory and statistical analyses. Authors EFLCB, FSM and PJG wrote the first draft of the manuscript. Authors EFLCB and LMA interpreted the results and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Jatropha curcas (Euphorbiaceae) is a multiple purpose lacticiferous plant with potential for biodiesel production and medicinal uses. There is in the literature different analyses about the toxic and cytogenotoxic effects of *J. curcas* extracts, but few information about latex toxicity. In addition, few models were employed to evaluate the toxicity response to *J. curcas* latex, and the toxicity in *in vivo* mammal's model has not been tested yet. The cytotoxic, mutagenic and antimutagenic potential of *J. curcas* latex were investigated using mouse bone marrow erythrocytes. The results indicated a cytotoxic and mutagenic potential of this latex to mammalian cells. But, when *J. curcas*

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latex was co-administrated with doxorubicin (DXR – chemotherapy medication), a reduction in the number of micronuclei was observed, indicating an interaction between *J. curcas* latex and DXR. The interaction of latex with DXR can cause a reduction in the activity of this drug and impair the treatment of its users. Moreover, there is a lack of data on herb–drug interactions, what should be more investigated to safeguard the wellbeing of patients.

Keywords: Doxorubicin; herb-drug interactions; micronucleus; mouse bone marrow erythrocytes; plant polyphenols.

1. INTRODUCTION

Jatropha curcas (Euphorbiaceae), popularly known as physic nut, is a perennial lactiferous. Many authors consider *J. curcas* to be one of the most promising oilseeds for biofuel production [1]. Interest in this species has been increasing, due to *J. curcas* rapid growth and easy propagation, contributing to its use as an alternative energy source, and the identification of several secondary metabolites in this plant with medicinal importance [1,2]. Crude extracts, essential oils and isolated compounds from *J. curcas* are used in a wide range of pharmacological activities, such as anti-inflammatory, antioxidant, antimicrobial, antiviral, molluscicide, larvicidal, anticancer, antidiabetic, procoagulant, anticoagulant, hepatoprotective, analgesic, healing and abortifacient [3-6].

But some folk communities report some side effects when using this plant, such as nausea, impotency, sterility, dizziness and hallucination [7]. Maybe this symptomatology is due to secondary metabolites present in this plant. It has been reported the presence of alkaloids, saponins, tannins, terpenoids, steroids, glycosides and phenolic compounds, including flavonoids in *J. curcas* leaf and stem methanolic extracts. One of the most common biological properties of alkaloids is their toxicity against cells [4].

Concerning the different *J. curcas* parts used in medicine, the latex has been shown biotechnological potential for the development of new drugs [8]. *J. curcas* latex is used in traditional medicine as antiangiogenic, anti-inflammatory, anticoagulant, antimicrobial, anticancer and healing [3]. Corroborating the traditional use, *J. curcas* latex showed one of the highest antioxidant activities in a comparison study with methanolic extracts from different parts of *J. curcas* [9]. The free radical and NO scavenging activities presented by *J. curcas* latex correlated well with the high levels of phenolic, flavonoid and saponin present in this latex. The anti-inflammatory effect of *J. curcas* extracts was attributed to their strong iNOS inhibition [9]. It was also demonstrated that *J. curcas* latex possess both procoagulant and anticoagulant activities, depending on the latex fraction being used [10]; and angiogenic and antiangiogenic activities depending on the latex concentration used [11-13]. The latex also presented antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pyogenes*, *Micrococcus* luteus, *Lactobacillus acidophilus*, *Candida albicans* and *Trychophyton* sp. [14,15].

Proteins with biological activities were isolated from *J. curcas* latex, namely curcain, curcacycline A and curcacycline B [16,17]. Curcain may be responsible for wound healing; curcacycline A presented moderate inhibition of (i) classical pathway activity of human complement and (ii) proliferation of human T-cells, besides antimicrobial and cytotoxic activities; and curcacycline B presented cytotoxic activity [18]. It was demonstrated the latex cytotoxicity against human cell lines, such HT-29 (colon adenocarcinoma) and Chang liver cell (cervix carcinoma) in a dose-dependent manner [9]. Curcacycline A also demonstrated cytotoxicity against ovarium cancer cell [18]. Moreover, it was observed toxic, cytotoxic and genotoxic effects of *J. curcas* latex on *Allium cepa* model [19]. However, the *J. curcas* latex toxicity in *in vivo* mammal's model has not been tested yet. In this context, we tested the *J. curcas* latex cytogenotoxic and anti-cytogenotoxic potentials using the mouse bone marrow micronucleus test. We also performed a phytochemical prospection to identify the secondary metabolites present in the latex.

2. MATERIALS AND METHODS

2.1 *Jatropha curcas* Latex Obtainment

The *J. curcas* latex 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 [20]. The cuts were made into the bark with a knife and had approximately 10 cm length and 0.5 cm depth.

2.2 Phytochemical Screening

We evaluated the presence of the following secondary metabolites: alkaloids, anthraquinones, coumarins, flavonoids and tannins, using methodologies previously described [21,22].

2.3 Animals Maintenance

This study was approved by the Ethics Committee on the use of animals at the Pontificia Universidade Católica de Goiás (Protocol nº 0021-1/2016). Forty-eight healthy male outbred mice of the species *Mus musculus* belonging to the Swiss Webster strain were used. The mice had body weight varying from 30 to 40 g and they were 45 to 60 days old on the day of the experiment. The animals were placed in standard individual polypropylene cages with solid floors that were covered with sterilized wood chips according to international standards. The animals were housed in an environment with an average ± SD temperature of 24±2°C and a relative humidity of 55±5%. The light-dark cycle was 12 h:12 h, and water and food were available *ad libitum*.

2.4 Mouse Bone Marrow Erythrocytes Test

The 48 animals were divided in 8 groups with 6 animals each. Experiments were performed to evaluate the mutagenicity and anti mutagenicity potential of *J. curcas* latex cotreatment with doxorubicin (DXR). To evaluate the mutagenicity, 3 groups were intraperitoneally (ip) treated with 10, 50 or 100 mg/kg bw *J. curcas* latex. A negative control group was ip treated with 1 ml/100 mg/kg bw of sterile distilled water. To test the antimutagenic potential of *J. curcas* latex, 3 groups of animals were ip co-treated with 10, 50 or 100 mg/kg bw *J. curcas* latex and 2 mg/kg bw of doxorubicin (DXR). A positive control group was ip treated with 2 mg/kg bw of DXR. For all experiments, after 24 h, mice were anesthetized with thiopental (30 mg/kg bw) and euthanized by cervical dislocation. Mice femurs were dissected and the bone marrow gently flushed out with fetal calf serum, and centrifuged (300 g, 5 min). The bone marrow cells were smeared on glass slides, coded for blind analysis, air-dried, and stained with quick panoptic (New Prov®). To determine cytotoxic activity, we evaluated the frequency of PCE in relation to normochromatic erythrocyte (NCE) frequency. To detect micronucleated polychromatic erythrocytes (MNPCE) frequency, we prepared two slides for each mouse, and scored 1000 polychromatic erythrocytes (PCE) per slide. The slides were visualized by optical microscopic (Olympus BH2, Tokyo, Japan).

2.5 Antioxidant Activity (AOA)

The scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was performed according to the adapted method described by Sánchez-Moreno and colleagues [23]. The samples were diluted in the same solution resulting in the concentrations 0.4 to 2 mg/ml for *J. curcas* latex. After, 0.1 ml of each solution was mixed with 3.9 ml of a 60 µM DPPH solution. After an incubation time of 30 min at room temperature, the absorbances were measured at 515 nm (Asample). The blank was performed with methanol without DPPH (Ablank). A control solution was performed using 3.9 ml DPPH solution and 0.1 ml of methanol (Acontrol). The scavenging activity of each solution was determined according to the following equation:

$$AOA\ (\%) = 100 - \frac{(Asample - Ablank)x\ 100}{Acontrol}$$

AOA was finally expressed as IC_{50}, which means the concentration (mg/ml) of the extract required to cause a 50% decrease in initial content of the DPPH solution. The assays were performed in triplicate. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tannic acid and ascorbic acid were used as positive controls.

2.6 Statistical Analysis

In order to analyze the cytotoxic and mutagenic activity of *J. curcas* latex, we used one-way analysis of variance (ANOVA), followed by a multiple comparison procedure (Tukey test). For this, Microsoft® Excel® for Mac 2011 was used to calculate average and standard deviation of the data and PAST version 1.94 [24] was used to compare the means. The results are presented as mean ± standard deviation and were considered statistically significant when $p < 0.05$.

3. RESULTS

3.1 Phytochemical Investigation

The phytochemical screening of *J. curcas* latex revealed the presence of anthraquinones, flavonoids and tannins. But alkaloids and coumarins were not identified in this latex sample.

3.2 Cytotoxicity Evaluation of *Jatropha curcas* Latex

Different concentrations of *J. curcas* latex used in this work promoted a dose-dependent PCE/NCE ratio decrease in comparison with the negative control (Fig. 1). This result indicates a cytotoxic potential of this latex to mammalian cells. When *J. curcas* latex was co-administrated with DXR, no significant difference in comparison to the positive control was observed, with exception of 50 mg/kg bw co-adiministration, indicating a weak cytotoxic modulation potential of the *J. curcas* latex on DXR (Fig. 1).

3.3 Mutagenicity Evaluation of *Jatropha curcas* Latex

The frequency of MNPCE in mice treated with 50 or 100 mg/kg bw *J. curcas* latex was higher than the negative control, indicating a mutagenic potential of *J. curcas* latex in concentrations higher than 10 mg/kg bw (Fig. 2). But, when *J. curcas* latex was co-administrated with DXR, a dose-dependent MNPCE reduction was observed, indicating a direct or indirect interaction between *J. curcas* latex and DXR (Fig. 2).

3.4 *J. curcas* Latex Antioxidant Potential

Using DPPH radical scavenging method, *J. curcas* latex presented a good antioxidant potential (IC_{50} = 0.87 mg/ml) when compared with four different positive controls: BHT (IC_{50} = 0.20 mg/ml), BHA (IC_{50} = 0.10 mg/ml), ascorbic acid (IC_{50} = 0.09 mg/ml), and tannic acid (IC_{50} = 0.04 mg/ml), demonstrating that this latex can act as a free radical scavenger.

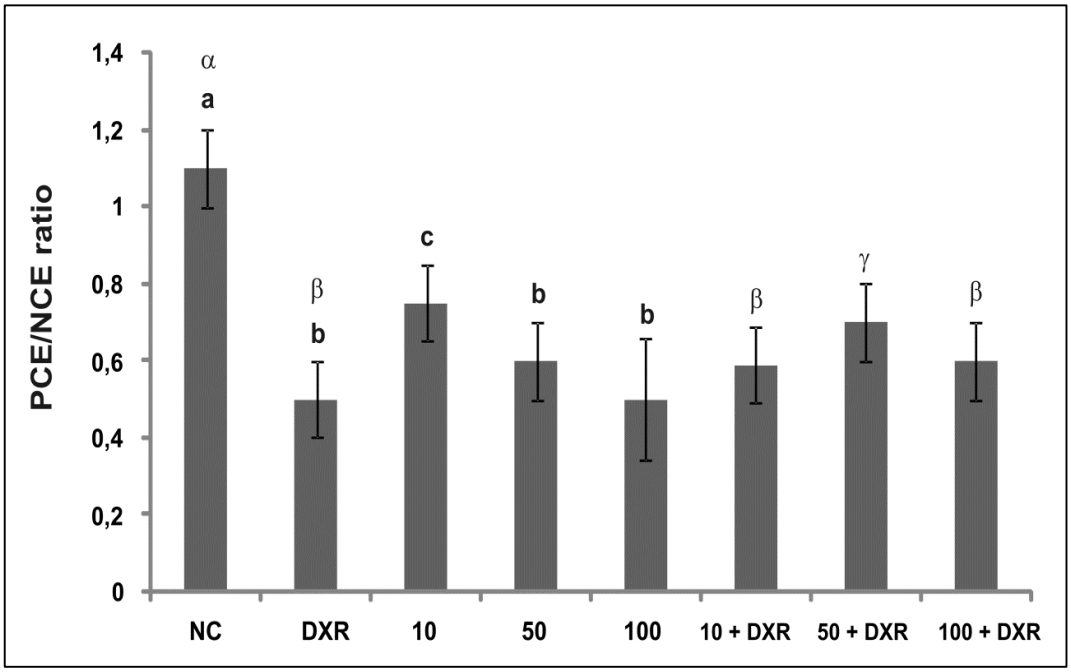

Fig. 1. *Jatropha curca*s latex (anti-)cytotoxicity against mouse bone marrow erythrocytes

Mice were treated with different doses of J. curcas latex (10, 50 or 100 mg/kg bw) along or not with doxorubicin (DXR) to investigate anti-cytotoxicity or cytotoxicity, respectively. These conditions were compared with negative (NC) and positive (DXR) controls. The frequency of polychromatic erythrocytes (PCE) in relation to normochromatic erythrocyte (NCE) was evaluated. Bars with different roman or greek letters represent statistically significant differences (p < 0.05) between cytotoxic or anti-cytotoxic treatments, respectively and controls

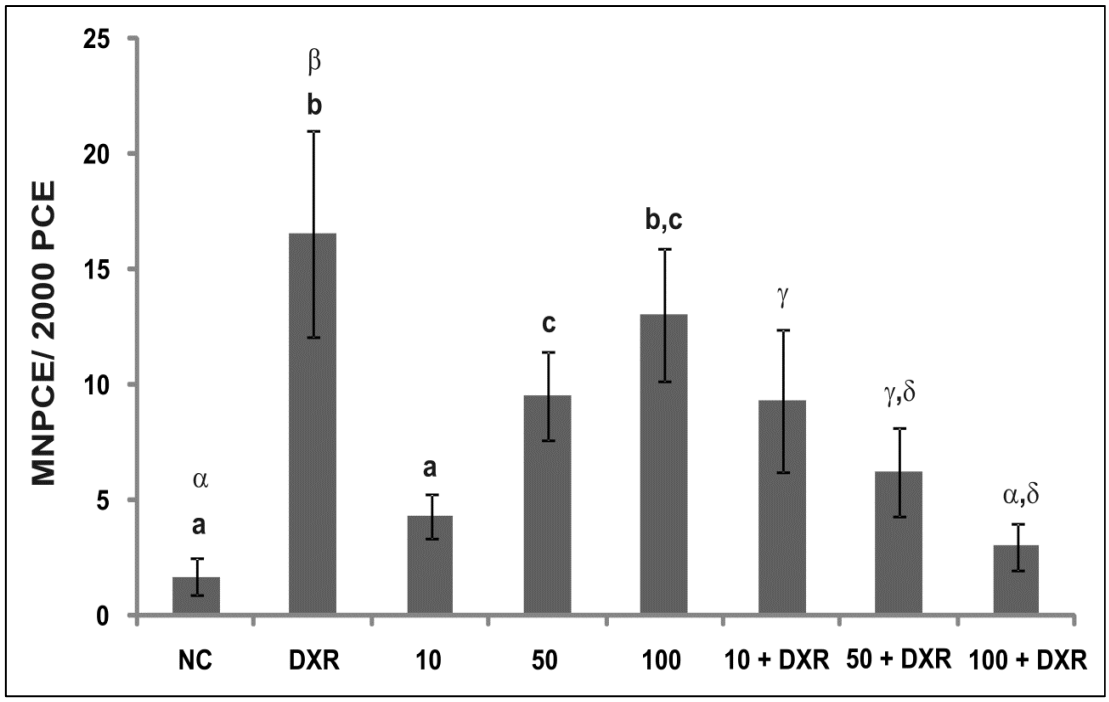

Fig. 2. *Jatropha curca*s latex (anti-)mutagenicity against mouse bone marrow erythrocytes
Mice were treated with different doses of J. curcas latex (10, 50 or 100 mg/kg bw) along or not with doxorubicin (DXR) to investigate anti-mutagenicity or mutagenicity, respectively. These conditions were compared with negative (NC) and positive (DXR) controls. The frequency of micronucleated polychromatic erythrocytes (MNPCE) in relation to 2000 polychromatic erythrocytes (PCE) per animal was evaluated. Bars with different roman or greek letters represent statistically significant differences ($p < 0.05$) between mutagenic or anti-mutagenic treatments, respectively and controls

4. DISCUSSION

In this study, the *in vivo* micronucleus assay was used to assess cytotoxic and genotoxic potential of *J. curcas* latex. This assay is used for the identification of genetic changes induced by the tested compound to the chromosomes or the mitotic apparatus of cells by the analysis of erythrocytes as sampled in the bone marrow and/or peripheral blood cells of animals [23]. The data presented here showed that *J. curcas* latex possess cytotoxic, mutagenic and anti-mutagenic activities.

J. curcas latex cytotoxicity was observed by PCE/NCE ratio decrease in comparison with the negative control. When a toxic agent affects the normal proliferation of bone marrow cells, there is a decrease in the number of immature erythrocytes (PCE) in relation to the number of mature erythrocytes (NCE), reflecting bone marrow toxicity and cell depression [25]. This reduction could be due to (i) direct cytotoxicity or (ii) micronuclei formation and heavy DNA damages leading to cell death or apoptosis [26]. *J. curcas* and *Jatropha gossypiifolia* latex also demonstrated cytotoxicity against *Allium cepa* meristematic root cells [19,27].

J. curcas latex mutagenicity was observed here by MNPCE frequency increase. *J. curcas* latex mutagenicity was also demonstrated against *Allium cepa* meristematic root cells by the increase in the number of cells containing micronucleus (MN) [19]. MNs are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division. MN can be induced by defects in the cell repair machinery and accumulation of DNA damages and chromosomal aberrations. A variety of mutagens may induce MN formation leading to cell death, genomic instability, or cancer development [28]. Chemical mutagens can act mainly as alkylating agents, base analogs, and intercalating agents [29]. Natural compounds, such as flavonoids and tannins, have been also related with mutagenic potential

[30]. Here, we demonstrated the presence of both flavonoids and tannins in *J. curcas* latex. So, these classes of compounds could be involved in *J. curcas* latex mutagenicity.

Interestingly, when the antimutagenic potential of *J. curcas* latex was investigated, it was demonstrated that this latex could be also antimutagenic. The *J. curcas* latex was associated with doxorubicin (DXR), an anthracyline drug widely used for treatment of various solid tumors. DXR acts (i) intercalating into DNA and disrupting topoisomerase-II-mediated DNA repair and (ii) generating free radicals that damage cellular membranes, DNA and proteins [31]. A great variety of antimutagenic agents act through multiple mechanisms to provide protection against mutagens. Antimutagens can (i) act as a potent antioxidant, removing reactive oxygen species (ROS); (ii) inhibit the ROS formation; (iii) stimulate the detoxifying enzymes; (iv) convert into molecules that display antioxidant activity; (v) inhibit the enzymes responsible for the biotransformation of mutagenic compounds, leading to the inhibition of promutagens bioactivation; (vi) direct interact with the mutagen before it induces DNA damage; (vii) prevent mutagenic compounds from reaching target sites; (viii) bind or insert into the outer membrane transporters and lead to the blockage of a mutagen to be transferred into the cytosol; (ix) rapid eliminate the mutagenic compounds from the cells before the induction of genetic material damage; and (x) modulate DNA repair enzymes [25]. Natural antimutagens may belong to the following major classes of compounds: flavonoids, phenolics, carotenoids, coumarins, anthraquinones, tannins, terpenoids and saponins [32]. Here, we demonstrated that *J. curcas* latex present flavonoids, anthraquinones and tannins and present a good antioxidant potential.

The dual role of *J. curcas* latex presented in this work was also observed in other studies with plant extracts. *Calendula officinalis* flower extracts produced both genotoxic and antigenotoxic effects against diethylnitrosamine in rat liver cell cultures [33]. *Amaranthus spinosus* leaf aqueous extracts presented genotoxic effects against *Allium cepa* meristematic root cells, but also presented ability to inhibit the oxidative damage induced by the direct mutagen hydrogen peroxide [34]. Pycnogenol®, a standardized plant extract obtained from the bark of *Pinus pinaster*, induced DNA damage and increased MN frequency in Chinese Hamster Ovary (CHO) cells, although revealed a reduction in the frequency of MN and the extent of DNA damage induced by H_2O_2 [35].

In this way, certain compounds exhibit dual nature and display both mutagenic and antimutagenic effects. Such compounds are called “Janus mutagens”, referring to the Roman god Janus who had one head with two faces looking in opposite directions [25]. Compounds with redox capabilities, can act either as a free-radical scavenger or a free-radical producer, based on the chemical concentration, redox state of the test system, and the properties of the specific physiologic pathway being investigated [36]. The majority of these substances are plant products or extracts [32].

Plant polyphenols present both mutagenic and anti-mutagenic roles. Polyphenols can act as a mutagen (i) directly binding to DNA, (ii) generating ROS, or (iii) inhibiting topoisomerases enzymes [30]. On the other hand, phenolics are able to act as antimutagens (i) interfering with the cytochrome P450-mediated metabolism of the mutagens, (ii) directly interacting with active mutagen metabolites, or (iii) exhibiting antioxidant properties [37], as observed in this work. This opposite effect is a feature that should be considered when using plant polyphenols as therapeutic agents [33]. Moreover, there is a lack of data on herb–drug interactions that could present both risks (adverse drug events) and benefits (through enhancement).

5. CONCLUSION

In summary, the opposite effect of *J. curcas* latex should be considered when using this species as therapeutic agents, since the latex may interfere with the activity of allopathic drugs such as DXR in cancer patients. The interaction of latex with DXR can cause a reduction in the activity of this drug and impair the treatment of its users. In this way, more rigorous scientific research is urgently needed to guide clinical practice as well as to safeguard the wellbeing of patients.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by the Ethics Committee on the use of animals at the Pontificia

Universidade Católica de Goiás (Protocol nº 0021-1/2016).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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