Effect of *Astrocaryum aculeatum* (tucumã) on doxorubicin toxicity: *in vivo* experimental model

Efeito da *Astrocaryum aculeatum* (Tucumã) na toxicidade da Doxorrubicina: modelo experimental *in vivo*

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Abstract

Objective: To obtain the oil of *Astrocaryum aculeatum* (A.a), and evaluate its genotoxicity/antigenotoxicity activities using the micronucleus test in peripheral blood cells.

Methods: The oil of *Astrocaryum aculeatum* was obtained by hydraulic pressing. The animals used were healthy Swiss male mice, at 6-7 weeks of age; there were six per group. The genotoxic and antigenotoxic activity of concentrations were 500, 1,000 and 2,000 mg/kg per 0.5 mL (oral), followed or not followed by intraperitoneal injection of doxorubicin (0.3 mL-15 mg/kg by body weight), in addition to a negative group (water) and dimethyl sulfoxide (600 µL). Peripheral blood samples were collected 24h and 48h after treatment.

Results: A statistically significant reduction was identified in the frequency of micronuclei in polychromatic cells ranging from 34.72% to 38.19% for 24-hour treatments, and from 63.70% to 66.12% for 48 hour.

Conclusion: The fixed oil of tucumã presented antigenotoxic potential for the concentrations used in acute treatments.

Resumo

Objetivo: Obter o óleo do *Astrocaryum aculeatum* (A.a) e avaliar a genotoxidade/antigenotoxidade pelo teste do micronúcleo em células do sangue periférico.

Métodos: O óleo da A.a foi obtido por prensagem hidráulica. Os animais foram camundongos *Swiss*, machos e saudáveis com 6-7 semanas de idade, 6 por grupo. Teste genotóxico e antigenotóxico as concentrações foram de 500, 1.000 e 2.000 mg/kg por 0,5 mL (via oral), seguidas ou não de injeção intraperitoneal de doxorrubicina (0,3mL - 15 mg/kg por peso corporal), além do grupo negativo (água) e dimetilsufóxido (600 µL). As amostras de sangue periférico foram coletadas 24h e 48h após o tratamento.

Resultados: Houve redução estatisticamente significativa na frequência de micronúcleos em células policromáticas que variou de 34,72% à 38,19% para os tratamentos de 24h, e de 63,70 à 66,12% para os de 48h.

Conclusão: O óleo fixo do tucumã apresentou potencial antigenotóxico para as concentrações em tratamentos agudos.

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Introduction

The *Astrocaryum aculeatum* (*tucumã*) is a fruit of yellow color with reddish tones, which is from a palm tree of the *Arecaceae* Family that reaches 10 to 25 meters high, 15 to 30cm in diameter,[1] usually solitary, stem with dark thorns, ascending leaves, erect flowers and broadly distributed in Amazon, which presents great biodiversity of the genus *Astrocaryum.*[1,2] This is an exceptionally tolerant species to acidic and nutrient poor soils, which is characteristic of the Amazonian region.[3]

The fruit has an ovoid shape, whose fibrous mesocarp provides a high nutritional content. The pulp is well appreciated, and consumed *in natura* by the population, or as a filling in sandwiches, tapiocas, custard, ice cream, and flour.[4]

de Rosso & Mercadante[5] described 24 carotenoids; 21 were chemically identified in the *tucumã*. *Tucumã* analysis show one of the highest concentrations of pro-vitamin A, representing 52 mg/100 g of pulp, with a potential antioxidant that block the harmful effects caused by free radicals. Carotenoids are essential for cell differentiation, embryonic development, vision, as well as many other functions, including potential therapeutic benefits [5,6]

According to Ambrósio et al., (2006),[7] β-carotene is a powerful antioxidant that protects against cardiovascular diseases by inhibiting the oxidative process of low-density lipoprotein (LDL). Important polyphenols, such as quercetin, are also present in *tucumã*, which is one of the main flavonoids present in the human diet. Flavonoids are compounds found in plants, which also have antioxidant properties.[7,8]

Chemotherapeutic doxorubicin (DXR) is an anthracyclic antibiotic and a potent, broad spectrum, antitumor medicine, frequently used in combination with different medicines in the treatment of acute leukemia, lymphomas and solid tumors, such as breast, ovarian, and endometrial. Its toxicity can be caused in different ways: its planar agglutinated portion can be inserted between adjacent base pairs in the DNA, modifying the ability of nuclear helicases to dissociate the DNA double-strand and the topoisomerase II enzyme. Acting as an aggressor for this enzyme, it modifies its normal purpose to induce DNA damage, which occurs by loss of one or two electrons, generating reactive compounds with the potential to damage macromolecules and lipid membranes.[9]

Chemotherapy is a method that uses chemical compounds for treatment of diseases by biological agents, called chemotherapeutic. When used for cancer, it is called antineoplastic chemotherapy, and according to the Federal Nursing Council, the nurse is the one who can administer these medications. The nurse must know that there are natural ways to assist with this treatment,[10] in which the oil of *tucumã* presents with considerable potential for the support of chemotherapy, as vitamin A produces less side effects, and prevents a break in the continuity of treatment. The present study may provide a theoretical basis to support advanced nursing practice in cancer patients.[11]

The consumption of fruits rich in antioxidants prevents excessive oxidation by the body itself, or in actions of medications such as doxorubicin, which promotes the production of free radicals that, if not controlled, can cause cellular damage which may lead to the development of several chronic and degenerative diseases.[9] As the search for natural antioxidants has increased greatly in recent years, mainly for applications in the pharmaceutical, cosmetic and nutritional sectors, the hypothesis for this study is that the consumption of *tucumã* should be encouraged.

The objective of this study is to obtain the oil of *Astrocaryum aculeatum* (*A.a*) (tucumã) and to evaluate the genotoxicity/antigenotoxicity using the micronucleus test in peripheral blood cells.

Methods

Obtaining the fixed oil from Astrocaryum aculeatum

The oil was supplied by the Institute of Scientific and Technological Research of the State of Amapá (IEPA). The fruit was washed dry and the seed was removed to obtain the mesocarp, which was distrib-

uted onto trays for dehydration in a greenhouse, with air recirculation at 50°C for 24 hours, and then was cooled to room temperature.

The resulting mass was quantified at 28.21% of the total initial mass. The dry material was subjected to hydraulic pressing, with 15 tons capacity (SIWA model FM3), obtaining 22.21% of fixed oil.

Chemical agent inducing DNA damage

The chemotherapeutic agent, doxorubicin (DXR, Rubidox®), was used as a micronucleus inducer in peripheral blood cells, as a positive control. The chemical inducer was dissolved in distilled water and intraperitoneally administered (0.3 mL/animal) at the concentration of 15 mg/kg body weight, established according to studies of Franke et al.,[12] and Venkatesh et al.[13]

Animals and Treatments

Swiss male mice were used for the experiments; they were 6-7 weeks of life, with a body weight of approximately 30g; they were obtained from the Biothermium of the Multidisciplinary Center for Biological Research in the Laboratory Animal Science Area (CEMIB) of the University of Campinas (UNICAMP). The research was conducted in accordance with international protocols, regarding the use and care of laboratory animals. The animals were kept in grid boxes with dimensions of 30x19x-12cm in a experimental room, under the following controlled conditions: temperature (22±2°C), humidity (50±10%), 12 hours of light-dark cycle, with *ad libitum* access to feed and water. The treatment protocols performed in this study were approved by the Committee of Ethics in the Use of Animals of UNIFAP, protocol 0012/2015.

Experimental design

The animals were divided into different groups, with six mice in each treatment group. The concentrations of the fixed oil *of Astrocaryum aculeatum* (A.a) were 500, 1,000 and 2,000 mg/kg of body weight, administered by gavage (0.5 ml) for observation of the genotoxic processes. The oil dilutions were made, respectively, in 0.5 ml of distilled water, with the solvent dimethylsulfoxide (DMSO) in the concentrations 150µl, 300µl and 600µl, respectively.

For antigenotoxic evaluation, immediately after administration of the *A. a*, the animals were treated with an intraperitoneal injection of DXR (0.3 ml), at a dose of 15 mg/kg of corporal weight. The solvent group is represented by treatment with DMSO at the concentration of 600 µl, because it was the dosage used in animals receiving 2,000 mg/kg of body weight of A.a. Peripheral blood samples from the acute treatment groups were collected after 24 and 48 hours (Table 1).

Table 1. Experimental groups and acute treatment protocols for the micronucleus test

Treatment	Group	Dosage (mg/kg)
Negative control	1a,b	-
DMSO	2a	-
A. a I	3a	500
A. a II	4a	1,000
A. a III	5a	2,000
DXR	6a,b	15
DMSO + DXR	7b	As in (2) and (6)
A. a. I + DXR	8b	As in (3) and (6)
A. a II + DXR	9b	As in (4) and (6)
A. a III + DXR	10b	As in (5) and (6)

DMSO - Dimethyl sulfoxide; A. a. - *Astrocaryum aculeatum*; DXR - Doxorubicin; [a] protocols for genotoxicity; [b] protocols for antigenotoxicity

Micronucleus test

The levels of micronucleated polychromatic erythrocytes (MNPCEs) in the peripheral blood were sampled based on the technique of MacGregor et al.,[14] and adapted, consisting of the following procedures:

- The tip of the animals' tails were cut and blood was dripped directly onto the dry slides;
- The material was smeared with a cover slip;
- The material was fixed in methanol for five minutes, after drying;
- The material was stained with Giemsa in the next day, according to the Manual for the Laboratory Diagnostic of Malaria (2005). Giemsa powder 0.75g was used for each 100 ml of the solution - 35 ml Glycerol and 65 ml Methanol, in a ratio of 1:10 (Giemsa/buffered water), for 20 minutes.

- Less mature micronuclei in polychromatic erythrocytes (PCEs) that were stained in blue were analyzed. More mature Nitrous Erythrocytes (NCEs) showed little or no influence from the stain, and were not analyzed.

Analysis of the glass slides

The glass slides of each animal were coded and analyzed within a short time in a blind test, in order to eliminate analysis errors. The analysis was performed by three observers, using a balanced system; that is, an equal number of cells were analyzed on different glass slides, for each animal of the study, by each observer.

The glass slides were first analyzed under a 40x mean magnification optical microscope to find fields of good technical quality, where the cells were well spread, undamaged and properly stained. After these fields were located, the observers followed the cell analysis process to identify the presence of the micronucleus, using a 100x magnification (immersion lens).

The micronucleus distribution among the cells may have value in identification of the mechanism, so, for its determination, 2,000 PCEs per animal were analyzed in the peripheral blood samples (24h and 48h), and a total of 400 erythrocytes per animal were analyzed to calculate the Nuclear Division Index (IDN)[15-17] using the formula:

$$IDN = \frac{PCE}{PCE + NCE}$$

The percentage of frequency reduction of PCEMNs was calculated according to Waters et al. (1990),[18] to determine the effectiveness of treatments for antigenotoxic effect, using the following formula:

$$\% \text{ of reduction} = \frac{A - B}{A - C}$$

A corresponds to the DXR group (positive control), B corresponds to the groups treated with *A. a.* + DXR (antigenotoxic groups), and C corresponds to the group treated with water (negative control).

Statistical analysis

The data were analyzed statistically using the analysis of variance (ANOVA) for completely randomized experiments, with the calculation of the F statistic and respective "p-values". In cases where $p < 0.05$, the treatment means were compared using the Tukey method, with the calculation of the minimum significant difference for $\alpha = 0.05$, using the Graph Pad Prism 6 program.

Results

The results obtained for the treatments in Swiss mice with different doses of the fixed oil of *Astrocaryum aculeatum*, and/or these combined with the intraperitoneal administration of DXR in the dosage of 15 mg/kg of body weight, and their respective controls are shown in table 2.

Table 2. Frequencies of micronucleated polychromatic erythrocytes (PCEMNs) from the peripheral blood cells of animals submitted to treatment of different doses of A.a., and their respective controls

Treatment (mg/kg of body weight)	PCEMNs total		PCEMNs (mean ± SD)	
	24 h	48 h	24 h	48 h
Control	27	26	4.50 ± 0.55	4.33 ± 0.52
DMSO	31	34	5.16 ± 0.63	5.66 ± 0.82
A. a. I (500 mg)	29	29	5.16 ± 0.98	4.83 ± 0.41
A. a. II (1000 mg)	29	29	4.83 ± 0.75	4.83 ± 0.75
A. a. III (2000 mg)	28	28	4.66 ± 0.52	4.66 ± 0.52
DXR mg	171[a]	274[a]	28.50 ± 1.38	45.66 ± 1.63
DMSO + DXR	167[a]	267[a]	27.83 ± 0.75	44.66 ± 1.05
A. a. I + DXR	121[a,b]	116[a,b]	20.16 ± 1.16	19.33 ± 0.52
A. a. II + DXR	118[a,b]	111[a,b]	19.66 ± 1.03	18.50 ± 0.55
A. a. III + DXR	116[a,b]	110[a,b]	19.33 ± 1.37	18.33 ± 0.52

A total of 2,000 PCEs were analyzed per animal, for a total of 12,000 cells per treatment; DXR: doxorubicin (15mg/kg body weight); [a]significant difference for control group ($p<0.05$); [b] Significant difference for the DXR group ($p < 0.05$).

Simultaneous administration of a single oral dose of each fixed oil concentration of *Astrocaryum aculeatum* per gavage and intraperitoneal injection of DXR, resulted in a significant reduction in the frequency of PCEMNs. Values ranging from 34.72% to 38.19% for the 24-hour treatments (Figure 1), and from 63.70 to 66.12% for the 48-hour treatments where found (Figure 2), when compared to the group treated with DXR alone. The gradual increase of the fixed oil concentration of *Astrocaryum aculeatum* resulted in a proportional increase in the reduction of genotoxicity in 24 hours; however the evaluation of the treatments at

48 hours indicated no dose-response relationship (Table 2). The frequency of PCEMNs was lower in animals treated with DMSO + DXR than in those treated with DXR alone, but these differences were not statistically significant, and the IDN did not show any indication of cytotoxic potential in all groups evaluated (Table 3).

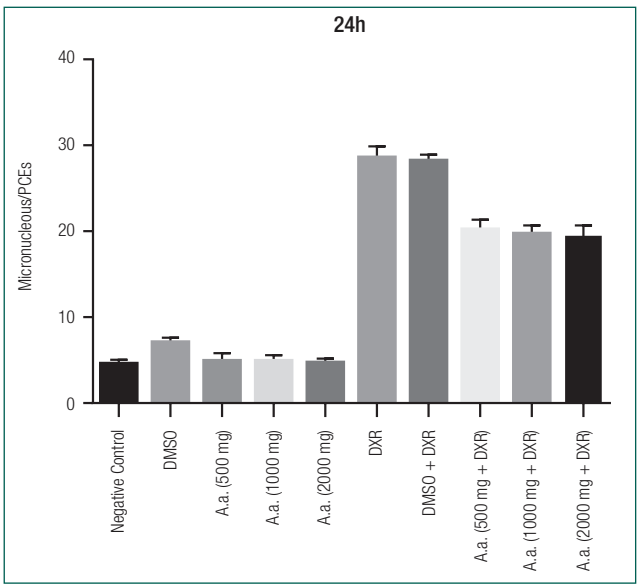

Figure 1. Frequencies of PCEMNs after 24 hours of treatment, with different doses of A.a. and DXR, and their respective controls

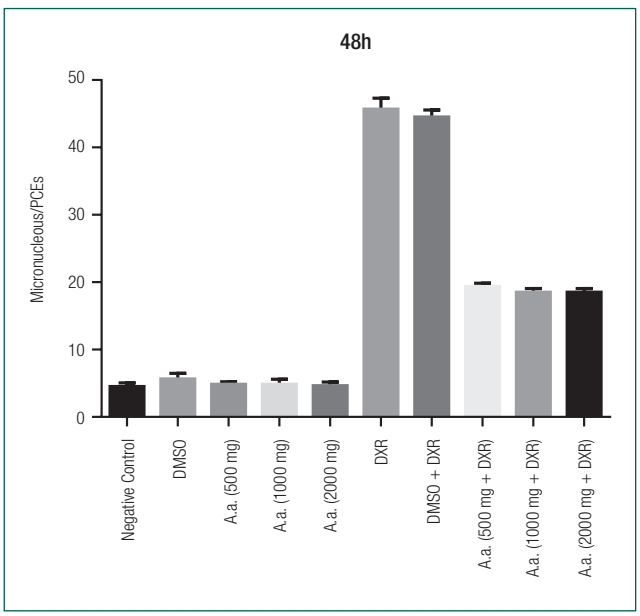

Figure 2. Frequencies of PCEMNs after 48 hours of treatment, with different doses of A.a. and DXR, and their respective controls

Table 3. Results of A.a. and DXR treatments and their respective controls (at 24 and 48 hours)

Treatment (mg/kg body weight)	IDN		PCEMNs/1,000 PCEs		REDUCTION (%)	
	24 h	48 h	24 h	48 h	24 h	48 h
Control	0.011 ± 0.51	0.010 ± 0.51	2.25	2.16	-	-
DMSO	0.013 ± 0.54	0.011 ± 0.54	2.58	2.83	-	-
A. a. I	0.013 ± 0.54	0.011 ± 0.83	2.42	2.42	-	-
A. a. II	0.014 ± 0.75	0.013 ± 0.75	2.42	2.42	-	-
A. a. III	0.013 ± 0.83	0.013 ± 0.75	2.33	2.33	-	-
DXR	0.013 ± 1.03	0.012 ± 0.89	14.25	22.83	-	-
DMSO+DXR	0.013 ± 0.75	0.010 ± 0.51	14.08	22.25	-	-
A. a. I + DXR	0.012 ± 1.16	0.011 ± 0.81	11.33	9.66	34.72	63.70
A. a. II + DXR	0.013 ± 0.75	0.011 ± 0.83	10.08	9.25	36.80	65.72
A. a. III + DXR	0.012 ± 0.75	0.010 ± 0.51	9.66	9.16	38.19	66.12

Discussion

The present study investigated the genotoxic and antigenotoxic effect of *tucumã* oil, an Amazonian fruit rich in carotenoids and other bioactive compounds, such as polyphenols.[5,12-19] The findings of this study demonstrated that *A.a.* presents characteristics similar to previously reported results on studies of *tucumã*, even in its various derivative forms in which it was examined.[7,8,19,20]

Substances, such as carotenoids and polyphenols found in the oil of *tucumã*, were previously evaluated by de Rosso and Mercadante,[5] and Gonçalves et al.,[8] who associated these compounds with the elimination of free radicals. These affirmations are corroborated with this study, because *A.a.* is described as rich in carotenoids and flavonoids; both compounds are known as antioxidants in the literature.

Several therapeutic properties of bioactive compounds, such as flavonoids, especially quercetin which is abundant in *tucumã*, have been extensively studied in the last decade, due to their antioxidant effects, anticancer potential, and protective effects on renal, cardiovascular and hepatic systems.[19-21, 23]

The antimicrobial properties of the *tucumã* are reported by Jobim et al.[21] These antimicrobial effects of the fruit are associated with its chemical composition, which includes several types of polyphenols. According to Daglia,[22] polyphenols are secondary metabolites produced by superior plants that have antibacterial, antiviral and antifungal properties.

However, in addition of the variability in bioactive compounds of different natures present in the fruit, the benefits derived from *tucumã* can be attributed to the antioxidant properties distinct from its several carotenoids. de Rosso and Mercadante[5] list 60 species of carotenoids present in different fruits, including 24 in the *tucumã*.

The bioactive molecules found in the *tucumã* present great antioxidant capacity.[24-26] However, the rich concentration of these molecules in the fixed oil of the fruit is not a guarantee that it will be able to completely reverse the free radicals [19] of a living organism exposed to molecules that can damage the genetic content of the cells.

Chemotherapeutic DXR, used in this study, is an anthracyclic antibiotic and a potent broad spectrum antitumor medicine, often used in the treatment of acute leukemia, lymphomas and tumors.[9] Doxorubicin is a micronucleus inducer, and is frequently used in mutagenicity tests as a positive control. It is reported as a metabolically active molecule for a free radical state that interacts with molecular oxygen to generate superoxide radicals.[27]

The results of the present study demonstrated a significant decrease in the frequency of micronuclei in polychromatic erythrocytes, which corroborates the studies of Sagrillo et al.,[19] and de Souza Filho,[20] which recognized the protective potential of *tucumã* in cells submitted to oxidative stress, which is a producer of free radicals. The results obtained in the evaluations of the treatment groups of this study that used *A. a.* associated with DXR, to test the antigenotoxic effect, indicated a significant reduction of 36.57 ± 1.74% for treatments of 24 hours and 65.18 ± 1, 29% for those of 48 hours.

The results of the genotoxic effect for the three concentrations tested (*A. a.* I 500 mg, *A. a.* II 1,000 mg and *A. a.* III 2,000 mg) as well as the DMSO group, when compared with the negative control group, demonstrated the absence of a toxic effect on DNA. These results corroborate[19,20] that different concentrations of *tucumã* provided a protective effect for the cell to oxidative stress, indicating some carotenoids, such as β-carotene, as an inducer of increased resistance to oxidative DNA damage.[20]

de Souza Filho et al.,[20] in their findings, suggested a relative genotoxic effect of *tucumã* on human peripheral blood mononuclear cells (PBMC), at concentrations higher than 500 µg/mg, when tested *in vitro* by the comet assay; however they recognized the methodological limitations related to their study, and emphasized that the results from studies that use only *in vitro* protocols to analyze the potential genotoxic effect of *tucumã* cannot be directly transferred to *in vivo* models.

Sagrillo et al.,[19] extracted the pulp and peel of *tucumã* in six different concentrations (100, 300, 600, 900, 1,200, and 1,500 ug/ml) to test its ability to treat oxidative stress induced in human lymphocyte cell cultures, and obtained best results with antioxidants effects at the lowest concentrations tested.

It was verified that there was a significant difference in the reduced rates of micronuclei when comparing treatment times (24 and 48 hours; a 32% difference was found with the 48-hour treatment. Studies that evaluate the genotoxic/antigenotoxic effect of *tucumã in vivo* were not found to compare results to this research, which makes it the reference for future investigations.

Conclusion

The results of this research demonstrate an evident protective potential for cellular DNA, at all the concentrations used, proving its antigenotoxic potential; however the genotoxic possibility was not demonstrated to be produced by the fixed oil of *tucumã* in every concentration studied. Therefore, the fixed oil of *tucumã* is an efficient antigenotoxic agent, producing satisfactory effects as a protector of cellular DNA damage, both at 24 and 48 hours after administration, achieving better results after 48 hours.

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Collaborations

Carneiro ABA, Pinto EJS, Ribeiro IF, Magalhães MRG and Monteiro Neto MAB contributed to the study design, analysis and data interpretation, relevant critical review of the intellectual content, and final approval of the version to be published.

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