Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: V. Ventura de Souza, M. Sossai Spadeto, R. Abreu Guedes, W.R. Clarindo, C.R. de Carvalho, J.A. Severi, T. da Silva Souza (2020) Toxicity of *Aristolochia* decoction: a relevant herbal in folk medicine. *Caryologia* 73(3): 111-120. doi: 10.13128/caryologia-880

Received: March 13, 2020

Accepted: July 14, 2020

Published: December 31, 2020

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Toxicity of *Aristolochia* decoction: a relevant herbal in folk medicine

Victor Ventura de Souza[1], Micheli Sossai Spadeto[2], Roselena Abreu Guedes[4], Wellington Ronildo Clarindo[1,2], Carlos Roberto de Carvalho[3], Juliana Aparecida Severi[4], Tatiana da Silva Souza[1,2,*]

[1] *Departamento de Biologia, Centro de Ciências Exatas, Naturais e da Saúde, Universidade Federal do Espírito Santo, Alegre – ES, Brasil*
[2] *Programa de Pós-Graduação em Genética e Melhoramento, Centro de Ciências Agrárias e Engenharias, Universidade Federal do Espírito Santo, Alegre – ES, Brasil*
[3] *Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa – MG, Brasil*
[4] *Departamento de Farmácia, Centro de Ciências Exatas, Naturais e da Saúde, Universidade Federal do Espírito Santo, Alegre – ES, Brasil*
*Corresponding author. E-mail: tatianas.souza@hotmail.com

Abstract. Ethnopharmacology studies report the use of *Aristolochia* (Aristolochiaceae) species as medicinal plants in various parts of the world. However, the acids aristolochic (AAs), secondary metabolites present in all species of *Aristolochia*, have cytogenotoxic activity and they are a potent carcinogen to rodents and humans. The aim of the current research was to perform to initial screening for the toxicity of *Aristolochia labiata* and *Aristolochia triangularis* decoctions through germination and growth rate, flow cytometry, mitotic index and cytogenetics analysis in *Allium cepa*. The decoctions were prepared from 2, 4, 8, 16 and 32 g L^{-1}. Decoctions at concentrations 4 g to 16 g L^{-1} significantly reduced the germination rate of *Allium cepa*. Seeds exposed to 32 g L^{-1} decoctions did not germinate. All decoctions reduced the growth rate of onion seedlings. Decoctions at 4 g L^{-1} to 16 g L^{-1} inhibited mitotic index. Highest concentrations of decoctions (8 g L^{-1} and 16 g L^{-1} for *Aristolochia labiata*; 16 g L^{-1} for *Aristolochia triangularis*) showed statistically significant increase in frequency of *Allium cepa* nuclei in the G_0/G_1 phase. Both decoctions induced the formation of heteropycnotic nuclei. Qualitative phytochemical prospecting of decocts were performed and alkaloids secondary compounds were the largest presence in both species, indicating that the AAs may be related to the observed toxicity. Caution is recommended in the consumption of decoctions from *Aristolochia labiata* and *Aristolochia triangularis* stems.

Keywords: Aristolochic acid, cytogenotoxicity, flow cytometry, heteropycnotic nuclei, *Allium cepa* test.

INTRODUCTION

Large part of the population of developing countries, especially traditional communities, depends on herbal medicine for primary health care

(WHO, 1978). The use of medicinal plants has been stimulated by Brazilian government aiming at the sustainable use of Brazilian biodiversity and the improvement of the public health system (Brasil, 2006).

Ethnopharmacology studies report the traditional knowledge on the use of *Aristolochia* (Aristolochiaceae) species as medicinal plants in various parts of the world (Heinrich *et al.,* 2009; Michl *et al.,* 2013). In Brazil, Albuquerque *et al.* (2007) verified that *Aristolochia labiata* (flowers, leaves and whole plant) is used by traditional communities in the caatinga region to relieve or cure menstrual colic and uterine inflammations. Decoction, infusion and maceration of leaves and stems of *Aristolochia triangularis* Cham. have traditionally been used to treat gynecology and urinary, gastro-intestinal, respiratory and musculoskeletal and joint diseases (Araujo & Lemos, 2015; Bolson *et al.,* 2015). The ethnobotanical survey showed that the *Aristolochia triangularis* is used as tea for the cure of diseases and/or culturally defined symptoms. Also, the species is used for the cure of spiritual diseases in the form of baths (Silva, 2008). Araujo and Lemos (2015) and Bolson *et al.* (2015) document that *Aristolochia triangularis* had the highest use values among the species cited by residents of traditional communities in the Northeast and South of Brazil, respectively.

Some medicinal plant compounds have their toxicological properties well documented in the literature. Despite the therapeutic effects of *Aristolochia* species, these plants present the Aristolochic acids (AAs) which are nitrophenanthrenes carboxylic acids. Aristolochic acids I (AAI) and II (AAII) present known cyto- and genotoxic activity and they are a potent carcinogen to rodents and humans (Chang *et al.,* 2007; Slade *et al.,* 2009; Hwang *et al.,* 2012; Bunel *et al.,* 2016; Youl *et al.,* 2020), causing a specific nephropathy associated with renal cancer (Li *et al.,* 2018; Sborchia *et al.,* 2019). As a consequence of their toxic effects, the consumption *of Aristolochia* species and its derivatives is prohibited in many countries such as Australia, Canada and United Kingdom (IARC, 2002; Neinhuis *et al.,* 2005). However, in Brazil its consumption is not regulated, being common to find dry parts of the plant in popular markets (Silva, 2008), pharmacies and stores of natural products, ready to be prepared. AAs toxicity becomes clinically significant after long periods of ingestion (Yamani *et al.,* 2015). Nevertheless, the knowledge concerning about toxic effect of decoctions of *Aristolochia* species is very scarce (Amat *et al.,* 2002).

Allium cepa test is commonly used to evaluate the toxic potential of medicinal plants and their metabolites (Akinboro & Bakare, 2007; Oloyede *et al.,* 2009). The germination rate of the seeds and the final growth of the seedlings are used to evaluate the phytotoxicity of the several compounds (Macedo *et al.,* 2008). Besides, *Allium cepa* test has also been accomplished to detect chromosomal abnormalities generated during the cell cycle (Vicentini *et al.,* 2001). This analysis is possible because *Allium cepa* has few chromosomes (2n = 16) with relatively large total length (Grant 1982). Still, *Allium cepa* test shows a similar sensitivity to other systems, like human lymphocytes (Fiskesjö 1985), and a correlation of 82% to carcinogenicity tests in rodents (Rank & Nielsen, 1994).

Flow cytometry (FCM) has broadly contributed to improve knowledge on the plants and animals' cell cycle and has been employed in the biomedical field, pharmacology, oncology and ploidy level determination (Jayat & Ratinaud, 1993). Currently, FCM applied in plants represent a very powerful tool to detect the cytotoxicity and DNA damage caused by drugs and environmental contaminants (Citterio *et al.,* 2002; Monteiro *et al.,* 2010; Andrade-Vieira *et al.,* 2012).

Considering the wide use of *Aristolochia labiata* and *Aristolochia triangularis* as medicinal plants, this study was conducted to analyze the toxicogenetic effects of their decoctions in root meristematic cells of *Allium cepa*. From the preliminary results obtained in this study, renal and testicular histopathology on mice is ongoing. These data are expected to help elucidate the effects of *Aristolochia* decoctions.

MATERIALS AND METHODS

Plant materials

Aristolochia labiata was collected in the forest garden of the Universidade Federal de São João Del-Rei (UFSJ), located in the city of São João del Rei, Minas Gerais State, Brazil. *Aristolochia triangularis* was collected in the Health Ministry located in the city of Venda Nova do Imigrante, Espírito Santo State, Brazil. A voucher specimen of the plants has been deposited at the herbarium of UFSJ, under reference number UFSJ132 (*Aristolochia labiata*) and UFSJ5571 (*Aristolochia triangularis*). Plant names were checked and updated with the Royal Botanic Gardens, Kew online website www.theplantlist.org.

Seeds of *Allium cepa* (Isla®; batch number: 774758; 90% of germination) were used as a test organism in current research because they are genetically and physiologically homogeneous (Leme *et al.,* 2008).

Preparation of decoctions

Stems of *Aristolochia labiata* and *Aristolochia triangularis* were boiled for 5 min in 1 L of distilled water (dH_2O). Considering that 8 g L^{-1} is the dosage commonly used in popular medicine (Simões *et al.,* 1995), 2, 4, 8, 16 and 32 g L^{-1} concentrations were prepared.

Qualitative phytochemical prospecting of both decoctions was carried out from the methodology proposed by Matos (1997). For this, the presence of the following metabolites was evaluated: phenols and flavonoids (sulfuric acid test), tannins (iron chloride III), saponins (foam index), coumarin (NaOH solution), alkaloids (Mayer and Wagner reagent), anthraquinone (ethyl ether + ammonia) and terpenoids (Liebermann–Burchard test).

Seed germination and seedling growth rates

To the test hypothesis that *Aristolochia* decoctions are phytotoxic (affects seed germination and seedling growth) to *Allium cepa*, onion seeds were exposed for 28 days to aqueous extracts. Culture medium MS (Murashige & Skoog, 1962) were supplemented with: 30 g of sucrose of L^{-1}, 0.4 g of L-glutamine, 0.04 g of L-cysteine, 7 g of $agar^{-1}$, pH = 5.7 ± 0.1. Culture media were autoclaved for 20 min at 121°C under 1 atm. Immediately after autoclaving, the decoction extracts were filter-sterilized and added to the tissue culture media. Colchicine at 0.025% was used as positive control (PC) and MS medium without addition of decocts as negative control (NC). In laminar flow, *Allium cepa* seeds were disinfected in 70% ethanol solution for 30 s and then with 1.5% $NaOCl_2$ solution for 20 min, and washed three times in autoclaved dH_2O for 1 min (Mursarurwa *et al.,* 2010). Fifteen seeds were inoculated in eight flasks containing 15 mL of the culture medium.

The inoculated seeds were maintained in a growth room with photoperiod 16/8 hours in light under 24 ± 1ºC. Seed germination (percentage of germinated seeds in each treatment after initial exposure) was recorded on the 28th day. In this time, the final length of the seedlings was measured for each treatment using a caliper rule.

Mitotic and chromosomal abnormalities

Onion seeds were germinated in Petri dishes lined with moistened filter paper with the different concentrations of decoctions. In Addition, dH_2O was used as negative control (NC) and the 0.025% colchicine as positive control (PC). Germination occurred in B.O.D. at 24°C for 72 h. *Allium cepa* roots with 1–2 cm were excised, fixed in ethanol: glacial acetic acid (3:1, v/v) and stored at -20°C. After 24h, the roots were hydrolyzed in 1N HCl at 25°C for 20 min, and subsequently the root meristems were stained with 2% acetic orcein, covered with foil and crushing the material. The slides were examined under a light microscope with 100x objective. Ten slides were prepared for each treatment being analyzed 500 cells/slide. Cytotoxic potential of decoctions was evaluated by mitotic index (number of dividing cells/total observed cells). Genotoxic effect was measured by the chromosomal abnormalities index (CA). Mutagenicity analysis was performed by micronuclei (MN) frequency.

Flow cytometry

The meristems of *Allium cepa* root after germination for 72 hours in a Petri dish were fixed in ethanol: glacial acetic acid (3: 1, v / v) were stored for 24 hours at -20 ° C, transferred to 70% ethanol and stored at -20°C. FCM analyzes were performed with 5 replicates for each decoction concentration and negative control (dH_2O), being three root meristems for each replicate. Root apical meristems were excised, washed 3 times for 10 min in dH_2O. Nuclei isolation and staining were performed according to the protocol proposed by Silva *et al.* (2010). Nuclei suspensions were analyzed by flow cytometry PAS® Partec flow cytometer (Partec® GmbH, Munster, Germany) after calibration of the equipment. The frequency of nuclei in G_0/G_1, S and G_2/M phases was measured to verify the cytotoxicity of the decoctions in each specific cell cycle period.

Statistical analyses

Statistical analyses were performed using the Bioestat 5.3 software. The Shapiro-Wilk test was used to verify the normality of the samples. As normality criteria were not satisfied, the non-parametric Kruskal-Wallis test with subsequent Dunn test ($p<0.05$) was performed.

RESULTS

Qualitative phytochemical analysis of decoctions is shown in Table 1. Decoctions presented phenols (detected by sulfuric acid) and alkaloids (detected by Mayer and Wagner reagents) as secondary metabolites. Since the alkaloid secondary compounds were the largest presence in both species, other metabolites were not detected.

Table 1. Qualitative phytochemical analysis of *Aristolochia labiata* and *Aristolochia triangularis*

Reagents	Metabolite class	*Aristolochia labiata*	*Aristolochia triangularis*
Sulfuric acid	Phenols	+	+
Sulfuric acid	Flavonoides	-	-
Iron chloride III	Tannins	-	-
Foam index	Saponins	-	-
NaOH solution	Coumarin	-	-
Mayer reagent	Alkaloids	+++	+++
Wagner reagent	Alkaloids	+	+
Ethyl ether + ammonia	Anthraquinone	-	-
Lieberman Burchard	Terpernóides	-	-

(-) absence; (+) presense

Table 2. Effects of decoctions of to *Aristolochia labiata* and *Aristolochia triangularis* on seed germination and seedling growth of *Allium cepa*

Species	Samples	Germination (%)	Seedling growth (cm)
Aristolochia labiata	MS	87.5	8.48 ±4.21
	Colchicine 0.025%	75.0	0.93± 0.25*
	2 g l^{-1}	87.5	1.46 ±0.92*
	4 g l^{-1}	62.5*	1.44± 0.37*
	8 g l^{-1}	62.5*	0.88±0.32*
	16 g l^{-1}	50.0*	0.67± 0.22*
	32 g l^{-1}	NG	NG
Aristolochia triangularis	MS	87.5	8.48 ±4.21
	Colchicine 0.025%	75.0	0.93± 0.25*
	2 g l^{-1}	75.0	0.53±0.19*
	4 g l^{-1}	50.0*	0.82 ±0.14*
	8 g l^{-1}	50.0*	0.45±0.50*
	16 g l^{-1}	50.0*	0.40±0.43*
	32 g l^{-1}	NG	NG

MS: negative control -culture medium (Murashige and Skoog, 1962). Colchicine 0.025%: positive control. NG: did not germinate. * Significant difference in relation to negative control ($p < 0.05$) - Kruskal-Wallis test.

Table 2 shows the effects of the decoctions of *Aristolochia labiata* and *Aristolochia triangularis* on seed germination and seedlings of *Allium cepa*. Seeds of the control group germinated satisfactorily. Decoctions of both species at concentrations 4, 8 and 16 g L^{-1} significantly reduced the germination rate of *Allium cepa*. Seeds exposed to 32 g L^{-1} decoctions did not germinate. All decoctions concentrations reduced the onion seedlings growth rate (Table 2, Figure 1A, 1B).

Cell proliferation rate was 13.34% in the control group (Table 3). Decoctions at 2.0 g L^{-1} did not promoted significant reduction of mitotic index. Decoctions at 4, 8 and 16 g L^{-1} inhibited cell proliferation in *Allium cepa* (Table 3). Changes in *Allium cepa* cell cycle

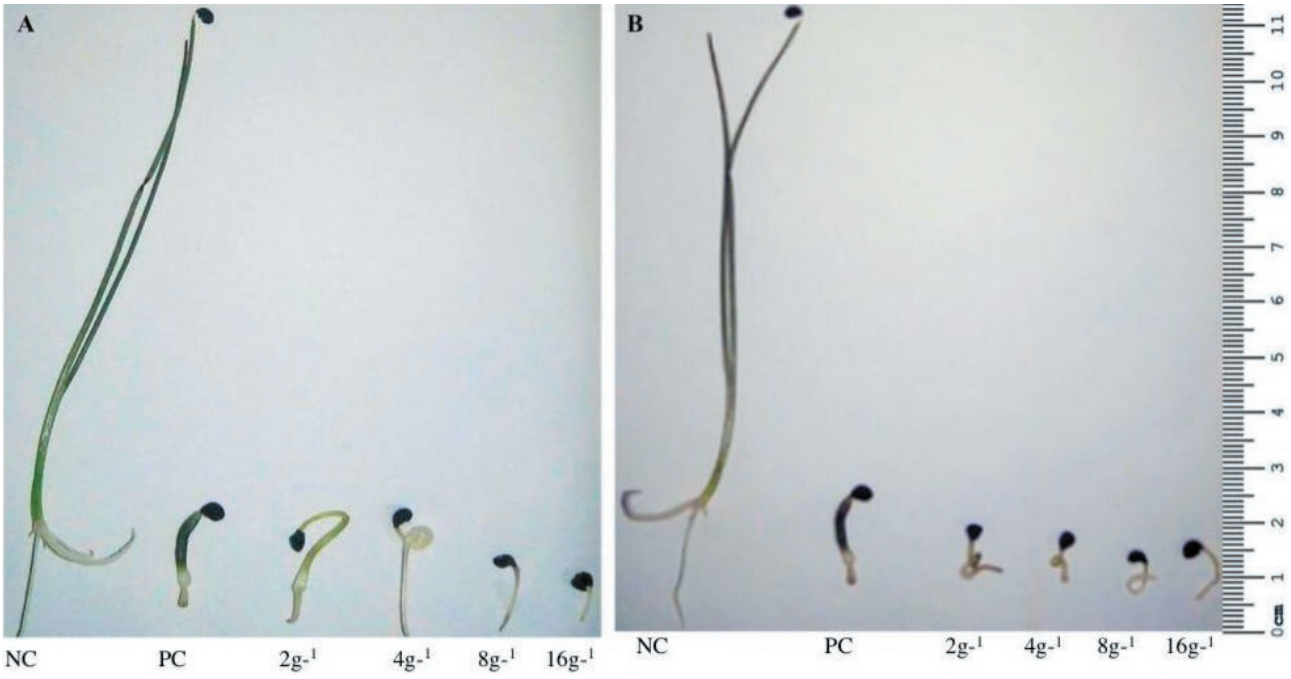

Figure 1. Seedlings of *Allium cepa* after 28 days of exposure to the decoctions of (A) *Aristolochia labiata* and (B) *Aristolochia triangularis*. NC = negative control - culture medium (Murashige and Skoog, 1962); PC = positive control (0.025% colchicine).

Table 3. Mitotic index (% percentage ± standard deviation) of *Allium cepa* root meristem cells exposed to decoctions of *Aristolochia labiata* and *Aristolochia triangularis*

Species	Samples	Mitotic index %	Number of cells in each cell cycle phase				
			Interphase	Prophase	Metaphase	Anaphase	Telophase
Aristolochia labiata	Distilled water	13.34 ± 4.72	4333	335	126	137	69
	Colchicine 0.025%	10.52 ± 3.61	4286	343	149	150	72
	2 g l^{-1}	6.92 ± 3.56	4691	75	144	81	9
	4 g l^{-1}	1.02 ± 0.86*	4949*	9*	21*	15*	6*
	8 g l^{-1}	1.10 ± 2.30*	4941*	11*	29*	13*	6*
	16 g l^{-1}	0.76 ± 0.68*	4960*	12*	6*	15*	7*
	32 16 g l^{-1}	NG	NG	NG	NG	NG	NG
Aristolochia triangularis	Distilled water	13.34 ± 4.72	4319	339	128	142	72
	Colchicine 0.025%	10.52 ± 3.61	4286	343	149	150	72
	2 g l^{-1}	8.38 ± 3.18	4951*	15	14	14	06
	4 g l^{-1}	2.20 ± 3.94*	4888*	51*	24*	24*	13*
	8 g l^{-1}	3.34 ± 3.69*	4803*	69*	73*	53*	02*
	16 g l^{-1}	3.84 ± 2.14*	4961*	16*	06*	11*	06*
	32 16 g l^{-1}	NG	NG	NG	NG	NG	NG

Distilled water: negative control. Colchicine 0.025%: positive control. NG: did not germinate.* Significant difference in relation to negative control ($p < 0.05$) - Kruskal-Wallis test.

are also shown in Table 4 and representative histograms are shown in Figure 2. According to FCM analyses, decoctions of both species promoted a concentration-dependent increase in frequency of *Allium cepa* nuclei in the G_0/G_1 phase. This increase was statistically significant for the highest concentrations of decoction (8 and 16 g L^{-1} for *Aristolochia labiata*; 16 g L^{-1} for *Aristolochia triangularis*). Thus, the frequency of nuclei in the S and G_2/M phase tended to decreased, parking cells in interphase. *Aristolochia labiata* decoctions at 8 and 16 g L^{-1} caused a greater number of cell damage; the highest dose

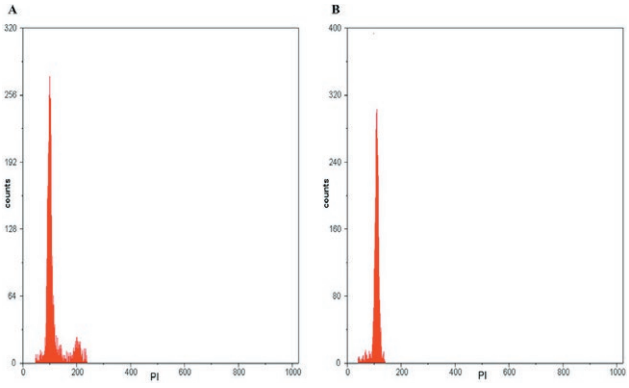

Figure 2. Histogram representative of the meristem of *Allium cepa*. A) Histogram representing the negative control. Note the presence of the G_0/G_1 peak (channel 100) G_2/M (channel 200), and between the particles at different times is S phase of the cell cycle, showing that the negative control has particles at all stage of G_0/M. B) Histogram representing the treatment of 16 g l^{-1} of *Aristolochia labiata*, observe particles in the peak (channel 100) demonstrating that the cells are in G_0/G_1, but there is no peak at (channel 200), which confirms the no progression of nuclei for the G_2/M phase, indicating that the extracts prevent cell proliferation.

Table 4. Frequency (%) of *Allium cepa* nuclei in cell cycle phases after treatment with *Aristolochia labiata* and *Aristolochia triangularis* decocts

Species	Samples	$\%G_0/G_1$	%S	$\%G_2/M$
Aristolochia labiata	Distilled water	70.08 ± 6.91	18.37 ± 4.03	11.00 ± 3.62
	2 g l^{-1}	88.96 ± 1.90	8.08 ± 1.36	3.00 ± 0.93
	4 g l^{-1}	91.34 ± 2.93	6.46 ± 2.63	2.00 ± 1.07
	8 g l^{-1}	97.16 ± 2.91*	2.57 ± 2.81*	0.27 ± 0.60*
	16 g l^{-1}	99.85 ± 0.32*	0.14 ± 0.32*	0.00 ± 0.00*
	32 g l^{-1}	NG	NG	NG
Aristolochia triangularis	Distilled water	74.08 ± 4.52	11.02 ± 4.17	14.89 ± 1.93
	2 g l^{-1}	84.72 ± 2.83	8.82 ± 1.86	6.46 ± 4.54
	4 g l^{-1}	86.23 ± 3.61	8.12 ± 1.47	5.63 ± 3.24
	8 g l^{-1}	87.18 ± 4.03	7.22 ± 2.84	5.59 ± 1.48
	16 g l^{-1}	92.28 ± 2.09*	4.34 ± 1.17*	3.38 ±1.24*
	32 g l^{-1}	NG	NG	NG

Distilled water: negative control. NG: did not germinate. * Significant difference in relation to negative control ($p < 0.05$) - Kruskal-Wallis test.

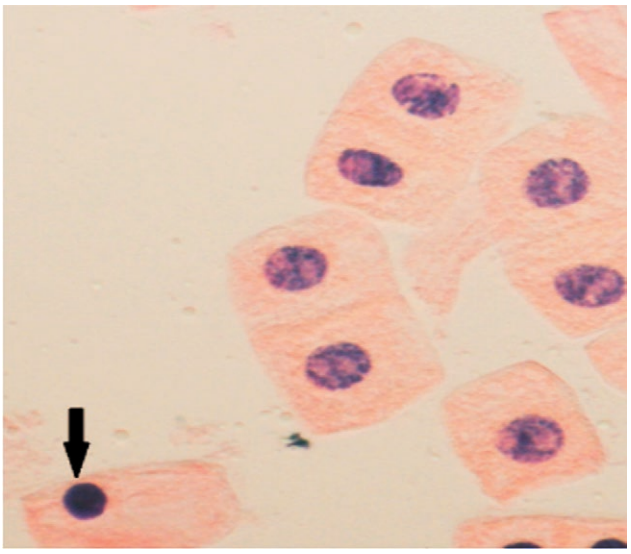

Figure 3. Heteropicnotic nucleus (arrow) in *Allium cepa* meristematic root cells after exposure to the decoctions of *Aristolochia labiata*. 1000x magnification.

Table 5. Percentage of chromosomal alterations (CA) and heteropycnotic nucleus observed in *Allium cepa* cells exposed to decoction of *Aristolochia labiata* and *Aristolochia triangularis*

Species	Samples	%CA	%Heteropycnotic nucleus
Aristolochia labiata	Distilled water	0.04 ± 0.12	0.00 ± 0.00
	Colchicine 0.025%	5.88 ± 2.81*	0.00 ± 0.00
	2 g l^{-1}	0.12 ± 0.16	0.00 ± 0.00
	4 g l^{-1}	0.00 ± 0.00	8.84 ± 1.53*
	8 g l^{-1}	0.00 ± 0.00	15.8 ± 13.03*
	16 g l^{-1}	0.00 ± 0.00	29.60 ± 29.02*
	32 g l^{-1}	NG	NG
Aristolochia triangularis	Distilled water	0.04 ± 0.12	0.00 ± 0.00
	Colchicine 0.025%	5.88 ± 2.81*	0.00 ± 0.00
	2 g l^{-1}	0.00 ± 0.00	13.4 ± 7.95*
	4 g l^{-1}	0.00 ± 0.00	17.2 ± 19.57*
	8 g l^{-1}	0.00 ± 0.00	17.1 ± 13.02*
	16 g l^{-1}	0.00 ± 0.00	19.12 ± 22.2*
	32 g l^{-1}	NG	NG

Distilled water: negative control. Colchicine 0.025%: positive control. NG: did not germinate.
* Significant difference in relation to negative control ($p < 0.05$) - Kruskal-Wallis test.

reached a level of absence of core peak in G_2/M phase. *Aristolochia triangularis* decoction at 16 g L^{-1} significantly reduced the number of nuclei in S and G_2/M phase.

Due to severe cytotoxicity, chromosomal abnormalities and micronuclei were not observed. Heteropycnotic nuclei (Figure 3) were analyzed separately. Decoctions of both species at 4, 8 and 16 g L^{-1} significantly increased the frequency of this abnormality. *Aristolochia triangularis* decoction at 2 g L^{-1} also induced heteropycnotic nuclei on the *Allium cepa* root cells (Table 5).

DISCUSSION

The genus *Aristolochia* presents a wide range of physiologically active compounds classified in five main categories: terpenes, phenols, alkaloids, flavonoids and lignoids (Pacheco *et al.,* 2009). Phytochemical analyses indicated mainly the presence of alkaloids in both decoctions studied and in a lower extent, phenols. Phenols bring advantages to the plant, as they are related to attraction of pollinators and protection against herbivory among others (Piesik *et al.,* 2011). Species of Aristolochiaceae are rich in alkaloids (Schmeiser *et al.,* 2001), including AAs that have attracted intense research interest because of cyto- and genotoxic properties of AAI and AAII (Wu *et al.,* 2005; Chang *et al.,* 2007; Slade *et al.,* 2009; Bastek *et al.,* 2019).

The amounts of AAI and AAII in decoctions were not determined. However, as the AAs are slightly soluble in water (O'Neil, 2001), we believe that possibly they are present in decoctions, which is the way by which people consume species of *Aristolochia*. Hwang *et al.* (2012) quantified AAs in aqueous extracts of *Aristolochia manshuriensis* Kom. The genotoxicity of the extracts was detected by bacterial reverse mutation assay and micronucleus in mice bone marrow erythrocytes. According to the authors, the genotoxicity of *Aristolochia manshuriensis* is directly related to the AAs.

Allium cepa test has been used as first cytogenotoxic screening of medicinal plant extracts (Dias & Takahashi, 1994; Fachinetto *et al.,* 2007; Meneguetti *et al.,* 2014; Mendes *et al.,* 2012) because the results are reliable and similar to those performed with in mammals (Rank & Nielsen, 1994), contributing to the safe use of these herbs (Mendes *et al.,* 2012).

Studies concerning toxicological activity of *Aristolochia* extracts are very scarce, in spite of their use in several countries (Amat *et al.,* 2002). In the current research, the toxic effects of decoctions of *Aristolochia labiata* and *Aristolochia triangularis* on *Allium cepa* were evaluated. Decoctions of both species at 32 g L^{-1} were phytotoxic because prevented the germination of onion seeds. The other concentrations tested promoted delayed germination and growth and mitodepressive

effects on *Allium cepa* root cells. Akinboro and Bakare (2007) also documented a relationship between macroscopic and microscopic parameters for *Allium cepa* root cells exposed to toxic aqueous extracts herbs. The growth inhibition of the seedlings was always accompanied by the reduction of the number of cells in division. Corroborating our results, Gatti *et al.* (2004) showed that extracts of *Aristolochia esperanzae* Kuntze delayed seed germination and root growth of *Lactuca sativa* L. and *Raphanus sativus* L. According to Baličević *et al.* (2015), extracts of *Aristolochia clematitis* reduced the germination and growth of *Tripleurospermum inodorum* L. (weeds), and the concentration of 100 g L^{-1} inhibited the germination of seeds of these plants. Aqueous extract at 25 g L^{-1} of *Aristolochia triangularis* presented antimitotic action to meristematic cells of *Allium cepa* (Amat *et al.,* 2002). Watanabe *et al.* (1988) documented that AAs are potent inhibitors of seed germination.

FCM analyses also showed a presence of compounds in the decoctions that delayed the progression of the cell cycle. The decoctions tested caused a concentration-dependent increase of *Allium cepa* nuclei in G_0/G_1. Consequently, *Aristolochia* decoctions promoted a reduction of *Allium cepa* nuclei in S and G_2/M phases. These results could reflect the activation of G_0/G_1 checkpoints in response to DNA damage. Plant cells have a p53-independent control of proliferation (Pelayo *et al.,* 2003). The checkpoint pathways transduce antimitogenic signals that lead to the temporary interruption of the cycle. Thus, the repair mechanisms can act before the irreversible transition to the subsequent cycle phase (Pelayo *et al.,* 2003; Junqueira & Carneiro, 2012). Also, heteropycnotic nuclei were observed in response to decoctions exposure. These markers of cell death are characterized by condensation of the nucleus (Andrade-Vieira, *et al.,* 2012), making it inoperative for failure of the enzyme synthesis (Manjo & Joris, 1995; Levin *et al.,* 1999). The activation of cell death mechanisms is the last resource to avoid proliferation of cells containing abnormal DNA. In this way, we could infer that DNA damage was not repaired in G_1, and in response, cell death pathways were activated.

Some studies also documented that *Aristolochia* extracts and AAs promoted cell cycle arrest and cell death in mammalian cell lines. Li *et al.* (2006) reported that AAI may cause DNA damage and cell cycle delay in porcine proximal tubular epithelial cell lines through a wild-type p53-independent pathway, prior to apoptosis or necrosis. Chang *et al.* (2007) found that cell cycle distribution determined by flow cytometry showed an increase of human urinary tract epithelium cells in the G0/G1 phase after exposure to AAs mixture (41% AA I and 56% AA II). Proteins levels that block the cell cycle (p53, p21 and p27) have increased. Additionally, there was a decrease in cyclinD1/cdk4 complex, which control proteins required for the progression of the cycle (Chang *et al.,* 2007). Li and Wang (2013) verified that methanol extract from *Aristolochia debilis* Siebold & Zucc. stems inhibited proliferation of human colon cancer cells by inducing sub-G_1 arrest. The authors also showed that *Aristolochia debilis* induced apoptosis in HT-29 cells by upregulation of Bax and corresponding downregulation of Bcl-2 expression as well as ROS production.

Allium cepa test was suitable for screening initial toxicity of *Aristolochia labiata* and *Aristolochia triangularis* decoctions. Our studies report similar results for other test systems. In vivo research on renal and testicular histopathology of decoctions in mice is ongoing.

CONCLUSION

Allium cepa test was used to evaluate decoctions from *Aristolochia labiata* and *Aristolochia triangularis* stems. Phytochemical analysis indicated mainly the presence of alkaloids in both decoctions studied. The decoctions promoted inhibition of onion seed germination and seedling growth. The mitodepressive effect of both decoctions was determined by mitotic index and FCM analyses. The induction of heteropycnotic nuclei suggests that decoctions promote cell death. We suggest that the AAs may be related to the observed toxicity. Caution is recommended in the consumption of decoctions from *Aristolochia labiata* and *Aristolochia triangularis* stems.

ACKNOWLEDGEMENTS

The authors thank Fundação de Apoio à Pesquisa e Inovação do Espírito Santo for undergraduate scholarship. We also thank Damielle Leite Figueiredo for technical support and Ariane Tonetto Vieira for editing images.

REFERENCES

Akinboro A, Bakare AA. 2007. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. Journal of Ethnopharmacology 112, 470–475.

Albuquerque UP, Medeiros PM, Almeida ALS, Monteiro JM, Neto EMFL, Melo JG, Santos JP. 2007. Medicinal

plants of the *caatinga* (semi-arid) vegetation of NE Brazil: a quantitative approach. Journal of Ethnopharmacology 114, 325–354.

Amat GA, Yajia EM, Gonzalez CF, Lorca GL, Sanchez-Gonzalez F, Riglo AG, Veron JR. 2002. Evaluation of cytological parameters induced by aqueous extracts of seven plants used as antihypertensive agents in argentine folk medicine. Acta. Farmacéutica Bonaerense 21, 37-42.

Andrade-Vieira LF, de Campos JMS, Davide LC. 2012. Effects of Spent Pot Liner on mitotic activity and nuclear DNA content in meristematic cells of *Allium cepa*. Journal of Environmental Management 107, 140-146.

Araujo JL, Lemos JR. 2015. Estudo etnobotânico sobre plantas medicinais na comunidade de Curral Velho, Luís Correia, Piauí, Brasil. Biotemas 28, 125-136.

Baličević R, Ravlić M, Mišić M, Mikić, I. 2015. Allelopathic effect of *Aristolochia clematitis* L. Proceedings. 50th Croatian and 10th International Symposium on Agriculture. Opatija, Croatia, 54–58.

Bolson M, Hefler SR, Chaves EID, Gasparotto Junior AG, Cardozo Junior, EL. 2015. Ethno-medicinal study of plants used for treatment of human ailments, with residents of the surrounding region of forest fragments of Paraná, Brazil. Journal of Ethnopharmacology 161, 1–10.

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, Schiff S, Ślusarczyk J, de Souza CP, Srivastava A, Sutan A, Papini A (2018). An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. Caryologia, *71*(3), 191-209.

Brasil. Política Nacional de Plantas Medicinais e Fitoterápicos, 2006. <http://portalsaude.saude.gov.br> (accessed May, 2019).

Bunel V, Antoine MH, Stévigny C, Nortier J, Duez P. 2016. New *in vitro* insights on a cell death pathway induced by magnolol and honokiol in aristolochic acid tubulotoxicity. Food and Chemical Toxicology 87, 77-87.

Chang HR, Lian JD, Lo CW, Huang HP, Wang CJ. 2007. Aristolochic acid-induced cell cycle G1 arrest in human urothelium SV-HUC-1 cells. Food and Chemical Toxicology 45, 396-402.

Citterio S, Aina R, Labra M, Ghiani A, Fumagalli P, Sgorbati S, Santagostino A. 2002. Soil genotoxicity assessment: a new strategy based on biomolecular tools and plant bioindicators. Environmental Science & Technology 36, 2748-2753.

Dias FL, Takahashi CS 1994. Cytogenetic evaluation of the effect of aqueous extracts of the medicinal plants *Alpinia nutans Rose (*Zingiberaceae) and *Pogostemun heyneanus Benth (*Labiatae) on wistar rats and *Allium cepa* Linn (Liliaceae) root tip cells. Revista Brasileira de Genética 17, 175-180.

Fachinetto JM, Bagatini MD, Durigon J, Silva ACF, Tedesco SB. 2007. Efeito anti-proliferativo das infusões de *Achyrocline satureioides* DC (*Asteraceae*) sobre o ciclo celular de *Allium cepa*. Revista Brasileira de Farmacognosia 17, 49-54.

Fiskesjö G. 1985. The *Allium* test as a standard in the environmental monitoring. Hereditas 102, 99-112.

Gatti AB, Perez SCJGA, Lima MIS, 2004. Atividade alelopática de extratos aquosos de *Aristolochia esperanzae* O. Kuntze na germinação e no crescimento de *Lactuca sativa* L. e *Raphanus sativus* L. Acta Botanica Brasilica 18, 459-472.

Grant WF. 1982. Chromosome aberration assays in *Allium cepa.* Mutation Research 99, 273-291.

Heinrich M, Chana J, Wanke S, Neinhuis C, Simmonds MSJ. 2009. Local uses of *Aristolochia* species and content of nephrotoxic aristolochic acid 1 and 2—A global assessment based on bibliographic sources Journal of Ethnopharmacology 125, 108–144

Hwang YH, Kim T, Cho WK, Yang HJ, Kwak DH, Ha H, Song KH, Ma JY. 2012. *In Vitro* and *In Vivo* Genotoxicity Assessment of *Aristolochia manshuriensis* Kom. Evidence-Based Complementary and Alternative Medicine 2012, 1-9.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans: Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monogr

Eval Carcinog Risks Hum 82:1-556, 2002.

Jayat C, Ratinaud MH. 1993. Cell cycle analysis by flow cytometry: principles and applications. Biolology of the Cell 78, 15-25.

Junqueira L.C.; Carneiro, J. Biologia celular e Molecular, 2012. 9 Ed. Rio de Janeiro: Guanabara Koogan, 376p.

Leme DM, Angelis DF, Marin-Morales MA. 2008. Action mechanisms of petroleum hydrocarbons present in waters impacted by an oil spill on the genetic material of *Allium cepa* root cells. Aquatic Toxicology 88, 214–219.

Levin S, Bucci TJ, Cohen SM, Fix AS, Hardisty JF, Legrand EK, Maronpot RR, Trump BF. 1999. The nomenclature of cell death: recommendations of an ad hoc Committee of the Society of Toxicologic Pathologists. Toxicologic Pathology 27, 484–90.

Li Y, Liu Z, Guo X, Shu J, Chen Z, Li L. 2006. Aristolochic acid I-induced DNA damage and cell cycle arrest in renal tubular epithelial cells in vitro. Archives of Toxicology 80, 524–532.

Li C, Wang MH. 2013. *Aristolochia debilis* Sieb. et Zucc. induces apoptosis and Reactive Oxygen Species in the HT-29 human colon cancer cell line. Cancer Biotherapy and Radiopharmaceuticals 28, 717-724.

Li W, Chan CK, Wong YL, Chan KJ, Chan HW, Chan W. 2018. Cooking methods employing natural anti-oxidant food additives effectively reduced concentration of nephrotoxic and carcinogenic aristolochic acids in contaminated food grains. Food chemistry, 264, 270-276.

Macedo MFS, Herbert AACN, Queiroz JDF, Argolo ACC, Saturnino ACRD, Coelho LCBB, Medeiros SRB. 2008. Determining the genotoxicity of an aqueous infusion of *Bauhinia monandra* leaves. Revista Brasileira de Farmacognosia 18, 509-516.

Manjo G, Joris I. 1995. Apoptosis, oncosis, and necrosis. An overview of cell death. American Journal of Pathology 146, 3–15.

Matos FJA. 1997. Introdução à fitoquímica experimental, Fortaleza. UFC (Eds), 1, pp. 44-46.

Mendes SS, Andrade JA, Xavier MA, Secundo Junior JA, Pantaleão SM, Estevam CS, Ferrari SF. 2012. Genotoxicity test of *Maytenus rigida* and *Aristolochia birostris* in the radicular meristem of the onion, *Allium cepa.* Revista Brasileira de Farmácia 22, 76-81.

Meneguetti DUO, Lima RA, Silva JB, Silva RP, Pagotto R, Facundo VA. 2014. Análise citotóxica e mutagênica do extrato aquoso de *Maytenus guyanensi*s Klotzsch Ex Reissek (Celastraceae) Chichuá (Xixuá) amazônico. Ciência e Natura 36, 31-39.

Michl J, Jennings HM, Kite GC, Ingrouille MJ, Simmonds MSJ, Heinrich M. 2013. Is aristolochic acid nephropathy a widespread problem in developing countries? A case study of *Aristolochia indica* L. in Bangladesh using an ethnobotanical–phytochemical approach. Journal of Ethnopharmacology 149, 235–244.

Monteiro MS, Rodriguez E, Loureiro J, Mann RM, Soares AMVM, Santos C. 2010. Flow cytometric assessment of Cd genotoxicity in three plants with different metal accumulation and detoxification capacities. Ecotoxicology and Environmental Safety 73, 1231-1237.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15, 473–497.

Neinhuis C, Wanke S, Hilu KW, Müller K, Borsch T. 2005. Phylogeny of Aristolochiaceae based on parsimony, likelihood and Bayesian analyses of trnL-trnF sequences. Plant Systematics and Evolution 250, 7–26.

Oloyede A, Okpuzor J, Omidiji O. 2009. Cytological and toxicological properties of a decoction used for managing tumors in Southwestern Nigeria. Pakistan Journal of Biological Sciences 12, 383-387.

O'Neil MJ. 2001. Solubility: Slightly soluble in water; soluble in acetic acid, acetone, aniline, alkalis, chloroform, diethyl ether and ethanol; practically insoluble in benzene and carbon disulfide *The Merck Index*, 13th Ed., Whitehouse Station, NJ, Merck & Co., pp. 48, 134.

Pacheco AG, Oliveira PM, Piló-Veloso D, Alcântara AFC. 2009. ^{13}C-NMR Data of diterpenes isolated from *Aristolochia* species. Molecules 14, 1245-1262.

Pelayo HR, Pincheira J, Giménez-Abián1 JF, Clarke DJ, De La Torre C. 2003. p53-Independent checkpoint controls in a plant cell model. Biological Research 36, 381-388.

Piesik D, Pánka D, Dalaney KJ, Skoczek A, Lamarski R, Weaver DK. 2011. Cereal crop volatile organic compound induction after 22 mechanical injury, beetle herbivory (*Oulema* spp.), or fungal infection (*Fusarium* spp.). Journal of Plant Physiology 168, 878 – 886.

Rank J, Nielsen MH. 1994. Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. Mutation Research 312, 17-24.

Sborchia M, De Prez EG, Antoine MH, Bienfait L, Indra R, Valbuena G, Arlt VM. 2019. The impact of p53 on aristolochic acid I-induced nephrotoxicity and DNA damage in vivo and in vitro. Archives of toxicology, 93(11), 3345-3366.

Schmeiser HH, Bieler CA, Arlt VM, Stiborova M, Wiessler M. 2001. Ingredients of *Aristolochia* plants are risk factors for cancer. Research Report 1999/2000, DFKZ (German Cancer Research Centre), Heidelberg, p. 135-136.

Silva IM. 2008. A etnobotânica e a medicina popular em mercados na cidade do Rio de Janeiro. Thesis, Escola Nacional de Botânica Tropical, do Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro.

Silva TCR, Abreu IS, Carvalho CR. 2010. Improved and reproducible flow cytometry methodology for nuclei isolation from single root meristem. Journal of Botany 2010, 1-7.

Simões CMO, Mentz LA, Schenkel EP. *Plantas da medicina popular no Rio Grande do Sul*. Porto Alegre: Editora da Universidade,1995, p.173.

Slade N, Mollc UM, Brdar B, Zoric A, Kovi B. 2009. p53 mutations as fingerprints for aristolochic acid – an environmental carcinogen in endemic (Balkan) nephropathy. Mutation Research 663, 1–6.

Vicentini VEP, Camparoto ML, Teixeira RO, Mantovani MS. 2001. *Averrhoa carambola* L., *Syzygiumcumini* (L.) Skeels and *Cissussicyoides* L.: medicinal herbal tea effects on vegetal and test systems. Acta Scientiarum 23, 593-598.

Watanabe K, Miyakado M, Iwai T, Izumi K, Yanagi K. 1988. Isolation of aristolochic acid and aristolic acid from *Cocculus triolobus* DC as potent seed germination inhibitors. Agricultural and Biological Chemistry 52, 1079-1082.

WHO - World Health Organization/UNICEF. Primary health care: report of the International Conference on Primary Health Care, Alma-Ata, URSS, 1978. Geneva, 1978. p. 61.

Wu TS, Damu AG, Su CR, Kuo PC. 2005. Chemical constituents and pharmacology of *Aristolochia* species. In: Attaur-Rahman, editor. Studies in natural product chemistry (bioactive natural proudcts), vol. 32. Amsterdam: Elsevier; pp. 855–1018.

Yamani A, Bunel V, Antoine MH, Husson C, Stévigny C, Duez P, Elachouri M. Nortier, J., 2015. Substitution between *Aristolochia* and *Bryonia* genus in North-Eastern Morocco: Toxicological implications. Journal of Ethnopharmacology 166, 250–260.

Youl EN, Husson C, El Khattabi C, El Mere S, Declèves AE, Pochet S, Antoine MH. 2020. Characterization of cytotoxic effects of aristolochic acids on the vascular endothelium. Toxicology in Vitro, 104811.