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Genotoxicity and cytotoxicity of *Erythrina velutina* Willd., Fabaceae, on the root meristem cells of *Allium cepa*

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Abstract: The effects of the decoction of *Erythrina velutina* Willd., Fabaceae, were investigated using the root meristem cells of *Allium cepa* L., Amaryllidaceae. Ten concentrations of the aqueous extract (0.125 to 1.25%) of this medicinal plant were analyzed at both macroscopic and microscopic levels. All concentrations showed root growth inhibition after 96 h treatment. Although there were no significant differences between the mitotic indexes of any concentration and the control, there were changes in the frequencies of cell stages at three different concentrations. Additionally, the presence of five different cells abnormalities was recorded: chromosome bridging, lagging chromosomes, chromosome fragments, disturbed metaphase and disturbed anaphase. These results suggest inhibitory and genotoxic activity of the decoction of E. velutina on *Allium cepa.*

Introduction

In Brazil, as in many other countries, medicinal plants are widely used in the treatment and prevention of various diseases. Most of these plants are used according to folk traditions developed by natives or brought to the country by Europeans, Africans and Asians. They have been considered an effective treatment for fever, insomnia, wounds, diarrhea, infections, respiratory problems, and many other illnesses (Rates, 2001; Akinboro & Bakare, 2007). However, some medicinal plants can produce compounds that are harmful to the organism. The indiscriminant use of these plants has increased concerns regarding the presence of toxic substances and their provocation of adverse effects (Saad et al., 2006). The study of medicinal plants is important not only for the confirmation of therapeutic uses, but also for the identification of potentially toxic, carcinogenic or teratogenic components (Varanda, 2006; Akinboro & Bakare, 2007).

Of the many medicinal plants found in Brazil, the genus *Erythrina* (Fabaceae) is widely used. *Erythrina* plants are distributed in the tropical and subtropical regions of the World, and most *Erythrina* species are native to the New World (Pillay et al., 2001; Ribeiro et al., 2006). In Brazil, these plants are used in folk medicine due to their tranquilizing effects (Lorenzi & Matos, 2002). Many chemical compounds have been isolated from a variety of *Erythrina* species, demonstrating that this genus is a rich source of biologically active substances. *Erythrina* species are known to produce flavonoids, isoflavonoids and terpenes. They are also known to be an important source of alkaloids, such as erytrine, erysotrine, erysotamidine, ermelanthine, and cocculine (Parsons & Williams, 2000; Pillay et al., 2001; Ganguly et al., 2006; Ribeiro et al., 2006).

The genus includes *Erythrina velutina*, known as “mulungu” in Brazil. This species is endemic to the floodplains of the semi-arid regions of northeastern Brazil, where it is commonly used by the population to treat insomnia and other disorders of the central nervous system (Lorenzi & Matos, 2002; Vancocelos et al., 2003; Dantas et al., 2004). There are many recent studies of *E. velutina*, most of which demonstrate its therapeutic

value for the organism, such as sedative and anxiolytic-like effects (Dantas et al., 2004; Raupp et al., 2008) and anti-nociceptive and anticonvulsant activities (Marchioro et al., 2005; Vasconcelos et al., 2007). However, few reports are available on the toxicological properties of Mulungu, especially its genotoxic effects. Therefore, this study investigated the genotoxicity of decoctions of different concentrations from the leaves of *Erythrina velutina* Willd. on the root meristem cells of *Allium cepa* Linn.

Materials and Methods

Collection of the medicinal plant

Erythrina velutina Willd., Fabaceae, was selected on the basis of its wide use by the population in Northeastern Brazil and its local availability. It was collected on the premises of the Federal University of Sergipe (UFS), Brazil, and was taken to the University Herbarium (ASE) for identification, where it is registered under the number 13026.

Preparation of the decoction

The decoction was prepared by cooking 100 g of dried *E. velutina* leaves in 4 L of distilled water and then leaving them to stand for cooling before straining. From this decoction, ten different concentrations were produced by diluting the initial one containing 2.5% botanical material in distilled water. Regarding this initial value, resulting dilutions contained the extracted compounds of 0.125 to 1.25% of these leaves.

Allium cepa *assay*

Onion bulbs were obtained at a local market and chosen according to their size (approximately 3.5 cm diameter) and appearance. The outer scales and old roots were removed carefully, and the bulbs were washed, dried and kept in a refrigerator at 4 ºC until the start of the experiment. For each concentration, including the negative control (distilled water), five bulbs were used. They were placed in flasks filled with each solution as far as the root growth region, and kept under laboratory conditions.

For root growth inhibition, evaluation of root length was conducted during 96 h. Ten roots were measured per bulb at 12 h intervals using a pair of calipers.

For the evaluation of the induction of chromosomal aberrations, the roots were left to grow to 1.5-2 cm. The root tips were then removed and fixed in ethanol:glacial acetic acid (3:1, v/v). To prepare the slides, the roots were placed in two Petri dishes with distilled water for 5 min, and then hydrolyzed in 1N HCl for 15 min. They were then squashed and placed in the slides with a drop of 45% acetic acid for 5 min. The roots were then stained with 15% acetoorceine for 15 min and cover slips were lowered carefully, to exclude air bubbles. The cover slips were sealed to the slides with clear fingernail polish (Grant, 1982). For each concentration and the control, ten slides were analyzed (1000 cells per slide) in a "blind" test at x1000 magnification. In addition to the evaluation of the induction of chromosomal aberrations, the Mitotic Index (MI) was estimated.

Quantification of alkaloids

The quantification was performed according to the colorimetric method proposed by Teles et al. (1995).The analyses of the reaction mixture with the plant extract in the concentrations of 0.05, 0.1, 0.15, 0.2 and 0.26 mg/mL were done in triplicates at 560 nm in a spectrophotometer. The data were plotted in comparison to the standard curve of pilocarpine at the same concentrations of the extracts.

Statistical analysis

The root length data are given as the mean±S.D. The mitotic index and the frequency of aberrant cells (%) were calculated as the number of dividing cells per 1000 observed and based on the proportion of aberrant cells scored at each concentration, respectively (Akinboro & Bakare, 2007). The data were analyzed by a one-way ANOVA followed by the Tukey post-test. In all cases, a value of $p<0.05$ was considered significant.

The data from the quantification of alkaloids were analyzed by Student's t-test. A value of $p<0.01$ was considered significant.

Results

In addition to the common cytogenetic parameters, such as mitotic index and chromosome abnormalities, root growth was also used as macroscopic parameter. Table 1 shows the results of the 96-h root growth inhibition test. There were no significant differences between treated groups and the control between 12 h and 60 h. At this point, differences started to appear between the 1.25% concentration and the control (at 72 h) and by the end of monitoring, all concentrations, even 0.125%, differed statistically from the control.

Statistical analysis indicated no remarkable changes in mitotic activity (Table 2). However, exposure to the decoction of *E. velutina* caused changes in the frequencies of different cell stages at 0.375, 0.625 and

1.25% concentrations when compared to the control.

Also, the decoction induced many types of aberrant cells in the *Allium cepa* root tips. The mitotic abnormalities included chromosome bridge, lagging chromosomes, chromosome fragments, disturbed metaphase (including stickiness and cc-metaphases) and disturbed anaphase. The percentages and photographs of these abnormalities are presented in Table 2 and Figure 1, respectively. The percentage of aberrant cells caused by the 0.5% and 0.625% concentrations

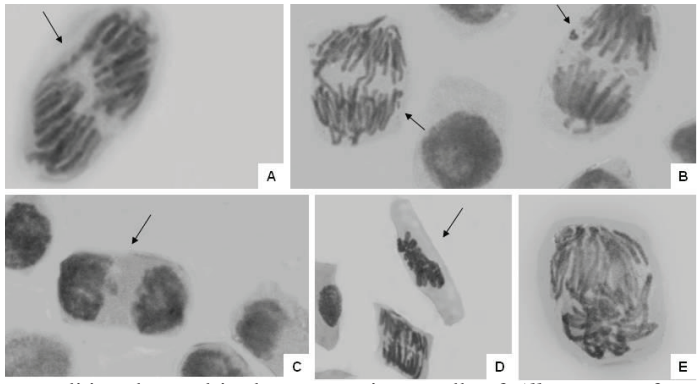

Figure 1. Chromosomes abnormalities observed in the root meristem cells of *Allium cepa* after treatment with the decoction of *Erythrina velutina*. A: Chromosome bridge; B: Chromosome fragment; C: Lagging chromosome; D: Disturbed metaphase: cc-metaphase; E: Disturbed anaphase.

Table 1. Effects of the decoction of *Erythrina velutina* on the root growth of *Allium cepa.*

Con.(%)	Mean root length (±S.D.) at time:							
	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
Control	0.51±0.48	1.01±0.63	1.83±0.49	2.71±1.02	4.18±1.88	6.18±3.06	9.96±5.58	12.72±7.68
0.125	0.14±0.31	1.14±0.67	2.38±0.94	2.85±1.35	3.70±1.53	4.32±1.65	5.48±1.77	6.54±1.99*
0.25	0.38±0.53	1.30±1.00	1.99±1.56	2.44±1.86	2.98±2.17	3.63±2.01	4.28±2.03*	5.24±2.32*
0.375	0.00±0.00	0.67±0.93	1.24±0.79	1.55±1.10	2.16±0.99	2.58±1.05	3.30±1.57*	4.14±1.98*
0.5	0.32±0.45	1.18±0.68	1.65±0.98	1.91±1.15	2.68±0.85	3.10±0.78	3.68±0.95*	4.29±1.10*
0.625	0.16±0.36	0.58±0.79	1.49±0.91	1.91±1.17	2.92±0.97	3.08±1.09	3.68±1.41*	4.30±1.55*
0.75	0.23±0.51	0.95±0.90	1.84±0.60	2.14±0.63	2.50±0.64	2.77±0.59	2.97±0.62*	3.33±0.72*
0.875	0.35±0.48	0.89±0.83	1.57±0.92	1.66±1.01	1.92±1.18	2.10±1.42	2.17±1.31*	3.11±1.34*
1	0.28±0.63	1.00±0.93	1.52±1.39	1.85±1.70	2.31±1.68	2.67±1.94	3.29±1.78*	3.64±1.94*
1.125	0.00±0.00	0.22±0.48	1.51±0.27	1.58±0.24	1.89±0.16	2.25±0.18	2.80±0.35*	3.32±0.53*
1.25	0.00±0.00	0.00±0.00	0.00±0.00	0.46±0.63	0.74±0.69	0.84±0.78*	1.05±0.98*	1.17±1.09*

* Significantly different from the control (p<0.05).

Table 2. Cytological effects of the decoction of *Erythrina velutina* on the cells of *Allium cepa.*

Con. (%)	Total cells examined	Total mitosis	% Prophase	% Metaphase	% Anaphase-Telophase	Mitotic Index (Mean±S.D.)	Types of abnormalities (%)					Abnormalities
							C.B.	L.C.	C.F.	D.M.	D.A.	(%)
Control	10000	2720	94.41	1.44	4.15	27.20±11.6	0.5	-	-	0.33	0.17	0.01
0.125	10000	1600	82.56	3.5	13.94	16.00±5.9	0.09	0.04	0.05	0.65	0.18	0.06
0.25	10000	3140	93.34	1.56	5.1	31.40±15.4	0.22	0.16	0.03	0.35	0.24	0.04
0.375	10000	3439	90.46	2.18*	7.36*	34.39±15.5	0.04	0.02	0.02	0.92*	0.02	0.06
0.5	10000	2301	87.27	3	9.73	23.01±8.4	-	0.02	-	0.90*	0.08	0.10*
0.625	10000	1833	79.65	4.04*	16.31*	18.33±9.5	0.01	0.01	0.01	0.75*	0.17	0.08*
0.75	10000	3362	99.37	0.15	0.48	33.62±20.6	-	0.25	-	0.63	0.13	0.01
0.875	10000	2909	95.15	1.31	3.54	29.09±11.9	0.03	0.13	0.05	0.74	0.05	0.04
1	10000	1936	92.35	1.76	5.89	19.36±20.3	0.03	0.09	-	0.74	0.06	0.04
1.125	10000	2691	93.65	1.3	5.05	26.91±11.3	-	-	-	0.8	0.2	0.03
1.25	10000	2692	83.73	1.78	14.49*	26.92±18.6	0.06	0.02	-	0.74	0.19	0.05

C.B., chromosome bridge, L.C., lagging chromosome, C.F., chromosome fragment, D.M., disturbed metaphase, D.A., disturbed anaphase. *Significantly different from the control (p<0.05).

were significantly different from that of the control. As for the different types of abnormalities, significant differences in disturbed metaphases were found at three different concentrations, with stickiness metaphases the most frequent among the latter abnormality.

The colorimetric method used to evaluate the concentration of alkaloids in the extract of *Erythrina velutina* showed a presence of 0.480% of total alkaloids.

Discussion

Erythrina velutina is a widely used medicinal plant in the Brazilian Northeast. While a number of studies have been conducted on the species, its mutagenic effects have not yet been tested. By using the *Allium cepa* assay, these effects were evaluated in the present study, using both macroscopic (root growth) and microscopic (mitotic index and mitotic aberrant cells) parameters.

On the macroscopic level, there was inhibition of root growth after 60 h when compared to the control, which can usually be associated with a decrease in the mitotic index (Tkalec et al., 2009). However, this effect was not observed in the present study, as there were no remarkable changes in the mitotic indexes. According to Parsons & Williams (2000), the alkaloids present in *Erythrina* have a wide range of biological properties, including the inhibition of protein and DNA synthesis in certain cells. This could lead to a delay or even interruption of the cell cycle, which would cause root growth inhibition and interrupt cell phases in mitosis, impeding the full cycle. This could also result in a relative increase of the number of cells in particular stages at some doses.

The colorimetric method showed that the plant extract has a significant amount of alkaloids, since there were no statistical differences between the extract, which contains many different substances, and the pilocarpine, a pure alkaloid.

At the microscopic level, aberrant mitotic cells and mitotic activity were also analyzed. The exposure to the decoction of *E. velutina* caused changes in the frequencies of different cell stages at 0.375, 0.625 and 1.25% concentrations, and in the frequencies of aberrant cells at 0.5% and 0.625% concentrations when compared to the control. Inside the range of tested concentrations, the ones causing cell aberrations were intermediate. We can postulate that lower concentrations may not present a sufficient amount of alkaloids, or other substances, to cause aberrations in the cell chromosomes. On the other hand, at higher concentrations, the amount of alkaloids in the extract might be high enough to cause activation of some intrinsic protective mechanism of the cell.

Alkaloids are genotoxic but their mechanisms of action are still not so clear. Fu et al. (2002) studied the mechanism of action of pyrrolizidines, which are alkaloids known to cause DNA binding, DNA cross-linking, DNA-protein cross-linking, mutagenicity and carcinogenicity. According to this study, pyrrolizidine alkaloids require metabolic activation in order to exert genotoxicities. Metabolizing enzymes are responsible for the alkaloids metabolism that generates metabolites that leads to formation of DNA adducts. Coulombe et al. (1999) also studied these alkaloids and discovered that a significant proportion of the cross-links induced by PA are protein-associated. Also according to Coulombe et al. (1999), some substances are sufficiently reactive to compete with pyrroles for reaction with DNA. Therefore, it would be logical to assume that an adequate cellular content of these substances is protective against potential genotoxic substances. It is highly likely that this mechanism can be general to a large number of tumorigenic alkaloids.

The concentrations that caused changes in the frequencies of different cell stages were also intermediate, except for one which is the highest concentration we used in this study. These differences could be due to cell arrest or delay caused by changes in the cell microtubes. According to Ye et al. (1998) an alkaloid known as noscapine binds to the tubulin of microtubes, alters its conformation and arrests mammalian cells in mitosis. In the study presented here, it could be considered that at higher concentrations some substances are able to compete with the alkaloids in *E. velutina* preventing the cell arrest in metaphase. However, they might not be able to compete with the alkaloids entirely leading to cell arrest in the last phases of the cell cycle.

The chromosome aberration assay is one of the few direct methods capable of measuring mutations in systems exposed to putative mutagenic or carcinogenic substances (Rank et al., 2002; Leme et al., 2008). Although the type of chromosome aberration induced by a specific substance may not be the same in plant and animal cells, if a particular chemical will induce chromosome aberration in one group, it will generally do so in the other as well (Grant, 1978).

The substance tested caused chromosome irregularities in the meristem cells of *Allium cepa*. Some concentrations that differed from the control were also the ones that apparently got stuck in certain phases of the mitotic cell cycle. Just as the alkaloids present in *E. velutina* can affect the cell cycle, its inhibition of proteins can affect the spindle function and cause a variety of abnormalities, such as lagging chromosomes, vagrants, disturbed metaphases and anaphases, and chromosome stickiness (Tkalec et al., 2009).

The appearance of vagrant chromosomes implies mitotic spindle disturbance, an aberration that could cause delayed prophase and/or metaphase (Tkalec et al., 2009). Chromosome stickiness is regarded as a physiological effect caused by the affected proteins of the chromosome. It may cause incomplete separation of daughter chromosomes as a result of the cross-linkage of chromoproteins (Kong & Ma,

1999; Tkalec et al., 2009). Chromosome bridges may be due to chromosomal stickiness and the subsequent failure of free anaphase separation. These bridges are usually formed by joined sister chromatids that stay together until late anaphase or telophase. If these connections become too strong, chromatids can break at or near the points of connection at anaphase (Gömürgen, 2005; Türkoglu, 2008).

The presence of cc-metaphase indicates effects on the organization of chromatin, which may be related to an imbalance of the proteins responsible for the structure of nuclear chromatin (Kurás et al., 2006). As for the lagging chromosomes, they are usually due to failure of the chromosomes to move to either of the poles (Tkalec et al., 2009).

Taking into account that cytological assays in plants serve as an excellent monitoring system for the detection of substances that may pose a genetic hazard (Grant, 1978), the present test shows that the decoction of *Erythrina velutina* can have genotoxic effects at some doses. Therefore, caution is required when using this plant for the preparation of teas and other medicinal products. We also recommend further cytogenetic studies using different test-systems in order to establish adequate procedures for the medicinal use of this plant by the local population.

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