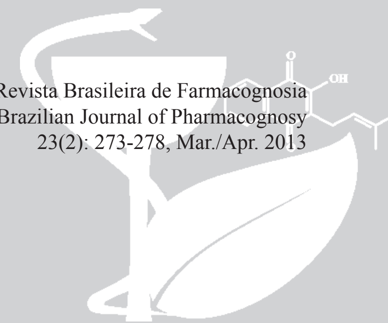

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Investigation of protective effects of *Erythrina velutina* extract against MMS induced damages in the root meristem cells of *Allium cepa*

Article

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Abstract: *Erythrina velutina* Willd., Fabaceae, is a medicinal plant that can be found in the tropics and subtropics, including in the semi-arid northeastern Brazil. It is commonly used in folk medicine to treat anxiety, agitation and insomnia. *E. velutina* has been known to present analgesic, anti-inflammatory and antibacterial activities, however, it is unknown if this plant present a protective effect on DNA. We assessed the antigenotoxic effect of *E. velutina* against the genotoxic effects induced by MMS in the root meristem cells of *Allium cepa*. Three concentrations of the aqueous extract (100, 200 and 400 mg/L) of this medicinal plant were used in three different types of treatment (pre-, post- and simultaneous). The effects of the extracts on the root meristem cells of *A. cepa* were analyzed at both macroscopic and microscopic levels. Protective effects were observed at higher concentrations in pre-treatment and in simultaneous treatment. The results suggest that *E. velutina* may present antigenotoxic properties and demonstrate its chemopreventive potential.

Keywords:
Allium cepa
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Erythrina velutina
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root growth

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Introduction

Mankind has always been exposed to many chemicals present in food and in pharmaceutical products, including those used in folk medicine. Many potential carcinogens are present in the human diet, and due to their large number and variety it is difficult to avoid them. An alternative strategy currently in use is to consume anti-carcinogenic/anti-mutagenic substances that could prevent or reverse some of the effects produced by carcinogens (Romero-Jiménez et al., 2005).

It has been documented that some natural compounds in foods and beverages for human consumption have an anti-mutagenic or anti-carcinogenic effect (Hamss et al., 1999; Oliveira et al., 2002). Many natural compounds of plant origin are known to have chemopreventive properties (Abraham, 2001; Razo-Aguilera et al., 2011). Dietary in take of such chemopreventive compounds have suggested an effective strategy to minimize the deleterious effects of genotoxic substances and carcinogens, protecting the organism against the development of various diseases including cancer (Abraham, 2001; Abdullaev et al., 2003).

An example of a chemopreventive strategy is the use of a group of natural products known as flavonoids. They are polyphenolic compounds that occur naturally in foods of plant origin. Flavonoids are generally non-toxic and demonstrate a variety of biological activities such as anti-allergic, anti-inflammatory, anti-oxidative, free radical remover, anti-mutagenic and modulator of enzyme activities (Heo et al., 2001; Agati et al., 2012).

*Erythrina velutin*a Willd., Fabaceae, plants have more than one hundred species and are known to be commonly used in folk medicine. These plants are also known to be a rich source of alkaloids and flavonoids, particularly isoflavones, pterocarpos, flavanones and isoflavanonas (Chacha et al., 2005). Some of these flavonoids have shown a variety of biological activities such as antimicrobial, anti-HIV, antibacterial, anti-inflammatory and anti-plasmoidal (Hedge et al., 1997; Andayi et al., 2006; Rukachaisirikul et al., 2007; Lee

et al., 2009; Vasconcelos et al., 2011). The species *Erythrina velutina* can be found in the tropics and subtropics, including in the semi-arid northeastern Brazil where it is often used to treat diseases of the central nervous system (CNS), as well as anxiety, agitation and insomnia (Lorenzi & Matos, 2002; Dantas et al., 2004; Raupp et al., 2008). The traditional use also indicates that this plant has analgesic, anti-inflammatory and antibacterial activities (Pillay et al., 2001). However, its protective effect on DNA has not been cited in the literature. Therefore, this study investigated whether the *E. velutina*. Extract could present protective effects against MMS induced damages in the root meristem cells of *Allium cepa.*

Materials and Methods

Plant material

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, Brazil, and was taken to the University Herbarium for identification, where it is registered under the number 13026.

Preparation of the aqueous extract

Dried leaves of *E. velutina* were triturated to produce a fine powder, pulverized, weighted and then decocted in distilled water. A 1000 mL of distilled water were added for every 400 g of the powder and the aqueous extracts were left to boil at 100 °C for 10 min. After cooling, the extracts of *E. velutina* were filtrated in vacuum. After filtration, the extracts were lyophilized and stored at 5 °C for later use. For the experiments, the following concentrations were used: 100, 200 and 400 mg/L.

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 and the positive controls, 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. Three kinds of treatments were given: pre-, post- and simultaneous, totalizing fifteen bulbs for each kind of treatment.

Allium cepa *root growth assay*

In pre-treatment, the roots were first treated with different concentrations of the aqueous extracts (100, 200 and 400 mg/L) for 48 h followed by treatment with 10 mg/L of methylmethanesulfonate (MMS, Sigma-Aldrich, CAS 66-27-3) for another 48 h. Inpost-treatment, the roots were first treated with 10 mg/LMMS for 48 h followed by the aqueous extracts for another 48h. In simultaneous treatment, for each concentration of the extract, 10 mg/L MMS was added. The treatment of roots with 10 mg/LMMS and Milli-Q water served as positive and negative controls, respectively.

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

Allium cepa *chromosomal aberration assay*

In pre-treatment, the roots were first treated with different concentrations of the aqueous extracts (100, 200 and 400 mg/L) for 20 h followed by treatment with 10 mg/L MMS for another 20 h. In post-treatment, the roots were first treated with 10 mg/L MMS for 20 h followed by the aqueous extracts for another 20 h. In simultaneous treatment, for each concentration of the extract, 10 mg/L MMS was added. The treatment of roots with 10 mg/L MMS and Milli-Q water served as positive and negative controls, respectively.

After treatments, the root tips were then removed and fixed in ethanol:glacial acetic acid (3:1, v/v) for 24 h and then transferred to 70% alcohol and stored at 4 °C. 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, fifteen slides were analyzed (2000 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.

Analysis of phytochemicals

The phytochemical prospecting was performed according to Matos (1997). It aimed to detect the occurrence of several chemical constituents present in the extract of the leaves of *E. velutin*a.

Statistical analysis

The root length data are given as the mean±SD. The mitotic index and the frequency of aberrant cells (%) were calculated as the number of dividing cells per 2000 observed and based on the proportion of aberrant cells scored at each concentration, respectively (Akinboro & Bakare, 2007). The data were analyzed usingthe Kruskal-Wallis test. In all cases, a value of $p<0.05$ was considered significant.

Results

Table 1 shows the results of the *Allium cepa* root growth test. Statistical analysis indicated no significant differences between treated groups and the controls.

The cytological effects were also examined. Table 2 shows the results of the mitotic activity and the chromosome aberrations found in the root meristem cells of *Allium cepa* under the different treatments.

Statistical analysis indicated changes in the mitotic activity. The exposure to the extract of *Erythrina velutina* Willd., Fabaceae, at the higher concentration (400 mg/L) of pre-treatment caused an increase in the mitotic activity and in the frequencies of different cell stages (prophase and anaphase-telophase) when compared to the positive control.

The types of chromosomal aberrations found in various stages of mitosis were: micronucleus, chromosomal bridge, lagging chromosome and chromosome fragments (Figure 1). According to statistical analysis, there were significant differences in the number of aberrations found at the concentrations of 200 mg/L and 400 mg/L of the extractin pre-treatment and at the higher concentration (400 mg/L) in simultaneous treatment. At these concentrations, the protective effect could be observed.

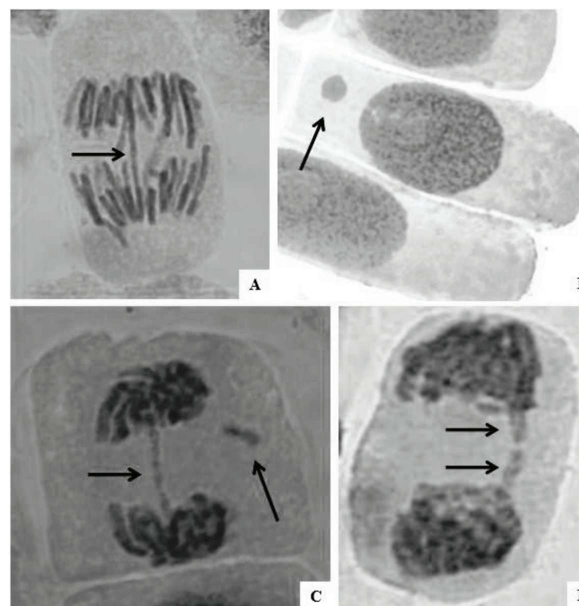

Figure 1. Chromosomes abnormalities observed in the root meristem cells of *Allium cepa* in various stages of mitosis; A. Chromosome bridge; B. Micronucleus; C. Chromosome bridge and chromosome fragment; D. Lagging chromosomes.

Significant differences between the negative control and different concentrations of the extract in different treatments could also be observed.

Phytochemical analysis indicated the presence of relevant groups of secondary metabolites. The phytochemical study of the aqueous extract of the leaves of *Erythrina velutina* showed the presence of the following chemical groups: aurones, chalcones,

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

Treatment	Mean root length (+SD) at time:			
	12 h	24 h	36 h	48 h
Negative control	0.70+0.96	1.88+1.99	3.78+3.99	5.85+6.19
Positive control	0.35+0.46	0.67+0.65	1.42+1.21	2.32+1.82
PRE100	0.19+0.42	0.77+0.84	1.97+1.19	3.24+1.03
PRE200	0.05+0.10	0.46+0.61	0.99+0.84	1.69+1.14
PRE400	0.09+0.19	0.57+0.65	1.18+1.32	1.62+1.39
POST100	0.71+0.81	1.61+1.63	3.38+2.22	5.38+3.56
POST200	1.06+0.54	2.07+0.28	3.5+0.52	4.17+0.71
POST400	1.18+1.79	2.44+2.79	4.40+3.92	5.55+4.79
SIM100	0.18+0.28	0.60+0.92	1.38+1.79	2.29+2.82
SIM200	0.54+0.84	1.98+1.87	3.25+2.89	4.48+4.04
SIM400	0.23+0.51	0.66+1.02	1.43+1.48	2.05+1.92

PRE100: pre-treatment (100 mg/L), PRE200: pre-treatment (200 mg/L), PRE400: pre-treatment (400 mg/L), POST100: post-treatment (100 mg/L), POST200: post-treatment (200 mg/L), POST400: post-treatment (400 mg/L), SIM100: simultaneous treatment (100 mg/L), SIM200: simultaneous treatment (200 mg/L), SIM400: simultaneous treatment (400 mg/L).

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

Treatment	Total cells examined	Total mitosis	MI (%)	Prophase(%)	Metaphase (%)	Anaphase-Telophase (%)	Types of abnormalities				Total Abnormalities
							MN	CB	LC	CF	
Negative control	30000	679	2.26	50.96	18.55	30.49	0	2	6	1	9
Positive control	30000	252	0.84	34.52	26.99	38.49	147	3	0	3	153
PRE100	30000	529	1.76	47.45	21.36	31.19	33	2	1	2	38
PRE200	30000	98[a]	0.32	37.76[a]	25.51[a]	36.73[a]	2[b]	0	1	0	3b
PRE400	30000	719[b]	2.39	44.92[b]	23.09	31.99[b]	27[b]	5	7	8	47
POST100	30000	179[a]	0.59	27.93[a]	22.91	49.16	140[a]	1	0	1	142[a]
POST200	30000	261	0.87	41.76	22.61	35.63	43[a]	2	2	4	51[a]
POST400	30000	197	0.65	25.38[a]	23.35	51.27	50[a]	1	0	1	52[a]
SIM100	30000	330	1.10	22.73	34.24	43.03	34[a]	0	0	0	34
SIM200	30000	459	1.53	45.32	22.22	32.46	32[a]	1	0	0	33
SIM400	30000	286	0.95	43.36	25.17	31.47	8[b]	1	0	0	9[b]

PRE100: pre-treatment (100 mg/L), PRE200: pre-treatment (200 mg/L), PRE400: pre-treatment (400 mg/L), POST100: post-treatment (100 mg/L), POST200: post-treatment (200 mg/L), POST400: post-treatment (400 mg/L), SIM100: simultaneous treatment (100 mg/L), SIM200: simultaneous treatment (200 mg/L), SIM400: simultaneous treatment (400 mg/L), MI; mitotic index, MN: micronucleous, CB: chromosome bridge, LC: lagging chromosome, CF: chromosome fragment; [a]Significantly different from the negative control ($p<0.05$). [b]Significantly different from the positive control ($p<0.05$).

flavonols, flavanones, leucoanthocyanidins, saponins and tannins.

Discussion

Natural products, particularly those originated from plants, have been an important source of therapeutic agents. Due to the increasingly intensive use of medicinal plants, studies on their properties are needed for contributing to a safe and effective use (Akinboro & Bakare, 2007; Fachinetto et al., 2007). Many plants used in the human diet may contain substances that are potential carcinogens and/or present anti-carcinogenic/anti-mutagenic effects that could prevent or reverse some of the effects produced by carcinogens (Romero-Jiménezet al., 2005). Therefore, it is important to assess both their genotoxic properties and their potential to protect genetic material from damage caused by chemicals. The anti-genotoxicity test is regularly used and it can reveal the protective effect against changes in the genetic material (mutations) induced by chemicals and other substances (Rani et al., 2005).

E. velutina was tested to investigate its protective effects against MMS induced damages using *Allium cepa* as a plant bioassay. The *Allium* test is a well-established assay and it is validated by the International Programme on Chemical Safety (IPCS, WHO) and the United Nations Environment Programme (Cabrera & Rodriguez, 1999; Rani et al., 2005). In addition to the common cytogenetic parameters, such as mitotic index and chromosome abnormalities, root growth was also used as macroscopic parameter.

In some treatments, differences were observed between the negative control and the some of the treatments, showing a genotoxic effect. However, this result may be expected in some concentrations as demonstrated by Silva et al. (2011). Protective effects were also observed at higher concentrations in pre-treatment (200 and 400 mg/L) and in simultaneous treatment (400 mg/L). In the same plant, different chemicals are present. Thus, depending on the concentration of phytochemicals and the interaction between them, the same plant can produce a genotoxic or an antigenotoxic effect depending on the concentration of the extract and the type of treatment used.

The presence of a protective effect in pre and simultaneous treatment show that the antimutagenic compounds present in *E. velutina* act as desmutagenic agents. This means that the antimutagenic substances act directly on the compounds that induce DNA mutations, inactivating them chemically or enzymatically by inhibiting metabolic activation of pro-mutagenic or sequestering reactive molecules (Kada et al., 1978). Many antimutagenic compounds found in foods are antioxidants and act sequestering the oxygen free radicals when administered as pre- or simultaneous treatment with the agent that induces DNA mutations.

As observed, this protection occurs at higher concentrations. This result indicates that the active compounds that show protective effect are present in greater amounts in these concentrations. As shown by the analysis of phytochemicals, *E. velutina* presents in its composition many compounds that are known to possess antimutagenic effect, such as aurones (Zampini et al., 2008; Kaur et al., 2009), flavonoids (Zhai et al., 1998; Perez-Carreon et al., 2002), saponins (Lee et al., 1999) and tannins (Kaur et al., 2000). The protective effect of *E. velutina* could be due to the

presence of flavonoids or the compounds, altogether, could possible act synergistically. Particularly, flavonoids have been extensively studied and have shown many pharmacological properties including anti-inflammatory and hepatoprotective and are able to interact with free radicals and substancesproduced by oxidative stress (Hosseinimehr et al., 2010). They can also exhibit immunoregulatory, anti-tumor and anti-radiation effects (Qi et al., 2011).

In conclusion, the present results suggest that the aqueous extract of *Erythrina velutina* may present antigenotoxic properties. This demonstrates a pharmacological importance of this plant and its chemopreventive potential. However, further experiments using different test-systems are required to establish adequate procedures for the medicinal use of this plant and to better characterize its properties.

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Authors' contributions

DSBSS, BB, ACFSG, BO contributed in collecting plant sample, running the laboratory work, analysis of the data and drafted the paper. CSE, VAN, ALLMS, ASD contributed to preparation of extract and phytochemicals analysis. SMP, RS designed the study, supervised the laboratory work and contributed to critical reading of the manuscript.

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