Growth Inhibition and Pro-apoptotic Action of Eleusine indica (L) Gaertn Extracts in Allium test

Amanda A. de Oliveira1* and Natália Faria Romão2

1Department of Pharmaceutical Sciences, Lutheran University Center of Ji-Paraná, Brazil.
2Department of Biological Sciences, Lutheran University Center of Ji-Paraná, Brazil.

Authors’ contributions

This work was carried out in collaboration between both authors. Author AAO designed and conducted the experiment, data analysis, and wrote the manuscript. Author NFR supervised the execution and reviewed the writing. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/2015/EJMP/18099

Editor(s):
(1) Marcello Iriti, Faculty of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
(2) Petigrosso, Lucas Ricardo, National University of Mar del Plata, Argentina.

Reviewers:
(1) Atef Mahmoud Mahmoud Attia, Biochemistry Department, Biophysical Laboratory, National Research Centre, Egypt.
(2) Petigrosso, Lucas Ricardo, National University of Mar del Plata, Argentina.

Complete Peer review History: http://www.sciencedomain.org/review-history.php?id=1085&id=13&aid=9034

ABSTRACT

Background: E. indica is a gramineae largely used in Brazilian traditional medicine for treatment of respiratory diseases. Previous research work has shown this plant to have glicoflavonoids constituents.

Place and Duration of Study: The study was carried out during four months in 2014 in the Laboratory of Molecular Biology, Department of Pharmacy, Lutheran University of Brazil (CEULJI/ULBRA), Ji-Paraná, Rondônia, Brazil.

Aims: This study aimed to determine possible mutagenicity and/or cytotoxicity activity of E. indica, using the Allium test to investigate root growth, mitotic index and micronuclei formation.

Methodology: We applied the Allium test to analyze the effects of E. indica compounds on genetic material. The plant aerial parts were dried, pulverized and prepared as aqueous extract. The experiment consisted in 5 assays: E. indica extracts in concentrations of 25%, 50%, and 100%; positive control (CuSO4); and negative control (distilled water). Each assay was performed with 10 repetitions.

Results: Initial results showed inhibition of meristems growth and stable mitotic index. Differential analysis indicated influence in cell division process with a significant number of cells in metaphase.
that did not progress to the next mitotic cycle stage, and no significant presence of micronuclei.

**Conclusion:** Our results strongly suggest the plant having cytotoxic compounds with microtubule affinity interaction without mutagenicity activity.

**Keywords:** Medicinal plants; aqueous extract; cytotoxicity; Allium cepa; micronuclei. Eleusine indica.

### 1. INTRODUCTION

Medicinal plants have been used in diseases’ treatment in the form of teas and infusions since the beginning of human history [1-3]. The Amazon region has a legacy of great genetic variability in plants and many of them are not yet cataloged species. A large array of plant species that have been used for a long time in traditional medicine have not yet been studied. One species that occurs in this region is the grass E. indica, pertaining to the Poaceae family, popularly known as Indian goosegrass; it is characterized as an upright plant, consisting of stem, hairless and striated, with heights ranging from 30 to 70cm, annual, herbal, with reproduction by seed, and with the ability to grow in any type of soil, preferably in environments with high temperatures and humidity [4].

In studies on medicinal purposes, this plant is cited for treatment of diseases related to the respiratory tract [5]. This fact was already confirmed in earlier work through the isolation of two flavones: the vitexin and the schaftoside, both with significant positive results in asthma treatment [6,7]. Studies have reported E. indica use in inflammatory processes, as nasal decongestant, as therapeutic for osteoporosis and plantar fasciitis, and in the form of infusion or decoction against pneumonia, influenza, and fever [8-11].

The compounds isolated from E. indica belong to the group of flavonoids which comprises more than 6000 substances known to have close relation with physiological processes in plants. Flavonoids regulate growth and development playing a role as a protective mechanism against pathogens and solar radiation. This substances’ group has extensive ubiquity and diverse biological activities, which can be beneficial or harmful, and only a limited number of them have been studied with this regard [12,13,9].

The Allium test is an excellent biomarker for first screening of genotoxicity and mutagenicity of medicinal plants due to its low cost, reliability, and compliance with other tests. It is also appropriated to study cytotoxic effects of medicinal plants, whereas the Allium cepa roots are in direct contact with the tested substance, allowing the evaluation in different concentrations. Chromosomal changes and division of meristematic cells in onion root are often used as an alert signal to advert people about the consumption of certain products [14,1]. Therefore, this study aimed to determine possible mutagenicity and/or citotoxicity activity of E. indica, using the Allium test to investigate root growth, mitotic index and micronuclei formation.

### 2. MATERIALS AND METHODS

#### 2.1 Collection of E. indica

*E. indica* samples were collected in the medicinal garden of Itapirema High School (EFA) in the city of Ji-Parana (Brazil) (Coordinates: 10°50’28.7"S, 62°01’47.3"W) during flowering stage (March, 2014). The plant was identified by the botanist Joseane B Barbosa, M.Sc, who was in charge of the university (CEULJI/ULBRA) Herbarium in which the voucher specimen was deposited under the number 007.

#### 2.2 Phytochemical Compounds Extraction

The extracts were prepared following the folk medicine usage of *E. indica*. The fresh aerial parts were oven dried with air circulation for 24 hours at 44°C, and subsequently pulverized. The phytochemical compounds extraction was made by decoction. Distilled water (pH=7.2) was heated up to 90°C, then *E. indica* pulverized parts were added in a proportion of 100g per 1L during 15 minutes [15]. Thereafter, the solution was cooled to room temperature and filtered in filter paper (PRO Lab. Quantitative Filter Paper). The resultant solution consisted in the 100% concentration assay, here and after denominated Eleusine₁. For the 50% concentration assay, distilled water was added to the Eleusine₁ solution in 1:1 ratio, here and after denominated Eleusine₂. Finally for the 25% concentration assay, distilled water were used in 3:1 ratio, here and after denominated Eleusine₃.
2.3 Allium Test

The A. cepa (2n = 16) specimens were acquired in the local popular market, all of small size, uniform, same origin, not sprouted and healthy. The bulbs were germinated with the bottom dipped in a 30mL solution which were the 3 E. indica assays, the negative and positive control for a period of 72 hours at 24°C. The experiment had as negative control (NC), distilled water, and as positive control (PC), copper sulfate (CAS No. 7758998; Sigma Chemical Co., St Louis, MO, USA) at a concentration of 0.006g/L\(^{-1}\)[16,17].

The experiment was conducted using 10 replicates for each assay. After the exposure period, the meristems were collected and placed in a solution of 3:1 (v/v) methanol/acetic acid at a temperature of 4°C during 12 hours for chemical fixation. Afterwards, the hydrolysis was performed in a 1 mol/L\(^{-1}\) Hydrochloric acid (HCl) solution for 5 minutes in water bath at 60°C followed by distilled water wash.

Slides were made in duplicate and stained with Panotipo Rapido LB Kit (Record No. 80002670065; Reny Lab Chemical and Pharmaceutical Co., Barbacena, MG, Brazil). The squash was performed after the coverslip addition [18]. Slide analysis was done by optical microscopy with 1000x magnification. The variables studied were: number of micronuclei formation per 1000 cells per slide, root number and root length measured after 5 days of exposure, and mitotic index (MI). The MI was calculated as shown in Equation 1, where CM is a number of cells in mitosis and TC is a total number of analyzed cells.

\[ MI = \frac{CM}{TC} \times 100 \]

2.4 Statistical Analysis

For statistical analysis, the collected data was treated in the statistical software R Core Team, version 3.0.2. The computed tests were ANOVA followed by Tukey’s test and the nonparametric Pearson correlation test, with an adopted significance level of \(P \leq 0.05\).

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characterization

The root number and root length levels are presented in Table 1. These results show that all E. indica assays caused significant root growth inhibition in comparison with negative and positive control assays. The inhibitions in root number and root length were greater in increasing concentrations of E. indica extracts. Average root length was 3.99 with a Standard Deviation (SD) of 1.71 (3.99±1.71 cm) in negative control and 1.4±0.55 cm in positive control. However, average root length in Eleusine\(_3\) assay decreased significantly in comparison to negative control (Table 1). Average root length in treatment assays also decreased significantly according to concentration. Statistical analysis shows that the values observed in Eleusine\(_2\) and Eleusine\(_3\) assays are significant (\(P \leq 0.05\)) and the values of Eleusine\(_1\) are highly significant (\(P < 0.001\)). When comparing data from treatment assays among themselves, no significant correlation was found (\(r = 0.27\) and \(P > 0.05\))\(^2\).

Inhibition in germination process is not contingent upon extract concentration and dose escalation may not interfere in the inhibitory process [19]. Studies with Cymbopogon citratus popularly known as lemon grass and also belonging to the Poaceae family, found the plant aqueous extract having compounds with allelochemical effects on Lactuca sativa root growth [20]. The influence of some plants on root growth occurs by the production of secondary metabolic compounds that act inhibiting or promoting the germination process and the cell division process [21]. The allelochemicals are released into the atmosphere by plants in various ways: by volatilization, root exudation, leaching and decomposition of waste vegetable structures [21,22].

3.2 Mitotic Index

The number of cells in mitosis and respective mitotic index are presented in Table 2, the results are not considered statistically significant (\(P > 0.05\)). Considering that root growth was inhibited, we analyzed the mitotic cell phase by cell differential analysis and we found that E. indica assays had a large number of cells in metaphase, as shown in Fig. 1, with severe depression values for anaphase and telophase which was proven by comparison with negative and positive controls (\(P \leq 0.001\)).

\(^{1}\)ANOVA and Tukey’s test.
\(^{2}\)Pearson Correlation coefficient.
Apoptosis is an essential process to maintain the development of living beings, as it is important to eliminate unnecessary or defective cells. During apoptosis, the cell undergoes morphological changes including cell shrinkage, loss of adhesion to the extracellular matrix and to neighboring cells, chromatin condensation, internucleosomal DNA fragmentation and formation of apoptotic bodies. This biological phenomenon, in addition to playing an important role in controlling many vital processes, is associated with numerous diseases such as cancer [31].

### Table 1. Average root number and root length in treatments and control groups assays

| Treatment groups                  | Concentrations | Average root number ± SD | Average root lengths (cm) ± SD |
|-----------------------------------|----------------|--------------------------|--------------------------------|
| Negative Control                  | Distilled water| 8.2±1.8                  | 3.99±1.71                     |
| Positive Control (CuSO₄)          | 0.006g/L⁻¹     | 3.2±0.6                  | 1.4±0.55                      |
| Eleusine₃                         | 25g/L⁻¹        | 2.3±0.92                 | 0.71±0.45*                    |
| Eleusine₂                         | 50g/L⁻¹        | 2.45±1.08                | 0.75±0.47*                    |
| Eleusine₁                         | 100g/L⁻¹       | 1.99±0.87                | 0.49±0.29***                  |

* Significant value (P<0.05); ** Highly significant value (P<0.001).

** MI measures the proportion of cells in the cell cycle M-phase and its inhibition can be interpreted as cellular death or a delay in the cell proliferation kinetics [23]. Reduction in the mitotic activity could be due to DNA synthesis inhibition or a blocking in the cell cycle G2 phase, preventing the cell from entering in mitosis [24]. Mitodepressive effects of some herbal extracts, including the ability to block DNA synthesis and nuclear proteins, were reported earlier [25,26]. Several other herbal extracts have been reported to inhibit mitosis [27-29].

The same effect was observed in earlier studies with chamomile inflorescence, with significant increase in the MI, and cell death concomitant with increased testing doses [30]. In a study conducted by [20], different results were reported for C. citratus extracts, in which root growth inhibition decreased MI. Flavonoids may interfere in the mitotic process, therefore induce apoptosis depending on its nature and concentration [13].

Cell differential analysis determined the absence of membrane cell boundaries in E. indica assays as shown in Fig. 1a. In cells with membrane absence the chromatin was in apoptotic process as in Fig. 1b. initially sparse and in transcription, beginning a rapid process of condensation and inactivation. Coarse lumps of heterochromatin were formed into the nucleus, with some changes in nuclear boundary. The inactivation of genetic material led to dismantling the cytoskeleton and to its disorganization, as in Fig. 1c, causing cell deformation on its contours and undoing its joints [31].

### 3.3 Micronuclei

The negative control assay showed 5.2±0.63 micronuclei formation, and the positive control had value of 28.4±1.77 micronuclei, both for every 2000 cells. In E. indica assays results for Eleusine₃, Eleusine₂ and Eleusine₁ were: 7.6±1.83, 7.2±3.76, and 9.1±1.66 micronuclei respectively, for every 2000 cells. The results reveal that aqueous extract of E. indica produces no mutagenic effect in meristems of A. cepa once the numbers indicate low significance (P>0.05) in comparison with the negative control assay; additionally, no chromosomal aberrations were observed.

### Table 2. Number of cells in mitosis and mitotic index by treatment

| Treatment     | Interphase Prophase | Mitotic phase Metaphase | Anaphase | Telophase | Mitotic index |
|---------------|---------------------|-------------------------|----------|-----------|---------------|
| Negative control| 16357              | 1265                    | 1227     | 580       | 571           | 18.22         |
| Positive control| 16128              | 1500                    | 1338     | 540       | 440           | 19.36         |
| Eleusine₃     | 16122              | 1778                    | 1898     | 122***    | 80            | 19.39         |
| Eleusine₂     | 16288              | 1680                    | 1897     | 99***     | 36            | 18.56         |
| Eleusine₁     | 16042              | 1922                    | 1885     | 100***    | 51            | 19.79         |

*** Highly significant value regarding to the negative and positive control groups (P<0.001)
While studying Brachiaria brizanthiae, also a Poaceae, [32] reported scarce micronucleus' formation, that occurs due to the meiotic behavior with slight formation of diploid accessions in the first division which is corrected in meiosis II leading to a normal cell division process. Bio-guided studies with C. citratus in Allium test did not lead to micronuclei formation [29]. In a study with Aloe vera extract, an average of 5.25 micronuclei was reported, which suggests that E. indica, although not belonging to the same family of A. vera, also leads to low significance values for micronucleus formation [1]. The low number of micronucleus formation induced by E. indica extract may be linked to its flavonol compounds: vitexin and schaftoside, which are antioxidants agents that act by sequestering free oxygen radicals [1,12].

4. CONCLUSION

Ultimately, our results indicate that when applied in higher concentrations E. indica extract has cytotoxic compounds with microtubule affinity interaction without mutagenicity activity. We used crude extracts of E. indica aerial parts. Experiments with crude extracts are appropriate because traditional medicinal herbs are generally used in the same way. Our findings in this study suggest that therapeutic use of E. indica requires additional investigation with isolated compounds regarding microtubules interaction in the cell cycle.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sturbelle RT, Pinho DSD, Restani RG, Oliveira GRD, Garcia GDL, Martino-Roth MDG. Evaluation of mutagenic and antimutagenic activity of Aloe vera in Allium cepa test and micronucleus test in human binucleated lymphocytes. Revista Brasileira de Farmacognosia. 2010;20(3):409-415.
2. Çelik TA, Aslantürk ÖS. Evaluation of cytotoxicity and genotoxicity of Inula viscosa leaf extracts with Allium test. Journal of Biomedicine and Biotechnology; 2010.
3. Zink T, Chaffin J. Herbal health' products: what family physicians need to know. American family physician. 1998;v58(5):1133-1140.
4. Stubbendieck JL, Hatch SL, Butterfield CH. North American range plants. U of Nebraska Press; 1992.
5. Karim S, Rahman M, Shahid SB, Malek I, Rahman A, Jahan S, Jahan FI, Rahmatullah M. Medicinal plants used by the folk medicinal practitioners of Bangladesh: a randomized survey in a village of Narayanganj district. American Eurasian Journal Sustainable Agricola. 2011;5:405-414.
6. Corrêa MFP, Melo GOD, Costa SS. Natural products from plant origin potentially useful in the asthma therapy. Revista Brasileirade Farmacognosia. 2008;18:785-797.
7. De Melo GO, Muzitano MF, Legorama Machado A, Almeida TA, De Oliveira DB, Kaiser CR, Koatz VL, Costa SS. C-glycosyl flavones from the aerial parts of *Eleusine indica* inhibit LPS-induced mouse lung inflammation. Planta Medica. 2005; 71(4):362-363.

8. Romano B, Pagano E, Montanaro V, Fortunato AL, Milic N, Borrelli F. Novel insights into the pharmacology of flavonoids. Phytotherapy Research. 2013; 27(11):1588-1596.

9. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011;82(4):513-523.

10. Biswas KR, Khan T, Monalisa MN, Swarna A, Ishika T, Rahman M, Rahmatullah M. Medicinal plants used by folk medicinal practitioners of four adjoining villages of Narail and Jessore districts, Bangladesh. American Eurasian Journal of Sustainable Agriculture. 2011;5:23-33.

11. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. Journal of Ethnobiology and Ethnomedicine. 2006;2:45.

12. Talhi O, Silva AMS. Advances in C-glycosyl flavonoid Research. Current Organic Chemistry. 2012;16(7):859-896.

13. Agati G, Azzarello E, Pollastri S, Tattini M. Flavonoids as antioxidants in plants: location and functional significance. Plant Science. 2012;196:67-76.

14. Fiskesjö G. The Allium test as a standard in environmental monitoring. Hereditas. 1985;102(1):99-112.

15. Qin SH, Zhang JM, He YX, HE Y, Liao W, Fu CM. Optimization of decoction conditions of compatibility of *Aconitum carmichaelii* and *Glycyrrhiza uralensis* based on dynamic change of chemical components. Chin J Exp Tradit Med Form. 2012;18(24):21-22.

16. Halliwell B. Free radicals and antioxidants: updating a personal view. Nutrition reviews. 2012;70(5):257-265.

17. Sies H, et al. Oxidative stress: introductory remarks. Oxidative stress. 1985;1:8.

18. Pakrashi S, Jain N, Dalai S, Jayakumar J, Chandrasekaran PT, Raichur AM, Chandrasekaran N, Mukherjee A. In vivo genotoxicity assessment of titanium dioxide nanoparticles by *Allium cepa* root tip assay at high exposure concentrations. PloS one. 2014;9(2):e87789.

19. Cheema ZA, Farooq M, Wahid A. Allelopathy: current trends and future applications. Springer Science & Business Media; 2012.

20. Souza SAM, Stein VC, Cattelan LV, Bobrowski VL, Rocha BHG. Utilização de sementes de alface e de rúcula como ensaios biológicos para avaliação do efeito citotóxico e alelopático de extratos aquosos de plantas medicinais. Rev. Biol. Ciências da Terra. 2005;5(1):0.

21. Weston LA, Mathesius U. Flavonoids: their structure, biosynthesis and role in the rhizosphere, including allelopathy. Journal of chemical ecology. 2013;39(2):283-297.

22. Weston LA, Ryan PR, Watt M. Mechanisms for cellular transport and release of allelochemicals from plant roots into the rhizosphere. Journal of experimental botany. 2012;63(9):3445-3454.

23. Rojas E, Herrera LA, Sordo M, Gonsebatt ME, Montero R, Rodriguez R, Ostrosky-Wegman P. Mitotic index and cell proliferation kinetics for identification of antineoplastic activity. Anti-Cancer Drugs. 1993;4(6):637-640.

24. Sudhakar RKN, Gowda KN, Venu G. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. Cytologia; 2001.

25. Mercykutty VC, Stephen J. Adriamycin induced genetic toxicity as demonstrated by the Allium test. Cytologia. 1980;45(4):769-777.

26. Schulze E, Kirschner M. Microtubule dynamics in interphase cells. The Journal of cell biology. 1986;102(3):1020-1031.

27. Çelik TA, Aslantürk ÖS. Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. Biologia. 2007;62(3):292-296.

28. Çelik TA, Aslantürk ÖS. Anti-mitotic and anti-genotoxic effects of *Plantago lanceolata* aqueous extract on *Allium cepa* root tip meristem cells. Biologia. 2006; 61(6):693-697.

29. Akinboro A, Bakare AA. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. Journal of Ethnopharmacology. 2007; 112(3):470-475.

30. Romero-Jimenez M, Campos-Sanchez J, Analla M, Muñoz-Serrano A, Alonso-Moraga A. Genotoxicity and anti-genotoxicity of some traditional medicinal herbs. Mutation Research/Genetic
Toxicology and Environmental Mutagenesis. 2005;585(1):147-155.

31. SHI H, Kwok RT, Liu J, Xing B, Tang BZ, Liu B. Real-time monitoring of cell apoptosis and drug screening using fluorescent light-up probe with aggregation-induced emission characteristics. Journal of the American Chemical Society. 2012;134(43):17972-17981.

32. Felismino MF, Pagliarini MS, Valle CBD. Meiotic behavior of interspecific hybrids between artificially tetraploidized sexual Brachiaria ruziziensis and tetraploid apomictic B. brizantha (Poaceae). Scientia Agricola. 2010;67(2):191-197.

© 2015 Oliveira and Romão; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1085&id=13&aid=9034