

Bioindicator of Genotoxicity: The *Allium cepa* Test

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1. Introduction

In Brazil many species of medicinal plants are used in popular medicine. However, their indiscriminate and uncontrolled use can cause more harm to public health than good, thus the knowledge about these plants, from their cellular levels to their action on living organisms is important. The economic potential of the germplasm of medicinal species is an asset that should be preserved and developed, making it a more affordable alternative form of therapy for use by the general population, while conserving plant genetic diversity. To make this a reality, studies on the characterization of these plants on many levels, biological and/or agronomical, are essential, including the capacity of their extracts acting on other living organisms.

Cytogenetic studies of plant species report possible alterations of plant chromosomes due to mutagenic substances in their composition or resulting from their metabolism. The study of mutagens in eukaryotic nuclei has been observed by cytological methods and it is known that the mutation may result from the action of radiation, drugs and viruses, as well as the intrinsic stability of nucleic acids. Therefore, mutagens can be detected cytologically by cellular inhibition; disruption in metaphase; induction of chromosomal aberrations, numerical and structural, ranging from chromosomal fragmentation to the disorganization of the mitotic spindle, and consequently of all subsequent dependent mitotic phases.

Studies on the genotoxicity of medicinal plant extracts should be prioritized; in this way we invest research efforts towards public health. The analysis of chromosomal alterations serves as a mutagenicity test and is one of the few direct methods to measure damages in systems exposed to possible mutagens or carcinogens. To enable the evaluation of the effects or damages that mutagenic agents might cause, it is necessary for the sample to be in constant mitotic division, seeking to identify the toxic effects and alterations occurring over a cell cycle. In order to do so, there is the *Allium cepa* test, which has been widely used for this purpose (Silva et al., 2003).

The mitotic index and replication index are used as indicators of adequate cell proliferation (Gadano et al., 2002), which can be measured by the plant test system *Allium cepa*. Cytotoxicity tests, using plant test systems *in vivo*, such as *Allium cepa*, are validated by several researchers, who jointly performed animal testing *in vitro* and the results obtained are similar (Vicentini, et al. 2001; Teixeira et al. 2003), providing valuable information for human health. El-Shahaby et al. (2003) stress the importance of using the *Allium cepa* test for detecting toxicity/genotoxicity and evaluating environmental pollution.

As previously mentioned, in Brazil, medicinal plants are used for treating illnesses alternatively, and the test is important for alerting the general population about possible genotoxic risks, which can be caused in eukaryotic plant organisms, such as the onion. We intend to focus on the use of this test as a bioindicator of genotoxicity aiming at human health, demonstrating that even in an indirect way, it is possible to prevent and to avoid environmental contamination by the abusive use of substances that cause chromosomal aberrations. On the other hand, the results obtained through the application are surprising and show that certain plants are antimutagenic and would allow the reversion of genotoxic processes.

We emphasize the advantages of this test as a useful, low-cost system, as well as value the knowledge from plant cytogenetic techniques, which enable its development. Leme & Marin-Morales (2009) reported that studies of sensitivity and correlation of the *Allium cepa* (onion) test system and other test systems are fundamental for the more accurate evaluation of environmental risks, as well as the extrapolation of data to other organisms such as humans. The tests for risks to human health are performed by using various test systems and among these, the *Allium cepa* test.

2. Description and importance of the *Allium cepa* test

The *Allium cepa* test has been used by many researchers mainly as a bioindicator of environmental pollution (Bagatini et al. 2009; Leme & Marin-Morales, 2009), testing crude extracts of cyanobacteria (Laughinghouse, 2007), as well as to evaluate the genotoxic potential of medicinal plants (Camparoto et al. 2003; Knoll et al. 2006; Fachinnetto et al. 2007; Lubini et al. 2008; Fachinnetto et al. 2009; Fachinnetto & Tedesco, 2009; Dalla Nora et al. 2010), because this test uses a model that is adequately sensitive to detect innumerable substances that cause chromosomal alterations.

The *Allium cepa* test is important since it is an excellent model *in vivo*, where the roots grow in direct contact with the substance of interest (i.e. effluent or complex medicinal mix being tested) enabling possible damage to the DNA of eukaryotes to be predicted. Therefore, the data can be extrapolated for all animal and plant biodiversity. The analysis of chromosomal alterations can be equal to the test of mutagenicity mainly for the detection of structural alterations; however, it is possible to observe numerical chromosomal alterations, as well. The *Allium cepa* test is one of the few direct methods for measuring damage in systems that are exposed to mutagens or potential carcinogens, and enables the evaluation of the effects of these damages through the observation of chromosomal alterations. For this undertaking, it is necessary that the sample remain in constant mitotic division, seeking to identify the toxic effects and alterations over a cell cycle; and the *Allium cepa* test has been widely used for this purpose. It is advantageous to use the *Allium cepa* test system since its

main component is a vascular plant, making it an excellent genetic model for evaluating environmental pollutants, detecting mutagens in different environments and evaluating many genetic endpoints (point mutations to chromosomal alterations). *Allium cepa* is distinctive in regards to its efficiency in detecting genetic damage and was introduced by Levan, in 1938, for helping observe disturbances in the mitotic fuse due to colchicine action.

Relevant studies by Fiskesjö (1985), showed the importance of the *Allium cepa* test system for evaluating genotoxicity, demonstrating that *Allium cepa* cells contain an oxidase enzyme system capable of metabolizing polycyclic hydrocarbonates. Even though other test systems have been shown to be sensitive for this detection, the results of the *Allium cepa* test should be considered as an alert for other organisms (i.e. bioindicators). Studies on sensibility and correlation among test systems are fundamental for a more accurate evaluation of environmental risks and for extrapolating the data to other groups of target organisms. A high sensitivity and good correlation with mammal tests and the same sensitivity as test systems of algae and human lymphocytes exist when compared with *Allium cepa*.

Furthermore, Rank & Nielsen (1993) performed adaptations for evaluating complex mixtures and Ma et al. (1995) adjusted the test for assessing mutagenicity and micronucleus analyses (MN) in F1 cells. Some researchers show certain restriction in regards to using plant test systems for evaluating certain classes of carcinogens, which require complex metabolization systems for the activation of its genotoxic action. However, Rank & Nielsen (1994) showed a correlation of 82% between the *A. cepa* test and the carcinogenicity test in rodents and concluded that the same was even more sensitive than the Ames test. Vincentini et al. (2001) reported that the *Allium cepa* test system is well accepted for the analysis of cytotoxicity and genotoxicity because the roots are in direct contact with the tested substance, allowing evaluation of different concentrations and times. The results by Camparoto et al. (2002) were similar when estimating the effects of infusions of *Maytenus ilicifolia* Mart. and *Bauhinia candicans* Benth with the test of *Allium cepa* and bone marrow cells of Wistar rats. Studies by Knoll et al. (2006) used the plant model of *Allium cepa* to test several populations of *Pterocaulon polystachyum* DC (known as quitoco in the southern region of Brazil) at different concentrations and obtained precise results on cell division inhibition, whose effects were attributed to the presence of flavonoids in the infusions tested and the authors demonstrated that with an increase in the concentration of the infusions of *P. polystachyum*, lower mitotic index values were recorded.

The use of medicinal plants for treating illnesses is an exploratory practice that is widely diffused in Brazil (Rosa & Ferreira, 2011) and due to this intense medicinal use, studies using bioindicators of toxicity and mutagenicity, such as the *in vivo* test of *Allium cepa* are necessary for contributing to their safe and efficient use. The plant test system of *Allium cepa* is as an ideal bioindicator for the first screening of genotoxicity, helping with studies that prevent damages to human health (Bagatini et al., 2007). Lubini et al. (2008) studied two species of the genus *Psychotria*, *Psychotria leiocarpa* Cham. & Schltdl. and *Psychotria myriantha* Mull. Arg. Using *Allium cepa* to test infusions at two concentrations of these species it was possible to verify the antiproliferative activity of the species *P. leiocarpa* and *P. myriantha*, and the results indicated that both species possessed the capacity to inhibit cell division and *P. myriantha* possessed genotoxic activity. We can indicate the use of *P. leiocarpa* in high concentrations as potentially therapeutic for inhibiting the cell cycle in eukaryotic organisms.

Studies by Souza et al. (2010) demonstrated that the species *Artemisia verlotorum* Lamotte (known in Brazil as infalivina) has antiproliferative and genotoxic capacity on the *Allium cepa* cell cycle. The authors found 32.2% of the chromosomal alterations in the highest concentration of the tested aqueous extract, 48 g/L. It was found that with an increase in the aqueous extract of *A. verlotorum*, there was higher inhibition of cell division, and consequently lower values of MI.

3. Methodology of the *Allium cepa* test

The *Allium cepa* test consists in obtaining onion bulbs cultivated without the application of herbicides or fungicides. After obtaining the bulbs, they should be scraped at the root to promote the emergence of new roots. Other bioassays can be performed with *Allium cepa* seeds placed to germinate in a BOD (biochemical oxygen demand) incubator with controlled temperature, which are used for allelopathy testing and also genotoxicity assessment. To set-up the experiment allowing rootlets to grow, all bulbs should be placed initially in a small 50 mL plastic cup (Figure 1), containing distilled or tap water (being that it is potable), for approximately 03 to 04 days so rootlets can emerge. After this period, the bulbs should be transferred to other clean and dry containers including the treatments. The plastic cups for water control treatment can be reused, to minimize the effect of plastic on the environment. In general, we use 5 groups of bulbs of *Allium cepa* for each treatment, with one being a negative control in water and another for the positive control in methylsulfonylmethane (MMS) or glyphosate. The issue of positive control is cited by Rank (2003) using methylsulfonylmethane and by Souza et al. (2010) using glyphosate. Through the studies developed to date at the Laboratory of Plant Cytogenetics and Genotoxicity (LABCITOGEN) of the Department of Biology at the Federal University of Santa Maria, various concentrations of glyphosate have shown chromosomal changes directly to the rootlets. Its residual effect on the environment after use is not proven. After rooting, the bulbs of the two control groups should remain in the water (negative control) and in the respective positive control, and the remaining should be transferred to the chosen treatments, which can be solutions of essential oil, leaf extracts by infusion, root or stem extracts by decoction, or samples of industrial and/or hospital effluents. These should remain in the dark for exactly 24 hours.

It is emphasized that in a case where a product such as ethanol PA is used for the dilution of the oil in which the rootlets of *Allium cepa* will be immersed, the same should consist of one of the treatments. The main idea is to minimize the error, allowing the results to express the effect of the substances that are interacting. Therefore, when possible, test them separately. After the rootlets are submitted for 24 hours in the individual treatments, they should be collected and immediately fixed in ethanol: acetic acid (3:1), also for 24 hours.

Afterwards, the rootlets should be removed from the fixing solution and transferred to ethanol 70% and be kept under refrigeration (4°C) until used. It is important to emphasize that all glassware used to keep the rootlets should be identified with a number or sample name and/or treatment, as well as date, using small tags written in pencil on the inner and outer part of the glass, thus avoiding any kind of mistake with the samples. The technique of bulb use is described by Fiskesjö (1994) and adapted by researchers who use the test, such as Knoll et al. (2006) and Fachinetto et al. (2009). In the next stage, slides are prepared,

analyzing 1000 cells per bulb, totaling 5000 cells per treatment or variations of these values, such as 500 cells per bulb, totaling 2500 cells. It has already been proven that one rootlet is enough for observing the damage caused to the DNA of *Allium cepa*, observing the cells after the treatment with mutagenic agents.

The researcher should have a large sample so that errors can be minimized. We believe that at least 200 cells be analyzed per bulb, totaling 1000 cells per treatment, in case of optimizing time, considering such samples as pilot or initial experiment. Slide preparation can follow the technique by Guerra & Souza (2002) where the whole technique of squashing and staining of the root tip for obtaining cells with good visualization is explained. In the routine of LABCITOGEN, for slide preparation, the rootlets are hydrolyzed in HCl for 5-6 minutes and rinsed in distilled water. Then, only the meristematic region of the rootlets (eliminating the rest and the root cap) should be fragmented, under the lens of a stereoscopic microscope using histological needles, previously stained with 2% acetic orcein. For Raven et al. (1988) the distance in relation to the apical meristem, where we find the location with the majority of cell division varies among species, and within a single species, depending on the root age. The combination of apical meristem plus the portion of the root where the cell divisions occur is called the cell division region or zone of division, including the quiescent region. In relation to staining, other stains can also be used, such as acetic orcein 1%, acetic carmine 1 and 2%, as well as Giemsa. During squashing and staining, a cover slip is used and carefully squeezed with filter paper to remove excess stain. Then, holding one end of the cover slip, it should be tapped with the end of the histological needle several times to spread the cells. Excessive force when tapping can tear the cells; this should not be confused with a morphological alteration.



Fig. 1. Sample of an onion bulb showing the rootlets already grown. Arrow indicates the plastic cup, in which the bulb can be placed for testing. Scale bar=2cm



Fig. 2. Organization of the experiment with *Allium cepa*, containing 4 groups of 5 onion bulbs, LABCITOGEM, UFSM, Santa Maria, RS, Brazil. Scale bar=2cm

The slides are then evaluated using a LEICA microscope with 400X magnification and the cells are observed during the interphase and cell division, in prophase, metaphase, anaphase, and telophase. Cell counts are carried out considering visual fields scanning the whole slide. The counts should be written down on a table, which is shown in Table 1. Following, the sum of all cells in interphase and in division is carried out and then the mitotic index (MI) is calculated for each treatment. During the cell count, they should be divided into two categories: regular (do not present damages in the chromosomes) and irregular (present damages in the chromosome, such as: chromosomal breakages, simple or multiple anaphasic bridges, micronucleus, laggard or lost chromosomes). The statistical analysis should be preferably performed using the χ^2 test with a $p < 0.05$ level of probability using a statistical program, e.g. BioEstat 4.0 (Ayres & Ayres, 2003) or BioEstat 5.0 (Ayres & Ayres, 2009). The mitotic index can be calculated through the number of cells observed in prophase + metaphase + anaphase + telophase (Love & Love, 1975). According to Sehgal et al. (2006), the mitotic index can be recorded using the formula:

$$\text{Mitotic index} = \frac{P + M + A + T}{\text{Total number of cells}}$$

For Ozmen & Summer (2004) the mitotic index was expressed in terms of divided cells/total number of cells and in this study, it was observed that the chromosomal aberrations were determined by scoring cells with bridges, fragments, sticky chromosomes and polar deviation in three randomly picked zones and micronucleus formation in 1,000 cells per slide. In their study, Ozmen & Summer (2004) used *Allium cepa* to test extracts of *Plantago lanceolata*. The researchers used two groups of ten bulbs which were placed to germinate in the dark at 22°C,

and the same were observed after 48h, removing the bulbs which had roots that only developed a little, and the rest were treated with water with 0.7% H₂O₂ for 1h. At the end of the treatment with H₂O₂, the roots were washed and then treated with two different concentrations of extracts (15 g/L and 30 g/L) of *Plantago lanceolata* for 24 hours. In this study the onions were placed to root in the dark, because the plant extracts were photosensitive. In the same way, other researchers placed the onions to root in the dark (Fachinetto et al., 2008) to test nanocoated tretinoin, since this was also photosensitive. So, the *Allium cepa* test can be undertaken with rooting the bulbs in either the dark or light.

Rank & Nielsen (2003) explain in their study the anaphase-telophase method of *Allium cepa* and made important considerations, such as genotoxic chemicals used for many purposes in manufacturing processes that can be found in environmental compartments such as air, water, soil, and sediments. The chemical can enter the environment from discharged wastewater, air emissions, during product consumption and from domestic and industrial waste sites. The important advantage of the *Allium cepa* test is that it is a "low budget" method, which besides being fast and easy to handle also gives reliable results.

To analyze cell division in *Allium cepa*, it is necessary to correctly identify all the phases that are considered for the calculation of the mitotic index. In Figure 3, there are cells in interphase and in cell division. Normally, most cells are found in interphase, thus the mitotic index value obtained from the negative control in water is variable.

The regular phases of *Allium cepa* cell division are presented and shown in Figure 4-A, B, C and D. In these phases of mitosis, there is an example of regular cells when the roots only grow in distilled water, not presenting chromosomal abnormalities. Figure 4-A shows prophase with chromosomes quite visible, which is characteristic of this phase; 4-B is metaphase with chromosomes arranged in the equatorial plate of the cell awaiting subsequent movement to the opposite poles during anaphase; 4-C shows anaphase with the chromosomes moving to the opposite poles of the cell in a stable way; 4-D is the phase that finalizes the mitotic cell division, telophase, showing the chromosomes already organized on opposite poles of the cell, awaiting the following step of cytokinesis, forming 2 new daughter cells.

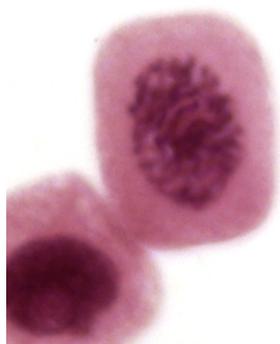


Fig. 3. *Allium cepa* cells obtained from 2cm-rootlets, after 24h under control in distilled water, fixed, stained and prepared according to the routine of LABCITOGEN, UFSM, Santa Maria, RS, Brazil. The arrow indicates cells in interphase. Scale bar= 10 μ

S1B1T5											
S2B1T5											
S3B2T5											
S4B2T5											
S5B3T5											
S6B3T5											
S7B4T5											
S8B4T5											
S9B5T5											
S10B5T5											

S=Slide, B=bulb, T=treatment, Inter=interphase, Pro=prophase, Meta=metaphase, Ana=anaphase, Telo=telophase

Table 1. Illustrative Framework for use in annotating results of an experimental design with 5 bulbs of *Allium cepa* and 5 distinct treatments



Prophase= the chromosomes are visible and tangled

A



Metaphase= the chromosomes are arranged in the equatorial plate

B

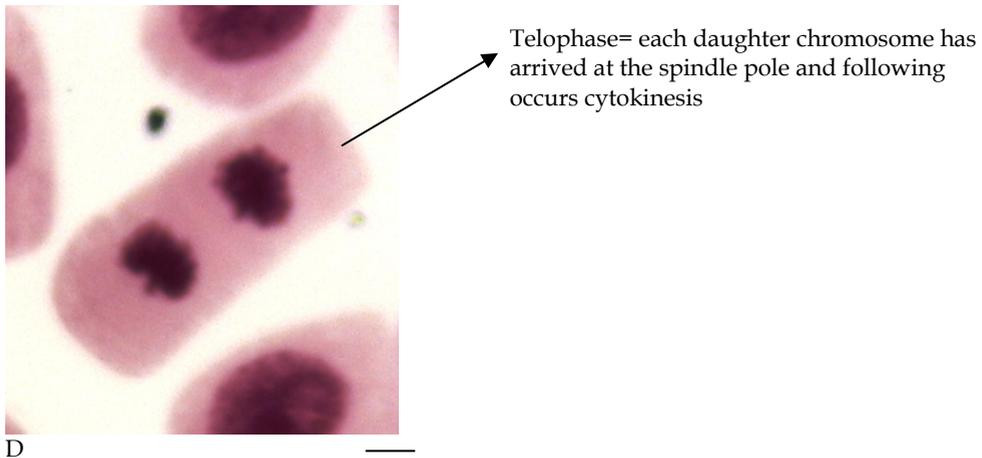
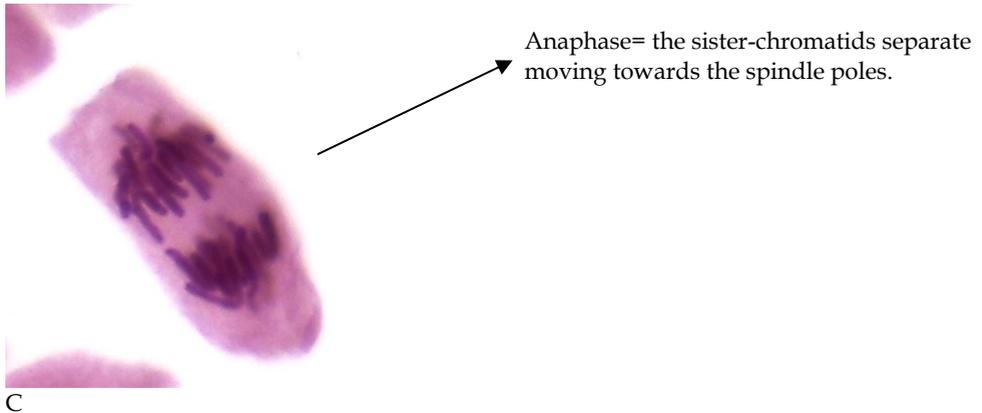


Fig. 4. *Allium cepa* cells in regular or normal division. A-interphase, B- prophase, C- metaphase, D- telophase. Scale bar= 10 μ

4. Use as a bioindicator in detecting the genotoxicity of hospital and industrial effluents

Cytogenetic tests are desirable for identifying the damaging effects of substances known in various concentrations under different exposure times for evaluation and influence on living organisms (Al-Sabti & Kurelec, 1985; Al-Sabti, 1989; Abdou et al., 1989; Arrigoni et al., 1989; Kakand Kaul, 1989; Kumar & Sinha, 1989; Rao, 1989; Chauhan & Sunderaraman, 1990; El-Khodary et al., 1990; Panda et al., 1990; Singh et al., 1990; Kumar et al., 1991; De-Serres, 1992). These tests provide data for understanding the harmful effects on tested organisms and are commonly used for biomonitoring pollutants, in addition to evaluating the effects of toxic and mutagenic substances on organisms in their natural habitat (Degrassi & Rizzoni, 1982; Al-Sabti & Kurelec, 1985; Dixit & Nerle, 1985; Fiskesjö, 1988; De Marco et al., 1988; Al-Sabti, 1989).

Industrial effluents have become one of the biggest problems in many developing and developed countries. It is known that these effluents, when not treated properly, can cause mutagenic or toxic effects directly on humans, affecting human health, resulting in diseases, such as cancer, congenital malformations, and cardiovascular diseases (Grover & Kauer, 1999). Studies by Siddiqui et al. (2011) were undertaken to validate plant-based tests for assessing the toxicity of water in India. In this study, the authors reported that plant-based bioassays have become increasingly popular for toxicological and eco-toxicological evaluations, and that the main reasons for the widespread use of the methods are simplicity, sensitivity and low cost, as well as the positive correlation with other toxicity tests.

Leme & Marin-Morales (2009) carried out an extensive review on the *Allium cepa* test and its use in environmental contamination, where they reported that vascular plants are recognized as excellent genetic models for detecting environmental mutagens and are frequently used in monitoring studies. *Allium cepa* is among the plant species used to evaluate DNA damages, chromosomal alterations and disturbances in the mitotic cycle. Furthermore, they reported that the test has been used to evaluate a large number of chemical agents, increasing its environmental application and it is a test characterized by being cheap. They also commented how the *Allium cepa* has advantages over other tests by the short preparation time for testing samples and although plants have low concentrations of oxidase enzymes, their results are consistent and can serve as a warning for other biological systems, since the target is DNA, which is common in all. In this review, they demonstrate that all types of effluents are also considered as complex mixtures and that the main results are of cytotoxicity, genotoxicity, and mutagenicity. In their review, Leme & Marin-Morales (2009) summarized data from several researchers on a large range of environmental contaminants and their genotoxic, cytotoxic or mutagenic effects on *Allium cepa*, such as pesticides, herbicides, metals and heavy metals.

Hospital effluents can cause severe problems to live organisms when not properly treated, and in developing countries, such as Brazil, environmental contamination by these effluents is not uncommon. This contamination is due to mutagenic compounds found within the effluent. Biomonitors (i.e. *Allium cepa*) can be used to alert the surrounding population of environmental contamination and genotoxic substances that have been released into the water. In a study by Bagatini et al. (2009), the *Allium cepa* test was used to evaluate the genotoxicity of a hospital effluent in Santa Maria, Rio Grande do Sul State, Brazil. During the study, chromosomal disruptions, anaphasic bridges, and micronuclei during telophase were observed, indicating environmental toxicity risk.

Laughinghouse (2007) studied the cytotoxic effects of crude extracts of cyanobacteria (blue-green algae), which can cause water pollution and the damages of direct toxin-producing strains can be tested using the *Allium cepa* test. The comparison of the toxic and genotoxic effects among species is fundamental for evaluating the biological risk of pollutants, particularly for compounds persistent in the environment (Bolognesi et al., 1999). The occurrence of blooms in continental waters used for human consumption causes essentially two problems for treatment. On one hand, being very small organisms, they can pass through filters at Water Treatment Plants (WTPs), reaching high densities in the distribution network. On the other, their toxins are not removed by the usual treatments (coagulation, flocculation, filtration, and disinfection), being even resistant to boiling. Besides these aspects, the traditional treatments can increase the risk of forming organochlorine

compounds from the group of trihalomethanes, which act as carcinogenic compounds when water rich in organic matter is treated with chlorine. It is important when there is a higher density of toxic cyanobacteria, not to resort to using pre-chlorination, but to use activated charcoal filters and ozone, which remove the toxins in the water more efficiently (Schmidt et al., 2002; Antoniou et al., 2005; Azevedo, 2006). The accidental ingestion of waters with high levels of toxins (acute ingestion) can cause intoxications, characterized by gastroenteritis with diarrhea, vomiting, nausea, abdominal cramps and fever, hepatitis with anorexia, asthenia and vomiting, or death (Jochimsen et al., 1998). The continued ingestion of low doses of toxins (chronic ingestion) can lead to chronic liver disorders. In fact, there should be more studies showing what are the risks of chronic exposure are since there are many factors that can lead a person to be subjected to low doses of these toxins. These situations can also be triggered by the ingestion of mollusks, as filter feeders that accumulate non-lethal doses of these products in their tissues, which are passed along the food chain, finally to humans (Kuiper-Goodman et al., 1999; Azevedo, 2006; Carvalho, 2006).

The decrease in water quality, especially of environments used for public water supply, irrigation and recreation, is of concern. The increase in eutrophication in these systems by higher nutrient loads (especially phosphorus and nitrogen) have favored the predominance of toxigenic cyanobacteria threatening human and animal health, aside from elevating the cost of water treatment. Thus, as a result of eutrophication, many countries, have suffered from an increase of toxic blooms, which is a severe problem to public health (Werner & Laughinghouse IV, 2006).

5. Use as a 'warning' bioindicator in detecting genotoxicity of medicinal plants

Various species of medicinal plants are used in popular medicine for the treatment of illnesses. However, the presence of cytotoxic and mutagenic substances in their composition or resulting from their metabolism can cause damage to human health. The mutagenic effects result in chromosomal alterations detected during the cell cycle through cytogenetic analysis. What does genotoxicity mean? It refers to the capacity of clastogenic agents causing lesions in the genetic material. Genotoxic agents can be defined functionally for possessing the ability to alter DNA replication and genetic transmission. The evaluations of genotoxicity include, mainly, damage in the DNA, mutations and chromosomal alterations. The observation of cells in interphase and cell division is used as an indicator of adequate proliferation of the cells, which can be measured through the *Allium cepa* test system.

The studies by various authors, such as Vicentini et al. (2001) and Camparoto et al. (2002) were performed with the *Allium cepa* test to test the genotoxicity of complex mixtures, in reality known as teas or extracts. The evaluation of the genotoxic effects of the plant extracts has been studied by Chauan et al. (1999), who indicated the sensitivity of the *A. cepa* system and correlated it with the mammal test system, validating its use as an alternative test for monitoring the potential genotoxicity of environmental chemicals and pesticides.

Meristematic onion cells and rat cells were used as test systems to verify the effects of genotoxicity of extracts (infusions) of medicinal plants such as *Maytenus ilicifolia* Mart and

Bauhinia candicans Benth. by Camparoto et al. (2002) demonstrating that there was not a significant difference in the decrease of the mitotic index in both cases studied; there was only a decrease in the mitotic index of the meristematic cells in the onion, whose bulbs were treated with a higher concentration (10 x higher than the one used by the population in the form of medicinal tea) of *Bauhinia candicans*. These studies indicated that the use of these plants could be continued, as long as they are always used in the recommended dosage.

Lubini et al. (2008) analyzed the genotoxicity of two species of *Psychotria* (*P. leiocarpa* and *P. myriantha*) through the *Allium cepa* test and the results indicated that both species possess capacity to inhibit cell division and *P. myriantha* possesses genotoxic activity. Çelik et al. (2006) studied extracts of *Plantago lanceolata* L. and their results showed that aqueous extracts reduced mitotic index and chromosome aberrations in treatment groups compared to controls. The results of the presented study are therefore important since they suggested the anti-genotoxic effect of the *P. lanceolata* leaf extract. In order to reach certain conclusions about this subject, however, further research should be performed with different test systems.

Extracts are most commonly prepared by infusion or decoction, depending on the part of the plant used. In infusion, extraction is carried out when the plant material is maintained in boiling water, in a covered container, for a certain period of time. Infusions can be applied to plant parts of soft structure, which should be beaten, cut or pulverized roughly according to their nature so that they can be easily penetrated and extracted with water. However, decoction consists of maintaining the plant material in contact, during a certain period of time, with a boiling solvent (usually water). It is a technique of restricted use, since many active substances are altered by a prolonged period of heating and it is customary to employ it with rigid/woody plants (Simões et al., 2011).

Worldwide, many species of medicinal plants are used to treat illnesses. Most of these species are not thoroughly studied, especially regarding the presence of toxic/mutagenic substances in their composition or arising from their own metabolism, thus damaging the health of the population. The presence of mutagenic substances in the plant species that might cause chromosomal alterations can be detected during the cell cycle of a species. The *Allium cepa* test system is frequently used for evaluating the potential genotoxicity of medicinal plant extracts through the analysis of meristematic cells from root-tips treated with medicinal infusions (teas). The knowledge of the potential genotoxicity of these medicinal species, through the analysis of the *Allium cepa* cell cycle serves as an indicator of safety for the population, which uses medicinal teas as their only medical treatment. Bagatini et al. (2007) reviewed this subject and indicated the importance of the *Allium cepa* test as a preliminary screening of genotoxicity in medicinal plant infusions.

6. Types of results and interpretation through the analysis of plant cytogenetics

The results, which can be obtained by analyzing the rootlets subjected to different treatments of interest to researchers, are performed by cytogenetic analysis during cell division. The *Allium cepa* cell cycle can be taken into consideration after 24hrs, and is

divided into interphase and cell division, understanding the prophase, metaphase, anaphase, and telophase phases. Some authors, such as Singh (2002) consider all phases of cell division starting with interphase. In the case of the *Allium cepa* test for the interpretation of the results, the subdivision is necessary, because those cells which are in interphase are not considered as cells in division. According to Love (1949) the mitotic index can be calculated from the formula:

$$MI = \frac{\text{total number of cells observed (cells in interphase + number of cells in division)}}{\text{number of cells in interphase}} \times 100$$

To get a percentage, multiply the result by 100. The slide must be prepared from the meristematic region (by removing the root cap) and the squashing technique should be used (Guerra & Souza, 2002), soon after stained with acetic orcein 2% or another stain that has the same affinity for DNA packaged as chromosomes. The observation of the cells through the microscope can be interpreted based on the regular cell division of *Allium cepa*. Figure 5-A, 5-B, 5-C, 5-D, 5-E, 5-F represents the phases involved in the analysis of irregular mitotic division, being respectively, A and B- irregular anaphase, C- irregular metaphase, D- binucleate cell, E-adherent cell, and F- binucleate cell.

Depending on the tested substances, such as, herbicides used in agricultural practice or leftover drugs discarded after use, it is possible to observe if these substances are mutagenic or even antimutagenic. If they are mutagenic it is possible to immediately see this through the structural damages such as those in Figures 6-A, 6-B, 6-C, 6-D, 6-E, 6-F, 6-G, where there are chromosomes with breaks, simple anaphasic bridges, multiple anaphasic bridge, adhesions, laggard chromosomes, disorganization of the metaphase, and binucleated cells. If they are antimutagenic it is necessary to verify the reversion of the mutations occurred.

The use of antimutagens and anticarcinogens every day is the most efficient procedure to prevent human cancer and genetic illnesses. There are many ways in which the action of mutagens can be reduced or prevented. Rangunathan & Paneersel Vam (2007) studied the antimutagenic potential of curcumin on chromosome aberrations in *Allium cepa*. These authors recorded that turmeric has long been used as a spice and food coloring agent in Asia. The antimutagenic potential of curcumin was evaluated using *Allium cepa* root meristem cells. The authors found that curcumin insignificantly induced chromosomal aberrations. However, the authors noticed that this spice had an antimutagenic potential against sodium azide (known to induce chromosomal aberrations). Thus, the mechanism of action remains unknown, though curcumin presented an antimutagenic potential demonstrated by the *Allium cepa* test.

Other important studies, such as Rossato et al. (2010), took into account the different cultivation environments of medicinal plants used for alternative medicine and then tested by *Allium cepa*. These authors evaluated the antiproliferative effect of *Pluchea sagittalis* (Lam.) Cabrera infusions at three different concentrations (2.5, 5 and 25 gdm⁻³) of leaves of *P. sagittalis* grown in three environments (in vitro, greenhouse, and field). From this study, they concluded that the leaf infusions of the studied species have a large proliferative capacity and the plant cultivation system affects the values of mitotic index, i.e. the proliferative capacity of cells.

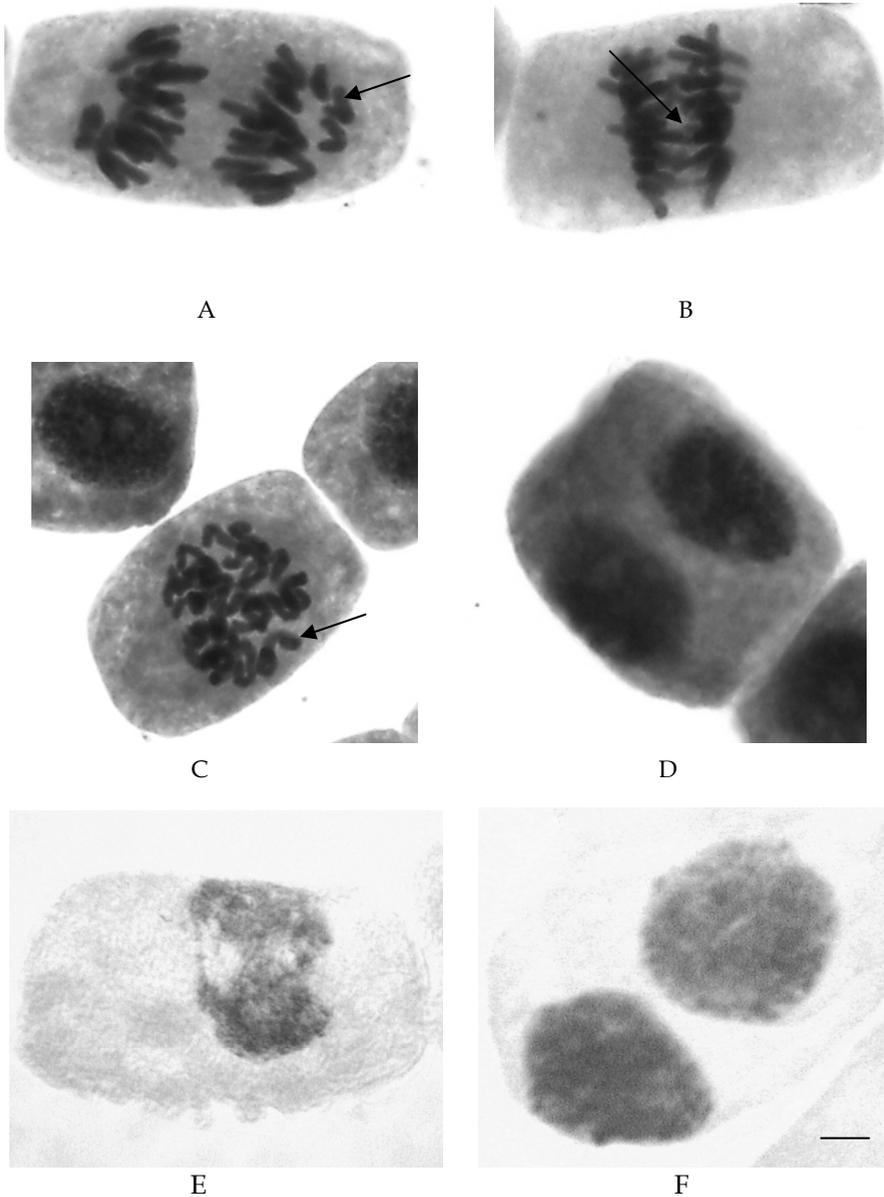


Fig. 5. *Allium cepa* meristematic cells showing the alterations due to the action of clastogenic agents. A- irregular anaphase, Arrow indicating lost chromosomes, B- irregular anaphase, with anaphasic microbridges, C- irregular metaphase, with unorganized chromosome, also known as C-metaphase, showing chromosomes with no orientation on the equatorial plate, D- irregular cell, binucleate, with an elliptical aspect, E- adherent nucleus, F- irregular cell, binucleate, with a round aspect. Scale bar= 10 μ

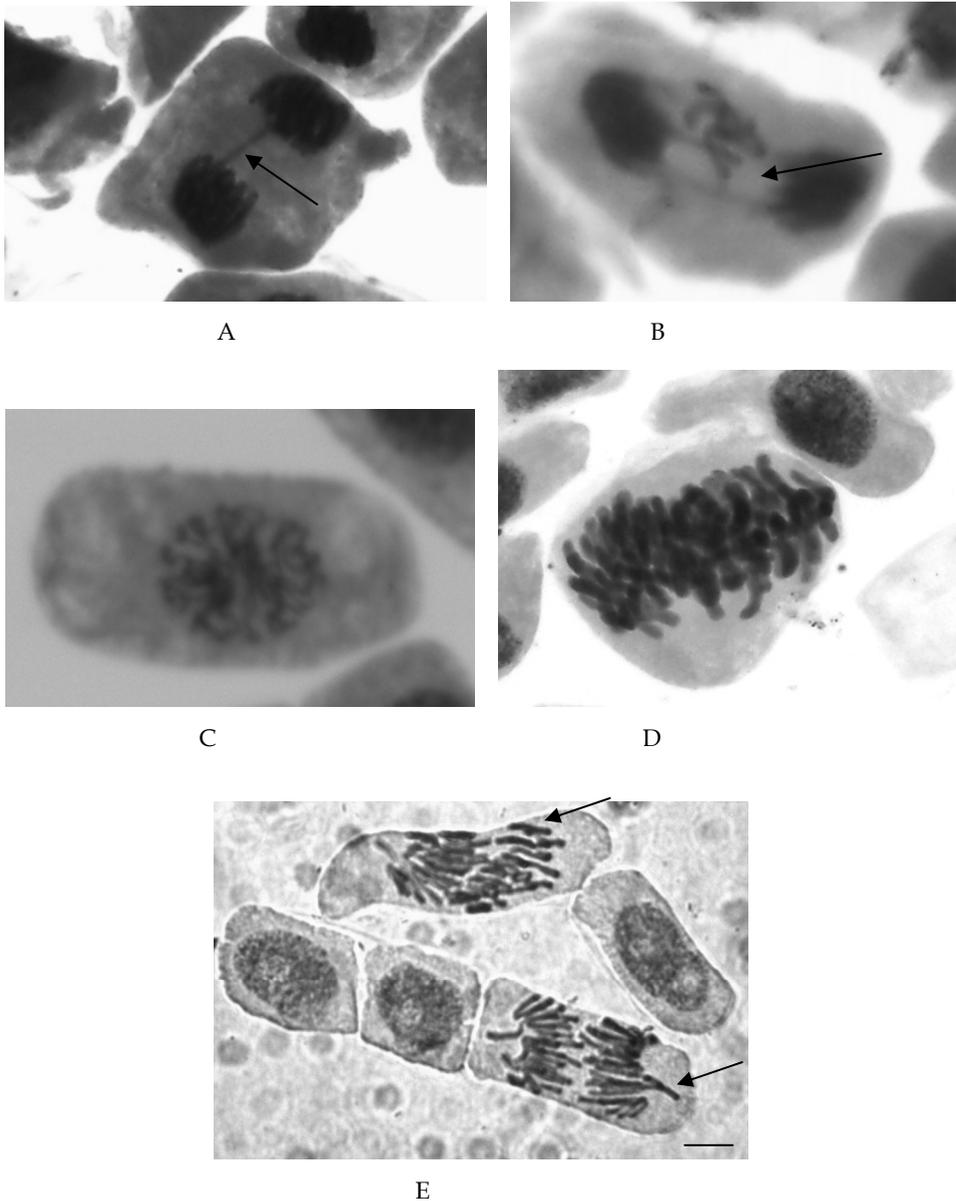


Fig. 6. Meristematic cells of *Allium cepa* showing the alterations due to clastogenic agents. A- cell in irregular anaphase, arrow indicates anaphasic bridge, B- anaphase with a bridge and arrow indicating lost chromosomes, C- irregular prophase, showing decompressed chromosomes, D- metaphase with numerical alteration, due to duplication of the number of chromosomes, E- cells in irregular anaphase, arrows indicating chromosomal breakage. Scale bar=10 μ

7. Conclusions

We conclude that the *Allium cepa* test is an excellent bioindicator of chromosomal alterations that serve as an alert for the population that uses medicinal teas indiscriminately, and that its constant use in the analysis of the treatment of industrial and hospital effluents is extremely adequate. Currently, due to major concern with environmental pollution, the *Allium cepa* test has occupied an important place for the prevention and prediction of environmental impact that will be caused by the use and disposal of substances including drugs and herbicides.

Although the test is merely a first assessment of genotoxicity, it always shows important scientific discoveries, and new adaptations of the test might reveal innumerable possibilities of its use, avoiding the use of animals for testing. More increments and analysis, as the sophistication of the method progresses, will lead us to get the most use for the benefit of the planet.

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