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Evaluation of the genotoxicity of aqueous extracts of leaves and stems of *Dolichandra unguis-cati* (L.) L.G. Lohmann (Bignoniaceae) using the *Allium cepa* test

Abstract

The province of Misiones is characterized by its great plant diversity and the use of medicinal plants is common, 80% of its boundaries are international, it borders the Republic of Paraguay to the west and the Republic of Brazil to the north and to the east, in both is also traditional the use of medicinal plants. Where in most cases they are marketed without previous toxicity studies. *Dolichandra unguis-cati* (Bignoniaceae) a species commonly known as "cat's claw", used in folk medicine to treat multiple conditions. The objective of this work is to analyze the genotoxic effects of aqueous extracts obtained from infusions of leaves and stems of *D. unguis-cati* using the *Allium cepa* test. The concentrations studied inhibited the growth of the roots, decreased the mitotic index with respect to a control and did not produce relevant genotoxic effects.

Keywords: genotoxicity, *D. unguis-cati*, aqueous extracts, *A. cepa test*

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Florentín P Alejandro, Altamirano Carlos G

Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Argentina

Correspondence: Altamirano Carlos G, Laboratorio de Farmacobotanica "Dr. Anibal G. Amat". Facultad de Ciencias Exactas, Químicas y Naturales. Universidad Nacional de Misiones, Argentina, Email carls-altamirano@live.com

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Introduction

D. unguis-cati (L.) L.G. Lohmann belongs to the family Bignoniaceae, it is a liana, with climbing stems with strong supportive roots, branches conspicuously lenticulate. With leaflets (2) lanceolate, oval-elliptic or oval-lanceolate, often with a tri-hook tendril, especially when young. Of solitary or 2-3 flowered flowers, bisexual, yellow or yellow-orange. Fruit is a lineal capsule, compressed, flat seeds, uniseriate in each loculo.[1] In popular medicine, it is used against venereal diseases (for example, syphilis), malaria,[2] for the treatment of rheumatism, bronchitis, flu and headache,[3] for the treatment of snake bite, diarrhea, fever, inflammatory reactions, to induce diuresis[4]. It was found that different extracts of ethyl acetate and ethanol from the aerial part without flowers were rich in phenolic compounds, the ethanol extracts showed significant anti-inflammatory activity in vitro tests, but nevertheless did not show great antitumor activity.[4]

There are few toxicity studies in *D. unguis-cati*, the acute and subacute toxicity of hydroalcoholic leaf extracts in rats was studied and behavioral changes, biochemical, hematological and histopathological parameters were determined, the results revealed no significant alterations and the tissues its normal physiological parameter.[5] The objective of this work is to evaluate the genotoxicity of aqueous extracts of the leaves and stems of *D. unguis-cati* (L.) L.G. Lohmann through the *Allium cepa* test. The *A. cepa* test is an excellent biological model, where the roots grow in direct contact with the substance of interest, which allows to predict the possible damage of the genetic material. Therefore, the results obtained can be extrapolated for all animal and plant biodiversity. Several extracts of plants of popular use have been studied by means of the test of *A. cepa*, in some it was found chromosomal aberrations, as for example extracts of *Maytenus laevis*,[6] like the extracts of *Psidium guajava*,[7] the mixture of Extracts of *O. gratissimum* and *M. lucida* inhibited cell division by blocking the cell cycle at the interphase.[8] In others, no significant genotoxic effects were found, such as in the alcoholic extracts of epicarp of *Crescentia cujete*.[9]

Materials and methods

Collection of *D. Unguis-cati*

D. Unguis-cati was collected in the city of Posadas to Lat.: -27.363626, Long.: -55.895259 and identified by means of routine taxonomic techniques. The herbal material was deposited in the Herbarium of the Department of Pharmacy in the Pharmacobotany Laboratory "Dr. Anibal Gumersindo Amat", from the Faculty of Exact, Chemical and Natural Sciences, National University of Misiones.

Conditioning of plant material

The bulbs of *A. cepa* were acquired in the local commerce, units of a homogeneous size were selected, of good aspect and integrity.

The leaves and stems of *D. unguis-cati* were cleaned, and then dried in an oven at 45°C for 48 hours. Subsequently they were crushed in manual mortar, until a homogeneous powder size was obtained.

Preparation of extracts of *D. unguis-cati*

The selected extractive method was the infusion, according to the Argentine National Pharmacopoeia 6th Edition, placing low sodium mineral water boiling, in contact with the necessary amount of previously ground drug, for the course of 20 minutes. Subsequently, the residue was filtered and washed until the volume necessary for each experience was completed.[10]

Pretreatment of bulbs

From the bulbs, their outermost cataphilles were removed, the disc

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was scraped with care not to damage the meristematic zone, and they were washed with running water for 2 hours, to eliminate any residue that could be contained in the commercial activity.[11]

Test of *A. cepa*

The *Allium cepa* test was performed in duplicate, using low sodium mineral water for all dilutions and controls. It was divided into two trials (Stage 1 and Stage 2), where in each experience 5 bulbs of *A. cepa* were selected for each concentration to be tested (testing 3 different concentrations in each experience), and 5 bulbs for control (considering a total of 20 bulbs per experience).[12] For both Stage 1 and Stage 2, the bulbs were placed in mineral water low in sodium for 24 hours, at room temperature, protected from light, until developing roots that reached an average length of 1cm. For the tests a device designed for the experiment was used (Figure 1) wiht glass containers to support the bulbs, submerging the disks, provided with a constant air supply by means of 2 aerators, the air is distributed by hoses towards each one of the containers. The different concentrations tested and the low sodium mineral water were replaced every 24 hours. The extracts of *D. unguis-cati* were prepared at the moment of being used by means of infusions, also using mineral water low in sodium.

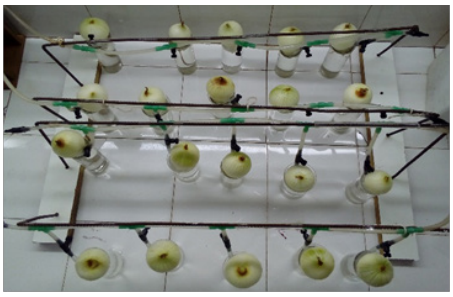


Figure 1 Experiment design.

Stage 1 and stage 2

After 48 hours since the rootlets were submerged, Stage 1 was carried out. In this instance, concentrations of 12.5g/L, 25g/L and 50g/L were tested. In Stage 2, concentrations of 12.5g/L, 20g/L and 30g/L were tested. In both stages, the bulbs were in contact with the extracts for 48 hours and in Stage 2, in addition, the 24-hour recovery stage in low-sodium water was continued in all bulbs.[12]

Obtaining genotoxicity biomarkers

To observe the state of mitosis in the meristems of the rootlets of *A. cepa* exposed to the extracts, histological preparations were made *in situ*. For this, the roots were harvested, they were fixed in Farmer[13] for 24 hours in a refrigerator at -3°C and then moved to 70°C alcohol where they were kept until the time of Preparation.[11] Under the magnifying glass, the meristematic zone was selected, placed on a slide with 2% acetic orcein and taken directly to the flame. Then the coverslip was placed and a slight pressure was exerted on the preparation ("squash"). Subsequently, it was observed in a BA2000 motic optical microscope and photographs were taken with a Motic10 camera for subsequent analysis.[12]

Mitotic index

The mitotic index were calculated as the ratio between dividing cells and total cells. A total of 1000 cells per root were counted, taking 2 roots for each bulb of each of the 20 bulbs in total, used for each experience.[12]

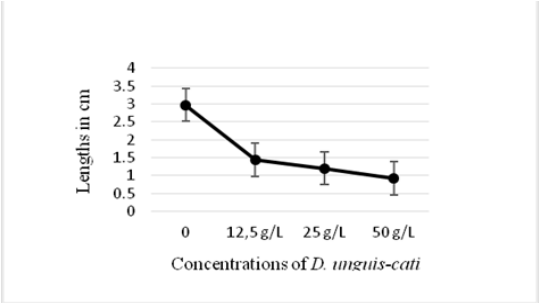
Statistical analysis

The statistical analysis was performed through Statgraphic 6.0.

Results

Stage 1 was carried out in order to obtain the concentration 50, which is the concentration of extract necessary for the growth in length of the roots of *A. cepa*, to reach 50% in comparison with the roots of the controls. The results obtained are shown in (Graph 1). The roots lengths less than 0.5cm were discarded from the analysis. As can be seen in Graph 1, the concentrations of 12.5g/L, 25g/L and 50g/L were tested, being the 50g/L dose the one that achieved the greatest decrease in root growth. High concentrations were tested in this instance to obtain the toxicity curve, and determine the ideal concentrations, where genotoxicity biomarkers are subsequently searched (in Stage 2).

Graph 1 shows the results obtained in Stage 1, the average lengths and the standard deviation of rootlets exposed to the different concentrations of extracts, and the control in low sodium water are observed. With the results obtained in this stage, we proceeded to design Stage 2 considering the concentration of 12.5g/L, analyzing the biomarkers of genotoxicity above this concentration, taking into account the behavior presented by the curve in this experience.



Graph 1 Average of results obtained in Stage 1.

Stage 2 and evaluation of extracts genotoxicity

In Stage 2, concentrations of 12.5g/L, 20g/L and 30g/L were tested, microscopic anomalies (chromosomal aberrations) and mitotic indices were analyzed for each of the concentrations studied. A total of 1000 cells per root were counted, from each bulb of each concentration analyzed (Figure 2) (Table 1).

Table 1 Summary of the results obtained in E1 and E2

Concentrations of *D. unguis-cati* extracts used in the experiment	Average lengths in cm (Stage 1)	Mitotic Indexes in% (Stage 2)	Observed chromosomal aberrations (Stage 2)
0,00 g/L	2,97±0,86	15.00±4.02	Not observed
12,50 g/L	1,44±0,52	12,00±2.50	Not observed
20,00 g/L	-	11,00±2,00	Not observed
25,00 g/L	1,20±0,46	-	Not observed
30,00 g/L	-	8,02±1,00	Not observed
50,00 g/L	0,92±041	-	Not observed

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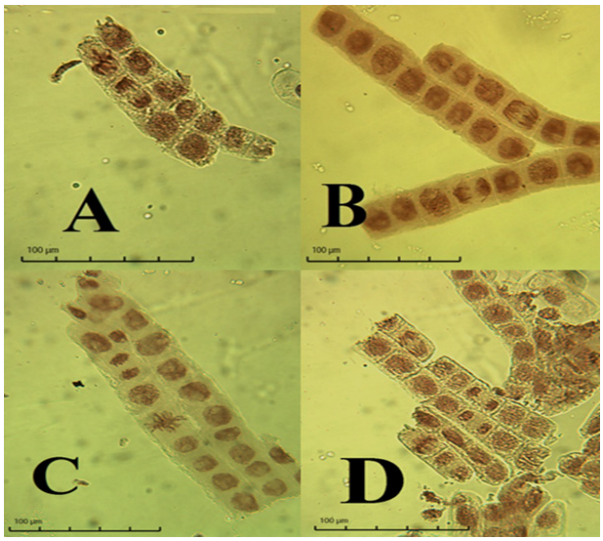

Figure 2 A, controls; B, C and D, roots exposed to C1=12.5 g/L, C2=20 g/L and C3=30 g/L respectively. Scale 100 µ.

Discussion

In Stage 1 it was observed how the length of the roots was affected by the presence of the extracts, the decrease in the growth in length of the roots is greater as the concentration of the extracts increases. In Stage 2, the highest concentration (30g/L) showed the highest inhibition of mitosis (mitotic index=8.02%) with respect to the control (mitotic index=15%), there is a notable inhibition of mitosis with regarding control, approximately 45%. The concentrations (20g/L) (mitotic index=11) and (12.5g/L) (mitotic index=12) also inhibited mitosis with respect to the control by 26% and 20% respectively. This decrease in the mitotic index as the concentration of the extracts increases coincides with the results of other medicinal extracts, for example extracts of *Psidium guajava*,[7] of *Solidago microglossa*[14] and of *Pterocaulon polystachyum*.[15]

Conclusion

In Stage 1, the concentration that turned out to be the most cytotoxic is that of 50g/L (the concentration that most inhibited the growth of the roots), which decreased the growth of the roots by 69.03% with respect to the control. In Stage 2, the highest concentration 30g/L showed the greatest inhibition of mitosis (mitotic index=8.02%), with respect to the control (mitotic index=15.00%). Where at higher concentrations, most of the dividing meristematic cells remained in interphase. Although the mitotic indexes decreased depending on the increase in the concentration of the extract, the cells in different mitotic phases found did not present micronuclei, anaphase bridge, delayed chromosomes or other types of biomarkers of genotoxicity in the studied preparations of the roots exposed in the different concentrations of *D. unguis–cati*. This inhibitory effect of mitosis could be due to a mechanism of defense of the meristematic cells of *A. cepa*, which under unfavorable conditions stop the cell cycle, which then continues in the recovery period in contact with low sodium water, without developing none of the chromosomal aberrations. It should be noted that the concentrations that have been used in the experiment are not the usual concentrations of *D. unguis-cati* in folk medicine. It is concluded based on the results obtained, that the extracts of the leaves and stems of *D. unguis-cati* in the concentrations studied do not present genotoxic effects. The need for future research in the same and in other biological models is proposed, with the objective of determining the safety limit of the aqueous extracts of the leaves of *D. unguis-cati*.

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None.

Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. Burkart A. *Flora Ilustrada de Entre Rios (Argentina)*. Parte V: Dicotiledóneas Metaclamídeas (Gamopétalas). A: Primulales a Plantaginales. Instituto Nacional de Tecnología Agropecuaria (INTA); 1979.
2. Lorenzi H, Matos FJDA. Plantas medicinais no Brasil: nativas e exóticas. 2nd ed. São Paulo: Instituto Plantarum; 2008.
3. Duke JA. *Duke's handbook of medicinal plants of Latin America*. CRC Press; 2008.
4. Aboutabl EA, Hashem FA, Sleem AA, et al. Flavonoids, anti-inflammatory activity and cytotoxicity of *Macfadyena unguis-cati* L. *African Journal of Traditional, Complementary and Alternative Medicines*. 2008;5(1):18–26.
5. Brondani JC, Reginato FZ, da Silva Brum E, et al. Evaluation of acute and subacute toxicity of hydroethanolic extract of *Dolichandra unguis-cati* L. leaves in rats. *J Ethnopharmacol*. 2017;202:147–153.
6. Freyre S, Estrada M, Bolaños H. Estudio preliminar de la citotoxicidad y la genotoxicidad de un extracto de origen vegetal conocido como palmo rosado en células meristemáticas de *Allium cepa*. *Memorias*, 2009;5(12):12–17.
7. Fernández V, Sales L, Gómez A, et al. Evaluacion citotoxica de *Psidium guajava* L. utilizando como bioensayo el *Allium test*. *Steviana*. 2009;1(1).
8. Oyedare BM, Bakare AA, Akinboro A. Genotoxicity assessment of water extracts of Ocimum gratissimum, Morinda lucida and Citrus medica using the Allium cepa assay. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*. 2009;8(2).
9. Espitia-Baena JE, del Rosario Duran-Sandoval H, Fandiño-Franky J, et al. Química y biología del extracto etanólico del epicarpio de Crescentia cujete L.(totumo). *Revista Cubana de Plantas Medicinales.* 2011;16(4):337–346.
10. Farmacopea Nacional Argentina. 6th ed. Buenos Aires; 1978. 76 p.
11. Fiskesjo G. The Allium test in wastewater monitorin. *Environmental Toxicology and Water Quality*. 1993;8(3):291–298.
12. Mudry MD, Carballo MA. *Genética Toxicológica*. De los Cuatro Vientos Editorial: Buenos Aires, Argentina; 2006.
13. Zarlavsky GE. *Histología Vegetal: Técnicas simples y complejas*. Sociedad Argentina de Botánica: Buenos aires; 2014. 198 p.
14. Bagatini MD, Fachinetto JM, Silva ACFD, et al. Cytotoxic effects of infusions (tea) of Solidago microglossa DC.(Asteraceae) on the cell cycle of Allium cepa. *Revista Brasileira de Farmacognosia.* 2009;19(2B):632–636.
15. Knoll MF, Silva ACFD, Canto-Dorow TSD, et al. Effects of Pterocaulon polystachyum DC.(Asteraceae) on onion (Allium cepa) root-tip cells. *Genetics and Molecular Biology.* 2006;29(3):539–542.