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Antiproliferative and genotoxic effects of *Mikania glomerata* (Asteraceae)

GRACIELI DALLA NORA[1], TAMARA PASTORI[1], HAYWOOD DAIL LAUGHINGHOUSE IV[2,3], THAIS SCOTTI DO CANTO-DOROW[1], SOLANGE BOSIO TEDESCO[1*]

1. Department of Biology, Universidade Federal de Santa Maria, UFSM, Santa Maria, RS, Brazil.
2. Department of Botany, National Museum of Natural History Smithsonian Institution, Washington, DC, USA.
3. Department of Environmental Science and Technology, College of Agriculture and Natural Resources, University of Maryland, College Park, MD, USA.

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ABSTRACT: *Mikania glomerata* is a plant used in Brazilian traditional medicine, known as 'guaco'. It possesses anti-inflammatory properties and the aqueous extracts of its leaves are indicated for the treatment of diseases of the respiratory tract. This study aimed at evaluating the antiproliferative and genotoxic effect of *Mikania glomerata* leaf infusions on the cell cycle of onion. The material used was collected in the native environment from Rio Grande do Sul State, Brazil. Aqueous extracts through infusions were prepared in two concentrations: 4g/L (usual concentration) and 16g/L (4x more concentrated) of each of the populations. Two groups of four onion bulbs for each plant population were used plus a control group. The rootlets were fixed in ethanol-acetic acid (3:1), conserved in ethanol 70% and slides were prepared using the squashing technique colored with orcein 2%. The cells were observed and analyzed during cell cycle. Per group of bulbs, 2000 cells were analyzed, and the mean values of the cell number of each of the phases of the cell cycle were calculated, determining the mitotic index (MI). Statistic analyses of the data were carried out by the x^2 ($p= 0.05$) test. We conclude that *M. glomerata* presents both antiproliferative and genotoxic activity.

Introduction

Medicinal plants are used worldwide for the treatment of diseases, and the majority has not been sufficiently studied, in relation to their cytotoxic/mutagenic potential, which can be monitored using the *Allium cepa* test, for their secure and efficient usage.

Among species used are those pertaining to the genus *Mikania*, with about 430 species distributed around the pantropics, temperate America, and southern Africa, but with its two major diversity centers in the highlands of southeastern Brazil and the eastern foothills of the Andes, ranging from Bolivia to Colombia (King and Robinson, 1987; Holmes, 1995). Around 171 species have been cited for Brazil (King and Robinson, 1987). Although presenting a large number of species, the genus *Mikania* has been little studied in Brazil. The only study known is that from Barroso (1958) and of species lists in some regional floras, like Cabrera and Vittet (1963) and Cabrera and Klein (1989) for Santa Catarina State and Angely (1956) for Paraná State, among others. For the state of Rio Grande do Sul, we only know of species lists for certain regions of the State, in addition to the survey of species of two sections of *Mikania* (Ritter *et al*., 1992). The genus *Mikania* belongs to the Family Asteraceae Backer, Tribe Eupatorieae.

*Address correspondence to: Solange Bosio TEDESCO.
E-mail: *stedesco@smail.ufsm.br*
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Mikania glomerata has been a widely used plant in Brazilian traditional medicine for centuries. This species is native to southern Brazil, where it is known as 'guaco', and is recognized for its described anti-inflammatory properties (Suyenaga *et al*., 2002). Leaf extracts are indicated for treating diseases of the respiratory tract, aiding in the combat of coughs, due to its chemical composition (Freitas, 2006), including the treatment of asthma and bronchitis (Teske and Trentini, 1997; Silva *et al*., 2006; Soares *et al*., 2006; Agra *et al*., 2008). It's also used in mouthwash and gargles for mouth and throat inflammations, along with applying its dye locally on bruises or in compressions on parts affected by traumatisms or rheumatic pains (Lorenzi and Matos, 2002). Studies also demonstrated its anti-allergenic (Fierro *et al*., 1999) and anti-microbial (Pessini *et al*., 2003; Amaral *et al*., 2003; Santos *et al*., 2003; Duarte *et al*., 2004) activities, in addition to analgesic (Ruppelt *et al*., 1991), anti-inflammatory (Falcão *et al*., 2005), antioxidant (Vicentino and Menezes, 2007), and anti-diarrheic (Salgado *et al*., 2005) activities. The principal substance that confers this identity to 'guaco' is coumarin (1,2-benzopyrone), for being present in large quantities in the leaves (Oliveira *et al*., 1984; Leite *et al*., 1992; Leal *et al*., 1996).

Due to the importance and medicinal use, this study was aimed at evaluating the antiproliferative and genotoxic effect of infusions of *Mikania glomerata* leaf infusions on the cell cycle of *Allium cepa*.

Material and Methods

Leaves sampling

The experiment was developed in the Laboratory of Plant Cytogenetics and Genotoxicity (LABCITOGEN) of the Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil. The plant material (leaves) were collected from plants growing naturally on the Campus of the Universidade Federal de Santa Maria, municipality of Santa Maria, RS, Brazil Lat (DMS) = 29º42'53.92"S e Long (DMS) = 53º43'03.25"O and plants naturally growing in woods in the township of Rincão da Várzea, municipality of Pinhal Grande Lat (DMS) = 29º20'04.05"S e Long (DMS) = 53º18'37.21"O. Herbarium vouchers of the specimens are deposited in the Herbarium SMDB (Santa Maria Department of Biology), of UFSM.

Preparation of aqueous extracts

Dry leaves of *Mikania glomerata* were placed in boiling water, infusing for 10 min, and then the aqueous extracts were strained and allowed to cool at room temperature. The infusions were prepared in two concentrations 4g/L and 16g/L, constituting the treatments T1 and T2, respectively, for each of the 2 populations studied. Two groups of four bulbs of *Allium cepa* from

TABLE 1.

***Allium cepa* meristematic cell numbers in the different cell cycle phases, treated with different aqueous extracts of *Mikania glomerata* collected in the municipalities of Santa Maria and Pinhal Grande, RS, Brazil.**

Species	Treatment	Interphase	Prophase	Metaphase	Anaphase	Telophase
	Control	1866	75	23	18	18
M. glomerata	[4g/L]	1898	41	20	15	26
(Santa Maria)	[16g/L]	1980	15	3	1	1
	Control	1866	75	23	18	18
M. glomerata	[4g/L]	1910	48	20	7	15
(Pinhal Grande)	[16g/L]	1976	23	1	0	0

Treatment time: Control = 0h (t zero), distilled water; Treatments: treatment 1 [4g/L], treatment 2 [16g/L] = 24h

commercial origin were used per treatment for each sampling location of *Mikania* and 01 control group in distilled water. The experiment was set-up by placing all the bulbs in distilled water to root; afterwards the same bulbs were submitted to the treatments with the aqueous extracts T1 and T2 for 24h, while the control groups remained in distilled water. Following, the rootlets were collected and fixed in ethanol-acetic acid (3:1) for 24h. After this period, they were taken out of the fixator, maintained in alcohol 70%, and conserved in the refrigerator until slide preparation.

Effects of aqueous extracts on the cell cycle of Allium cepa

In average, two rootlets per bulb were used during the preparation of the slides, which were hydrolyzed in HCl 1N for 5 min, washed in distilled water, and colored with acetic orcein 2%. The slides were prepared according to the squashing technique (Guerra and Souza, 2002) and examined with light microscopy at 400x for cell cycle phase (interphase, prophase, metaphase, anaphase, and telophase) observation. Per group of bulbs, 2000 cells were analyzed, calculating the mean values of the cell numbers in each of the cell cycle phases of *A. cepa,* and determining the mitotic index (MI).

Statistical analysis of the data was carried out by the x^2 test (p= 0.05), using the statistical program BioEstat 3.0 (Ayres, 2003).

Results

Table 1 presents the total number of analyzed cells and the number of cells in each of the different cell cycle phases from the *Allium cepa* cells, which were treated with *M. glomerata*.

In Table 2, the total number of analyzed cells, as well as the number of cells that were observed in interphase and in different phases of cell division during the *A. cepa* cell cycle is presented. In this same table, we also show the mitotic index rates and the percentage proportion of cells with inhibited cell division in relation to the control.

For the species *M. glomerata* from the municipality of Santa Maria, the rates referring to the MI were 7.18% for the control, 5.37% for T1, and 1.01% for T2, with a significant difference between the control and treatments (x^2=84.643), between the treatments, (x^2=56.849), and between the control and T2 (x^2=87.769); however, not differing significantly between the control and T1 (x^2=4.611). The amount of cell cycle irregularities (CCI), also relating to the cells in division, were 0% for control, 11.25% for T1, and 4% for T2, differing significantly between the control and the treatments (x^2=43.571), though not differing significantly between T1 and T2 (x^2=0.721).

Table 3 presents the total number of cells in division and the chromosomal alterations observed in the analyzed cells. Through the analysis of the mitotic index (MI) and the cell cycle irregularities (CCI) from

TABLE 2.

Cell numbers in the cell cycle (interphase, prophase, metaphase, anaphase, and telophase) of *Allium cepa* root-tips treated with aqueous extracts of *Mikania glomerata*, collected in the municipality of Santa Maria and Pinhal Grande, RS, Brazil.

Species	Treatment	Number of total cells	Cells in Interphase	Cells in Division	Mitotic Index (%)
	Controle	2000	1866	134	7.18[a]
M. glomerata	[4g/L]	2000	1898	102	5.37[b]
Population I	[16g/L]	2000	1980	20	1.01[c]
	Controle	2000	1866	134	7.18[a]
M. glomerata	[4g/L]	2000	1910	90	4.71[b]
Population II	[16g/L]	2000	1976	24	1.21[c]

Averages followed by the same letter do not differ significantly at the 5% level, by the x^2 test.
Treatment time: Control = 0h (t zero), distilled water; Treatments: treatment 1 [4g/L], treatment 2 [16g/L] = 24h

rootlets of *A. cepa*, CCI were found from cells in cell division submitted to the aqueous extracts of *M. glomerata*, like chromosomal breakages, retardations, and fragment formations; anaphasic bridges; and micronuclei in telophase (Figs. 1a, b, c, d, e). No CCI were observed during interphase. There was a significant difference from the control, in relation to the appearance of CCI, when compared with the treatments (Table 3).

For the species *M. glomerata* from Pinhal Grande, the MI values (Table 2) of the *Allium cepa* cells were 7.18% for the control, 4.71% for T1, and 1.21% for T2. These results differed significantly between the control and the treatments (x^2=77.359), between the treatments (x^2=39.331), and between the control and T2 (x^2=79.732), despite not differing significantly between the control and T1 (x^2 =9.156). The CCI values (Table 3) in relation to the cells in division were 0.8% for the control, and 0% for the treatments, differing significantly between the control and the treatments (x^2=28.245), while not differing between T1 and T2 (x^2=4.632).

We observed that the cell division inhibition in the *A. cepa* roots exposed to T2 from *M. glomerata* from Santa Maria was 80.33%, and for the bulbs treated with T2 from *M. glomerata* from Pinhal Grande was 72.83% (Table 2).

Discussion

Mutagenic agents can be cytologically detected by the inhibition of the cell cycle, interruption in metaphases, induction of numerical and structural chromosomal alterations, and exchanges within sister-chromatids, among others (Vieira and Vicentini, 1997).

In this study, the evaluation of the effects of aqueous extracts of *M. glomerata* using the *A. cepa* test system demonstrated that the species significantly inhibited cell division, in both of the analyzed concentrations (Table 2). This indicates the antiproliferative capacity of the aqueous extracts of *M. glomerata*. Besides this, *M. glomerata*, collected in both locations, presented chromosomal aberrations in significant proportions in T1 and T2 (Table 3), which involves genotoxic activity of the species. In Figure 1, mitotic irregularities caused by clastogenic agents in the plant system treatments *in vivo* can be observed, known as anaphasic bridges (Figs. 1a, b, d), chromosomal breakages (Figs. 1a, b, d, e, f), disoriented or lost chromosomes (Figs. 1a, b, f), binucleated cell (Fig. 1c).

In population I and II, chromosomal aberrations rates of 39.2% and 20% were found, respectively, in the usual concentration for consumption of medicinal teas. In population I, in infusions 4 times more concentrated occurred 39,2% of chromosomal aberrations in cells found in cell division. However, in the plant populations collected in Pinhal Grande, the same results did not take place for the same analyzed standard. There was no evidence in the Pinhal Grande population of genotoxic activity in the 16g/L concentration, which can be explained by the genetic variability existent in this population from another location or it can be a result from a larger number of prophases (Table 3). We observed that from all the cells that presented chromosomal aberrations, they never appeared during prophase.

We emphasize that plants collected in distinct locations produce different quantities of secondary metabolites, and this reflects during the use of their teas in different concentrations. It is important to consider the apoptotic mechanisms that should have probably been

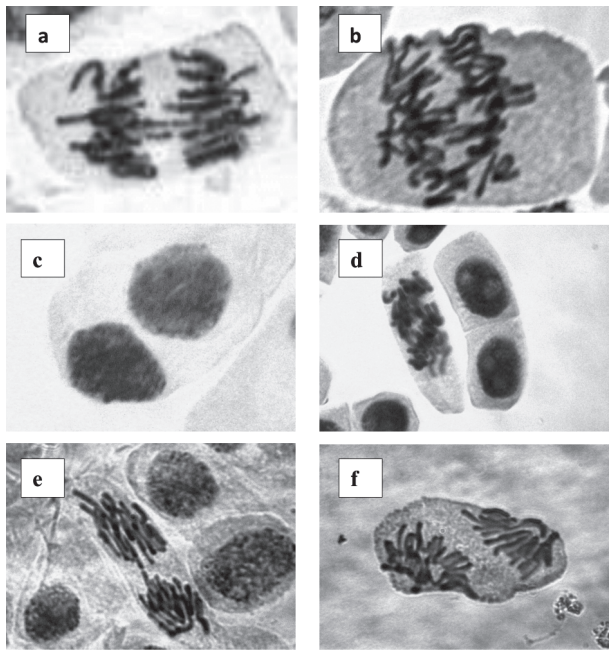

FIGURE 1. *Allium cepa* cells treated with aqueous extracts of *M. glomerata*. **a**- Anaphase with bridge, chromosomal breakages and **b**- Irregular anaphase with chromosomal breakages and retardation (extracts from the Santa Maria population 4g/L), **c**- Binucleated cell (submitted to the 16g/L treatment of the Santa Maria population), and **d**- Anaphase with bridges, breakages, and lagging chromosomes (submitted to the 16g/L treatment of the Santa Maria population); **e**- Anaphase with a bridge and chromosomal breakages and **f**- Irregular anaphase with chromosomal breakages (extract from the Pinhal Grande population, 4g/L).

activated in larger concentrations in plants from Pinhal Grande, due to the environmental differences. Studies demonstrated that plants of *M. glomerata* cultivated in greenhouses through micropropagation and vegetative reproduction presented differences in the quantity of coumarin in their leaves (Teixeira *et al*., 2003; Vicentini *et al*., 2001). Therefore, reforcing that in this case, the fact of chromosomal aberrations not appearing in population II in the more elevated concentrations can be attributed to the genetic variability of the metabolite production in higher concentrations. Nevertheless, the existence of genotoxic activity of the compounds found in 'guaco' leaves was evidenced, principally in the usual concentration for consumption.

Studies by Bolina *et al.* (2009) demonstrated that *M. glomerata* possesses coumarin (1,2-benzopyrone), triterpenes/steroids, and flavanoic heterosides within in its chemical makeup. In the study by leaves (Oliveira *et al*., 1984) the presence of alkaloids, saponins, tannins, and polyphenols in the aerial parts of *M. glomerata* was reported. There are many compounds present in the species that can be responsible for inhibiting cell division and causing chromosomal aberrations, nonetheless, the principal compound found in the leaves is coumarin.

The mitotic index and replications index are used as indicators of adequate cell proliferations (Gadano *et al*., 2002), which can be measured using the plant testing system *A. cepa*. The method of chromosomal aberrations in *Allium cepa* root-tips is validated by the International Programme on Chemical Safety (IPCS) and the United Nations Environmental Programme (UNEP), as an efficient test for the analysis and monitoring *in situ* of genotoxicity of environmental substances (Cabrera and Rodriguez, 1999: Silva *et al*., 2004).

Cytotoxicity tests using plant system tests *in vivo*, like *A. cepa*, have been validated by various researchers which conducted animal tests *in vitro* together with the plant tests, obtaining similar results (Teixeira *et al*., 2003; Vicentini *et al*., 2001). The chromosomal and meristematic root-tip cell division aberrations of the onion are frequently used to alert the population about consuming a product (Vicentini *et al*., 2001). Another study using the methanol extract of dried latex and the crude dried latex of *Calotropsis procera* demonstrated anti-mitotic activity in the *Allium cepa* model (Sehgal *et al.*, 2006).

Researchers Fachinetto *et al*. (2007) demonstrated the antiproliferative capacity of *Achyrocline satureioides* (Lam.) DC infusions that in high concentrations also inhibited cell division of *A. cepa.* In the same way, Lubini *et al*. (2008) verified that *P. myriantha* Müll. Arg. and *P. leiocarpa* Cham. & Schlecht. infusions possess

TABLE 3.

Mitotic aberrations in the *A. cepa* root-tips treated with the aqueous extracts of *Mikania glomerata*, collected in the municipality of Santa Maria and Pinhal Grande, RS, Brazil.

Species	*M. glomerata (Pop. I)*			*M. glomerata (Pop. II)*		
Treatment		Control	[4g/L]	[16g/L]	Control	[4g/L]	[16g/L]
Number of cells in Division		134	102	20	134	90	24
Cell aberrations	*Breakage*	-	1	-	-	4	-
	Chromosome retardation	-	15	2	-	1	-
	Bridges	-	3	-	-	2	-
	Micronucleus	-	3	1	-	2	-
	Binucleated	-	-	2	-	-	-
	Disorganized division	-	18	-	-	9	-
Total cells with aberrations		-	40	5	-	18	-
Cell Aberrations (%)		0	39.2%	25%	0	20%	0

Treatment time: Control = 0h (t zero), distilled water; Treatments: treatment 1 [4g/L], treatment 2 [16g/L] = 24h

antiproliferative effects on the *A. cepa* cell cycle, along with aqueous extracts of *P. myriantha* expressing genotoxic activity. Kumar and Singhal (2009) evaluated the response of germinating mung beans and *Vigna radiata* (syn. *Phaseolus aureus*) to several drugs and plant extracts. The growth retardation brought about by drugs was found associated with a significant reduction in mitotic index in the root tip meristematic tissue.

Through the analysis of the results of infusions in different concentrations of two populations of the species *M. glomerata* by the test system *A. cepa,* it's possible to verify the antiproliferative activity of the species as well as its genotoxic activity. The indication of using *M. glomerata* as a potential therapeutic to inhibit the eukaryotic cell cycle is not valid, since it possesses genotoxic activity. More studies are necessary to establish the secure use of *M. glomerata* as a medicinal tea.

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