Genetics and Molecular Biology, 29, 3, 539-542 (2006)

www.sbg.org.br

Research Article

Effects of *Pterocaulon polystachyum* DC. (Asteraceae) on onion (*Allium cepa*) root-tip cells

Michelle Frainer Knoll, Antonio Carlos Ferreira da Silva, Thais Scotti do Canto-Dorow and Solange Bosio Tedesco

Departamento de Biologia, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

Abstract

In Brazil leaf infusions of the plant *Pterocaulon polystachyum* DC (Asteraceae) are used to treat digestive problems. We used plants from six *P. polystachyum* populations to prepare fresh aqueous leaf infusions at the concentration normally used in Brazil (2.5 g L^{-1}) and at twice (5 g L^{-1}) and four times (10 g L^{-1}) this concentration. We rooted onion bulbs in a water control or one of the infusions for 24 h and used the *in vivo* onion (*Allium cepa*) root-tip cell test to investigate the potential mutagenicity of the infusions by calculating the mitotic index for the control and the experimental treatments and comparing them using the Chi-squared test (χ^2, p = 0.05). There was a highly significant decrease in the mitotic index of root-tip cells treated with infusion as compared to those exposed to water only. These results indicate that *P. polystachyum* infusions present cytotoxic and anti-proliferative activity and therefore have therapeutic potential.

Key words: medicinal plants, mutagenicity, onion (*Allium cepa*) test, *Pterocaulon polystachyum*.

Received: June 14, 2005; Acepted: November 11, 2005.

Introduction

Medicinal plants are used in both the pharmaceutical industry and as alternative non-prescription medicines, although systematic investigations of the therapeutic potential of such species are often lacking. Plant biodiversity is extensive but only 15% to 17% of known plant species have been systematically studied for their medicinal properties (Simões *et al.*, 2001).

In developing and non-industrialized countries, including Brazil, plant infusions are commonly used to treat a wide range of diseases (Teixeira *et al*., 2003). However, it has also been pointed out that while some medicinal plants may suppress the effects of mutagens others may contain toxic substances or provoke mutagenic effects (Vicentini *et al*., 2001) so mutagenicity testing is required before the use of a specific medicinal plant can be endorsed.

Brazil has one of the highest levels of biodiversity and potential genetic resources in terms of medicinal plants which can serve as primary sources for the manufacture of synthetic pharmaceuticals. One of the many plants used as popular non-prescription medicines In Brazil is *Pterocaulon polystachyum* ('quitoco' in Portuguese), which is found in southern Brazil, Paraguay, northeastern Argentina and Uruguay (Burkart, 1974) and is widely distributed in the southern Brazilian state of Rio Grande do Sul where infusions of its leaves are used in the treatment of various digestive disorders (Lopes, 1995), for the direct application to injuries and to repel insects (Zardini, 1983).

The effects of mutagens on eukaryotic nuclei can be assessed cytologically by observing inhibition of cell growth or division, interruption of metaphase or the induction of numerical and structural chromosomal aberrations and changes among sister and other chromatids (Vieira and Vicentini, 1997).

Cytotoxicity and environmental pollution (El-Shahaby *et al*., 2003) have been assessed by the *in vivo* onion (*Allium cepa*) root-tip cell test, which is known to give similar results to *in vitro* animal cytotoxicity tests (Teixeira *et al*., 2003; Vicentini *et al*., 2001, Chauhan *et al*., 1999). Onion root-tip cytotoxicity tests are based on the analysis of various parameters including atypical nucleolus patterns (*e.g.* heteromorphic pairing of nucleoli) and the appearance of micronuclei as a consequence of disordered mitosis and chromosomal breakdown (Grover and Kaur, 1999).

It is known (Vera, 2001) that *P. polystachyum* leaves contain compounds such as coumarin with known cytotoxic activity (Mongelli *et al.*, 2000; Riveiro *et al.*, 2004). The aim of our study was to use the *in vivo* onion (*Allium cepa*) root-tip cell test to investigate the cytotoxic and mutagenic potential of leaf infusions prepared from various *P. polystachyum* populations.

Send correspondence to Solange Bosio Tedesco. Departamento de Biologia, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Av. Roraima 1000, Cidade Universitária, Camobi, 97105900 Santa Maria, RS, Brazil. E-mail: stedesco@smail.ufsm.br.

Material and Methods

Plant material

The experiments were conducted at the Plant Cytogenetics Laboratory of the Federal University of Santa Maria (Universidade Federal de Santa Maria, UFSM), Rio Grande do Sul (RS) state, Brazil. We collected plants from different *Pterocaulon polystachyum* (*Asteraceae*) populations from six sites (populations P1 to P6) in Rio Grande do Sul: Boca do Monte, Santa Maria (P1); Paraíso do Sul, Santa Maria (P2); São Sepé road BR 392 (P3);São Pedro road intersection (P4); São Sepé (P5) Camobi road BR287, Santa Maria (P6). Voucher samples from each population were deposited in the UFSM herbarium.

Infusion preparation and onion root-tip cell test.

Fresh young leaves from the branches of *P. polystachyum* grown in your natural habitat were placed in boiling water for five minutes and the extracts filtered through filter-paper and cooled to room temperature. The infusions were prepared at the concentration normally used in Brazil (2.5 g L^{-1}) and at twice (5 g L^{-1}) and four times (10 g L^{-1}) this concentration.

For the onion root-tip cell test we used 18 *Allium cepa* bulbs divided into three groups (one for each infusion concentration) of six onion bulbs for each *P. polystachyum* population. For each infusion, all bulbs were rooted in water for three days after which four bulbs were placed in the infusion for 24 h, the remaining two bulbs being left in the water where they had rooted for a further 24 h to act as controls which had received no infusion. After 24 h the root-tips, measuring from five to ten millimeters, from both the control and experimental bulbs were collected and fixed in 3:1 (v:v) ethanol-acetic acid for 24 h before being placed in 70% (v/v) aqueous ethanol and refrigerated until needed. An average of five slides was made for each bulb using five root-tips which were hydrolyzed in 1N hydrochloric acid for five minutes and washed in distilled water, the fragmented meristematic region being stained with 2% (w/v) acetic orcein. Five fields were assessed for each slide and the number of interphase, prophase, metaphase, anaphase and telophase cells recorded using bright-field optical microscope with a 40X objective. For each *P. polystachyum* population we assessed 6000 cells for each treatment and for the controls and the mean values for the different cell-cycle phases and the mitotic index (MI) was calculated. Statistical analysis was performed using the Chi-squared (χ^2) test at $p = 0.05$.

Results

The number of interphase, prophase, metaphase, anaphase and telophase cells are shown in Table 1. For the 10 g L^{-1} infusion made from *P. polystachyum* population 1 leaves the number of anaphase cells was low and there were no prophase and metaphase or telophase cells, while for the 10 g L^{-1} population 2 infusion there were no telophase cells.

Table 2 shows the number of interphase and dividing cells along with the mean mitotic index (MI). For population 1 infusions the control mitotic index (7.5%) was significantly higher than for any of the extracts and these also differed significantly from each other with the lowest mitotic index being 0.03% for the 10 g L^{-1} infusion, similar results also occurring with population 2. For population 3 infusions and the control the mitotic index ranged from 7%

Table 1 - Number of onion root-tip cells in different phases of the cell cycle. The onions were placed for 24 h in water (control) or aqueous infusions of 2.5, 5 or 10 g per litre of fresh leaves from six different *Pterocaulon polystachyum* populations.

Pop	Number of cells in different phases of the cell cycle				
	Interphase	Prophase	Metaphase	Anaphase	Telophase
Population 1					
Control	5549	125	81	127	118
2.5	5725	72	52	61	90
5	5952	18	8	10	12
10	5998	0	0	2	0
Population 2					
Control	5361	1.004	416	581	588
2.5	5592	178	78	75	77
5	5844	52	24	43	37
10	5978	11	7	4	0
Population 3					
Control	5580	161	78	60	121
2.5	5690	199	68	87	97
5	5590	206	56	59	89
10	5549	129	50	42	89
Population 4					
Control	5663	80	90	57	110
2.5	5714	107	40	27	61
5	5680	128	66	32	94
10	5765	118	48	26	94
Population 5					
Control	5546	110	116	75	153
2.5	5829	84	53	44	91
5	5695	113	63	43	86
10	5728	52	42	34	43
Population 6					
Control	5602	150	68	72	102
2.5	5813	100	70	42	79
5	5839	35	49	21	56
10	5709	34	50	34	69

Pop: *Pterocaulon polystachyum* population and grams of leaves per litre of infusion.

Table 2 - Number of interphase and dividing cells and the mitotic index of onion root-tip cells. The onions were placed for 24h in water (control) or aqueous infusions of 2.5, 5 or 10 g per litre of fresh leaves from six different *Pterocaulon polystachyum* populations. The total number of cells analyzed for each treatment was 6000.

Pop	Number of cells		
	Interphase cells	Dividing cells	Mitotic index (%)
Population 1			
Control	5549	451	7.5a
2.5	5725	275	5.05b
5	5952	48	0.8c
10	5998	2	0.03d
Population 2			
Control	5361	639	10.64a
2.5	5592	408	6.8b
5	5844	156	2.6c
10	5978	22	0.4d
Population 3			
Control	5580	420	7a
2.5	5549	451	7.5a
5	5590	410	6.8a
10	5690	310	5.16b
Population 4			
Control	5663	337	5.6a
2.5	5714	286	4.7ab
5	5680	320	5.3a
10	5765	235	4b
Population 5			
Control	5546	454	7.5a
2.5	5728	272	4.5b
5	5695	305	5b
10	5829	171	2.8bc
Population 6			
Control	5602	392	6.6a
2.5	5709	291	4.8b
5	5839	161	2.6c
10	5813	187	3.1c

Pop: *Pterocaulon polystachyum* population and grams of leaves per litre of infusion.
Means followed by the same letter do not differ significantly by the chi-square test at p = 05.

to about 5% and decreased as the concentration of the infusion increased, but in this case there was no significant difference between the control and the 2.5 g L^{-1} infusion ($\chi^2 = 1.19$, $\alpha = 0.05$) and between the 2.5 g L^{-1} and 5 g L^{-1} infusions ($\chi^2 = 2.1$, $\alpha = 0.05$). The mitotic index for the population 4 infusions and the control ranged from 5.6% to 4% but there was no apparent relationship between mitotic index and infusion concentration, although there was a significant difference between the control group and the 10 g L^{-1} infusion for which the mitotic index was about 4% ($\chi^2 = 19.1$, $\alpha = 0.05$). For this population, as with population 3, the mitotic indices for the 2.5 g L^{-1} and 5 g L^{-1} infusions did not differ ($\chi^2 = 2.0$, $\alpha = 0.05$). For population P5 infusions and control the mitotic index ranged from 7.5% to 2.8% and the mitotic index for the control group differed significantly from that of all the treatments, but again the 2.5 g L^{-1} and 5 g L^{-1} infusions did not differ between themselves ($\chi^2 = 1.97$, $\alpha = 0.05$). The mitotic index of population 6 infusions and control ranged from 6.6% to 2.6%, with the mitotic index values for the control group being significantly different from those for all the infusions, although in this case there was no significant difference between the values for the 10 g L^{-1} and 5 g L^{-1} infusions ($\chi^2 = 2.0$, $\alpha = 0.05$).

Discussion

Our results show that the higher the 10 g L^{-1} *P. polystachyum* infusions produced the greatest inhibition of onion root-tip cell division (Table 1) and decreased mitotic index (Table 2) but there was no statistically significant intra or interpopulational variability for the *P. polystachyum* populations from different sites in regard to their cytotoxic effects.

Decreased mitotic index caused by *P. polystachyum* infusions have previously been reported by Mongelli *et al.* (1999) who studied Argentinean populations of this plant which have been used to treat cancers, these authors having demonstrated that *P. polystachyum* contains compounds which interact with the DNA. Our results regarding the cytotoxic activity of *P. polystachyum* support the work of Mongelli *et al*. (1999) and Riveiro *et al*. (2004) who have attributed these effects to the presence of coumarins.

Camparoto *et al*. (2002) have shown that *Maytenus ilicifolia* and *Bauhinia candicans* can cause decreased cell division in both onion root-tip and rat bone-marrow cells and decreased mitotic index in onion root-tip cells.

Riveiro *et al*. (2004) have reported that *P. polystachyum* extracts have antiproliferative and differential activities on human leukemic cells, indicating that such extracts may have therapeutic potential in the treatment of leukemia.

Our results not only support the antiproliferative and cytotoxic activity of *P. polystachyum* infusions but also show that at the concentrations studied there were no mutagenic effects on *in vivo* onion root-tip cells as witnessed by the fact that despite the large number of cells assessed there were no visible chromosome aberrations in either the treated or control cells.

Taken together our results indicate that not only is the onion root-tip test useful in assessing infusions prepared from different *P. polystachyum* populations but that such

infusions have cytotoxic and anti activities and may have potential for the therapeutic inhibition of the cell cycle in eukaryotic organisms, although further studies will be necessary to establish its use as an antimutagenic agent for use in the treatment of humans with proliferative diseases.

Acknowledgments

We are grateful Profa Dra. Maria Rosa Chitolina Schetinger of the Federal University of Santa Maria (Universidade Federal de Santa Maria) for a critical reading of the manuscript.

References

Burkart AF (1974) Flora Ilustrada de Entre Rios (Argentina). Coleccion Cientifica del I.N.T.A. v. 6. I.N.T.A., Buenos Aires, 554 pp.

Camparoto ML, Teixeira RO, Mantovani MS and Vicentini VEP (2002) Effects of *Maytenus ilicifolia* Mart. and *Bauhinia candicans* Benth infusions on onion root-tip and rat bone-marrow cells. Genet Mol Biol 25:85-89.

Chauhan LKS, Saxena PN and Gupta S K (1999) Cytogenetic effects of cypermethrin and fenvalerate on the root meristem cells of *Allium cepa*. Environmental and Experimental Botany 42:181-189.

Lopes AMV (1995) Plantas Usadas na Medicina Popular do Rio Grande do Sul. Caderno Técnico. Imprensa Universitária, Santa Maria, 74 pp.

El-Shahaby AO, Abdel Migid HM, Soliman MI and Mashaly IA (2003) Genotoxicity screening of industrial wastewater using the *Allium cepa* chromosome aberration assay. Pakistan J Biol Sci 6:23-28.

Grover IS and Kaur S (1999) Genotoxicity of wastewater samples from sewage and industrial effluent detected micronucleus assays. Mutation Res 426:183-188.

Mongelli E, Coussio J and Ciccia G (1999) Medicinal species of the Solanaceae family: Primary screening of cytotoxicity. Acta Horticulturae 501:177-179.

Mongelli E, Pampuro S, Coussio J, Salomon H and Ciccia G (2000) Cytotoxic and DNA interaction activities of extracts from medicinal plants used in Argentina. J Ethnopharmacol 71:145-151.

Riveiro O (2004) Induction of cell differentiation in human leukemia U-937 cells by 5-oxygenated-6,7-methylenedioxycoumarins from *Pterocaulon polystachyum*. Cancer Letters 210:179-188.

Simões CMO, Schenkel EP, Gossmann G, Mello JCP, Mentz LA and Petrovick PR (2001) Farmacognosia: Da Planta ao Medicamento. 3 edition. Editora da Universidade Federal do Rio Grande do Sul,Porto Alegre, 821 pp.

Teixeira RO, Camparoto ML, Mantovani MS and Vicentini VEP (2003) Assessment of two medicinal plants *Psidium guajava* L. and *Achillea millefolium* L., in *in vitro* and *in vivo* assays. Gen Mol Biol 26:551-555.

Vera N, Bandon A, Catalan CA, Gedris TE and Herz W (2001) New coumarins from *Pterocaulon polystachyum*. Planta Medica 67:674-677.

Vicentini VEP, Camparoto ML, Teixeira RO and Mantovani MS (2001) *Averrhoa carambola* L., *Syzygium cumini* (L.) Skeels and *Cissus sicoydes* L.: Medicinal herbal tea effects on vegetal and animal test systems. Acta Scientiarum 23:593-598.

Vieira D and Vicentini VEP (1997) Estudo do efeito mutagênico do floxacin em *Allium cepa*. Rev Brasil Genét (Supplement) 20:115-115.

Zardini EM (1983). Etnobotánica de Compuestas argentinas con especial referencia a seu uso farmacológico. Acta Farmacêutica Bonaerense 3:77-79.

Associate Editor: Marcelo Guerra