BIOCELL
2013, 37(2): 23-28

ISSN 0327 -9545
PRINTED IN ARGENTINA

Post-treatment with plant extracts used in Brazilian folk medicine caused a partial reversal of the antiproliferative effect of glyphosate in the *Allium cepa* test

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Key words: medicinal plants, *Psychotria brachypoda*, *Psychotria birotula*, cell proliferation, apoptosis

ABSTRACT: Species of the genus *Psychotria* are used for multiple purposes in Brazilian folk medicine, either as water infusions, baths or poultices. This study was aimed to evaluate the genotoxic and antiproliferative effects of infusions of *Psychotria brachypoda* and *P. birotula* on the *Allium cepa* test. Exposure to distilled water was used as a negative control, while exposure to glyphosate was used as a positive control. The interaction of extracts (as a post-treatment) with the effects of glyphosate was also studied. Results showed that glyphosate and the extracts of both *P. brachypoda* and *P. birotula* reduced the mitotic index as compared with the negative control (distilled water). Surprisingly, however, both extracts from *P. brachypoda* and *P. birotula* caused a partial reversal of the antiproliferative effect of glyphosate when used as a post-treatment. Glyphosate also induced the highest number of cells with chromosomal alterations, which was followed by that of *P. birotula* extracts. However, the extracts from *P. brachypoda* did not show any significant genotoxic effect. Post-treatment of glyphosate-treated samples with distilled water allowed a partial recovery of the genotoxic effect of glyphosate, and some of the *Psychotria* extracts also did so. Notably, post-treatment of glyphosate-treated samples with *P. brachypoda* extracts induced a statistically significant apoptotic effect. It is concluded that *P. brachypoda* extracts show antiproliferative effects and are not genotoxic, while extracts of *P. birotula* show a less potent antiproliferative effect and may induce chromosomal abnormalities. The finding of a partial reversion of the effects of glyphosate by a post-treatment with extracts from both plants should be followed up.

Introduction

The economic potential of medicinal species that are native to Brazil is huge and these species are considered a natural resource worthy of preservation and optimization of their use (Pereira *et al*., 2006).

The flora of Rio Grande do Sul features *Psychotria brachypoda* (Müll. Arg.) Britton and *P. birotula* Smith & Downs mut. Char. (Rubiaceae) (Dillenburg and Porto, 1985) among several other medicinal species.

The most popular internal uses of infusions of *Psychotria* species include bronchial, gastrointestinal and female reproductive disorders, as well as being considered an aid in the pre- and postpartum periods. External uses as poultices and baths are also popular for the treatment of fever, headaches and earaches and for skin and eye disorders (Adjibadé, 1989 *apud* Paranhos, 2003; Lajis *et al*., 1993; Perry, 1980).

P. brachypoda produces the alkaloid psycholatine, with high pharmacological potential, because it has analgesic activity of the opioid, anxiolytic and antipsychotic types, interacting with receptors of several

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Received: December 22, 2011. Revised version received: August 11, 2013. Accepted: August 18, 2013.

neurotransmitter systems in the central nervous system (Fragoso, 2007). *P. birotula* contains pyrrolidinoindole alkaloids, along with meso-chimonanthine and chimonanthine, also used pharmaceutically (Brand *et al*., 2009).

According to Silva *et al*. (2004) tea consumption would suppress the effects of mutagenic agents, but Vicentini *et al*. (2001) reported that teas and herbal infusions may contain toxic mutagenic substances. So, studies of toxicity and mutagenic activity are needed to contribute to the safe use of these folk medicines.

Thus, this study was aimed to assess the antiproliferative, genotoxic, and antimutagenic effects of the extracts of *P. brachypoda* and *P. birotula*, using the *Allium cepa* test. This test, first introduced by Levan (1938), was used since it has been recognized by the

TABLE 1.

Number of cells in either interphase or the mitotic phases, and on the mitotic index (*Allium cepa* test; 5000 cells were analyzed per treatment).

Treatment	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI (%)
Distilled water*	4734	154	42	29	41	5.32[a]
3% glyphosate**	4823	48	79	30	20	3.54[b]
P. brachypoda extract, 5 g/L	4944	16	7	13	20	1.12[c]
P. brachypoda extract, 20 g/L	4984	10	0	3	3	0.32[d]
P. birotula extract, 5 g/L	4921	37	15	12	15	1.58[c]
P. birotula extract, 20 g/L	4827	85	57	16	35	3.86[b]
3% glyphosate, followed by distilled water	4812	66	49	36	37	3.76[a,b]
3% glyphosate, followed by *P. brachypoda* extract, 5 g/L	4794	69	58	42	37	4.12[a,b]
3% glyphosate, followed by *P. brachypoda* extract, 20 g/L	4806	61	47	37	49	3.88[a,b]
3% glyphosate, followed by *P. birotula* extract, 5 g/L	4799	68	28	31	74	4.02[a,b]
3% glyphosate, followed by *P. birotula* extract, 20 g/L	4774	71	45	28	82	4.52[a,b]

MI values followed by the same letter do not differ significantly at the 5% level (Chi-square test).
* Negative control; ** Positive control

International Programme on Chemical Safety (IPCS, WHO) and the United Nations Environment Programme (UNEP) as an efficient test for the analysis and monitoring *in situ* of the genotoxicity of environmental substances. It has also been validated by comparison with animal tests (Teixeira *et al*., 2003; Vicentini *et al*., 2001).

Materials and Methods

Leaves of *P. brachypoda* and *P. birotula* were collected in the municipality of Dom Pedro de Alcântara (Rio Grande do Sul, Brazil) in September 2010, i.e., during the period of vegetative development. The species were identified according to Mori *et al.* (1989).

TABLE 2.

Number of cells with chromosomal alterations or apoptosis (*Allium cepa* test; 5000 cells were analyzed per treatment).

Treatment	Bridges in anaphase or telophase	Chromosomal break up	Laggard chromosome	Total altered mitoses	Apoptotic cells
Distilled water*	0	0	0	0[a]	0[c]
3% glyphosate**	22	40	40	102[c]	0[c]
P. brachypoda extract, 5 g/L	2	0	5	7[a]	0[c]
P. brachypoda extract, 20 g/L	1	0	3	4[a]	0[c]
P. birotula extract, 5 g/L	11	0	23	34[b]	0[c]
P. birotula extract, 20 g/L	30	0	47	77[b,c]	0[c]
3% glyphosate, followed by distilled water	7	16	18	41[b]	0[c]
3% glyphosate, followed by *P. brachypoda* extract, 5 g/L	37	9	21	67[b,c]	19[b]
3% glyphosate, followed by *P. brachypoda* extract, 20 g/L	43	0	10	53[b]	59[a]
3% glyphosate, followed by *P. birotula* extract, 5 g/L	31	2	18	51[b]	0[c]
3% glyphosate, followed by *P. birotula* extract, 20 g/L	37	0	30	67[b,c]	0[c]

Means followed by the same letter do not differ significantly at the 5% level (Chi-square test).
* Negative control; ** Positive control

The leaves were dried at room temperature for 90 days, and then aqueous extracts were prepared by infusion for 10 minutes (tea) in two concentrations for each species (5 and 20 g of dried leaves per liter).

The meristem cells of *A. cepa* rootlets were used to evaluate the effect on the mitotic index (MI). Eleven groups of 5 bulbs were placed for 4 days in distilled water to allow root development before the different treatments were applied. The following treatments were used: (1) distilled water (negative control) for 24 h; (2) 3% glyphosate (positive control) for 24 h; (3) *P. brachypoda,* 5 g/L extract for 24 h; (4) *P. brachypoda,* 20 g/L extract for 24 h; (5) *P. birotula,* 5 g/L extract for 24 h; (6) *P. birotula,* 20 g/L extract for 24 h; (7) 3% glyphosate for 24 h, followed by 24 h post-treatment in water; (8) 3% glyphosate for 24 h, followed by 24 h post-treatment in *P. brachypoda* extract, 5 g/L; (9) 3% glyphosate for 24 h, followed by 24 h post-treatment in *P. brachypoda* extract, 20 g/L; (10) 3% glyphosate for 24 h, followed by 24 h post-treatment in *P. birotula* extract, 5 g/L; (11) 3% glyphosate for 24 h, followed by 24 hours post-treatment in *P. birotula* extract, 20 g/L.

At the end of the different treatments the roots (2 cm samples, meristematic region) were collected and fixed in ethanol: acetic acid (3:1) during 24 hours and then stored in 70% alcohol in the refrigerator. Afterwards the samples were hydrolyzed in HCl 1N for 5 minutes and were stained with acetic orcein 2% after squashing the meristematic region with a glass rod (Guerra and Souza, 2002). The slides were studied with a light microscope LEICA 400X. One thousand cells were counted per bulb and the MI and the percent occurrence of chromosomal alterations were calculated.

We counted 5000 cells for each group of bulbs, analyzing cells in mitosis (prophase, metaphase, anaphase, and telophase) and interphase, and also recording the number of apoptotic cells observed during the count of 5000 cells.

Statistical analysis of the effect of treatments was performed by the Chi-square test, using BioEstat 5.O (Ayres, 2007).

Results

Table 1 shows the effect of the different treatments on the number of cells in interphase and the different phases of cell division, as well as on the MI values.

Glyphosate, as wells as extracts from both *P. brachypoda* and *P. birotula* caused significant decreases in the MI as compared with the distilled water control, but both doses of *P. brachypoda*and the lower dose of *P. birotula* were more effective than glyphosate.

The antiproliferative effect of glyphosate treatment was not modified by the post-treatment with distilled water for 24 h, but post-treatment with both doses of *P. brachypoda* and *P. birotula* extracts caused a partial reversal of the effect of glyphosate.

Table 2 shows the number of cells in apoptosis and cells with different chromosomal alterations induced by treatments. The alterations observed were laggard chromosomes (Fig. 1A), anaphase (Figs. 1B and 1C) and telophase bridges (Fig. 1D), chromosomal break up (Fig. 1E) and apoptosis (Fig. 1F).

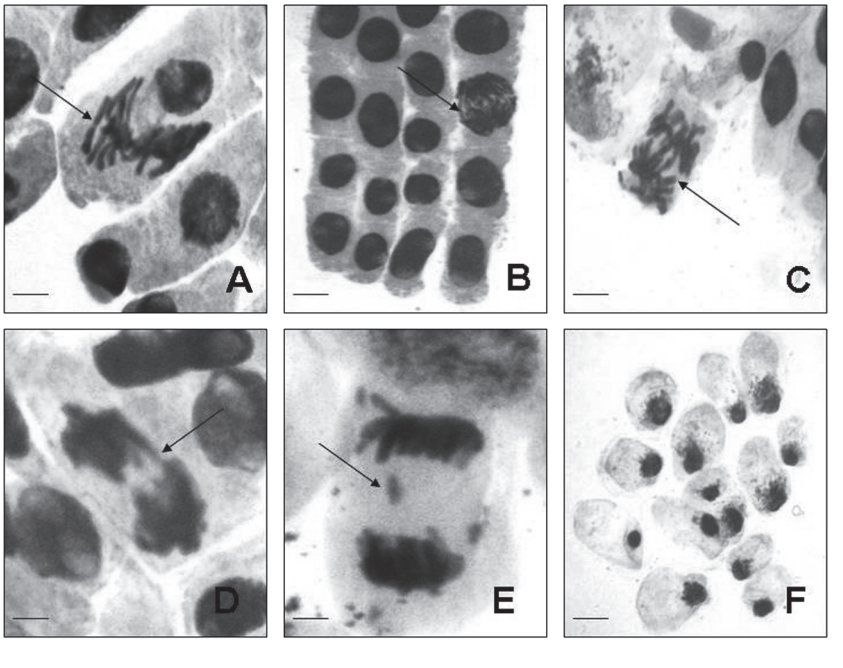

FIGURE 1. Chromosomal alterations in cells of *Allium cepa* under different treatments. **A)** Metaphase showing a laggard chromosome (*P. birotula,* 5 g/L). **B)** Anaphase bridges (*P. birotula,* 20 g/L). **C)** Anaphase bridges (*P. brachypoda,* 5 g/L). **D)** Telophase bridge (3% glyphosate). **E)** Chromosomal break up: a fragment is indicated in a telophase cell by an arrow (3% glyphosate followed by extract of *P. birotula,* 5 g/L). **F)** Apoptotic cells (3% glyphosate followed by extract of *P. brachypoda,* 20 g/L). Scale bars indicate 10 μm.

Glyphosate treatment induced the highest number of cells with chromosomal alterations, while treatment with *P. brachypoda* extracts did not differ from the distilled water controls. The extracts of *P. birotula*, however, induced a significantly higher number of alterations than either distilled water or *P. brachypoda* extracts. Post-treatment with distilled water, as well as with two extracts (*P. brachypoda*, 20 mg/L, and *P. birotula*, 5 mg/L) caused a partial reversal of the genotoxic effect of glyphosate.

Also, glyphosate treated samples showed a significantly higher number of cells in apoptosis when post-treated with both *P. brachypoda* extracts, and this apoptotic effect was dose dependent.

Discussion

Glyphosate is known for its antiproliferative action and for inducing chromosomal alterations in meristematic cells of *A. cepa* (Souza *et al*., 2010). These effects were confirmed in the current study.

We are reporting here that water extracts from both *P. brachypoda* and *P. birotula* significantly reduced the mitotic index as compared with the negative control (distilled water). Surprisingly, however, extracts from both *P. brachypoda* and *P. birotula* caused a partial reversal of the antiproliferative effect of glyphosate when used as a post-treatment. Glyphosate also induced the highest number of cells with chromosomal alterations, which was followed by that of *P. birotula* extracts. However, the extracts from *P. brachypoda* did not show any genotoxic effect. Post-treatment of glyphosate-treated samples with distilled water allowed a partial recovery of the genotoxic effect of glyphosate, and some of the *Psychotria* extracts also did so. It is concluded that *P. brachypoda* extracts show antiproliferative effects and are not genotoxic, while extracts of *P. birotula* show a less potent antiproliferative effect and may induce chromosomal abnormalities.

Antiproliferative activity has also been reported in the congeneric species *P. myriantha* Müll. Arg and *P. leiocarpa* Cham. & Schltdl. (Rubiaceae) by Lubini *et al*. (2008), as well as for a number of Asteraceae (e.g., *Achillea millefolium* L., Teixeira *et al*., 2003; *Pterocaulon polystachyum* DC, Knoll *et al*., 2006; *Achyrocline satureioides* (Lam.) DC, Fachinetto *et al*., 2007; *Solidago microglossa* DC, Bagatini *et al*., 2009; *Baccharis trimera* (Less.) DC and *B. articulata* (Lam.) Pers., Fachinetto and Tedesco, 2009; *Mikania glomerata*, Dalla Nora *et al*., 2010) and also for representatives of the Myrtales (*Psidium guajava* L., Teixeira *et al*., 2003) and the Cupressaceae (*Thuja occidentalis*, Jorge *et al*., 2009).

It should also be mentioned that genotoxic activity was shown by extracts from *P. birotula* in the current study, although the effect was significantly lower than that of glyphosate. On the contrary, extracts from *P. brachypoda* did not produce any significant genotoxicity. Lubini *et al*. (2008) also found genotoxicity in extracts from *Psychotria myriantha*, but not in those of *P. leiocarpa*.

Notably, post-treatment with extracts from *P. brachypoda* and *P. birotula* (at some but not all doses) caused a partial reversal of the antiproliferative and genotoxic effects of glyphosate. Post-treatment with distilled water was ineffective. These results are interesting but difficult to interpret at the present stage of knowledge: indeed, a reversal of an antiproliferative action of glyphosate by an extract which is in itself antiproliferative is intriguing and may indicate that the antiproliferative action of glyphosate and *Psychotria* extracts are using different mechanisms.

Also notably, post-treatment of glyphosate treated bulbs with both concentrations of *P. brachypoda* was followed by induction of apoptosis in a statistically significant number of cells.

Finally, it should be mentioned that a partial but significant reversal of the genotoxic effect of glyphosate was produced by post-treatment with extracts from *P. brachypoda* (20 g/L) or *P. birotula* (5 g/L). However, post-treatment with distilled water was similarly effective.

Acknowledgements

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

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