Genetics and Molecular Biology, 25, 1, 85-89 (2002)

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Effects of *Maytenus ilicifolia* Mart. and *Bauhinia candicans* Benth infusions on onion root-tip and rat bone-marrow cells

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Abstract

Medicinal plants are widely used to treat various diseases, and in Brazil the plants *Maytenus ilicifolia* Mart. and *Bauhinia candicans* Benth are commonly used in popular medicine. However, there are a large number of compounds in plants which can produce alterations in genetic material, and this study was conducted to investigate any possible mutagenic and cytotoxic effects that *M. ilicifolia* and *B. candicans* infusions may have on the cell cycle and chromosomes. Infusions were prepared with *in natura* leaves to give two concentrations of infusions, one at the concentration normally used by the population in general and the other at 10 times this value (*i.e.* 3.5 and 35 mg/mL for *M. ilicifolia* and 0.465 and 4.65 mg/mL for *B. candicans*). Onion (*Allium cepa* L.) root-tip cells (RTC) and Wistar rat bone-marrow cells (BMC) were used as test systems in *in vivo* assays. The *M. ilicifolia* infusions at both concentrations, and the *B. candicans* infusion at the lower concentration, had no statistically significant depressive mitotic effect on RTC. A statistically significant depressive mitotic effect on RTC was found with the more concentrated (4.65 mg/mL) *B. candicans* infusion as compared with a negative control. In BMC, infusions of *B. candicans* and *M. ilicifolia* produced no statistically significant increase in the number of chromosome alterations or rates of cell division as compared to controls. The significance of these findings are discussed in the light of the use of these plants as therapeutic agents.

Key words: Allium cepa test, *Bauhinia candicans*, chromosome damage, *Maytenus ilicifolia*, medicinal herbs, mutagenicity test, Wistar rat bone marrow cells.

Received: February 4, 2000; accepted: March 25, 2002.

Introduction

Plants have always been used as a common source of medicines, both in traditional remedies and in industrialized products. In Brazil, the majority of the population uses traditional natural preparations derived from plant material for treating a variety of disease, and because of this it is extremely important that genotoxicity tests are applied to the active ingredients of these preparations in order to assess their mutagenic potential. The plants *Maytenus ilicifolia* Mart. (Celastraceae) and *Bauhinia candicans* Benth (Leguminosae) are commonly used in popular Brazilian medicine. The fresh or dry leaves of *M. ilicifolia* are used as an infusion to alleviate stomach pain and nausea and to treat ulcers and gastritis, this plant containing phenols, gallic tannins and other tannic compounds as well as epigallocatechinic derivatives. The leaves and flowers of *B. candicans* are used as an infusion to treat hypocholesterol and non-insulin dependent diabetes, the tissues of this plant containing sterols, flavonoids, pinetol, coline, trigoneline and citosterol. The research reported in this paper used onion (*Allium cepa* L.) root-tip cell and Wistar rat (*Rattus norvegicus*) bone-marrow cell assays to assess whether these plants have any effect on mitotic index or the occurrence of chromosome aberrations.

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Materials and Methods

Plant material

Maytenus ilicifolia Mart. and *Bauhinia candicans* Benth were obtained from the Irenice Silva medicinal plant garden (State University of Maringá, Brazil). Infusions

were prepared in the same way as is normally done when they are used by the general population. The *M. ilicifolia* leaves (*in natura*) were boiled for five minutes in tap water, and the infusion covered and allowed to cool. The *B. candicans* leaves (*in natura*) had boiling water poured over them and were left to stand for ten to fifteen minutes, strained and the infusion allowed to cool. The infusions from both plants were prepared at two concentrations, one corresponding to that normally used by the general population and the other at a concentration ten times higher, *i.e.* 3.5 and 35.0 mg/mL for *M. ilicifolia* and 0.465 and 4.65 mg/mL for *B. candicans*.

Root-tip cells

Onion bulbs (*Allium cepa* L.) were placed in flasks containing aerated water at room temperature until rooted, after which root sample were taken to act as time-zero (t_{zero}) controls. Some bulbs were then placed in the infusions prepared above (controls were put in water) for 24 h, after which more roots were removed and the bulbs returned to water for 24 to observe if there was recovery from any possible damage. The roots were fixed and stained using the Feulgen reaction and permanently mounted on slides. The slides were examined `blind' (*i.e.* without knowing their treatments) using an optical microscope with a 40X objective. For each bulb 1000 cells were analyzed, *i.e.* a total of 6000 cells each for the control, treatment and recovery groups. Cells with morphological structural alterations were recorded and the mitotic index (MI) of the cells calculated. The statistical evaluation was performed using the χ^2 test at a probability level of 0.05.

Bone marrow cells

Wistar rats (*Rattus norvegicus*) with a body weight (b.w.) of about 100 g were obtained from the Central Animal House at the State University of Maringá, three male and three female rats being used for each group (treatments and control). For each group, live rats were injected intraperitoneally for 24 h with 1 mL of one of the infusions prepared above, positive control animals being treated with 1.5 mg of cyclophosphamide (CP)/100g b.w. All rats were injected with 0.5 mL/100 g b.w. of a 0.16% colchicine solution an hour-and-a-half before sacrifice, bone marrow cells being obtained by modification of the method of Ford and Hamerton (1956). Chromosome analysis was carried out using optical microscopy and a 100X immersion lens. For each rat, 100 metaphases were examined in a `blind' design, giving a total of 600 metaphases each for the control and treatment groups. Mitotic index values were calculated for the 5000 cells by sex (a total of 10000 cells/group). Statistical evaluation was performed using he χ^2 test at a probability level of 0.05.

Results

Root-tip cells

Table 1 shows the total number of cells analyzed, mean mitotic index values and the number of cells at the different phases of the cell cycle (interphase, prophase, metaphase, anaphase and telophase) in root-tip cells treated with *M. ilicifolia* and *B. candicans* infusions.

In the case of *M. ilicifolia* the mitotic index of the root-tip cells decreased after 24 h in each concentration of extract, with a lower mean mitotic index occurring at the higher concentration, this effect remaining even after the bulbs were subjected to a 24 h recovery period in water, although the results were not significant according to the χ^2 test.

For *B. candicans* the mitotic index of the root-tip cells also decreased after 24 h in each concentration of extract with the difference being statistically significant only for the higher concentration of 4.65 mg/mL ($\chi^2 = 4.86$). After recovery in water for 24 h there was a small increase in mitotic index, which although not significant, was well below the mitotic index of the t_{zero} controls ($\chi^2 = 3.38$).

Neither of the *M. ilicifolia* infusions, nor the 0.465 mg/mL *B. candicans* infusion, produced a permanent significant depressive mitotic effect on the root-tip cells, although there was a statistically significant temporary inhibition of cellular division in the more concentrated *M. ilicifolia* infusion (35.0 mg/mL), but this effect was reversible.

Rat bone-marrow cells

Table 2 shows the mean total mitotic index, total analyzed metaphases and the number of chromosome alterations observed in male and female Wistar-rats treated with infusions of *M. ilicifolia* and *B. candicans* and in positive and negative control rats. Compared with the negative controls, there was no statistically significant increase in the number of chromosome alterations in bone-marrow cells from animals treated with any of the infusions prepared from either *M. ilicifolia* or *B. candicans*, nor was there any alteration in the cellular division index.

Discussion

Although frequently used by the general population as medicines, plant infusions are phyto-complexes of varying composition and contain alkaloids, flavonoids, tannins and other complex compounds that are produced by plants as protective mechanisms and which may be toxic or non-toxic when isolated in pure form.

In spite of the infusions having caused a decrease in cellular division in root-tip cells compared with non-treated controls only the higher concentration of *B. candicans*

Table I - Mitotic index of onion root-tip cells treated with *Maytenus ilicifolia* (*Mi*) and *Bauhinia candicans* (*Bc*) infusions.

Groups[1]	Treatment time[2]	Mitotic index	Number of cells in different phases				
			Interphase	Prophase	Metaphase	Anaphase	Telophase
Control	Control	8.4	5497	236	104	95	68
	Treated	7.8	5528	176	134	95	67
	Recovery	7.8	5532	253	104	71	40
Mi (3.5)	Control	9.7	5417	330	115	91	47
	Treated	8.0	5520	274	109	59	38
	Recovery	6.9	5586	182	134	60	38
Mi (35.0)	Control	10.4	5378	353	123	93	53
	Treated	4.8	5711	169	76	25	19
	Recovery	4.5	5730	132	86	31	21
Control	Control	4.4	5736	155	50	27	32
	Treated	3.0	5820	109	43	13	15
	Recovery	3.5	5791	109	67	17	16
Bc (0.465)	Control	4.9	5705	155	66	38	36
	Treated	3.7	5779	112	72	25	12
	Recovery	4.1	5754	145	50	34	17
Bc (4.65)	Control	7.4	5555	273	93	42	37
	Treated	1.4*	5918	38	25	15	4
	Recovery	2.4	5858	82	38	12	10

[1]The number of cells analyzed in each group was 6000. Concentration of infusion in mg/mL in parenthesis.
[2]Treatment time: Control = 0 h (t_{zero}), Treated = 24 h, Recovery = 24 h.
*Statistically significant.

Table II - Mitotic index and chromosome aberrations of bone-marrow cells of Wistar rats treated with *Maytenus ilicifolia* (*Mi*) and *Bauhinia candicans* (*Bc*) infusions.

Groups[1]	Mitotici index	Total alterations	Types of aberration			
			Gaps		Breaks	
			Chromatidic	Chromosomic	Chromatidic	Chromosomic
Control	1.32	2 (0.3)[2]	0	1	1	0
Positive control	1.32	79 (13.2)	6	0	46	27
Mi (3.5)	1.86	1 (0.2)	0	1	0	0
Mi (35.0)	1.67	0	0	0	0	0
Bc (0.465)	1.57	2 (0.3)	2	0	0	0
Bc (4.650)	1.53	0	0	0	0	0

[1]The number of cells analyzed in each group was 600. Positive control rats were injected with 1.5 mg cyclophosphamide/100 g body weight. Concentration of infusion in mg/mL in parenthesis.
[2]Percentage alterations.

showed a statistically significant inhibitory effect, although this cytotoxic effect was reversible with slight recovery in cell division after 24 h recovery in water. It is possible that a high concentration of any chemical will have an effect (inhibitory or stimulatory) on the cell cycle, as has been shown for caffeine in *Drosophila prosaltans* (Itoyama *et al.*, 1997), mefloquine in human blood lymphocytes (Grisolia *et al.,* 1995), *Alpinia mutans* and *Pogostemun heyneanus* extracts in *A. cepa* root-tip cells (Dias and Takahashi, 1994) and glaucolide B extracted from *Vemonia eremphila* Mart. in human lymphocytes (Burim *et al.,* 1999).

Yen and Chen (1994) studied the relationship between the chemical composition of tea leaves and their extracts and their antimutagenic activity. They found that the principal components of tea leaves and their extracts are catechins, which seem to be responsible for antimutagenic activity, which varied from 4.3% in black tea to 26.7% for green tea. Green tea is produced from non-fermented *Thea*

sinensis leaves (the most popular beverage in the orient) much used in Japan as an antipyretic, diuretic and antioxidant, and which has been shown to have antimutagenic and antitumor effects *in vitro* and *in vivo* (Kada *et al*., 1985; Shimoi *et al.*, 1986; Jain *et al*., 1989; Wang *et al*., 1989). It has also been reported that the mortality rate due to human cancers in areas of tea cultivation is significantly lower than in areas where tea is not grown (Sasaki *et al*., 1993), and that the catechin present in green tea suppresses the action of many environmental mutagens (Nakamura *et al.,* 1997). Black tea also has high antimutagenic activity *in vitro,* which varies according to the extent of fermentation of the tea during the manufacturing process (Yen and Chen, 1994; Apostolides *et al.,* 1996). It has also been reported that black and green teas, without caffeine, have a chemo-preventive dose-dependent effect in preventing liver and lung cancer in rats (Cao *et al*., 1996).

Horikawa *et al.* (1994) assessed the activity of six Chinese medicinal herbs on *Salmonella* and found that tannin and catechin compounds were responsible for the inhibition of mutagenicity caused by benzo[a]pirene.

Bauhinia candicans contains flavonoids (the second most common group of metabolites in the vegetable kingdom) and their very low toxicity makes them attractive compounds for use as therapeutic agents (Martins *et al*., 1995). In a study involving the mutagenic effects of 2-(2-furyl)-3-(5-nitro-2-furyl) (AF-2), Ohtsuka *et al.* (1995) investigated the antimutagenic effects of nine active compounds from the Chinese medicinal herb, *sho-saiko-to* and found that the main active antimutagenic compounds were the saponins and the flavonoids. According to Bu-Abbas *et al.* (1996) the high concentration of flavonoids in green tea compared with black tea may mean that these compounds are one of those responsible for the antimutagenic and (possibly) anticarcinogenic properties of tea and its fermented products.

It may be that the presence of flavonoids and tannins in *M. ilicifolia* and *B. candicans* was the reason why these medicinal plants did not show cytotoxic and clastogenic effects when tested in our system. It is also possible that extracts of these plants may have antimutagenic effects in different test systems, since the literature cited above indicates that plants containing flavonoids and tannin-like compounds can have such an effect.

Our results indicate that the consumption of infusions made from *M. ilicifolia* and *B. candicans* can be continued, although they should be used with caution always exactly following the traditional methods of preparation, especially with regards to the concentration of the infusions and the duration of treatment, so that the infusions have the desired pharmacological effects without toxicity. Medicinal plants can be very useful, but it is still necessary for the general population to take care not to use such plants indiscriminately.

Acknowledgments

The authors thank Professor Irenice Silva for her collaboration in the choice and identification of the plants.

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