

CYTOGENETIC EVALUATION OF THE EFFECT OF AQUEOUS EXTRACTS OF THE MEDICINAL PLANTS *Alpinia nutans* ROSC (ZINGIBERACEAE) AND *Pogostemon heyneanus* BENTH (LABIATAE) ON WISTAR RATS AND *Allium cepa* LINN. (LILIACEAE) ROOT TIP CELLS

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ABSTRACT

Aqueous extracts of two medicinal plants, *Alpinia nutans* Rosc (Zingiberaceae) and *Pogostemon heyneanus* Benth (Labiatae) were tested *in vivo* on two biological systems for clastogenic and turbagenic activity.

Neither extract was effective in inducing structural or numerical chromosome aberrations in rat bone marrow cells at the following concentrations (weight of leaves used to make extract): 150 up to 1200 mg/kg body weight for *A. nutans*, and 160 up to 1280 mg/kg body weight for *P. heineanus*.

When tested on onion (*Allium cepa*) root-tip cells, the extracts were highly toxic at the highest concentrations (more than 20 mg/ml for *A. nutans* and more than 16 mg/ml for *P. heyneanus*). At lower concentrations, the two extracts had an inhibitory effect on onion cell development.

INTRODUCTION

The use of medicinal plant extracts for the treatment of human diseases is an ancient practice which has greatly increased in recent years. However, little information is available about the risks that these products may pose to health. Many of these plants contain compounds which are known to cause diseases or even death in animals and humans (Evans and Osman, 1974; Kangwanpong *et al.*, 1981; Panigrahi and Rao, 1982) or to act as natural mutagens and carcinogens (Hartwell and Abbott, 1969; Ames, 1983; Sugimura, 1986; Sakamoto-Hojo *et al.*, 1988; Schimmer and Kühne, 1990; Vargas *et al.*, 1990).

Aqueous extracts of *Alpinia nutans* Rosc and *Pogostemon heyneanus* Benth leaves are used by the population of Northern Brazil for their sedative action (van den Berg, 1982). Despite their widespread use, their mode of action on living organisms is still unknown. Thus, mutagenic evaluation of these extracts is necessary to estimate the risk that their use may pose to human health.

MATERIAL AND METHODS

Sample collection and preparation

The plants were obtained from street market vendors in Belém (PA) and identified by Dr. Elaine Elisabetski, Federal University of Para.

Weighed dried ground leaves were boiled in distilled water, filtered and the pH was adjusted to 6.8. The different concentrations were obtained according to a weight/volume ratio, indicated as mg leaves per ml distilled water.

Wistar rat bone marrow system

Wistar rats (*Rattus norvegicus*) were obtained from the Animal Rearing Facility of the Faculty of

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Medicine of Ribeirão Preto, USP. Males and females animals weighing approximately 100 g received *ip* 1 ml of the extracts at the following concentrations: 150, 200, 600 and 1200 mg/kg body weight for *A. nutans*, and 160, 320, 640 and 1280 mg/kg body weight for *P. heyneanus*. Ten animals (five males and five females) were used per concentration. The control group received distilled water.

The highest concentrations employed were obtained using a large volume of leaves (12 g for *A. nutans* and 12.8 g for *P. heyneanus*) in 100 ml of water (Preston *et al.*, 1987). The remaining concentrations were obtained by dilution in distilled water.

The animals were sacrificed 24 hours after treatment and 0.5 ml of 0.16% colchicine was injected 90 minutes before sacrifice.

Metaphase chromosomes were obtained from bone marrow by the technique of Ford and Hamerton (1956), modified in the Department of Genetics of the Faculty of Medicine of Ribeirão Preto, USP. Fifty cells per animal were analyzed by light microscopy for the presence of numerical and structural chromosome aberrations.

Data obtained from control and treated animals were compared by analysis of variance and by linear regression. The values were transformed to $y = \arcsin\sqrt{p}$, where "p" represents the proportion of cells with chromosome aberrations (Sokal and Rohlf, 1969; Beiguelman, 1988).

A. cepa root tip cells

Commercially obtained onion bulbs were cleaned and allowed to germinate in dark flasks containing running water. After germination, the bulbs were transferred to flasks containing the different concentrations of aqueous extracts and left there for 24 hours.

The concentrations chosen for treatment were inferior to the dose that totally inhibited cell division, which was 2 g/ml for *A. nutans* and 1.6 g/ml for *P. heyneanus*. The following concentrations were used: 0.30, 0.60, 1.25, 2.50, 5.00, 10.00 and 15.00 mg/ml for *A. nutans*, and 0.03, 0.06, 0.12, 0.50, 2.00, 4.00, and 8.00 mg/ml for *P. heyneanus*.

After treatment at concentrations from 5.0 to 15.0 mg/ml of *A. nutans* and from 0.03 to 8.0 mg/ml of *P. heyneanus*, which inhibited cell division and reduced the mitotic index, the bulbs were returned to water for a recovery period of 24 hours.

Five bulbs were used for each treatment and control. Roots were collected three times from each bulb: before treatment (control), after 24 hours of treatment in aqueous solution (treatment) and after 24 hours of recovery in water (recovery). Roots were fixed in 3:1 ethanol:acetic acid for two hours and stained by the Feulgen reaction. Cell preparations were obtained by squashing the root-tip in

45% acetic acid. A total of 1000 cells/bulb were scored for the analysis of mitotic and phase indices.

Data obtained by the analysis of controls and treated *A. cepa* root-tip cells were compared by the Student *t*-test and by the Wilcoxon test (Sokal and Rohlf, 1969; Beiguelman, 1988).

RESULTS

Effects of A. nutans and P. heyneanus aqueous extracts on rat bone marrow cells

The frequency of chromosome aberrations in rat bone marrow cells treated with *A. nutans* aqueous extracts and controls ranged from 1.2 to 4.4%, but this variation was not statistically significant between treatments ($F_{4,45,0.05} = 2.49 < F_{Tab} = 2.61$) and did not differ from controls (Table I).

Table I - Number of chromosome aberrations in bone marrow cells from Wistar rats treated with different concentrations of *Alpinia nutans* and *Pogostemon heyneanus* aqueous extracts and their controls.

Concentration (mg/kg)	Chromosome aberrations (chromatid)	
	Gaps	Breaks
<i>A. nutans</i>		
0	5	5
150	1	5
300	11	12
600	18	4
1200	9	5
<i>P. heyneanus</i>		
0	4	2
160	9	6
320	6	5
640	6	1
1280	3	0

It was also observed that the *P. heyneanus* extract did not induce a significant increase in the frequency of chromosome aberrations among the different treatment groups or between them and the controls ($F_{3,45,0.05} = 1.09 < F_{Tab} = 2.84$) (Table I).

No significant differences in frequency of chromosome aberrations were observed between females and males or between treatments with the two extracts (Table II).

Table II - Number of cells with chromosome aberrations and mean frequency in bone marrow cells from Wistar rats treated with different concentrations of *Alpinia nutans* and *Pogostemon heyneanus* aqueous extracts and their controls.

Concentration (mg/ml)	Animals										Mean frequency
	Males					Females					
	A ₁	A ₂	A ₃	A ₄	A ₅	A ₆	A ₇	A ₈	A ₉	A ₁₀	
<i>A. nutans</i>											
0	1	2	1	1	0	0	2	2	0	1	0.020
150	1	3	0	0	0	0	2	0	0	0	0.012
300	1	1	1	8	4	4	1	0	1	1	0.044
600	3	5	2	4	1	1	2	0	1	3	0.044
1200	5	3	1	1	1	1	0	1	1	0	0.028
<i>P. heyneanus</i>											
0	1	0	1	1	1	0	1	0	1	0	0.012
160	0	0	0	2	1	5	1	4	0	2	0.030
320	0	2	1	0	2	1	1	1	1	2	0.022
640	1	0	0	2	1	1	0	0	1	1	0.014
1280	0	1	0	0	0	0	1	0	1	0	0.006

Among the chromosome aberrations detected in the various treatments with the two extracts, chromatid gaps and breaks were the most frequent, and occurred only at the rate of one per cell. Variations in the frequencies of numerical chromosome alterations were small and random.

Effect of *A. nutans* and *P. heyneanus* aqueous extracts on *A. cepa* root-tip cells

When *A. cepa* bulbs were treated with *A. nutans* extracts at concentrations higher than 20 mg/ml, the roots lost their turgidity, stopped growing and died. Cytologic analysis of cells treated at concentrations of 0.6 up to 10.0 mg/ml showed that cell division was inhibited, as confirmed by the MI values, which were lower than those for the controls. These differences (Table III) were dose dependent and significant. When the bulbs were allowed to recover in water after treatment, the cells reacquired the ability to divide (Figure 1).

When the bulbs were treated with the *P. heyneanus* extract, the frequency of meristematic cells was reduced both at high and low concentrations. The mitotic indices decreased considerably when compared to control bulbs, demonstrating a drastic inhibition of cell division (Table IV). In view of the cytotoxic effect of these concentrations, the bulbs were placed in water for 24 hours after treatment, and partial recovery of cell division was observed (Figure 2).

Table III - Phase and mitotic indices in *Allium cepa* root-tip cells treated with different concentrations of *Alpinia nutans* extracts and their respective controls.

Concentration (mg/ml)	Phase indices (%)				MI (%)
	PI	Mel	AI	TI	
Control	49.06	21.18	13.81	15.95	14.92
0	58.05	19.89	9.76	22.30	11.06
Control	50.67	20.86	10.88	17.59	13.42
0.30	57.11	19.11	9.88	13.80	9.42
Control	49.75	24.69	11.97	13.58	16.20
0.60	39.55	29.51	14.14	16.80	9.76*
Control	47.95	27.05	10.53	14.47	13.68
1.25	40.95	28.02	15.95	15.09	9.28
Control	50.94	26.12	6.24	16.69	13.78
2.50	51.70	20.45	7.39	20.45	3.52*
Control	52.46	22.13	8.52	16.89	12.20
5.00	39.63	46.95	3.05	10.37	3.28*
Control	36.21	26.44	14.37	22.99	10.44
10.00	15.56	40.00	14.44	30.00	1.80*

PI = Prophase index; Mel = Metaphase index; AI = Anaphase index; TI = Telophase index.

*Significantly lower than controls (Student *t*-test).

0.60 = (0.005 < P < 0.01);

2.5 = (0.005 < P < 0.01);

5.0 = (0.002 < P < 0.005);

10.0 = (0.001 < P < 0.002).

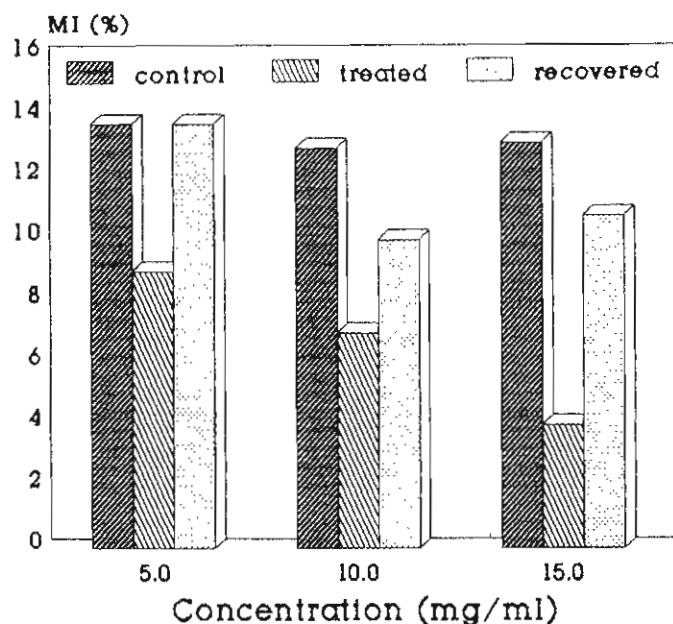


Figure 1 - Mitotic indices of *Allium cepa* root-tip cells treated for 24 hours with different concentrations of *Alpinia nutans* aqueous extract, recovered for 24 hours in water, and their controls.

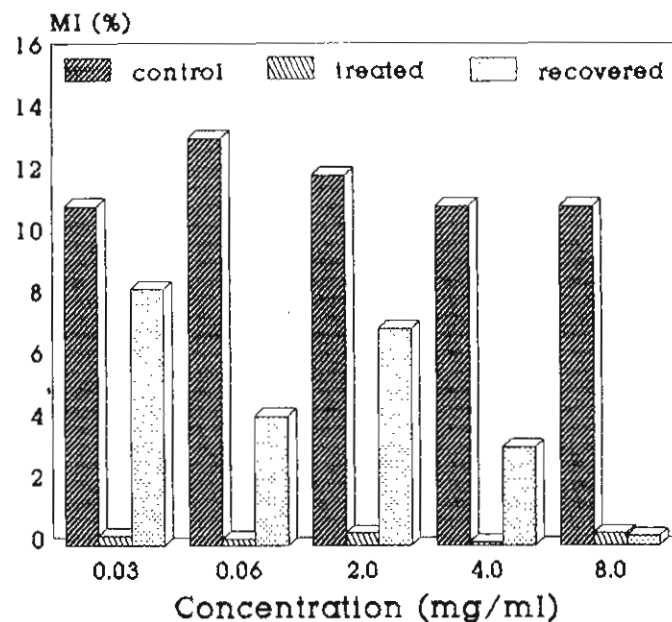


Figure 2 - Mitotic indices of *Allium cepa* root-tip cells treated for 24 hours with different concentrations of *Pogostemon heyneanus* aqueous extract, recovered for 24 hours in water and their respective controls.

Table IV - Phase and mitotic indices in *Allium cepa* root-tip cells treated with different concentrations of *Pogostemon heyneanus* extracts and their respective controls.

Concentration (mg/ml)	Phase indices (%)				MI (%)
	PI	Mel	AI	TI	
Control	46.31	51.27	7.52	22.42	13.56
0	39.27	27.71	10.09	22.94	10.90
Control	50.53	18.72	10.03	20.72	14.96
0.06	0	0	0	0	0*
Control	56.86	18.53	8.77	15.84	14.14
0.12	1.0	0	0	0	0.04*
Control	48.51	22.71	9.87	18.91	16.82
0.5	41.86	28.66	7.42	22.06	9.70*
Control	39.25	23.93	14.18	22.64	9.70
2.0	29.46	38.17	6.22	26.14	4.82*

PI = Prophase index; Mel = Metaphase index; AI = Anaphase index; TI = Telophase index.

*Significantly lower than controls (Student *t*-test).

0.06 = ($P < 0.001$); 0.12 = ($P < 0.001$); 0.5 = ($0.01 < P < 0.02$);

2.0 = ($0.01 < P < 0.02$).

DISCUSSION

Aqueous extracts of the leaves of *P. heyneanus* and *A. nutans* had no clastogenic effect on Wistar rat bone marrow cells (Table I). The variability observed among

animals can be attributed to intraindividual differences (Table II). Similar results have been obtained with the extracts of *Luffa operculata* (Medeiros and Takahashi, 1987) and *Stryphnodendron obovatum* Benth (Vicentini-Dias and Takahashi, 1993), also used in Brazilian folk medicine.

Aqueous extracts are complex mixtures that may be both mutagenic and antimutagenic. The rhizome juice of ginger (*Zingiber officinale*) contains mutagenic substances such as [6]-gingerol. However, it also contains antimutagenic component(s) against tryptophan pyrolysates (Kada *et al.*, 1978; Morita *et al.*, 1978) and [6]-gingerol in strain Hs 30 of *Escherichia coli* (Nakamura and Yamamoto, 1982). Furthermore, several plant extracts have also shown an antimutagenic effect against potent mutagens. The tannic acid present in green and black tea showed an antimutagenic effect against mitomycin C, ethyl nitrosourea (ENU) and 4-nitroquinoline 1-oxide when tested on mice (Sasaki *et al.*, 1990; Imanishi *et al.*, 1991). In addition, chlorophylline, alpha-linoleic acid and beta-carotene inhibited the activity of the powerful mutagen thiotepa when tested on Chinese hamsters (Renner, 1990). Aqueous extracts of these four herbs (*Oldenlandia diffusa*, *Scutellaria barbata*, *Astragalus membranaceus* and *Ligustrum lucidum*) significantly inhibited aflatoxin B1 (AFB1) binding to DNA, reduced AFB1-DNA adduct formation and also significantly decreased the formation of organosoluble metabolites of AFB1 (Wong *et al.*, 1992).

When *A. nutans* and *P. heyneanus* aqueous extracts were tested on *A. cepa* root-tip cells to evaluate their action on the kinetics of the cell cycle, a decrease in

their mitotic indices was observed, which was more marked at concentrations above 2.5 mg/ml of the *A. nutans* extract and 2.0 mg/ml of the *P. heyneanus* extract. At these concentrations the extracts proved to be extremely cytotoxic and inhibited *A. cepa* root growth. C-mitosis formation was not recorded in any of the assays performed, and the cytotoxic effects observed gradually disappeared when the bulbs were allowed to recover in water. The action on cell division probably resulted from a reversible toxic action of the extracts. The absence of structural aberrations of the break type also suggests that the extracts acted exclusively as a toxic agent on the formation of the mitotic spindle, with no chemical action on DNA or on the DNA-protein complex.

Aqueous extracts of *A. nutans* and *P. heyneanus* had a depressive effect on the division of meristematic cells of *A. cepa*, but did not increase the frequency of chromosome aberrations in rat bone marrow cells.

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RESUMO

Os extratos aquosos de duas plantas medicinais, *Alpinia nutans* Rosc e *Pogostemon heyneanus* Benth foram testados *in vivo*, quanto às suas atividades clastogênica e aneugênica, em dois sistemas biológicos.

No sistema de medula óssea de ratos Wistar nenhum dos dois extratos mostrou-se eficiente em induzir alterações cromossômicas estruturais ou numéricas nas concentrações testadas que foram: *A. nutans* (150, 300, 600 e 1200 mg/kg/p.c.) e de *P. heyneanus* (160, 320, 640 e 1280 mg/kg/p.c.).

Quando testados no sistema de células meristemáticas de *A. cepa*, os extratos apresentaram grande toxicidade nas concentrações mais altas (superiores a 20 mg/ml para *A. nutans* e a 16 mg/ml para *P. heyneanus*). Em concentrações mais baixas tiveram efeito inibidor sobre o ciclo celular.

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