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Brief Communication

Mutagenic and Antimutagenic Evaluation of the Juice of the Leaves of *Bryophyllum calycinum* (*Kalanchoe pinnata*), a Plant with Antihistamine Activity

Gisela Umbuzeiro-Valent,[1]* Deborah A. Roubicek,[1] and Eva Maria A.B. Haebisch[2]

[1]*Mutagenicity and Cytotoxicity Sector, CETESB, Environmental Sanitation Technology Company, São Paulo, Brazil*

[2]*Biomedical Sciences Institute, University of São Paulo, São Paulo, Brazil*

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INTRODUCTION

Bryophyllum calycinum Salisb (*Kalanchoe pinnata*, *B. pinnatum*) Crassulaceae, is a plant widely dispersed throughout Brazil. The aqueous extract of its leaves was found to cause significant inhibition of cell-mediated and humoral responses in mice, and was shown to protect them when infected with *Leishmania amazonensis* [Rossi-Bergmann et al., 1994; Da Silva et al., 1995, 1996]. Analgesic activity of the crude extract of *B. calycinum* was also verified [Kohn et al., 1996], and the juice of its leaves exhibit histamine-blocking activity in rodents. The juice contains flavonoid compounds, carbohydrates, and mineral salts. A flavonoid fraction obtained by partitioning the juice between n-butanol and water contained the substance responsible for the antihistamine activity [Nassis et al., 1992].

Because the juice obtained by pressing the leaves of *B. calycinum* is used in folk medicine for treatment of wounds, bruises, burns, insect bites and other skin diseases, and a prior study [Obaseiki-Ebor et al., 1993] showed an inhibitory effect on the mutagenicity of direct-acting mutagens, this study was performed to investigate the mutagenic and antimutagenic activity of the juice of *B. calycinum*, either with or without metabolic activation, using the Salmonella/mammalian microsome assay (Ames test).

MATERIALS AND METHODS

Preparation of the Juice of *B. calycinum* and Chemicals

The plant was collected in São Paulo, SP. The juice, which was obtained by pressing the leaves, was sterilized by double filtration through a 0.45 μm (Millipore) membrane and stored refrigerated.

4-Nitroquinoline-1-oxide (4NQO; Sigma, St. Louis, MO), 2-nitrofluorene (2NF; Aldrich Chemical Co., Milwaukee, WI), and 2-aminoanthracene (2AA; Sigma), dissolved in dimethylsulfoxide (DMSO; Sigma), were used as the mutagens; the chemical stock solutions were freshly prepared for each experiment.

Salmonella/mammalian microsome assay (Ames test)

The mutagenicity assay was performed using in situ concentration procedure (plate incorporation technique) diluting the sample in more concentrated top agar, according to Coriel Institute for Medical Research [1986]. The strains of *Salmonella typhimurium* used were TA1535, TA1537, TA98, and TA100, and the S9 mix was prepared according to Maron and Ames [1983] using the Aroclor-1254-induced rat liver S9 fraction, purchased lyophilized (MOLTOX; Molecular Toxicology Inc., Boone, NC). The doses were 0.1, 0.2, 0.5, 1.0, and 2.0 mL of the sterilized juice per plate; each dose was tested in triplicate. The positive controls employed were 4NQO at 0.25 μg without S9 and 2AA at 2.5 μg per plate with S9.

The antimutagenicity assays were performed using the plate incorporation procedure, *S. typhimurium* strain TA98, 2AA with metabolic activation, and 4NQO and 2NF without metabolic activation. Dose-finding experiments with these mutagens were conducted to determine the appropriate doses to use in the antimutagenicity tests.

In order to determine any possible interference between the juice of the leaves of *B. calycinum* and the S9 mix, two different experiments were done, using a two-step preincubation procedure [De Flora et al., 1992, modified]. Briefly, we first incubated 2AA with S9 for 20 min at 37°C, then we added the different doses of the juice and allowed it to stand for another 20 min at 37°C (condition A). After that we added the bacteria and plated

*Correspondence to: Gisela Umbuzeiro-Valent, Setor de Mutagênese e Citotoxicidade, CETESB, Companhia de Tecnologia de Saneamento Ambiental, Rua Prof. Frederico Hermann Jr., 345, São Paulo, SP 05489-900, Brazil. E-mail: giselav@cetesb.br

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TABLE I. Results of the Plate Incorporation and the Two-Step Preincubation under Condition A (2AA + S9; Juice) Procedures

	S. typhimurium TA98 + S9 Revertants/plate					
	Plate incorporation			Two-step preincubation[1]		
Dose of *B. calycinum* juice/plate	Mean ± SD[2]	MR[3]	%Inhib	Mean ± SD	MR	%Inhib
DMSO	44 ± 6.5	—	—	48 ± 8.9	—	—
2AA[4]	1163 ± 46.5	26.3	—	377 ± 74.6	7.8	—
2AA + 0.10 mL	1074 ± 17.0	24.3	8%	463 ± 50.2	9.6	0%
2AA + 0.25 mL	555 ± 70.0	12.5	54%	132 ± 19.1	2.7	75%
2AA + 0.50 mL	155 ± 26.8	3.5	90%	97 ± 14.1	2.0	85%
2AA + 1.00 mL	71 ± 6.9	1.6	98%	69 ± 7.0	1.4	94%
2AA + 1.50 mL	55 ± 4.4	1.2	99%	59 ± 7.1	1.2	97%
2AA + 2.00 mL	57 ± 17.3	1.3	99%	61 ± 14.6	1.2	96%

[1]Two-step preincubation: 2AA + 0.5 mL S9, 20 min, 37°C, followed by addition of *B. calycinum* juice, 20 min, 37°C.
[2]SD = standard deviation.
[3]MR = mutagenic ratio (spontaneous and induced revertants/spontaneous revertants).
[4]2AA = 2.5 μg/plate.

TABLE II. Results of the Plate Incorporation and the Two-Step Preincubation under Condition B (Juice + S9; 2AA) Procedures

	S. typhimurium TA98 + S9 Revertants/plate					
	Plate incorporation			Two-step preincubation[1]		
Dose of *B. calycinum* juice/plate	Mean ± SD[2]	MR[3]	%Inhib	Mean ± SD	MR	%Inhib
DMSO	49 ± 14.9	—	—	55 ± 2.2	—	—
2AA[4]	1211 ± 113.9	24.9	—	427 ± 98.9	7.7	—
0.10 mL + 2AA	1227 ± 95.9	25.2	0%	786 ± 58.0	14.2	0%
0.25 mL + 2AA	265 ± 30.2	5.5	78%	276 ± 9.8	5.0	35%
0.50 mL + 2AA	66 ± 12.7	1.4	95%	44 ± 5.7	0.8	90%
1.00 mL + 2AA	47 ± 9.5	1.0	96%	36 ± 8.7	0.7	92%
1.50 mL + 2AA	48 ± 6.8	1.0	96%	50 ± 8.1	0.9	88%
2.00 mL + 2AA	62 ± 5.7	1.3	95%	58 ± 1.2	1.0	86%

[1]Two-step preincubation: juice of *B. calycinum* + 0.5 mL S9, 20 min, 37°C, followed by addition of 2AA, 20 min, 37°C.
[2]SD = standard deviation.
[3]MR = mutagenic ratio (spontaneous and induced revertants/spontaneous revertants).
[4]2AA = 2.5 μg/plate.

it on minimum agar plates. The other experiment was performed in the same way, changing *B. calycinum* for 2AA in the first step (Condition B).

The results of the antimutagenicity experiments were submitted to the ANOVA test and the percent inhibition was calculated according to Ong et al. [1986].

In all experiments the background lawns were carefully evaluated for toxicity using a stereomicroscope.

RESULTS AND DISCUSSION

The juice showed no mutagenic activity in any of the four strains tested. Doses higher than 0.5 mL without S9 and higher than 1.0 mL with S9 were toxic for the strain TA1537 (data not shown).

During the mutagenicity experiments, we observed the formation of turbidity after the addition of S9 to the tube containing the juice, especially at doses of juice higher than 0.5 mL. This observation led us to suspect a possible false-negative response due to the interaction of the S9 mixture with the juice, causing inactivation of the metabolic activation system. This hypothesis was tested with the antimutagenicity experiments (two-step preincubation procedures under conditions A and B), and the results are presented in Tables I and II. The dose of 2.5 μg of 2AA/plate was adequate to evaluate the antimutagenicity of the juice in both plate incorporation and preincubation procedures, with mutagenic ratios of 22.8 and 8.5, respectively (data not shown). The similar responses obtained for the two-step preincubation procedures and the plate incorporation assays showed that there is no inhibitory effect of the S9 mix caused by the juice.

At a dose of 0.25 mL, the juice caused a statistically significant reduction in the mutagenicity of 2AA ($P \leq 0.05$) and the percent of inhibition was more than 90% at higher doses in all conditions tested.

We also verified no antimutagenic activity of the juice when tested against 4NQO and 2-NF (data not shown), suggesting the possibility of a specific mechanism for aro-

matic amines and/or other classes of chemicals. Other experiments must be carried out to confirm this hypothesis and clarify the processes involved on the antimutagenic activity of *B. calycinum*.

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E. Zeiger