ANTIMUTAGENIC EFFECTS OF EIGHTEEN PHILIPPINE PLANTS

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ABSTRACT

Expressions from *Cassia alata* L., *Anona squamosa* L., *Lagerstroemia speciosa* (L.) Pers., *Allium sativum* L., *Psidium guajava* L., *Capsicum annuum* L., *Allamanda cathartica* L., *Symphytum officinale* L., *Leucaena leucocephala* Lamk., *Plumeria acutifolia* Poir., *Garcinia mangostana* L., *Phaseolus aureus* Roxb., *Quisqualis indica* L., *Blumea balsamifera* (L) D.C., *Allium cepa* L., *Spondias purpurea* L., *Carmona astusa* (Vohl) Masam, and *Mentha cordifolia* L., reduced the induction of micronucleated polychromatic erythrocytes by mitomycin C, dimethylnitrosamine and tetracycline showing that these plants have antimutagenic effects. Heating did not affect the antimutagenic property of the plants.

INTRODUCTION

Somatic mutation causes cancer and germinal mutation may lead to genetic diseases and these phenomena may be brought about by environmental chemicals such as pesticides, food additives, synthetic drugs, atmospheric and water pollutants. The search therefore, for substances that reduce or remove the genotoxicity of environmental chemicals is a global concern.

In our laboratory, the antimutagenic effects of vitamins (Sylianco, 1981) mineral ions (Sylianco and Daya, 1984) and plants (Sylianco and Blanco, 1981; Gagalac and Sylianco, 1981; Sylianco *et al,* (in press) have been studied. This report is part of our continuing studies on the antimutagenic activity of plants.

MATERIALS AND METHODS

Genotoxicity to bone marrow cells and antimutagenic effects of expressions of plants given in Table 1 were studied using the micronucleus test (Schmid, 1976). The mutagens were administered intraperitoneally while the expressions were given orally by gavage. About 0.5 mL of expressions was used per 20 g body weight of the mouse. Five mice of Strong A strain were used for each plant per test system.

Three mutagens were used: mitomycin C, dimethylnitrosamine, and tetracycline. Two administrations were made, 30 and 6 hours before the animals were sacrificed by cervical dislocation. Immediately after sacrificing the animal, both femurs were removed and the bones were freed from the muscles. The bone marrow was flushed out of the bones by means of fetal calf serum in a syringe. By gentle aspirations and flushings,

the marrow was forced out through the opening around the needle. The suspension was centrifuged at 1000 rpm for 5 minutes and the supernatant drawn off by means of a Pasteur pipette. The cells in the sediment were carefully mixed by repeated aspirations with the capillary part of a new, dry siliconized pipette.

A small drop of the viscous suspension was put on the end of the slide and spread by pulling the material behind a polished cover glass held at an angle of 45°. After staining, slides were scored in a microscope at high magnification for the number of micronucleated polychromatic erythrocytes per thousand.

RESULTS AND DISCUSSION

Expressions from *Cassia alata, Anona squamosa, Lagerstroemia speciosa, Allium sativum, Psidium guajava, Capsicum annuum, Allamanda cathartica, Symphytum officinale, Leucaena leucocephala, Plumeria acutifolia, Garcinia mangostana, Phaseolus aureus, Quisqualis indica, Blumea balsamifera, Allium cepa, Spondias purpurea, Carmona astusa, and Mentha cordifolia* reduced the formation of micronucleated polychromatic erythrocytes induced by mitomycin C, dimethylnitrosamine and tetracycline (Table 2). Heat did not have any effect on the antimutagenic potential of these plants (Table 3).

The mutagens used in this study affected somatic cells by fragmenting the chromatin material of bone marrow cells as shown by the appreciable formation of micronucleated polychromatic erythrocytes. The fragments of the chromatin material lag behind after telophase and when the nucleus is expelled, some fragments are left behind to form micronuclei in the cytoplasm of the cells.

The expressions from the plants tested reduced appreciably the formation of micronucleated polychromatic erythrocytes. This implies that expressions of these plants contain antimutagens. It is possible that the antimutagens are vitamins (Sylianco *et al*, 1981) or mineral ions (Sylianco and Daya, 1984) since plants are known to contain these substances. It is also possible that substances of undetermined structures are causing the antimutagenic effects.

Heating the expressions to 100°C for 10 minutes had no effect on the antimutagenic activity implying that the antimutagens present are heat resistant. This removes the possibility of vitamins C and B acting as antimutagens since these substances are readily destroyed by heat.

Mitomycin C is an anticancer agent and also a carcinogen. It is a bifunctional alkylating agent of DNA (Flamm and Mehlmann, 1978). It readily affects the DNA of cancer cells but it is non-selective and also affects the DNA of normal cells; thus it is mutagenic. Its genotoxicity can be reduced by expressions from the plants tested but whether the effect on cancer cells can be reduced, is not known.

Dimethylnitrosamine is an alkylating agent of DNA (Magee and Hultin, 1962). It can readily release carbocations upon metabolism and has been shown to be a carcinogen

(Zeiger and Legator, 1971). It is found in fried bacon (Fazio *et al,* 1971), cigarette smoke (Mcglashin *et al,* 1968) and nitrite-treated meats (Ender and Ceh, 1968). Exposure to dimethylnitrosamine can be widespread and unavoidable even to non-smokers who are exposed to cigarette smoke. Its genotoxicity, however, can be reduced by plant foods and other plant preparations.

Tetracycline is an antibiotic that is widely used. It is a mutagen as well as a teratogen (Sylianco and Blanco, 1984). Its mutagenicity and teratogenicity can be reduced by vitamin C and vitamin E. In this study, raw and heated expressions from plants reduced its genotoxicity therefore, this property cannot be attributed to the vitamin C in plant expressions since vitamin C is destroyed by heating.

Mineral ions have been shown to be effective antimutagens (Sylianco and Daya, 1984). Plant preparations can contain mineral ions which can lessen the genotoxicity of mitomycin C, dimethylnitrosamine and tetracycline.

There is a possibility that amino acids which can be found in plant expressions can lessen the genotoxicity of mutagens. Cysteine was shown to be antimutagenic to an antimoebic drug (Flores and Sylianco, 1983) and basic and acidic amino acids were also shown to be antimutagenic (unpublished data).

The findings that some plants possess antimutagenic activity are very relevant. Some are utilized as fruits and vegetables and since we cannot have an environment completely free from mutagens, it is comforting to know that some constituents of our daily diet can reduce genotoxicity of carcinogens. This knowledge is also significant economically since vitamins as dietary supplements have become very expensive.

Future studies are recommended for further identification of antimutagens found in plants. It is possible that substances other than those of vitamins and amino acids can be identified.

SUMMARY AND CONCLUSIONS

Expressions from *C. alata, A. squamasa, L. speciosa, A. sativum, P. guajava, C. annuum, A. cathartica, S. officinale, L. leucocephala, P. acutifolia, G. mangostana, P. aureus, Q. indica, B. balsamifera, A. cepa, S. purpurea, C. astusa* and *M. cordifolia,* reduced the mutagenicity potential of mitomycin C, dimethylnitrosamine and tetracycline and exhibited antimutagenic effects.

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Table 1. Common and scientific names and parts used

Common Name	Scientific Name	Parts Used
"Akapulko"	*Cassia alata* L.	Leaves
"Atis"	*Anona squamosa* Linn.	Seeds
"Banaba"	*Lagerstroemia speciosa* (L) Pers	Leaves
"Bawang"	*Allium sativum* L.	Head
Bell Pepper, green	*Capsicum annuum* Linn.	Fruit
Bell Pepper, red	*C. annuum*	Fruit
"Campanilla"	*Allamanda cathartica* Linn.	Flowers
Comfrey	*Symphytum officinale* Linn.	Leaves
"Ipil-ipil"	*Leucaena leucocephala* Lamk.	Seeds
"Kalatsutsi"	*Plumeria acutifolia* Poir.	Leaves
Mangosteen	*Garcinia mangostana* Linn.	Rind
Mongo	*Phaseolus aureus* Roxb.	Seeds
"Niyog-niyogan"	*Quisqualis indica* L.	Seeds
"Sambong"	*Blumea balsamifera* (L) DC	Leaves
"Sibuyas", white	*Allium cepa* Linn.	Bulbs
"Sineguelas"	*Spondias purpurea* Linn	Fruit
"Tsaang gubat"	*Carmona astusa* (Vohl) Masam.	Leaves
"Yerba buena"	*Mentha cordifolia* Linn.	Leaves

Table 2. Antimutagenic effects of some plants against mitomycin C, dimethylnitrosamine and tetracycline

	Number of micronucleated polychromatic erythrocytes per thousand		
	Mitomycin C (3mg/kg)	Dimethylnitrosamine (15 mg/kg)	Tetracycline (150 mg/kg)
Mutagen	9.41 ± 1.90	9.14 ± 1.30	7.86 ± 0.83
plus *C. alata*	2.83 ± 0.69	1.99 ± 0.49	1.84 ± 0.63
plus *A. squamosa*	3.76 ± 0.72	3.33 ± 0.72	3.00 ± 0.97
plus *L. speciosa*	4.49 ± 0.83	1.88 ± 0.38	1.99 ± 0.41
plus *A. sativum*	1.78 ± 0.35	2.01 ± 0.88	2.16 ± 0.87
plus *P. guajava*	4.33 ± 1.93	2.32 ± 0.23	2.18 ± 0.67
plus *C. annuum* (green)	6.94 ± 2.53	3.08 ± 0.16	3.24 ± 0.75
plus *C. annuum* (red)	3.58 ± 0.73	2.77 ± 0.38	3.39 ± 0.72
plus *A. cathartica*	1.51 ± 0.19	2.83 ± 0.79	2.03 ± 0.57
plus *S. officinale*	2.04 ± 0.38	3.11 ± 0.19	2.41 ± 0.31
plus *L. leucocephala*	3.86 ± 1.55	2.41 ± 0.57	2.68 ± 0.32
plus *P. acutifolia*	1.33 ± 0.60	2.33 ± 0.47	1.77 ± 0.76
plus *G. mangostana*	6.06 ± 2.10	2.88 ± 0.69	1.66 ± 0.33
plus *P. aureus*	2.77 ± 1.01	2.57 ± 0.84	2.33 ± 0.33
plus *Q. indica*	1.68 ± 0.28	2.41 ± 0.50	1.78 ± 0.50
plus *B. balsamifera*	4.73 ± 1.32	2.88 ± 0.19	1.99 ± 0.57
plus *A. cepa*	3.88 ± 1.44	2.44 ± 0.55	2.49 ± 0.42
plus *S. purpurea*	2.52 ± 0.57	3.55 ± 1.01	3.16 ± 0.10
plus *C. astusa*	3.34 ± 0.97	1.88 ± 0.19	1.93 ± 0.56
plus *M. cardifolia*	3.39 ± 0.43	2.39 ± 0.64	2.16 ± 0.42
Negative control	2.10 ± 0.37	2.30 ± 0.97	1.98 ± 0.65

Table 3. Effect of heat on the antimutagenic effects of some plants

Scientific Name	No. of micronucleated polychromatic erythrocytes per thousand	
	Raw	Heated
C. alata + mitomycin C	2.83 ± 0.69	2.13 ± 0.98
A. sativum + mitomycin C	1.78 ± 0.35	2.24 ± 0.83
C. annuum + mitomycin C	3.58 ± 0.73	2.22 ± 0.32
S. officinale + mitomycin C	2.04 ± 0.38	1.79 ± 0.64
P. aureus + mitomycin C	2.77 ± 0.11	3.88 ± 0.69
B. balsamifera + dimethylnitrosamine	2.88 ± 0.19	3.10 ± 1.05
A. cepa + dimethylnitrosamine	2.44 ± 0.55	2.16 ± 1.67
S. purpurea + dimethylnitrosamine	3.55 ± 1.01	3.44 ± 1.16
C. astusa + dimethylnitrosamine	1.88 ± 0.19	2.16 ± 0.05
M. cordifolia + dimethylnitrosamine	2.39 ± 0.64	2.83 ± 1.03
L. leucocephala + tetracycline	2.49 ± 0.42	1.74 ± 0.56
P. acutifolia + tetracycline	1.77 ± 0.76	2.44 ± 0.19
G. mangostana ± tetracycline	1.66 ± 0.33	2.32 ± 0.50
P. aureus + tetracycline	2.33 ± 0.33	2.11 ± 0.50
Q. indica + tetracycline	1.78 ± 0.50	1.56 ± 0.58
B. balsamifera + tetracycline	1.99 ± 0.57	2.49 ± 1.17