PRELIMINARY STUDY ON THE DESMUTAGENIC AND ANTIMUTAGENIC EFFECT OF SOME NATURAL PRODUCTS

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ABSTRACT

Extracts of some plant parts were assayed for their antimutagenic/desmutagenic action against ultraviolet (UV) radiation, N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), benzo(a)pyrene (B[a]P) and 3-amino-1-methyl-5H-pyrido(4-3-b) indole (Trp-P-2) by using *Escherichia coli* WP-2 and *Salmonella typhimurium* TA98. Mutagenicity of B[a]P and Trp-P-2 was decreased considerably by lemon, black and green tea; of MNNG by lemon, black tea, green tea, *Psidium guajava, Terminalia chebula, Ziziphus jujuba, Cassia fistula* and of UV radiation by *Psidium guajava, Terminalia chebula, T. arjuna, Ziziphus jujuba, Eucalyptus* sp., *Aegilops* sp. and *Accacia arabica*. The frequent use of these plant products may help to reduce the genetic hazards of these mutagens.

INTRODUCTION

It is now widely recognized that most, if not all cancers, may be due to various environmental and dietary factors. Wynder and Gori[1] estimated that over half of all female cancer deaths and 40% of all male cancer deaths may be related to food factors. Sugimura *et al*[2] suggested that there are hundreds of mutagens in the food (some naturally present and some which might develop during processing of various food materials) taken by us which can cause DNA damage and tumor initiation. This carcinogenic hazard is due not only to the presence of genotoxic agents but also to lack of antimutagenic/anticarcinogenic agents in deficient diets. One of the best ways to minimize the effect of mutagens and carcinogens is by identifying the antimutagens (substances which suppress or inhibit the process of mutagenesis by acting directly on the mechanism of cell) and desmutagens (substances which somehow destroy or inactivate, partially or fully the mutagens, thereby affecting less cell population) in our diets and increasing their use. In the present study various natural products of economical and edible importance have been evaluated for their antimutagenic and desmutagenic activity by using *Escherichia coli* WP-2 and *Salmonella typhimurium* TA98 bacterial strains.

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MATERIAL AND METHODS

Media and soft agar

For reverse mutation assay of *E. coli* WP-2 semi-enriched minimal agar medium (SEM) was prepared by dissolving 1 g of $(NH_4)_2SO_4$, 10 g of KH_2PO_4, 0.1 g of $MgSO_4.7H_2O$ and 0.5 g of trisodium citrate. $2H_2O$ in distilled water (neutralized (pH 7.0) with KOH) and mixed with 4 g of glucose, 15 g of Difco agar, 0.16 g of nutrient broth in 1 litre distilled water.

For *S. typhimurium* TA98, modified Vogel Bonners medium (MB medium) was prepared by dissolving 1 g of $(NH_4)_2SO_4$, 10 g of KH_2PO_4, 0.1 g of $MgSO_4.7H_2O$, 0.5 g of trisodium citrate, 4 g of glucose, 15 g of Difco agar and 80 mg of Difco nutrient broth powder in 1 litre distilled water[3,4]. Soft agar was prepared by dissolving 0.7 g Difco agar and 0.6 g NaCl (for *S. typhimurium* TA98 0.78 mg histidine and 1.22 mg biotin were also added) in 100 ml distilled water, autoclaved and stored at 40°C until use.

Preparation of S-9 mixture

Liver microsomal fraction (S-9 mix) was prepared[5] from Chinese hamsters and stored at −80°C. The mix contained 0.3 ml S-9, 33 μmol KCl, 8 μmol $MgCl_2$, 4 μmol NADP, 5 μmol glucose 6-phosphate and 100 μmol Na_2HPO_3 per ml.

Preparation of plant extracts

Methanol extracts of plant materials were prepared by boiling 20 g dry material in 200 ml metha-

nol for 4 hr at 60°C followed by vacuum evaporation under reduced pressure. Tea extract was obtained by adding 200 ml boiling water to 20 g dry tea leaves in a glass beaker and by lyophlizing the filtrate. Lemon extract was obtained by lyophlizing lemon juice.

Preculture of bacteria

E. coli B/rWP-2[3] and *S. typhimurium* TA98 were grown in nutrient broth (Difco 8 g/l) supplemented with NaCl (5 g/l) for 14 hr.

Antimutagenic assay against UV radiation

The overnight cultured cells were washed twice with phosphate buffer (pH 7.4), resuspended in phosphate buffer and irradiated in a Petri dish (9 cm dia) by a germicidal lamp (15 W) for 15 sec with intermittent stirring. The fluence of UV lamp was 1.5 J/m^2/sec. Plant extracts were dissolved in distilled water or dimethly sulphoxide (DMSO). The plant extract solution 100 μl (50 μl, if solvent is DMSO), phosphate buffer (0.5 ml) and 0.1 ml mutagenized cells (1–2.10^8/ml) were well mixed in a tube and then poured on SEM plates using 2 ml soft agar. To determine the cellular viability, the suspension of mutagenized cells was diluted 10^{-6} and 0.1 ml was poured in the same way as described above. The revertants and viable cells were counted after incubation at 37°C for two days.

Desmutagenic assay against N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)

MNNG 1.5 μg/0.1 ml, 0.5 ml phosphate buffer, 50–100 μl plant extract and 0.1 ml bacterial cells (1–2.10^8 cells/ml) were incubated at 37°C with constant stirring for 20 min and then poured on SEM plates using 2 ml soft agar. To determine the cellular viability, diluted cells (10^{-6} fold) were also incubated, plated similarly and observed after 48 hr incubation at 37°C.

Desmutagenic assay against benzo(a)pyrene (B[a]P) and 3-amino-1-methly-5H-pyrido (4-3-b) indole (Trp-P-2)

B[a]P (5 μg/0.1 ml) and Trp-P-2 (1.0 μg/0.1 ml) were separately mixed with 0.5 ml S-9 mix, 50–100 μl plant extract and 0.1 ml bacterial cell suspension, and incubated at 37°C for 20 min with constant stirring followed by pouring on MB plates with the help of soft agar (2 ml). Cell viability was also determined by the same aforementioned method. Revertants and survivals were counted after incubation at 37°C for 2 days. All samples were tested twice in duplicate. The relative mutagenic activity (RMA) was determined by the formula:

$$\text{RMA} = \frac{m/s}{M/S} \times 100$$

where m and M are respectively the numbers of revertants in the presence of and absence of extract, and s and S are numbers of survival in the presence of and absence of extract.

RESULTS AND DISCUSSION

The mutagenic effect of MNNG, Trp-P-2 and B[a]P was decreased considerably by lemon (32.10, 1.35 and 14.00 RMA respectively—tables 1–3); black tea (36.25, 1.05 and 15.90 RMA respectively—tables 1–3); green tea (38.1, 31.2 and 27.0 RMA respectively—tables 1–3). Trp-P-2, a pyrolytic product may form during the cooking of protenaceous food like fish and meat. Its mutagenicity is very high and is comparable with Aflatoxin-B$_1$ (one of the most potent carcinogen). It is of great significance that lemon, black tea and green tea which are widely consumed all over the world showed very good desmutagenic activity against Trp-P-2 besides MNNG (direct acting mutagen) and B[a]P (requiring metabollic activation). The increased use of these food products may help to reduce the genetic hazards of these mutagens.

The mutagenicity of MNNG was reduced considerably by the following plant materials: leaf extract of *Psidium guajava* (59.3 RMA), fruit extract of *Terminalia chebula* (60.5 RMA), bark extract of *Ziziphus jujuba* (66.7 RMA), leaf extract of *Cassia fistula* (49.4 RMA) (table 1). UV-induced mutagenesis was decreased by *Psidium guajava* (28.5 RMA), *Terminalia chebula* (36.7 RMA), *T. arjuna* (30.1 RMA), *Eucalyptus* (41.2 RMA), *Aegilops* sp. (76.3 RMA), *Accacia arabica* (22.3 RMA) (table 4). The economical as well as edible importance of these natural products is well known. So the frequent use of these plant products may provide a shield against genetic damage due to these mutagens.

It has been suggested[6] that antimutagens/desmutagens act in two ways: (a) those agents which act directly on mutagens through, (i) chemical reaction; (ii) enzymatic reaction; (iii) adsorption by high molecular substances e.g. fibres, (b) those agents which inhibit generation of active mutagenic form through: (i) inhibition of metabolic activation; (ii) inhibition of chemical reaction that generate mutagens from precursors.

Table 1 *Desmutagenic effect of some natural products against mutagenic effect of MNNG in E. coli WP-2*

Plant	Economic importance	Part from which extract was made	Dose mg/plate	Mean number of revertants	Mean number of survival	Relative mutagenic activity	Survival percentage
Control				154.5	172.5	100.0	100.0
Camellia (Black tea)	Common beverage	Processed leaves*	4	52.0	156.5	36.2	90.7
Camellia (Green tea)	Common beverage	Processed leaves*	4	59.5	172.0	38.1	99.7
Control				386	42	100.0	100.0
Citrus (Lemon)	As taste maker in food and drinks	Fruit (juice*)	20	108	36	32.1	86.9
Control				80	124	100.0	100.0
P. guajava	Table-4	Leaf**	5	33	87	59.3	70.0
T. chebula	do	Fruit**	5	45	116	60.5	93.5
Z. jujuba	do	Bark**	5	41	96	66.7	77.3
Cassia fistula	seeds are of medicinal importance	Leaf**	5	49	105	49.4	97.7

* Lyophilyzed extract; ** Extract in methanol.

Table 2 *Desmutagenic effect of some natural products against mutation-induced by Trp-P-2 in S. typhimurium TA 98*

Plant	Part from which extract was made	Dose mg/plate	Mean number of revertants	Mean number of survival	Relative mutagenic activity	Survival percentage
Control			2352	69	100.0	100.0
Camellia (Black tea)	Processed leaves*	4	50	76	1.9	109.4
Camellia (Green tea)	Processed leaves*	4	731	73	29.4	105.8
Citrus	Fruit (Juice*)	5	55	67	2.4	97.1

* Lyophilized extract.

Table 3 *Desmutagenic effect of some natural products against mutation-induced by B[a]P in S. typhimurium TA 98*

Plant	Part from which extract was made	Dose mg/plate	Mean number of revertants	Mean number of survival	Relative mutagenic activity	Survival percentage
Control			142	72	100.0	100.0
Camellia (Black tea)	Processed leaves*	4	25	78	15.9	108.3
Camellia (Green tea)	Processed leaves*	4	41	77	27.0	106.9
Citrus (Lemon)	Fruit (Juice*)	5	20	71	14.0	97.9

* Extract was lyophilyzed.

Table 4 *Antimutagenic effect of some natural products against UV-induced mutagenicity in E. coli WP-2*

Plant	Economic importance	Part from which extract was made	Mean number of revertants	Mean number of survival	Relative mutagenic activity	survival percentage
Control			216	89	100.0	100.0
P. guajava	Fruits are edible	Leaf*	57	82	28.5	92.7
T. chebula	As drug against gastric disorders and as spice	Fruit*	83	92	36.7	104.0
Z. jujuba	Fruits are eaten	Bark*	23	66	14.3	74.6
		Leaf*	88	98	37.0	110.2
T. arjuna	As spice	Bark*	68	92	30.1	104.0
Eucalyptus	Medicinal as well as commercial	Bark*	97	96	41.2	108.5
Aegilops	Fruits are edible	Leaf*	197	106	76.3	119.2
Accacia	Its gum is used in the preparation of some sweets	Bark*	34	63	22.3	70.6

* Extract in methanol; The dose in the cases was 5 mg/plate.

The desmutagenic action of lemon, black tea and green tea against Trp-P-2, MNNG, B[a]P and of *Z. jujuba, C. fistula* and *P. guajava* against MNNG-induced mutagenesis might be due to some chemical reaction or by inhibition of metabolic activation. The decrease in UV induced mutagenicity due to *P. guajava, T. chebula, T. arjuna, Z. jububa, Eucalyptus* sp. and *Accacia* might be because of some enzymatic action which reverted the formation of pyrimidine dimers. It will be of interest to find out the exact mode of action after purifying the active component of these natural products.

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