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MUTAGENICITY AND ANTI-MUTAGENICITY OF SELECTED SPICES

K. K. SOUDAMINI, M. C. UNNIKRISHNAN, K. SUKUMARAN AND R. KUTTAN*

Amala Cancer Research Centre,
Amala Nagar P.O.,
Thrissur - 680 553

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Abstract: Using *Salmonella typhimurium* strains TA 100 and TA 1535, the mutagenicity and anti-mutagenicity of extracts of several spices were checked. Spices like pepper, pippali, ginger and mustard increased the number of revertants indicating their mutagenic potential. Garlic extract on the other hand was found to inhibit the mutagenicity produced by direct acting mutagens such as N-methyl N'-nitro-N-nitrosoguanidine and sodium azide. Asafoetida and turmetic extract were found to inhibit microsomal activation dependent mutagenicity of 2-acetamidofluorene. Similar results were also obtained using curcumin and eugenol which are phenolics present in turmeric and clove respectively. These results indicated that some of the spices may ameliorate the effect of environmental mutagens especially present in the food.

Key words: mutagenicity anti-mutagenicity
2-acetamidofluorene spices

INTRODUCTION

Spices are major ingredients in the culinary art in most parts of the world although use of particular spices vary. They give the characteristic aroma and taste of the food and some of them give specific colour for the preparation. The effect of intake of spices has not been studied well enough, although studies have been carried out both *in vivo* and *in vitro* using some of the isolated ingredients from it. Capsaicin isolated from pepper and 4-alkyl-1, 2-methylenedioxy benzene (Safrole) and quercetin present in several spices have been shown to be mutagenic (1-3). However, other spices tested did not show any mutagenicity in *Salmonella* test system (4).

Spices have been shown to have anti-oxidant activity (5, 6) and are being used as food preservatives. Turmeric *(Curcuma longa)* has been shown to inhibit superoxide production and lowered lipid peroxides (7). Because of the antioxidant activity, use of these extracts in ameliorating the carcinogen induced neoplasms has been reported (8). In the present study we have determined the mutagenic and anti-mutagenic activity of some of the spices by *Salmonella typhimurium* assay.

METHODS

Salmonella typhimurium strains TA 100, 98 and TA 1535 were originally procured from Prof. B.N. Ames, University of California, Berkeley, U.S.A. and after subculturing in nutrient broth were dispensed in small vials and freezed in presence of dimethyl sulfoxide (9%) and kept at —90°C. The bacterial culture was inoculated in fresh nutrient broth and grown for 16 h at 37°C before the experiment.

Nutrient broth was purchased from Hi-media Laboratories, Bombay and noble agar Difco Laboratories. 2-acetamidofluorene was purchased from Sigma Chemicals, St. Louis, Mo. Curcumin was obtained as a gift from KANCOR, Angamaly and eugenol from Romali, Bombay. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was a gift from Dr. S.V. Bhide, Cancer Research Institute, Bombay. All other chemicals were of analytical reagent quality. All spices were bought from the local market where the storage time was approximately

*Corresponding Author

1-4 months and powdered and kept tightly closed in the refrigerator (4°C). One gram of the powder was extracted with 200 ml of 70% ethanol, overnight at 25°C with stirring. It was filtered and concentrated in vacuum at 25°C using a rotory evaporator (Yorco, India). The syrupy liquid was weighted and redissolved in 10 ml of 70% ethanol so that the solution contained extract from 100 mg of the dry powder per millilitre.

Mutagenicity testing : Histidine requiring strains of *Salmonella typhimurium* (TA 100 and TA 1535) were grown in nutrient broth. Cells (10^8) were plated in minimal agar plates (containing 0.5 mM Histidine/Biotin). The concentrations of the spice extract used depended upon the toxicity it produced to test organism. A non-toxic concentration of 50 mg and 25 mg/plate were used for most of the spices except for pippali as well as pepper (25 mg and 10 mg/plate) and turmeric (2.5 mg and 1 mg/plate). The plates were incubated for 48 h at 37°C and number of revertants formed were counted using a colony counter. All experiments were done twice with three plates per each concentration. Each data is expressed as the mean of six plates with standard deviation. Statistical significance was determined by students' 't' test. Sodium azide (1 µg/plate) and MNNG (1 µg/plate) were used as positive controls and untreated plates were considered as negative controls.

Anti-mutagenicity testing : Anti-mutagenicity of extrcts of spices were tested using strains of *Salmonella typhimurium* strains TA 1535 and TA 100. A non-toxic concentration of spice extracts were incorporated into minimal agar plate (containing a 0.5 mM Histidine/Biotin) (9). Plates were spread with a solution (100 µl) of mutagens such as sodium azide (1 µg/plate) or MNNG (1 µg/plate). Five min later, bacteria (10^8 cells, 100 µl) were spread on the plate using a glass spreader and incubated for 48 h at 37°C. After the incubation, the number of revertants formed in mutagen treated plates and mutagen with extracts of spices treated plates were counted. Anti-mutagenicity was expressed as the difference in the number of colonies. Each data is the mean of six plates with standard deviation.

Effect of spice extracts on the activation of 2-acetamidofluorene : Male rats (150-180 g) were given sodium phenobarbital (0.1%) in drinking water for 4 days to induce liver microsomal enzymes (9). Animals were killed on the 5th day and livers were removed aseptically and microsomal fraction (S9 fraction) was prepared (10).

The activation of 2-acetamidofluorene was done by the incubaton of S9 fraction (200 µl) with 2-acetamidofluorene (50 µg in 50 µl dimethyl sulfoxide). The following co-factors and buffers were used during the incubation. NADP (0.1 M, 25 µl), glucose 6-phosphate (1 M, 25 µl) KCl (1.65 M)-$MgCl_2$ (0.4 M) salt solution (10 µl), phosphate buffer (0.2 M, pH 7.4, 200 µl).

Logarithmically growing *Salmonella* culture (TA 98, 10^8 cells) were added to the above mixture and incubated at 37°C for 45 min and further poured over minimal agar plate (containing 0.5 mM Histidine/Biotin) (10). The plates were further incubated for 48 h at 37°C for the development of revertant colonies. The effect of spices of 2-acetamidofluorene was determined by incubating the extracts of spices with the activation mixture. After incubation, the number of revertants formed were counted in the case of (a) 2-acetamidofluorene alone, (b) 2-acetamidofluorene after activation, (c) 2-acetamidofluorene, activated in presence of extracts of spices. Difference in the number of colonies in 2-acetamidofluorene activated plates and 2-acetamidofluorene activated in presence of extracts of spices indicated the anti-mutagenicity of spice extract. Each data is expressed on the average of six values with standard deviation. Effects of curcumin and eugenol present in turmeric and clove respectively on the mutagenicity of activated 2-acetamidofluorene were also evaluated in a similar manner using different concentration.

RESULTS

Mutagenicity of spices : The mutagenicity of the extracts of spices were checked in TA 1535 and TA 100 at two different concentrations. Ginger showed mutagenicity in both TA 1535 and TA 100 at both concentrations as seen from the increased

TABLE I : Mutagenicity of extracts of spices to *Salmonella typhimurium*. (Data represents mean of 6 reading ± SD).

Spice extract	*Concentration (mg/plate)*	*Average number of revertants/plate* TA 1535	TA 100
Asafoetida	50 mg	20 ± 3.3	147 ± 6.9
(Ferula asafoetida)	25 mg	10 ± 5.8	151 ± 4.6
Garlic	50 mg	18 ± 5.0	170 ± 7.9
(Allium sativum)	25 mg	17.1 ± 1.9	^{xxx}185 ± 4.2
Ginger	50 mg	^{xxx}53 ± 2.9	^{xxx}200 ± 8.6
(Zingiber officinale	25 mg	53 ± 2.6	181 ± 5.2
Black Pepper	25 mg	^{xxx}32 ± 12.1	160 ± 5.9
(Piper nigrum)	10 mg	18 ± 2.27	170 ± 12.4
Pipplali	25 mg	^{xxx}92 ± 5.1	158 ± 9.5
(Piper longum)	10 mg	46 ± 6.4	162 ± 4.6
Mustard	50 mg	^{xxx}64 ± 3.6	161 ± 8.2
(Brassica campastris)	25 mg	23 ± 2.5	161 ± 6.0
Sesame	50 mg	35 ± 3.9	161 ± 8.4
(Sesamum indicum)	25 mg	21 ± 3.0	169 ± 6.9
Turmeric	2.6 mg	14 ± 1.7	150 ± 5.8
(Curcuma longa)	1.0 mg	14 ± 2.2	142 ± 7.1
Spontaneous revertants		17 ± 2.0	159 ± 2.6

P = ^{xxx} <.001; ^{xx} < .01; ^{x} <.05 compared to SP

number of revertants. Pippali (Piper longum) showed mutagenicity only in the case of TA 1535 but did not show effect in TA 100. Other spices which showed mutagenicity at 50 mg using TA 100 were black pepper and mustard but these extracts were not mutagenic at lower concentration or when TA 1535 was used. As could be expected, the mutagenicity of spices were much lower than the established mutagen such as sodium azide and MNNG.

Anti-mutagenicity of extracts of spices using MNNG as mutagen : As shown in Table II, garlic extract showed remarkable anti-mutagenicity to MNNG induced mutation as seen by the reduction in revertants even at lower concentration and as the concentration increased, the number of revertants formed were further reduced. However, when TA 100 was used instead of TA 1535, this effect could not be well documented. The only other extract which showed a reduction in revertants was that of ginger. However, at lower concentration, ginger extract did not show any anti-mutagenic activity. Other extracts of spices did not have any ati-mutagenic activity and extract of pippali showed mutagenicity as seen by the increased number of revertants.

Anti-mutagenicity of extracts of spices using sodium azide as mutagen : Ginger extract inhibited the sodium azide induced mutation considerably in TA 1535 as well as in TA 100 (Table III). Garlic also showed lowered number of revertants in TA 1535 at higher concentration but not at lower concentration. Pippali showed increased revertants indicating mutagenic potential of the extract. Other extracts of spices except sesame did not show any significant effect as mutagenic or anti-mutagenic agents.

Anti-mutagenicity of extracts of spices using 2-acetamidofluorene as mutagen : Turmeric was found to be the most effective in inhibiting the activation of 2-acetamidofluorene at concentration of 2.5 mg to 1 mg per plate (Table IV). At this concentration turmeric extract was not found to be toxic to the cells and hence the reduced number

TABLE II : Anti-mutagenicity of extracts of spices to *Salmonella typhimurium* TA 1535 and TA 100 against MNNG as mutagen. (Data represents mean of 6 reading ± SD)

Spice extract	*Concentration (mg/plate)*	*Average number of revertants/plate* TA 1535	TA 100
Asafoetida	50 mg	648 ± 8.1	891 ± 18.0
(Ferula asafoetida)	25 mg	633 ± 5.6	851 ± 18.0
Garlic	50 mg	^{xxx}459 ± 11.7	^{x}830 ± 22.9
(Allium sativum)	25 mg	536 ± 5.6	854 ± 20.8
Ginger	50 mg	^{xxx}502 ± 12.5	943 ± 23.3
(Zingiber officinale)	25 mg	622 ± 16.1	810 ± 15.2
Black Pepper	25 mg	699 ± 16.1	917 ± 18.2
(Piper nigrum)	10 mg	656 ± 14.0	885 ± 21.7
Pipplali	25 mg	724 ± 19.4	906 ± 23.4
(Piper longum)	10 mg	764 ± 15.9	871 ± 16.8
Mustard	20 mg	689 ± 16.1	^{x}835 ± 14.7
(Brassica campastris)	25 mg	664 ± 15.1	844 ± 19.3
Sesame	50 mg	667 ± 22.9	875 ± 13.0
(Sesamum indicum)	25 mg	673 ± 41.3	857 ± 17.5
Turmeric	2.5 mg	655 ± 15.0	857 ± 18.9
(Curcuma longa)	1.0 mg	654 ± 13.9	862 ± 15.6
MNNG 1 µg/plate alone		602 ± 13.9	865 ± 18.9
Spontaneous revertants		17 ± 2.0	159 ± 2.6

P = ^{xxx} < 001; ^{xx} < .01; ^{x} <.05 compared to MNNG

of colonies obtained was due to the inhibition of activation. Turmeric extract alone or acetamidofluorene alone did not have any effect in the assay at this condition. Asafoetida extract also was found to have an effect on the activation of acetamidofluorene. However, garlic extract which had anti-mutagenic activity when direct acting mutagens were used, did not have any effect in acetamidofluorene activation. Pippali and pepper showed increased mutagenicity.

We have also checked the effect of curcumin and eugenol (present in turmeric and clove respectively) for their inhibitory action on microsomal activation of acetamidofluorene and following mutagenicity (Table V). As seen in the table eugenol at concentrations 100 µg/plate inhibited the mutagenicity by 50%. Similar result was also seen in the case of curcumin which inhibited the acetamidofluorene induced mutagenicity by 50% at concentration 50 µg/plate.

DISCUSSION

In the present study we have evaluated the mutagenicity and anti-mutagenicity of some of the extracts of spices which are being used not only in India but several parts of the world. These spices have not only the aroma and colour but also have reported medicinal properties and are useful in the preservation of food materials (11).

Spices have been reported to produce cytotoxiticy to cultured cells but their tumour reducing activity was rather poor (12). Moreover, spices like turmeric and its active ingredient curcumin, garlic and its active ingredient diallyl sulphide and asafoetida have been shown to inhibit carcinogenesis in mice (8, 13).

Black pepper, pippali and ginger showed mutagenicity while other spices did not. The active ingredient in pepper, i.e., piperine has been shown to be mutagenic (1). However, the mutagenic

TABLE III : Anti-mutagenicity of extracts of spices to *Salmonella typhimurium* TA 1535 and TA 100 against sodium azide as mutagen. (Data represents mean of 6 reading ± SD)

Spice extract	*Concentration (mg/plate)*	*Average number of revertants/plate* TA 1535	TA 100
Asafoetida	50 mg	163 ± 9.6	594 ± 12.4
(Ferula asafoetida)	25 mg	137 ± 7.8	559 ± 14.4
Garlic	50 mg	^{xx}115 ± 5.0	577 ± 12.4
(Allium sativum)	25 mg	148 ± 5.4	584 ± 16.0
Ginger	50 mg	^{xxx}89 ± 4.2	^{x}528 ± 11.4
(Zingiber officinale)	25 mg	84 ± 3.4	544 ± 9.0
Black Pepper	25 mg	165 ± 6.7	592 ± 16.2
(Piper nigram)	10 mg	157 ± 7.1	593 ± 13.2
Pipplali	25 mg	210 ± 5.9	651 ± 23.1
(Piper longum)	10 mg	285 ± 8.26	624 ± 17.5
Mustard	20 mg	133 ± 5.1	574 ± 22.1
(Brassica campastris)	25 mg	133 ± 14.1	599 ± 10.5
Sesame	50 mg	123 ± 8.5	574 ± 13.2
(Sesamum indicum)	25 mg	^{xx}108 ± 8.9	584 ± 16.1
Turmeric	2.5 mg	127 ± 5.0	570 ± 16.1
(Curcuma longa)	1.0 mg	133 ± 8.5	557 ± 14.7
Sodium Azide 1 µg plate/alone		132 ± 9.0	574 ± 21.0
Spontaneous revertants		17 ± 2.0	159 ± 2.6

P = ^{xxx} < 001; ^{xx} < .01; ^{x} <.05 compared to MNNG

potential of these extracts as compared to known mutagen such as MNNG and Sodium azide used in this study as positive controls were lower.

Studies to determine the anti-mutagenic activity of these extracts indicated that extracts made from garlic inhibited the mutagenicity produced by direct acting mutagens such as sodium azide and MNNG while turmeric and its active ingredient inhibited the activation of the mutagens such as acetamidofluorene by rat liver S9 fraction and thereby inhibited its mutagenic and possibly carcinogenic potential. In the case of acetamidofluorene the activation needed the oxidation mediated by P-450 enzyme to an aminoxide and this step was inhibited by turmeric, curcumin and eugenol (14).

The ingredients responsible for the inhibition of mutagenicity and carcinogenicity of these spices are not completely understood. Curcumin present in the turmeric was shown to be anti-carcinogenic in mice and inhibited the promotion induced by 12-0-terradecanoyl phorbol-13-ecetate (TPA) (15). This was followed by a decrease in the TPA-induced ornithine decarboxylase. Similar results were also found in the case of ferulic acid which has been reported to be present in asafoetida and turmeric (15). Several sulfur containing compounds have been isolated and identified from garlic. Diallyl sulphide present in garlic has been shown to be anti-carcinogenic in animals by inhibiting promotion (16). Asafoetida also contained sulfur containing compounds similar to garlic as well as phenols such as umbelliferon. Recently turmeric also was found to contain a water soluble peptide which inhibited lipid peroxidation (17).

We do not know the mechanism of inhibition of mutagenesis by these spice extrat excepting their free radical scavenging action. Other mechanisms can also explain the action of spices

TABLE IV : Anti-mutagenicity of extracts of spices to *Salmonella typhimurium* TA 98 against 2-Acetamidofluorene (2-AAf) as mutagen. (Data represents mean of 6 reading ± SD)

Spice extract	*Concentration (mg/plate)*	*Average number of revertants/plate* TA 1535
Treated with 2-AAF and Spice Extracts		
Asafoetida	10mg	108 ± 5.3
(Ferula asafoetida)	5 mg	113 ± 5.9
Garlic	10 mg	132 ± 83
(Allium sativum)	5 mg	153 ± 7.9
Ginger	10 mg	120 ± 5.7
(Zingiber officinale)	5 mg	137 ± 8.8
Black Pepper	25 mg	182 ± 8.8
(Piper nigram)	10 mg	147 ± 7.7
Pipplali	25 mg	163 ± 6.4
(Piper longum)	10 mg	138 ± 5.5
Mustard	10 mg	146 ± 7.0
(Brassica campastris)	5 mg	144 ± 9.3
Sesame	10 mg	96 ± 10.3
(Sesamum indicum)	5 mg	121 ± 8.1
[b]Turmeric	2.5 mg	^{xxx}67 ± 85
(Curcuma long)	1.0 mg	^{xxx}72 ± 12.6
2-Acetamidofluorene alone (50 µg/plate)		149 ± 19.0
Spontaneous revertants		29 ± 34

P = ^{xxx} < 001; ^{xx} < .01; ^{x} <.05 compared to AAF

TABLE V : Anti-mutagenicity of curcumin and eugenol to *Salmonella typhimurium* TA 98 against 2-acetamidofluorene (2-AAF) as mutagen. (Data represents mean of 6 reading ± SD)

Experiment	*Test system*	*Concentration (mg/plate)*	*Average number of revertants/plate*
I	2-AAF 50	432 ± 31	
	+ Eugenol	20	422 ± 28
	+ Eugenol	100	^{xxx}195 ± 22
	+ Eugenol	200	^{xxx}118 ± 13
	+ Eugenol	400	^{xxx}30 ± 2
II	2-AAF	50	457 ± 12
	+ Curcumin	50	248 ± 3.6
	+ Curcumin	100	222 ± 1.7
	+ Curcumin	250	^{xxx}131 ± 2.6
	+ Curcumin	500	^{xxx}83 ± 2.5

Spontaneous revertants (27 ± 3 for experiment I and 25 ± 2 for experiment II) not subtracted.

P = ^{xxx} <0.001 compared to AAF alone.

as anti-mutagens. For example the spices may induce the elimination of the carcinogens by induction of glutathione-S-transferase activity and Phase II enzymes (18). Moreover, some of the ingredients in the spices may form adducts with carcinogens as has been clearly demonstrated with ellagic acid and benzo (e) pyrene (19).

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