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Research Article

Radioprotection Imparted by Four Spices in a Bacterial System

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ABSTRACT: **Introduction:** A wide variety of substances derived from herbs and spices have been shown to possess potent antioxidant, anti-inflammatory, anti-mutagenic and anticarcinogenic activities. The study of the modulation of genotoxicity by the extracts of some such spices would thus be meaningful and interesting. **Objective**: Aqueous extracts of four extensively used spices viz. *Trachyspermum copticum* (Ajwain), *Piper nigrum* (Black pepper), *Syzyquim aromaticum* (Clove) and *Pimenta dioica* (Allspice) have been employed to study the modulation of genotoxicity, if any, induced by a potent DNA damaging agent viz. ultraviolet radiation (UV C), in *Salmonella typhimurium* cells. **Methods***:* Induction of *umu*-gene was assayed in *Salmonella typhimurium* cells in presence or absence of varying amounts of the spice extract after a fixed dose of UV C exposure. The percentage survival of the *Salmonella typhimurium* cells exposed to UV C was computed by the study of the colony forming units on LB-Agar plates. **Results**: All the aqueous extracts of Ajwain, Black Pepper, Clove and Allspice was found to impart radio-protection to *Salmonella typhimurium* cells against UV C induced DNA damage in a dose–dependent manner. Clove has been found to impart a very high degree of radioprotection as compared to the other spice extracts and also at a much lower concentration. This has been reflected by both the assay methods mentioned above. **Conclusions**: Our results indicate that aqueous extracts of all four spices studied, impart radioprotection against UV induced DNA damage in *Salmonella typhimurium* cells in a dose-dependant manner. All the spice extracts mentioned above, excepting clove, presumably due to its antibacterial activity, were found to support the growth of the *Salmonella typhimurium* cells. This may be indicative that the extracts are imparting protection by effectively reducing the UV dose upon absorption of a part of UVC by the constituents of the spice extracts excepting clove.

KEYWORDS: Spice extract, Radioprotection, Bacterial assay system.

INTRODUCTION

Phenolic substances derived from herbs and spices are potent antioxidants and exhibits anti-inflammatory, anti-mutagenic and anticarcinogenic activities in living systems.[1] These extracts may have the potential to modulate the genotoxic activity caused by harmful radiations and chemicals to which the human population is incessantly exposed to. The study of the modulation of genotoxic activity by some commonly used Indian spices will therefore be meaningful and interesting. As a first step to this, a number of such spices have been employed to study their potential role in the modulation of genotoxic activity induced by a harmful radiation viz. UVC, using *Salmonella typhimurium* as a model test system. The *umu*-gene expression assay as devised by Oda et al[2] in *Salmonella typhimurium* TA 1535/pSK1002 cells have been widely used and accepted as a short term bacterial assay system for the screening of genotoxic agents. In the above noted strain a *umu*C'-*lac*Z fusion gene is carried in a multi copy plasmid, thus improving the sensitivity of the system for the detection of *umu* gene expression (the gene that is believed to be responsible for induced mutagenesis). Measurement of the amount of β-galactosidase produced in these cells under different conditions would therefore reflect the extent of *umu* gene induction under those conditions. The present study aims at investigating on the modulation of DNA damage in TA 1535/pSK1002 cells induced by a fixed dose of UVC (which produces a significant amount of DNA damage) with varying doses of crude extracts of four commonly used Indian spices viz. Ajwain (*Trachyspermum copticum)*, Black Pepper (*Piper nigrum*), Clove (*Syzyquim aromaticum*), and Allspice (*Pimenta dioica*). A study of the survival of the cells

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containing different volumes of the spice extracts and exposed to a fixed dose of UVC producing a high degree of DNA damage (resulting in around 10^{-4} % survival of the cells) has been undertaken in order to detect the modulation if any in the DNA damage induced by the potentially lethal dose of UV radiation without any fail. The effect of the extracts on the growth of *Salmonella typhimurium* cells were also investigated.

MATERIALS AND METHODS

Bacterial strain

Bacterial strain used in the study was *Salmonella typhimurium* TA 1535/pSK1002. The strain was determined through the kind courtesy of Dr. S. Nakamura, Japan.

Bacterial media

The culture media used in this study were i) LB media containing 0.5% yeast extract (Difco), 1% Tryptone (Difco) and 1% NaCl in distilled water, the pH of the medium was adjusted to 7.2. ii) LB Agar plates containing the above noted media supplemented with 1.5% Bacto Agar.

Chemicals

ONPG used in the studies was obtained from Sigma Aldrich Chemical Co. All other chemicals used were obtained from Merck Chemicals and were of GR grade.

Spice extract

All the four spices viz. clove, black pepper, allspice and ajwain were procured from the local market. 10g each of the grounded spices were soaked in 20 ml 0.85% NaCl and kept overnight at 4° C. The extracts were then filtered through a 0.22 nm membrane filter (Millipore Corporation).

umu-gene induction assay:

2 ml aliquots of saline suspended log phase cells supplemented with 700 µl of saline alone or containing different volumes of the spice extracts were exposed to 16.04 J/m^2 of ultraviolet radiation in an UV chamber. The dose rate of the UV tubes as calculated by UV-actinometry was 4 .01 J/m^2. The units of β-galactosidase in these cells (which correlates with the *umu* gene activity) is measured following the method of Miller[3].

Survival study

The surviving fraction of the *Salmonella typhimurium* TA 1535/pSK1002 cells exposed to germicidal UV in the chamber as referred to above at an UV dose of 32.08 J/m^2 in absence or presence of different amounts of the spice extracts were computed by the study of the colony forming units (c. f. u.) in LB Agar plates. Treating the percentage survival of the UVC exposed cells without any spice extract as the control, the survival of the UVC exposed cells containing various amounts of the spice extracts were computed in order to monitor the extent of modulation of DNA damage effected by these agents. The percentage survival was calculated as follows:

$$\text{Survival percentage} = \frac{\text{c.f.u. in the UVC exposed cells with or without the spice extract}}{\text{c.f.u. in the unexposed cells}} \times 100$$

Effect of the spice extracts on bacterial growth:

LB media, saline or water extracts of the spices (each 400ml) were added to 2 ml of saline suspended log phase *Salmonella typhimurium* cells and incubated at 37° C with shaking. The optical density at 600 nm for all the three samples were recorded at different time intervals in order to study the effect of these extracts on the growth of the organism, vis-à-vis the saline suspended control.

RESULTS

umu- gene induction assay

Fig. 1 shows the units of β-galctosidase produced in TA 1535/pSK1002 cells exposed to a fixed dose of 16.04 J/m^2 of ultraviolet radiation in presence or absence of varying amounts of the extract of the spices under consideration. Three sets of such experiments performed with the individual spices revealed similar patterns. It is evident that each of the spices studied viz. black pepper, clove, ajwain and allspice exhibited a gradual decrease in the units of β-galactosidase with increasing concentrations of the extracts vis-à-vis the UVC exposed control without any spice extract. As the

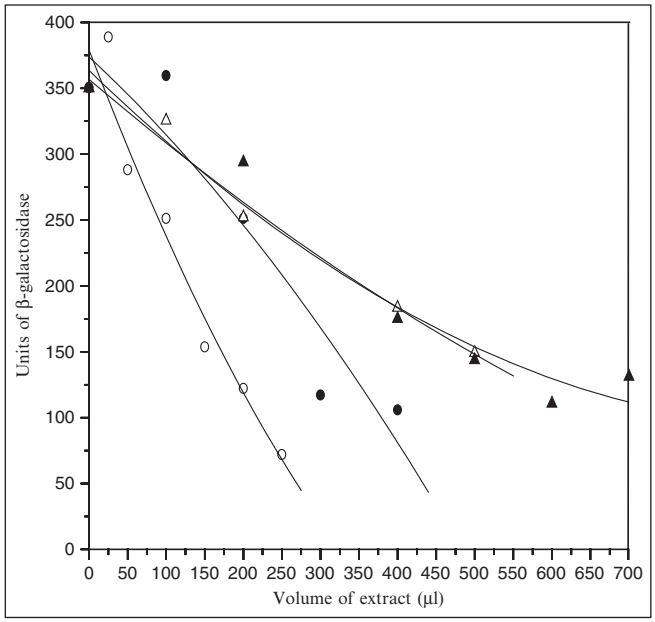

Figure 1: umu-gene expression in Salmonella typhimurium cells containing no or fixed volumes of extracts of Allspice (▲), Ajwain (Δ), Black Pepper (●) and Clove (○) after a fixed dose of UV radiation.

amount of β-galactosidase correlates with the *umu* gene induction in the cells (as referred to above), the decrease in the β-galactosidase level with increase of the spice extracts is indicative of the decrease in the level of umu gene induction in the cells after UVC exposure. This amounts to radioprotection rendered by the spice extracts against ultraviolet induced DNA damage. The effect was found to be dose-dependant. 250 µl of clove extract was found to impart a very high degree of radioprotection as compared to that imparted by the other spice extracts. Allspice, ajwain and black pepper extracts also exhibited considerable radioprotection, but at a much higher concentration of the extracts added.

Survival study

Figure 2 represents the survival of the TA 1535/pSK1002 cells in presence or absence of varying amounts of the extracts after an exposure of the cells to a much higher dose (32.08 J/m^2) of UVC radiation. It is evident that the extracts considerably protects the cells against the lethal action of germicidal UV in a dose-dependant manner. The percentage survival of the UVC exposed cells goes on increasing from a basal level of about 10^{-4}% with increasing concentrations of the spice extracts. The survival level reaches 89.75 % in presence of 300 µl of clove extract and declines thereafter to 50.85 % for 700 µl of the extract. The survival of the UVC exposed cells with increasing amounts of allspice, ajwain and black pepper extracts gradually goes on increasing up to 700 µl of the extracts and attains the values of 90.95%, 63.1% and 55.46% respectively.

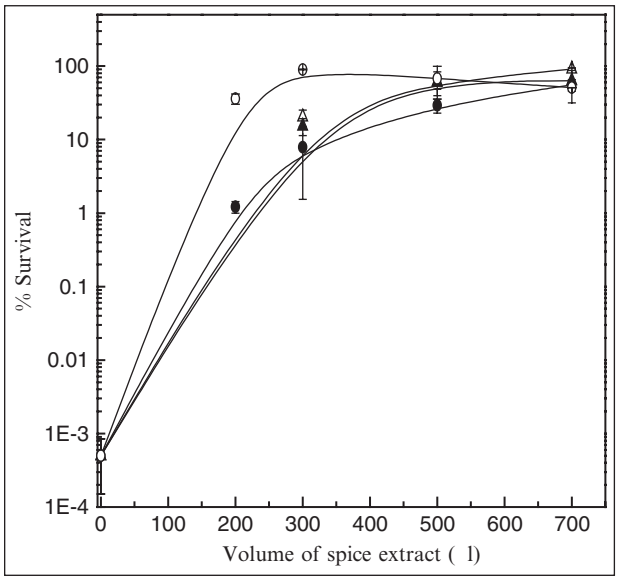

Figure 2: Survival of *Salmonella typhimurium* cells containing varying amounts of extracts of Allspice (▲) , Ajwain (Δ), Black Pepper(●) and Clove (○) after exposure to a fixed dose of UVC radiation. The error bars as indicated by the vertical lines have been calculated from individual data of multiple determination.

Figures 3, 4, and 5 indicates that the water extracts of ajwain, allspice and black pepper respectively enhances the growth of *Salmonella typhimurium* cells to an extent comparable to LB media. This is evident from the increase of the optical density of the cells at 600 nm from a starting value of about 0.2 to a value of more than 1.0 after a period of 250 minutes of incubation at 37°C. The saline suspended cells, however, maintains the same value of the optical density as the initial one all through this period. Clove, however, do not show any such growth enhancement, as is evident from the growth curves presented in Figure 6. All these experiments have been repeated at least twice to confirm the results represented in Figures 3, 4, 5,and 6. The nature of the growth profiles however remain the same for all such observations.

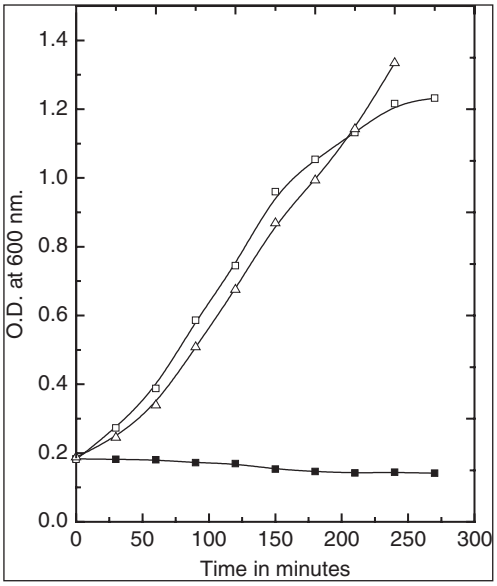

Figure 3: Growth of *Salmonella typhimurium* cells in saline (■), LB media (□) and water extract of Ajwain (Δ).

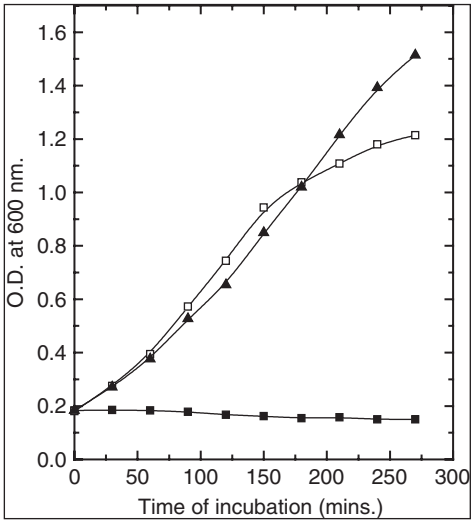

Figure 4: Growth of *Salmonella typhimurium* cells in saline (■), LB media (□) and water extract of Allspice (▲).

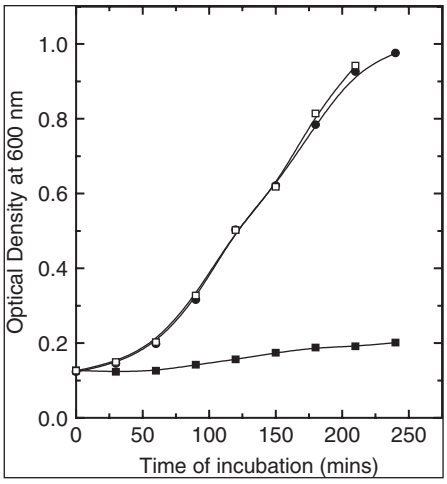

Figure 5: Growth of *Salmonella typhimurium* cells in saline (■), LB media (□) and Black pepper extract in water (●)

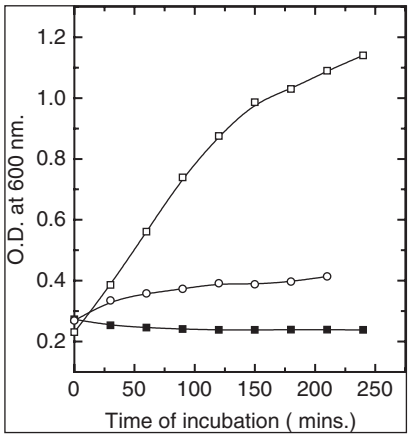

Figure 6: Growth characteristic of *Salmonella typhimurium* cells in saline (■), LB media (□) and water extract of Clove (○).

DISCUSSION

A number of herbs and spices possess antimicrobial activity. Aqueous extract of black pepper did not exhibit antibacterial activity against *B. subtilis*, but showed good inhibitory activity against *Staphylococcus aureus*[4]. It was observed by Samsuddeen et al[5] that although ethanol extracts of clove and black pepper were active against *Salmonella* spp and *Staphylococcus aureus*, the water extracts were not. Acetone extracts of ajwain showed more antibacterial activity as compared to the aqueous extract.[6] Ethanol extract and hot water extract of black pepper showed significant antimicrobial activity, whereas cold water extract showed no activity at al.[7, 4] The efficiency of plant extract as antibacterial agent, however, was dependent on the solvent of extraction.[7] This is in agreement with the results of Agatemore,[8] who found that ethanolic extracts were more potent than the respective aqueous extracts against food borne microorganisms.

Both the *umu*-gene induction assay and survival studies proves that all of the extracts under observation impart protection in a dose-dependant manner against the UV induced damage in *Salmonella typhimurium* cells. Clove was found to impart a very high degree of radioprotection to *Salmonella typhimurium* cells against UVC at a much lower concentration of the extract as compared to the other three spice extracts used. Amongst the other three spice extracts ajwain showed a slightly higher degree of radioprotection for 700 µl of the spice extract as compared to allspice and black pepper.

The antimutagenic property of black pepper, allspice, ajwain and clove has previously been reported.[9-12] It has also been reported that black pepper imparts radio-protection to γ-ray irradiated bacterial cells.[13]. Our results indicate that aqueous extracts of all four spices studied imparts radioprotection against UV induced DNA damage. As ultraviolet radiation mostly induces cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PPs), the protection imparted by the spices under study against UVC may be due to the components in the spice extracts, which are protecting against the damage, by way of absorption of the UV radiation or repairing the damage induced by UV. Nevertheless, considering the fact that at least three of the spice extracts enhances the growth of the bacterium under study, it may be reasonable to conclude that in these cases absorption of UVC radiation by some component of the extracts (as it usually occurs in case of rich growth media) may lead to the radioprotection of the cells against UVC induced DNA damage by effectively reducing the dose obtained by the bacterium. The mechanism of radioprotection imparted by clove extracts (which does not enhance growth of the organism) may be due to some component which effectively repairs the ultraviolet induced DNA damage. Further studies to elucidate these mechanisms would be meaningful and interesting.

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