Publications
Chemopreventive Effects of *Cuminum cyminum* in Chemically Induced Forestomach and Uterine Cervix Tumors in Murine Model Systems

Gagandeep, Sivanandhan Dhanalakshmi, Ester Méndiz, Agra Ramesha Rao, and Raosaheb Kathalupant Kale

Abstract: Lately, a strong correlation has been established between diet and cancer. For ages, cumin has been a part of the diet. It is a popular spice regularly used as a flavoring agent in a number of ethnic cuisines. In the present study, cancer chemopreventive potentials of different doses of a cumin seed–mixed diet were evaluated against benzo(a)pyrene [B(a)P]-induced forestomach tumorigenesis and 3-methylcholanthrene (MCA)-induced uterine cervix tumorigenesis. Results showed a significant inhibition of stomach tumor burden (tumors per mouse) by cumin. Tumor burden was 7.33 ± 2.10 in the B(a)P-treated control group, whereas it reduced to 3.10 ± 0.57 (P < 0.001) by a 2.5% dose and 3.11 ± 0.60 (P < 0.001) by a 5% dose of cumin seeds. Cervical carcinoma incidence, compared with the MCA-treated control group (66.67%), reduced to 27.27% (P < 0.05) by a diet of 5% cumin seeds and to 12.50% (P < 0.05) by a diet of 7.5% cumin seeds. The effect of 2.5 and 5% cumin seed–mixed diets was also examined on carcinogen/xenobiotic metabolizing phase I and phase II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase (LDH), and lipid peroxidation in the liver of Swiss albino mice. Levels of cytochrome P-450 (cyt P-450) and cytochrome b$_{5}$ (cyt b$_{5}$) were significantly augmented (P < 0.05 by the 2.5% dose of cumin seed diet. The levels of cyt P-450 reductase and cyt b$_{5}$ reductase were increased (significance level being from P < 0.05 to P < 0.01) by both doses of cumin. Among the phase II enzymes, glutathione S-transferase specific activity increased (P < 0.005) by the 5% dose, whereas that of DT-diaphorase increased significantly (P < 0.05) by both doses used (2.5 and 5%). In the antioxidant system, significant elevation of the specific activities of superoxide dismutase (P < 0.01) and catalase (P < 0.05) was observed with the 5% dose of cumin. The activities of glutathione peroxidase and glutathione reductase remained unaltered by both doses of cumin. The level of reduced glutathione measured as nonprotein sulfhydryl content was elevated (significance level being from P < 0.05 to P < 0.01) by both doses of cumin. Lipid peroxidation measured as formation of MDA production showed significant inhibition (P < 0.05 to P < 0.01 by both doses of cumin. LDH activity remained unaltered by both doses of cumin. The results strongly suggest the cancer chemopreventive potentials of cumin seed and could be attributed to its ability to modulate carcinogen metabolism.

Introduction

Dietary habits and nutrition are known to play an important role in the prevention of cancer. Numerous epidemiological studies have shown that a diet high in vegetables and fruits is associated with reduced risk of most cancers (1,2). Further, the differences in cancer rates are known to be correlated with dietary patterns. The lower incidence of fat-related cancers in Japanese and Mediterranean people compared with Americans was found to be linked with their dietary habits (3). As a whole, the traditional Mediterranean diet is considered to be a cancer-low-risk diet relative to the rest of Europe, North America, and other economically developed countries (4). High incidences of stomach tumor in certain parts of Japan have been ascribed to the consumption of preserved fish and the scarcity of fresh vegetables and fruits (5).

Spices are an integral part of the diet, especially in the Indian subcontinent. There are numerous reports of spices being potent chemopreventive agents such as *Curcuma longa* mustard seeds, and *Garam masala* (mixture of spices). *C. longa* was found to possess chemopreventive effects against skin cancer, forestomach cancer, colon cancer, and oral cancer in mice (6). Mustard seeds were shown to reduced tumor incidence and tumor burden in 7,12-dimethylbenz(a)anthracene (DMBA)-induced skin papillomagenesis and transplacental and translactational carcinogenesis in Swiss albino mice (7,8). *G. masala* has shown cancer chemopreventive efficacy against DMBA-induced transplacental and translactational carcinogenesis in mice (9). Cumin (*Cuminum cyminum* Linn., family Umbelliferae) is one of the most wide

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ly used spices. Our work focuses on the chemopreventive mechanisms of cumin seeds, an important dietary ingredient in the Indian diet since ancient times.

Cummin is a small, slender annual herb having seedlike fruits locally known as jeera. The cumin seed is extensively used in mixed species and for flavoring curries, soups, sas-sages, bread, and cakes. It is an ingredient of curry powder and various pickle preparations. Analysis of cumin seeds in the Indian diet since ancient times.

The seeds yield on distillation a volatile oil (oil content, 36.6; fiber, 12.0; mineral matter, 5.8; calcium, 1.08; phosphorus, 0.49; iron, 31.0 mg/100 g; carotene calculated as vitamin A, 870 I.U./100g; and vitamin C, 3 mg/100 g. The yields seed on distillation a volatile oil (oil content, 2.0-4.0%) having cumaldehyde, C10H12O (p-isopropyl-benzaldehyde), as the chief constituent. In addition to aldehyde, the oil contains p-cymene, pinene, dipentene, cumene, cuminic alcohol, &-phellandrene, and &-terpenone (10). Glycosides, namely, 3-O-β-D-glucopyranoside, 4-O-β-d-glucopyranoside, and 1-O-β-D-fructofuranoside, were isolated from the cumin fruit (11).

In indigenous medicine, cumin seeds have long been considered a stimulant, carminative, stomachic, and astringent and useful in diarrhea, dyspepsia (10) as well as in relieving sleeplessness, common cold, and fevers (12). Cumin has proven antibacterial activity (13) and hypolipidemic (14) and antihyperglycemic (15) effects. Ethereal extract of cumin has been shown to be comparable with that of standard antibiotics (17). Cumin in the diet was effective in decreasing the levels of phosphatases and sucrose, implying its role in stimulation of digestion (18). On administration in the diet, it was able to stimulate the activities of arylhydroxylase, cytochrome P-450 (cyt P-450), cytochrome b5 (cyt b5), and N-demethylase in rats (19). Essential oil of cumin has the potency to enhance the activity of glutathione S-transferase (GST) in mouse and to suppress the formation of aflatoxin B1-induced DNA adduct formation (20,21). Cuminaldehyde, isolated from cumin, when administered in high concentration was shown to inhibit ascorbate/Fe2+-induced lipid peroxidation in rat liver microsomes (22) and also found to scavenge superoxide radicals in the xanthine-xanthine oxidase system (23).

Cumin was shown to protect the colon in the presence of carcinoen through decreased activity of β-glucuronidase and mucinase (24). It was also shown to inhibit significantly 3'-methyl-4-dimethyl aminoazo benzene–induced hepatocarcinogenesis and benzo(a)pyrene [B(a)P]-induced neoplasia (25). Thus, very little work has been carried out on the chemopreventive effects of cumin. Therefore, we have evaluated the cancer chemopreventive potentials of cumin seeds adopting B(a)P-induced forestomach tumorigenesis and methylcholanthrene (MCA)-induced uterine cervix tumor genesis in a murine model system. Further, the modulatory influence of cumin seeds on the hepatic xenobiotic detoxification enzyme system (phase I and phase II) was assessed. In addition, modulation of the antioxidant system comprising of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and reduced glutathione (GSH) was also evaluated. Our study in two tumor model systems strongly suggests the cancer preventive potential of cumin seeds, which could be attributed to the modulation of enzymes involved in carcinogen metabolism.

Materials and Methods

Chemicals

B(a)P, MCA, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), GSH, oxidized glutathione (GSSG), pyrogallol, 2,6-dichlorophenolindophenol (DCPIP), potassium ferricyanide, triton X-100, ethylenediamine tetracetic acid (EDTA), bovine serum albumin (BSA), sodium pyruvate, thiobarbituric acid (TBA), reduced nicotinamide adenine dinucleotide (NADH), and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma Chemical Co. Sodium carbonate, potassium dihydrogen ortho-phosphate, trichloroacetic acid, (Cl)2COOH, potassium chloride (KCl), and potassium sodium (+)-tartrate were from Qualigens Fine Chemicals, a division of Glaxo India Limited (Mumbai-400025 India). Sodium hydroxide, sodium chloride (NaCl), sulfuoric acid, hydrochloric acid (HCl), and Tris buffer GR were from E. Merck India Limited (Mumbai-400018 India). A yellow variety of beeswax was obtained from Mysore (India) and filtered in a molten state (60°C) two times to remove dust particles.

Modulator

Cumin seeds were bought from the local market (AGMARK brand). Seeds were crushed and added in the standard feeds powder according to the desired concentration (2.5%, 5%, or 7.5%) and pellets were formed. Pellets with different concentrations of modulator (seeds) were stored in neat, clean bags and kept in the feed storeroom of the Jawaharlal Nehru University under strict sanitary conditions until the end of the experiment.

Animals

Random-bred female Swiss albino mice (7–8 wk old) were used for the present study. They were maintained in the air-conditioned animal facility (Jawaharlal Nehru University, New Delhi) with a 12-h light/dark cycle and provided (unless otherwise stated) with standard food pellets and tap water ad libitum. All animals were cared for according to the Principles of Laboratory Animal Care of the National Institutes of Health and under strict adherence to the Indian Animal Ethics Committee.

To justify the potential of any drug that can be used in the prevention of cancer, the drug has to go through various stages. One of the initial steps is to check the efficacy of the
drug in the mammalian system of animals other than human beings. Over time the murine model system has become one of the ideal systems to work with in the field of cancer biology. This system has helped in increasing our understanding of various steps in the process of carcinogenesis and its prevention. However, at the same time there is a possibility of individual differences such as immune response and genetic susceptibility toward the drug that may lead to variations in the results. To avoid this an adequate number of animals is kept in each group.

In this study three independent experiments were carried out. Experiments I and II were aimed at evaluating the chemopreventive potential of cumin in forestomach tumorigenesis and uterine cervix tumorigenesis in a mouse model system. In the third experiment, the modulatory influence of cumin seeds on the hepatic enzymes involved in xenobiotic metabolism was undertaken.

**Experiment I**

**Influence of cumin seeds on B(a)P-induced mouse forestomach tumorigenesis.**

**Preparations of chemicals and modulator:** B(a)P was dissolved in peanut oil and the concentration was adjusted to 1 mg B(a)P per 0.1 ml of peanut oil. Diet of cumin seeds (2.5% and 5%) was prepared and fed to the animals.

**Experimental design:** The experiment was performed as described by Azuine and Bhide (26). This is a modified method originally described by Wattenberg et al. (27). The animals were sorted into the following groups:

<table>
<thead>
<tr>
<th>Groups and Treatment</th>
<th>Initial</th>
<th>Final</th>
<th>Total Tumor Incidence (%)</th>
<th>Tumor Burden (papilloma per mouse)</th>
<th>Inhibition of Tumor Burden (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B(a)P</td>
<td>32.1 ± 2.69</td>
<td>33.2 ± 2.52</td>
<td>100</td>
<td>7.33 ± 2.10</td>
<td>0.00</td>
</tr>
<tr>
<td>B(a)P + 2.5% diet of C. cyminum</td>
<td>30.5 ± 2.25</td>
<td>32.7 ± 2.68</td>
<td>71.43</td>
<td>3.10 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.71</td>
</tr>
<tr>
<td>B(a)P + 5% diet of C. cyminum</td>
<td>30.8 ± 2.66</td>
<td>35.0 ± 2.90</td>
<td>64.29</td>
<td>3.11 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.57</td>
</tr>
<tr>
<td>5% diet of C. cyminum</td>
<td>31.2 ± 2.01</td>
<td>36.7 ± 2.20</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Values are expressed as mean ± SD of 12–15 animals.

<sup>b</sup>: P < 0.001 represents significant changes against control.
nated threads and were kept on 7.5% cumin mixed diet throughout the experiment after laparotomy.

Group V (n = 8): Animals of this group were kept on 7.5% cumin mixed diet until the end of the experiment.

Group VI (n = 8): Animals of this group were inserted intracervically with wax-only–impregnated threads and were kept on a normal diet throughout the experiment.

Animals were weighed initially, at fortnightly intervals, and at autopsy. Twelve weeks after the insertion of cotton threads, the surviving animals were killed and their uterine cervices were fixed in Bouin’s fluid. Then the tissues were processed for histopathological assessment of pre-cancerous and cancerous lesions in the cervical epithelium.

Experiment III

Modulation of hepatic xenobiotic metabolizing enzymes, sulfhydryl contents, lipid peroxidation, and antioxidant enzymes by cumin seeds.

Experimental design: Animals were randomly sorted into the following groups:

Group I (n = 8): Animals were put on a normal diet for 15 days; this group of animals served as a negative control.

Group II (n = 8): Animals were put on 2.5% cumin seeds mixed diet for 15 days.

Group III (n = 8): Animals were put on 5% cumin seeds mixed diet for 15 days.

Preparation of homogenates and cytosol and microsome fractions: Animals were sacrificed by cervical threads and were kept on 7.5% cumin mixed diet until the end of the experiment.

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Shimadzu UV-160 spectrometer. GSH was used as a standard to calculate millimoles of -SH content per gram of tissue.

**Glutathione reductase:** GR was determined by the procedure described by Carberg and Mannervick (37). Reaction mixture (final volume 1 ml) contained 0.2 M sodium phosphate buffer (pH 7.0), 2 mM EDTA, 1 mM GSSG, and 0.2 mM NADPH. The reaction was started by adding 25 µl of cytosol, and the enzyme activity was measured indirectly by monitoring the oxidation of NADPH following a decrease in OD/min for at least 3 min at 340 nm. One unit of enzyme activity has been defined as nanomoles of NADPH consumed per minute per milligram of protein based on an extinction coefficient of 6.22 mM⁻¹ cm⁻¹.

**Glutathione peroxidase:** GPx activity was measured by the coupled-assay method described by Paglia and Valentine (38). Briefly, 1 ml of the reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA, 0.24 U/ml yeast GR, 0.3 mM glutathione (reduced), 0.2 mM NADPH, 1.5 mM H₂O₂, and cytosol sample. Reaction was initiated by adding NADPH, and its oxidation was monitored at 340 nm by observing the decrease in OD/min for 3 min. One unit of enzyme activity has been defined as nanomoles of NADPH consumed per minute per milligram of protein based on an extinction coefficient of 6.22 mM⁻¹ cm⁻¹.

**Catalase:** CAT was estimated at 240 nm by monitoring the disappearance of H₂O₂ as described by Aebi (39). The reaction mixture (volume 1 ml) contained 0.02 ml of suitably diluted cytosol in phosphate buffer (50 mM, pH 7.0) and 0.1 ml of 30 mM H₂O₂ in phosphate buffer. CAT enzyme activity has been expressed as moles of H₂O₂ reduced per minute per milligram of protein.

**Superoxide Dismutase:** SOD was assayed utilizing the technique of Marklund and Marklund (40), which involves inhibition of pyrogallol autoxidation at pH 8.0. A single unit of enzyme was defined as the quantity of SOD required to produce 50% inhibition of autoxidation.

**Lipid peroxidation:** Lipid peroxidation in the microsomes was estimated spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method, as described by Varshney and Kale (41) and is expressed in terms of malondialdehyde (MDA) formed per milligram of protein. In brief, 0.4 ml of microsomal sample was mixed with 1.6 ml of 0.15 M Tris KCl buffer to which 0.5 ml of 30% TCA was added. Then 0.5 ml of 52 mM TBA was added and placed in a water bath for 45 min at 80°C, cooled in ice, and centrifuged at room temperature for 10 min at 3,000 g. The absorbance of the clear supernatant was measured against a blank of distilled water at 538.1 nm in a spectrophotometer (Hitachi U-2000).

**Lactate dehydrogenase:** LDH was assayed by measuring the rate of oxidation of NADH at 340 nm according to the method of Bergmeyer and Bernt (42). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.5), 0.5 mM sodium pyruvate, 0.1 mM NADH, and the required amount of cytosolic fraction to make a final volume of 1 ml. The reaction was started at 25°C by addition of NADH, and the rate of oxidation of NADH was measured at 340 nm. The enzyme activity was calculated using an extinction coefficient of 6.22 mM⁻¹ cm⁻¹. One unit of enzyme activity is defined as that which causes the oxidation of 1 µmol of NADH per minute.

**Protein determination:** Protein was determined by the method of Lowry et al. (43) using BSA as the standard at 660 nm.

**Statistical Analysis**

In experiment I, the B(a)P-treated group was considered a positive control. Groups treated with B(a)P + cumin (2.5% or 5%) were considered experimental groups (cases). The group treated with a 5% dose of cumin without B(a)P treatment was considered a negative control. In each group the number of papillomas per mouse (tumor burden) was counted at the end of the experiment. After calculating the mean and the standard deviation, the Mann-Whitney rank sum test was performed to find the significance between the cumin-treated groups (cases) and the positive control group.

In experiment II, the MCA-treated group was considered a positive control. Groups treated with MCA + cumin (5% or 7.5%) were considered experimental groups (cases). The group treated with the high dose of cumin (7.5%) without MCA treatment was considered a negative control. Tumor incidences in different groups were evaluated. The χ² method was used to find the significance between the cumin-treated groups (cases) and the positive control group.

In experiment III, the control group was kept on a normal diet. Other groups received 2.5% and 5% diet of cumin. These groups served as experimental groups (cases). In these groups the levels of cyt P-450, cyt bs, GSH, and LP as well as the specific activities of cyt P-450R, cytochrome bsR, GST, DTD, SOD, CAT, GPx, GR, and LDH were determined in the liver of mice. After calculating the mean and the standard deviation, the Mann-Whitney rank sum test was performed to find the significant differences between the control and cumin-treated groups (cases).

**Results**

**Mouse Forestomach Tumorigenesis**
(Experiment I)

Table 1 depicts the effects of cumin seed supplementation in the diet on B(a)P-induced forestomach tumorigenesis. No
significant difference was noticed in the weight gain profile of animals treated with either dose of cumin seed mixed diet (2.5% and 5%) or in the positive control group of mice. All of the animals in the positive control group developed forestomach papillomas by B(a)P treatment. The mean number of papillomas per mouse (tumor burden) in this group was 7.33 ± 2.10. In contrast, in animals treated with B(a)P + 2.5% cumin dose and B(a)P + 5% diet of cumin seed, tumor burden reduced to 3.10 ± 0.57 (P < 0.001) and 3.11 ± 0.60 (P < 0.001), respectively. The percentage inhibition of tumor multiplication was 57.71 and 57.57 by the low (2.5%) and high (5%) doses of cumin seed treatment, respectively. Relative to positive control the total tumor incidence decreased by 28.57% with the low (2.5%) and high (5%) doses of cumin seed. A 5% diet of cumin seed (negative control group) did not result in any tumor.

Uterine Cervix Carcinogenesis (Experiment II)

The effects of cumin seeds on MCA-induced tumor-igenesis in the uterine cervix of mice are given in Table 2. Apparently, no toxic effect was noticed in the animals, which were kept on cumin mixed diet (5% and 7.5%). Only a small number of animals died (most likely due to postoperative complications) in each group. There was no appreciable change in the body weight gain (data not shown) in experimental animals.

The animals that were inserted intracervically with MCA plus wax-impregnated threads were kept on a normal diet (group II). This group displayed 66.67% tumor incidence in their uterine cervixes. Animals inserted intracervically with wax-only-impregnated threads did not show any tumor in their uterine cervixes (group VI). The negative control group (group V) also did not show any tumor incidence. The animals of group III, inserted intracervically with MCA plus wax-impregnated threads and treated with 5% dose of cumin seed mixed diet, showed 27.27% tumor incidence in their uterine cervixes. However, animals of group IV, which were inserted intracervically with MCA plus wax-impregnated threads and treated with 7.5% dose of cumin seed mixed diet, showed only 12.50% tumor incidence in their uterine cervixes.

Carcinoma in situ and invasive carcinoma type were used to calculate cancer incidence. The incidences of hyperplasia and dysplasia did not show any correlation with the type of treatment given to different groups.

Hepatic Studies (Experiment III)

Phase I system: Cyt P-450 and cyt b5 function as important cofactor components of mono-oxygenase enzyme. The levels of cyt P-450 and cyt b5 were augmented by the lower dose of cumin by 1.25- and 1.24 (P < 0.05)-fold, respectively, when compared with respective controls. The specific activities of cyt P-450R and cyt b5R were induced by the 2.5% dose to 1.31- and 1.24 (P < 0.01)-fold and by the 5% dose to 1.41 (P < 0.01)- and 1.33 (P < 0.005)-fold, respectively, over that of the control value (Table 3).

Phase II system: The specific activity of GST was elevated significantly only by the 5% dose of cumin to 1.26 (P < 0.005)-fold when compared with control. DTD was induced by both lower and higher doses of cumin, by a magnitude of 1.12- and 1.09 (P < 0.05)-fold, respectively (Table 3).

Antioxidant parameters: The modulatory effect of cumin on the enzymes involved in antioxidant function and levels of GSH is given in Table 4. The specific activities of SOD and CAT were elevated to 1.33 (P < 0.01)- and 1.22 (P < 0.05)-fold, respectively, only by the 5% dose. The activities of GPX and GR remained unaltered by both doses of cumin. The level of GSH was induced significantly by 2.07 (P < 0.05)- and 2.12 (P < 0.01)-fold with the 2.5% and 5% doses, respectively, in comparison with the control (Table 4).

Lipid peroxidation: Lipid peroxidation measured as formation of MDA production showed significant inhibition by both doses of cumin. Lipid peroxidation was reduced by 0.83-fold (P < 0.01) with the 2.5% diet and 0.82-fold (P < 0.05) with the 5% diet of cumin in comparison with the control group (Table 4).

Table 2. Chemopreventive Action of Cuminum cyminum on MCA-Induced Cervical Carcinogenesis in a Murine Model System

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (route)</th>
<th>Effective No. of Mice</th>
<th>No. of Mice Normal or with Cervical Lesions</th>
<th>Carcinoma</th>
<th>Dysplasia (%)</th>
<th>Tumor Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Nil</td>
<td>10</td>
<td>4 N</td>
<td>H+ H++ H+++ D+ D++ D+++ CIS InvC</td>
<td>0 10</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>MCA-wax</td>
<td>15</td>
<td>1 N</td>
<td>H+ H++ H+++ D+ D++ D+++ CIS InvC</td>
<td>2 8</td>
<td>13.33</td>
</tr>
<tr>
<td>III</td>
<td>MCA-wax</td>
<td>11</td>
<td>1 N</td>
<td>H+ H++ H+++ D+ D++ D+++ CIS InvC</td>
<td>2 8</td>
<td>27.27</td>
</tr>
<tr>
<td>IV</td>
<td>MCA-wax 5% dose</td>
<td>16</td>
<td>7 N</td>
<td>H+ H++ H+++ D+ D++ D+++ CIS InvC</td>
<td>3 1</td>
<td>31.25</td>
</tr>
<tr>
<td>V</td>
<td>MCA-wax 7.5%</td>
<td>16</td>
<td>7 N</td>
<td>H+ H++ H+++ D+ D++ D+++ CIS InvC</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>Wax</td>
<td>6</td>
<td>3 N</td>
<td>H+ H++ H+++ D+ D++ D+++ CIS InvC</td>
<td>0 0</td>
<td>0</td>
</tr>
</tbody>
</table>

a: N, normal; H, hyperplasia; D, dysplasia; CIS, carcinoma; Inv C, invasive carcinoma. +, mild; ++, moderate; ++++, severe.
b: P < 0.05 represents significant changes against MCA control.
Table 3. Modulatory Influences of Two Different Doses of Cuminum cyminum in the Diet on Mice Hepatic Phase I and Phase II Drug Metabolizing Enzyme Levelsa

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cyt P450f</th>
<th>Cyt b5g</th>
<th>Cyt P450 R5</th>
<th>Cyt b5 Rg</th>
<th>GSTa</th>
<th>DTDg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.357 ± 0.022 (100)</td>
<td>0.434 ± 0.040 (100)</td>
<td>0.185 ± 0.027 (100)</td>
<td>2.616 ± 0.387 (100)</td>
<td>2.18 ± 0.148 (100)</td>
<td>0.030 ± 0.0012 (100)</td>
</tr>
<tr>
<td>2.5% diet of C. cyminum</td>
<td>0.445 ± 0.051 (125)</td>
<td>0.540 ± 0.054 (124)</td>
<td>0.242 ± 0.029 (131)</td>
<td>3.237 ± 0.353 (124)</td>
<td>2.10 ± 0.186 (96)</td>
<td>0.033 ± 0.0030 (112)</td>
</tr>
<tr>
<td>5% diet of C. cyminum</td>
<td>0.349 ± 0.050 (98)</td>
<td>0.476 ± 0.020 (110)</td>
<td>0.261 ± 0.023 (141)</td>
<td>3.492 ± 0.411 (133)</td>
<td>2.74 ± 0.229 (126)</td>
<td>0.032 ± 0.0024 (109)</td>
</tr>
</tbody>
</table>

a: Values are expressed as mean ± SD of 6–8 animals. Values in parentheses represent relative change in parameters assessed (that is, levels of activity in livers of mice receiving test substance to activity in liver of control mice). Treatment duration, 14 days.
b: nmol/mg protein.
c: μmol of NADPH oxidized/min/mg protein.
d: μmol of NADH oxidized/min/mg protein.
e: μmol CDNB-GSH conjugate formed/min/mg protein.
f: μmol of DCPIP reduced/min/mg protein.
g: P < 0.05 represents significant changes against control.
h: P < 0.01 represents significant changes against control.
i: P < 0.005 represents significant changes against control.

Table 4. Modulatory Influence of Two Different Doses of Lipid Peroxidation, and Lactate Dehydrogenasea

<table>
<thead>
<tr>
<th>sgs</th>
<th>GSHa</th>
<th>SODa</th>
<th>CATa</th>
<th>GPxa</th>
<th>GRe</th>
<th>LRa</th>
<th>LDHa</th>
</tr>
</thead>
<tbody>
<tr>
<td>rol vehicle</td>
<td>12.72 ± 1.41 (100)</td>
<td>5.28 ± 0.567 (100)</td>
<td>83.64 ± 3.39 (100)</td>
<td>16.6 ± 2.1 (100)</td>
<td>29.5 ± 1.8 (100)</td>
<td>1.12 ± 0.014 (100)</td>
<td>2.28 ± 0.24 (100)</td>
</tr>
<tr>
<td>2.5% diet of C. cyminum</td>
<td>26.33 ± 4.00 (207)</td>
<td>5.26 ± 0.465 (100)</td>
<td>72.94 ± 8.95 (87)</td>
<td>16.9 ± 2.0 (102)</td>
<td>27.9 ± 3.7 (95)</td>
<td>0.930 ± 0.14 (83)</td>
<td>2.15 ± 0.20 (94)</td>
</tr>
<tr>
<td>5% diet of C. cyminum</td>
<td>26.98 ± 3.74 (212)</td>
<td>7.04 ± 0.548 (133)</td>
<td>102.3 ± 11.1 (122)</td>
<td>17.1 ± 1.8 (103)</td>
<td>33.3 ± 3.8 (113)</td>
<td>0.916 ± 0.13 (82)</td>
<td>2.06 ± 0.24 (90)</td>
</tr>
</tbody>
</table>

a: Values are expressed as mean ± SD of 6–8 animals. Values in parentheses represent relative change in parameters assessed (that is, levels of activity in livers of mice receiving test substance to activity in liver of control mice). Treatment duration, 14 days.
b: nmol GSH/g tissue.
c: specific activity expressed as μmol/mg protein.
d: μmol H2O2 consumed/min/mg protein.
e: nmol NADPH consumed/min/mg protein.
f: nmol malondialdehyde formed/mg protein.
g: μmol/mg protein.
h: P < 0.05 represents significant changes against control.
i: P < 0.01 represents significant changes against control.

Lactate dehydrogenase: LDH is a biochemical indicator of cellular damage. There was no significant change in the activity of LDH by both doses of cumin used (2.5% and 5%; Table 4).

Discussion

Cumin is one of the most extensively used condiments in the world. It has a peculiar, strong, and heavy odor and is widely used in ayurvedic medicine for the treatment of various disorders such as dyspepsia, diarrhea, and jaundice. The chemical entities, which primarily establish its characteristically pungent flavor, are found in the oil of cumin. The chief constituent of the oil is cumaldehyde, C9H12O (p-isopropylbenzaldehyde).

An attempt has been made in this study to examine the chemopreventive potentials of cumin seeds using experimental carcinogenesis as well as its modulatory action on detoxifying and antioxidant enzymes. Dietary administration of cumin exerted a strong chemopreventive effect against B(a)P-induced forestomach and MCA-induced cervix tumors in Swiss albino mice. B(a)P, employed for initiating stomach cancer, is widely distributed in the environment, in cigarette smoke, automobile exhaust, etc. B(a)P, like many other polycyclic aromatic hydrocarbons, also needs metabolic activation before it can exert carcinogenicity. The phase I system (cyt P-450, cyt b5, cyt P-450R, and cyt b5R) is involved in this activation process (44–47). The modulation of these enzymes can have a significant effect on carcinogenicity and mutagenicity (48,49). B(a)P is initially converted into 7,8-diol, which is then converted to anti-7,8-dihydroxy-9,10-epoxy,7,8,9,10-tetrahydrobenzapyrene (anti-BPDE). The ultimate carcinogen, anti-BPDE is an inducer of DNA damage by virtue of its capacity in forming DNA adducts, predominantly at the N2 position of guanine. Anti-BPDE could be readily inactivated by GSH through conjugation (50).
As phase I enzymes play a key role in the biotransformation of carcinogens, their modulation by 2.5 and 5% doses of cumin was evaluated. Cumin significantly increased the specific activity of cyt P-450 at the 2.5% dose level. Many proven chemopreventive substances, for example, indole 3-carbinol, are known to act via induction of cyt P-450 (51). The other components of the phase I system (cyt b5, cyt P-450R, and cyt b2R) function in a synergistic manner, facilitating the transfer of an electron to cyt P-450, accepted from NADPH or NADH, thus enabling the proper functioning of the cyt P-450 system. Although cyt b5 was significantly increased by the 2.5% dose only, reductases were significantly increased by both doses of cumin used.

Phase II enzymes, namely, GST and DTD, facilitate the detoxification of carcinogens and their excretion. The main function of GST is to catalyze the conjugation of electrophilic xenobiotics/carcinogens to the endogenous nucleophile GSH, thus protecting the cellular components from toxic compounds (52). Reduced mutagenic response to DMBA and AFB1 in Fisher 344 rat hepatocytes has been correlated with the induction of GST (53). The chemopreventive action of cumin against B(a)P and MCA carcinogenesis can be due to increased activity of GST. As B(a)P 4,5-epoxide and anti-BPDE have been shown to be good substrates of GST (54,55). GST and glutathione detoxify the ultimate carcinogenic metabolite of B(a)P, that is, benzo(a)-pyrene-7,8-diol-9,10-epoxide, and may thus inhibit the deleterious effects of B(a)P (56).

DTD, another phase II enzyme, is a flavoprotein and catalyzes the two-electron reduction of quinones, quinone imines, azo dyes, and other nitrogen oxides (57,58). Through redox cycling, quinones contribute to oxidative stress due to generation of reactive oxygen species (ROS). Thus, major metabolic function of this enzyme might be to reduce the formation of ROS from redox reactions. The induction of DTD, which mediates the two-electron reduction of quinones leading to the formation of hydroquinones, is important in attenuating the toxicity of quinone metabolite. Dithioliones and their analogs (Oltipraz), known to be chemopreventive against a variety of chemical carcinogens, are effective inducers of DTD (59). Thus, inducers of DTD can be assumed to play important roles in cancer chemoprevention. Cumin seeds significantly increased the specific activity of DTD at both dose levels (2.5% and 5%).

An increasing body of evidence indicates that oxidative stress is an important element of mutagenesis, which is a basis for carcinogenesis (60). Hydroxyl radical produced as a result of oxidative stress initiates a chain of reactions that leads to the process of lipid peroxidation and generates in turn the highly mutagenic singlet oxygen. Glutathione is known to be an effective quencher of this singlet oxygen (61,62). GSH is one of the major cellular defenses against ROS generated endogenously or the electrophilic metabolites of carcinogen generated during phase I biotransformation. The electrophilic functional groups are conjugated with glutathione to form amphiphilic thioether either spontaneously or through GSTs (63). Cumin seeds significantly enhanced the GSH content at both doses of cumin used, providing probable protection against oxidative stress.

The other major cellular defense against oxidative stress is the SOD-CAT system. SOD dismutates pairs of superoxide anions by oxidizing one to oxygen and reducing the other to hydrogen peroxide. Hydrogen peroxide, which is also considered to be mutagenic, is degraded to H2O2 and oxygen by CAT. Metabolism of the carcinogens and the applications of the tumor promoters have been known to generate activated oxygen species. Because the SOD-CAT system is effective in detoxifying these free radicals, as well as H2O2, agents that cause enhancement in the activities of the SOD-CAT system would be of great use in protection against activated oxygen species and peroxide molecules. Both SOD and CAT were significantly enhanced by the higher dose of cumin (5%). GPx, another enzyme of antioxidant defense, is involved in the detoxification of hydrogen peroxide when its cellular concentration is quite low (64). GR could be regarded as an auxiliary mechanism that facilitates the regulation of GSH homeostasis. GPx and GR level remained constant at both dose levels of cumin used.

Peroxidation, initiated in membranes by hydroxyl radical, is self-propagating and yields mutagenic ROS, including singlet oxygen and lipid hydroperoxides. Because cumin significantly enhances some of the components of the antioxidant defense mechanism and also the specific activity of GST, a reduction in lipid peroxidation is highly expectable. Our experiment demonstrated that cumin significantly inhibited lipid peroxidation at both doses used in the experiment.

Apoptosis is considered to be a complement to mitosis in the maintenance and growth of tissues. It is known that mutations affecting regulation of cell cycle and apoptosis lead to carcinogenesis. Cumin is likely to prevent mutations as it has increased the antioxidant potential of animals through the enhanced activities of GST, DTD, SOD, and CAT as well as the levels of GSH (Table 3 and 4). Further, cumin might have also prevented the mutations by scavenging the free radicals and in turn making them unavailable for interactions with critical targets such as DNA. This possibility is supported by the significant inhibition of lipid peroxidation by both doses of cumin (Table 4). Thus, it could be inferred that cumin might have exerted cancer chemopreventive effects through checking mutations, which disrupts the normal process of cell cycle and apoptosis.

LDH, a cytoplasmic marker enzyme, is well known as an indicator of cell damage induced by several factors, including xenobiotic compounds and radiation (65,66). The cumin treatment did not result in any change in LDH activity, signifying its nontoxic nature.

The present study has clearly demonstrated the cancer-preventive action of cumin seeds using chemically induced tumorigenesis in murine model systems. The findings also suggested that, apart from the abilities to modulate metabolic activities of phase I and phase II systems to induce detoxification of carcinogen and to enhance antioxidant potentials, other mechanisms such as modification of cell cycle and/or modifications of apoptotic pathways might have played an
important role in the cancer-preventive properties of cumin. At the given dose levels cumin appears to be safe as it could not cause any adverse effects on the animals as evidenced by the level of lipid peroxidation, specific activity of LDH, food intake, and general body weight gain (of the animals) during the course of the experiment. Therefore, it could be inferred that cumin seeds might have contributed to the cancer chemopreventive effects in the human population. Cumin seeds seem to possess most of the characteristics of an ideal cancer chemopreventive agent, that is, orally effective, less or no toxicity, cost-effectiveness, and higher efficacy. However, its preventive potential needs to be tested further in other tumor model systems alone or in combinations with different chemopreventive agents.

Acknowledgments and Notes
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Submitted 11 July 2003; accepted in final form 22 October 2003.

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