Spices In Cancer Prevention: An Overview
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Abstract
Culinary herbs are herbaceous plants that add flavour and colour to all types of meals. Spices have been part of Indian folk and traditional medicine since ancient times. But recently, extensive work is being conducted all over the world to demonstrate the anti-mutagenic and anticarcinogenic potential of some of the commonly consumed spices. This renewed interest in natural medicines today is mainly due to the fact that many chronic diseases including cancer still remain difficult to cure. As such, attempts are being made to identify naturally occurring anticarcinogens, which may lead to new strategies for cancer prevention. This article is an attempt to consolidate some of the works that has been carried out on spices and their active principles in order to prove their probable cancer preventive properties. It is now becoming clear that the beneficial properties in spices are due to the presence of potent phytochemicals in them. Plants have the capacity to synthesize a diverse array of chemicals. In plants, these compounds function to attract beneficial and repel harmful organisms, serve as photoprotectants, and respond to environmental changes. In humans, they can have complementary and overlapping actions, including antioxidant, antimutagenic and antiinflammatory effects, modulation of detoxification enzymes, and induction of apoptotic activity and so on. Thus, incorporation of these spices in our regular diet may prove to be beneficial for our health. Nevertheless, the effect of spices in the context of total diet still remains to be evaluated. It is still not clear that mechanisms that appear to influence disease risk in animals, often fed high doses of these spices, can be extrapolated to humans consuming realistic amounts of these spices as part of their daily diet.

INTRODUCTION
A diet rich in plant foods may provide protection against several chronic diseases including cancers (1). The associative evidence comes from case-control, cohort as well as epidemiologic studies. But most of the initial studies in this field have concentrated on fruits and vegetables. The importance of herbs and spices, which have been used as flavour enhancers since ancient times in different cuisines all over the world, is only beginning to surface. Spices as defined by the US Food and Drug Administration are “aromatic vegetable substances, in the whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition (2). The flavours are provided by the essential oils and oleoresins present in spices. The terpenes and terpene derivatives are probably the most important class of aroma compounds, with monoterpenes contributing to the fragrance of 90% of spices (3). Monoterpenes occur in many different plants, and the characteristic aroma of a spice results from a specific mixture of monoterpenes, not a specific compound. Spices have also been used since antiquity to preserve food due to the presence of antioxidant phytochemicals. By virtue of these powerful phytochemicals, herbs and spices are known to exhibit an array of biochemical and pharmacological activities including antioxidant and anti-inflammatory properties that are believed to contribute to their anticarcinogenic and antimutagenic activities. In different herbs, a wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcuminoids, and phthalides have been identified. Many phytochemicals are present in plants as glycosides (i.e., with a sugar moiety attached). Generally, glycosides are nonvolatile and lack fragrance, for example glucosinolates of the cabbage family. Cleaving the glycosidic bond yields the aglycon, which itself may be volatile and fragrant.

Major phytochemicals found in some of the commonly used spices are given in Table I.

Usually viewed as flavor enhancers, spices can even protect against a wide range of cancers, heart disease and other chronic diseases (4). The National Cancer Institute has identified several commonly used herbs as possessing cancer-preventive properties. The list includes some spices
of Labiatae family (basil, mints, oregano, rosemary, sage, and thyme); spices of the Zingiberaceae family (turmeric and ginger); and spices of the Umbelliferae family (anise, caraway, celery, chervil, cilantro, coriander, cumin, dill, fennel, and parsley) (5).

Pre-clinical studies in a variety of cancer cell lines including breast, cervical, colon, gastric, hepatic, leukemia, oral epithelial, ovarian, pancreatic, and prostate have consistently shown that curcumin possesses anti-cancer activity in vitro and in pre-clinical animal models. The robust activity of curcumin in colorectal cancer has led to five phase I clinical trials being completed showing the safety and tolerability of curcumin in colorectal cancer patients (6).

Recently FSP (fenugreek seed powder) and diosgenin (major steroidal saponin constituent of fenugreek) was also found to have cancer preventive property. It was found that, by comparison with control, continuous feeding of FSP and diosgenin suppressed total colonic ACF in azoxymethane induced F344 rats. Diosgenin was also able to reduce the number of multicrypt foci. Similarly, results from a study conducted to assess the affect of cumin on experimentally induced forestomach and uterine cervix tumorigenesis in Swiss albino mice showed a significant inhibition of benzo(a)pyrene [B(a)P]-induced stomach tumor burden (tumors per mouse) by cumin (7). Cervical carcinoma incidence was also found to be reduced in the cumin treated group compared with the 3-methylcholanthrene (MCA)-treated control group (8). Results from yet another experiment show that chilli supplementation promotes colon carcinogenesis, whereas cumin or black pepper suppresses colon carcinogenesis in the presence of the procarcinogen DMH (9).

Dietary isothiocyanates (ITCs, which are abundantly present in mustard seeds) may play an important role in the prevention of human cancers. Several recent epidemiological studies have already shown that dietary consumption of ITCs inversely correlates with the risk of developing lung, breast and colon cancers (10).

Garlic (Allium sativum) is a popular spice, a remedy for a variety of ailments and is also known for its medicinal uses as an antibiotic, antithrombotic and antineoplastic agent. Epidemiological and animal studies have shown that garlic consumption reduces the incidence of cancer e.g. in the stomach, colon, breast and cervix (11).

It is quite evident from these observations that the identification of spices harbouring potent phytochemicals, which have the capacity to interfere with carcinogenic processes, has been receiving increased interest. Given the wide range of botanical species and plant parts from which spices are derived, spices can contribute significant variety and complexity to the human diet.

The major classes of phytochemicals that contribute to the aroma of spices and examples of their sources are provided in Table 1 below.

**Table 1:** Some of the Commonly Consumed Spices and their Constituents

<table>
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<th>Name of the Spice</th>
<th>Family</th>
<th>Commercial Part</th>
<th>Main Constituents</th>
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| Anise (Pimpinella anisum) | Umbelliferae    | Dried and crushed seeds | trans-anethole, α-pinen, camphor, β-pinen, linalool, 4-oxo-α-anethole, 
                                      |                                         | 3,4-dimethoxybenzaldehyde, 2,5,6,7-tetramethoxy- 
                                      |                                         | 3,4-dihydroxyacetophenone (17). |
| Black Mustard (Brassica nigra) | Brassicaceae   | Swed grains     | Allylthiocyanate (obtained from seeds by maceration in warm water during 
                                      |                                         | production) (19). |
| Black Pepper (Piper nigrum) | Piperaceae     | Dried, crushed, fruits of the pepper | α-phenyl (peppermint), α-pinene, camphor, carvone, α-phellandrene, 
                                      |                                         | | 
| Cardamom (Elettaria cardamomum) | Zingiberaceae | Fruit (Capsule) | The volatile oil fraction containing, linalool, 36.8%, 1,8-cineole, 21.5%, 
                                      |                                         | | terpinen-4-ol (3). |
| Cinnamon (Cinnamomum zeylanicum) | Lactoraceae | Bark | cinnamaldehyde, eugenol, trans-cinnamic acid, phenolic compounds, condensed 
                                      |                                         | | tannins, calcium, magnesium, proanthocyanidins, other monoterpenes 
                                      |                                         | | including limonene and α-pinene, sesquiterpenes including pinene, 
                                      |                                         | | camphene, caryophyllene, eugenol, eugenyl acetate, eugenol phenyl 
                                      |                                         | | ether, eugenyl esters. |
| Cloves (Syzygium aromaticum) | Myrtaceae     | Dried flower buds| The oil itself is dominated by eugenol, α-caryophyllene, α-symplocene, 
                                      |                                         | | acetyl eugenol, eugenyl acetate, caryophyllene, 
                                      |                                         | | caryophyllene. |

It is quite evident from these observations that the
Factors that may be relevant to the prevention of the carcinogenic process are: anti-oxidant action including the ability to induce phase-II detoxifying enzymes, anti-inflammatory action and the ability to induce apoptosis.

ANTI-OXIDANT ACTIVITY

Oxidative damage can result when the critical balance between free radical generation and antioxidant defenses is unfavorable. It has been hypothesized that oxidative damage plays a key role in cardiovascular disease, cancer initiation, cataract formation, the aging process, inflammatory diseases, and a variety of neurologic disorders (27). If not quenched by antioxidants, these highly reactive compounds will react with and potentially alter the structure and function of several cellular components, such as lipid-containing cell membranes, lipoproteins, proteins, carbohydrates, RNA, and DNA. To protect against the deleterious effects of free radicals, our body has developed an antioxidant defense system. As part of this defense system, anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) etc., play a vital role in the efficient detoxification of harmful free radicals.

Naturally occurring antioxidants are being extensively analysed for their ability to protect DNA against such injury.

EXPERIMENTAL EVIDENCE TO DEMONSTRATE THE ANTIOXIDANT PROPERTY OF SPICES AND THEIR CONSTITUENTS.

Chemical analysis of about several foodstuffs comprising of cereals, pulses, oilseeds, vegetables, fruits and beverages was carried out to determine antioxidant phenolics and flavonoids in commonly consumed Indian foods. Amongst spices high flavonoid content (> 100 mg/100 gm) was found in fenugreek seeds, mustard seeds, cinnamon, red chili powder, cloves and turmeric whereas cumin, cardamom were found to contain medium levels (50-100 mg) of flavonoid (28). Experiments have also been conducted to investigate the effect of spice principles on scavenging of superoxide anion. Spice principles eugenol (clove) and cumin aldehyde (cumin) dose-dependently inhibited the superoxide anions, as measured by nitrobluetetrazolium (NBT) reduction in xanthine-xanthine oxidase system (29).

The antioxidant activity in the etheric, methanolic and aqueous cinnamon extracts was measured by the b-carotene/linoleic acid system, at 50 degrees C and absorbances reading at 470 nm every 15 min intervals for 120 min. It was found that extracts inhibited the oxidative process in 68%; 95.5% and 87.5% respectively (30). These findings have been further supported by the following studies carried out in animal models.

IN VIVO STUDIES

The anti-oxidant effect of cinnamon (Cinnamomum velum) and cardamom (Amomum subulatum) was proved by an in vivo study according to which the antioxidant enzyme activities were found to be significantly enhanced whereas GSH content was markedly restored in rats fed a fat diet with spices. In addition, these spices were also found to partially counteract increase in lipoprotein conjugates and hydroperoxides (31).
Chithra et al. studied the antiperoxidative effect of coriander seeds (Coriandrum sativum) in rats administered a high-fat diet. They observed a significant decrease in the levels of lipid peroxides, free fatty acids, and glutathione as compared to the control groups. In yet another study, coriander seed oil and its fractions exhibited the strongest radical scavenging activity toward the stable galvinoxyl radical compared to black cumin and niger seed oils.

In a study conducted to test the anti-oxidant activities of water and alcoholic extract (1:1) of some commonly used spices (garlic, ginger, onion, mint, cloves, cinnamon, and pepper), cloves exhibited the highest antioxidant activity. All the spices dose-dependently inhibited oxidation of fatty acid, linoleic acid in presence of soybean lipoxygenase.

In another study, lipid peroxidation measured as formation of MDA production showed significant inhibition by treatment with cumin against benzo(a)pyrene [B(a)P]-induced forestomach tumorigenesis and 3-methylcholanthrene (MCA)-induced uterine cervix tumorigenesis. Similarly, another study also found that Mediterranean spices which included cumin and saffron could inhibit lipid peroxidation.

Gagandeep et al. showed that the levels of cytochrome P-450 (cyt P-450) and cytochrome b5 (cyt b(5)) were significantly augmented in Swiss albino mice by cumin seed diet. Among the phase II enzymes, glutathione S-transferase specific activity increased by a higher dose of cumin.

Saffron was found to have modulatory effects on some phase II detoxifying enzymes like GST and GPx, as well as CAT and SOD in mice initiated by 7-12 dimethyl benz[a]anthracin (DMBA) and promoted with croton oil. In another experiment, black pepper and piperine were also found to have the same kind of influence on the detoxifying enzymes in Wister rats fed a high fat diet.

Enhanced GST, Cyt. b5 and SH levels were observed in mice treated with cloves (0.5%, 1% and 2% w/w in the diet). Significant reduction in Cyt P-450 and MDA levels was observed in all groups at 30 days duration.

Dietary supplementation of curcumin (2%, w/v) to male ddY mice for 30 days significantly increased the activities of glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and catalase. Parallel to these changes, curcumin feeding to mice also resulted in a considerable enhancement in the activity of phase II-metabolizing enzymes viz. glutathione S-transferase and quinone reductase.

One in vitro study suggests that induction of detoxifying enzymes may account for chemoprotective properties of mustard seeds. Almost two fold increase in the activity of GST was observed in Benzo(a)pyrene induced DNA damage in human derived cells.

**ANTI-MUTAGENIC ACTIVITY**

Inhibiting the activity of mutagens can be one of the most important ways to prevent the initiation of the carcinogenic process. Thus, screening of substances having antimutagenic activity may be considered as a potential target for chemopreventive approach.

**EXPERIMENTAL EVIDENCE TO DEMONSTRATE THE ANTIMUTAGENIC PROPERTY OF SPICES AND THEIR CONSTITUENTS.**

**IN VITRO STUDIES**

In one study, it was found out that eugenol, a compound present in many spices such as cloves, cardamom etc., significantly inhibited tobacco-induced mutagenicity at concentrations of 0.5 and 1 mg/plate. Eugenol also inhibited the nitrosation of methylurea in a dose-dependent manner. Essential oils from common spices such as nutmeg, ginger, cardamom, celery, xanthoxylum, black pepper, cumin, and coriander were found to inhibit DNA adduct formation by aflatoxin B1 in vitro very significantly and in a dose-dependent manner.

Another study investigated the antimutagenic activity of the hot water extracts of caraway, coriander and black pepper seeds against mutagenicity induced by various carcinogens such as N-methyl-N'-nitro-N-nitrosoguanidine and dimethylnitrosamine by the Ames assay. The tested samples (equivalent to 1-2 mg of spice powder) reduced the mutagenicity induced by 2.7 nmole (397 ng) of N-methyl-N'-nitro-N-nitrosoguanidine by more than 84%, and that induced by dimethylnitrosamine (1.48 mg) or ICR-170 (10 ng) by 30-60%.

The antimutagenic activity of coriander juice against the mutagenic activity of 4-nitro-o-phenylenediamine, m-phenylenediamine and 2-aminofluorene was investigated using the Ames reversion mutagenicity assay (his- to his+) with the S. typhimurium TA98 strain as indicator organism. Aqueous crude coriander juice significantly decreased the mutagenicity of metabolized aromatic amines.
Another experiment was carried out to examine the protective effect, if any, of mustard juice against B(a)P-induced DNA damage in human derived cells. Treatment of the cells with small amounts of mustard juice (0.1-1.25 microl/ml) and B(a)P reduced the genotoxic effect of the carcinogen in a dose-dependent manner (39).

**IN VIVO STUDIES**

At 100 and 200 mg/kg body wt doses, curcumin (the main yellow bioactive component of turmeric) has been shown to reduce the number of aberrant cells in cyclophosphamide-induced chromosomal aberration in Wistar rats (44). Turmeric also prevents mutation in urethane (a powerful mutagen) models (45).

In the wing Somatic Mutation and Recombination Test (SMART) in Drosophila melanogaster, black pepper was found effective in reducing the mutational events induced by the alkylating agent ethyl carbamate. Suppression of metabolic activation or interaction with the active groups of mutagens could be mechanisms by which the spice exerts its antimutagenic action (46).

**Anti-inflammatory Property**

Epidemiological and experimental data support the use of nonsteroidal anti-inflammatory drugs, including specific inhibitors of cyclooxygenase 2 (COX-2), as chemopreventive agents in a number of epithelial cancers, including colon, mammary, esophageal, lung, and oral cavity (47). Non-steroidal Anti-inflammatory drugs (NSAIDs), however, even aspirin, is toxic with ulceration the greatest side effect if they are overused. Therefore, the search for plant-based sources of anti-inflammatory compounds that are relatively safe to use and could provide avenues for long term cancer prevention is still on.

**EXPERIMENTAL EVIDENCE TO DEMONSTRATE THE ANTI-INFLAMMATORY PROPERTY OF SPICES AND THEIR CONSTITUENTS.**

**IN VITRO STUDIES**

Cinnamaldehyde (component of cinnamon) has been shown to be effective in inducing cell apoptosis in a number of human cancer cells. Using the XTT assay, cinnamaldehyde exhibited a powerful antiproliferative effect on PLC/PRF/5 cells (61).

Specific induction of apoptosis by 1,8-cineole (one of the main components in bay leaves and cardamom) was observed in human leukemia Molt 4B and HL-60 cells. The fragmentations of DNA by cineole to oligonucleosomalsized fragments that is a characteristic of apoptosis were concentration- and time-dependent in Molt 4B and HL-60 cells (62).

These findings suggest that natural mediators of apoptosis may play important role in the prevention of cancer.

**APOPTOTIC ACTIVITY**

Induction of apoptosis in tumor cells, a form of physiological death in unwanted or dysfunctional cells, is an appealing therapeutic approach (58). Escape from apoptotic signals often accompanies tumor progression. The response to chemo- or radiation therapy in some cancers correlates with the induction of apoptosis within the tumors (59, 60).

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cell death by DNA-damage in human cancer cell lines, TK-10, MCF-7 and UACC-62 (63). Recently, curcumin has been shown to cause apoptosis in mouse neuro 2a cells (64). In another investigation, the results from flow cytometry assay indicated that curcumin induced ROS and Ca+2 productions, decreased the levels of MMP and increased the activity of caspase-3, leading to cell apoptosis. Western blot assay also revealed that curcumin increased the levels of Bax and the release of cytochrome c, and decreased the levels of Bcl-2 in the HL-60 cells (65).

Results from the in vitro experiments indicated that diosgenin inhibits cell growth and induces apoptosis in the HT-29 human colon cancer cell line in a dose-dependent manner (66). Another constituent of fenugreek, Protodioscin PD displayed strong growth inhibitory effect against HL-60 cells. Morphological change showing apoptotic bodies and the fragmentation by PD of DNA to oligonucleosomal-sized fragments which is a characteristic of apoptosis, suggests that growth inhibition by PD of HL-60 cells results from the induction of apoptosis by this compound in HL-60 cells (67).

Isothiocyanates (present in mustard seeds) were found to induce apoptosis and/or cause arrest in the cell-cycle progression in 2 human bladder carcinoma lines (UM-UC-3 and T24) in an experiment conducted by Tang et al (68).

In another study conducted to investigate the effect of garlic extract, flow cytometry assay; Western blotting and cDNA microarray were applied in human colon cancer colo 205 cells. Results indicated that garlic extract, when administered to the colo 205 cell cultures, reduced the percentage of viable cells, induced apoptosis, increased the levels of Bax, cytochrome c and caspase-3, but decreased the level of Bcl-2. Thus it was concluded that crude extract of garlic could induce apoptosis in colo 205 cells through caspase-3 activity, by means of a mitochondrial-dependent mechanism (69).

PROPOSED MECHANISM OF ACTION

From the present discussion, it can be deduced that any of the mechanisms described above singly or in combination may be responsible for the anti-tumorigenic potential of the spices. Till date turmeric has drawn the attention of many researchers and literature suggests that a large number of laboratories all around the world are working to elucidate its pharmacological activities. The anticarcinogenic potential of turmeric is relatively well established now. Among various mechanisms, induction of apoptosis plays an important role in its anticarcinogenic effect. It induces apoptosis and inhibits cell-cycle progression, both of which are instrumental in preventing cancerous cell growth in rat aortic smooth muscle cells (70). The antiproliferative effect is mediated partly through inhibition of protein tyrosine kinase and c-myc mRNA expression and the apoptotic effect may partly be mediated through inhibition of protein tyrosine kinase, protein kinase C, c-myc mRNA expression and bcl-2 mRNA expression (71). Recently, curcumin has also been shown to induce apoptosis by acting as topoisomerase II and also by impairing the ubiquitin–proteasome system through the mitochondrial pathway. Curcumin causes rapid decrease in mitochondrial membrane potential and release of cytochrome c to activate caspase 9 and caspase 3 for apoptotic cell death (65, 66). Furthermore, curcumin is also recognized as a potent anti-inflammatory agent having cancer chemopreventive activity (72). Curcumin offers its anti-inflammatory effect through inhibition of NFkB activation (73). The anti-inflammatory role of curcumin is also mediated through downregulation of cyclooxygenase-2 and inducible nitric oxide synthase through suppression of NFkB activation (74).

It appears that significant portion of the chemopreventive effects of isothiocyanates (which are the chief components of mustard seeds) may be associated with the inhibition of the metabolic activation of carcinogens by cytochrome P450s (Phase I), coupled with strong induction of Phase II detoxifying and cellular defensive enzymes. Inductions of Phase II cellular enzymes are largely mediated by the antioxidant responsive element (ARE), which is regulated by the transcriptional factor, Nrf2. Moreover, apoptosis and modulation of cell cycle appear to be yet another potential chemopreventive mechanisms elicited by isothiocyanates. Finally, modulation of other critical signaling mediators, including the NF-kappaB and AP-1 by a wide array of chemopreventive agents including isothiocyanates may also contribute to the overall chemopreventive mechanisms (75).

Experiments to test the chemopreventive efficacy of other spices are only at the initial stages. It is quite apparent that almost all the spices which are being commonly used are potent antioxidative agents, i.e., they can neutralize free radicals and protect our body against oxidative damage. Unless protected by antioxidants, macromolecules such as RNA, DNA, proteins, and lipids are damaged by free radicals. Increasing evidences revealed that oxidative damage is involved in the pathogenesis of carcinogenesis (76, 77). Oxidative mechanisms plays a potential role in different stages of carcinogenesis such as initiation, promotion, and
research on spices and spice-principles as natural expression (inhibition of bcl-2 and by induction of caspase-3 protein). Several other spice principles are also underway. For example, administration of black pepper (1 g, single dose) or piperine (single or multiple doses) resulted in an approximately 2-fold increase in plasma concentrations of the P-glycoprotein substrates phenytoin and rifampin.

In the past few years' experimental data in favour of the anti-inflammatory, anti-mutagenic and apoptotis-inducing activity of spices have been piling up, but their underlying mechanisms of action is yet to be explored. It can however, be hypothesized that the active ingredients in the spices, such as, phenols, terpenes, isothiocyanates etc. are responsible for their pharmacological properties. For instance phenolic compounds, e.g., eugenol (an important component of clove) have been suggested to inhibit cyclooxygenase-2 (COX-2), an important biomarker for several cancers, by competitive inhibition. In fact, several mechanisms are suggested to be involved by which the phenolic compounds inhibit the enzymatic activity of COX-2, including interference or competition for the arachidonic acid binding site and by reducing the enzyme activity of the oil and supercritical CO2 extract of Elettaria cardamomum L. In a study, piperine, which is a major constituent of black and long pepper, was identified as an inhibitor of both human P-glycoprotein and CYP3A4. Many drug-drug interactions can be explained by inhibition of P-glycoprotein and/or CYP3A4. Experimental data indicate that piperine might affect disposition of drugs that are substrates for both P-glycoprotein and CYP3A4. For e.g., administration of black pepper (1 g, single dose) or piperine (single or multiple doses) resulted in an approximately 2-fold increase in plasma concentrations of the P-glycoprotein substrates phenytoin and rifampin.

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In the ongoing discussion it is clear that research on spices and spice-principles as natural chemopreventers is gaining impetus all over the world. Although a large amount of facts and figures regarding spices and their cancer preventive properties have been mounting since the past decade, but there are many horizons that still remain unexplored.

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