CHAPTER - II

REVIEW OF LITERATURE
Plants and plant products used extensively in Indian diet and traditional medicines are increasingly being screened for their role in modulating the activity of environmental mutagens. The property of preventing mutagenesis/carcinogenesis has been reported in many plant products. In screening for antimutagenic effects, extracts of different plant parts have been used, ranging from leafy vegetables, fruits, seeds and underground storage organs to whole plants. A number of medicinal plants and Ayurvedic formulations have been tested for the mutagenic/antimutagenic/antitumour effects on various test systems. The following review is confined to mutagenic, antimutagenic and anticarcinogenic/antitumour studies of plant products, particularly dietary constituents like spices.

A great majority of mutagens and carcinogens can only act through interaction with environmental factors. Exposure of the human population to mutagens and carcinogens is not limited to occupational settings since mutagens have been found in airborne particles, diesel engine emissions, beverages and food (Ames, 1983). Food is possibly the most chemically complex substance to which humans are exposed, undergoing various effects during storage, processing and cooking, often to form genotoxic compounds. Cooking of proteinaceous foodstuffs leads to the generation of amino acid pyrolysates and quinoline compounds, which have been shown to be mutagenic in bacterial assays (Sugimura and Sato, 1983) and carcinogenic in experimental animals (Sugimura, 1985). Nutritionally essential metals too can induce diverse genotoxic effects at high doses (Sharma and Talukder, 1987). Total prevention from exposure to these harmful products is not always feasible. The simultaneous production of antimutagenic and desmutagenic factors has added to the possibility of identifying such factors and using the information to modify or alleviate toxic effects.
Review of Literature

Awareness of human exposure to mutagenic and carcinogenic agents has led to a greater interest in natural antimutagenic and anticarcinogenic factors in dietary ingredients. A number of agents, known to suppress mutations and carcinogenesis in various test systems has been identified from different plant systems. Further a large amount of work has been carried out with the major individual components or isolated fractions of vegetable extracts. These include crude vegetable extracts and plant derived products such as plant pigments, flavonoids, vitamins and fibres.

Experiments with crude aqueous extracts of a large number of vegetables have been found to modify the effects of clastogenic chemicals in several tests systems both in vivo and in vitro and positive correlations between chromosomal aberrations, mutagenicity and carcinogenicity have been recognized. Effect of pretreatment with various vegetable juices, both fresh and boiled on 7, 12-dimethylbenzanthracene (DMBA) induced chromosomal aberrations in rat bone marrow cells in vivo was studied by Ito and his colleagues (Ito et al., 1986). The vegetables included onion, burdock, cabbage, eggplant, Welsh onion, lettuce, carrot, celery and pumpkin. Chromosomal aberrations (breaks, gaps and exchanges) were suppressed by both fresh and boiled juices from all the vegetables and significantly so from onion, burdock, eggplant, cabbage and Welsh onion. The degree of suppression of mutagenic effects was same whether the juices were force-fed or given freely as drinking water. Short-term in vivo mouse bone marrow micronucleus test was carried out to evaluate the role of carrot and spinach in modulating the genetic damage induced by the commonly used chemotherapeutic drug, cyclophosphamide (CP) (Abraham et al., 1986). The vegetable juices, whether administered before, after or simultaneously with various concentrations of CP, significantly suppressed the induction of micronuclei in the treated animals.
Extracts of *Phyllanthus emblica* L. fruits, a rich source of vitamin C and used in many Ayurvedic and Unani systems of medicine, when administered orally to Swiss albino mice together with known clastogens (ZnCl$_2$-metallic salt, ethyl parathion-insecticide, metanil yellow-food additive) reduced the cytotoxic effects to a greater degree than a combination of the mutagens with vitamin C alone (Giri and Banerjee, 1986).

Populations are exposed mainly to environmental and dietary complex mixtures rather than to a single chemical. Thus, the counteraction of mutagenic effects of a large number of such mixtures as coal dust, tobacco (nitroso compounds), red wine and red grape juice (flavonoids), airborne and diesel emission particles (nitropyrenes), cigarette smoke, fried beef and fried shredded pork (aromatic amines and other polycyclic hydrocarbons) by chlorophyllin was extensively studied (Ong *et al*., 1986). Investigations carried out in bacterial mutation assays using *Salmonella typhimurium* TA 98, revealed that chlorophyllin itself was neither toxic nor mutagenic, it inhibited the mutagenic activity of each of the complex mixtures in a dose-dependent manner, especially of the extracts of airborne particles, cigarette smoke, fried beef and fried shredded pork. The antimutagenic property of chlorophyllin was also found to be heat resistant.

Plant polyphenolic acids (ellagic, caffeic, chlorogenic and ferulic acids) have been observed to act as antimutagens towards Benzo(a)Pyrene and its mutagenic metabolites in bacterial mutation assays (Wood *et al*., 1982). The phenolic compounds hydroxychavicol and eugenol separated from betel leaf chewed widely in India, exhibited a dose-dependent suppression of DMBA-induced mutagenesis (Amonkar *et al*., 1986).
Nineteen vitamins including some derivatives (retinoids, riboflavin, folic acid, menadione, cyanocobalamin, ascorbic acid, pyridoxine, pyridoxamine, pyridoxal, thiamin, nicotinamide, pantothenic acid, FAD and FMN were tested for their ability to suppress the mutagenic activity of aflatoxin (AFB1) (Battacharya et al., 1987). The vitamins modified the microsome mediated mutagenic activation in Salmonella typhimurium strain TA100, with the first seven vitamins giving a significant difference. Ascorbic acid was found to inhibit mutagenicity of AFB1 only at high dose. This finding however contradicts the reports of ascorbic acid being mutagenic at high doses. Nevertheless, vitamin C has been extensively studied as an antimutagen and anticarcinogen and has shown to decrease the chromosomal damage induced by cyclophosphamide in human leucocyte cultures (Gebhart et al., 1985).

In bacterial mutation assays using Salmonella typhimurium TA98, fibres of a large number of vegetables (cabbage, burdorck, sweet pepper spinach, carrot, onion, bamboo shoot, Japanese radish) were found to inactivate pyrolysate mutagens derived from amino acids, by adsorbing them (Kada et al., 1984).

2.1. MUTAGENICITY STUDIES

Cereals, pulses, vegetables, fruits and spices are natural foods which are rich in number of chemical compounds like flavonoids, alkaloids and furans. Some of the compounds are known mutagens. The chemical components of foods are altered by pre-ingestion processes like cooking, storage, or by the addition of certain food additives (Sugimura, 1982). The major components of foods which are known mutagens are shown in the Table 2.1.
Table 2.1. Classes of Mutagens in Foods*

<table>
<thead>
<tr>
<th>Natural food contaminants:</th>
<th>Flavonoids, furans, alkaloids.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food contaminants:</td>
<td>Pesticides, package materials, solvents, nitroso compounds, polycyclic aromatic hydrocarbons.</td>
</tr>
<tr>
<td>Food additives:</td>
<td>Food colours, food flavours, preservatives, sweeteners, anti-oxidants, miscellaneous food additives.</td>
</tr>
<tr>
<td>Mutagens generated by food processing:</td>
<td>Products of heating, smoking, boiling, curing, irradiation, solvent extraction.</td>
</tr>
<tr>
<td>Mutagens generated by food storage:</td>
<td>Malonaldehyde, fumigation products, mycotoxins.</td>
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Dietary components and food dishes commonly consumed in South India were screened (Sivaswamy et al., 1991) for their mutagenic activity. Kesari powder, calamus oil, palm drink, toddy and Kewra essence were found to be strongly mutagenic; garlic, palm oil, arrack, onion and paralyzed portions of bread toast, chicory powder were weakly mutagenic, while tamarind and turmeric were not mutagenic. Certain salted, sundried and oil fried food items were also mutagenic, Cissus quadrangularis was mutagenic, while ‘decoctions’ of cumin seeds, aniseeds and ginger were not showed mutagenicity. Several perfumes, essential oils and colouring agents, which are commonly used were also screened and many of them exhibited their mutagenic potential by inducing the reverse mutation in Salmonella typhimurium tester strains.

The cytotoxicity of the extracts from eight different spices used in Indian diet was determined (Unnikrishnan and Kuttan, 1988) using Dalton’s lymphoma ascites tumour cells and human lymphocytes in vitro and Chinese Hamster Ovary cells
(CHO) and Vero cells in tissue culture. Alcoholic extracts of the spices were found to be more cytotoxic to these cells than their aqueous extracts. Ginger (Zingiber officinale), pippali (Piper longum) and black pepper (Piper nigrum) were the most cytotoxic but asafoetida (Ferula asafoetida), mustard (Brassica compastris), garlic (Allium sativum), sesame (Sesamum indicum) and horsegram (Dolichos biflorus) were much less cytotoxic.

Extracts of caraway, coriander and black pepper seeds were not mutagenic for S. typhimurium strains-TA98 and TA100 (Higashimoto et al., 1993). However, the aqueous and methanolic extracts treated with nitrite were mutagenic for strain TA100. Black pepper showed highest mutagenicity and other two were moderate.

Chillies and their principal alkaloid Capsaicin along with turmeric powder were mutagenic in S. typhimurium (Nagabhushan and Bhide, 1986). The relationship between mutagenicity and the pungent properties of spices were studied (Azizan and Blevins, 1995 using S. typhimurium strains and assessed that there is no relationship between these two aspects. They observed that among six compounds associated with the pungent properties of specific spices (capsaicin, thymol, borneol, allyl isothiocyanate, eugenol and cinnamaldehyde), only capsaicin was mutagenic in S. typhimurium strain TA 100.

Pepper is widely incorporated in the diet of Asian and Western countries and it is also an important constituent of more than 150 Ayurvedic formulations (Karthikeyan and Rani, 2003). John and Abraham (1991) reported the ability of black pepper to induce chromosomal aberrations in Swiss albino mice. In another study spices like black pepper (P. nigrum), pippali (P. longum), ginger (Z. officinale) and mustard (B. nigra) increased the number of revertants in S. typhimurium strains indicating their mutagenic potential (Soudamini et al., 1991, 1995).
Review of Literature

Vijayalaxmi (1980) studied genetic effects of turmeric and curcumin in mice and rats using MN test, chromosomal aberration test and dominant-lethal assay. The results obtained with all three test systems showed that neither turmeric nor curcumin had any adverse cytogenetic and mutagenic effects when incorporated into the diets of mice and rats, in amounts which are normally consumed by man. Acute toxicity studies conducted (Shankar et al., 1980) on different species of animals including non-rodents (guinea pigs and monkeys) revealed that turmeric or its alcoholic extract consumption is not toxic even at very high level. Further, Kaushik et al. (1993) studied mutagenicity of turmeric on root tip cells of *Vicia faba*. They concluded that turmeric extract has strong mutagenic potential and is radiomimetic in higher concentration at chromosome levels.

Among the various kinds of spices tested, the aqueous extracts of dill weed (*Anethum graveolens* L.) and dill seeds (*A. sowa* D.C.) exhibited a mutagenicity to *S. typhimurium* strains TA98 and TA100 (Fukuoka et al., 1980). A well known spice asafoetida (*Ferula narthex* Boiss) was found to be responsible for altering gestation period, litter size and sex ratio of the litter in albino rats (Borkar et al., 1996). Study results of Ungsurungsie et al. (1982) showed mutagenic activity of crude extracts and water-heated/water-macerated residues of Ceylon cinnamon (bark of *Cinnamomum zeylanicum* Nees) in *Bacillus subtilis* strains H17 (rec+) and M45 (rec-).

2.2. ANTIMUTAGENICITY STUDIES

Food contains certain chemical components most of which have neither nutritional value nor any role in the normal metabolic processes. The components which are generally removed in the process of refining include fibres, polyphenols (which impart colour), saponins, lectins, tannins, coumarins, amines, flavonoids and
anthocyanins. Recent studies have revealed that most of these compounds have some beneficial effects like reducing blood cholesterol and triglycerides and other useful pharmacological properties like antidiabetic, antifertility, anticarcinogenic, antiallergic and antimutagenic effects. Wattenberg (1983) reported that foods contain large number of anticarcinogens and antimutagens i.e., compounds which counteract the effect of carcinogens. These compounds are polyphenols, aromatic isothiocyanates, methylated flavonoids, coumarins, plant sterols, selenium salts, protease inhibitors, ascorbic acid, tocopherols and retinols which are known to inhibit cancer formation. Epidemiological studies have shown that vegetarians have a lower risk of cancer than the non-vegetarians, as raw, green vegetables contain most of the above compounds. Wattenberg suggested that these compounds can reduce the risk of cancer.

Mustard is a spice used for flavouring and as a source of edible oil in India and all over the world. The leaves of this plant are consumed as vegetable. Mustard belongs to cruciferous family, other members of which are cabbage, broccoli, cauliflower, etc. All these vegetable extracts have the property of inactivating the mutagenicity of food mutagens like tryptophan pyrolysate (Kada et al., 1978). The active principle of mustard, dithiolthiones can protect against liver toxicity induced by some chemicals. The antimutagenic property of mustard assessed in experimental animals, showed significant effects. Same report says that leafy vegetable cabbage and a known spice ginger contain antimutagenic factor(s) against tryptophan pyrolysate in bacterial strains. Further, they carried out a screening for bio-antimutagens in several plant specimens and found that the homogenate of Japanese green tea (Camellia sinensis) gave the highest bio-antimutagenic activity in the Bacillus subtilis NIG 1125 strain (Kada et al., 1985).
Shinohara and Kuroki (1987) studied antimutagenic substances in vegetables and fruits on *S. typhimurium*. The dialyzates from broccoli, burdock, cucumber, eggplant, komatsuna, peaman, spinach, sarcocarps, pericarps and envelopes of apple, amanatsu and natsudaidai showed antimutagenicity on Trp-P-2 (mutagen isolated from pyrolyzed tryptophan). This antimutagenic activity was retained even after heating them at 100°C showing heat-stability.

Post treatment with vanillin, anisaldehyde, cinnamaldehyde and coumarin was effective in reducing the mutation frequencies induced by UV or X-rays in Chinese hamster V79 cells (Imanishi *et al.*, 1990). In another study chromosome aberrations were suppressed when vanillin, cinnamaldehyde or anisaldehyde was given orally to mice after X-ray irradiation. Further, dose-dependent decrease in MNPCEs was observed (Sasaki *et al.*, 1990).

Wu *et al.* (1990) reported that roasted ginger has inhibiting tendency on gastric ulcer in rats while the dry ginger has no such effects and suggests that water soluble constituents of the dry ginger have changed in the roasting process. Most of the heated vegetables showed greater inhibitory activity than unheated samples against the mutagenicity induced by chemicals in *S. typhimurium* system (Yamaguchi, 1992). Further the hot water extracts of caraway, coriander and black pepper reduced the mutagenicity induced by N-methyl-N-nitro-N-nitrosoguanidine significantly but did not show significant result against other mutagens tested in *S. typhimurium* (Higashimoto *et al.*, 1993).

Ruan *et al.* (1992) investigated antimutagenic effect of eight natural foods on moldy foods in a high liver cancer incidence area using Ames test. The results showed that these extracts (sesame, chest nut, dad-lily, laver, red Chinese date, bamboo shoots, kelp and green tea) had marked inhibitory effects on the mutagenic activity.
induced by AFB or metabolic extracts from *Aspergillus versicolor* or *A. ochraceus*. Study results of Abbas *et al.* (1994) shows that aqueous extracts of green tea possess marked antimutagenic potential against a variety of important dietary and environmental mutagens in Wistar albino rats.

Garlic was found to inhibit the mutagenicity induced by direct acting mutagens such as N-methyl N’-nitro-N-nitrosoguanidine (MNNG) and sodium azide. Asafoetida, turmeric, curcumin (phenol present in turmeric) and eugenol (phenol present in clove) were found to inhibit microsomal activation dependent mutagenicity of 2-acetamidofluorene (Soudamini *et al.*, 1995). Further, Mitscher *et al.* (1996) reviewed natural antimutagenic agents and tumour chemopreventive agents. They concluded that spices like garlic (*A. sativum*), caraway (*Carum carvi* L.), coriander (*C. sativum*), black pepper (*P. nigrum*) and turmeric (*C. longa*) are showing antimutagenic activity in bacterial strains. But dietary curcumin and the glycoflavanoid hesperiden (both turmeric constituents) showed anticarcinogenic activity against various carcinogens.


copticum) using Ames test and *in vivo* bone marrow micronucleus test against various mutagens/carcinogens.

Shukla *et al.* (2002) evaluated the antimitogenic potential of certain dietary constituents like black tea extract, diallyl sulfide (component of garlic, *A. sativum*) and curcumin (component of turmeric, *C. longa*). They found that the antigenotoxicity of these dietary constituents towards cyclophosphamide induced chromosomal aberrations and micronuclei. In another study El Hamss *et al.* (2003) used the wing Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* to study the modulating action of bell pepper (*Capsicum annum*) and black pepper (*Piper nigrum*) in combination with the alkylating agent Methyl methanesulfonate (MMS) and the promutagen agent ethyl carbamate (EC). They observed that bell pepper was effective in reducing the mutational events induced by EC and MMS and black pepper was effective only against EC.

It has been reported that some kinds of plant essence, such as cinnamaldehyde, coumarin, umbelliferone, anisaldehyde, vanillin and tannic acid had antimitogenic effects on mutations induced by UV or chemicals in *Escherichia coli* (Ohta *et al.*, 1983a,b, 1986, 1988; Shimoi *et al.*, 1985). Further, Sukumaran and Kuttan (1995) observed inhibition of tobacco-induced mutagenesis by eugenol (constituent of clove) in Ames Salmonella/microsome assay and also noticed that eugenol inhibited the nitrosation of methyl urea in a dose-dependent manner. It was reported that capsaicin (a major pungent and irritating ingredient of hot pepper chilli) and diallyl sulfide (a thioether found in garlic) suppress vinyl carbamate (VC) and N-nitrosodimethylamine (NDMA) induced mutagenesis or tumourigenesis in *S. typhimurium* (surh *et al.*, 1995).
Simultaneous treatment of ICR170, a strong mutagen with safrole had no significant effect on mutations in Chinese hamster V79 cells. Pre-treatment and post-treatment with safrole slightly enhanced the frequency of mutations induced by ICR170 (Kuroda et al., 1992). Study results of Farag and Abo-Zeid (1997) using Ames test proved that the mutagenicity of some spices due to the presence of safrole (ingredient of star anise, *Illicium verum* Hook.; cumin, *C. cyminum*; black pepper, *P. nigrum* and ginger, *Z. officinale*) can be destructed during drying of the washed seeds or during cooking either with or without any additional treatment as irradiation. Further they found that boiling whole seeds or powder of black pepper during cooking for few minutes (1-5 min) were more efficient in decreasing safrole content.

Allylisothiocyanate (AIT), a potent component of wasabi (*Wasabi japonica*) and horseradish (*Cholearia arnoracia*) showed antimutagenic activity toward 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline [MeIQx], a well-known mutagen/carcinogen in broiled fish and meat. They also decreased His+ revertant colonies of 3-chloro-4-dichloromethyl-5-hydroxy-2(5H)-furanone (MX) in the Ames test (Kinae et al., 2000). It was inferred that chlorophyll can successfully suppress the mutagenic activities of capsaicin and 2-aminoanthracene together with other antimutagenic factors that were present in the acetone extract of *Capsicum annum* in *S. typhimurium* TA 100 (Azizan and Blevins, 1995).

Vinitketkumnuen et al. (1994) found that ethanolic extract of lemon grass, *Cymbopogon citratus* Stapf (a Thai medicinal plant commonly used in the diet and in medicine) modifies mutation in *S. typhimurium* strains TA98 and TA100 which are induced by various known mutagens. *Ocimum sanctum* (Sanskrit: Tulasi), a sacred and well known medicinal plant in India affords in vivo protection against radiation induced cytogenetic damage in mice (Ganasoundari et al., 1996). A survey of
scientific literature for the last 30 years has identified a number of medicinal plants and their components with antimutagenic and anticarcinogenic properties. This includes spices like garlic (*A. sativum*), mustard (*Brassica juncea*), turmeric (*C. longa*) and ginger (*Z. officinale*) (Devasagayam and Tilak, 2002).

It has already reported that curcumin, the major pigment in turmeric (important constituent of *curcuma longa*), possesses good anti-oxidant (Ruby *et al.*, 1994; Soni *et al.*, 1997a), anti-inflammatory (Ruby *et al.*, 1998) and antitumour activity (Kuttan *et al.*, 1985; Menon *et al.*, 1995). Further it was tested for its antimutagenic and anticarcinogenic activity (Nagabhushan and Bhide, 1987) and found that potent inhibitors of mutagenesis in bacterial strains (Ruby *et al.*, 1996a). Both natural and synthetic curcumin showed antiinflammatory and cholesterol reducing properties in mice (Soudamini and Kuttan, 1992; Ruby *et al.*, 1998) and in man (Soni and Kuttan, 1992). Further they found curcumin to inhibit lipid peroxidation and superoxides (Nishigaki *et al.*, 1992; Inoue *et al.*, 1992; Thresiamma *et al.*, 1995).

Using Ames test, curcumin itself a non-mutagen, inhibited the mutagenic effects of chilli extract and capsaicin (Nagabhushan and Bhide, 1986). Similarly, curcumin was reported to inhibit the activity of known environmental mutagens which require metabolic activation, although it was reported to be ineffective against mutagens which do not require metabolic activation (Nagabhushan *et al.*, 1987; Nagabhushan and Bhide, 1987).

Turmeric and curcumin along with CP increased the life span of animals when compared to animals treated with CP alone (Soudamini *et al.*, 1992). Histopathological study revealed that both turmeric and curcumin are inhibitors of aflatoxin induced toxicity in experimental ducklings. Further extracts of turmeric,
garlic and asafoetida inhibited the aflatoxin production considerably (Soni et al., 1992). Further, Turmeric and curcumin inhibited aflatoxin toxicity in ducklings (Soni et al., 1993). In another study turmeric, curcumin, asafoetida and garlic were found to inhibit the mutagenesis induced by aflatoxin in Salmonella testers strains (Soni et al., 1997b). There was a significant time-dependent reduction in the number of radiation-induced micronucleated polychromatic erythrocytes in mice with a single gavage doses of 5, 10 or 20 mg/kg b.w. curcumin in peanut oil (Abraham et al., 1993).

Kuttan (1994) highlighted the antioxidant, antiinflammatory, liver protecting, antimutagenic, anticarcinogenic and antiteratogenic activities of turmeric and its constituent curcumin. Further, Shishu et al. (2002) studied the antimutagenic potential of various constituents of turmeric against the heterocyclic amine mutagens that are generated during cooking of muscle meats such as beef, fish and chicken using Ames test. Results indicated that natural curcuminoids are highly effective in a dose-dependent manner.

Turmeric has been attributed a number of medicinal properties in the traditional system of medicine. The major claims have been for use as antiseptic, cure for poisoning, eliminating body waste products, for dyspepsia, respiratory disorders and cure for a number of skin diseases including promotion of wound healing. Curcumin, curcuminoids and essential oils are the major active constituents. The main activities have been found to be anti-inflammatory, hepato-protective, antimicrobial, wound healing, anticancer, antitumour and antiviral (Srimal, 1997). Paper further added on the proper evaluation of antiviral properties of curcumin, particularly against HIV.
2.3. ANTICANCER/ANTITUMOUR STUDIES

The association between diet and cancer has received increasing attention and support in the last two decades as a result of data compiled by epidemiologists, clinicians and laboratory scientists. Dietary practices may either increase or decrease cancer risk depending on the intake of nutrients as well as non-nutrients and their interactions at several stages of carcinogenesis. Dietary substances can alter carcinogen metabolism, \textit{in vivo} host response, damage to macro molecules, immune surveillance and modify promotion and progression of neoplasia (Krishnaswamy, 1991). The case control approach revealed that vitamin A, E, zinc and selenium have potential effects on cancer risk. However, several non-nutrient components in leafy vegetables and spices appear to offer protective role by inhibiting the process of carcinogenesis. Of these, turmeric, mustard, onion and garlic appear to be promising agents. Nutrient intervention (chemoprevention) of precancerous lesions appears to be an attractive alternative to prescription for prevention of cancer. Dietary modifications though difficult, could be more interesting as both the nutrients and non-nutrients can have an aggregate effect on cancer prevention.

In 1969, the International Agency for Research on Cancer (IARC) initiated the Monographs Programme to evaluate the carcinogenic risk of chemicals to humans. Results from short-term mutagenicity tests were first included in the IARC Monographs in the mid-1970s based on the observation that most carcinogens are also mutagens, although not all mutagens are carcinogens. Experimental evidence at that time showed a strong correlation between mutagenicity and carcinogenicity and indicated that short-term mutagenicity tests are useful for predicting carcinogenicity (Waters et al., 1999). Although the strength of these correlations has diminished over
the past 30 years with the identification of putative nongenotoxic carcinogens and understanding mechanisms of carcinogenesis.

As effort continue toward better understanding of the various mechanisms involved in the induction of human cancer, results from short-term genetic tests will continue to provide valuable information for discriminating mutagenic and non-mutagenic mechanisms. Results from mammalian assays in vivo, followed by those from mammalian assays in vitro are considered more relevant than those from non-mammalian assays (Waters et al., 1999).

Life style including dietary habits is one of the most important factors responsible for different types of cancer. The role of diet in human cancer has prompted many to analyse the food items for possible mutagens and carcinogens (Sivaswamy et al., 1991). The limited data on herbs, spices and condiments come mainly from a few human case-control studies and some experimental animal studies. Human studies are limited by difficulties in quantifying intakes of individual items that are typically consumed in small quantities (Anonymous, 1997). A few studies have provided results relevant to spices in particular and herbs in general. These include in vitro studies and animal experiments.

Strong evidence suggests that consumption of fruits and vegetables results in decreased incidence of all types of cancer. They are known to contain variety of non-enzymatic antioxidants, namely carotenoids, tocopherols, ascorbic acid and plant polyphenols which exert their antimutagenic activity, even after subjected to the cooking process (Mathur, 1997). Diet consisting of food containing carbohydrates, proteins and fats but lacking fruits and vegetables, the level of DNA damage is higher than for diets including fruits and vegetables, which are rich in natural antioxidants (Simic and Bergtold, 1991).
The role of dietary factors on the development of tumours in animals (Boone et al., 1990; Freedman et al., 1990) and of antioxidants in reducing tumour incidence (Wattenberg, 1978) and the underlying free radical mechanisms (Simic, 1989) are supporting to the above observation and conclusion. Fruits and vegetables lower the level of oxidative DNA damage most likely reflects the interaction of plant antioxidants with O2, thereby reducing the level of H2O2 and OH radicals. Other reactions may play a major role (e.g., inhibition of peroxyl radicals) but the correlations are not apparent (Simic and Bergtold, 1991).

Kuttan et al. (1985) reported that turmeric extract as a potential anticancer agent. This property is due to curcumin, a cytotoxic component present in turmeric (C. longa). Further the animal experiments showed that both turmeric extract and curcumin decreased the incidence of tumour formation in experimental mice. An ethanolic extract of turmeric as well as an ointment of curcumin (its active ingredient) were found to produce remarkable symptomatic relief in patients with external cancerous lesions (Kuttan et al., 1987). In another study Soudamini and Kuttan (1988) reported that both aqueous and alcoholic extracts of turmeric were cytotoxic to various cell lines in vitro and tumour reducing in Swiss albino mice bearing Dalton's lymphoma ascites tumour.

Comparable anti-tumour effects were observed in animal studies with turmeric. A 1% dietary turmeric inhibited the formation of BP-induced forestomach tumours in female Swiss mice by 58% and lowered the incidence of spontaneous mammary tumours in C3H Jax mice by 60% (Nagabhushan and Bhide, 1987). Anti-tumour effect of curcumin is supported in another study of skin carcinogenesis in mice. Repeated applications of turmeric extract and curcumin in the promotion phase
produced a reduction in the expression of papillomas in mouse skin induced by 7,12 dimethylbenzanthracene (Soudamini and Kuttan, 1989).

Strong antioxidant effects of several components of turmeric result in an inhibition of carcinogenesis. Extracts of the spice may play a role as chemoprotectant, which limit the development of cancers (Liberti, 1993). Curcumin showed a reasonable increase in the lifespan of Ehrlich tumour bearing mice (Ruby et al., 1994). Further it also showed a significant reduction on solid tumours in mice when injected intraperitoneally.

Later studies of Ruby et al. (1995, 1996b) showed that natural and synthetic curcuminoids (especially those having the phenolic structure) possess the anticancer and antioxidant activities evinced by curcumin and also act as potent antipromoters. They increased the life span of animals bearing Ehrlich ascites carcinoma. Further, dietary administration of food additives such as turmeric, garlic and curcumin to rats treated with aflatoxin B₁ inhibited the development of hepatocellular neoplasm (Soni et al., 1997b).

Dhar et al. (1968) screened ethanolic extracts of 285 botanically identified plant materials including spices with 61 tests including anticancer tests. Results obtained includes anticancer potential of several plants. They noticed that a number of plants showed activity with the crude extract which could not be confirmed on fractionation.

Out of 20 spices/leafy vegetables screened for their influence on the carcinogen-detoxifying enzyme, glutathione-S-transferase (GST) in Swiss mice, spices like cumin seeds, poppy seeds, asafoetida and turmeric showed protective activity against carcinogenesis (Aruna and Sivaramakrishnan, 1990). They significantly suppressed (in vivo) the chromosome aberrations caused by B(a)P in
mouse marrow cells. But spices like fenugreek seeds, coriander seeds and ginger did not show significant result.

Chemical components isolated from vegetables and spices such as cauliflower, citrus fruits, tomatoes, green chillies, pineapples, strawberries, garlic, onion, soya and red chillies were studied for their anticancer activities (Madhavankutty, 1994). Components like sulphoraphane, indole-3-crbinol, flavonoids, coumaric acid, chlorogenic acid, allylic sulphides, genistein, and capsaicin showed anticancer ability.

Tumour reducing activity of extracts of eight commonly used spices in India were studied in mice transplanted intraperitoneally with Ehrlich ascites tumour (Unnikrishnan and Kuttan, 1990). Oral administration of extracts of black pepper (*P. nigrum*), asafoetida (*Ferula asafoetida*), pippali (*Piper longum*) and garlic (*Allium sativum*) could increase the percentage of life span but intraperitoneal administration of spice extracts did not produce any significant reduction in tumour growth except of sesame (*Sesamum indicum*). Asafoetida and garlic were not only found to increase the life span of tumour bearing animals but also was shown to inhibit chemical carcinogenesis. Other spices like ginger (*Z. officianale*), mustard (*Brassica comasparris*) and horsegram (*Dolichos biflorus*) did not show any positive response against ascites tumour.

Painting and feeding of mice with black pepper extract results in a significant increase of the number of tumour bearing mice. Further, feeding of mice with powder of black pepper in diet has no impact on carcinogenesis (Shwaireb *et al.*, 1990). Force feeding of d-limonene (a pepper terpenoid) for a long time to the mice showed anticarcinogenic activity against above constituents and methylcholanthrene (MCA). But piperine (one of black pepper alkaloid) was ineffective. In another study El-Mofty *et al.* (1988, 1991) speculated that one or more constituents of black pepper may be
responsible for tumour induction in the organs of the Egyptian toad, *Bufo regularis* fed with suspension of black pepper. Further studies showed metastatic deposits of hepatocellular carcinomas in the spleen, kidney, fat body and ovary. Wrba *et al.* (1992) observed that in mice, injection of safrole and tannic acid (constituents of black pepper) during the pre-weaning period induced tumours in different organs. Piperine could inhibit the pulmonary metastasis induced by B16F10 melanoma cells in mice and also observed significant increase in life span of tumour bearing animals treated with piperine (Pradeep and Kuttan, 2002).

Among the vegetables/spices, those belonging to the allium family have received increased attention in recent times. Onion and garlic are commonly consumed through the diet. They contain sulphur compounds like diallylsulphide and diallyl disulphide. Onion (*Allium cepa* L.) extract was found to be cytotoxic to ascites cells (MFS-180) under *in vitro* condition and also it could check the ascites tumour growth in mice when administered along with transplantation of tumour cells (Nerkar *et al.*, 1981). Study results of Unnikrishnan *et al.* (1990) showed chemoprotection of garlic (*Allium sativum*) extract towards cyclophosphamide toxicity in mice with an increase in life span. Garlic extract alone does not have any tumour reducing activity, but it reduced the toxicity of cyclophosphamide and increased its therapeutic efficacy significantly. Further, it was found that garlic extract reverse the toxicity induced weight loss in animals and free radical scavenging significantly.

Samman *et al.* (1998) observed that mint (*Mentha arvensis* L.) has a chemopreventive effect against shamma (Shamma, a complex mixture of powdered tobacco, slaked lime, ash, oils, spices and other additives, has been linked to oral cancer in Saudi Arabia) induced carcinogenesis in hamsters. Menon *et al.* (1998) studied two dietary soybean isoflavones, genistein and daidzein for the inhibition of
lung metastasis induced by B16F-10 melanoma cells in mice. Genistein inhibited lung
tumour nodule formation and also increased the life span of the tumour-bearing
animals. But daidzein had no significant effect on the reduction of lung metastasis.
Sukumaran and Kuttan (1991) studied antitumour potential of ferns in mice bearing
Ehrlich ascites tumour using animal survival studies.

In recent years, several observations have strongly implicated nitrate, nitrite
and nitrosamines in the development of tumours in man. Nitrates are ubiquitous in
water and food. Though nitrates are not harmful it can be reduced to nitrite under
certain circumstances both food and in the body which combine with amines in food
to form nitroso compounds. The nitrate content of foods show that it is high in spices
followed by vegetables particularly the non-tuberous variety followed by roots and
tubers (Gundimeda et al., 1993). In vitro studies shows several spices such as pepper,
red chillies, and cumin yield significant amount of nitroso compounds. Substances
such as turmeric and tomato can inhibit in vitro, nitrosations (Krishnaswamy and
Polasa, 1995). Vitamin C is a potent inhibitor of in vivo nitrosations. High nitrite
containing foods with high salt intake may increase the risk of gastric cancer.

In an attempt to find natural products with antitumour/radiosensitizing
properties, extracts of medicinal plants were screened in experimental tumour
systems. It was found that Withania somnifera, popularly known as Ashwagandha
(Sanskrit) in India, has properties that may prove useful in clinical cancer therapy.
Sharada et al. (1996) studied the antitumour and radiosensitizing effects of Withaferin
A (WA), a steroidal lactone from W. somnifera on mouse Ehrlich ascites carcinoma in
vivo. Increase in life span and tumour free survival were studied up to 120 days.
Important findings of the study were the higher in vivo tumour killing when WA
treatment was combined with irradiation. The drug inhibited tumour growth and
increased survival, which was dependent on the WA dose per fraction rather than the total dose.

The alcoholic extract of the dried Withania roots as well as the active component Withaferin A isolated from the extract showed significant antitumour and radiosensitizing effects in experimental tumours in vivo, without any noticeable systemic toxicity (Umadevi, 1996). Further, Withaferin A showed significant growth inhibitory and cytocidal effects on exponentially growing mouse Ehrlich ascites carcinoma in vivo (Umadevi, 1996). Shohat et al. (1967, 1970) found that Withaferin A obtained from Withania leaves inhibited the in vivo growth of Ehrlich ascites carcinoma in the mouse. In another study Umadevi et al. (1992, 1993) observed that ethanolic extract of Withania roots was very effective against transplantable mouse sarcoma-180 solid tumour in vivo and that the tumour killing effect significantly increased by combining Ashwagandha treatment with irradiation and hyperthermia. Further, crude extract was also effective in prolonging the life span of mice bearing Ehrlich ascites tumour (Umadevi et al., 1994).

Rao and Umadevi (1996a) studied in vivo response of mouse sarcoma-180 to different doses of Cisplatin in combination with radiation (RT) and hyperthermia (HT). On the basis of tumour cure (CR), volume doubling time (VDT), regrowth delay (RD) and animal survival up to 120 days; it was concluded that combination of a moderate dose of HT with low dose of cisplatin could enhance the tumour cure and prolong survival of mice. Further, in vivo response of mouse sarcoma-180 to multimodality treatment using AK-2123 (AK), hydralazine (HDZ), irradiation (RT) and hyperthermia (HT) was analysed (Rao and Uma devi, 1996b). Multimodality approach using AK, RT and HT with the inclusion of HDZ was more effective than...
the bimodality and the trimodality treatments without HDZ based on CR, VDT, RD and animal survival up to 120 days.

Umadevi and Ganasoundari (1995) studied radioprotective effect of leaf extract of *Ocimum sanctum* using Swiss albino mice. Animal survival was studied up to 30 days and the aqueous extract was more effective in increasing survival than the alcohol extract. Further, intraperitoneal (ip) administration gave the best protection (70% survival) and other routes (im, iv, and po) were less effective and produced (37-47%) survival. Menon *et al.* (1997) reported increase in life span of mice bearing metastatic tumour treated with herbal drugs like brahma rasayana and aswagandha rasayana.
Table 2.2. Summary of studies on the mutagenic potential of different dietary substances.

<table>
<thead>
<tr>
<th>Exposure/Test material</th>
<th>Test object</th>
<th>Test system</th>
<th>Mutagenicity (+/-)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenol</td>
<td><em>S. typhimurium</em> TA100, TA102, TA1535</td>
<td>Revertants</td>
<td>-</td>
<td>Sukumaran and Kuttan, 1995.</td>
</tr>
<tr>
<td>Mustard and horse radish</td>
<td><em>S. typhimurium</em> TA100</td>
<td>Revertants</td>
<td>-</td>
<td>Kassie <em>et al.</em>, 1996.</td>
</tr>
<tr>
<td>Chilli oleoresin &amp; Capsaicin</td>
<td><em>S. typhimurium</em></td>
<td>Revertants</td>
<td>-</td>
<td>Gupta <em>et al.</em>, 2000</td>
</tr>
</tbody>
</table>

**Note:** CAT = Chromosomal aberration test; DLT = Dominant lethal test; MN = Micronucleus test; SSA = Sperm-shape abnormality test.
Table 2.3. Summary of studies on antimutagenicity of different dietary substances.

<table>
<thead>
<tr>
<th>Exposure/Test material</th>
<th>Analysed biomarker (mammals)</th>
<th>Analysed biomarker (other than mammals)</th>
<th>Mutagens used</th>
<th>Protective effect/Antimutagenicity (+/-)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophylls (Aquatic plants)</td>
<td>-</td>
<td>Ames test – <em>S. typhimurium</em></td>
<td>B(a)P</td>
<td>-</td>
<td>Sato <em>et al.</em>, 1990.</td>
</tr>
<tr>
<td>Turmeric (<em>C. longa</em>) and Curcumin</td>
<td>Wistar rats-liver (DNA)</td>
<td>-</td>
<td>B(a)P</td>
<td>+</td>
<td>Mukundan <em>et al.</em>, 1993.</td>
</tr>
</tbody>
</table>

*Contd....*
## Review of Literature

<table>
<thead>
<tr>
<th>Exposure/Test material</th>
<th>Analysed biomarker (mammals)</th>
<th>Analysed biomarker (other than mammals)</th>
<th>Mutagens used</th>
<th>Protective effect/Antimutagenicity (+/-)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tea (Camellia sinensis &amp; C. assamica)</td>
<td>Review Article</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Gupta et al., 2002.</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Wistar rats-CAT</td>
<td>-</td>
<td>CP</td>
<td>+</td>
<td>Taneja et al., 2002.</td>
</tr>
</tbody>
</table>

**Note:**
- AF = Aflatoxin; 2-AAF = 2-acetamidofluorene; B(a)P = Benzo (a) pyrene; CIS = Cisplatin; CP = Cyclophosphamide; DAB = Dimethylaminoazobenzene; DMBA = 7,12-dimethylbenzanthracene; IQ = 2-amino-3-methylimidazo (4,5f)quinoline; GST = Glutathione S-transferase; MMC = Mitomycin; MG = Methylglyoxal; MMS = Methylmethane sulfonate; MNNG = N-methyl-N-nitro-N-nitroso guanidine; 4-NPDA = 4-nitro-O-phenylenediamine; NQO = 4-nitroquinoline, 1-oxide; SA = Sodium azide; URE = Urethane; CAT = Chromosomal aberration test; MN = Micronucleus test; SSA = Sperm-shape abnormality test.
Table 2.4: Summary of studies on anticarcinogenicity/antitumour activity of dietary substances.

<table>
<thead>
<tr>
<th>Exposure/Test material</th>
<th>Carcinogens/Tumour</th>
<th>Test object/Animals</th>
<th>Organs</th>
<th>Anticarcinogenicity (+/−)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamoyl methanes (analogs of curcumin)</td>
<td>DLA cells</td>
<td>Albino mice and CHA cell culture</td>
<td>Cells</td>
<td>−</td>
<td>Rao et al., 1987.</td>
</tr>
<tr>
<td>Curcumin</td>
<td>BMBA, TPA</td>
<td>Albino mice</td>
<td>Skin</td>
<td>+</td>
<td>Nishio et al., 1992.</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>BHC</td>
<td>Dawley rats and golden hamsters</td>
<td>Cheek pouch</td>
<td>−</td>
<td>Bhide et al., 1993.</td>
</tr>
<tr>
<td>Chilli, cumin &amp; black pepper</td>
<td>DMH</td>
<td>Wistar rats</td>
<td>Colon</td>
<td>Chilli (−), cumin &amp; black pepper (+)</td>
<td>Nalini et al., 1998.</td>
</tr>
</tbody>
</table>

Note: BHC = Benzene hexachloride; CHO = Chinese hamster ovary; DLA = Dalton’s lymphoma ascites; DMBA = 7,12-dimethylbenzanthracene; DMH = 1,2-dimethylhydrazine; 20-MC = 20-methylcholanthrene; TPA = 12-O-tetradecanoylphorbol-13-acetate.