Introduction
The food that we eat has a very significant bearing on our physical, mental and spiritual health. According to Ames (1983), the human diet contains a variety of naturally occurring mutagens and carcinogens. The preponderance of certain foods in some countries has been linked to the incidence of certain specific types of cancers in their populations. It is for this reason that dietary mutagens have attracted considerable attention and a number of studies have been undertaken to elucidate the link between dietary practices and cancer. The differences in cancer rates of various population groups are generally ascribed to environmental and lifestyle factors such as smoking, dietary carcinogens and promoters. These differences may be also, to a large extent due to insufficient amounts of anti-carcinogens and other preventive factors in the diet (Maugh, 1979). Studies have suggested that a greater intake of fiber rich cereals, fruits and vegetables and a lower consumption of fat rich products and alcohol is advisable (Doll and Peto, 1981; Peto and Schneiderman, 1981).

In the recent years, a large number of dietary components and constituents have been evaluated in microbial and animal test systems. However, there is still a lack of definitive evidence about their carcinogenecity and mechanisms of action. A large number of chemical carcinogens and mutagens are known to form covalent adducts with DNA and there is a large body of evidence implicating DNA as a critical target in chemically induced cancer (Miller, 1978; O'Connor, 1981). To understand carcinogenesis at the molecular level it is essential to determine the conformational changes in the target macromolecules and relate the
findings to probable aberrations in the functioning of modified macromolecules. In the recent years, there is a growing interest in oxygen radicals and lipid peroxidation as a source of damage to DNA and subsequent promotion of cancer (Harman, 1981; Gensler and Bernstein, 1981; Totter, 1980; Tappel, 1980). In addition, emphasis has also been given to the fact that DNA damage may be caused by endogenous metabolites produced during the body's normal metabolic processes (Burcham and Marnett, 1994). The mammalian systems have evolved a plethora of defense mechanisms against mutagens and carcinogens, the most important of which are against oxygen radicals and lipid peroxidation.

There is growing recognition that cancer induction by occupational and industrial factors accounts for a relatively small percentage of all human cancers. It has been suggested by Doll and Peto (1981) that about 35% of all cancer deaths in the United States may be linked to the diet. According to them, there are five possible ways by which diet may affect the incidence of cancer:

i) ingestion of powerful direct acting carcinogens or their precursors

ii) affecting the formation of carcinogens in the body

iii) affecting transport, activation or deactivation of carcinogens

iv) affecting "promotion" of cells that are already initiated; and

v) overnutrition

Normal individual consumption of potentially mutagenic substances per day from foods and beverages is estimated to be between 1-2 gm. In addition, the
endogenous conditions favour the formation of still more mutagens in vivo in humans (Ohshima and Bartsch, 1981).

MUTAGENS AND CARCINOGENS IN DIETARY PLANT MATERIALS

It is obvious that food is a very complex substance to which humans are exposed. Most people perceive food substances of natural origin as free of risk. Such acceptance is largely based on faith because our objective knowledge on this topic is relatively poor. A large number of chemicals are synthesized by plants, presumably as a defense against a variety of invasive organisms such as bacteria, fungi and insects (Kapadia, 1982; Clarke, 1982; Pamukcu et al., 1980; Stich et al., 1981a). The number of these toxic chemicals is extremely large and new plant chemicals are being continuously discovered (Jadhav et al., 1981; Griesebach and Ebel, 1978). It has been known for many years that plants show experimental carcinogenic activity for several species and various tissues. Wide use of short term tests for detecting mutagens (Ames, 1979; Stich and San, 1981a) and a number of animal cancer tests on plant substances have contributed to the identification of many natural mutagens and carcinogens in the human diet (Kapadia, 1982). Food mutagens are major xenobiotic, genotoxic substances. They include, as typical examples, aflatoxin B1 from Aspergillus flavus, nitrosamines in fermented foods, polycyclic aromatic hydrocarbons in heated foods and heterocyclic amines in heated meat and fish (Sugimura, 1982 and 1986; Ames, 1983). Most of these are metabolically oxidized by cytochrome P₄₅₀ and
then esterified to ultimately reactive forms to produce DNA adducts through electrophilic and nucleophilic reactions (Miller and Miller, 1977). Some examples of most frequently ingested compounds are discussed below.

Safrole and Estragole are related compounds that occur in certain species and essential oils and are weak hepatocarcinogens (Fenaroli, 1971; Guenther and Althausen, 1949). Studies have implicated 1'-hydroxysafrole and 1'-hydroxyestrargole as proximate carcinogenic metabolites of safrole and estragole, respectively (Drinkwater et al., 1976; Borchert et al., 1973). Eugenol and anethole are structurally related to safrole and estragole and are widely used as flavouring agents or as food additives. Black pepper contains small amounts of safrole and large amounts of a closely related compound piperine (Concon et al., 1979). Extracts of black pepper caused tumours in mice at a number of sites at a dose equivalent to 4 mg of dried pepper per day given for 3 months.

Ivie et al. (1981) have reported that linear furocoumarins (psoralens), which are widespread in plants of the Umbelliferae family, are potent light activated carcinogens and mutagens. Three of the most common furocoumarins are psoralen, xanthotoxin and bergapten. In addition to Umbelliferae, psoralen also occurs in plants from several other families (Ivie, 1978). Psoralens are potent photosensitizers and highly mutagenic in the presence of activating long wavelength UV light. They readily intercalate into duplex DNA where they form
light induced mono- or di- adducts with pyrimidine bases. Psoralen, in the presence of light, is also effective in producing oxygen radicals (Ya et al., 1982).

Pyrrolizidine alkaloids are naturally occurring carcinogens and have been found in some 50 species of the families Compositae, Boraginaceae and Leguminosae (Schoental, 1982), which are used as foods or herbal remedies. Several of these alkaloids are hepatotoxic and certain hepatotoxic pyrrolizidine alkaloids are also carcinogenic (Hirono et al., 1977; Schoental, 1976). However, a number of these alkaloids have been reported to be mutagenic (Clark, 1960) in Drosophila and Aspergillus system (Alderson and Clark, 1966). Mori et al. (1985) have used a hepatocyte primary culture DNA repair test to screen seventeen pyrrolizidine alkaloids for their DNA damaging property. This test is highly responsive to pyrrolizidine alkaloids (Williams et al., 1980). Among the results obtained by these authors is the indication of a species difference in liver bioactivation of these alkaloids. This implies that there may be species difference in the carcinogenic potential of pyrrolizidine alkaloids.

Edible mushrooms contain various hydrazine derivatives in relatively large amounts. Most hydrazines that have been tested have been found to be carcinogenic and mutagenic. The most common commercial mushroom Agaricus bisporus contains about 300 mg of agaritine, the glutamyl derivative of the mutagen 4-hydroxymethyl-phenylhydrazine, per 100 gm of mushrooms as well as smaller amount of the closely related carcinogen N-acetyl-4-
hydroxymethylphenylhydrazine (Toth et al., 1982). Some agaritine is metabolized by the mushroom to a diazonium derivative, which is a potent carcinogen and is also present in the mushroom in smaller amounts. Many hydrazine carcinogens may act by producing oxygen radicals (Hochstein and Jane, 1981).

A number of 1,2-dicarbonyl compounds, e.g. maltole, kojic acid, ethyl maltole, diacetyl and glyoxyl have been found to be mutagenic in the Salmonella/microsome assay. Several compounds in this class are of toxicological interest because they occur in various foods. For example, maltole is a product of carbohydrate dehydration and is present in soyabean, coffee and baked cereals such as bread. Kojic acid is a metabolite of many micro-organisms including several fungi used in food production, while diacetyl is an aromatic component of butter, beer, coffee, etc. (Fishbein, 1983).

A number of furans, such as 2-methylfuran, 2,5-dimethylfuran, furfural, 5-methylfurfural and 2-furylmethylketone are found in numerous food products including meat, milk products, various nuts, tea and coffee (Maga, 1979). Stich et al. (1981b) have reported that these furans induced relatively high frequencies of chromatid breaks and chromatid exchanges when they were exposed to cultured Chinese hamster ovary (CHO) cells in the absence of a liver microsomal preparation. The clastogenic doses of many of the furans were relatively high (100-3900 ppm), whereas the concentration in food products was relatively low. However, Stich et al. (1981b) cautioned that the furans are not the only genotoxic
chemicals in the complex mixture of heated, roasted or boiled food products. Even if the furans do not pose a serious health hazard by themselves due to their small amounts in most food items, they do contribute significantly to the total genotoxicity of many consumable foods and beverages.

In addition to pyrrolizidine alkaloids, certain glycoalkaloids found in potato such as solanine and chaconine have been reported to be highly toxic as they are strong inhibitors of choline esterase (Jadhav et al., 1981). Pyrrolizidine alkaloids and other glycoalkaloids can reach levels that can be lethal to humans in potatoes that are diseased or exposed to light (Katsui et al., 1982).

Cyclopropenoid fatty acids, present in cotton seed and other oils have been reported to be carcinogenic and mitogenic having various toxic effects in farm animals. Among these, sterculic acid and malvalic acid are widespread in the human diet. They are also potentiators of carcinogenicity of aflatoxins (Hendricks et al., 1980). Human exposure to these fatty acids results from the consumption of products of animals fed on cotton seed. Another major toxin in cotton seed is gossypol which accounts for about 1% of its dry weight. Gossypol causes male sterility through formation of abnormal sperm and is carcinogenic as well (Xue, 1980). It is a potent initiator and also promoter of carcinogenesis in mouse skin (Haroz and Thomassan, 1980). Gossypol has been tested in China as a possible male contraceptive as it is inexpensive and causes sterility during use. Its mode of action as a spermicide is presumably through the production of oxygen radicals.
A number of quinones and their phenolic precursors are found in the human diet and have been shown to be mutagens (Stich et al., 1981b; Brown, 1980; Levin et al., 1982). Quinones are quite toxic as they can act as electrophiles or accept a single electron to yield the semi-quinone radicals which can react directly with DNA or generate superoxide radicals (Morimoto et al., 1983; Kappus and Sies, 1981). Many dietary phenols can autoxidise to quinones generating hydrogen peroxide at the same time. The amounts of these phenols in human diet are appreciable. Catechol, which is mainly derived from metabolism of plant substances, is a potent promoter of carcinogenesis and an inducer of DNA damage (Carmella et al., 1982).

In addition, there are many other dietary compounds that have been shown to be mutagenic and carcinogenic in various test systems. Allylisothiocyanate, a major flavour ingredient of mustard oil, is one of the main toxins of mustard seeds and has been shown to be a carcinogen in rats (Dunnick et al., 1982). Phorbol esters, present in plants of Euphorbiaceae family, are potent promoters of carcinogenesis and cause nasopharyngeal and oesophageal cancers (Hecker, 1981). A variety of carcinogens and mutagens are present in mold contaminated food grains, nuts and fruits. Some of these, such as various aflatoxins, are amongst the most potent carcinogens and mutagens known (Hirono, 1981; Tazima, 1982). Nitrosoamines and other nitroso compounds formed from nitrate and nitrites in food have been directly related to the incidence of stomach and oesophageal cancer. Nitrates are present in large amounts in spinach, radish, lettuce and beans.
Although alcohol is not a constituent of a normal human diet, in view of its widespread use, it would be relevant to mention its toxic role. Alcohol has long been associated with the cancer of mouth, pharynx and liver (Tuyns et al., 1982). Alcohol metabolism generates acetaldehyde, which is a mutagen and possibly a carcinogen (Stich and Rosin, 1983; Campbell and Fantel, 1983). It also generates radicals that produce lipid hydroperoxides and other mutagens and carcinogens (Winston and Cederbaum, 1982; Videla et al., 1982).

**ADDITIVES IN FOOD AND COSMETICS**

Sodium nitrite is extensively used as a preservative in meat, fish and cheese. A possible formation of nitrosamines from amines, present in or derived from the diet, occurs by reaction with nitrous acid at acidic pH. In humans, gastric juice attains a pH of nearly 1.0. Such high concentration of hydrogen ions gives rise to the nitrosyl cation NO\(^+\), which is a highly reactive nitrosylating agent. Nitrous acid itself is a known mutagen for various bacterial and fungal cells. Its mutagenecity is presumably related to the deamination of adenine and cytosine (Fishbein et al., 1970). Sodium bisulphite is used as a bacteria inhibitor in a variety of beverages and as a preservative in canned fruits and vegetables. The bisulphite anion reacts, rather specifically, with uracil and cytosine, within single stranded regions of DNA and RNA. It is also mutagenic to bacteria and bacteriophages (Singer, 1983). EDTA and its alkali salts are widely used as sequestrants in various foods. They are useful as antioxidants due to their property
of forming poorly dissociable chelate complexes with trace quantity of metal ions such as copper and iron in fats and oils. EDTA has been shown to induce chromosome aberration and breakage in various plant species.

Sodium chloride in the form of common salt is one of the commonest food additives around the world. However, research has shown that areas where people ingest more sodium chloride show an elevated incidence of gastric cancer (Hirayama, 1968). Such salt intake causes mucous membrane damage to stomach as evidenced by the existence of malondialdehyde in extracts of gastric mucous membranes after sodium chloride administration (Takahashi et al., 1991). It is, therefore, likely that sodium chloride – lipid peroxidation – malondialdehyde – mutation scenario plays a major role in carcinogenesis of the stomach (Sugimura et al., 1996).

Saccharin was synthesized in the last century and since then it has been widely used as an artificial sweetener. Reports on the mutagenecity and carcinogenecity of saccharin are conflicting and there is some suggestion that these activities are thought to be due to impurities present in saccharin preparations (Kramers, 1975). The possibility of an in vivo conversion of saccharin into a mutagenic metabolite has also been suggested (Batzinger et al., 1977). Another artificial sweetener, which was widely used but is now banned in USA and many other countries, is cyclamate. Cyclamate induces chromosome breakage in cells of several plants and animal species. It is converted in vivo into
cyclohexylamine, which is also an inducer of chromosome breaks (Fishbein et al., 1970).

Under normal atmospheric conditions fats and oils slowly become oxidized. Antioxidants such as octyl gallate, prevent this oxidation process. They are also added to other non-fat foods such as cut foods to prevent discouloration (Vander Heijden et al., 1986). Typical products containing octyl gallate include the following: fats, oils and margarine, dried milk, peanut butter, snack foods, chewing gum and soap bases.

Octyl gallate in peanut butter has reportedly caused contact dermatitis in a man whose occupation involved mixing peanut butter with octyl gallate (Van Ketel, 1978). Allergic reactions to other gallic acid esters have also been reported. These include allergic contact dermatitis due to propyl gallate in lip balm (Wilson et al., 1989), lip stick (Cronin, 1980) and other topically applied cosmetics (Heine, 1988). There is a reported case of allergy to octyl gallate causing stomatitis (Pemberton et al., 1993). The eight gallates investigated up to now (methyl, ethyl, propyl gallate etc.) by experimental sensitization are moderate to strong contact sensitizers. Also an increase of side chain length is closely correlated with an increase in sensitizing potential. If the gallates come to be used to a greater extent in topical products because they are more effective than other antioxidants and possibly due to the fact that propyl gallate has excellent light protective properties,
we will certainly encounter more cases of gallate hypersensitivity in the future (Hausen and Beyer, 1992).

OXYGEN FREE RADICALS AND HUMAN DISEASES

Oxygen is an essential element for aerobes as it is the terminal acceptor of electrons during respiration, which is the main source of energy in these organisms. The generation of highly reactive oxygen metabolites is an integral part of the normal cellular metabolism that includes mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation and fertilization (Roth, 1997). It has been proposed that known harmful effects of oxygen were due to the formation of free radicals derived from it (Gilbert, 1981). The presence of free radicals can be advantageous for cells. In fact, they are being continuously produced in organisms and many of them are necessary to carry out certain biological reactions. For example, recent research has indicated that reactive oxygen species (ROS) can directly affect the cellular signalling apparatus and thereby control of gene expression. It further illustrates the role of ROS as potential inter and intracellular signalling molecules (Palmer and Paulson, 1997). In addition, hydroxyl radicals are thought to act as site specific oxidants in plants, targeted to play a role in loosening the cell wall during cell expansion, fruit ripening and organ abscission (Fry, 1998). However, when there is a free radical over-production or antioxidant defense systems are weakened for any reason,
cellular damage can appear (Valenzeula and Videla, 1989; Halliwell et al., 1992). Cellular DNA may be subjected to oxidative damage resulting from attack by free radicals of exogenous or endogenous origin (Collins et al., 1997). Ionizing radiations, tobacco smoke, pesticides, pollutants or some medications are exogenous sources of free radicals. Intracellular systems also produce oxygen free radicals. The autooxidation of small soluble molecules such as catecholamines, flavins, tetrahydopterins, quinones and thiols in the cellular cytoplasm may produce oxygen free radicals by concomitant O₂ reduction (Fridovich, 1983; Proctor and Reynolds, 1984). Reduced flavins and ascorbic acid upon autooxidation produce superoxide anion. This radical further accepts an electron from a reducing agent, such as thiols to yield peroxide (H₂O₂). There is in vitro evidence that H₂O₂ may then react with certain chelates of copper and iron to yield the highly reactive hydroxyl free radical (OH·) (Wolff et al., 1986). That the superoxide anion actually appears in metabolism is confirmed by the ubiquitous occurrence of superoxide dismutase. Several cytoplasmic enzymes, for example, xanthine oxidase and aldehyde dehydrogenase generate oxygen free radicals as products of their catalytic cycles. Reactions catalyzed by lipoxygenase and cyclooxygenase in the synthesis pathway of leukotrienes, thromboxanes and prostaglandins involve oxygen free radical production. Cyclooxygenase is also able to metabolize certain xenobiotics to more toxic species which may react with oxygen and yield very reactive oxygen species (Yamammoto, 1991; Riendeau et al., 1989). A main source of superoxide anion (O₂⁻) is the respiratory burst of phagocytic cells when they are activated. Compounds that stimulate the
biosynthesis of peroxisomes induce over-production of hydrogen peroxide. Peroxisomes have a great capacity to form hydrogen peroxide because they contain a high concentration of oxidases (Brunk and Cadenas, 1988; Del Rio et al., 1992). When the respiratory chain is highly reduced and its activity is dependent on ADP availability, radicals may be formed at sites different from cytochrome oxidase. Data show that autooxidation of ubiquinone and NADH dehydrogenase produces superoxide radicals (Beyer, 1990; Freeman and Grapo, 1982). Cytochromes P<sub>450</sub> and B<sub>5</sub> of the microsomic electron transport systems generate superoxide radicals during their catalytic cycle. Cytochrome reductases involved in redox reactions of cytochromes P<sub>450</sub> and B<sub>5</sub> can also produce superoxide radicals and hydrogen peroxide when they undergo autooxidation (Sevanian et al., 1990). The high reactivity of free radicals results in their having a short half-life, as well as a short radius for action. These free radicals react in a way that long chains of propagation are established causing biological effects far from the system which produce the first radical. All the cellular components, lipids, proteins, nucleic acids and carbohydrates may be damaged by reactions with oxygen free radicals, giving rise to metabolic and cellular disturbances.

Oxygen free radicals have been shown to oxidize lipids. Hydroxyl and hydroperoxyl radicals, but neither superoxide nor hydrogen peroxide, are able to attack unsaturated fatty acids of phospholipids and other membrane lipid compounds initiating, in this way, lipid peroxidation. Lipid peroxidation causes severe damage to the membrane structure and, consequently, alters its fluidity and ability to function correctly (Gutteridge and Halliwell, 1990; Niki et al., 1991;
Alcohols, aldehydes, volatile hydrocarbons and hydroperoxides, the final products of peroxidation, inhibit protein synthesis and are also able to alter vascular permeability, inflammatory response and chemotactic activity (Southorn and Powis, 1988; Blake et al., 1987; Del Maestro et al., 1981). In addition, malondialdehyde, a by-product resulting from peroxidation of fatty acids with three or more double bonds and a main indicator of lipid peroxidation has also been found to cause cross-linking and polymerization of membrane components as well as to react with DNA nitrogenated bases (Nielson, 1981; Valenzuela, 1991).

Oxidized proteins increase their hydrophobicity and sensitivity to proteolysis. Free radicals may react with amino acids containing unsaturated or sulfur groups. These reactions give rise to structural disturbances in proteins as well as cross-linking and aggregation phenomena, which are favoured by inter and intra molecular disulfide bond formation (Gebicki and Gebicki, 1993; Stadtman, 1992). Proteins are fragmented by free radicals involving peptide bond hydrolysis following oxidation of proline residues by hydroxyl radical and superoxide anion (Wolff and Dean, 1986). Oxidative radical damage occurs to a large extent in nucleic acids through alterations in both their bases and their deoxyribose sugars. The components of DNA most susceptible to free radical action are the thymine and cytosine bases followed by adenine and guanine and finally the deoxyribose sugar. However, for double stranded DNA, the deoxyribose moiety is modified more frequently than the bases, due to its external location (Davies et al., 1990; Dample, 1990). Carbohydrates are also targets of oxygen free radicals.
Consequently glycosylated proteins are more sensitive to oxygen damage (Freeman and Grapo, 1982; Sies, 1985).

There are many diseases that involve radical reactions in mammalian systems. According to Foga et al. (1997), ROS may play an important role in HIV-1 pathogenesis and HIV-1 gp120 induced neurotoxicity. Free radicals also play a major part in inflammation (Randerrath et al., 1992; Kunz et al., 1991), the process, which is the response of the host organism to injury. It involves enhanced vascular permeability with edema formation and leukocyte infiltration into the damaged area. Inflammatory response is advantageous for organisms. However, abnormal overactivation of phagocytes with consequent exacerbation of reactive oxygen metabolite production may damage surrounding tissues and change the viscosity of the extracellular fluid. This is the case in gout and autoimmune diseases such as myasthenia gravis, systemic lupus erythematosus, dermatomyositis etc. (Southorn and Powis, 1988; Halliwell and Gutteridge, 1989). Patients with rheumatoid arthritis suffer from neutrophil accumulation in their joints (Greenwald and Moy, 1980). These neutrophils overproduce the ROS responsible for the depolymerisation of hyaluronic acid, a glycosaminoglycan necessary for maintaining synovial fluid viscosity in joints.

A great number of ocular complaints are associated with oxidative damage. Amongst the components of the eye, the retina is the most sensitive to free radical oxidations. This is due to the fact that macular membranes in the retina have the highest concentrations of polyunsaturated fatty acids of any known
tissue. Furthermore, not only is oxygen turnover in retina very high but mitochondria are abundant in its cells. These factors render the macula highly susceptible to free radical damage, particularly to lipid peroxidation (Gerster, 1991). Retrolental fibroplasia (retinopathy of prematurity) is a complication derived from use of increased oxygen tensions in incubators for premature babies. Hyperoxia inhibits the growth of the retinal blood vessels. The lipid peroxidation and an increased production of thromboxanes \( A_2 \) induced by free radicals may be responsible for this condition.

The lung is an organ greatly effected by free radical production. Long periods of exposure to high oxygen pressures damage the lungs of different animal species, causing many diseases (pulmonary emphysema, bronchopulmonary dysplasia, adult respiratory distress syndrome) and even death (Webster and Nunn, 1988; Jackson, 1985). Oxygen free radicals and other toxic products formed by the lung cells themselves and by activated neutrophils that accumulate in the lungs when pure oxygen is breathed, may possibly contribute to the hyperoxidant damage. Inflammation in lungs is characterised by an influx of polymorphonuclear leukocytes (PMN) that release a variety of reactive oxygen species. These ROS have been implicated in epithelial cell DNA damage (Knapen et al., 1999). Tobacco has been suggested as being a contributory factor in the appearance of lung pathologies. Smoking impairs the ability of antiprotease to protect lung elastin from neutrophil proteases because free radicals contained in cigarette smoke inactivate this protein. The final result is the destruction of lung connective tissue elastin (Pryor and Dooley, 1985). Studies suggest that oxygen
free radicals might also be involved in the development of atherosclerotic plaques and ischemia-reperfusion injuries (Lehr et al., 1992; Gulati et al., 1992).

Number of theories have been proposed to explain the nature of aging and one such is the free radical theory (Sohal, 1993; Seppi et al., 1991; Ji et al., 1990). According to the free radical theory of aging, these very reactive species, produced continuously during normal metabolism, eventually accumulate, damaging DNA and other macromolecules. This is due to progressive defects in the defense systems against reactions that generate free radicals. The result is the appearance of degenerative lesions and cellular death. Then the organism ages and finally dies.

It has been suggested that certain promoters of carcinogenesis act by generation of oxygen radicals, this being a common property of these substances. It is also been suggested that oxidative macromolecular damage may play a role in the teratologic mechanism of xenobiotics that are bioactivated to a reactive intermediate (Wells et al., 1997). Fat and hydrogen peroxide are among the most potent promoters of carcinogenesis (Welsch and Ayslworth, 1983). Other well-known cancer promoters are lead, calcium, phorbol esters, asbestos and various quinones. Many carcinogens that do not require the action of promoters and are by themselves able to induce carcinogenesis (complete carcinogens), also produce oxygen radicals (Demopoulos et al., 1980). These include nitroso compounds, hydrazines, quinones and polycyclic hydrocarbons. Much of the toxic effect of ionizing radiation damage to DNA is also due to the formation of oxygen radicals.
(Totter, 1980). The mechanism of action of promoters involves the expression of recessive genes and an increase in gene copy number through chromosome breaks and creation of hemizygosity (Kinsella, 1982; Varshavsky, 1981). Promoters also cause modification of prostaglandins which are intimately involved in cell division, differentiation and tumour growth (Fischer et al., 1982). Most data on radical damage to biological macromolecules concern with the effects of radiation on nucleic acids because of the possible genetic effects.

**ANTICARCINOGENS**

One of the theories of etiology of cancer holds that the major cause is due to damage to DNA by oxygen radicals and lipid peroxidation (Ames, 1983; Totter, 1980). The protective defense mechanisms against mutagens and carcinogens include the shedding of surface layer of the skin, cornea and the alimentary canal. The major sources of endogenous oxygen radicals are hydrogen peroxide and superoxide that are generated as side products of metabolism (Pryor, 1976-1982). In addition, oxygen radicals also arise from phagocytosis after viral and bacterial infection or in inflammatory reactions (Tauber, 1982). The exogenous oxygen radicals are contributed by a variety of environmental agents (Pryor, 1976-1982). The enzymes that protect cells from oxidative damage are superoxide dismutase (SOD), catalase, D.T.diaphorase (Lind et al., 1982), glutathione peroxidase and glutathione transferases (Warholm et al., 1981).
A substantial body of data from many epidemiological and laboratory studies support the idea that dietary factors have a profound impact on the prevention of many cancers in humans (Block et al., 1992; Stavric, 1994). Diet can modify the pathophysiological processes of various metabolic disorders and can be an effective preventive strategy for various disease processes most of which have known to involve oxidative damage. Both the nutrient and non-nutrient components of the diet have been recognized for their antioxidant and chemopreventive benefits. The possible chemopreventive mechanisms include carcinogen blocking activities, antioxidant/anti-inflammatory activities and antiproliferative/antiprogressive activities. Carcinogenesis blocking activities encompass inhibition of carcinogen uptake, inhibition of carcinogen formation or activation, deactivation or detoxification of carcinogens, prevention of carcinogen binding to DNA and enhancement of the level of fidelity of DNA repair. Antioxidant/anti-inflammatory activity includes scavenging of reactive electrophiles and oxygen radicals and inhibition of arachidonic acid metabolism. Antiproliferative/antiprogressive activities comprise modulation of signal transduction, modulation of hormonal and growth factor activity, inhibition of polyamine metabolism, induction of terminal differentiation, restoration of immune responses, enhancement of inter cellular communication, restoration of tumour suppressor function, induction of apoptosis, telomerase inhibition, correction of DNA methylation imbalances, inhibition of angiogenesis, inhibition of basement membrane and activation of antimetastasis genes (Krishnaswamy and
Raghuramulu, 1998; Kelloff et al., 1996). Some small molecules in the human diet act as antioxidative agents and presumably have an anticarcinogenic effect. Some of these compounds are discussed below.

*Resveratrol*, a phytoalexin found in grapes and other food products has been shown to possess chemopreventive activity. Recent studies have shown that resveratrol acts as antioxidant and antimutagen and mediates anti-inflammatory effects besides inhibiting cyclo-oxygenases and hydroperoxide functions. In addition, it inhibits the development of preneoplastic lesions in carcinogenic treated cells in various cultures (Jang et al., 1997). *Tocopherol* (vitamin E) is an important trap of oxygen radicals in membranes (Pryor, 1976-1982) and has been shown to decrease the carcinogenic effect of quinones, adriamycin and daunomycin which are toxic because of free radical generation (Ames, 1983). Protective effect of tocopherols against radiation induced DNA damage and dimethylhydrazine induced carcinogenesis have also been observed (Beckman et al., 1982). *β-Carotene* is a potent antioxidant present in the diet and is important in protecting lipid membranes against oxidation. Singlet oxygen is a highly reactive form of oxygen which is mutagenic and is mainly generated by pigment mediated transfer of energy of light to oxygen. Carotenoids are free radical traps and are remarkably efficient as quenchers of singlet oxygen (Packer et al., 1981). *β-Carotene* and similar polyenes are also the main defense in plants against singlet oxygen generated as a by-product of the interaction of light and chlorophyll (Krinsky and Deneke, 1982). Carotenoids have been shown to be
anticarcinogens in rats and mice and may also have a similar effect in humans (Mathews-Roth, 1982, Peto and Schneiderman, 1981). Glutathione is present in food and is one of the major antioxidants and is antimutagenic in cells. Dietary glutathione is an effective anticarcinogen against aflatoxins (Novi, 1981). The cellular concentration of glutathione is influenced by dietary sulfur amino acids (Tateishi et al., 1981). Selenium, which is present in the active site of glutathione peroxidase, is another important dietary anticarcinogen. Glutathione peroxidase is essential for destroying lipid hydroperoxides and endogenous hydrogen peroxide and therefore helps to prevent oxygen radical induced lipid peroxidation (Flohe, 1982). Several heavy metal toxins such Cd$^{2+}$ (a known carcinogen) and Hg$^{2+}$ decrease glutathione peroxidase activity by interaction with selenium (Flohe, 1982). Other antioxidants include uric acid and ascorbic acid. The latter has been shown to be anticarcinogenic in rodents treated with UV light and benzo(α)pyrene (Hartman, 1982). Uric acid is present in high concentrations in the blood of humans and is a strong antioxidant (Ames et al., 1981). A low uric acid level has been considered a risk factor in cigarette caused lung cancer, however, too high levels may cause gout.

Flavonoids are widely distributed plant secondary metabolites found in the edible portions of a majority of food plants and beverages. These polyphenolic, low molecular weight compounds possess a wide range of physiological and pharmacological properties which have the potential of being exploited for the therapeutic purpose (Asad et al., 1998). Flavonoids have been reported to possess
The polyphenol Tannic acid is present in green tea and several plants, including Embellica phyllanthus. The latter has been credited with several therapeutic properties in anaemia, jaundice, uremia, cholesterolemia, etc. (Tripathi et al., 1988; Thakur, 1985), some of which may be due to antioxidative properties of tannic acid against DNA damage (Devasagayam et al., 1995) and lipid
peroxidation (Ramnathan and Das, 1992). Flavonoids as well as the related polyphenol tannic acid significantly inhibit single strand breaks in plasmid pBR322 DNA induced by singlet molecular oxygen \(({^1}O_2)\). The protective ability of these compounds were both time and concentration dependent (Devasagayam et al., 1995). Hamamelitannin which contains two galloyl groups and hamamelose has a strong scavenging activity against superoxide anion radicals (Masaki et al., 1993). Studies showed that several flavonoids, tannic acid, gallic acid and fat soluble vitamins inhibited Hela and Raji lymphoma cell growth and are known to possess anticarcinogenic properties (Mukhtar et al., 1988). All tannic acid extracts tested so far are antitumour promoters but their efficacy may vary considerably depending on their origin and length of their polygalloyl chain (Gali et al., 1993).

_Capsaicin_ (trans-8-methyl-N-vanillyl-6-noneamide) is the major pungent principal of hot peppers of the genus _Capsicum_ (Park et al., 1998). Apart from its use as a food additive in various spicy cuisines, capsaicin is also utilized for therapeutic purposes to treat various peripheral painful conditions such as rheumatoid arthritis and diabetic neuropathy (Surh and Lee, 1996). Recent studies have revealed various antigenotoxic and anticarcinogenic effects of capsaicin suggesting this compound as another important dietary phytochemical with a potential chemopreventive activity (Surh et al., 1998). Joe and Lokesh (1994) have demonstrated that capsaicin lowers the generation of ROS by activated macrophages. Other workers have demonstrated that capsaicin inhibits cyclophosphamide (CP) induced chromosomal aberrations and DNA strand
breaks. This protective action of capsaicin against CP-induced toxicity may possibly be linked with its already reported desensitization effect against chemical irritant induced damages (De et al., 1995). The antimutagenic and anticarcinogenic properties of capsaicin are believed to be due to its inhibition of xenobiotic metabolizing enzymes (Miller et al., 1993). In several instances it has been reported that capsaicin modulates microsomal cytochrome P450 dependent monooxygenase activities, thereby affecting metabolism of carcinogens and other xenobiotics (Miller et al., 1993; Surh et al., 1995; Modly et al., 1986; Teel, 1991; Miller et al., 1993; Zhang et al., 1993). Capsaicin was found to interact with rat hepatic mixed function oxidases as demonstrated by inhibition of ethylmorphine demethylase activity (Miler et al., 1993). The compound also suppresses the activity of rat epidermal aryl hydrocarbon hydroxylase that is responsible for the metabolism of benzo(α)pyrene and other polyaromatic hydrocarbons. Metabolism and subsequent covalent DNA binding of benzo(α)pyrene in human and murine keratinocytes were attenuated by capsaicin. In addition, capsaicin has been shown to display inhibitory effects on metabolism, mutagenecity and/or covalent DNA binding of aflatoxin B1 and the tobacco specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridal)-1-butanone (NNK) (Zhang et al., 1993; Jang et al., 1989; Teel, 1993). Extracts of hot red peppers also displayed productive effects against aflatoxin B1 induced bacterial mutagenesis (Kim et al., 1991). Capsaicin, along with its saturated analogue dihydrocapsaicin (DHC) has been shown to inhibit cytochrome P4502E1, an isoform that catalyzes metabolic activation as well as detoxification of many low molecular weight carcinogens.
(Guengerich et al., 1991). In agreement with these findings, both compounds inhibited the mutagenecity or tumourigenecity of vinyl carbamate or dimethyl nitrosoamine which are preferentially activated by cytochrome P4502E1. Capsaicin also ameliorated the peroxidative changes in rat hepatic and pulmonary tissues induced by chloroform CCl4 or dichloromethane (Day and Ghosh, 1992). Capsaicin pretreatment also protects against the free radical induced pulmonary damage in rats exposed to such gaseous chemical irritants as sulfur dioxide and nitrogen dioxide (Day and Ghosh, 1989). Recent studies have revealed that capsaicin induces apoptotic cell death- the most potent natural defense against cancer- in human gastric cancer cells (SNU-1) (Kim et al., 1997).

POSSIBLE ROLE OF ENDOGENOUS SUBSTRATES IN OXIDATIVE DNA DAMAGE

It is well established that aerobic organisms produce oxygen species during the course of normal metabolism. These include superoxide anion, hydrogen peroxide and hydroxyl radical (Fridovich, 1989; Sies, 1991; Weiss, 1989). Oxidative DNA damage by these species has been implicated in a number of human diseases, including cancer. The other diseases where such damage may play an important role are rheumatoid arthritis (Gridley et al., 1993), Alzheimer’s disease, Parkinson’s disease and amyotrophic lateral sclerosis (ALS) (Sanchez-Ramos and Ames, 1994; Mecocci et al., 1994; Robberecht et al., 1994). In
addition, oxygen radical toxicity is considered one of the causes of human male infertility (Aitken and Fischer, 1994).

Recent studies have shown that considerable DNA damage may be caused by endogenous metabolites generated during the body's normal metabolic processes (Burcham and Marnett, 1994). For example, it has been shown that malondialdehyde which is the ubiquitous product of lipid peroxidation and eicosonoid metabolism reacts with cellular DNA to form a propanodeoxyguanosine adduct (Chaudhury et al., 1996). Evidence indicates that the adduct exists at significant levels in the hepatic DNA of rats and humans and is an effective premutagenic lesion in E. coli (Burcham and Marnett, 1994; Chaudhaury et al., 1994).

In this laboratory studies have been carried out on the mechanism of interaction with DNA of several endogenous metabolites and antioxidants of plant and animal origin. These include flavonoids (Rahman et al., 1989 & 1990, Fazal et al., 1990; Said Ahmad et al., 1992 & 1994), tannic acid (Bhat and Hadi, 1992, 1994a & 1994b; Khan and Hadi, 1998), uric acid (Shamsi and Hadi, 1995), bilirubin (Asad et al., 1999) and L-DOPA (Husain and Hadi, 1995). Of these, uric acid and bilirubin are present in the human extracellular fluid and are considered to have an antioxidant function. However, Stowe and Prutz (1987) have suggested that many biological antioxidants are themselves capable of causing oxidative DNA damage.
L-DOPA (L-3,4-dihydroxyphenylalanine) is an important metabolite in various metabolic reactions. It is formed by the decarboxylation of tyrosine and in turn undergoes decarboxylation to form the neurotransmitter dopamine. Dopamine, which accounts for 90% of the total catecholamines serves as the precursor of hormones adrenalin and noradrenalin. The neurological disorder, Parkinson's disease is associated with an under production of dopamine in the human brain (Lehninger et al., 1993). It is for this reason that L-DOPA is an effective drug in the treatment of Parkinson's disease. Studies have shown, however, that dopamine can condense with acetaldehyde, a product of ethanol metabolism, to generate 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroxyquinoline (Salsolinol) (Dostert et al., 1988). Salsolinol is considered to be involved in the etiology of Parkinson's and Huntington's diseases and has been detected in the cerebrospinal fluid of Parkinsonian patients (Moser and Kompf, 1992). Thus, dopamine may be considered a precursor of an endogenous neurotoxin. Other workers have shown that L-DOPA, dopamine and 3-O-methyl-DOPA caused extensive base modification in DNA in the presence of H$_2$O$_2$ and the traces of copper ions (Spencer et al., 1994). These authors have proposed that copper ion release in the presence of L-DOPA and its metabolites may be an important mechanism of toxicity in ALS and Parkinson's disease. Studies in this laboratory has established that L-DOPA in the presence of Cu(II) alone is capable of causing strand scission in DNA in vitro and that this breakage results from the generation of reactive oxygen species. That this reaction is biologically active was
demonstrated by the inactivation of Lambda bacteriophage by L-DOPA-Cu(II) system (Husain and Hadi, 1995).

L-DOPA also serves as precursor of melanin, the UV blocking agent in skin. Melanin is synthesized from DOPA by a spontaneous polymerization of DOPA chrome, an oxidised product of DOPA (Nicolaus, 1986). Melanin has been shown to absorb free radicals and active oxygen species (Sarna et al., 1984; Korytowski et al., 1987) but when it does so, free radicals and active oxygen species are also produced. Melanins are unique amongst the biological molecules in that they continuously emit a free radical signal (Sealy, 1984). The free radicals associated with this signal are called "melanin free signals". When melanins are irradiated with UV or when they absorb superoxide anion radical the melanin free signal is enhanced and depending on the conditions, superoxide anion radical, hydroxyl radical and hydrogen peroxide are generated (Korytowski et al., 1987). Melanin mediated radical production is potentially lethal to cells (Menon et al., 1985).

Uric acid is present in human plasma at a relatively high concentration (upto 0.6 mM) and is capable of scavenging hydroxyl radicals, lipid hydroperoxides, singlet oxygen and oxo-heme reductants. In fact, it has been hypothesized that uric acid may be partially responsible for the relatively long life span of humans (Ames et al., 1981). Studies in our laboratory have shown that uric acid in the presence of Cu (II) and molecular oxygen caused DNA breakage
and that this reaction involve active oxygen species such as the hydroxyl radical. Further, uric acid-Cu (II) system was able to inactivate bacteriophage Lambda thereby showing that it is biologically active (Shamsi et al., 1995).

Bilirubin is the end product of heme catabolism. In its bound form with albumin, bilirubin is considered one of the naturally occurring antioxidants of human extracellular fluids (Halliwell and Gutteridge, 1990). It has been the subject of interest because of its toxicity under the conditions of hyperbilirubinemia. The physiological concentration of bilirubin is 5-17 μM (Bloomer et al., 1971). Under certain diseased conditions such as kernicterus and jaundice, the plasma bilirubin levels may reach considerably higher values. Concentrations greater than 300 μM are associated with the risk of development of neurological disorders due to deposition of bilirubin in brain and its enhanced toxic effects on cellular functioning in this tissue (Meuwissen and Heirwagh, 1982; Schenker et al., 1986). Very recent studies in this laboratory have shown that bilirubin forms a complex with Cu(II) and that this complex generates reactive oxygen species due to the redox cycling of the metal ions. The ROS generated, particularly hydroxyl radicals, cause DNA strand breaks in vitro (Asad et al., 1999).