Review Article

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Bioactive phytochemicals with emphasis on dietary practices

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Diet can modify the pathophysiological processes of various metabolic disorders and can be an effective preventive strategy for various disease processes most of which are known to involve oxidative damage. Both nutrient and non-nutrient components of the diet have been recognized for their anti-oxidant and other potential benefits. Plant foods contain phytochemicals such as flavonoids, phenolic acids, *etc*., which show biological activity. Some common foods used in Indian culinary practices were assessed for their anti-oxidant, anti-mutagenic and anti-carcinogenic effects and vitamin D activity and evaluated for their plausible biological effects. Green leafy vegetables had the highest anti-oxidant activity followed by wheat and rice. Cooking decreased this activity. Eugenol, the active principle of clove, was shown to offer protection against CCl_4 induced hepatotoxicity in rats. It also showed anti-peroxidative activity in addition to decrease in O_2 formation. Studies on the anti-carcinogenic effect of turmeric/curcumin revealed that both are potent anti-mutagens *in vivo* and reduce the adducted DNA levels in liver of rats challenged with B(a)P. In another study, Syrian hamsters receiving turmeric/curcumin through diet or local paint on cheek pouch had lower tumour burden as well as adducted DNA level against 7-12-DMBA challenge. Turmeric/curcumin were found to be better anti-tumour agents when given in the post initiation phase of carcinogenesis. The beneficial effect of turmeric was found to be due to its anti-oxidant potential. Studies on humans at risk of palatal cancer due to reverse smoking showed that turmeric (1 g/day) for 9 months had a significant impact on the regression of precancerous lesions. Onion and garlic also possess anti-mutagenic principle. Further studies on the bioactive phytochemicals in plants showed that certain plants belonging to Solanaceae (*Cestrum diurnum, Lycopersicon esculentum* and *Solanum melongena*) have calcinogenic potential and vitamin D like activity. In view of the vast data on bioactive principles from plants, it is suggested that dietary prevention coupled with other life-style changes is perhaps the right answer for prevention of cancer and other chronic diseases in India.

Key words Allium vegetables - anti-oxidant - carcinogenesis - chemoprevention - curcumin - DNA adducts - mutagenesis - non-nutrient - phytochemicals - tumourigenesis - turmeric - xenobiotics

For the most part of this century, health concerns in the field of human nutrition have been centered around deficiency disorders of macro- and micro-nutrients with emphasis on the role of essential nutrients in health and disease. There is an increasing consensus among scientists that both nutrients and non-nutrient components of the diet may play a significant role in several chronic and metabolic disorders such as cardiovascular diseases, cancer, cataract, diabetes, aging, *etc*[1]. Many of these disorders have a long latency period between dietary exposures, inception and disease manifestations. Diet can modify the patho-physiological processes and hence primary prevention through dietary modifications has great promise for determining the health and nutrition trends and pattern of diseases.

India today faces diet related disorders at both ends of the spectrum[2]. While on the one hand, we have nutrient deficiency disorders which have their origin in utero, and the impact of which continues in later years in all age groups, on the other hand, chronic diseases in adults seem to be on the increase in India[3]. More recent research efforts elsewhere recognize the anti-oxidant, immune potentiating and hormone like activities of nutrients and non-nutrients besides their regulatory role in certain biological functions[4,5]. Thus we have come a long way from the empirical healing of diseases by foods to more sophisticated research on structure activity relationships, biochemical functions, molecular biology and biotechnology[6]. It is only from the last half of the century that foods are being scientifically investigated and evaluated for their disease prevention and therapeutic effects. Consequently, new terminologies such as functional foods, medicinal foods and neutraceuticals have been introduced in nutritional sciences. In fact, food is now viewed as a miracle medicine[7] and food based approaches *viz*., food and food technology including fortification, for both nutritional deficiencies and prevention of chronic diseases is the focus of attention[6,8].

Most dietary guidelines for chronic diseases stress on plant foods for better health[9]. Plant foods such as cereals, vegetables, fruits, pulses (legumes), nuts, spices and beverages such as tea and wine contain many biologically active micronutrients other than vitamins and minerals. These are known as phytochemicals or phytoprotectants.

As the incidences of chronic diseases are on the rise in India and since Indians are basically vegetarians and consume plant foods of diverse variety, it was considered essential to evaluate and assess the potential benefits of some common foods used in Indian culinary practices. The plant foods were assessed for their anti-oxidant, anti-mutagenic and anti-carcinogenic effects and vitamin D activity and evaluated for their plausible biological effects.

ANTIOXIDANTS IN HEALTH AND DISEASE

Pro-oxidants and anti-oxidants in recent years have been implicated in the pathogenesis of several diseases[10,11]. The reactive oxygen species (ROS) damage the biomolecules such as DNA, proteins, carbohydrates and lipids and affect the enzyme processes and genetic machinery. Almost every organ or system in the body is affected by oxidant stress (Table I). The functional degeneration of somatic cells during aging also contributes to the progress of diseases which in turn also affects the level of endogenous oxidants and other mutagens produced[12]. The oxidation products of the biomolecules accumulate with age. The various endogenous and exogenous sources of oxidative damage are indicated in Fig. 1. Oxidative stress is a disturbance in the pro-oxidant to anti-oxidant balance, in favour of the former. Therefore in order to survive, aerobic organisms have developed an anti-oxidant defense system. These include both nutrients and non-nutrient components as well as some enzyme systems (Fig. 2).

Several chronic diseases such as coronary heart disease, cancer, cataract, diabetes, *etc*., have been positively correlated with low vegetable and fruit consumption[13]. Although vitamins such as E and C, selenium (Se) and carotenoids have been extensively studied for their beneficial effects, it is only recently that the anti-oxidant defense offered by these nutrients has received recognition[14]. Further, the phytochemicals, which primarily serve in plant protection, are now considered as the vitamins of the 21st century[15]. There are more than a dozen classes of non-nutrient phytochemicals which show anti-cancer activity (Table II). Several mechanisms have been suggested for their impact on the process of carcinogenesis (Table III)[16].

Table I. Free radical mediated diseases

Aging	Genetic disorders
Amyotrophic lateral sclerosis	Inflammatory disorders
Arthritis	Muscular dystrophy
Asthma	Parkinson's dementia
Atherosclerosis	Pulmonary fibrosis
Autoimmune diseases	Radiation injury
Bronchopulmonary dysplasia	Skin disease (porphyria)
Cancer	Senile dementia
Cataract	Stroke
Drug induced GIT/liver disorders	

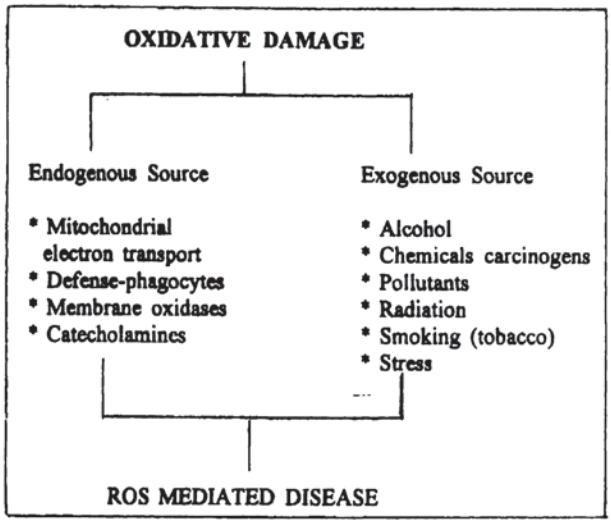

Fig. 1. Sources of oxidative damage.

Table II. Non-nutrient anti-oxidants

Simple phenols	-	Vanilin
Flavonoids	-	Catechin, Epicatechin gallate, Quercetin, Chrysin, Cyanidin
Phenolic acids	-	Ferulic acid, Caffeic acid, p-coumaric acid
Coumarins		
Lignans		
Essential oils		
Terpenoids	-	mono, di, tri terpinoids
Glycocides		
Peptides	-	Carnosine, Anserine
Protease inhibitors		
Inositol hexaphosphate		
Sterols		
Isothiocyanates, Thiocyanates, Conjugated linoleic acid, Phytoestrogens		

Among the most investigated non-nutritive chemopreventors are plant phenols, flavonoids, coumarins, benzyl-isothiocyanates, *etc*. Phenolic acids like caffeic acid, ferulic acid, gallic acid and ellagic acid have been investigated in great detail[17]. These compounds in general influence the quality, acceptability and stability of foods by acting as flavourants, colourants and anti-oxidants. Flavonoids are a group of polyphenolic compounds which have cancer blocking property. Flavonoids have a common skeleton of diphenyl pyrons, two benzene rings (A and B) linked through a heterocyclic pyran or pyrone ring (C). The basic ring structure allows a multitude of substitution patterns giving rise to flavonoids, flavones, catechins, anthocyanadines and isoflavonoids (Table II). They are low molecular weight compounds having three phenolic rings

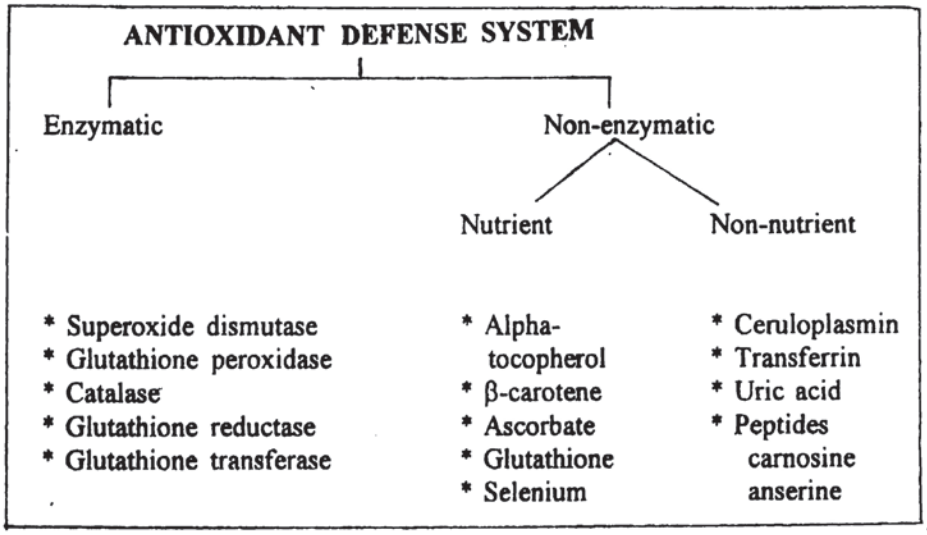

Fig. 2. Antioxidant defense system.

Table III. Carcinogenesis - action of preventive agents[16]

Inhibitors of carcinogen	:	Inhibit nitrosamine formation *e.g.* ascorbic acid, ferulic acid, caffeic acid
Blocking agents	:	Block the activities of enzymes which biotransform a procarcinogen to carcinogen (Cyt P_{450}) *e.g.* isothiocyanates, diallylsulphide, ellagic acid, ferulic acid
Inducing agents	:	↑ GST activity (detoxification) *e.g.* isothiocyanates, sulpharaphane, limonene, terpinoids, curcumin
Scavenging agents	:	Physical reaction with carcinogenic electrophiles (conjugation/trapping) *e.g.* vitamins C & E, flavonoids
Suppressing agents	:	↓ Tumour progression/promotion (↓ metabolic processes) *e.g.* isoflavones, phytoestrogens, epigallocatechin gallate (EGCG), selenium (Se)

Superscript refers to the reference no. in the list of References

(pyrone) based on flavon nucleus. The conjugated ring structure and the heterogenous group allow the phenols to actively scavenge and stabilize free radicals. The carboxylic group inhibits lipid oxidation by metal chelation[17].

Dietary sources and intake

Flavonoids are present in a wide variety of fruits, vegetables, nuts, whole seeds, spices, tea and wine. Quercetin is the major flavonoid in vegetables, fruits and wine. The daily intake of flavonoids in the West is estimated to be in the range of 0.5 to 1.0 g while in USA and Holland, it is calculated to be 24 mg/day[18]. In India, the plant foods can provide more phytochemicals provided the intake of vegetables and fruits satisfies the recommended daily intake.

Total anti-oxidant activity

As a first step towards generating data on total anti-oxidant activity, studies at the National Institute of Nutrition (NIN), Hyderabad, concentrated on cereal grains, pulses and vegetables. An *in vitro* technique of inhibition of iron induced peroxidation of linoleic acid was used (Annual Report, NIN, Hyderabad, 1997-98). The results expressed as alpha-tocopherol equivalents are indicated in Table IV. The green leafy vegetables had the highest anti-oxidant activity followed by that of wheat and rice. Cooking decreased the anti-oxidant activity considerably. Further work is in progress on the effect of food processing on the anti-oxidant activity. Attempts are being made to generate a database on the different classes of anti-oxidants present in food.

Table IV. Total anti-oxidant activity of cereals and green leafy vegetables

Food items	µg of alpha-tocopherol equiv/g fresh weight
Rice (*Oryza sativa*)	80-102
Whole wheat (*Triticum aestivum*)	220-500
Spinach (*Spinacia oleracea*)	750-890
Amaranth (*Amaranthus gangeticus*)	620-810
Coriander leaves (*Coriander sativum*)	610-750
Fenugreek leaves (*Trigonella sativum*)	520-690
Gogu (*Hibiscus cannabinus*)	700-810
Mint (*Mentha spicata*)	630-720
Ponnaganti (*Alternanthera sessilis*)	500-630
Ambati chukka (*Rumex vesicarius*)	530-610

Anti-oxidant activity of cloves

Spices find extensive use as fragrance and flavouring agents in Indian and oriental cuisines. Natural anti-oxidants are receiving greater attention in view of the reports about the toxic effects of synthetic ones[19-21]. Various spice principles are now being extensively studied.

Eugenol is the principal constituent (70-90%) of the essential oil of clove and is also present in many essential oils of plants especially basil, cinnamon and nutmeg. The antiseptic, analgesic and antibacterial properties of eugenol render it useful in dental and surgical pastes. It is also a component in many Ayurvedic preparations and has been used in the treatment of flatulent colic, chronic diarrhoea and other gastrointestinal disorders[22].

Eugenol was shown to offer protection against CCl_4-induced hepatotoxicity in rats[23]. Treatment with doses of 1 and 5 mg eugenol/kg b.wt offered significant protection against CCl_4 induced liver damage. This protection was evident only when eugenol was given along with or soon after CCl_4 injection; probably because of it fast elimination.

Eugenol was also shown to inhibit the lipoxygenase mediated lipid peroxidation in a liposomal membrane system as assessed by linoleic acid hydroperoxide and thiobarbituric acid reactive substances (TBARS) formation *in vitro*[24]. Eugenol also inhibited non-enzymatic lipid peroxidation induced by Fe^{2+}-ascorbate and Fe^{2+}-H_2O_2. The effect of eugenol was also studied on xanthine oxidase-xanthine — Fe^{3+} ADP mediated lipid peroxidation which represents enzyme induced peroxidation[25]. It was found that eugenol inhibited the lipid peroxidation in a dose dependent manner as assessed by formation of TBARS. It also inhibited xanthine oxidase (XO) activity. The antiperoxidative effect of eugenol was about 35 times more and inhibition of XO was about 5 times higher than allopurinol (known inhibitor). Hence it may be concluded that O_2 formation is also inhibited by eugenol.

Cancer process

Cancer is usually observed in a population of cells that have acquired the ability to multiply and spread without restraint. It occurs in three stages of initiation, promotion and progression[26]. During the initiation phase the DNA sequences are altered which finally lead to changes in gene expression (epigenetic changes). The immune surveillance system and DNA repair mechanisms can however correct the damage. The biotransformations can also conjugate the oxidative metabolites and prevent their binding to critical molecules. The conjugated products are inactive and tend to get excreted.

Anti-cancer activity

Studies on the promotion, progression and ultimate manifestations of cancer suggest that several cancers are due to environmental causes and are life-style related. The major impetus for cancer control through chemoprevention has originated from studies on diet and cancer. Several chemoprevention trials using nutrients and non-nutrients are in progress throughout the world[27].

Though the incidence rates of cancer in India are lower than that in Western countries, with the current population of over 960 million, it is estimated that the absolute number of cancer patients may be around 6-7 lakhs[28]. With the present growth rate, there will be at least a one million new cases added annually by the turn of this century. The diversity in religion, socio-economic status and food habits in India may all contribute to cancer rates as well as sites affected. Diet and cancer have been shown to be closely associated[13]. It was therefore considered necessary to study the diet and its anti-carcinogenic effects.

Turmeric as anti-cancer agent

Spices such as turmeric, ginger, garlic, pepper and cumin seeds are used individually and also as a spice mixture in several Indian food preparations. Turmeric, an Indian spice is used for its colour, flavour and digestive properties. Further, it has been documented to have medicinal properties. It is also used as cosmetic for its brilliant yellow colour and characteristic perfume. It belongs to genus *Curcuma* of Zingiberaceae family and consists of many species[29]. Its oleoresin is used in developing countries in the food industry. In this form, it is free from microbial contaminants and is therefore used in ready to eat and other convenient foods.

The chemical composition indicates that it has starch (40-50%), protein (6-10%), fat (5-8%), fibre (3-5%), fixed (7%) and volatile oils 4 per cent and ash 3-7 per cent. In addition, it has several micronutrients such as carotenes, thiamin, riboflavin, niacin, vitamin C, iron, zinc, calcium, magnesium and selenium in various concentrations[30].

The yellow colour of the turmeric is due to its functional compound curcumin, the yield of which varies from 2-5 per cent. Elucidation of the chemical structure of curcumin showed that it was a derivative of methane substituted by two ferulic acid residues[31]. Natural curcuminoids-diferuloyl methane (curcumin I), P-hydroxy cinnamoyl methane (curcumin II) and *bis* P-hydroxy cynnamoyl methane (curcumin III). have all been isolated from the water extract of *Curcuma longa*. In the curcumin mixture, curcumin I is present to a greater extent than curcumin II and III. The structure of curcumin has also been confirmed by synthesis and is predictive of the anti-oxidant activity of polyphenolic compounds.

With incomplete repair, specific alterations occur in the DNA during cell proliferation within the micro-environment which leads to formation of initiated cells. The initiated cells are morphologically unchanged and remain in a latent or dormant state for a considerable period and only in the presence of appropriate promoters, they tend to become neoplastic. Following DNA damage, oncogene activation or inhibition of suppressor genes may occur.

Prevention of carcinogenesis at this stage involves elimination of potential carcinogens and inhibition of metabolic activation of pro-carcinogens, inactivation of ultimate carcinogens, competent blocking of DNA repair and immune surveillance.

The promotion of the cancer process can last up to a decade in human beings. It is an extremely complex process with a variety of cellular changes which may involve gene expression, cell proliferation and transformation of initiated cells into a population of preneoplastic cells. Growth factors, hormones and nutrition are actively involved in this process. They are accompanied by structural changes and alterations in the organization of cells in the tissue with increased growth rate (mitosis). The process of promotion offers the greatest potential for intervention or prevention. Anti-oxidant mechanisms and those which maintain appropriate membrane stability are important at this stage.

The next stage of progression of premalignant to malignant lesions occurs due to additional chromosomal damage and dysregulation of controlling factors. These cells become aggressive and invade healthy tissues and also migrate into distant organs to form metastatic foci of cancerous cells. Actually the precancerous cells are a clonal expansion of the cells altered with the gene expression and the promoted cells progress to neoplasia and anaplasia which ultimately lead to metastasis[26].

Impact of turmeric on carcinogenesis

Turmeric has been investigated for its effects on initiation, promotion and progression of carcinogenesis. Our first studies on turmeric were directed towards establishing its effects as an antimutagen[32]. In these studies, it was considered necessary to align the animal and human response to diet. In order to study the effect of environmental factors which are highly suspected to be involved, *in vivo* studies on carcinogen exposure were conducted in experimental rats. The anti-mutagenic activity of turmeric was assessed by the classical Ames assay which quantitated the urinary mutagens. As polycyclic aromatic hydrocarbons have been widely studied and since these have a widespread occurrence and distribution in the environment as byproducts, the antimutagenic potential of turmeric was assessed against the ubiquitous pollutant, benzopyrene [B(a)P]. Animals were kept on turmeric based diet for 3 months during which time, they were exposed to 5 mg dose of B(a)P at the end of 1, 2 and 3 months. Urine samples were collected after the B(a)P injection for quantitating the mutagens. The TA98 and TA100 mutants were used. The assay was conducted both in the presence and absence of S9 fraction. These rats were also exposed to an inducer of microsomal mixed function oxidase namely 3-methyl-cholanthrene.

The frequency of revertants in the urine of animals fed turmeric at 1, 5 and 10 per cent in the diet for one month were significantly reduced when compared to controls and both with and without S9. Similar observations were made when turmeric was given for 2 and 3 months. There were no additional benefits of prolonged feeding and there were no differences between 1, 5 and 10 per cent of turmeric. Therefore, in order to study dose related anticarcinogenic effects, lower dose of turmeric at 0.1 and 0.5 per cent in the diet were fed to rats for one month before they were exposed to the carcinogen. There was a significant decrease in the mutagenic response to B(a)P in both tester strains with and without S9 and the extent of reduction was less when compared to 1 per cent turmeric treated groups. When a lower dose of B(a)P was used, the mutagen load was lower and turmeric could effectively counteract the effects of carcinogen Similar results were observed with methylcholanthrene. No histopathological changes were observed in any of the organs in animals except that some of the animals developed subcutaneous malignant fibrous histocytoma at the injection site after 3 months. None of the animals in the group receiving 10 per cent turmeric developed tumours. These results supported the antimutagenicity of turmeric observed by Nagabhushan and Bhide[33] and Nagabhushan *et al* [34]. Investigations with 0.01, 0.03

and 0.06 per cent of curcumin incorporated into the diet also clearly indicated a significant impact even at 0.01 per cent curcumin (Annual Report; NIN, Hyderabad, 1991-92).

The various studies conducted at the NIN on turmeric and its active principle curcumin suggest that it can impact on all the stages of carcinogenesis (Fig.3). It prevents activation of carcinogens and attack of electrophiles on DNA, acts as anti-oxidant and antipromoter, retards the conversion of preneoplasia in addition to repairing the damage to DNA.

The results of both human and animal experiments reinforce the plausibility of its use as a preventive strategy against polycyclic aromatic hydrocarbons

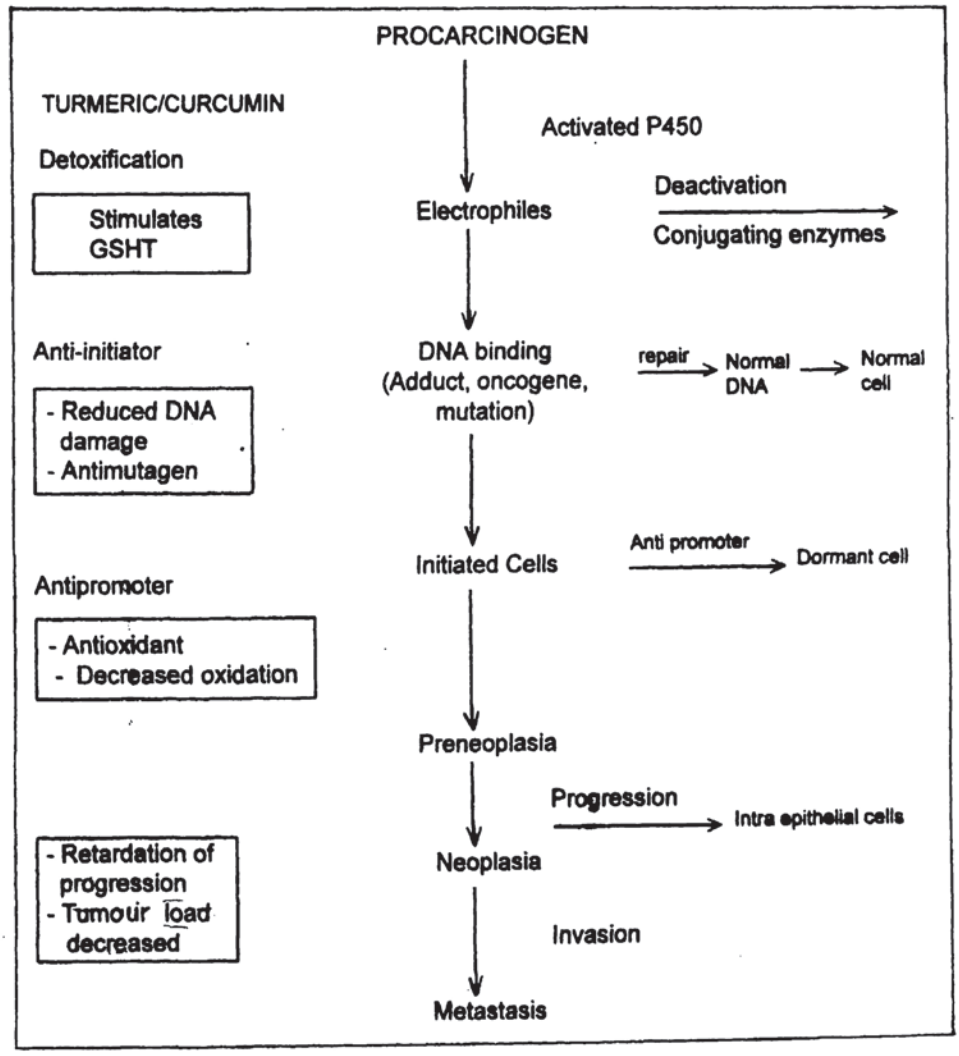

Fig. 3. Role of turmeric/curcumin on cancer prevention.

induced cancer. They could be used mostly as the preventive agents in the diet or curcumin can be used as a pharmacological tool in high risk populations. It would however be wise to rely on natural means of protection — a dietary prescription for a population approach as compared to experimental chemoprevention programmes.

Turmeric and curcumin on BP-DNA adducts

One of the primary events in chemical carcinogenesis is the formation of covalent carcinogen-DNA adducts and as a consequence of covalent interactions *in vivo*, point mutations may arise. Therefore, an experiment was conducted to study the adducts in carcinogen treated animals which received either turmeric or curcumin in the diet. The effects of turmeric and curcumin on BP-DNA adducts were quantitated using ^{32}P post labelling assay[35]. The results of the study indicated that with both turmeric at 0.1, 0.5 and 3 per cent and curcumin at 0.03 per cent in the diet, adducts were significantly reduced when compared to the control groups. In hepatic tissue, both the number of adducts as well as f moles adducted nucleotide/µg DNA was significantly decreased in all the treated groups.

Turmeric/curcumin on xenobiotic metabolism

As ingested environmental chemicals need to be eliminated from the body to protect against their toxicity, the drug metabolising system has developed. The liver is the key organ for metabolising carcinogens, drugs, *etc*. Certain extra-hepatic tissue such as the intestine, kidney and lung also possess xenobiotic metabolising enzymes. The liver and the gastrointestinal tract act as the first line of defense against lipophilic substances. These inhibitors of carcinogenesis are reported to enhance the host detoxification mechanisms and act as blocking agents[36]. Therefore, enzymes such as aryl hydrocarbon hydroxylase (AHH), uridinediphosphoglucuronyl-transferase (UDPGT) and glutathione-S-transferase (GST) were assessed in rats after 4 wk of turmeric and/or curcumin feeding[37].

The AHH was not altered by turmeric or curcumin feeding either in the liver or in the gastrointestinal tract. UDPGT activity was also found to be similar in all groups. However, GST activity was significantly elevated both in the liver and intestine at 0.1 per cent of turmeric and 0.03 per cent of curcumin in the diet. The stimulation ranged from 25-30 per cent. The increased GST activity may to some extent explain the anti-carcinogenic properties of curcumin and turmeric. An increased GST activity in animals fed high amounts of the cruciferous groups of vegetables and fruits agrees well with their anti-carcinogenic effects on tumours of the forestomach induced by B(a)P[38]. The non-stimulatory effect of turmeric and curcumin on AHH as against the inducing effects on GST would ultimately positively decrease the load of carcinogen on the body.

Turmeric/curcumin in experimental tumourigenesis

As oral cancers in India occur most commonly in men and women and tobacco chewing and smoking have been identified as risk factors, it was considered necessary to assess the effects of curcumin and turmeric in experimental tumourigenesis in Syrian golden hamster cheek pouch challenged by 7-12-DMBA. Cheek pouches were painted with DMBA as well as turmeric and curcumin for induction and retardation of tumours along with incorporation of turmeric and curcumin in stock diets at concentrations of 1 and 0.03 per cent of turmeric and curcumin respectively. Some groups received turmeric/curcumin only in the diet while the others received turmeric/curcumin both in the diet as well as by local delivery[39]. At the end of 14 wk, the animals were sacrificed and oral pouches were examined for tumour load and malignancy by histopathology. DNA adducts were also estimated by ^{32}P post labelling assay. Neoplastic changes were graded using standard procedures. When turmeric/curcumin was given in the diet or applied locally the animals appeared to have lesser percentage of microscopic tumours as compared to control groups where 90 per cent had such tumours. The incidence of the microscopic tumours was only 42 per cent in the animals which received turmeric in the diet and as local paint. The polyploid neoplasms were of variable number and size with the animals receiving turmeric by both oral and local route having the least tumour burden. The average neoplastic grading (score) was not different between groups except in animals fed or painted turmeric/curcumin. In animals which received curcumin, most of the tumours did not go beyond grade I. However, DNA adducts as measured by ^{32}P

post labelling assay were significantly reduced in all the experimental groups given turmeric/curcumin through diet or paint, compared to positive control animals. The results suggest that the turmeric/curcumin may act as anti-proliferators and anti-promoters. The tumours in animals which received turmeric/curcumin were not only similar, but less severe and mostly intra-epithelial neoplasms. Boone and co-workers[40] have suggested that intra-epithelial neoplasia are of critical importance for cancer chemoprevention. The lesions become aneuploid as they become more severe and progress towards neoplasia. The intraepithelial neoplasia progress to more serious lesions (dysplastic) giving rise to carcinoma *in situ*. Reactive oxygen species can induce progression of tumours. Thus turmeric appears to act both as anti-promoter and anti-initiator.

As the experiment did not delineate the effects of turmeric/curcumin as anti-initiator and anti-promoter very successfully further experiments on forestomach tumours induced by B(a)P in mice were conducted using turmeric and curcumin during initiation or in the promotion period (Annual Report: NIN, Hyderabad, 1996-97).

None of the animals progressed beyond papillary hyperplasia and papilloma including the positive control group. While papilloma was seen in 80 per cent of the positive controls, the inhibition was 67 and 50 per cent respectively in the groups which received turmeric and curcumin during initiation and it was 50 and 100 per cent respectively in groups which received turmeric/curcumin in the post initiation period.

The area occupied by papillomas was also significantly reduced in both turmeric/curcumin treated groups and it appears as if both turmeric and curcumin act in both the initiation and promotion phases. However, the trend of results indicated a better effect when turmeric/curcumin were used in the post initiation phase. Further long-term experiments are in progress. It is essential to mention here that turmeric significantly reduced formation of nitrosocompounds under *in vitro* simulated gastric conditions (Annual Report; NIN, Hyderabad, 1993-94). Several other groups observed similar effects on hamster cheek pouch models, forestomach, colon, skin and mammary tumours[41].

Turmeric/curcumin as anti-oxidant

Turmeric and curcumin have been shown to be anti-oxidants[42-44]. The anti-oxidant effects of turmeric and curcumin were evaluated in an animal model where oxidant damage was induced by paracetamol and DMBA. Markers such as TBARS — an indicator of lipid peroxidation, glutathione (GSH) — an anti-oxidant in liver homogenate and superoxide dismutase (SOD) and glutathione peroxidase were evaluated. The elevation of TBARS was much lower in turmeric/curcumin treated rats when compared to the controls and so was the case with SGOT and SGPT indicating that the liver damage due to paracetamol metabolite N-acetylaminoparaaminobenzoquinone (NAPQi) was counteracted by curcumin[42]. The glutathione tripeptide was depleted whether or not curcumin or turmeric were incorporated into the diet and no change was observed in SOD or glutathione peroxidase. It is suggested that the oxidative metabolite of paracetamol namely NAPQi is detoxified to some extent through the increased activity of GST. Curcumin with its phenolic groups together with beta-diketone structure is probably responsible for its high biological activity[43].

Curcumin on DNA repair : DNA repair is one of the important mechanisms of protecting the system from the onslaught of genotoxic agents. Therefore, the effect of curcumin was studied on the single strand breaks (SSb) in DNA of *Saccharomyces cerviciae* exposed to UV radiation, 8-methoxypsoralen and benzopyrene. The single strand breaks in DNA were estimated by alkaline elution technique and were found to be significantly reduced in the yeast cells in the presence of curcumin *in vitro* (Polasa *et al*, unpublished data). *In vivo* studies in the forestomach mucosa of mouse exposed to B(a)P showed that SSbs were much less in curcumin treated animals[45]. More recently, a single cell microgel electrophoresis has been extensively used to detect DNA damage in individual cells imbedded in agarose to assess DNA repair.

Curcumin was used at various concentrations to assess its repair capacity against DNA damage induced by B(a)P. DNA damage induced by B(a)P in lymphocytes of smokers and non-smokers and in women was effectively counteracted by curcumin. suggesting that in addition to its anti-initiating.

detoxifying and anti-oxidant activities, it also has the ability to repair DNA (Annual Report; NIN, Hyderabad, 1997-98).

Curcumin has been reported to be effective in preventing colon tumourigenesis and mammary cancer against azoxymethane or 7,12-DMBA[41,46]. There are also reports to show that curcumin when applied topically inhibited TPA-induced oxidation of DNA bases in epidermis in CD-1 mice[47].

Studies in humans : As ultimately the effects of turmeric/curcumin need to be assessed in humans, its antimutagenicity was evaluated in human smokers who are known to excrete large amounts of mutants. Turmeric was administered at a dose of 1.5g/day for 30 days which was calculated by extrapolating the dose used in experimental animals. The observations suggested that turmeric given by the oral route was a strong inhibitor of urinary mutagens even within a short period of 15 days. Almost 50-70 per cent subjects had a significant impact on the revertants with turmeric feeding. The liver and kidney functions were not altered[48]. Further, a clinical trial was done in a group of reverse smokers who are at high risk of palatal cancers in a specific area of Andhra Pradesh. A dose of 1 g/day of turmeric was administered for a period of 9 months and the results suggest that it had a significant impact on the regression of precancerous lesions such as red and white patches over the palatal regions and also decreased micronuclei and DNA adducts in oral epithelial cells which are markers for genomic damage (Annual Report; NIN, Hyderabad, 1994-95).

Effect of cooking turmeric/curcumin

As turmeric in the Indian culinary practices is usually either boiled or fried, it was considered essential to assess its antimutagenic properties after heating or frying (Annual Report; NIN, Hyderabad, 1993-94). Based on the measure of SOS DNA repair system in *Escherichia coli* PQ37 the effects of boiled and fried turmeric on the genotoxic response were evaluated. It was evident that turmeric whether fried or boiled, evoked a similar SOS response in the tester strain indicating that cooking at high temperatures was unlikely to destroy the antimutagenic potential of turmeric.

Other anti-cancer agents

Allium species of vegetables are now identified as protective agents in the diet for several cancers[49]. The diallyl sulphide and allylmethane trisulphite are the components of the allium species which induce microsomal monoxygenase enzymes and GST.

Studies on onion and garlic have been initiated at NIN, Hyderabad and antimutagenic potentials have been demonstrated (Annual Report; NIN, Hyderabad, 1993-94). Onion and garlic fed to Wistar rats at doses of 0.1 to 1 per cent through diet had potent antimutagenic effects. Detoxifying enzymes were also estimated in certain tissues and stimulation was observed in cytosolic GST and quinone reductase activity. The effects on xenobiotic metabolism may account for its antimutagenic properties. Green leafy vegetables have been assayed on similar lines for their detoxifying potential and prevention of DNA damage. The observations suggest that they have potent anticancer activity. All these observations are in line with epidemiological observations on consumption of vegetables and fruits as protective agents for cancers at various sites[13] and our own observations have revealed that low intake of vegetables and fruits increase the upper aerodigestive tract cancers[50].

Other studies from India and abroad

There have been extensive studies on turmeric/curcumin which demonstrate an array of protective actions of this spice and its active principle.

Hepatoprotective activity

Both curcumin and turmeric have been shown to protect liver against a variety of toxicants *in vitro* as well as *in vivo*. They include CCl_4, aflatoxin B1, paracetamol, iron and cyclophosphamide in mouse, rat and duckling. Curcumin has been shown to strongly inhibit cytochrome P 450 1A in liver, an isoenzyme involved in the bioactivation of several toxins including B(a)P[51].

Hypolipidaemic/hypoglycaemic activity

Ethanolic extracts of *Curcuma longa* exhibit hypoglycaemic activity. With an intraperitoneal dose of 10 mg/rat, the reduction in the blood glucose levels at 3 and 6 h after the administration of the

extract was 37.2 and 54.5 per cent respectively. Curcumin, when fed to the rats at the level of 1.5 g/kg diet, for 7 wk showed both hypocholesterolaemic and hypolipidaemic activities. Some workers have also observed a marked increase in hepatic cholesterol 7-alpha-hydroxylase activity suggesting a higher rate of cholesterol catabolism with curcumin. Experimentally (turmeric 4 mg/kg/day = curcumin 0.4 mg/kg/day) and clinically (equivalent to 20 mg/kg/day of curcumin) turmeric extract has been found to decrease serum lipid peroxides which play an important role in the pathogenesis of normal senescence and age related diseases like atherosclerosis. In a clinical trial conducted in China, turmeric (50 g/day) was found to be as effective as clofibrate[51].

Anti-inflammatory activity

Curcumin has anti-inflammatory effect in acute, sub-acute and chronic models of inflammation in mice and rats as tested by the paw edema test and also a beneficial effect in adjuvant induced arthritis in rats. In clinical trials, curcumin did not produce any side effect up to 1600 mg/kg/day for 4 wk and showed positive response in patients with rheumatoid arthritis and osteoarthritis[51].

Effect on gastrointestinal tract/respiratory system/infections

Curcumin and turmeric have been reported to increase bile flow and also have antiulcer and anti-gall stone properties. Encouraging results have been found in clinical trials conducted with the volatile oil of curcumin in patients with bronchial asthma. Curcuma extract has also been reported to have an antiallergic activity[51].

Turmeric when applied locally is effective in wound healing[51]. Similar use of turmeric was also found in Chinese medicine and all ancient Indian systems of medicine.

Alcoholic extracts of turmeric/curcumin have been shown to possess antifungal activity. Recently curcumin has been demonstrated to inhibit the long terminal repeats of HIV-1 virus gene expression and also HIV-1 integrase which is essential for integration of double stranded DNA copy of the viral RNA genome into a host chromosome and for HIV replication[52]. Thus, curcumin could be active as an anti-HIV drug.

CALCINOGENIC PRINCIPLE IN PLANTS

Apart from phytochemicals, certain nutrients are well known to protect against cancers. Case control and cohort studies on vitamin D document a decrease in the risk of colorectal cancer on high intake of vitamin D which is accompanied by elevated levels of vitamin D in the serum. Vitamin D deficiency can occur in the Indian population, particularly in over-crowded, ill-ventilated and polluted environments. Hence attempts were made to explore the vitamin D activity of some plants.

Involvement of certain plants in the development of calcitonic disease in grazing animals has been well documented[53,54]. Plant species belonging to Solanaceae (*Solanum malacoxylon, Cestrum diurnum, etc.*) and Gramineae (*Trisetum flavescens*) have been identified in most cases as the cause of calcitonic disease. Calcitonic disease in grazing cattle has also been reported from India but the causative agent is not defined[55]. The leaves of these plants were shown to have calcinogenic property which mimics the biological activity of active metabolite of vitamin D_3 *i.e.* 1,25-dihydroxy vitamin D_3 [$1,25(OH)_2D_3$][56]. The active principle in *S.malacoxylon* and *C.diurnum* has been identified as a glycoside of $1,25(OH)_2$ D_3.

The plant *C.diurnum* is naturalised in India and is seen growing along fences, road sides and in neglected fields and pastures. Owing to its abundant occurrence and being naturalised in a subtropical country with plenty of sunlight; and with a reported incidence of calcitonic disease in grazing animals in India, it was felt necessary to undertake studies for the presence of vitamin D like activity in locally available species of *C.diurnum*.

Most of the calcinogenic plants so far discovered belong to either the Solanaceae or the Gramineae families and since some of the vegetable plants also belong to family Solanaceae it was felt of interest to screen some of the vegetable plants for this active principle.

Active principle in *C. diurnum*

Leaves of *C. diurnum* grown in Hyderabad, were tested in rachitic and vitamin D deficient rat models.

Vitamin D dependent serum and bone parameters and calcium transport by the gut which were altered in vitamin D deficiency were restored to control levels by incorporating *C.diurnum* leaf powder at 2 per cent level in the diet. Similarly in rachitic rats, the extent of new bone calcification was significantly improved either by giving *C.diurnum* leaf in the diet or by vitamin D administration. An approximate estimation of vitamin D like activity indicated that the plant had about 100,000 IU cholecalciferol equivalents/kg dry leaf powder, which is three times higher than the values reported earlier[53]. This may be because of long exposure to sunlight of the plants grown in this part of the world.

The nature of active principle in C.diurnum : The nature of the active principle in *C.diurnum* leaves was investigated using various physicochemical and biological techniques. The chloroform extract of the leaves (containing free vitamin D_3 and its metabolites) and the chloroform-methanol (1:2) extract (containing glycosidic vitamin D_3 and its metabolites) of the residue after glycosidase treatment were partially purified by column chromatography. Fractions corresponding to authentic vitamin D_3, 25-hydroxy vitamin D_3 (25 OH D_3), were found to be biologically active. These fractions were also analysed and quantified by HPLC. For the first time we demonstrate the presence of free vitamin D_3, 25 OH D_3 and 1,25 $(OH)_2$ D_3 in a calcinogenic plant. Apart from the free forms, fractions corresponding to glycosidic metabolites were also found. However, concentration of free metabolites were much higher than that of the glycosidic forms.

Calcinogenic principle in common vegetable plants

Commonly consumed vegetable plants were screened for the calcinogenic principle. The fruit and leaves of tomato (*Lycopersicon esculentum*), brinjal (*Solanum melongina*) and potato (*Solanum tuberosum*) were screened for the active principle using various biological and physicochemical tests.

The calcinogenic activity was present in the leaves but not in the fruit of the tomato plant. The chloroform extract of the leaves (containing free vitamin D and its metabolites) and the ethanol extract of the residue (containing the glycosidic forms) were partially purified by column chromatography. The fractions corresponding to authentic vitamin D_3, 25 OHD_3 and 1,25 $(OH)_2D_3$ and their glycoside forms were identified. Free vitamin D_3 was observed to be the major active principle and the concentration of free forms of the metabolites was higher than the corresponding glycosides. The active principle was demonstrated using a vitamin D deficiency animal model.

No activity could be detected in the fruit of the brinjal plant. Administration of dried leaf powder at 2 per cent level in the diet of vitamin D deficient rats resulted in a significant increase in intestinal calcium transport, but surprisingly, it did not restore the serum calcium levels to normal. It is possible that bone was spared from resorption to restore calcium levels to normal. These results indicate that the active principle present in the leaves of brinjal plant could be an analog of $1,25(OH)_2D_3$. Further work needs to be done to confirm the nature of the active principle which can have potential for the treatment of osteoporosis.

No calcinogenic activity could be detected in potato.

Chemoprevention vs dietary prevention

Prescriptive and proscriptive approaches for cancer prevention would definitely have an impact on the incidence of upper aerodigestive and cervical cancers in India. A wide range of plant products as suggested in this review, are source of anti-oxidants and act as modifiers of the carcinogenic process, appear to be the right approach for modifying cancer risk in the population. A chemopreventive approach can be targeted at the high risk population using intermediate biological end points as the surrogate indicators for cancer prevention as has been in the case of turmeric. However, dietary prevention coupled with other life-style changes though demanding, is perhaps the right answer for prevention of cancer and chronic diseases in India.

Conclusions

In the field of nutrition, foods which enhance and promote health and prevent diseases are attracting the attention of scientists, consumers and

industrialists. The current emphasis is also on cost effective health care strategies and highlights the importance of dietary changes to optimise health. In addition to nutrients, non-nutrients are the centre of attraction as they seem to provide additional benefits by way of the phytochemicals. Several plant foods, herbs and spices seem to have higher anti-cancer, anti-oxidant, antihypocholesterolaemic, hypoglycaemic, hypercalcaemic and detoxifying activities which confer additional benefits for improving health. Studies in our laboratory have shown that green leafy vegetables have very high anti-oxidant activity as also the spice principles such as eugenol and curcumin. Spices such as turmeric, onion and garlic were shown to be potent antimutagens in both *in vitro* and *in vivo* situations. These were also shown to induce tissue GST activity. Extensive studies on turmeric/curcumin have shown that the tumour burden as well as DNA adducts in Syrian hamster cheek pouches painted with 7-12-DMBA — a known carcinogen, were significantly reduced. In humans, both turmeric/curcumin inhibited the urinary mutagen levels. They were also shown to reduce oral precancerous lesions in reverse smokers. Apart from these, calcinogenic or vitamin D like activity was identified in leaves of *C. diurnum*, *Lycopersicon esculentum* and *Solanum melongina*.

Results from clinical trials with nutrients as supplements have not provided positive evidence of substantial protective effects either against cardiovascular disease or cancer. However, there is enough evidence to suggest that plant foods decrease the risk of these diseases and obviously the wide variety of protective phytochemicals providing aggregatory and synergistic effects appear to be more important than individual nutrients or combination of nutrients for prevention.

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