
Natural Products for Chemoprevention

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SUMMARY

Natural products have a long history of use in the service of mankind for the prophylaxis and treatment of several diseases and cancer is not an exception. For such a dreadful disease, apart from conventional modalities like surgery, radiotherapy and chemotherapy a few other approaches are also available or being tried. It has been reported that 1/3rd of cancer patients use some form of complementary and other alternative medicines. In the recent times, considerable attention has been focussed on the identification and development of natural products for chemoprevention. By systematic and rigorous screening processes many of the potential chemopreventives have shown considerable safety and efficacy in preclinical evaluation and are in the stages of clinical testing. In this article chemopreventive agents from natural sources, difficulties involved in their development, their characteristics, diverse chemical constituents, mechanisms of action, efficacy and adverse effects in experimental systems and future directions are reviewed. Special attention has been focussed on Ashwagandha, Tulsi, carotenoids, vitamins, soybean, ω -3 fatty acids, green tea, resveratrol and anthocyanidins as chemopreventives.

INTRODUCTION

Cancer, a disease of multifactorial origin, is one of the major health problems. According to a

WHO report 1998, it is the third largest cause of death worldwide.¹ It is the second leading cause of death in US. It has been estimated that > 1500 Americans die per day of cancer.² The global cancer trends have been summarized in the booklet 'Global Action against Cancer' launched by WHO/UICC (2003). Marlin comments that the most important issue for all of us is that countries with relatively low cancer rates will see them increase enormously if nothing is done. The booklet predicts that the number of cancer deaths worldwide will increase from 6.2 million to 10 million within 20 years. South America, The Caribbean, South East Asia, Northern Africa and West Asia will have the highest increase.

Basically, cancer is of genetic origin in which cell cycle is disrupted as a result of imbalance in tumour induction and tumour suppressor genes, the tumour induction genes (proto-oncogenes) taking an upperhand leading to uncontrolled proliferation, invasion, dedifferentiation and loss of function and metastasis. Mutation in a cell may not necessarily lead to induction of cancer, accumulation of mutations coupled with promoters, will convert a normal cell to a cancerous cell. Recent reports indicate that disruption of circadian rhythm is also associated with cancers both experimentally and clinically.^{3,4} It is not only the disruption of circadian rhythm, several other factors also influence the genesis of cancer, chemical carcinogens being the most important in this respect which cause approximately 80% of all cancers.

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It has now been well established that cancer is a multistep process occurring via initiation, promotion and progression. It has been proved experimentally in mouse skin model of chemical carcinogenesis.

VARIOUS APPROACHES IN CANCER

The conventional methods to treat cancer are surgery, radiotherapy and chemotherapy. Recently several newer approaches / drugs have emerged which include chemoprevention (synthetic and natural agents), drugs inducing differentiation, antimetastatic drugs, antiangiogenic drugs, hypoxic tumour cell specific agents, antitelomerase drugs, aromatase inhibitors, biological response modifiers, radiosensitizers, antisense therapy, gene therapy and cancer vaccines, etc. Other strategies like reduction of side effects of anticancer agents using 5-HT₃ receptor antagonists, haematopoietic growth factors, interferons and interleukins are also being tried with varying success rate. Drugs modifying circadian rhythm have also proved beneficial.

Combination therapy has resulted in higher success rate than a single drug in resistant tumours. Enhanced survival and quality of life of the patient has been reported with appropriate therapies. Strategies are also being directed towards selective drug delivery to tumour cells while sparing normal cells.

The synthetic chemotherapeutic agents, though widely used in cancer, suffer from the drawbacks of high toxicity (suppression of bone marrow, nausea, vomiting, alopecia, impotence, immunosuppression etc.), are costly and not within the reach of a common man. It seems probable to overcome the above shortcomings with the use of drugs of natural origin, however, maintenance of their continuous supply is a difficulty, active principles may be lost during the processing, yield of the active principles is low and their standardization is difficult. Afterall, natural products are considered to be

relatively safer and have long history of their use traditionally for various diseases in general. However, their traditional claims need to be scientifically validated.

WHO estimated that 80% of the world population in the developing countries mainly relies on traditional medicines for their healthcare needs, most of them are plant derived. The estimated plant species on the earth are 3,50,000 and several of them may be the source of useful anticancer drugs. Natural products account for the development of 44% of all new drugs.⁵ It has been reported that 1/3rd of cancer patients use some form of complementary and alternative medicine.^{6,7}

NATURAL PRODUCTS : NEED FOR SYSTEMATIC SCREENING

Keeping in view the high burden of cancer, high toxicity of synthetic drugs and the advantages of natural products over the synthetic ones, there is an urgent need to research and develop the anticancer drugs from the natural sources. Natural products can be systematically screened both in-vitro and in-vivo experimental models of cancer, subjected to toxicological evaluation followed by clinical trials and marketing.⁸ Postmarketing surveillance studies continue after marketing of a drug. These studies provide clear-cut indication of safety and efficacy of a drug in actual practice.

As a result of screening process, a number of anticancer drugs from natural sources have been developed and marketed for clinical use e.g. vincristine, vinblastine, paclitaxel, etoposide etc. There are also a number of promising agents under extensive preclinical and clinical evaluation. In this chapter an attempt has been made to give an overview of the safety and efficacy of some natural products in chemoprevention of cancer.

CONCEPT OF CHEMOPREVENTION

The term 'chemoprevention' was coined by Sporn and Newton in 1979 and is one of the desirable strategies to prevent the impact of cancer in human population by administration of chemical agents (synthetic or natural).⁹ This approach has been shown to inhibit, reverse or halt the process of carcinogenesis. Since carcinogenesis is a relatively quick process in contrast to cancer (which occurs over a long period of time), chemoprevention intervention at the carcinogenesis stage when only few mutations are present will serve as a primary prevention measure).¹⁰ However, a lot of difficulties are also faced in the development of chemopreventives. The major difficulties are listed below.

DIFFICULTIES INVOLVED IN THE DEVELOPMENT OF CHEMOPREVENTIVE AGENTS

1. Lack of adequate animal models for testing chemopreventive efficacy in certain major cancer sites e.g. lung (squamous cell carcinomas), prostate, ovary, brain, pancreas and estrogen receptor negative breast cancer.
2. There is a problem of compliance and longer duration required for observation of cancer end points.
3. Lack of surrogate end point biomarkers.
4. Lack of perfect correlation between in-vitro experiments in cancer cells, in-vivo experiments in animals and that the clinical trials.
5. Clinical trials are costly and large population size is required for the results to be valid scientifically.
6. Non-specific normal tissue toxicity of the agent.
7. Difficulty of early screening of cancer and selection of a chemopreventive agent.
8. Lack of industry participation.

CHARACTERISTICS OF AN IDEAL CHEMOPREVENTIVE

According to Ito et al an 1997,¹¹ an ideal chemopreventive must fulfil the following characteristics :

1. It must be able to inhibit initiation, promotion and progression.
2. It must be able to block nitrosamine formation.
3. It must be free from genotoxicity.
4. It must be free from carcinogenicity.
5. It must not be a carcinogen precursor.
6. It must lack enhancing activity at any stage of carcinogenesis.
7. It must be free from general toxicity.
8. It must be easily and commercially available.
9. It must have little or no toxic effect in normal & healthy cells.
10. It must have low cost.

However, none of the chemopreventives fulfils all the above characteristics. There is scope to try towards this novel goal to fulfill, if not all, most of the above characteristics. It has been reported that currently more than 50 drugs are under clinical trials as chemopreventives.¹² According to Soria et al 2003, around 2000 natural and synthetic agents have shown to be effective as chemopreventives in experimental systems. The agents which have been and studied in various clinical trials are – retinoids, N-acetyl cysteine, β -carotene, calcium, α -tocopherol (vitamin E), γ -tocopherol, curcumin, selenium, tamoxifen, finasteride, non-steroidal anti-inflammatory drugs (NSAIDs), green tea polyphenol and soybean etc.¹³⁻¹⁷

SOURCES OF NATURAL CHEMOPREVENTIVES AND DIVERSE NATURE OF ACTIVE INGREDIENTS

The chief sources of natural chemopreventives are vegetables, fruits, spices and plants.¹⁸ However, they may also be present in animal source.¹⁹ These chemopreventives are complex mixtures and a few have been isolated in the pure form and their structures characterized. Various categories of chemopreventives are carotenoids (β -carotene, α -carotene, leutin, lycopene etc), selenium, curcumin, volatile oils (d-limonene, eugenol), coumarins, saponins, organosulphurs (allicin), fatty acids (specially ω 3 fatty acids), amino acids and related compounds, phytoestrogens [isoflavonoids (daidzein, genistein), flavonoids, stilbenes (resveratrol) and lignans etc], glycosides, alkaloids, vitamins, anthocyanins etc. Among the phytoestrogens, mainly genistein and resveratrol have been studied, and are reported to reduce the incidence of various cancers.²⁰ However, soya and isoflavonoids do not provide protection against colon cancer.²⁰

MECHANISMS OF ACTION OF CHEMOPREVENTIVES

Progression of a normal cell to a cancerous cell is a multistep and decade long process (in most cases). Therefore, drugs with multiple mechanisms of action without destroying normal cells are desired. A better understanding of carcinogenesis has made it possible to study the mechanisms of chemopreventive action at the molecular level. In-vitro studies using cell lines, in-vivo studies using tumour implantation models, chemical carcinogen-induced experimental tumours, genetically modified animal models and clinical studies have further increased our understanding regarding mechanisms of chemopreventive action. The chemopreventives are known to act by inhibiting initiation, promotion and progression. The various mechanisms involved are – induction of

apoptosis, inhibition of phase I enzymes (thereby inhibiting metabolic activation of the carcinogen), induction of phase II enzymes (induction of hepatic and extra hepatic detoxification mechanisms), inhibition of free radical mediated damage to cellular DNA/cellular environment (antioxidant mechanisms – there could be differential action in-vitro and in-vivo), anti-inflammatory, inhibition of cyclooxygenase (s), affecting the release of fatty acids in cancer cells, modulation of DNA synthesis, inhibition of angiogenesis, interaction with signal transduction mechanisms (e.g. down regulation of cyclin dependent kinases etc.) and modulation of immune response etc. The drugs with different mechanisms of action may be tried in combinations with the hope of facilitating the action of each other and cutting their side effects to minimum. This approach will be useful in enhancing the compliance.

THE INDIVIDUAL CHEMOPREVENTIVE ASHWAGANDHA

Ashwagandha (*Withania somnifera*) is well known for its physical power enhancing activity. Apart from this, several other activities are also attributed to it. In MTT assay, *Withania somnifera* hydroalcoholic root extract (WSRE) exerted significant antiproliferative activity on HeLa cells. The encouraging results in this assay further prompted us to carry out chemopreventive studies in vivo. WSRE when administered at a dose of 400mg/kg body weight once daily by oral route to Swiss albino mice one week before 20-methylcholanthrene injection (subcutaneously into the thigh region) and continued for 15 weeks thereafter, resulted in significant reduction and delay in fibrosarcoma incidence, tumour volume and significant increase in survival when compared with control. The findings were supported by biochemical and histopathological studies. The 20-methylcholanthrene induced raised levels of lipid peroxidation product, malondialdehyde, in

mice liver were brought down significantly by the extract administration. The depleted levels of non-enzymatic antioxidant (reduced glutathione) and enzymatic antioxidants (superoxide dismutase, catalase and glutathione-S-transferase) in mice liver were significantly raised by WSRE administration in methylcholanthrene induced fibrosarcoma tumour bearing animals suggesting the antioxidant and detoxifying potential of the extract²¹. Massive necrosis of the fibrosarcoma was produced by WSRE administration. Other investigators have also reported the enhanced survival and chemopreventive activity of *W. somnifera* root extract against 20-methylcholanthrene induced fibrosarcomas in mice, however, by intraperitoneal administration.²²

In another experiment, 400mg/kg body weight of WSRE orally (3 times/week on alternate days), one week prior to 7,12-dimethylbenz(a)anthracene (DMBA) application (topical application on depilated back of mice twice weekly for 8 weeks) and then continued for 24 weeks thereafter, showed significant reduction in DMBA-induced papillomas. The findings were supported by physical, biochemical and histopathological studies. A significant reduction in delay and incidence of papillomas was observed by WSRE administration as compared with controls. Survival of mice was also significantly enhanced by the extract administration. Impaired antioxidative defense in skin papillomas was reversed to near normal levels by the extract administration. The severity of DMBA-induced papillomas was reduced markedly by the extract administration in the histopathological studies.²³ Other investigators have also reported the protective action of *W. somnifera* root extract against DMBA-induced carcinogenesis.²⁴ However, the route, frequency and duration of extract administration varied.

TULSI

Tulsi (*Ocimum sanctum*) is a sacred herb and worshiped by Hindu community. It possesses vast number of pharmacological activities²⁵. In our studies *Ocimum sanctum* seed oil (OSSO) showed significant antiproliferative activity against HeLa cells in MTT assay.²⁶ The activity of the oil appeared to be due to its prooxidant activity. The morphological examination of the cells revealed that the cell death occurred due to enlargement of viable cells with differentiation. Massive cellular necrosis was seen at the highest concentration of the oil (250g/ μ l). The oil was further screened for the chemopreventive activity in mice. Administration of 100 μ l/kg body weight of the oil once daily by oral route, one week prior to 20-methylcholanthrene injection in thigh region of Swiss albino mice and continuously for 15 weeks thereafter prevented and delayed the fibrosarcoma incidence, reduced the tumour volume and enhanced survival as compared with controls.²⁷ The chemopreventive activity was also supported by biochemical parameters (enzymatic and non-enzymatic antioxidants) and histopathological findings.

The oil at the dose of 100 μ l/kg body weight (3 times/week) also showed chemopreventive activity against DMBA-induced skin papillomas in Swiss albino mice.²⁶ In this model, a significant reduction in incidence of papillomas, number of papillomas and significant increase in survival time was observed in oil treated mice as compared with controls. Chemopreventive activity of the oil was found to be associated with the antioxidant mechanism. The histopathological studies revealed squamous cell carcinoma with vacuolar changes in tumour cells with focal areas of necrosis in the oil administered mice papillomas suggestive of protective action of the drug against DMBA-induced skin papillomas.

CAROTENOIDS

Carotenoids are the chemical compounds occurring widely in fruits, green and yellow leafy vegetables etc. Amongst the numerous carotenoids present in nature, special attention has been focussed on β -carotene with respect to its role in prevention of risk of lung cancer. In-vitro, carotenoids possess antioxidant properties and inhibit carcinogen-induced neoplastic transformation, inhibit plasma membrane lipid peroxidation and upregulate the expression of connexin-43 which is predicted to possess chemopreventive potential in-vivo. Various retrospective and prospective studies show an inverse relation between fruits and vegetable consumption and risk of developing lung cancer. Recent studies (ATBC and CARET trials), however, suggest an increased incidence and mortality from lung cancer in those receiving β -carotene supplementation.^{28,13} The explanations suggested for such an effect are – activation of carcinogenic mechanisms if high concentration of β -carotene interacts with highly oxidative tobacco smoke, and procarcinogenic effect of β -carotene resulting in changes in cytochrome P-450 in some circumstances. The role of β -carotene in reducing the risk of lung cancer in smokers remains to be established and cannot be recommended for chemoprevention in heavy/active smokers at high dose, rather it must be avoided in high doses in heavy smokers at high risk. β -carotene possesses both antioxidant and pro-oxidant properties, antioxidant properties being seen usually at low dose and pro-oxidant at high dose contributing to chemopreventive and cancer risk enhancing properties respectively. Evidence exists for the cocarcinogenic properties of β -carotene resulting in induction of carcinogen metabolizing enzymes and excessive production of oxidative stress. This compound could be harmful if given alone to smokers or to individuals exposed to environmental carcinogens.²⁹ Beta-carotene, a co-carcinogen or anticarcinogen, remains to be resolved.

Supplementation of β -carotene (50mg/day for more than 4 years) had no effect on developing the risk of second primary or recurrent head and neck cancer. There was insignificant increase in survival of lung cancer in both smokers and non-smokers.³⁰ However, in a placebo controlled chronic intervention trial in Columbian population with precancerous gastric lesions, supplementation of β -carotene (30mg/day), vitamin C (2g/day) or both with/without anti *H pylori* treatment resulted in significant regression of gastric lesions. The combination of β -carotene and vitamin C had maximum benefit.³¹

Erhardt et al, 2003 assessed the relation between plasma concentration of lycopene, β -carotene and α -tocopherol and colorectal adenomas in white subjects undergoing a complete colonoscopy (73 with adenomas, 63 without any polyps, 29 with hyperplastic polyps).³² Dietary history, alcohol consumption, smoking status were collected from all subjects. Plasma concentrations of these antioxidants were measured by HPLC. No significant difference in patients with adenomas and controls was observed with respect to body mass index, intakes of energy, fat, protein, carbohydrates, fibre, β -carotene and alcohol or prevalence of smoking. However, adenoma patients were slightly older. Median plasma lycopene concentration was significantly lower in adenoma patients than in controls (35%, $p < 0.016$) and that of β -carotene also lower in adenoma patients (-25.5%) than controls but not significantly. The smoking and plasma lycopene concentration of $< 70\mu\text{g/L}$ were the only risk factors for adenomatous polyps. No significant difference was observed in patients with hyperplastic polyps from that of controls in any of these antioxidants. The findings of this study suggested that high intake of lycopene from tomato (a strong antioxidant) protected against the risk of colorectal adenomas. The exact relation between plasma lycopene and tissue concentration (intestinal mucosa) is not known.

The possible explanation for such protective effect was explained as under-

The plasma lycopene concentration served as a marker for contents in colorectal mucosa. Lycopene is known to accumulate in human tissues reflecting chronic intake whereas plasma concentration, the acute intake. In view of the investigators, initiation-promotion process of colorectal neoplasia takes years to develop and therefore the long term administration would be beneficial. There is an inverse relation between plasma lycopene concentration and adenoma risk. High serum or plasma concentration is associated with decreased risk of lung cancer, stomach cancer, prostate cancer and colorectal cancer. From these studies it is inferred that consumption of fruits and vegetables in low quantities would be beneficial and for optimum benefit, a mixture of micronutrients may be used.

VITAMIN C

Epidemiological and laboratory studies suggest that high consumption of antioxidant rich fruits and vegetables reduce the risk of cancer. Vitamin C is a water soluble, antioxidant vitamin abundantly present in fruits and vegetables. It is used as a dietary supplement to prevent chronic diseases like cardiovascular diseases (hypertension), stroke, neurodegenerative disorders and cancer. It exerts chemopreventive effects without toxicity at doses higher than the currently recommended dietary allowance of 60mg/day. It is a chemopreventive in stomach and pancreatic cancer and acts by multiple mechanisms – inhibition of carcinogen formation, as an antioxidant (ROS induced DNA damage/genotoxicity) mainly in the promotion stage of carcinogenesis, possibly as a mild prooxidant to

trigger the antioxidant signal transduction, anti-inflammatory and restores cell-to-cell communication. An inverse relation has been observed between plasma vitamin C and cancer mortality. It is also suggested that the antioxidant rich whole diet is more useful than the individual component (multiple combined effects especially of several polyphenolics). Consumption of 5 servings of fruit and vegetables containing 200-280 mg vitamin C /day could be recommended.^{33,34}

VITAMIN E

It is a fat soluble vitamin having potent free radical scavenging activity. It inhibits oxidative DNA damage and prevents cancer in lung (smokers), skin and liver. However, there are reports suggesting negative association between vitamin E intake and skin and intestinal cancer (tumour promoter).³³

SOY

In the past few years special attention has been focussed on soy foods for their potential health benefits. Soybeans are considered as the versatile plant foods with high quality proteins and minimal saturated fat. Soybeans are the source of naturally derived phytoestrogens. The chief constituents of soy beans are isoflavones genistein, diadzien and a minor constituent glycitein. Soybeans and soy foods contain approximately 1-3mg of isoflavones per gram. Isoflavones are considered to be the natural selective estrogen receptor modulators. They are known to possess beneficial role not only in cardiovascular diseases and osteoporosis but also in cancer. Numerous studies strongly support their beneficial role in inhibiting various cancers in experimental models. Several investigators have demonstrated that a high soy intake during childhood is associated

with a reduced risk of breast cancer.^{35,15 16} However, the evidence is lacking that consumption of soy/isoflavone during adult life is protective against breast cancer in Western countries.³⁵

Consumption of soy has been associated with reduced risk of colon cancer in some human populations as well as preclinically, but the substantial evidence is lacking³⁶. Guo et al 2004, have demonstrated that dietary soy isoflavones and estrone protected ovariectomized female mice with intact (Wild type, WT) or disrupted estrogen receptor alpha (ER α KO) from azoxymethane-induced colon cancer.³⁷ This protective effect of soy protein was suggested to be independent of estrogenic compounds in the diet. The dietary fibre (cellulose and others) might have altered gut microflora and thereby reduced the tumour cell growth. The N Soy (Nova Soy) and E1 (Estrone) reduced tumourigenesis in both WT and ER α KO mice. This effect was independent of ER α . Another hypothesis is that N Soy and E1 mediated their protective effects via ER β . One more hypothesis is that N Soy and E1 altered ER-independent pathways in the colon, it could be through locally generated metabolites in case of protection provided by E1.

ω -3 FATTY ACIDS

ω -3 Fatty acids are abundantly present in some fish and fish oils. They are also present in some vegetable sources like soybean and canola (low erucic acid rape seed) and marine foods. Alpha-linolenic acid is an ω -3 fatty acid present in vegetable oils; eicosapentaenoic acid and docosahexaenoic are predominantly present in fish and seafoods.³⁸ There is now strong epidemiological and experimental evidence to suggest their chemopreventive role in breast, colon and possibly in prostate cancer without

serious toxicity.^{39,40} They exert their chemopreventive activity by suppressing neoplastic transformation, cell growth inhibition, enhanced apoptosis and by antiangiogenic mechanisms; the common feature of these biological activities being the inhibition of eicosanoid production from ω -6 fatty acid precursors.

The long chain n-3 fatty acids have been shown to increase cell permeability,⁴¹ affect hormone and growth factor function^{42,43} by altering the composition of lipid bilayers of cell membranes critical for their antitumour activity. For the optimal activity to be seen in clinical trials, it is important to select the appropriate source of supplementation and specific n-3 fatty acid because the composition, quality and yield of n-3 fatty acid may vary depending on the geographical source which ultimately affect the compliance of the individual and thereby the results of the study. They can be tried in clinical trials both for primary and secondary prevention of breast cancer risk. They can be tried in combination also (e.g. with selective COX-2 inhibitors in colon cancer).

GREEN TEA

The source of green tea is *Camellia sinensis*. It is a popular beverage also in certain parts of the world. Epidemiological studies suggest that the incidence of prostate cancer is lower in Japanese and Chinese population as they consume green tea regularly. These studies were validated scientifically by Adhami et al, 2003.⁴⁴ They observed that the chief polyphenolic principle of the green tea, (-)-epigallocatechin-3-gallate (EGCG) prevented prostate cancer (a very common cancer in elderly males) acting via multiple molecular targets- apoptosis, cell growth inhibition, cyclin kinase inhibitor WAF-1/p-21-mediated cell growth dysregulation etc. The cyclic DNA microarray analysis demonstrated that EGCG resulted in induction

of genes which functionally exhibited growth inhibitory effects and repression of genes belonging to G-protein network in LNCaP cells. In TRAMP model (transgenic adenomatous carcinoma of the mouse prostate), oral administration of 0.1% solution of green tea polyphenol in tap water for 24 weeks significantly inhibited P_{Ca} development and metastasis. In further studies by these authors, oral feeding of green tea polyphenol as a sole source of drinking fluid to TRAMP mice significantly inhibited the VEGF, MMP-2 and MMP-9. Overall, green tea and its constituents act on multiple targets in experimental systems. Clinical studies are required to verify their chemopreventive potential.

RESVERATROL

Resveratrol is a stilbene polyphenolic compound present in grapes, peanuts and pines. Red wine is its chief source. It has been demonstrated to possess growth inhibitory effect on colon cancer cell lines.^{45, 46} Oral administration of resveratrol reduced the tumour load in Lewis lung carcinoma bearing mice and azoxymethane-induced aberrant crypt foci in colorectal cancer in rat.^{47, 48} Oral administration of resveratrol in drinking water showed efficacy in inhibiting experimental fibrosarcoma in mice.⁴⁹

In a recent study, dietary resveratrol did not affect intestinal tumourigenesis in APC^{Min/+} mice at the 3 doses tested in two independent experiments.⁵⁰ The authors suggested that resveratrol was an ineffective modulator of COX-2 expression in tumours, the decrease in PGE₂ levels was insufficient to modify tumour integrity and the levels of free resveratrol did not attain a sufficient concentration to duplicate the antitumourigenic effects observed in-vitro.

ANTHOCYANINS

Anthocyanins widely occur naturally in coloured fruits and vegetables such as blue

berries, red cabbages, purple sweet potato etc. They possess antioxidant properties. Anthocyanidins are the flavonoids structurally related to flavones. In a recent study, they have been shown to inhibit tumour promotion in mouse JB6 cells. They inhibited TPA-induced MAPK (mitogen activated protein kinase), AP1 (activator protein-1), activation and cell transformation.⁵¹

In grapes, proanthocyanidins are found in highest quantity in seeds and contribute to organoleptic properties of wines. Bomser et al, 1999 reported the antitumour activity of proanthocyanidin fraction of grape seeds against DMBA-TPA-induced skin tumourigenesis in mice.⁵² They suggested that suppression of ornithine decarboxylase and myeloperoxidase was related to antiproliferative and anti-inflammatory mechanisms. Inhibition of TPA-induced mouse skin ornithine decarboxylase and protein kinase-C was subsequently reported by these investigators.⁵³

Singletary and Meline, 2001 have further reported the effect of grape seed proanthocyanidins (GSP) on colon aberrant crypts and breast tumours in a rat dual-organ tumour model.⁵⁴ They found that feeding female Sprague-Dawley rats, diets containing 0.1-1% GSP was associated with significant inhibition (72-88%) of AOM-induced aberrant crypt foci and ornithine decarboxylase activity (20-56%) in distal third of the colon. However, feeding these doses of proanthocyanidins did not result in inhibition of DMBA-induced rat mammary tumourigenesis, which was attributed in part to the lack of effect of feeding GSP on liver carcinogen metabolizing enzymes, cytochrome P450 1A and glutathione-S-transferase. The above findings warrant further evaluation of GSP as potential colon cancer preventive agents.

Apart from above chemopreventives several other natural products from various

plant sources have also been investigated in several experimental systems as shown in table 1.

Table 1
Chemopreventive activity of plant derived natural products in various experimental systems

Plant	Part/Constituent used	Experimental model	Mode of action and active principle involved	Investigators	Ref. No
<i>Aloe arborescens</i> var. <i>natalensis</i> (Ghrithkumari)	Whole leaf	Azoxymethane-induced aberrant crypt foci in rat colorectum	Inhibition of phase I enzymes and activation of phase II enzymes, free radical scavenging effect, anti-inflammatory, reduction of cell proliferation. Barbaloin, isobarbaloin, lectin/lectin like substances	Shimpo et al, 2001	55
	Whole leaf	N-ethyl-N-nitroso-N-nitrosoguanidine-induced tumourigenesis in mice	Inhibition of tumour promotion, reduction in erythrocyte polyamine level. Barbaloin, isobarbaloin, aloinin	Chihara et al, 2000	56
<i>Allium sativum</i> (Garlic)	Bulb extract	DMBA-induced hamster buccal pouch carcinogenesis	Decreased lipid peroxidation and enhanced enzymatic and non-enzymatic antioxidants. Organosulphur compounds and vitamin C	Balasenthil et al, 2000	57
<i>Crocus sativus</i> (Saffron)	Crude extract from stigmas	Human tumour cell lines, Ames test and Colony formation assay	HeLa cells most susceptible. Twelve components – crocin 1,2,3, picrocrocin, HTCC-diglycosilkaempferol trans crocin-3, safranal, crocetin, cis-crocetin-3.	Abdullaev et al, 2002	58
<i>Curcuma longa</i> (Turmeric)	Curcumin	Human mammary epithelial and breast carcinoma cell lines	Inhibition of human telomerase reverse transcriptase and telomerase	Ramchandran et al, 2001	59
	Curcumin	DMBA-TPA-induced mouse skin papillomas,	Suppression of extracellular signal regulated kinase activity and NF-kB	Chun et al, 2003	60
		Leukocyte COX-2 assay		Plummer et al, 2001	61
	Curcumin	DMBA-induced skin papillomas	Antioxidant	Soudamini and Kuttan	62
<i>Cymbopogon citratus</i> Stapf (Lemongrass)	Oil	Azoxymethane-induced aberrant crypt foci in rat colon	Antioxidant	Suaeyun et al, 1994	63

<i>Digitalis purpurea</i> subspecies <i>heywoodii</i> (<i>Digitalis</i>)	Leaf extract	Human cancer cell lines [human renal adenocarcinoma (TK-10), human breast adenocarcinoma (MCF-7) and human melanoma (UACC-62)]	Induction of apoptosis, possibly DNA-topoisomerase poison (methanol extract most effective). Cardiac glycosides	Lazaro et al, 2003	64
<i>Humulus lupulus</i> (Hops)	Chalcone compounds	Human breast cancer cells (MCF-7), human colon cancer cells (HT-29), human ovarian cancer cells (A-2780) Sulforhodamine assay	Inhibition of DNA synthesis (XN was the most potent of the 5 Hops flavonoids). Prenylated flavonoids	Miranda et al, 1999	65
<i>Ocimum sanctum</i> (Tulsi)	Leaf extract	DMBA-induced skin papillomagenesis in Swiss albino mice	Antioxidant and detoxification mechanisms	Prashar et al, 1994	66
<i>Ocimum gratissimum</i>	Clocimum oil	DMBA-induced skin papillomas	Antioxidant, elevation in hepatic and skin GST, sulfhydryl (-SH), cytochrome b ₅ activity	Singh et al, 1999	67
<i>Citrus aurantium</i>	Orange peel oil	DEN-induced hepatocarcinogenesis	Restoration of gap junctions lost by DEN-induced hepatocarcinomas, possibly upregulation of connexin genes. Limonene	Bodake et al, 2002	68
<i>Polygonum cuspidatum</i>	Resveratrol isolated from the roots	Lewis-lung carcinoma bearing C57BL/6 mouse model	Inhibition of DNA synthesis in LLC cells and inhibition of LLC-induced neovascularization and capillary like network and tube formation (angiogenesis) of human umbilical vein endothelial cells (HUVEC)	Kimura and Okuda, 2001	47

ADVERSE EFFECTS

In our studies, oral administration of WSRE at 400mg/kg body weight daily for 4 months to Swiss albino mice did not show any mortality and this dose was found to be the maximal tolerated dose. In studies by other authors, *Withania somnifera* alone did not show any toxicity on long term administration. Subacute toxicity studies for 90 days of *W somnifera* in combination with *Panax ginseng* (Ginseng) were conducted in rats at 3 dose levels.⁶⁸ Food consumption, body weight, haematological, biochemical and histopathological parameters

were recorded. A significant increase in body weight, food consumption, liver weight and improvement in haematopoiesis were noticed. Brain, heart, lung, liver, spleen, kidneys, stomach, testis and ovaries were normal on gross examination. The combination of these two drugs did not result in subacute toxicity. In a different study, *W somnifera* has shown beneficial effect (reversal of cyclophosphamide induced toxicity in mice), rather than harmful effect.⁶⁹

No significant mortality was observed in mice when seed oil was administered orally at

the dose of 100 μ l/kg body weight one week before DMBA application and continuously for 24 weeks thereafter. Similarly no mortality was observed in mice when 100 μ l/kg body weight of the oil was administered orally one week before methylcholanthrene injection and continued for 15 weeks thereafter.

β -carotene either alone or in combination with retinyl palmitate has shown an increase in the incidence of lung cancer in active smokers.

Vitamin C has shown adverse effect in the following target tissues – bladder, buccal pouch and forestomach owing to its prooxidant activity.³³

Phytoestrogens exhibit structural resemblance with diethylstilbestrol and are therefore a matter of concern for their adverse effects. Infertility syndrome in sheep, higher plasma levels of isoflavone in infants fed soy milk formula on long term (in contrast to infants fed on human or cow's milk) has been reported.²⁰ Immunosuppression and lymphocytopenia (with genistein) and lymphocytopenia (with ipriflavone) have also been reported in animal and human studies.

When soy foods and isoflavone supplements (either from soy or red clover) were used for short term therapies of vasomotor symptoms associated with menopause, no serious side effects were associated.

ω -3 Fatty acids are generally well tolerated. The suspected tendency for increase in bleeding time and impairment of wound healing in animal and clinical studies respectively point to the caution to be observed in their use. The dietary long chain polyunsaturated fatty acids when supplemented to healthy infants in a double blind randomized clinical trial had no adverse effect on plasma amino acid concentration and indicators of protein metabolism. However, some amino

acids in formula fed infants warrant further studies.⁷⁰

Green tea is generally free from toxicity.

CONCLUSIONS

It is evident that both, the products of vegetable as well as animal origin have been tried to test their chemopreventive efficacy in animal model systems, cell lines and clinical trials. The refinement of dose and duration has not been achieved in majority of clinical testing systems. Certain natural products like β -carotene and α -tocopherol have been shown to provide either no benefit or increase the incidence of lung cancer in active smokers putting a question mark on their chemopreventive efficacy. However, such results need further explanation. There is a lack of perfect correlation between in-vitro and in-vivo animal data and the clinical safety and efficacy data. That is why a drug effective in preclinical setting may not be effective in clinical setting. The heterogeneity of cancer cell origin in animal and human beings as well as pharmacokinetic and pharmacodynamic differences in handling of drugs in animal/cell lines and human beings may account for such unpredictable results. Even then, majority of in-vitro assays predict the chemopreventive efficacy in animal models and animal efficacy in turn possibly reflects clinical efficacy, thus facilitating drug development process.

As obvious from the adverse effects, natural products are not absolutely safe, but relatively safer, caution needs to be exercised in their use. If natural products are used judiciously in balanced state they will hold promise with respect to their beneficial effects on human health in general and as chemopreventives, especially high risk population. Thus chemoprevention remains a highly desired, yet unfulfilled goal, to reduce the impact of human cancers. It is still too early stage to recommend a particular natural product for cancer chemoprevention for want of adequate safety and efficacy data in major clinical trials.

FUTURE DIRECTIONS

Transgenic animal models, gene-knock out mouse models and carcinogen-induced models may be developed in future to test chemopreventive efficacy. Only a few such models have been used in past.^{71,72} There is a need to focus on strategies to optimize the risk-benefit profile of chemopreventive agents. For this, approaches like pharmacodynamic modeling, drug combinations (to reduce the dose of individual agent and to target resistant cancer cells) and systematic development of dietary compounds may prove useful.¹² The need for nutritional genomics, proteomics and metabolic profiling using highthroughput screening is highly felt in this post genomic era.⁷³ Active participation and collaboration of industries (e.g. agricultural and pharmaceutical industries) and academic institutions will lead to comprehensive and integrated strategies for research and development of natural products for prevention of cancer. With the efforts of agricultural scientists it has been possible to generate high yield and quality nutrients from various sources.

Several other measures like surrogate end points biomarkers/ intermediate end point of cancer incidence and quality of life improvement, computer assisted image analysis, high volume gene-chip technology, immunochemistry and fluorescence techniques are highly promising for scientific validation in future.^{12,74}

Attempts may be made in future with α -carotene (from carrots) and lycopene (from tomato) to observe their chemopreventive potential in lung cancer.

Screening of persons for baseline cellular antioxidant status and genotype, to determine beneficiaries from exogenous antioxidants supplementation has been recommended.^{75,76}

The individual and population or community based studies to define cancer risk and intervention studies need to be carried out. The results of intervention studies may not match completely with that of epidemiological studies. Though individual targeting seems to be a highly challenging task, it needs to be tried.

Rigorous preclinical and clinical toxicity studies are continuously required to develop safe and effective chemoprevention for early intervention. Postmarketing surveillance studies will give an indication of a rare adverse effect of a chemopreventive agent under actual conditions of clinical use and therefore, are necessary.

Selective drug delivery such as by inhalation route may be more effective in future than the oral route for lung chemoprevention as the drug may be delivered directly to the respiratory epithelium in the aerosolized form. However, the nature of the drug is limiting factor for such delivery to be applicable. In this attempt, retinyl palmitate has been delivered in the aerosolized form and has been shown to reverse human bronchial dysplasia and metaplasia in prospective pilot study without adverse effects.⁷⁷

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