5. Anticarcinogenic property of medicinal plants: Involvement of antioxidant role

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Abstract. Carcinogens are involved in carcinogenesis, mutagenesis and genotoxicity. One of the best ways to minimize the detrimental effects of carcinogens is by the use of medicinal plants i.e., natural anticarcinogens. Naturally occurring anticarcinogenic principles present in medicinal plants, human diet have protective effects against carcinogens. These include flavonoids, phenolics, coumarins, carotenoids, anthraquinones, tannins, saponins etc. Most of these have excellent antioxidant property which is partially or completely responsible for their anticarcinogenic effects. The present chapter attempts to furnish a brief overview on natural products conferring anticarcinogenicity.

Introduction

Cancer is characterized by a rapid and uncontrolled formation of abnormal cells which may mass together to form a growth or tumor, or proliferate throughout the body indicating abnormal growth at other sites. Cancer is considered as one of the most fearsome causes of morbidity and
mortality in all over the world. Carcinogens are the physical, chemical and biological agents that can cause cancer. A plethora of synthetic and natural substances, apart from various genotoxic physical and biological agents, are known to act as carcinogenic or co-carcinogenic agents. There is increasing evidence that aberrations in somatic cells are not only involved in the carcinogenesis but can also cause genetic disorders like atherosclerosis, heart diseases and several other degenerative disorders [1]. Since the carcinogens are involved in the initiation and promotion of several human diseases, including cancer, the significance of novel bioactive phytocompounds in counteracting these carcinogenic effects is now gaining credence. Such chemicals that reduce the carcinogenesis caused by physical and chemical carcinogens are referred to as anticarcinogenic agents [2].

Numerous studies have been carried out in last four decades in order to identify compounds that might protect humans against carcinogenesis and its consequences. There are continued efforts all over the world to explore the rich biodiversity of edible as well as medicinal plants and other edible non-toxic plants in pursuit of the most effective phytoantimutagens. These bioactive compounds belong to a variety of different chemical groups such as phenolics, pigments, allylsulfides, glucosinolates, tannins, anthocyanins, flavonoids, phytosterols, protease inhibitors, and phytoestrogens. Many of these substances elicit, apart from their anticarcinogenic properties, additional beneficial effects such as activation of the immune system and/or protection against cardiovascular diseases [3].

The group of chemicals that cause cancer in human and animals are collectively referred to as carcinogens. Environmental pollution is associated with increased risk of cancer. Prevention of cancer and other related diseases can be pursued by avoiding exposure to recognized carcinogens, by favoring the intake of protective factors and by fortifying physiological defense mechanisms. Moreover, there is an increasing awareness that certain naturally occurring substances in plants and other source have protective effects against environmental carcinogens and also endogenous carcinogens. Hence research work related to the discovery, characterization and use of anticarcinogenic agents is receiving considerable attention. A large number of experimental reports have begun to appear in the scientific literature, wherein increasingly more natural anticarcinogens have been identified, isolated and found to possess significant cancer chemopreventive properties. In the present chapter promise of these natural anticancinogenic agents and their antioxidant role in mediating this effect has been focused upon.
Carcinogenesis, carcinogens and anticarcinogens

Carcinogenesis is a complex multi-stage process induced by various types of carcinogens that ultimately lead to the development of cancer. The common consensus among cancer researchers is that carcinogenesis, the process of malignant growth of normal cells, proceeds through a series of steps involving spontaneous changes in genetic material – DNA which is prone to damage by environmental agents especially radiation and chemical mutagens. Many intrinsic and extrinsic factors, interacting with one another, influence this process of carcinogenesis. Carcinogenesis includes three main phases: initiation, promotion and progression.

Initiation

Initiation is an irreversible process, which begins when the cells in normal tissues are exposed to a carcinogen. The carcinogen exposure causes damage to their genomic DNA that remains un-repaired. It also involves the uptake of the given carcinogenic agent followed by its transportation and distribution to the organs where its metabolism occurs. The reactive metabolite of the carcinogenic agent interacts with the cellular DNA followed by subsequent alteration in the DNA molecule thereby fixing genotoxic damage to produce mutation.

Promotion

As the name indicates it is expansion of the damaged cells to form an actively proliferating multi-cellular pre-malignant tumor cell population.

Progression

The third step is called ‘progression’ which is an irreversible process in which malignant cells grow and invade into the surrounding normal tissues or organs. Molecular mechanisms of tumor progression are not fully understood, but mutations and chromosomal aberrations are thought to be involved.

The substances which can induce carcinogenesis are known as carcinogens. These include, physical agents like ultra violet (UV) and X-rays cause the deletion of nucleotide. These agents produce a variety of lesions in DNA including strand break base damage and dimerisation of bases. Many diverse environmental, industrial, dietary and natural chemicals are capable of inducing mutation and genotoxic effects. Endogenous carcinogens are a group of cancer-causing compounds produced in vivo from harmless
precursors. There is evidence that mutation in somatic cells causes cancer, genetic disorders and many other degenerative disorders including arthritis and connective tissue disorders, hepatic disorders, neurodegenerative disorders, cardiovascular disorders, diabetes, chronic inflammation, ageing etc. The carcinogenic effects of genotoxic chemicals are additive, cumulative and sometimes irreversible [4].

An anticarcinogenic agent can prevent the transformation of a pro-carcinogenic compound into carcinogen, inactivate the carcinogen or otherwise prevent the reaction between mutagen and DNA. Another kind of anticarcinogens may induce, repress or inactivate directly or indirectly the enzymes of the DNA repair recombination and replication pathways [5].

Many substances reported to be antimutagens have themselves been shown to be promutagenic or carcinogenic. Chemicals belonging to such a category are termed ‘Janus carcinogens and mutagens’ after the ancient Roman God ‘Janus’ who had been depicted as having one head with two faces, one looking forward and one looking backward. Several other recent reports have also addressed or emphasized the biphasic nature of many active substance reported to modulate the mutagenicity or carcinogenicity of heterocyclic amines. The majority of these modulating substances are plant products or extracts. Extensive study of the antimutagenicity literature showed that a number of chemicals have both antimutagenic mutagenic effects. For instance, β-carotene was the first presumptive anticarcinogen to be included in large-scale, clinical intervention trials, but the trials were terminated prematurely upon revelation that β-carotene treatment was associated with an increased cancer incidence rather than the expected decrease. Other examples include testosterone, β-oestradiol, diethylstilbesterol, vanillin etc [6].

Unfortunately, currently available cancer chemotherapeutic agents being not tissue specific, insidiously affect the host cells especially bone marrow, epithelial tissues, reticulo-endothelial system and gonads thereby increasing morbidity. Hence, effective alternative strategies are critically necessary to control malignancy.

Extensive research in the last few decades on the detection and characterization of anticarcinogenic compounds from edible, non-edible, and medicinal plants and marine organisms has demonstrated a great diversity. Major emphasis has been laid on the flavonoids, phenolics, carotenoids, coumarins, anthraquinones, tannins, terpenoids, saponins etc., all of which are secondary plant metabolites. Others include vitamins, hormonal steroids and food products and dietary supplements. In recent years, there has been an increased interest in identifying the anticarcinogenic constituents of both dietary and medicinal plants all over the world.
Potentially anticarcinogenic plants include a number of common or ethnic group restricted edible plants, including cereals, pulses, vegetables, and spices and medicinal herbs and health tonic plants. Consumption of dietary green leafy vegetable, fruits, carrots, nuts, beverages, and green tea etc. can impart necessary protection against the genotoxic effects of carcinogens present in food, drugs, cosmetics, industrial wastes etc. and thereby help in prevention of several types of cancers and other degenerative diseases like atherosclerosis, diabetes mellitus, ischaemic heart disease, rheumatoid arthritis, neurological disorders etc.

These natural products play important role, not simply in the primary prevention of cancer, but also in the prevention of cancer recurrence, which is of utmost importance in determining survival. The search for non/less-toxic and broad-spectrum natural anticarcinogenic remedies should be extended through systematic screening of the unexplored wealth of the plant kingdom.

**Mechanism of anticarcinogenesis**

The major mechanisms of anticarcinogenesis can be broadly described as under [7]:

1. Chemical or enzymatic inactivation.
2. Prevention of formation of active species.
3. Scavenging.
4. Antioxidant or free radical scavenging.

### 1. Chemical or enzymatic inactivation

Many carcinogens, which are reactive, acting not only on DNA but also on proteins and enzymes, can be directly inactivated by a range of different chemicals. Anticarcinogenic properties have been associated with both inhibitors and inducers of cytochrome P-450 enzymes such as indole-3-carbinol. Inducers of phase-II metabolic enzymes such as glutathione transferase tend to inhabit a wide range of target carcinogens. e.g. isothiocyanates such as benzyl isothiocyanate and antioxidants such as 2, 3-tert butyl-4-hydroxy-anisole (BHA).

### 2. Prevention of formation of active species

Many genotoxic mutagens or carcinogens require metabolic activation or bio-activation to an electrophilic from (the active species) that can react with the DNA. Although these processes commonly occur in the liver, there is
increasing evidence for metabolic activation by other tissues also, especially for the GIT. N-nitro compounds are often formed in the stomach through a reaction from nitrite and secondary or tertiary amines.

3. Scavenging

A number of anticarcinogens are able to scavenge dietary carcinogens through binding or adsorption. In general, the carcinogen remains intact during this process but is unable to react with DNA. Chlorophyllin and some dietary fibers appear to act in this way.

4. Antioxidant and free radical scavenging

Free radicals can damage DNA and cause mutagenicity and cytotoxicity and thus play a key role in carcinogenesis. It is believed that reactive oxygen species (ROS) can induce mutations and inhibit DNA repair process, that result in the inactivation of certain tumor suppressor genes, leading to cancer. This appears to be the most commonly operative anticarcinogenesis mechanism of medicinal plant constituents. A wide range of anticarcinogenic agents from medicinal plants have excellent antioxidant or free radical scavenging activity e. g. carotenoids, flavonoids and phenolic compounds. These agents can directly scavenge most oxidative and nitrosative free radicals generated during carcinogenesis process thus reduce oxidative stress and prevent carcinogen-toxicity to the vital organs like liver, heart, kidney and brain. These agents can also mediate anticarcinogenesis by augmenting the endogenous both non-enzymatic (glutathione) and enzymatic (glutathione S-transferase, glutathione reductase, superoxide dismutase, catalase) antioxidant defense mechanisms prevalent in the body thereby ameliorating the oxidative impact generated due to carcinogenesis [8].

Natural anticarcinogenic agents

Extensive research in the last few decades on the detection and characterization of anticarcinogenic compounds from edible, non-edible, and medicinal plants and marine organisms has demonstrated a great diversity. Several authors have suggested that natural antimutagens may belong to any of the following major class of compounds. Major emphasis has been laid on the flavonoids, phenolics, carotenoids, coumarins, anthraquinones, tannins, terpenoids, saponins, and several others all of which are secondary plant metabolites. More than 500 compounds belonging to at least 25 chemical classes have been recognized as possessing anticarcinogenic effects [9]. In recent years, there has been an increased interest in identifying the
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Anticarcinogenic constituents of both dietary and medicinal plants all over the world. The major classes of antimutagenic compounds are briefly described below.

**Vitamins**

Vitamins have been extensively studied for their anticarcinogenic potential. Vitamin C and E have been shown to be anticarcinogenic against doxorubicin induced chromosomal aberrations [10]. Vitamin A, C and E were found to be antimutagenic towards methyl azoxy methanol (MAM) induced mutagenesis in *Salmonella typhimurium* strain TA100 [11]. Vitamin C (ascorbic acid) when administered concurrently with a pesticide showed significant decrease in the frequency of pesticide induced mutations [12]. Vitamin C has also good protective effect on xenobiotic-induced organ toxicity. Anticarcinogenic activity of vitamin C is due to its remarkable antioxidant effect [13].

**Flavonoids**

Flavonoids are a class of phytochemicals that possess anticarcinogenic properties in addition to a wide range of biological activities. Flavonoids present an important class of potent antimutagens and anticarcinogens. This potential effect is due to excellent antioxidative effect of flavonoid compounds. Distinct structure activity relationship (SAR) were detected when 56 flavonoids, 32 coumarins, 5 naphthoquinones and 12 anthraquinones were tested for their antimutagenic potencies, with respect to mutagenesis induced by 2-nitrofluoro 3-nitro fluoranthene and 1-nitropyrene in *S. typhimurium* TA98. Among flavonoids, all flavones and many flavonoids with phenolic hydroxyl group like leuteolin, kaempherol etc exhibited anticarcinogenic property. Chalcones and dihydrochaleones were potent antimutagens [14]. Quercetin, the most widely occurring plant flavonoid has potent anticarcinogenic and xenobiotic-induced organ damage preventive properties which are mediated by amelioration of oxidative stress.

A number of known flavonoids including flavonoid glycosides and isoflavones were reported to possess significant anticarcinogenic activity. Citrus juice flavonoids are reported to possess significant anticarcinogenic and antimutagenic properties [15]. Fourteen flavonoids including flavones and flavonol derivatives exhibited antimutagenic effect against induction of micronuclei by benzo[a]pyrene (Bap) in polychromatic erythrocytes (PCEs) of mice [16].

Isolation of two new isoflavones, fremontin and fremontone from the root of *Psorothamnus fremontii* was reported, which are highly active in the
inhibition of mutagenicity of ethyl methane sulfonate (EMS) at all concentrations tested [17]. Antimutagenic effect of hispidulin and hortensin, the flavonoids from *Millingtonia hortensis* was seen when tested against 2-amino anthracene, aflatoxin B$_1$ induced mutation [18]. Other flavonoids include glaberene from *Glycyrrhiza glabra*, quercetin, myricetin, kaemferol, hesperidin and other flavonoids isolated from *Ocimum javonica*. Antimutagenic activity all these flavonoids has been tested using *S. typhimurium* against various types of mutagens [19].

**Phenolic compounds**

Phenolic compounds are a widely studied group of polyphenolic compounds from natural food and medicinal plants and are also implicated in various biological activities [20]. In board sense, flavonoids and tannins are included under this category. Polyphenols are well known plant antioxidant principles responsible for several important pharmacological properties namely anticarcinogenic (antimutagenic and chemopreventive), antimicrobial, antidiabetic, hepatoprotective, cardioprotective etc. Certain phenolic compounds such as ellagic acid found in strawberries, raspberries, grapes, walnuts, etc. have been found to be anticarcinogenic due to their excellent antioxidant potential [21]. Also, the compounds such as epicatechin, (–)-epicatechin gallate, (–)-epigallocatechins, (–)-epigallocatechin gallate have been reported to be responsible for the anticarcinogenic activity of green tea and black tea [22, 23]. The antigenotoxic properties of tea leaf extracts in *Salmonella* umu-test was reported [24]. The antimutagenic activity of green tea catechins was demonstrated against oxidative mutagens such as tertiary butyl hydroxide, hydrogen peroxide using *Salmonella typhimurium* 102 tester strains [25]. Anticarcinogenic effect of green tea against smoke-induced mutations in humans was investigated and it was found that green tea can block the cigarette smoking-induced increase in sister chromatid exchange frequency [26].

Phenolics present in turmeric and clove, namely curcumin and eugenol respectively were found to inhibit the mutagenicity produced by direct acting mutagens such as N-methyl-N'-nitro-N-nitrosoguanidine using *S. typhimuricem* strains TA100 and TA1535, and eugenol inhibited tobacco-induced mutagenesis in Ames test [27].

**Anthraquinones**

The anticarcinogenic activity of anthraquinones (aloe-emodin-anthraquinone isolated from *Aloe barborescence*), were reported [19]. Among compounds structurally related to anthraquinones, anthrone, acridone and xanthonic exerted anticarcinogenicity, anthrone being the most patent one.
All naphthaquinones were potent antimutagens, plumbagin and 2-methyl-5-hydroxy naphthoquinone showed exceptional anticarcinogenicity [14].

**Carotenoids**

Carotenoids are tetraterpenoid compounds showing good antioxidant property. Several studies on carotenoids have shown that they prevent activation of procarcinogens. The water insoluble residues of some carotenoid rich fruits and vegetables such as apricots, arranges, brussels, sprouts, carrots, yellow-red peppers and tomatoes when sequentially extracted with several solvents and tested for inhibition of mutagenicities induced by aflatoxin B₁, benzo[a]pyrene (BaP), imidazoquinoline and cyclophosphamide (CP) in histidine deficient strains of *S. typhimurium*, number of BaP or CP-induced micronuclear in PCEs in bone-marrow of mice was reduced significantly by the carotenoids viz. lycopene, canthaxanthin, lutein and β cryptoxanthin [28]. All carotenoids are potential dietary cancer chemopreventive agents owing to their underlying antioxidant property.

Antimutagenicity of carotenoids extracted from five different types of green peppers (*Capsicum sp.*) has been reported on *S. typhimurium* tester strain YG1024, against the mutagenicity of some nitroarenes [29]. Anticarcinogenic activity of β-carotenre, canthaxanthin, β-carotene-8-apo-β-carotenal and 8-apo-β-carotene methyl ester showed a dose dependent decrease in the mutagenicity compared with 1-methyl-3-nitro-1-nitrosoguanidine and benzo[a]pyrene in *S. typhimurium* tester strain [30].

**Diterpenoids**

Diterpenoid like erythroxylidiol isolated from *Aquillaria agallocha* demonstrated antimutagenic as well as antitumor activity [31]. Four novel dibenzoate diterpenes, pulcherrimins A, B, C, and D obtained from roots of *Caesalpinia pulcherrima*, were found to be active in DNA repair-deficient yeast mutant [32].

**Coumarins**

Coumarins are chemically 2H-1-benzopyran-2-ones, widely distributed in the plant kingdom. A wide range of structures with varying complexity occurs in angiosperms. Coumarins have been shown to behave both as antimutagenic as well as anticarcinogen. For instance, coumarin, umbelliferone, 8-methoxypsoralen isolated from different plant sources have been found to be antimutagenic [19]. Psoralen from *Psoralea corylifolia* and imperatorin and
Ostholt from *Selium monniere* have been described to inhibit chemical carcinogenesis induced by benzo[a]pyrene. Non-toxicity and high activity of several coumarins including psoralen from *Selium monniere* was observed in the inhibition of carcinogenesis produced by benzo[a]pyrene [33].

**Tannins**

Tannins come under the board category of polyphenolics. Several tannins have been found to reduce the carcinogenic activity of a number of carcinogens. Their anticarcinogenic and antimutagenic potential has been related to their antioxidative property, which is important in protecting cellular oxidative damage including lipid peroxidation [34]. The anticarcinogenic effect of tannic acid was studied *in vivo* using micronucleus test and it was found that the frequency of micronuclei induced by mitomycin C, ethyl nitrosourea or 4-nitroquinoline-1-oxide in mouse bone marrow cell was decreased by the oral administration of tannic acid 6 h before the mutagen injection, they also observed the antimutagenic effect of tannic acid *in vivo* in the mouse spot test using male PW and female C57BL/10 mice [35]. Anticarcinogenic effects of (+) catechin, ellagic acid and gallic acid against known carcinogens were also reported [36].

**Saponins**

Saponins are plant glycosides showing soap like frothing with water. As many as thirteen saponins have been isolated from and identified in *Calendula officinalis, C. arvensis, Hedera helix*. Four from *C. arvensis* and three from *H. helix* showed anticarcinogenic activity against benzo[a]pyrene and a mutagenic concentrate from a smoker with a dose response relationship in modified liquid incubation technique of the *Salmonella* assay [37]. Ginseng saponin metabolites introduced by human intestinal bacteria were found antigenotoxic against benzo[a]pyrene induced clastogenecity [38].

**Miscellaneous compounds**

Ajoene and one of the derivatives of allicin are the organosulphur compounds found in garlic extract with significant anticarcinogenic activity [39]. Alkaloids and triterpenoids were also reported to possess cancer chemopreventive actions [40]. Various other miscellaneous groups of phytocompounds, such as caffeine, trigonelline, and piperine, have been demonstrated to possess anticarcinogenic properties [6]. Xanthones such as euxanthone and 1,5 dihydroxy-8-methoxyxanthone isolated from *Visma*
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*amazonica* display considerable antimutagenic activity against 2-aminoanthracene and EMS [41]. Chemopreventive effect of *Indigofera aspalathoides* extract against 20-methylcholanthrene-induced carcinogenesis in Swiss mice was observed through the alleviation of oxidative stress [42].

An 80% ethanol extract of lemon grass (*Cymbopogon citrates*) was found to be antimutagenic to various known mutagens in *Salmonella* mutation assay [43]. This extract was known to inhibit the formation of azoxymethane induced DNA adducts and aberrant crypt foci in the rat colon [44]. Essential oil from lemon grass (*Cymbopogon citrates*) that is used as a constituent of ‘Lemongrass Tea’ in central and Southern parts of India was found to possess antimutagenic activity against lead nitrate and cyclophosphamide induced micronuclei and chromosomal aberration *in vivo* in Swiss albino mice. Its chief constituent citral, a monoterpenoid was reported to possess anticlastogenic activity [45].

Numerous references of anticarcinogenic activities of various plants and their constituents are found in the literature, and newer reports of pharmacological screening continually appear in the scientific literature.

**Plant derived food products as anticarcinogens**

Dietary components especially from plant origin exhibit a wide range of activities that can prevent carcinogenesis. Naturally occurring plant based substances in foods have been shown in laboratory experiments to serve as dietary anticarcinogens. Extensive works were carried out demonstrating the antimutagenic and anticarcinogenic potential of some commonly consumed culinary spices and vegetables such as turmeric, mustard, green leafy and allium species of vegetables [5]. A human intervention study with vegetable products containing different carotenoids showed that the supplementation of diet with tomato, carrot or spinach products resulted in significant decrease in lymphocyte DNA damage [46]. All of these food products are rich in polyphenolics (especially flavonoids) and carotenoids which are putative antioxidants.

The anticarcinogenic properties of two dietary supplements namely, garlic and mustard oil were observed against the clastogenic activity of sodium arsenite [47]. Garlic extract was found to inhibit the mutagenicity produced by direct acting mutagens such as N-methyl-N'-nitro-N-nitrosoguanidine and sodium azide using *S. typhimuum* strains TA100 and TA1535. This antimutagenic effect of garlic has been attributed to its organosulphur constituents as stated above [39]. Casein showed a strong antimutagenic activity *in vivo* and *ex vivo* in the DNA repair host mediated assay and liquid suspension assay, respectively [48]. Yogurt (a fermented
milk product) was reported to be antimutagenic [49]. Antimutagenic effect of guava (*Psidium guajava*) fruit was reported [50]. The mechanism of anticarcinogenic effects of mushrooms was found to be by direct chemical interaction with the carcinogens viz. aflatoxin B₁, benzo[α]pyrene and acridine or inhibition of the activation process in the case of procarcinogens [51]. Asafoetida and turmeric extracts were found to inhibit microsomal activation dependent mutagenicity of 2-acetamido fluorine; similar results were also obtained using Indian spinach leaf extract, curcumin and eugenol which are phenolics present in turmeric and clove respectively [27]. Alkyl-resorcinols, amphiphillic compounds commonly found in cereal grains demonstrated antimutagenicity in Ames test [52]. Recently the present author reported the ameliorative effect of pointed gourd (a common vegetable in India) against sodium arsenite induced organ toxicity in rats was mediated by alleviation of oxidative stress [53].

**Conclusion**

Many of the environmental pollutants, residues of pesticides and toxins present in food drugs and chemicals are common agents of carcinogenesis in human population. Hence there is a need to find natural anticarcinogenic agents having the potential to prevent or at least delay the onset and severity of genetic damage, which can be incorporated into the regular diet of an individual. Potentially anticarcinogenic plants include a number of common or ethnic group restricted edible plants, including cereals, pulses, vegetables, and spices and medicinal herbs and health tonic plants. Most of they possess very good antioxidative potential. Their anticarcinogenic effect can be partially or completely attributed to the alleviation of carcinogen-induced oxidative stress by multiple mechanisms. Consumption of natural antioxidant rich dietary green leafy vegetable, fruits, carrots, nuts, beverages, and green tea etc. can impart necessary protection against the genotoxic effects of carcinogens present in food, drugs, cosmetics, industrial wastes etc. and thereby help in prevention of cancer. The search for non-toxic and broad-spectrum natural anticarcinogens with antioxidative potential should be extended through systematic screening of the unexplored rich diversity of the plant kingdom.

**References**


