Introduction
INTRODUCTION

Cancer chemoprevention by plant derived small molecules

The use of traditional medicines and plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). An increasing reliance on the use of plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998). Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and wellbeing and the bioprospecting of new plant-derived drugs (Hoareau and DaSilva, 1999; Harvey, 2008). Furthermore, epidemiological studies have provided compelling evidence that link cancer risk and food habits. The consumption of fruits and vegetables as well as grains, has been strongly associated with reduced risk of cardiovascular diseases, cancer, diabetes, Alzheimer's disease, cataract and age related functional decline (Willett, 1994; Willett, 1995; Temple, 2000). Dietary consumption of foods and herbal medicines is a convenient method of administering potential chemopreventive phytochemicals in a cost-effective manner. Numerous reports suggest a protective role for a diet rich in fruits and vegetables (Terry et al., 2001; van Duyn and Pivonka, 2000; Steinmetz and Potter, 1996). Overall, diets high in vegetables and fruits (>400 g/d) may prevent at least 20% of all cancers. Some of the most convincing evidence of the health benefits of fruit and vegetable consumption relates to the reduced risk of gastrointestinal cancers, such as those associated with the mouth, pharynx, esophagus, stomach, colon, and rectum (Terry et al., 2001). The proposed mechanisms by which vegetables and fruits reduce cancer are multiple and complex. Various stages of carcinogenesis may be inhibited, and various in vitro or in vivo systems are used to model these inhibitory effects in preclinical studies. Therefore, it is logical to acquire compelling in vitro data prior to performing tests with animal models, and it is necessary to isolate and
characterize active chemical principles before moving on to the animal model and clinical studies. Figure 1 shows the sources of some of the commonly used plant sources and figure 2 shows the chemical structures of some of the plant derived molecules under different phases of investigation.

Several of these plant derived molecules are known to possess a wide spectrum of pharmacological properties. The area where plants and their constituents are considered to have a major impact on longevity and quality of life is in the chemoprevention of cancer. It has been recognized that the active principle of plant products are mainly secondary metabolites. Plants have an almost limitless ability to synthesize secondary metabolites, of which at least 12000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases these substances serve in plant defense mechanism against predation by microorganisms, insects and herbivores. The cancer inhibitory action of a variety of human nutrients derived from plants as well as non-nutritive plant derived constituents (phytochemicals) has been confirmed in different animal tumor models (Dragsted et al., 1993; Pezzuto, 1996) and has led to an increased emphasis on cancer prevention strategies in which these dietary factors are utilized. Polyphenols are widely distributed plant derived dietary constituents and have been implicated as the active components in a number of herbal and traditional medicines (Wollenweber, 1988).

Cells in humans and other organisms are constantly exposed to a variety of oxidizing agents termed as free radicals / reactive oxygen species (ROS). Damage to DNA by ROS has been widely accepted as a major cause of cancer (Ames, 1983). Due to highly reactive nature, they can cause oxidation of various biomolecules such as DNA, proteins and lipids resulting in cellular injury and death (Freidovich, 1999; McCord, 2000). Cells utilize a number of antioxidant defense systems (both enzymatic and non-enzymatic) to prevent the accumulation of ROS and to keep themselves in a state of redox homeostasis (Klaunig and Kamendulis, 2004; Clarkson and Thompson, 2000). However, under the condition of imbalance in redox status, high levels of ROS can induce apoptosis.
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(Lau et al., 2004), whereas chronic low levels of ROS promote cardiovascular diseases (Barchowsky et al., 1996) and carcinogenesis (Lau and Chiu, 2006).

![Spices](image1)

**Spices**

- Asian ginger (1) - (Alpinia galanga)
- Fennel (2) - (Foeniculum vulgare)
- Sesame seed (3) - (Sesamum indicum)
- Turmeric (4) - (Curcuma longa)
- Cloves (5) - (Eugenia caryophyllus)
- Red chili (6) - (Capsicum annum)
- Fenugreek (7) - (Trigonella foenum graecum)
- Poppy seed (8) - (Papaver somniferum)
- Gamboge (9) - (Garcinia hanburyi)
- Onion (10) - (Allium cepa)
- Onion seed (11) - (Nigella sativa)
- Holy basil (12) - (Ocimum sanctum)
- Pomegranate (13) - (Punica granatum)

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- Aloe (1) - (Aloe vera)
- Veldt-grape (2) - (Cissus quadrangularis)
- Picroliv (3) - (Picrorhiza kurroa)
- Himalayan fir (4) - (Abies webbiana)
- Chitrak (5) - (Plumbago zeylanica)
- Beauty berry (7) - (Callicarpa macrophylla)
- Pink trumpet tree (8) - (Tabebuia avellanedae)
- Bloodroot (9) - (Sanguinaria canadensis)
- Guggulu (10) - (Commiphora mukul)
- False pepper (11) - (Embelia ribes)
- Rohitukine (12) - (Dysosyllum binecitarifrum)
- Ashwagandha (13) - (Withania somnifera)
- Indigo (14) - (Polygonum tinctorium)
- Pinecone ginger (15) - (Zingiber zerumbet)

Figure 1: Some sources of natural cancer preventive compounds. (From: Gullet et al., 2010)
Fruits & Vegetables

- Cauliflower (Brassica oleracea)
- Mullberry (Morus nigra)
- Artichoke (Cynara cardunculus)
- Grapes (Vitis vinifera)
- Soybean (Glycine max)

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- Lacquer tree (Rhus verniciflua)
- Goldenseal (Hydrastis canadensis)
- God of thunder vine (Tripterygium wilfordii)
- Smoke tree (Cotinus coggygria)
- Evodia (Evodia rutaecarpa)
- Song gen (Phellinus linteus)
- Magnolia (Magnolia officinalis)

Others

- Cashew nut (Anacardium occidentale)
- Horse chestnut (Aesculus hippocastanum)
- Palm (Elaeis guineensis)
- Elephant's foot (Elephantopus scaber Linn)
- Hop (Humulus lupulus L.)
- Ginger lily (Hedychium coronarium)
- Cork bush (Mundulea sericea)
- Tropical rose mallow (Hibiscus vitifolius)
- Oleander (Nerium oleander)

Figure 1 (continued)
Thus, to prevent or slow down the oxidative stress induced by free radicals, sufficient amounts of antioxidants are needed to be consumed. The non-nutritive secondary metabolites are considered as effective antioxidants. These include terpenes and terpenoids, polyphenols, and carotenoids that may help protect the cells from oxidative damage, thereby lowering the risk of chronic diseases. Cancer development is a long term process and proceeds through sequential morphological changes from normal, preneoplastic, and premalignant lesions to highly malignant neoplasms. It is believed that constituents of plant products have the ability of inhibiting all three stages of chemical carcinogenesis, namely tumor initiation, promotion and progression (Jang et al., 1997).

Next to water, tea is the most popular beverage consumed in the world and is distinguished by the presence of a group of polyphenols called catechins. A growing body of evidence from laboratory animal studies demonstrates that tea consumption has an inhibitory effect on carcinogenesis at various organ sites. For example, oral administration of tea infusion can inhibit the development of experimental rodent skin tumors (Wang et al., 1992), growth of implanted tumor cells (Hera et al., 1989), invasion and metastasis of malignant tumors (Liu et al., 2001; Kuroda and Hara, 1999) and angiogenesis (Jung and Ellis, 2001; Cao and Cao, 1999). Four major green tea components are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC), all of which are also present in black and other teas. The mechanisms by which tea polyphenols may act include inhibition of mutagenesis (Muto et al., 1999; Okuda et al., 1984; Kuroda, 1996), genotoxicity (Sasaki et al., 1993; Xu et al., 1996), transformation (Komatsu et al., 1997; Komatsu et al., 1994), cell proliferation (Ahmad et al., 2000; Liang et al., 1999) and angiogenesis (Jung and Ellis, 2001; Cao and Cao, 1999). This compound has been shown to exert anticarcinogenic effects in a diverse array of animal and cell culture models. Surh and Chun (2007) have summarized mechanistic and anti-carcinogenesis studies conducted with curcumin, of which inhibition of tumor promotion is predominant. Curcumin inhibits 12-O-tetradecanoyl-phorbol-13-acetate-induced inflammation, hyperplasia, proliferation, ornithine decarboxylase,
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1'-Actoxychavicol acetate (1)  Anethole (2)  Sesamin (3)  Curcumin (4)  Eugenol (5)

Capsaicin (6)  Diosgenin (7)  Noscapine (8)  Gambogic acid (9)

Quercetin (10)  Thymoquinone (11)  Ursolic acid (12)  Ellagic acid (13)

Ayurvedic Medicine

Emodin (1)  Piceatannol (2)  Picroliv (3)  Pinitol (4)  Plumbagin (5)

Acetyl-11-keto-β-boswellic acid (6)  Betulinic acid (7)  β-Lapachone (8)  Sanguinarine (9)  Guggulusterone (10)

Embelin (11)  Flavopiridol (12)  Indirubin 3'-monoxime (13)  Withanolide (14)  Zerumbone (15)

Figure 2: Structure of a few natural cancer preventive compounds. (From: Gullet et al., 2010)
Fruits & Vegetables

- Indole 3-carbinol (1)
- Morin (2)
- Silymarin (3)
- Resveratrol (4)
- Genistein (5)

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- Butein (1)
- Berberin (2)
- Celastrol (3)
- Fisetin (4)
- Evodiamine (5)
- Hispolon (6)
- Honokiol (7)

Others

- Anacardic acid (1)
- Escin (2)
- γ-Tocotrienol (3)
- Isodeoxyelephantopin (4)
- Xanthohumol (5)
- Coronarin-D (6)
- Deguelin (7)
- Gossypin (8)
- Oleandrin (9)

Figure 2 (continued)
reactive oxygen species (ROS) generation, COX, and lipoxygenase in mice. Curcumin has synergistic activity with genistein (Verma et al., 1997), green tea (Khafif et al., 1998), and embelin (Sreepreeya and Bali., 2006), and enhances the efficacy of the anti-cancer drugs 5-fluorouracil (5-FU) (Koo et al., 2004) and gemcitabine (Kunnammakara et al., 2007), and the vinca alkaloid vinorelbine (Sen et al., 2005). Similarly, because of its potent antioxidant effect, lycopene has drawn much attention as a cancer preventive agent. Population studies have shown that high intake of lycopene is inversely associated with the incidence of certain types of cancers, including those of the digestive tract, prostate, and cervix. Consumption of tomato products may prevent disease progression in benign prostate hyperplasia (BPH) and prostate cancer (Schwarz et al., 2008; Kim et al., 2003). A combination of vitamin E, selenium, and lycopene dramatically inhibited prostate cancer development and increased disease-free survival in an animal model (Venkateswaran et al., 2004). The anti-tumor activity of thymoquinone (TQ) seems promising both for chemoprevention and preventing drug-induced toxicity. Additionally, this compound exhibits selectivity to cancer cells, since normal human pancreatic ductal epithelial cells and mouse keratinocytes are resistant to the apoptotic effects of TQ (Banerjee et al., 2009; Gali-Muhtasib et al., 2004).

There are many other nutritive and non-nutritive plants and natural compounds currently under investigation for their potential cancer chemopreventive effects. These include ellagic acid, some triterpenes (such as lupeol, betulinic acid, ginsenosides, oleanolic acid, etc), and ginkolide B. Among the triterpenes, lupeol (Chaturvedi et al., 2008) and betulinic acid (Cichewicz et al., 2004) have been extensively investigated for their chemopreventive activities (Chaturvedi et al., 2008) and showed a broad spectrum of activity against multiple cancer types in both cell culture and animal models. *Ginkgo biloba* extracts and its constituent ginkolide B also have been tested for their chemopreventive activities and showed some promise against several cancer types (Ye et al., 2007; DeFeudis et al., 2003). Further, natural compounds have been presumed to be safer than synthetic compounds due to their presence in the diet, wide availability and tolerability.
Moreover, since these molecules have simple molecular structures and relatively low toxicity, they offer a promise for designing new drugs as anticancer, anti-infective agents and many other therapeutic agents (Table 1).

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**Biosynthesis of secondary metabolites**

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as “secondary metabolites” of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Harborne, 1973; Okwu, 2004). Secondary metabolites of plants execute a vast array of important functions in plants (Croteu et al., 2000). They are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2004). These metabolites often have an ecological role in regulating the interactions between plants, microorganisms, insects and animals. They can be defensive substances, antifeedants, attractants and pheromones. The secondary metabolites are derived from branch points with primary metabolism. The major classes of secondary metabolites and some of their biological properties are discussed below.

- **Terpenoids and steroids**

The terpenoids form a large and structurally diverse family of natural products derived from C5 isoprene units joined in a head-to-tail fashion (figure 3). Typical structures contain carbon skeletons represented by \((\text{C}_5\text{)n}\), and are classified as hemiterpenes \((\text{C}_8)\), monoterpens \((\text{C}_{10})\), sesquiterpenes \((\text{C}_{15})\), diterpenes \((\text{C}_{20})\), sesterterpenes \((\text{C}_{25})\), triterpenes \((\text{C}_{30})\) and tetraterpenes \((\text{C}_{40})\) (Figure 1.2).
Terpenoids and steroids have been known for several centuries as components of fragrant oils obtained from leaves, flowers and fruits. Monoterpenes, with sesquiterpenes are the main constituents of essential volatile oils.

Essential oils have been seen to induce cytotoxicity in bacteria and eukaryotes as a result of collapse of the proton pump and depletion of the ATP pool (Richter and Schlegel, 1993; Novgorodov and Gadz, 1996; Vertesi et al., 1997; Knobloch et al., 1989; Sikkema et al., 1994; Hclander et al., 1998; Ultee et al., 2002; Di Pasqua et al., 2006; Turina et al., 2006). Essential oils have also been found to coagulate in the cytoplasm (Gustafson et al., 1998) and damage lipids and proteins (Ultee et al., 2002; Burt, 2004). Permeabilization of outer and inner mitochondrial membranes leads to cell death by apoptosis and necrosis (Yoon et al., 2000; Armstrong, 2006). It seems that chain reactions from the cell wall or the outer cell membrane invade the whole cell, through the membranes of different organelles like mitochondria and peroxisomes. These effects suggest a phenolic-like prooxidant activity (Sakagami and Satoh, 1997; Cowan, 1999; Fukumoto and Mazza, 2000; Sakihama et al., 2002; Burt, 2004; Barbehenn et al., 2005). Because of a great number of constituents, the essential oils seem to have no specific cellular targets (Catson et al., 2002).

Geraniol, a major component of geranium, rose, citronella and lemon oils, has been shown to possess insecticidal (Jeon et al., 2009; Traina et al., 2005), antimicrobial (Si et al., 2006; Inouye et al. 2001), antioxidant (Tiwari and Kakkar, 2009), anticancer (Burke et al., 1997; Wiseman et al., 2007) and anti-inflammatory properties (Ji et al., 2002). Isomer of geraniol, linalool, found in the oil of a garden herb, clary sage, has also been reported to have anti-inflammatory, antinociceptive and analgesic effects (Peana et al., 2003). The anti-inflammatory property of citrus, a constituent of lemon oil, has been shown to be a result of regulation of COX-2 activity. Herbs, including the mints, the sages and rosemary, are the source of many terpenes. Menthol possesses useful physiological properties including local anaesthetic and refreshing effects. A number of diterpenoids possess antitumour activity (Wang et al., 2011; Ji et al, 2011). One of these, paclitaxel, was originally obtained from the bark of the Pacific yew, Taxus brevifolia, but it is now made
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semi-synthetically from more readily available taxanes. Studies on the mechanism of action of paclitaxel revealed that it blocks depolymerization of microtubules (Schiff et al. 1979). It possesses powerful activity against a number of tumors and it is used in the treatment of breast and ovarian cancer. On the other hand, some of the diterpenoid constituents of Euphorbia species have powerful skin irritant and co-carcinogenic properties.

![Terpenoids and steroids biosynthesis pathway](image)

Figure 3: Terpenoids and steroids biosynthesis pathway.
Diosgenin, a steroidal sapogenin from the Mexican yam, and hecogenin, from sisal, are used as starting materials for the partial synthesis of the steroidal hormones (Xu et al., 2003). The steroidal alkaloids, such as solasodine, occur in plants of the Solanaceae, including tomato and potato. A number of plant steroids possess a useful pharmacological activity such as antiproliferative (Perrone et al., 2012) and cardioprotective activity (D'Urso et al., 2008). These include the digitalis glycosides (cardenolides) from the foxglove, Digitalis lanata. These are used in the treatment of heart failure.

**Alkaloids**

Some of the first natural products to be isolated from medicinal plants were alkaloids. The alkaloids are a highly diverse group of natural products related only by the presence of organic nitrogenous bases. They are derived from unrelated pathways (figure 4). Alkaloids are found mainly in plants, but also to a lesser extent in microorganisms and animals. One or more nitrogen atoms are present, typically as primary, secondary, or tertiary amines, and this usually confers basicity to the alkaloid. However, the degree of basicity varies greatly, depending on the structure of the alkaloid molecule, and the presence and location of other functional groups. Indeed, some alkaloids are essentially neutral. Alkaloids containing quaternary amines are also found in nature. The biological activity of many alkaloids is often dependent on the amine function being transformed into a quaternary system by protonation at physiological pHs. Many alkaloids have neuroactive properties and interact with the receptors at nerve endings. This is not surprising, since many alkaloids have fragments buried within their overall structure which resemble the natural substances (the neurotransmitters) that bind to these receptors. The antidepressant activity of piperine has been related to its ability to scavenge ROS in corticosterone-induced neurotoxicity in PC-12 cells (Mao et al., 2011). Piperine and the cis-cis isomer, chavicine, are found in the fruit of the pepper Piper nigrum, and are responsible for the sharp taste of pepper. The fruit of the betel palm, Areca catechu, produces a mild stimulant, arecoline (Yoganathan, 2002).
The coca plant, *Erythroxylon coca*, is known for the production of cocaine, has as a paralyzing effect on sensory nerve endings and produces a sense of euphoria. The roots, leaves and berries of a number of poisonous plants of the Solanaceae, including deadly nightshade (*Atropa belladona*), henbane (*Hyoscyamus niger*) and thorn apple (*Datura stramonium*), have been a rich source of therapeutically important tropane alkaloids (Balandrin et al., 1993). These plants provided some of the hallucinogenic ‘sorcerer’s drugs’ of the Middle Ages. Atropine and the related epoxide, scopolamine, are two examples with a powerful biological activity (Wu et al., 2011). Atropine dilates the pupils of the eye and its derivatives are used in ophthalmology. The tobacco plant, *Nicotiana tabacum*, produces the toxic alkaloid nicotine, which is the major neuroactive component of tobacco smoke (Purkis et al., 2011). Moreover, nicotine is suggested to exert either non-receptor-mediated biological effects or, more importantly, act on the different subtypes of nicotinic...
brain receptors, in particular those associated with the nigrostriatal dopaminergic pathway, thus presenting its therapeutic potential for Parkinson's disease (Iluriez et al., 2011). There are many alkaloids that are derived from simple phenylalkylamine C6–C3 units. The Chinese medicinal herb, *Ephedra sinica*, has been used for many years for the alleviation of bronchial problems (Li et al., 2004; Lee, 2011). The herbal composition GGEx18 from *Laminaria japonica*, *Rhizoma palmatum*, and *Ephedra sinica* has been shown to reduce obesity via skeletal muscle AMPK-stimulated expression of PPARα and its target enzymes for fatty acid oxidation (Shin et al., 2011). Extraction of the plant gave ephedrine and its epimer, pseudoephedrine. Alkaloids such as mescaline have been found in hallucinogenic plants such as the Mexican peyote cactus, *Lophophora williamsii*. The benzylisoquinoline alkaloids, which are formed via phenylalanine or tyrosine, are widespread. They have been found in various plants from the Annonaceae, Lauraceae, Rhamnaceae, Ranunculaceae and Papaveraceae families. The best known source of these alkaloids is the opium poppy, *Papaver somniferum*, in which they co-occur with the more complex morphine series (Ziegler et al., 2009). Typical examples include papaverine, which has been used as a muscle relaxant. Berberine is a yellow pigment from *Berberis* and *Mahonia* species which was used as a mild antibiotic in the treatment of sores (Imanshahidi and Hosseinzadeh, 2008). In addition to neuroactive properties, a number of naturally-occurring alkaloids are important drugs in the control of various cardiovascular conditions. Quinidine, isolated from the bark of *Cinchona* tree, is an important anti-arrhythmic drug. Reserpine is known to possess antihypertensive properties and papaverine is used as a peripheral vasodilator. In addition, theophylline, a xanthine alkaloid, is an important bronchodilator used to control asthma in children.

**Phenylpropanoids**

The shikimate pathway provides an alternative route to aromatic compounds, particularly the aromatic amino acids L-phenylalanine, L-tyrosine and L-tryptophan (figure 4). This pathway is employed by microorganisms and plants, but not by animals, and accordingly the aromatic amino acids feature among those essential amino acids for humans. Phenylalanine and tyrosine form the basis of
C6C3 phenylpropane units found in many natural products, e.g. cinnamic acids, coumarins, lignans, and flavonoids, and along with tryptophan are precursors of a wide range of alkaloid structures. In addition, it is found that many simple benzoic acid derivatives, e.g. gallic acid and p-aminobenzoic acid (4-aminobenzoic acid) are produced via branch points in the shikimate pathway.

Phenolic compounds, including their subcategory, flavonoids and other plant derived antioxidants have demonstrated protective effects in carcinogenesis. Epidemiologic studies have consistently demonstrated an inverse relationship between flavonoid consumption and risks for certain types of cancer (Russo, 2007). Numerous studies have reported flavonoid mediated antiproliferative effects against human and rodent ovarian, leukemic, intestinal, lung, breast and bladder cancer cells. For example, quercetin strongly suppresses transformed OVCA 433 human ovarian cancer cell growth. Moreover, quercetin inhibits normal proliferation in cultured primary ovarian adenocarcinoma tumor cells (Scambia et al., 1994 a, b). At low concentrations, quercetin inhibits DNA synthesis and growth in HL60 human promyelocytic leukemia cells (Uddin and Choudhry, 1995; Kang and Liang, 1997). The citrus flavonoid tangeretin suppresses HL60 proliferation (measured as tritiated thymidine incorporation into DNA) even more strongly (Hirano et al., 1995), while genistein is inhibitory at concentrations similar to conventional anticancer drugs such as doxorubicin and methotrexate (Hirano et al., 1994). Genistein, kaempferol and quercetin inhibit the proliferation of human colon cancer cells Caco-2 and HT29 (Aguiló et al., 1994; Kuo, 1996). Curcumin is cytostatic in several hormone dependent (MCF-7 and T-47D) and independent (SK-BR3, BT-20 and MDA-231) breast tumor cell lines (Mehta et al., 1997) while genistein and quercetin, in addition to their antiproliferative action, appear to alter the metastatic potential of rat breast adenocarcinoma cells, measured as a reduced ability to migrate within collagen matrix (Lu et al., 1996). Quercetin inhibits tritiated thymidine uptake and proliferation in several non-small-cell lung carcinoma cell lines and reduces bromodeoxyuridine incorporation in primary lung tumor slices (Caltagirone et al., 1997).
Introduction

- Polyketides and fatty acids

Polyketides constitute a large class of natural products grouped together on purely biosynthetic grounds. Their diverse structures can be explained as being derived from poly-β-keto chains, formed by coupling of acetic acid (C₂) units via condensation reactions,

\[ n\text{CH}_3\text{CO}_2\text{H} \rightarrow [\text{CH}_2\text{CO}]_n^- \]

Included in such compounds are the fatty acids, polyacetylenes, prostaglandins, macrolide antibiotics and many aromatic compounds, e.g. anthraquinones and tetracyclines.

Putative chemopreventive and anticancer activity of plant derived molecules

Carcinogenesis is an extremely complex process and generally described as initiation, promotion and progression. In carcinogenesis, cells undergo transformation, acquiring specific characteristics enabling the development of full malignancy. These features include self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Ultimately, these acquired characteristics of cancer are underpinned by genomic instability due to environmental stress. The loss of function in cells to repair DNA damage and ensure correct chromosomal segregation during mitosis enables evolving populations of premalignant cells to develop the characteristic hallmarks of cancer. These underlying principles represent prime targets for chemopreventive interventions. An anticancer agent should be able to interfere with one or more of the phases of carcinogenesis (Russo, 2007). Chemopreventive activity is often the result of a combination of several distinct intracellular events, as opposed to a single biological response (Surh, 2003; Milner, 2008). This has led to a substantial increase in interest in the anticancer and chemopreventive properties of plant...
derived products. The most important mechanisms proposed can be subdivided into the following aspects:

**Antioxidant activity**

In a normal living cell, a finely balanced redox homeostasis exists, whereby the level of ROS is counteracted by antioxidant defense systems. Increased levels of reactive species have been clearly implicated in the development of cancer with direct damage of DNA proposed as the primary mechanism (Evans et al., 2004). Antioxidants may be characterized as inhibiting the generation of ROS or direct scavenging of free radicals. Through this process, antioxidant compounds themselves become radicals, though much less reactive, preventing damage to cellular biomolecules (Evans et al., 2008). Antioxidant action also extends to numerous other cellular physiological processes, including modulation of signal transduction, regulation of gene expression of detoxifying and antioxidant enzymes, and factors affecting vascular homeostasis (Frankel and German, 2006). Interestingly, antioxidants also have a role as prooxidants (discussed below). Repeated mild stress may shift the defense system to a higher steady state, producing an adaptive beneficial effect on the cell (Moskaug et al., 2005). For example, luteolin, kaempferol, quercetin and myricetin at relatively low concentrations (50-100 μM), significantly reduce DNA strand breakage and oxidized pyrimidine levels in H2O2-stressed lymphocytes (Duthie et al., 1997a, b; Noroozi et al., 1998). Similarly, tea polyphenols decrease the incidence of hydroxyl radical-generated chromatid breaks in lymphocytes exposed to fluorescent light irradiation (Parshad et al., 1998). The number and positioning of the hydroxyl groups in the flavonoid structure appear to be important to the antioxidant and cytoprotective potential of the compound. There are also many studies with Caco-2 cells, which are generally accepted as a good model for normal human colonocytes, which indicate a cytoprotective ability of flavonoids against oxidative DNA damage (Raeissi et al., 1997; Ricchi et al., 1997; Venturi et al., 1997; Duthie and Dobson, 1999).
**Antiinflammatory activity**

Inflammation and chronic inflammatory mechanisms have been implicated in the development of major chronic conditions of old age, including cancer and atherosclerosis (Finch and Crimmins, 2004). It is generally accepted that significant links between inflammation and cancer exist. Chronic inflammatory states may be triggered by microbial infections, autoimmune disease, or inflammation of unknown origin, and 15–20% of all deaths from cancer are estimated to be linked with underlying infection or inflammatory responses. Non-steroidal anti-inflammatory drugs are also known to reduce the risk of developing certain cancers and the associated mortality (Balkwill and Mantovani, 2001; Johnson, 2007). EGCG has demonstrated an anti-inflammatory effect, through the inhibition of the NF-κB signaling pathway. It is able to increase IkB levels while inhibiting NF-κB nuclear translocation in mice at doses of 20–50 mg/kg body weight (Khan and Mukhtar, 2008). EGCG also inhibits the expression of iNOS and COX-2 and subsequent NO and PGE2 production (Chen and Zhang, 2007) without affecting COX-1 expression in vitro (Shankar et al., 2007). Quercetin has also been found to inhibit NF-κB, iNOS, and COX-2 activity, although at high doses, impacting its potential to be useful in vivo. The impact of prolonged exposures to micromolar concentrations of quercetin has also not been assessed (Davis et al., 2009; Ossola et al., 2009). Genistein is another phytochemical that inhibits NF-κB activation and COX-2 expression (Banerjee et al., 2008).

**Induction of cell cycle arrest**

In cancer, normal cell growth and behavior is lost and alterations in the regulation of cell cycle have been described (Hartwell and Kastan, 1994). Thus, any perturbation of cell cycle specific proteins by phytochemicals can potentially affect and block the continuous proliferation of tumorigenic cells (Waldman et al., 1997). Many of the naturally derived anti-cancer agents were originally discovered using assays largely based on testing for cytotoxic activity against cancer cell lines grown either *in vitro* or using *in vivo* models. These compounds have been shown
to exert their cytotoxic action through interaction with tubulin, and include agents, such as vinblastine, vincristine, colchicine, combretastatin and maytansine which promote the depolymerisation of tubulin (Jordon et al., 1991; Lobert et al., 1996), while, in the case of the taxanes, microtubules are "bundled" as a result of stabilization against depolymerization (Abdal et al., 2003). Other important examples are the camptothecin derivatives, topotecan and irinotecan, which exert their cytotoxic action through inhibition of topoisomerase I (Beretta et al., 2011).

There are over 2000 kinases so far identified from genomic studies and all have a common site, the position where the ATP is bound (Cragg and Newman, 2005). The moderately anti-tumor active flavonoid, quercetin, is an early example of a natural product compound class that ultimately led to cyclin dependent kinase (Cdk) inhibitors. This flavanoid resembles an ATP-mimic where the planar bicyclic chromone ring system is an isostere of adenine. Quercetin exerts its anti-tumor effect through blocking cell cycle progression at the G0/G1 interface, consistent with Cdk inhibition, and a close analogue, myricetin, shows an IC50 close to 10 µM versus Cdk2. Flavopiridol showed about a 100-fold more selectivity for Cdk's compared to its activity for tyrosine kinases, and was the first compound identified by the National Cancer Institute (NCI) as a potential anti-tumor agent that subsequently was proven to be a relatively specific Cdk inhibitor (Cragg and Newman, 2005).

**Induction of apoptosis**

Apoptosis is arguably one of the most potent forms of defense against cancer (Ghavami et al., 2009). The cellular mechanisms by which apoptosis occur are highly conserved, and mutations with the apoptosis machinery is common to virtually all cancers, underscoring its importance in carcinogenesis (Sun et al., 2004). Apoptosis typically progresses through 1 of 3 pathways: the extrinsic pathway, the intrinsic pathway, or the granzyme B (GrB) pathway (Boivin et al., 2009). Briefly, the extrinsic pathway involves induction via the activation of death receptors on the cell surface. The intrinsic pathway relies on an increase in mitochondrial permeability and cytochrome c release. The granzyme B pathway
involves the exposure of sensitive target cells to the cytotoxic cell protease granzyme B (Ghawami et al., 2009).

EGCG in particular is able to induce apoptosis in many cancer cells, whilst sparing the relevant normal cells, through an increase in pro-apoptotic Bcl-2 family proteins (Khan and Mukhtar, 2008). Similarly, quercetin is able to inhibit cellular proliferation in many cancers through the induction of the intrinsic apoptosis pathway. It activated both caspase-3 and -9 but not caspase-8 and also increased the proapoptotic Bcl-2 family proteins. Experiments in animals have also used diets with composition of quercetin (0.01–2%) that might be reasonable in humans (Murakami et al., 2008). Genistein also induces cell cycle arrest and apoptosis in various cell lines. This occurs via the mitochondrial dependent pathway with cytochrome c release and caspase-3 and -9 activation (Banerjee et al., 2008).

**Prooxidant effect**

Most of the pharmacological properties of putative plant derived anticancer agents are considered to reflect their ability to scavenge endogenously generated oxygen radicals or those free radicals formed by various xenobiotics, radiation etc. However, some data in the literature suggests that the antioxidant properties of these compounds may not fully account for their chemopreventive effects and therapeutic properties (Ahmad et al., 1992; Gali et al., 1992). Of particular interest is the observation that several antioxidants have been found to induce apoptosis in cancer cell lines but not in normal cells (Clement et al., 1998; Ahmad et al., 1997; Chang et al., 2008). It is generally understood that antioxidants counteract ROS production and inhibit the latter-induced oxidative DNA damage and therefore reduce the risk of cancer. On the other hand, growing experimental evidence suggests that antioxidant-mediated production of ROS (prooxidant action) may be responsible for their ability to induce apoptosis of cancer cells (Qian et al, 2009; Hadi et al., 2007; Hadi et al., 2000). On the basis of the studies carried out in this laboratory we have proposed a novel prooxidant action mechanism for the cytotoxic action of plant derived polyphenolic antioxidants,
which has implications for their chemopreventive properties against cancer. It has been reported that several chemopreventive agents that are antioxidants at some concentrations become prooxidants at other concentrations (Lee and Park, 2003). Moreover, the antioxidant/prooxidant ability of antioxidant depends on the milieu where it is present (El-Najjar et al., 2010). Compared with normal cells, preneoplastic cells and neoplastic cells have been shown to contain elevated levels of copper (Gupte and Mumper, 2008) and may be more sensitive to electron transfer with antioxidants to generate ROS. Therefore DNA damage induced by antioxidants in the presence of redox active metal Cu(II) may be an important pathway through which preneoplastic cells and neoplastic cells can be killed while normal cells survived (Ullah et al., 2009).

Essentially this would be an alternative, non-enzymatic and copper-dependent pathway for the cytotoxic action of certain anticancer agents that are capable of mobilizing and reducing endogenous copper. As such this would be independent of Fas and mitochondria mediated programmed cell death. It is conceivable that such a mechanism may also lead to internucleosomal DNA breakage (a hallmark of apoptosis) as internucleosomal spacer DNA would be relatively more susceptible to cleavage by ROS. Indeed such a common mechanism better explains the anticancer effects of polyphenolic antioxidants with diverse chemical structures as also the preferential cytotoxicity towards cancer cells.

**Gossypol**

Gossypol is a sesquiterpene aldehyde, which is formed metabolically through acetate via the isoprenoid pathway (Burgos et al., 1997). Gossypol is present in the seed, roots, and stem of the cotton plant (*Gossypium sp.*) (figure 5). It is the major constituent of cotton seed oil. It is a yellow pigment similar to flavonoids in structure. In the plant, it acts as a natural defensive agent against predators, provoking infertility in insects. In the 1970s, the Chinese government began researching the use of gossypol as a contraceptive. Their studies involved over 10,000 subjects, and continued for over a decade. They concluded that gossypol
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provided reliable contraception, could be taken orally as a tablet, and did not upset men's balance of hormones. Similar research into using it as an alternative to vasectomy still continues in Austria, Brazil, Chile, China, the Dominican Republic and Nigeria (Coutinho, 2002). The different biological activities exhibited by gossypol are discussed below.

**Antioxidant property**

Gossypol is a polyphenolic compound from the viewpoint of its chemical structure. Like many other phenolic chemicals, such as butylated hydroxytoluene (BHT), coumaric acid, gallic acid, quercetin, myricetin, catechin, galloatechin, etc., gossypol is an effective and potent natural antioxidant. For example, gossypol was found to be able to protect carotene in vitro against preformed fat peroxides many decades ago (Hove, 1944; Hove and Hove, 1944). Gossypol is able to inhibit rat liver microsomal peroxidation, caused by an incubation with ferric/ascorbate (IC50 < 0.1 µM) (Laughton et al., 1989). Gossypol also exhibited a significant positive effect on oil and biodiesel stability. With a concentration of 0.1% gossypol, the oxidative stability indices (OSI) of cottonseed oil biodiesel could increase to 17.2 h from 4.15 h at 110 °C (Fan et al., 2008). In some cases, modification of the functional groups on gossypol may not affect its original chemical and biological activities (Bickford et al., 1954; Hove, 1944). Bickford and coworkers (1954) also found the other Schiff base-formed gossypol derivatives, gossypol-urea, gossypol-aminobenzene-thiol, and gossypol-glycineindicates, have roughly equivalent antioxidative ability to gossypol on a molar basis. Gossypol bis(piperinocthylimine) and bis(morpholinocthylimine) also showed potent antioxidant action in human blood serum and rat brain synaptosomes. At equal concentrations, these substances suppressed the peroxidation of lipids in enzymatic and nonenzymatic systems regarding the oxidation of rat liver microsomes (Dalimov et al., 1989). On the contrary, in many other cases, the modification of phenolic hydroxyl groups on gossypol could significantly decrease the antioxidant abilities regarding free radical scavenging activity, reducing power, and DNA damage prevention activity (Wang et al., 2008), demonstrating that the hydroxyl groups are critical for the antioxidative activities. The relative capability
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of gossypol and its methylated derivatives to prevent DNA damage caused by ultraviolet light and hydrogen peroxide was consistent with the compounds' antioxidant effects. This suggests that gossypol's protection of DNA may occur partially by quenching free radicals, therefore alleviating oxidative stress. A previous study (Li et al., 2000) also found that gossypol demonstrated the ability, in a dose-dependent manner, to protect supercoiled plasmid DNA from damage caused by exposure to Fe³⁺/ascorbate.

Anti-parasitic, anti/protozoan activities

Malaria is a vector-borne infectious disease caused by protozoan parasites. Human malaria is usually caused by the infection of *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax* (Mendis, 2001). A series of gossypol derivatives with modified aldehydic groups and hydroxyl groups have been shown to inhibit the growth of *Plasmodium falciparum* (Razakantoanina et al., 2000; Royer et al., 1986). The derivatives with ethyl, propyl, or isopropyl side chains as well as gossylyc nitrile 1,1'-divalcrate with IC₅₀ values close to gossypol (IC₅₀ = 16 µM) showed stronger inhibition than other gossypol derivatives against the growth of *Plasmodium falciparum*. Similarly, the inhibition of LDH activity in *T. gondii* can also inhibit growth of the parasite in cultures (Dando et al., 2001). Montamat and coworkers (1982) reported that a 5-min exposure to 100 µM gossypol (~ 50 ppm) immobilizes cultures of *Trypanosoma cruzi*. Blanco et al. (1983) reported that a 30-min exposure to 25 µM gossypol (~ 12 ppm) immobilizes and alters the cell morphology of *T. cruzi*. Later, Kaminsky and Zweygarth (1989) reported that, for three separate *Trypanosoma brucei* strains (including one drug resistant strain), the IC₅₀ value for a 24-h gossypol exposure was > 10 ppm. In the study of *T. cruzi*, gossypol was also reported to inhibit some oxidoreductases (Gerez de Burgos et al., 1984; Montamat et al., 1982), such as, alpha-hydroxyacid and malate dehydrogenases, NAD-linked enzymes, and glutamate dehydrogenase, glucose-6-phosphate dehydrogenase, and NADP-dependent enzymes.
Anti-microbial, anti-viral activity

The anti-microbial properties of gossypol have been reported by several research groups. Gossypol has general antifungal activities with LD50 values from 20 to 100 ppm of pure gossypol (Bell, 1967), and has an inhibitory effect on microorganisms including aerobic spore formers and lactobacilli and some yeasts (Margalith, 1967). Vadehra et al. (1985) investigated the effects of gossypol on the growth of a variety of bacteria and on spore formation and germination in Bacillus cereus. It was found that gossypol had more potent antibacterial properties against gram positive organisms than gram negative bacteria such as Pseudomonas aeruginosa, Salmonella spp., Klebsiella pneumoniae, Shigella spp., Proteus spp., and Escherichia coli.

Subsequent research (Poprawski and Jones, 2001) found that fungi Paecilomyces fumosoroseus (associated with cutaneous and disseminated infections in dogs and cats) were highly tolerant to gossypol even at 500 ppm, but could be strongly inhibited at 1000 ppm of gossypol.

Lin et al. (1989 and 1993) reported that gossypol inhibited the replication of HIV-1 and found (-)-gossypol to be more inhibitory (IC50=5.2 µM) compared to the (+)-gossypol (IC50=50.7 µM). Besides HIV-1, gossypol also showed anti-viral activity in multiple enveloped viruses including herpes simplex virus type 2 (HSV-II), influenza virus, and parainfluenza virus (Vander Jagt et al., 2000). Later, Royer et al. (1991) found that gossypol and its derivatives, gossylic nitrile-1, 1'-diacetate, gossylic iminolactone, and gossylic lactone inhibited the replication of HIV-1 in vitro. Further, Royer and coworkers (1995) tested several other gossypol derivatives for inhibition of HIV: 1, 1'-Dideoxygossypol, 1, 1'-dideoxygossylic acid (DDGA), 8-deoxymemihemigossypol (DHG), and 8-deoxymemigossylic acid (DHGA). They found that DDGA was the most effective in inhibiting the replication of HIV in vitro with EC50 < 1 µM. Meanwhile, DDG was less effective than DDGA. DHG showed some anti-HIV activity, and DHGA was ineffective against HIV. Since all four gossypol derivatives were found to have much lower affinities for albumin than the parent compound gossypol, this would possibly enhance the anti-virus activity of the gossypol derivatives in vivo with less interference from in vivo proteins.
**Anti-cancer activity**

Gossypol is capable of inhibiting the growth of a variety of cancer cell lines including breast, colon, prostate, and leukemia cells (Balci et al., 1999; Benz et al., 1990; Huang et al., 2006; Zhang et al., 2003). These disruptions include inhibition of cytoplasmic and mitochondrial enzymes involved in energy production (Ueno et al., 1988) and uncoupling of oxidative phosphorylation (Flack et al., 1993; Abou-Dona et al., 1974). In addition, depletion of cellular ATP has been demonstrated in cultured tumor cells (Keniry et al., 1989). Gossypol also inhibits key nuclear enzymes responsible for DNA replication and repair, including DNA polymerase α (Rosenberg et al., 1986) and topoisomerase II, and blocks DNA synthesis in HeLa cells (Wang, 1984). Hou et al. (2004) found that gossypol at 50 µM for 6 h could induce DNA fragmentation in human promyelocytic leukemia cells (HL-60), a hallmark characteristic of apoptosis, and also induce the truncation of Bid protein, the loss of mitochondrial membrane potential, cytochrome c release from mitochondria into cytosol, and activation of caspases-3, -8, and -9. In human alveolar lung cancer cells, gossypol induced Fas/Fas ligand mediated apoptosis (Moon et al., 2008). The inhibitory activity of (-)-gossypol was related to the reduction of the cell cycle regulator, cyclin D1, and the induction of the cell proliferation inhibitor, TGF-β. In the study of human prostate cancer cells, it was found that (-)-gossypol-induced apoptosis was mediated by the regulation of Bcl-2 and caspase families (Huang et al., 2006). Another in vitro study (Mohammad et al., 2005) demonstrated (-)-gossypol had significant inhibitive effects against the growth of lymphoma cell line WSU-DLCL2 and fresh cells obtained from a lymphoma patient with no effect on normal peripheral blood lymphocytes. (-)-Gossypol also induced complete cytochrome c release from mitochondria, increased caspases-3 and -9 activity, and caused apoptotic death without affecting protein levels of Bcl-2, Bcl-X (L), Bax, and Bak. Recent research has revealed that (-)-gossypol acts as a BH3 mimetic, binding to the BH3-binding domain in various pro-apoptotic proteins of the Bcl-2 family, displacing pro-death partners to induce apoptosis (Balakrishnan et al., 2008; Meng et al., 2008). (-)-Gossypol exerts its antitumor activity through inhibition of the anti-
apoptotic protein Bcl-xL accompanied by an increase of pro-apoptotic Noxa and Puma (Meng et al., 2008). Further work needs to be done to ascertain gossypol's primary mechanism of action. Currently, (−)-gossypol is being evaluated in phase I and II clinical trials as a single agent in B-cell malignancies and prostate cancer, and in combination with other antitumor agents in a variety of hematologic, lymphoid, and solid-tumor malignancies (Warr and Shore, 2008).

Synthesis of gossypol derivative, apogossypolone

The toxicity of gossypol does not permit it to be an effective antitumor agent. Nevertheless, gossypol represents a potentially interesting lead compound (Kitada et al., 2003; Zhang et al., 2009) for the synthesis of novel anticancer agents. The design and synthesis of gossypol analogs has become an exciting research area. Extensive attempts to chemically modify gossypol to obtain therapeutic antitumor agents with greater potency and less toxicity have been performed. More than 50 new analogues of gossypol have been synthesized to date (Dodou et al., 2005; Zhang et al., 2009). Unfortunately, none of them has been used in the clinic as an antitumor agent because of undesirable side effects, insolubility and a lack of selectivity against tumor cells. Based on the binding affinity of gossypol to protein it was observed that the two reactive groups in gossypol can combine with lysine residues of proteins to form Schiff's bases, which have been assumed to cause the toxicity of gossypol in animals and humans (Zhan et al., 2009).

Apogossypolone (ApoG2) is a derivative of gossypol that was designed by Ascenta and later synthesized by Zhan et al. (2009) (figure 6) in order to reduce the nonspecific reactivity and toxicity of gossypol and is currently in the preclinical phase of testing. This modification involved the removal of two reactive aldehyde groups on the polyphenolic rings of gossypol and further addition of four quinonoid moieties. Current research shows ApoG2 is a potent inhibitor of Mcl-1 and Bcl-2 proteins. ApoG2 has recently been shown that it blocks binding of Bim and Bcl-2 and induces apoptosis in lymphoma cell lines with minimal toxicity (Mohammadianpanah et al., 2009). Further it has also been shown that ApoG2 induces apoptosis in follicular Small Cleaved Cell Lymphoma model, pre-B-acute
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Acetyl-CoA

\[ \text{Acetyl-CoA} \rightarrow \text{Acetoacetyl-CoA} \]

Oxoglutarate

\[ \text{Oxoglutarate} \rightarrow \text{HMG-CoA} \]

Hydroxymethylglutaryl-CoA

\[ \text{Hydroxymethylglutaryl-CoA} \rightarrow \text{Mevalonate} \]

Figure 5: Biosynthesis of gossypol.

**Figure 5: Biosynthesis of gossypol.**

ApoG2

40% NaOH, 95°C, 30 min

96% yield

98% H2SO4

98% yield

1

2

3

4

5

Figure 6: Synthesis of ApoG2. (From: Zhan et al., 2009)
lymphoblastic leukemia, mantle cell lymphoma, marginal zone lymphoma, as well as chronic lymphocytic leukemia. Therefore, ApoG2 could potentially be a more effective drug in the lymphoma clinic spanning a greater array of patients (Arnold et al., 2008). Further, ApoG2 alone or in combination with adriamycin has been shown to induce apoptosis in human hepatocellular carcinoma cells (HHC) by down regulating anti-apoptotic proteins Bcl-2, Mcl-1, and Bcl-XL, up-regulating pro-apoptotic protein Noxa, and promoting the activities of caspases-9 and -3. Tumor growth in hepatocellular carcinoma xenograft was inhibited in nude mice when ApoG2 was administered orally without causing damage to the normal tissues (Mi et al., 2008). ApoG2, also induces apoptosis and suppresses tumor growth in nasopharyngeal carcinoma xenografts (Sun et al., 2008).

**Thymoquinone**

Thymoquinone (TQ) (figure 2) is the bioactive constituent of the volatile oil of black seed (54%) and was first extracted by El-Dakhakhany (1963). TQ has been shown to exert anti-inflammatory, anti-oxidant and anti-neoplastic effects both in vitro and in vivo. Several methods have been used to quantify the levels of TQ in black seed oil, such as gas chromatography (Houghton et al., 1995), high performance liquid chromatography (HPLC) (Ghosheh et al., 1999) and differential pulse polarography (Michelitsch and Rittmannsberger, 2003). TQ is not the only bioactive constituent of black seed oil; other pharmacologically active constituents, identified by HPLC, include dithymoquinone, thymohydroquinone and thymol (Ghosheh et al., 1999). Other fractionated proteins of *N. sativa* ranging in their molecular mass from 10 to 94 kDa were purified by ion exchange chromatography and have also been found to be bioactive (Haq et al., 1999).

**Biological functions**

Many investigators have shown that the growth inhibitory effects of TQ are specific to cancer cells (Gali-Muhtasib et al., 2004a&b; Shoieb et al., 2003; Worthen et al., 1998). TQ showed significant anti-neoplastic activity against human pancreatic adenocarcinoma, uterine sarcoma and leukemic cell lines, while
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it is minimally toxic to normal cells (Worthen et al., 1998). The multidrug-resistant variants of human pancreatic adenocarcinoma, uterine sarcoma and leukemic cell lines, which are over 10-fold more resistant to the standard anti-neoplastic agents doxorubicin and etoposide as compared to their respective parental controls, were equally sensitive to TQ (Worthen et al., 1998). TQ also exerts anti-oxidant effects and inhibits inflammation in animal models and cell culture systems (Mansour et al., 2002). Despite this knowledge about the potent anti-cancer, anti-oxidant and anti-inflammatory effects of TQ, the molecular pathways involved in its activities are not well understood.

Effects on the immune system

Oxidative stress and inflammatory disorders are now widely known as the major pathogenetic factors of carcinogenic malignant transformation (Coussens and Werb, 2002). Several studies point to the effect of black seed and TQ on the immune system by modulating the levels of pro- and anti-inflammatory mediators (Badary et al., 2003). TQ has also been shown to inhibit inflammation and oxidative stress in cells (Houghton et al., 1995; Mansour et al., 2002). The anti-inflammatory effect of black seed has been found to be comparable to that of 100 mg/kg aspirin (Al-Ghamdi, 2001). The seeds produce an increase in the ratio of helper to suppressor T cells and enhance natural killer cell activity in healthy volunteers (El-Kadi et al., 1989). A recent study has shown that the intraperitoneal injection of black seed essential oil leads to significant anti-inflammatory activities against carrageenan induced paw edema in rats (Hajhashemi et al., 2004). Using a COX-2 assay, it has been shown that TQ (IC50 = 0.3 µM) is an inhibitor that is more potent than indomethacin (IC50 = 0.6 µM) of COX-2-catalyzed PGE2 production (Marsik et al., 2005). TQ treatment completely averted the acetic acid induced colitis in rats (Mahgoub, 2003). It has also been reported to inhibit TNF-β production in murine septic peritonitis by TQ (Haq et al., 1999). Furthermore, TQ was found to reduce the NO production in supernatants of lipopolysaccharide (LPS)-stimulated macrophages, without affecting their cell viability (El-Mahmoudy et al., 2002). In other studies, TQ has been reported to have potent superoxide anion scavenging abilities and to inhibit iron-dependent
microsomal lipid peroxidation (Badary et al., 2003). The generation of superoxide anion (O$_2^-$) by the xanthine/xanthine oxidase system was inhibited by TQ in a dose-dependent manner (IC$_{50}$ = 3.4 M). This is promising considering the fact that O$_2^-$ reacts with protein and non-protein sulfhydryls and polyunsaturated fats and initiates aromatic hydroxylation reactions, thus damaging cells and causing inflammation.

**Effect on apoptosis**

The induction of apoptosis in tumor cells is a key mechanism for the effectiveness of chemopreventive drugs. TQ has been shown to induce apoptosis by p53-dependent (Gali-Muhtasib et al., 2004b) and p53-independent (El-Mahdy et al., 2005) pathways. In HCT-116 human colon cancer cells, the pro-apoptotic effects of TQ are dependent on p53 (Gali-Muhtasib et al., 2004b), while in myeloblastic leukemia HL-60 cells, TQ-induced apoptosis is p53-independent and occurs through the activation of caspase 8, 9 and 3 (El-Mahdy et al., 2005). TQ treatment of HCT-116 cells resulted in a marked increase in p53 and p21 protein levels and in a significant inhibition of the anti-apoptotic Bcl-2 protein (Gali-Muhtasib et al., 2004b). Co-incubation with the specific p53-inhibitor, pifithrin-alpha, restored the mRNA and protein levels of Bcl-2, p53 and p21 to control levels and suppressed TQ-induced apoptosis. p53-null HCT-116 cells are less sensitive to TQ-induced apoptosis (Gali-Muhtasib et al., 2004b). Furthermore, TQ induces apoptosis in osteosarcoma cells (Shoieb et al., 2003) and neoplastic keratinocytes (Gali-Muhtasib et al. 2004a). By contrast, normal cells and primary mouse keratinocytes are resistant to the apoptotic effects of TQ (Shoieb et al., 2003; Gali-Muhtasib et al. 2004a), and no significant alteration of their morphology and proliferation is observed, confirming the selectivity of this compound to cancer cells (Gali-Muhtasib et al. 2004a).

**Effect on cell cycle**

There are only a few studies that have dealt with the effects of TQ on cell cycle and proliferation of cancer cells. Growth inhibition by TQ is associated with inhibition of DNA synthesis (Worthen et al., 1998) and induction of cell cycle
arrest (Shoieb et al., 2003). Recent studies have shown promising anti-neoplastic effects for TQ in neoplastic keratinocytes (Gali-Muhtasib et al. 2004a) and human colon cancer cells (Gali-Muhtasib et al., 2004b). The mechanism of anti-proliferation in papilloma cells was triggered by the induction of G1 cell cycle arrest and the restoration of p16 protein levels. Treatment of mouse papilloma cells with 30 μM TQ caused a 22% increase in the number of cells in the G1 phase within 24 h. TQ-induced G1 arrest was associated with sharp increases, from null to 10-fold, in p16 protein levels as early as 2h after treatment, and this increase was sustained for up to 24 h (Gali-Muhtasib et al. 2004a). The latter is of special interest considering the fact that the modulation of p16 expression increases tumor sensitivity to chemotherapeutic drugs (Hochhauscr, 1997). On the other hand, TQ treatment of the more aggressive mouse spindle cancer cells caused G2/M arrest (38% increase), which was accompanied by decrease in cyclin B1 protein expression (Gali-Muhtasib et al. 2004a). Growth inhibition of HCT-116 human colon cancer cells by TQ correlated with G1 phase arrest of the cell cycle (Gali-Muhtasib et al., 2004b). A significant up-regulation of p53 and p21 transcript and protein levels, as well as a significant decrease in Bcl-2 was seen, in TQ-treated cells. In another study, TQ-induced growth inhibition and cell cycle regulation were examined in cisplatin-sensitive and cisplatin-resistant canine osteosarcoma cells (Shoieb et al., 2003). TQ was four- to five-fold more cytotoxic to the resistant cell line than its correspondent parent line. It is noteworthy that cisplatin-resistant osteosarcoma cells are seven- to eight-fold more resistant to the cytotoxic effects of cisplatin compared to the parental cell line. TQ-induced death of cisplatin-sensitive osteosarcoma cells was found to be due to cell cycle arrest at G1 phase as determined by flow cytometry analysis. Treatment with 30 μM TQ for 24 h resulted in three-fold increase in the percentage of cells in G1 as compared to control (Shoieb et al., 2003). The mechanism of cell death of the resistant variant was not determined in this study. Despite this knowledge about the potent anti-cancer, anti-oxidant and anti-inflammatory effects of TQ, the molecular pathways involved in its activities are not well understood.