INTRODUCTION
INTRODUCTION

Dietary constituents and prevention of disease:

Dietary agents consist of a wide variety of biologically active compounds that are ubiquitous in plants and have been used in traditional medicine for thousands of years. Hippocrates recognized and professed the importance of various foods in human health approximately 2,500 years ago (Khan et al., 2008). 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). Heart diseases, cancer and stroke are the top three causes of death in most industrialized countries. It is estimated that one third of all cancer deaths can be avoided through appropriate dietary modifications (Doll and Peto, 1981; Willett, 1995). This convincing evidence suggests that a change in dietary behaviour such as increasing consumption of fruits, vegetables and grains is a practical strategy for significantly reducing the incidence of chronic diseases.

The biologically active chemicals found in fruits, vegetables and grains are termed as phytochemicals, many of which provide desirable health benefits beyond nutrition to reduce the risk of a number of chronic diseases (Liu, 2003). It is estimated that more than 5000 phytochemicals have been identified but a large percentage still remains unknown (Shahidi and Naczk, 1995) and they need to be identified before their health benefits can be fully understood. However, more and more convincing evidence suggests that the benefits of phytochemicals present in fruits and vegetables may be even greater than is currently understood because oxidative stress induced by free radicals is involved in the etiology of a wide range of

Cells in humans and other organisms are constantly exposed to a variety of oxidizing agents termed as free radicals / reactive oxygen species. These free radicals may play an important role in the origin of life and biological evolution, implicating their beneficial effect on the organisms (McCord, 2000). For example, phagocytes produce reactive oxygen species (ROS) as a defence mechanism against various infectious agents. ROS are inevitably generated along with cellular metabolism (Klaunig and Kamendulis, 2004; Poli et al., 2004). 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 defence 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 (Lau et al., 2004), whereas chronic low levels of ROS promote cardiovascular diseases (Barchowsky et al., 1996) and carcinogenesis (Lau and Chiu, 2006). They have also been found to induce genetic alterations including DNA damage, mutations, epigenetic changes and genomic instability (Lopez-Lazaro, 2007). The key factor is to maintain a balance between oxidants and antioxidants in order to sustain an optimal physiological condition within the body. To prevent or slow down the oxidative stress induced by free radicals, sufficient amounts of antioxidants are needed to be consumed.

Fruits and vegetables contain a wide variety of secondary metabolites that possess antioxidant properties. These include polyphenols and carotenoids that may help protect cellular systems from oxidative damage and also lower the risk of chronic diseases. There has been
considerable scientific evidence, both epidemiological and experimental, accumulated in the past three decades indicating that modification in life style including diet, can have a major effect on the risks of numerous cancers (Martinez and Giovanucci, 1997). Of particular relevance is the consistent cancer protective effect reported for individuals consuming increased quantities of fruits and vegetables compared to those with low intakes. The cancer inhibitory action by a variety of human nutrients derived from plants as well as of 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. There have been two major diet related prevention strategies that have been involved in combating cancer, i.e. cancer chemoprevention and dietary prevention with appreciable overlap existing between them. Generally, cancer chemoprevention is recognized as the pharmacological intervention with synthetic or naturally occurring chemicals to prevent, inhibit or reverse carcinogenesis or prevent development of invasive cancer (Sporn, 1991; Kelloff and Boone, 1996; Kelloff et al., 1997; Mayne and Lippman, 1997). On the other hand dietary prevention is recognized as the changes in food consumption pattern necessary to decrease cancer development (Schatzkin and Kelloff, 1995; Goodman, 1997). Plant derived polyphenolic compounds such as flavonoids, tannins, curcumin and the stilbene resveratrol possess a wide range of pharmacological properties, the mechanisms of which have been the subject of considerable interest. They are recognized as naturally occurring antioxidants and have been implicated as anticancer compounds (Mukhtar et al., 1998). In recent years, several reports have documented that plant polyphenolics, including curcumin, resveratrol
and gallocatechins such as gallic acid, epigallocatechin, epicatechin-3-gallate and epigallocatechin-3-gallate (EGCG) induce apoptosis in various cancer cell lines (Inoue et al., 1994; Jaruga et al., 1998; Clement et al., 1998). Gallocatechins are constituents of green tea, the consumption of which is considered to reduce the risk of various cancers such as those of bladder, prostate, esophagus and stomach (Ahmad et al., 1997). Resveratrol is present in human dietary material such as peanuts, grapes, mulberries and beverages such as red wine. Of particular interest is the observation that a number of these polyphenols including epigallocatechin-3-gallate, gallic acid and resveratrol induce apoptotic cell death in various cancer cell lines but not in normal cells (Inoue et al., 1994; Ahmad et al., 1997; Clement et al., 1998).

**Anticancer properties of plant derived polyphenols and antioxidants:**

Natural dietary agents have drawn a great deal of attention from both the scientific fraternity and the general public owing to their ability to suppress cancers (Khan et al., 2008). The wide array of phenolic substances and antioxidants present in fruits and vegetables can be divided into two main groups: cancer blocking and cancer suppressing agents. The former prevent carcinogens from hitting their cellular targets (initiation) by several mechanisms: (a) enhancing carcinogen detoxification, (b) modifying carcinogen uptake and metabolism, (c) scavenging reactive oxygen species (ROS) and other oxidative species, (d) enhancing DNA repair. Cancer suppressing agents inhibit cancer promotion and progression after the formation of pre-neoplastic cells by interfering with (a) cell cycle regulation, (b) signal transduction, (c) transcriptional regulation and (d) apoptosis (Surh,
Introduction

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 both human and rodent ovarian, leukemic, intestinal, lung, breast and bladder cancer cells. For example, quercetin (10 μM) 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 (IC_{50} 10 μM) and growth (IC_{50} 7.7 μM) 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, with an IC_{50} of 0.17 μM (Hirano et al., 1995) while genistein is inhibitory at concentrations similar to conventional anticancer drugs such as deoxorubicin and methotrexate (Hirano et al., 1994). Genistein, kaempferol and quercetin inhibit the proliferation of human colon cancer cells Caco-2 and HT29 (Agullo et al., 1994; Kuo, 1996) while naringenin and catechin do not (Kuo et al., 1996). Curcumin is cytostatic in several hormone dependant (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).

Very few studies have investigated the cytostatic ability of flavonoids both in malignant cells and in their untransformed counterparts. Although several polyphenols, most notably genistein, while showing considerable growth inhibition in HL-60 cells had little or no effect on mitogen-induced blastogenesis in normal human peripheral blood lymphocytes (Hirano et al., 1994). Similarly, tritiated thymidine uptake is inhibited in HL60 cells following exposure to tangeretin, but is unchanged in normal lymphocytes (Hirano et al., 1995). The polyhydroxylated flavonoids quercetin and taxifolin and the polymethoxylated flavonoids nobiletin and tangeretin inhibit HTB 43 squamous cell carcinoma and 9L gliosarcoma cell growth but are less effective in transformed human CCI embryonic fibroblast cells (Kandeswami et al., 1992). While these studies appear to suggest that the flavonoids display a tumor-specific action, it should be noted that comparisons were not made on cells derived from the same tissue.

In an elegant study by Chen et al. (1998), epigallocatechin-3-gallate (EGCG), the major polyphenol present in green tea, inhibited colorectal cancer and breast cancer growth more than in their respective normal counterparts. Similarly, EGCG reduced W138 human lung fibroblastic cell growth only weakly compared to its virally transformed (VA) counterparts. The IC50 value of EGCG was 120 μM in WI38 cells compared with only 10 μM in W138VA cells. Conversely, the flavonoids quercetin and genistein are equally toxic towards colonic cancer cells and non-transformed intestinal crypt cells (Kuo, 1996).

In addition to cell culture studies, the capacity of certain dietary...
polyphenols to protect against both chemically induced and spontaneous formation of tumors in animals is well established. For example, quercetin administered to rats in combination with dimethylbenz-(a)-anthracene (DMBA) or N-nitrosomethylurea (NMU) reduces the incidence and multiplicity of mammary tumors by 30% and 50% respectively (Verma et al., 1988). Quercetin and luteolin (10 g/Kg diet) decreases fibrosarcoma incidence (52% and 60% respectively) and tumor size in male Swiss albino mice following treatment with the model chemical carcinogen 20-methylcholanthrene (Elangovan et al., 1994). The citrus flavonoid naringenin inhibits the \textit{in vivo} development of DMBA induced mammary tumors in Sprague-Dawley rats (So et al., 1996).

Apart from polyphenols, several other bioactive compounds present as extra nutritional constituents of plants have shown to exert a protective effect against carcinogenesis. It has been demonstrated that capsaicin modulates microsomal cytochrome P450-dependent monooxygenase activities, thereby affecting metabolism of carcinogens and other xenobiotics (Miller et al., 1993; Surh et al., 1995). Also, another study by Morre et al. (1995) showed that capsaicin preferentially repressed the growth of some transformed cells of human origin, including HeLa, ovarian carcinoma, mammary adenocarcinoma and promyelocytic leukemia cells in culture. One of the most prominent effects curcumin (derived from turmeric) has on experimental carcinogenesis is its capability to inhibit tumor promotion (Conney et al., 1997; Huang et al., 1997). Curcumin has been reported to alleviate TPA (12-O-tetradecanoyl phorbol-13-acetate) induced skin tumor promotion and epidermal ODC (ornithine decarboxylase) mRNA expression (Lu et al., 1993) as well as ODC activity (Huang et al., 1988). Lycopene, a potent antioxidant carotenoid has been shown to protect against various forms of cancer,
including cancer of prostate (Clinton, 1998; Nguyen and Schwartz, 1999), cervix (Clinton, 1998; Weisburger, 1998), pharynx and esophagus (Krinsky, 1998; La Vecchia, 1998) and stomach (Krinsky, 1998; La Vecchia, 1998; Nguyen and Schwartz, 1999). It also inhibits tumor promotion and proliferation of cells in culture and animal models (Krinsky, 1998; Nguyen and Schwartz, 1999).

There are several suggested mechanisms by which polyphenols exert anticancer effects:

**Antioxidant effects:** Carcinogenesis is a multi-stage process of genetic change affecting proto-oncogenes or tumor suppressor genes in a single cell or a clone of cells. Such genetic alterations may be initiated by increased and persistent damage to DNA causing permanent alterations in the genetic message when the cell replicates its DNA and divides. Reactive O and N species are potential carcinogens as they can directly and indirectly induce structural alterations in DNA by oxidation, methylation, depurination and deamination reactions. The ability of certain polyphenols to inhibit oxidative DNA damage is well documented. 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).

Ex-vivo studies also suggest that the antioxidant potential of polyphenols may be anticarcinogenic. For example, the ability of plasma to inhibit oxygen free radical induced DNA damage to lymphocytes was increased by 20% 1 hour after consumption of 300 ml wine (Fenech et al., 1997). Moreover, indices of oxidized DNA in bladder mucosal cells of smokers inversely correlate with the level of phenolics measured in their urine (Malaveille et al., 1998).

**Modulation of enzyme activity associated with carcinogen activation and detoxification:** One of the mechanisms by which polyphenols may exert their anticarcinogenic effect is by modulating the enzyme systems that metabolize carcinogens or pro-carcinogens to genotoxins. In this way, the activation of the carcinogen may be inhibited, or it may be converted to a less reactive compound before it reacts with DNA and initiates carcinogenesis. The cytochrome P-450 superfamily of enzymes metabolizes a large number of procarcinogens to reactive intermediates, which bind covalently to DNA and can induce malignant transformation. The activity of some P-450s are either induced or inhibited by flavonoids. For example, naringenin and tangeretin are potent inhibitors of microsomal 7-ethoxyresorufin-O-deethylase (EROD) activity, which is a marker substrate for P450 1A (Obermeier et al., 1995). Similarly, quercetin inhibits EROD activity (IC$_{50}$ < 1 µM) in microsomes from human hepatoma HepG2 cells (Musonda et al., 1997). Pentoxyresorufin-O-dealkylase (PROD) activity is also decreased, indicating the ability of the flavonoids to inhibit P450 2B activity. Tangeretin inhibits nifedipine oxidase (P450 3A) in human liver microsomes (Obermeier
et al., 1995). Flavone and several hydroxylated derivatives (3-OH-, 5-OH-, 7-OH- and 3, 7-dihydroxyflavone) are found to be potent inhibitors of cDNA expressed human P450s 1A1 and 1A2 (IC_{50} < 1 μM), while galangin is a selective inhibitor of P450 1A2 (Zhai et al., 1998). The ability of flavonoids to inhibit P450 1A is directly related to their antimutagenic properties. Several flavones, including apigenin and luteolin and flavonols such as kaemferol, quercetin and myricetin, reduce the mutagenicity of the food-derived heterocyclic amine 3-amino-1-methyl-5H-Pyrido [4, 3-b] indole (Trp-P-2) in the Ames test (Salmonella typhimurium TA 98). Trp-P-2 is metabolized by P450 1A to the ultimate mutagen N-hydroxy-Trp-P-2 that binds to the DNA molecule and initiates carcinogenesis (Kanagawa et al., 1998).

Therefore, the effect of flavonoids on xenobiotic metabolizing enzyme is complex and highly dependent on a number of factors including the chemical structure of the flavonoid, the species under investigation and the model system being employed. Despite the considerable experimental evidence that certain polyphenols have potent anti-carcinogenic activity, epidemiological support is contradictory. For example, some ecological, cohort and case-control studies suggest that tea consumption lowers the risk of developing cancer whereas other investigations have failed to find such associations or have even indicated procarcinogenic effects (Blot et al., 1996). In addition, no correlation was observed between estimated flavonoid intake (determined in 1985) and cancer incidence (P = 0.54) and mortality (P = 0.51) at all sites after a 5-year period in 738 elderly Dutch men in the range of 65-84 years (Hertog et al., 1994). The inconclusive nature of the epidemiological studies may reflect a lack of information on the duration and amount of polyphenol intake, inadequate control of confounding and potential basis in recall and
reporting of intake patterns.

Much scientific research needs to be conducted before making science-based dietary recommendations. The discovery of agents which are effective, safe, non-toxic and the development of dose schedules that will allow their beneficial use is the principal need in the chemoprevention of cancer.

RESVERATROL:

Resveratrol (3,5,4'-trihydroxy-trans-stilbene; figure 1) is a polyphenol and has been classified as a phytoalexin for being synthesized in spermatophytes in response to injury, UV irradiation and fungal attack (Langcake and Pryce, 1976). It was first isolated from the roots of the oriental medicinal plant Polygonum capsidatum (Ko-Jo-Kon in Japanese) (Nonomura et al., 1963). The observation that resveratrol was one of the major active ingredient of folk plant, known for its remedial effects against a host of disease states (Nonomura et al., 1963) and was synthesized in response to fungal infection in grapevines (Vitis vinifera) (Langcake and Pryce, 1976) provided the early impetus for the interest in unraveling the biological properties of this compound. Since the first reported detection of resveratrol in grapevines in 1976 and then in wine in 1992 (Siemann and Creasy, 1992), most of the work is focused on resveratrol in grapevines. Epidemiological studies have revealed an inverse correlation between red wine consumption and the incidence of cardiovascular disease, a phenomenon commonly known as the “French Paradox” (Renaud and Lorgeril, 1992). Consequently, the early research on resveratrol was centered on its effects on metabolic pathways regulating cardiovascular systems such as lipid metabolism, promotion of vasorelaxation, anti-atherosclerotic properties and platelet
aggregation (Hao and He, 2004).

Besides its effects on the cardiovascular system, resveratrol exhibits a remarkable inhibitory potential in various stages of tumor development. The antitumor activity of resveratrol was first revealed by its ability to reduce the incidence of carcinogen-induced development of cancers in experimental animals (Jang et al., 1997).

![Chemical Structure of Resveratrol](image)

**Figure 1. Chemical Structure of Resveratrol**

Subsequently, resveratrol has been shown to exert numerous effects that may block tumor development at several discrete stages during the multigenic process of carcinogenesis (Hursting et al., 1999), involving interactions between resveratrol and multiple targets (Aggarwal and Shishodia, 2006). Resveratrol is able to block each step in the process of carcinogenesis by inhibiting several molecular targets such as kinases, cyclooxygenases, ribonucleotide reductase and DNA polymerases (Saiko et al., 2008a). Further, resveratrol induces $G_1$ phase arrest and triggers mitochondrial dependent, p53 dependent, ROS dependent, bcl-2 sensitive apoptotic response in tumor cells. It has also been shown to induce p53 accumulation and inhibition of NFkB (Surh, 1999; Saiko et al., 2008a).

Furthermore, promising data with the use of resveratrol have also been obtained regarding progressive neurodegenerative maladies such as
Alzheimer’s, Huntington’s, and Parkinson’s diseases. Because neurotoxicity is often related to mitochondrial dysfunction and may be ameliorated through the inclusion of metabolic modifiers and/or antioxidants, resveratrol may provide an alternative (and early) interventionist approach that could prevent further damage (Saiko et al., 2008a). Recently, Howitz’s group and others have described the anti-ageing properties of resveratrol. The molecule is the most potent activator of sirtuins transcription (Howitz et al., 2003). The prosurvival properties of resveratrol have been confirmed by showing its ability to increase aerobic capacity in mice by inducing genes for oxidative phosphorylation and mitochondrial biogenesis in a SIRT1-dependent manner (Lagouge et al., 2006). Thus, resveratrol exerts a range of beneficial effects on human health and disease (figure 2).

Resveratrol is found in several edible natural products such as grapes (Vitis spp.), peanuts (Arachis spp.) (Sanders et al., 2000) and berries (blue berries, cranberries and lingo berries, all Vaccinium spp.)
Introduction

(Rimando et al., 2004). Extracts from roots, heartwood bark and leaves of most of these plants are commonly used in traditional oriental medicine. The content of resveratrol in different sources varies widely, depending on factors such as cultivars, climate, fungal infections, UV exposure and wine making procedure. In beverages, red wine contains considerable amounts of resveratrol. Its concentrations measured in a sampling of red wine varieties ranged from 2 to 40 µM (Gusman et al., 2001).

EPIGALLOCATECHIN-3-GALLATE (EGCG):

Tea, (derived from the leaves of the plant *Camellia sinensis*), is the most popular beverage, consumed by over two-thirds of the world’s population. A number of beneficial health effects of green tea such as cancer chemoprevention and cardioprotective effects are attributed to its regular consumption (Khan et al., 2008). *Camellia sinensis*, a member of Theaceae family, is an evergreen shrub. It is processed in different ways in different parts of the world to give green, black or oolong tea. Commercial green tea is prepared by picking, lightly steaming or allowing the fresh tea leaves to dry at elevated temperatures thereby preserving 90 % of the polyphenols contained in fresh leaves from being degraded (Balentine et al., 1997). Dried tea leaves are mainly composed of phytochemicals known as polyphenols (30–36 %), most notably flavanols (including catechins), flavonoids, flavonodiols and phenolic acids. The majority of the polyphenols are flavanols, more commonly known as catechins (Ahmad and Mukhtar, 1999). The primary catechins in green tea are (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), (-)-epicatechin (EC), (+)-gallocatechin and (+)-catechin (Graham, 1992). The catechin in green tea that has gained the most attention with respect to the anticarcinogenic activity is the potent antioxidant EGCG. Figure 3 shows the
structure of EGCG. Much of the anticarcinogenic effect of green tea is attributed to EGCG. EGCG makes up about 10–50% of the total catechin content and appears to be the most powerful of all the catechins, responsible for the major health benefits associated with green tea consumption. EGCG has both anti matrix metalloproteinase and anti angiogenesis activities (Cao et al., 2002). A typical tea beverage, prepared in a proportion of 1g leaf to 100 mL water in a 3-min brew, usually contains 250–350 mg tea solids, comprising of 30–42% catechins and 3–6% caffeine (Balentine et al., 1997).

![Chemical Structure of EGCG](image)

Figure 3: Chemical Structure of EGCG

The health benefits of green tea are mainly attributed to its antioxidant properties and the ability of its polyphenolic catechins to scavenge reactive oxygen species (Yang, 1999). EGCG and structurally related green tea catechins were found to be strong inhibitors of lipid peroxidation in rat liver homogenates compared with such antioxidants as glutathione, ascorbic acid and tocopherol (Yoshino et al., 1994). Kidney slices from rats subjected to oral administration of EGCG (50 mg/kg body weight) for 7 days or fed with 3% green tea leaf powder for 50 days exhibited lesser extent of tert-butyl
hydroperoxide-induced lipid peroxidation, compared with the control animals (Sano et al., 1995).

Green tea consumption has been associated with a lower incidence of coronary artery disease in Japanese populations (Sano et al., 2004). Indeed, Miura et al. (2000) showed that oral intake of green tea extract by human volunteers increased resistance of plasma LDL to oxidation in vivo, an effect that lowers the risk of atherogenesis. Evidence also suggests that tea catechins affect discrete cell signaling pathways in neuronal cells, leading to a neuroprotective effect (Weinreb et al., 2003).

Epidemiologic, as well as laboratory studies, have revealed that increased consumption of green tea has been associated with reduced frequencies of several types of malignant tumors (Yang, 1997; Fujiki et al., 1998; Nakachi et al., 1998). Among many polyphenolic compounds isolated from green tea, EGCG is believed to be a key active constituent in terms of cancer chemopreventive potential (Fujiki et al., 1992; Komori et al., 1993; Fujiki et al., 1994). The strong antioxidative activity retained in this polyphenol has been confirmed in numerous in vitro and in vivo studies (Ho et al., 1992; Wei and Frenkel, 1993) and appears to contribute in part to the antimutagenic and anticarcinogenic effects of green tea. Several studies have demonstrated that EGCG can inhibit carcinogenesis at all stages: initiation, promotion and progression (Chung et al., 2003). This multifaceted inhibition of the tumorigenesis process is attributed to a combination of antioxidative, antiproliferative and pro-apoptotic effects (Gouni-Berthold and Sachinidis, 2004). EGCG has also shown to inhibit the process of angiogenesis, tumor metastasis and invasion in animal models (Jung and Ellis, 2001; Garbisa et al., 2001; Fassina et al., 2004).
ALOIN AND ALOE-EMODIN:

The *Aloe* plant – *Aloe barbadensis miller*, commonly known as Aloe Vera, has long been used as a traditional medicine and in the formulation of retail products such as laxatives, dietary supplements and cosmetics. Aloe extracts have also been used to treat inflammation (Hutter et al., 1996), cancers (Yoshimoto et al., 1987) and AIDS (Kahlon et al., 1991). It is also known to improve blood glucose levels (Ajabnoor, 1990), have antiviral action (Sydiskis et al., 1991) and modulates the immune response (Hart et al., 1989).

Many active components have been isolated from Aloe species and studied for their biological activities. Among them, aloin and aloe-emodin (Figure 4) have been identified as the main active components in Aloe. Aloin, also called barbaloin, is a bitter tasting yellow crystal and is the C-glycoside derivative of an anthraquinone (Saccu et al., 2001). The level of aloin in Aloe is highly variable and appears to depend on the species and strain of Aloe as well as the growing conditions. Aloin, which is localized in the outer rind of the Aloe plant, has been reported to constitute upto 30 % of the aloe plant’s dried leaf exudates (Groom and Reynolds, 1986). Aloe-emodin is a naturally occurring hydroxy anthraquinone derivative present in the leaves and roots of a number of plants. It usually occurs in combination with its glycosides or in a reduced form (anthrone) (Thomson, 1971). Aloe-emodin is present in low levels in plants such as Aloe and Senna and is thought to arise through oxidative decomposition of its glycosides rather than through direct biosynthesis (Grun and Franz, 1982). Although aloe-emodin is a minor constituent of most botanical raw materials, studies have shown that aloe-emodin is the pharmacologically active metabolite of aloin and sennosides (Lemli, 1988; Akao et al., 1996).
Introduction

Since plants and botanical ingredients containing aloin and aloe-emodin are widely used in traditional medicines and cosmetics, the toxicological properties of these compounds have been examined in a number of studies. The known pharmacological effects of aloe-emodin include antitumor, antifungal, antibacterial, antiviral and laxative activities. Aloin has been reported to be nonmutagenic using an *in vitro* assay (Brown and Dietrich, 1979). *In vitro* studies have provided evidence of aloe-emodin’s toxicity and suggest that aloe-emodin has preferential toxicity to carcinoma cells (Pecere et al., 2000; Lee et al., 2001; Wasserman, 2002). Furthermore, it has also

Figure 4: Chemical Structures of Aloin and Aloe-emodin
been shown that UV and visible light potentiate the toxicity of aloe-emodin and structurally related anthraquinones. Falvey and co-workers reported that exposure of human skin fibroblast to aloe-emodin and UV light elicited phototoxicity, which was associated with oxidative damage to both DNA and RNA (Vath et al., 2002; Warmer et al., 2003). Emodin, a structural isomer of aloe-emodin, has been reported to be toxic to leukemia cells after exposure to visible light (Hartley et al., 1990). Recently, it has been shown that aloe anthraquinones inhibited the LPS (lipopolysaccharide) induced inflammatory response in RAW 264.7 macrophages (Park et al., 2009). Since aloin and aloe-emodin contain a polyphenolic structure, these compounds may be responsible for the anti inflammatory effects of aloe (Korkina et al., 2003). The activity of aloe-emodin was comparable to that of kaempferol and to quercetin which are known potent inhibitors of inflammation (Park et al., 2009). Another study reported aloe-emodin as a new anti-angiogenic compound with inhibitory effects in vivo (Cardenas et al., 2006).

Biosynthesis of polyphenols in plants:

Resveratrol: It is produced as secondary metabolite in higher plants and serves to defend against pathogen attacks. The majority of polyphenolic compounds produced by plants are synthesized by a highly branched phenylpropanoid pathway. The initial compound is cinnamic acid, which arises from phenylalanine by the action of PAL (Phenyl-ammonia lyase). A series of hydroxylation, methylation and dehydration reactions leads to the formation of p-coumaric acid. 4-coumaroyl-CoA and three molecules of malonyl-CoA serve as precursor molecules which lead to the formation of a tetraketide which in turn forms resveratrol in the presence of the enzyme stilbene synthase (figure 5).
Introduction

![Biosynthetic pathway of Resveratrol](image)

PAL: Phenyl-ammonia lyase; C4H: Cinnamate-4-hydroxylase; 4CL: 4-coumaryl lyase; STS: Stilbene synthase

**Figure 5: Biosynthetic pathway of Resveratrol**
Introduction

Epigallocatechin-3-Gallate (EGCG): Flavonoids, especially the flavon-3-ols, (-)-epigallocatechin, (-)-epicatechin, (+)-gallocatechin and (+)-catechin are the most prominent metabolites present in tea. The precursor molecules in the flavon-3-ol biosynthetic pathway is 4-coumaroyl-CoA and malonyl-CoA which forms naringenin. Naringenin, in a series of reactions, leads to the formation of leucoanthocyanidins. Anthocyanidin synthase converts leucoanthocyanidins to anthocyanidins which finally leads to the formation of epigallocatechins by anthocyanidin reductase (Figure 6).

Aloin and Aloe-Emodin: Aloin and aloe-emodin are anthraquinones. Malonyl-Co-A plays an important role during the synthesis of stilbenes and flavonoids. It is also the starting point for the biosynthesis of anthraquinones via the formation and polymerization of malonyl-Co-A units by polyketide synthases (PKS). One acetyl-Co-A unit is extended by 7 malonyl-Co-A units via an octaketide chain under the influence of the enzyme octaketide synthase leading to the formation of anthraquinones (figure 7). The anthraquinones exhibit a particular substitution pattern i.e. they are substituted in both rings A and C. Aloe-emodin is typically substituted with hydroxyl groups in both the rings and a methylhydroxy group in ring C. The carbonyl group on the 10- position of aloe-emodin is converted to a hemiacetal with rhamnose forming aloin.
Coumaroyl CoA

3 Malonyl CoA → Chalcone synthase (CHS)

Naringenin

Flavanone 3β hydroxylase (FHT)

Dihydromyricetin

Dihydroflavonol 4-reductase (DFR)

Leucodelphinidin

Anthocyanidin synthase (ANS)

Delphinidin

Anthocyanidin reductase (ANR)

Epigallocatechin

Epigallocatechin-3-Gallate (EGCG)

Figure 6: Biosynthetic pathway of EGCG

(From: Punyasiri et al., 2004)
Absorption, metabolism and bioavailability:

Resveratrol: The absorption and transport of resveratrol have been studied in several models: isolated rat intestine (Andlauer et al., 2000; Kuhnle et al., 2000), rats and mice after oral administration (Asensi et al., 2002; Vitrac et al., 2003), human carcinoma Caco-2 cell line (Kaldas et al., 2003), human hepatocytes (Lancon et al., 2004) and healthy human subjects (Goldberg et al., 2003).

The human intestinal Caco-2 cells treated in vitro with resveratrol showed a high absorption of resveratrol which occurred principally by transepithelial diffusion. However, the transport of resveratrol was nonlinear with time, suggesting metabolism to be the rate limiting step with respect to bioavailability (Kaldas et al., 2003). In another study, resveratrol (25 mg/bw) was administered orally to six normal healthy volunteers. The absorption was at least 70 %, with peak plasma levels of resveratrol and metabolites of about 2 μM. Due to rapid and extensive metabolism, only trace amounts of unchanged resveratrol was found in the systemic circulation (Walle et al., 2004). Furthermore, resveratrol has a short initial
half life of ~ 8-14 min (Asensi et al., 2002). The bulk of an intravenous dose of resveratrol is converted to sulphate and glucouronide conjugates within ~ 30 min which have a half life of ~ 9.2 hours. Although modifications such as glucuronidation and sulphation aid in excretion, the undeniable in vivo efficacy of resveratrol, despite its low bioavailability has lead to speculation that its metabolites could retain some activity (Baur and Sinclair, 2006).

EGCG: Green tea catechins namely EGCG, EGC and EC have been extensively studied as cancer chemopreventive agents. However, the absorption and oral bioavailability of these catechins is low, resulting in systemic catechin levels in humans that are many fold less than the effective concentrations determined in in vitro systems (Chow et al., 2005). A study conducted on humans compared the pharmacokinetics of equimolar doses of pure EGCG and EGC in healthy volunteers. The average peak plasma concentrations after a single dose of 1.5 mmol/L were 5.0 μmol/L for EGC and 1.3 μmol/L for EGCG (Higdon and Frei, 2003). Another study, where decaffeinated green tea (20 mg tea solids/kg) was fed to human subjects, reported that the time needed to reach the peak plasma concentrations (of EGCG, EGC, EC) were in the range of 1.3-1.6 hours. The elimination half-lives were ~ 3.4, 1.7, and 2.0 hours respectively (Lee et al., 2002). It has also been shown that absorption of EGCG from the small intestine occurs largely via passive diffusion (Lambert et al., 2006). Furthermore, EGCG undergoes extensive biotransformation to yield methylated, sulphated and glucouronidated metabolites in mice, rats and humans (Lambert et al., 2006; Feng, 2006). Thus, intensive research on metabolism and bioavailability of tea catechins is required for future cancer chemoprevention studies with EGCG in vivo.

Aloin and Aloe-edomin: Aloin and aloe-edomin, as discussed earlier are polyphenolic constituents of aloe extract, which functions primarily as skin
conditioning agents. Aloe Vera extract appreciably penetrates the skin in vitro and in vivo (Boudreau and Beland, 2006). As a result, the topical application of aloe vera to the skin of humans may result in significant accumulation of aloe vera components both on the surface and within skin layers (Xia et al., 2007). In vivo studies have shown that orally administered aloin is poorly absorbed but is metabolized by esterases secreted by intestinal microflora to aloe-emodin, which is readily absorbed (Ishii et al., 1994). Once the C-glycoside of aloin is hydrolyzed, it forms aloe-emodin anthrone which is further auto-oxidized to the quinone form (Che et al., 1991).

**Anticancer and antitumor properties:**

**Resveratrol:** Resveratrol has been suggested as a potential cancer chemopreventive agent based on its inhibitory effects on diverse cellular events associated with tumor initiation, promotion and progression (Jang et al., 1997). It has also been shown to suppress the final steps of carcinogenesis, i.e. angiogenesis and metastasis (Delmas et al., 2006). Biochemical pathways involved in differentiation, transformation, cell cycle regulation and cell death induction have all been demonstrated as potential targets of resveratrol (Gusman et al., 2001; Joe et al., 2002). Resveratrol affects some of the intricate pathways operating in carcinogenic transformation of cells. These include intracellular generation of reactive oxygen species (ROS), activation of protein kinases, induction of enzymes such as cyclooxygenase (COX) and lipooxygenase, that generate proinflammatory mediators, activation of transcription factors etc. (Pervaiz, 2003).

Extensive literature on the anticancer activity of resveratrol, suggests a potential antiproliferative and apoptogenic use of resveratrol in various cellular models (Cucciolla et al., 2007). Depending on the concentration of
resveratrol used, studies have shown that resveratrol can either stimulate (Mizutani et al., 1998) or inhibit cell proliferation (Pervaiz, 2001). At relatively high concentration \( \cong 50 \mu M \), the effect generally is predominantly antiproliferative as demonstrated in a variety of cell lines such as HT29 and Caco-2 human colon cancer cells, Hep G2 cells, prostate cancer cells, MCF7 and HL60 cells etc (Joe et al., 2002; Saiko et al., 2008b; Colin et al., 2008; Benitez et al., 2009). The mechanisms for this growth inhibitory activity of resveratrol could be due to its ability to block ribonucleotide reductase (Fontecave et al., 1998; Saiko et al., 2008a), inhibit DNA polymerase (Tsan et al., 2002; Locatelli et al., 2005) or ornithine decarboxylase (Schneider et al., 2000; Ulrich et al., 2007). A number of studies have also established that resveratrol inhibits cellular proliferation by inducing cell cycle arrest in the G1/S phase (Bhat and Pezutto, 2002). In MCF-7 breast cancer cells and hepatic stellate cells, exposure to resveratrol resulted in accumulation of cells in the S phase caused by a decrease in the progression through the cell cycle or an inhibition of S to G2 phase transition (Souza et al., 2008; Marel et al., 2008). A number of protein targets of resveratrol have also been identified. In a variety of cellular models, resveratrol strongly upregulated p53 and p21 (tumor suppressor proteins) imposing a checkpoint on G1/S transition (Orallo et al., 2002; Mnjoyan et al., 2003; Alkhalaf, 2007). Furthermore, resveratrol activates a whole series of p53 responsive targets such as p21, p300/CBP, Apaf-1, p57(KIP2), Pig7, Pig8, Pig10, cyclin D and Bax that are related to cell cycle arrest and apoptosis, while it down regulated survivin, cyclin E, Bcl-2, Bcl-xl and cIAPs (Aggarwal et al., 2004; Narayanan, 2006). Resveratrol inhibits a key factor of cell survival, NF-\( \kappa \)B, through direct inhibition of I\( \kappa \)B kinase (Holmes-McNary and Baldwin, 2000). The inhibition of NF-\( \kappa \)B is associated with an antiproliferative action and with the induction of cell death (Estrov et al., 2003; Narayanan et al., 2003). NF-\( \kappa \)B controls the transcription of a variety of genes, including tumor promoting COX2, iNOS, matrix metalloprotease (MMP-9) and
endothelial adhesion molecules (Chen and Greene, 2004). In addition, dietary administration of resveratrol in DMBA induced tumor bearing rats reduced growth of tumor tissue and decreased transcription of NF-κB and its regulated genes COX2 and MMP9 (Banerjee et al., 2002). Inhibition of proliferation in human epidermoid carcinoma A431 cells have been shown to be associated with regulation of the JAK/STAT pathway, where resveratrol prevents phosphorylation of JAK, thereby inhibiting STAT1 phosphorylation (Madan et al., 2008). These observations suggest that resveratrol regulates the activation of transcriptional factors directed to clusters of genes responsible for inducing cell cycle arrest and eventually apoptosis.

Although some of the in vitro biological effects of resveratrol have not been corroborated in vivo, there is sufficient evidence to support the anti-proliferative and growth inhibitory activity in animal models of carcinogenesis. Earlier studies have shown that resveratrol treated mice developed fewer tumors in response to 7,12-dimethyl benz(a)anthracene (DMBA) and phorbol 12-myristate 13-acetate (PMA) in a two stage skin carcinogenesis model (Jang et al., 1997). Using HL60 human leukemia and T47D breast carcinoma cells, another study reported that there was a decrease in incidence of tumors in resveratrol treated mice which could possibly be due to the targeted killing of the tumor cells by resveratrol. Both cell lines exhibited classical hallmarks of apoptotic cell death (Clement et al., 1998; Pervaiz, 2001). Provinciali et al. (2005) have demonstrated that resveratrol supplementation delayed the development and reduced the metastasizing capacity of spontaneous mammary tumors in HER-2/neu transgenic mice. The antitumor effect of resveratrol was related to the downregulation of HER-2/neu expression and the induction of apoptosis in tumor cells.

A few reports have offered an interesting perspective on resveratrol actions on normal versus malignant cells. In one study, the IC 50 of resveratrol for
proliferation inhibition varied almost two fold: 34 µM in leukemia and 59 µM in hematopoietic cells. Human fibroblasts transformed with SV40 virus were sensitive to resveratrol modulation of pro versus anti-apoptotic genes whereas normal fibroblasts were not (Lu et al., 2001). These promising results contrast with those of other studies in which resveratrol exhibited similar effects on normal and neoplastic cells. Thus, being a natural constituent of wine, fruits and nuts and the fact that it has no deleterious effect on normal cells or tissues, resveratrol is under preclinical scrutiny for its therapeutic potential.

Additionally, experimental and epidemiological studies have shown that the micronutrients present in food can act as antimitotic agents, implicated in cancer initiation, promotion and progression, or mortality (Ames et al., 1995). Every antioxidant, including vitamin antioxidants are redox agents, protecting against free radicals in some circumstances and promoting free radical generation in others (Herbert, 1996). Studies have revealed prooxidant effects of antioxidant vitamins such as vitamin E (Burkitt and Milne, 1996) and vitamin C (Podmore et al., 1998), under certain circumstances. Moreover, earlier studies in our laboratory have established that several classes of plant derived polyphenolic compounds such as flavonoids (Rahman et al., 1990), tannins (Khan and Hadi, 1998), curcumin (Ahsan and Hadi, 1998) and capsaicins (Singh et al., 2001) are themselves capable of causing oxidative DNA breakage either alone or in the presence of transition metal ions. Similarly, although resveratrol is widely believed to be an antioxidant; there is evidence in literature to support its prooxidant properties, for instance the report on the concentration dependent induction of DNA strand breaks in ΦX-174 plasmid DNA by resveratrol (Win et al., 2002). Thus, it does appear that the anticarcinogenic activities of plant polyphenol, resveratrol may be related but not due entirely to their antioxidative and the above mentioned properties. A prooxidant action may be important in anticancer and apoptosis inducing properties of resveratrol.
Introduction

EGCG: Green tea, particularly its major polyphenolic constituent EGCG possess effective chemopreventive and therapeutic properties against various cancers (Mukhtar and Ahmad, 2000). It has been shown that EGCG inhibit carcinogenesis in a variety of tissues including lung, bladder, skin, small intestine, prostate and breast (Mimoto et al., 2000; Chen et al., 2004a; Mantena et al., 2005; Stuart et al., 2006; Thangapazham et al., 2007).

Numerous reports have demonstrated that the growth inhibitory or antiproliferative activity of EGCG in various types of tumor cells appears in part to be mediated via apoptosis (Valcic et al., 1996; Zhao et al., 2004; Raza and John, 2008). It has also been shown that EGCG induces cell cycle arrest and apoptosis in many cancer cells without affecting the normal cells (Ahmad et al., 1997; Yang et al., 2002). EGCG induced apoptosis in human epidermoid carcinoma cell line (A431), human carcinoma keratinocyte cell line (HaCaT), human prostate carcinoma cell line (DU145) and mouse lymphoma cell line (L5178Y) and such apoptosis inducing activity was related to cell cycle arrest in the G0-G1 phase (Ahmad et al., 1997). The growth of premalignant and malignant cells derived respectively from dysplastic leukoplakia and squamous carcinoma of oral epithelial origin was also inhibited by EGCG, and was found to be associated with cell cycle arrest in the G1 phase (Khafif et al., 1998a,b). Reports suggest that EGCG exerts its growth inhibitory effects through modulation of the activities of several key cell cycle regulatory proteins. Nihal et al. (2005) have shown that EGCG treatment of human melanoma cells resulted in significant dose-dependent decrease in cyclin D1 and CDK2 protein levels and induction of p16, p21 and p27. Kavanagh et al. (2001) reported that EGCG induced p27 in breast cancer cells, which caused growth arrest in G1/S phase. Hastak et al. (2005) have clearly demonstrated that EGCG activated growth arrest, primarily via a p53-dependent pathway that involved the function of both p21 and Bax such that down-regulation of either molecule conferred a growth advantage to prostate carcinoma cells. With regard to apoptosis,
EGCG has also been shown to activate caspase-3 and caspase-9, regulate mitochondrial functions (release of cytochrome c and Smac/DIABLO, and depolarization of mitochondrial membranes), and cleave PARP. These physiological events are critical for the mitochondrial-dependent apoptosis or cell-intrinsic pathway of apoptosis (Roy et al., 2005; Kuhn et al., 2005; Sen et al., 2006). EGCG has also been shown to modulate multiple signal transduction pathways in a fashion that controls the unwanted proliferation of cells, thereby imparting strong cancer chemopreventive as well as therapeutic effects (Khan et al., 2006). EGCG restrained carcinogenesis in a variety of tissues through inhibition of mitogen-activated protein kinases (MAPK), growth factor-related cell signaling, activation of activator protein 1 (AP-1) and nuclear factor-B (NF-κB), topoisomerase I, matrix metalloproteinases and other potential targets (Chen and Zhang, 2007). Maeda-Yamamoto et al. (2003) have reported that EGCG inhibited the phosphorylation of extracellular signal regulated kinases 1 and 2 (ERK1/2), and suppressed p38 MAPK activity in human fibrosarcoma HT1080 cells. EGCG has also been shown to inhibit NFκB activity in human colon and prostate cancer cells (Yan et al., 2004; Gupta et al., 2004; Song et al., 2006). Treatment of normal human epidermal keratinocytes with EGCG was found to inhibit UVB mediated activation of NFκB (Afaq et al., 2003). Furhtermore, EGCG has been shown to down-regulate the expression of COX-2 and iNOS by suppressing NF-κB activity (Surh et al., 2001). Several evidence has indicated that AP-1 plays a key role in cancer development and it is up-regulated during tumor promotion stage. It has been found that EGCG inhibited EGF- or TPA-induced cell transformation, as well as AP-1-induced transcriptional activity and DNA binding activity. This study also implicated that the inhibition of AP-1 activation occurred via the inhibition of a JNK-dependent pathway (Dong et al., 1997). Thus, AP-1 serves as another potential target, besides NF-κB, for the cancer preventive effects of EGCG.
EGCG can also prevent the cancer progression stage by influencing matrix metalloproteinases. Fassina et al. (2004) have found that EGCG (25-100 μmol/L) inhibited the MMP-2 and MMP-9 in endothelial cells. It has also been reported that EGCG inhibited the activity and expression of membrane-type matrix metalloproteinase 1-MMP (MT1-MMP), a protein responsible for the activation of MMP (Annabi et al., 2002). Thus, EGCG can inhibit or delay cancer invasion, metastasis, and angiogenesis via modulations in MMPs. It has also been demonstrated that EGCG selectively inhibited the activity of topoisomerase I (but not topoisomerase II), which play a role in DNA replication, transcription, and chromosome condensation in human colon cancer cell lines. The doses of EGCG necessary for this inhibition (10-17 μmol/L) were found to be lower than those necessary for inhibition of cell growth (IC50 = 10-90 μmol/L) (Berger et al., 2001). Besides inhibiting tumor promotion, EGCG administered intraperitonially caused growth inhibition and/or regression of experimentally induced skin papillomas in mice (Wang et al., 1992). EGCG also effectively suppressed the growth of human mammary cancer cells (MCF-7) in athymic mice (Liao et al., 1995). Thus EGCG, one of the major antioxidative polyphenol of green tea possesses a broad spectrum of anticarcinogenic effects.

Studies show that the anticarcinogenic activity of tea polyphenols are believed to be related, but not due entirely to their antioxidative properties. On the other hand, the prooxidant activity of tea polyphenols may play a role in inducing apoptosis (Yang et al., 2000). EGCG induced apoptosis of human lung cancer H661 cells could be inhibited by catalase (Yang et al., 1998) suggesting the role of ROS in apoptosis induction by EGCG. Another report has suggested that EGCG possess the chemical properties of a prooxidant. It has been shown that ROS generation caused by EGCG triggers apoptosis in human lymphoblastoid B cells (Noda et al., 2007). Thus, EGCG seems to be an attractive agent for chemopreventive/chemotherapeutic approach towards combating cancer.
Aloin and Aloe-emodin: The chemopreventive efficacy of aloin and aloe-emodin are in the process of being explored and the mechanisms of their anticancer effect are largely unknown. Reports have shown that aloe-emodin possess antiproliferative effects on some types of cancers, such as lung squamous, glioma and neuroectodermal cancer cells (Pecere et al., 2000; Lee et al., 2001; Mijatovic et al., 2005). Pecere et al. (2000) have shown that aloe-emodin has a specific *in vitro* and *in vivo* antineuroectodermal tumor activity. The growth of human neuroectodermal tumors was inhibited in mice without any significant toxic effect on the animals. It was also shown that aloe-emodin did not inhibit the proliferation of normal fibroblasts nor that of hematopoietic progenitor cells. The cytotoxicity lead to the induction of apoptosis. This was the first report that described the potential antitumor activity of aloe-emodin. Subsequently, several studies conducted with aloe-emodin reported its antiproliferative and apoptosis inducing ability in cancer cell lines. Chen et al. (2004b) demonstrated that aloe-emodin inhibited cell proliferation and induced G2/M arrest and apoptosis in human promyelocytic leukemia HL-60 cells. It has also been shown that aloe-emodin induced apoptosis in human gastric carcinoma cells by causing the release of apoptosis inducing factor and cytochrome c from mitochondria followed by activation of caspase-3 (Chen at al., 2007). Guo et al. (2008) reported that anticancer effect of aloe-emodin on gastric cancer cells involved suppression of c-myc expression leading to cell cycle arrest in G2/M phase. Another study was conducted to investigate the anticancer effect of aloe-emodin on two human liver cancer cell lines, Hep G2 and Hep B3. It was observed that aloe-emodin inhibited cell proliferation and induced apoptosis in both examined cell lines but with different antiproliferative mechanisms. In Hep G2 cells, aloe-emodin induced p53 expression, accompanied by induction of p21 expression that was associated with cell cycle arrest in G1 phase. In contrast, with p53 deficient Hep B3 cells, the inhibition of cell proliferation was mediated through a p21 promoted aloe-
emodin induced apoptosis by enhancing expression of Bax (Kuo et al., 2002).

Aloe-emodin has also been shown to exert photocytotoxic effect on tumor cells. Photoexcitation of aloe-emodin induced cytotoxicity and photooxidative damage of RNA and DNA in human skin fibroblasts through the formation of singlet oxygen (Vath et al., 2002; Wamer et al., 2003). Cardenas et al. (2006) have reported that photoexcited aloe-emodin was much more cytotoxic than unexcited aloe-emodin to human HT-1080 fibrosarcoma and U2-OS osteosarcoma cells. This remarkable result suggested that aloe-emodin could be a candidate for photodynamic therapy for some kinds of cancers (Brown et al., 2004).

Although aloe-emodin has been extensively investigated for apoptosis inducing effects, the precursor to aloe-emodin i.e. aloin, has been subjected to only minimal investigation for any cytotoxic effects. Recently, one of the studies reported that aloin induced apoptosis in Jurkat cells by specifically blocking G2/M phase (Buenz, 2008).

Thus, studies suggest that aloe-emodin represents a suitable chemotherapeutic drug candidate for treatment of some cancers whereas further studies need to be conducted in order to explore the chemopreventive properties of aloin.