INTRODUCTION
Dietary constituents and prevention of disease

Food provides not only essential nutrients needed for life but also other bioactive compounds for health promotion and disease prevention. Previous epidemiological studies have consistently shown that diet plays a crucial role in the protection of chronic diseases (Temple, 2000). Consumption of fruits and vegetables as well as grains, has been strongly associated with reduced risk of cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts and age related functional decline (Temple, 2000; Willett, 1995). 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 (Willett, 1995; Doll & Peto, 1981). This convincing evidence suggests that a change in dietary behavior 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 and have been linked to the reduction in the risk of major chronic disease. It is estimated that more than 5000 phytochemicals have been identified but a large percentage still remains unknown (Shahidi et al., 1995) and they need to be identified before their health benefits are fully understood. However, more and more convincing evidence suggests that the benefits of phytochemicals 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 chronic disease (Ames & Gold, 1991). Cells in humans and other organisms are constantly exposed to a variety of oxidizing agents some of which are necessary for life. These agents may be present in air, food or water or they may be produced by metabolic activities within the cells. The key factor is to maintain a balance between oxidants and
antioxidants to sustain optimal physiological conditions within the body. Overproduction of oxidant can cause an imbalance leading to oxidative stress, especially in chronic bacterial, viral and parasitic infections (Liu et al., 1995). Oxidative stress can cause damage to large biomolecules such as proteins, lipids and DNA resulting in an increased risk for cancer and cardiovascular disease (Ames & Gold, 1991; Liu et al., 1995; Ames et al., 1993). To prevent or slow down the oxidative stress induced by free radicals, sufficient amounts of antioxidants need 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 disease. 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 & Giavanucci, 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 (Kelloff & Boone, 1996; Mayne & Lipman, 1997; Sporn, 1991). On the other hand dietary prevention is recognized as the changes in food consumption pattern necessary to decrease cancer development (Goodman, 1997; Schatzkin
& Kelloff, 1995). 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., 1988). 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 (Jaruga et al., 1998; Clement et al., 1998; Inoue et al., 1994). 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 is 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).

**Biosynthesis of plant polyphenols**

Polyphenolic compounds are produced as secondary metabolites in higher plants. These compounds fulfill a vast array of important functions in plants, being involved in development and interactions with the environment (Croteau et al., 2000). For example stilbenes and coumarins serve to defend pathogen attacks, flavonoids act as UV irradiation protectents while isoflavone serve as flower pigments.

The majority of polyphenolic compounds produced by plants are synthesized by a highly branched phenylpropenoid pathway. The initial compound is cinnamic acid, which arises from phenylalanine by the action of PAL. Several simple polyphenols with the basic C6-C3 skeleton of...
phenylalanine are produced from cinnamate via a series of hydroxylation, methylation and dehydration reactions; these include p-coumaric acid, caffeic acid, ferulic acid, sinapic acids and other simple coumarins (Dixon et al., 1995). In addition, compounds such as styrenes, benzoic acid and derivatives, acetophenones and gingerols arise from hydroxycinnamic acid by chain shortening and lengthening without ring formation. Addition of cyclic esters at the side chains produces hydroxy coumarins and chromones and various condensation reactions with malonyl residue produce xanthones, stilbenes and flavonoids.

Figure 1. Biosynthesis of polyphenols in higher plants.

PAL, Phenyl-ammonia lyase; C4H, Cinnamate-4-hydroxylase; TAL, Tyrosine-ammonia lyase; 4CL, 4-coumaryl lyase.
Anticancer properties of plant polyphenols

Numerous studies have reported flavonoid mediated antiproliferative effects against human and rodent ovarian, intestinal, lung, breast and bladder cancer cells as well as leukemic 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., 1994a, 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 & Chawdhury, 1995; Kang & 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 doxorubicin 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., 1997). Curcumin is cytostatic in several hormone dependent (MCF-7 and T 47D) and independent (SK-BR3, BT-20 and MDA231) 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., 2001). 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). Quercetin also inhibits ML-3 murine hepatoma cell growth (Chi 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
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Inhibition in HL60 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 flavonoid quercetin and taxifolin and the polymethoxylated flavonoids nobiletin and tangeretin inhibit HTB 43 squamous cell carcinoma cells and 9L gliosarcoma cell growth, but are less effective in transformed human CCl human 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 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 fibroblast cell growth only weekly compared to their virally transformed (VA) counterparts. The IC₅₀ value of EGCG was 120 µM in W138 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 dimethyl-benz-(a)-anthracene (DMBA) or N-nitrosomethylurea (MNU) reduces the incidence and multiplicity of mammary tumor 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). Quercetin (20 g/Kg) also increases the survival and reduces tumor burden of mice (Balb/c) transplanted intrasplenically with ML-3 hepatoma cells.
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(Chi et al., 1997). The citrus flavonoid naringenin inhibits the in vivo development of DMBA induced mammary tumors in Sprague-Dawley rats (So et al., 1996). Several studies have described protective effects of tea polyphenols against carcinogenesis. Rats fed on a diet containing 10 g green tea catechins/kg have a considerably reduced mortality (7% reduced mortality) from mammary tumors following DMBA treatment compared with rats given carcinogen alone (66%) (Hirose et al., 1994). Similarly hamster fed on green tea polyphenols display fewer hyperplastic pancreatic duct lesions after treatment with N-nitroso-bis-(2-oxopropyl)-amine (Majima et al., 1998). In a comprehensive study, Yang et al. (1998) described the ability of both green and black tea infusions to inhibit N-nitrosodiethyl-amine-induced lung carcinogenesis in A/J mice.

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

Antioxidant effects

Carcinogenesis is a multi-stage process of genetic changes 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., 1997, 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 important to the antioxidant and cytoprotective potential of the compound. There are also many
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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 & 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 activities 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 procarcinogens 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 P-450 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 ability of the flavonoids to inhibit P450 2B activity. Tangeretin inhibits nifedepine 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 potent
inhibitors of cDNA expressed human P450s IA1 and IA2 (IC$_{50} < 1$ μM), while galangin is a selective inhibitor of P450 IA2 (Zhai et al., 1998). The ability of flavonoids to inhibit P450 IA 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* TA98). Trp-P-2 is metabolized by P450 IA 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 (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 biases in recall and reporting of intake patterns.

**Biosynthesis and natural sources of caffeic acid (CA)**

CA (3,4-dihydroxycinnamic acid) is a simple phenolic acid and a catechol. It possesses a single propenoic side chain on its aromatic ring that contains a dihydroxy catecholic moiety (Figure 2). In higher plants it is synthesized in phenylpropanoid pathway. The immediate precursor is p-coumaryl CoA (Figure 1). It is the major representative of dietary cinnamates. In foods, CA is esterified/conjugated mainly with quinic acid,
which yields chlorogenic acid (5-caffeo-y1quinic acid). Coffee is the major source of chlorogenic acid, i.e., 1 L of coffee provides 500-800 mg of chlorogenic acid, which corresponds to 250-400 mg of CA (Clifford, 1999). CA is also found in fruits, grains, vegetables and many other plants (Eberhardt et al., 2000). CA, free and esterified, is the most abundant phenolic acid and represents between 75 and 100% of the total hydroxycinnamic acid content of most fruits (Manach et al., 2004). It is also a constituent of wine (Shahidi et al., 1995; Macheix et al., 1990) and is particularly abundant in propolis beehives with 20%-25% content (Greenaway et al., 1987). Thus it is an important dietary constituent in humans and its intake has been estimated in the order of 206 mg/day in subjects drinking coffee (Radtke et al., 1998). Additionally, it accounts for up to 90% of total intake of phenolic acids in humans (Radtke et al., 1998).

![Chemical structures of caffeic acid, p-coumaric acid, and o-coumaric acid](image)

**Figure 2.**

**Absorption of caffeic acid**

There is less data on CA absorption in humans. The major problem in measuring the absorption of CA in humans is its bacterial degradation in the colon (Scheline, 1968). To overcome this problem Olthof et al. (2001) studied the absorption of CA in healthy ileostomy subjects and reported that the maximum of 95% of the ingested CA was absorbed from the small intestine in humans. In rat plasma, CA glucuronides and sulfates are the main plasma metabolites reaching their maximum concentrations of 0.12-0.34 μM/L within the first 0.5-1 hour after administration of 700 μmol/kg chlorogenic acid (Azuma et al., 2000). Using an experimental model Nardini et al. (1997) have demonstrated that 0.8% CA in the diet is associated with plasma concentrations of up to about 5.52 μM. Dietary CA is readily absorbed by humans and rat where it circulates in plasma at micromolar concentrations (Nardini et al., 1997;
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Jacobson et al., 1983). A study by Gumbinger et al. (1993) reports that ferulic and isoferulic acids are the tissular metabolites of CA. Within the acidic environ of stomach CA is absorbed by passive non-ionic diffusion. This is supported by the fact that CA and structurally related compound cinnamic acid were rapidly absorbed in rats (Fahelbum et al., 1977). The second mechanism for the absorption of CA might involve absorption by an active transport mechanism in the small intestine. Results from in vitro studies indicate that in the small intestine, an active Na⁺-dependent transport mechanism might be involved in the absorption of cinnamic acids such as CA (Wolffram et al., 1995; Ader et al., 1996). Mechanisms, passive absorption in the stomach as well as active absorption in small intestines might play a role in the absorption of CA in humans.

**Caffeic acid as an antioxidant**

CA has been reported to have a wide spectrum of biological effects such as anti-inflammatory properties (Fernandez et al., 1998), anti-tumor activity (Tanaka et al., 1993 & Hagiwara et al., 1996), inhibition of HIV replication (Kashiwada, Y. et al., 1995 & Kries, W. et al., 1990) and apoptosis inducing activity (Satoh & Sakagami, 1997; Orsolic et al., 2004). Many of the biological properties of CA are attributed to its antioxidant potential. Among natural phenolic compounds, the antioxidant activity of CA has been described extensively on liver microsomes or homogenates (Cholbi et al., 1991), erythrocytes and isolated rat hepatocytes (Annon et al., 1992; Liu et al., 1992). Its action as an oxygen radical scavenger and chain-breaking antioxidant is well documented (Laranjinha et al., 1994; Bors et al., 1984). According to Carton et al (2001) CA was a superior antioxidant compared with other phenolic acids such as p-coumaric and ferulic acids in inhibiting LDL-oxidation and therefore might protect against cardiovascular disease (Nardini et al., 1995). CA inhibits the oxidation of lipoproteins exposed to ferrylmyoglobin and to recycling alpha-tocopherol from alphatocopheroxyl radical (Laranjinha et al., 1995). An in vivo study has shown that dietary supplementation of CA in rats resulted in a statically significant increase of alphatocopherol both in plasma and lipoprotein (Nardini et al., 1995). Moreover, CA was found to be present in postprandial plasma in micromolar concentrations, doubling
plasma antioxidant capacity. These results demonstrate the physiological relevance of CA and its antioxidant action.

Antitumor, antimutagenic and apoptosis inducing activity of caffeic acid

Antimutagenic potential of caffeic acid has been described in several studies. In two short-term genotoxicity assays (Ames assay and Drosophila wing spot test), mutations were induced by aflatoxin B₁; CA was effective in reducing these mutational events whereas α-tocopherol did not show any antimutagenic action (Karekar et al., 2000). Similarly, the potential of CA to reduce acridine- and ofloxacin-induced genotoxicity was also evaluated by the Ames assay (Belicova et al., 2001). Nevertheless, both studies concluded that the capacity of CA to protect DNA results from the blockage of mutagenic action by means of CA interacting with a genotoxic compound (acridine orange), from the arrest of metabolic activation of a promutagen (aflatoxin B₁) or from scavenging ROS produced by a mutagen (ofloxacin), respectively (Karekar et al., 2000; Belicova et al., 2001).

CA may be considered a two faceted molecule (Nardini et al., 2001) as it shows proapoptotic effects at high concentrations (> 200 μM), and antiapoptotic properties at lower levels. This behavior may explain the wide spectrum of biological activities, sometimes conflicting, ascribed to CA, and is attributable to the ability of CA in inhibiting, in a concentration dependent fashion, the activity of different specific kinases, such as PKC (Nardini et al., 2000) and PTK, involved in fundamental signal transduction pathways. CA has been shown to be the most effective quencher of singlet oxygen among several simple phenolic acids tested (Foley et al., 1999). It has been reported to present a scavenging activity towards the hydroxyl radical (Chimi et al., 1991). It has also been shown to eliminate superoxide anion (Laughton et al., 1989; Zhou & Zheng, 1991). All these reactive oxygen species have been positively linked to tumor promotion and progression and therefore it may not be surprising to expect CA as having anti-tumor properties. Keunzing and co-workers (1984) showed that CA reacted rapidly and completely with equimolar quantity of sodium nitrite in simulated gastric fluid. In rats receiving aminopyrine and nitrite, but not in rats treated directly with N-nitrosodimethylamine (NDMA), CA blocked the elevation of serum NDMA levels. The
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authors suggested that dietary CA might play a role in the body’s defence against carcinogenesis by inhibiting the formation of N-nitroso compounds. CA has been shown to reduce the mutagenicity of terminal carcinogens of polycyclic aromatic hydrocarbons (Wood et al., 1982). CA, when concurrently administered with the carcinogen, inhibited the formation of tongue neoplasmas (squamaus cell papilloma and carcinoma) induced by 4-nitroquinoline-1 oxide in male F334 rats (Tanaka et al., 1993). The authors suggest possible application of CA for cancer chemoprevention in tongue in addition to other tissues (skin, lung, liver and esophagus). It has been shown to have an inhibitory effect on development of naturally occurring preneoplastic hepatocytic foci in long term feeding studies in male F344 rats (Hagiwara et al., 1996). CA has been reported to induce apoptotic cell death in human promyelocytic leukemia HL60 cells and that apoptotic effect was enhanced by CuCl₂ or deferoxamine mesylate, an iron chelator, but was reduced by FeCl₃ (Satoh et al., 1997). Oral administration of CA has also been reported to inhibit subcutaneous tumor growth whether given before tumor cell inoculation or after stabilization of tumor growth. The life span of mice treated with CA before tumor cell inoculation was significantly prolonged compared with controls (Orsolic et al., 2004). The same authors (Orsolic et al., 2005) in a recent study show direct anti-tumor activity of CA towards transplantable mammary carcinoma cells in mice when the compound is administered locally at the exact site of tumor growth. Their results confirmed that CA treatment delays tumor growth and significantly increases the survival period without mortality or body weight loss. In a novel study Chung et al. (2004) showed that CA inhibited hepatoma growth and metastasis achieving complete regression by exerting two novel but different mechanisms. Here CA suppressed the growth of HepG2 tumor xenografts in nude mice in vivo. The subcutaneous and oral administration of CA significantly reduced liver metastasis. The anti-tumor and anti-metastasis effects of CA were mediated through the selective suppression of MM-9 enzyme [which plays a major role in promoting angiogenesis and metastasis] activity and transcriptional down regulation by the dual inhibition of NF-kB as well as MM-9 catalytic activity. Further it has been shown in this study that CA suppresses the function of NF-kB by blocking its nuclear translocation which otherwise
would bind to the promoter region of MM-9 gene activating its transcription. According to these authors these findings have important implications for the therapeutic potential of CA, which may reduce the incidence of adverse events such as pain and tenderness in the joints and pain affecting shoulders and hands that appear in long term treatment with broad-spectrum MMP inhibitors in clinical studies. Xu et al. (2005) recently studied the effect of CA on several cell lines. Their results showed that CA inhibits proliferation of cancer cells, with IC\(_{50}\) between 100 µg/ml and 200 µg/ml. Further CA induced apoptotic cell death in human hepatocarcinoma cell line (BEL-7402) and changed cell cycle distribution by arresting cells in S phase. The \textit{in vivo} part of the same study showed that CA inhibited the tumor growth of mouse-transplanted hepatocarcinoma H22 and colorectal cancer C26 cells with an inhibition rate of 42-43% when the mice were treated with 1 g/kg CA for 10 days. Xu et al. (2004) studied inhibition of tumor angiogenesis and anti-metastasis effect of CA using various \textit{in vivo} and \textit{in vitro} metastasis assays. The \textit{in vivo} inhibitory effect of sodium caffeate on the metastasis and angiogenesis was examined in the Lewis lung carcinoma pulmonary metastasis model and chicken chorioallantoic membrane (CAM) model, respectively. The \textit{in vivo} results showed that CA (1 gm/kg i.p. for 14 days) inhibited pulmonary metastasis at a rate of 55%. The angiogenesis of CAM was inhibited by CA (200 µg/egg) at a rate of 70%. The \textit{in vitro} results showed that CA inhibited the proliferation of transformed human umbilical vein endothelial cells by inducing apoptosis. CA also reduced the adhesion and invasion ability of human high metastatic giant cell carcinoma of the lung cells and inhibited the secretion of MMP-2 and MMP-9 in these cells. Interestingly CA has been shown to inhibit apoptosis induced by oxidized low-density lipoproteins in cultured human endothelial cells (Viereia et al., 2000). CA in a few studies has been shown to have hazardous effects too. Administered at 2% in the diet for two years, CA was shown to induce hyperpalasias and tumors in the forestomach and kidney of F344 rats and B6C3F1 mice (Hagiwara et al., 1991). On the other hand, CA at 0.05% for 32 weeks inhibited squamous epithelial carcinoma of the rat tongue induced by 4-nitroquinoline-1-oxide given for 5 weeks (Tanaka et al., 1993).
Caffeic acid influences enzyme activities

CA has been shown to influence the activity of several key enzymes. The reported ability of CA to inhibit several protein kinases such as phosphorylase kinase (PhK), protein kinase C (PKC) and protein kinase A (PKA) suggest a more direct and specific involvement of this molecule in the modulation of cellular functions not necessarily associated with its antioxidant activity. All these kinases are known to trigger the major signal transduction systems in the course of a wide spectrum of cell responses. PhK is the key regulatory enzyme involved in the metabolism of glycogen, while both PKA and PKC are involved in the major signal transduction systems. A non-competitive inhibition of PhK by curcumin, a phenolic compound present in turmeric, has already been reported (Reddy et al., 1994). CA might interact with beta subunit of PhK or other regulatory subunits, possibly inducing a conformational change resulting in the inhibition of the activity, as suggested for curcumin (Reddy et al., 1994). The strong inhibition of PhK may have important implications for the anti-proliferate activity of these polyphenols (Nardini et al., 2000). CA is a strong inhibitor of 5- and 12-lipooxygenases (Koshihara et al., 1984), glutathione S-transferase (Chan et al., 1995) and xanthine oxidase (Schefferlie et al., 1993).

Biosynthesis and natural sources of \( \beta \)-coumaric acid

\( \beta \)-coumaric acid (4-hydroxycinnamic acid) is a simple phenolic acid having a propenoic side chain and a single phenolic hydroxyl group on its aromatic ring (Figure 2). It is synthesized in higher plants in the phenylpropanoid pathway and arises from tyrosine and phenylalanine. The immediate precursor is cinnamic acid which is hydroxylated by \( \text{C}4\text{H} \) at the para position to form \( \beta \)-coumaric acid (Figure 1). \( \beta \)-coumaric acid is widely distributed in many foods. The major sources are tea (1-2 mg/kg), spinach, Brussels sprouts and cereal brans (2-60 mg/kg), apples and berries (69-1700 mg/kg) (Clifford, 1999). Zhou et al. (2004) have studied the phenolic acid composition in rice in three cultivars. The phenolic profiles of both brown and milled rice were dominated by ferulic acid and \( \beta \)-coumaric acid. Alcoholic beverages- beer, white and red wines are also major sources of \( \beta \)-coumaric acid, latter being the richest among the three. The mean values of \( \beta \)-coumaric acid for white wine, red wine and beer are 2.2, 4.1 and 2.1
mg/L respectively (Gorinstein et al., 2000). Que et al. (2006) studied the phenolic contents of five Chinese rice wines and identified $p$-coumaric acid as one of the several phenolic constituents. Nardini et al. (2004) studied the composition of free as well as bound phenolic acids in hydrolysed and non-hydrolysed beer samples of three different brands- Italian, Austrian and German beers. The content of $p$-coumaric acid increased moderately (the increase was $\leq 50\%$ of the value measured in non-hydrolyzed samples) after hydrolysis in all beers except German beer, which showed 469% increase in $p$-coumaric acid content after hydrolysis.

**Absorption and metabolism of $p$-coumaric acid**

Absorption, distribution, metabolism and elimination of $p$-coumaric acid have not been extensively investigated. Since this dietary component is an acid, it is probably absorbed mainly in the stomach and in the first part of small intestine, as reported for other similar compounds (Fahelbum & James, 1977). It is possible that $p$-coumaric acid as such or as conjugated with glycine or glucuronic acid is excreted through gut, where it can be deconjugated and hydroxylated by the gut microflora, as reported for cinnamic and benzoic acids (Clifford, 1999). $p$-coumaric acid as well other hydroxycinnamates occur naturally in esterified forms (Macheix et al., 1990) and are not cleaved in gastric lumen (Rechner et al., 2001) nor the small intestine (Plumb et al., 1999), but in the colon by esterase activity of the gut microflora (Scheline, 1991). A study by Rechner et al. (2002) detected low levels of glucuronide derivatives of $p$-coumaric acid in a group of healthy volunteers who were put on polyphenol specific meal components. Yeh & Yen (2006) administered orally $p$-coumaric acid in rats (10 mg/kg of body weight), and it was found to be absorbed and distributed to the blood in intact form. Comparison of the time course of changes in plasma concentrations showed that $p$-coumaric acid was directly absorbed and distributed in the blood, and the plasma concentrations increased in the period up to 2 hr postadministration and then gradually decreased. These results show a close agreement with prior work, done by Konishi et al. (2003), showing that phenolic acids, especially $p$-coumaric acid, were directly absorbed and distributed to the blood.
Recently large amount of research has been done about the absorption of phenolic compounds. Most of the studies concerning absorption of phenolic compounds from diet are carried out on rats, while relatively, few papers deal with the measurement of phenolic acids and their metabolites in human urine. The measurements of human plasma levels of phenolic acids after phenolics or phenolic-rich food administration are reported by a limited number of studies. Nardini et al. (2006) studied the absorption in humans of several phenolic acids, with related structures, from beer, focusing on the measurement of plasma levels of free and conjugated forms of phenolic acids. Fasting subjects received in the morning 500 ml of beer in combination with 27 g of crackers. A significant rise in both free and conjugated (glucuronates and sulfates) phenolic acid levels in human plasma after beer administration, with maximum absorption peak at 30 min was observed. A significant increase in nonconjugated and total p-coumaric acid was observed 30 min after beer plus cracker administration with respect to time 0, the nonconjugated forms amounting to 54.4%, 54.7% and 59.5% of total at time 0, 30 and 60 min, respectively. Among conjugated derivatives, a significant increase in glucuronate derivatives was observed at 60 min after ingestion with respect to time 0, while the increase of sulfate conjugates measured at 30 and 60 min after ingestion did not reach statistical significance.

**Antioxidant properties of p-coumaric acid**

Little attention has been focused on the antioxidant potential/activity of simple phenolic acids which are at higher concentration in dietary plant materials than the polyphenolic flavonoids and anthocyanidins. P-coumaric acid has been shown to act as scavenger of thiol free radicals (D’aquino et al., 1994). Lodovicci et al. (2001) have also shown the radical scavenging activity of p-coumaric acid. Kumar et al. (2006) undertook an investigation on the bound and free phenolics present in turmeric (*Curcuma longa*). The results indicate that p-coumaric acid, besides curcumin, contributes significantly to the antioxidant activity of turmeric. These findings were further confirmed by determining the free radical scavenging ability of p-coumaric acid where it exhibited an IC$_{50}$ of 31.25 µg/mL. Nardini et al. (1995) analyzed the antioxidant capacity of p-coumaric acid on *in vitro* oxidative modification of human LDL catalyzed by Cu$^{2+}$. The study shows...
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that the presence of second hydroxyl group (CA) enhanced its antioxidant capacity. Castelluccio et al. (1995) have reported significant antioxidant activity of \( p \)-coumaric acid in terms of its ability to increase the resistance of LDL to cholesterol oxidation, lipid peroxidation and oxidative modification of apoprotein \( B_{100} \). Several other studies report potent inhibition of LDL oxidation \textit{in vitro} by \( p \)-coumaric acid (Morton et al., 2000). Vieira et al. (1998) showed that \( p \)-coumaric and caffeic acids synergistically interplayed with ascorbate in the protection of LDL from oxidation promoted by ferrylmyoglobin, a 2 electron oxidation product that was generated from the reaction of methemoglobin and \( \text{H}_2\text{O}_2 \). The authors conclude that there is a synergistic antioxidant activity of diet-derived \( p \)-coumaric and caffeic acids with ascorbate in the protection of LDL from oxidation and is of physiological relevance. Owen et al. (2000) also found that \( p \)-coumaric acid possesses antioxidant activity against ROS produced by hypoxanthine and xanthine oxidase. Abdel-wahab et al. (2003) studied the influence of \( p \)-coumaric acid on Doxorubicin (DOX)-induced oxidative stress in rat's heart. DOX is a quinine-containing anticancer antibiotic that is widely used to treat different types of human neoplastic diseases such as hematopoetic, lymphoblastic (Hitchcock-Bryan et al., 1986) and solid tumors (Bonadonna et al., 1996). The clinical use of this anticancer drug is greatly limited by its dose-dependent cardiotoxicity (Booser et al., 1994). The involvements of oxygen free radical, superoxide radical and oxidative stress have been strongly accepted as crucial factors in the pathogenesis of DOX-induced cardiac damage. The authors have attributed the protecting potential of \( p \)-coumaric acid to its free radical scavenging capability and therefore suggested the concomitant administration of \( p \)-coumaric acid with DOX cancer therapy.

\textbf{Antimutagenic and anticancer properties of \( p \)-coumaric acid}

Guglielmi et al. (2003) have demonstrated that \( p \)-coumaric acid at a dose of 50 mg/kg decreases effectively (by 50\%) basal oxidative DNA damage, measured as 8-OHdG levels and by Comet assay, in rat colonic mucosa. The authors showed that the protective effect of \( p \)-coumaric acid is related to the increased expression of GST-M2, an important isoform of GST, which is highly expressed in many tissues (Eaton & Bammler, 1999) and plays a protective role against endogenous oxidative stress in
many tissues (Baez et al., 1997). It is interesting to observe in this respect that epigallocatechin gallate, one of the major constituents of green tea polyphenols with interesting chemopreventive properties, specifically induces the GST-M2 isoform (Chou et al., 2000). Further Lodovici et al. (2001) have shown that p-coumaric acid reduced oxidative DNA damage induced in vitro by Fe and cumene hydroperoxide.

Virtually all human prostate cancers, regardless of grade and stage, lack expression of phase 2 enzyme, GSTP-1 (glutathione S-transferase) (Lin et al., 2001). Brooks et al. (2002) screened a diverse set of compounds for their ability to induce quinone reductase enzyme activity, a surrogate of phase 2 enzyme response, which compensates for the loss of GSTP-1 expression, in human prostate cancer cell line LNCaP. The authors have highlighted an intriguing possibility that p-coumaric acid in tomatoes may act in concert with lycopene to protect against prostate cancer by quenching free radicals and inducing carcinogen defenses in prostate cancer cells. Hudson et al. (2000) also have shown that brown rice (a staple diet in Asia) extract, in which p-coumaric acid was found to be one of the several phenolic constituents, inhibits proliferation and colony forming ability of cancer cells. The group further demonstrated that purified p-coumaric acid also possessed cytotoxic/cytostatic and anticlonogenic properties in human derived immortalized and tumorigenic breast (HBL 100) and colon (SW 480) cancer cells.

Plant cell walls are composed of polysaccharides (Harris & Ferguson, 1993). However, these also contain other components that may confer on them health promoting properties. In particular, un lignified primary cell walls of grasses and cereals and of other families of monocotyledons, and of families in the dicotyledon order Caryophyllales, contain the hydroxycinnamate ferulic acid, together with small amounts of p-coumaric acid ester-linked to the cell wall polysaccharides (Smith & Harris, 2001). In this connection it is noteworthy to note that Ferguson et al. (2003) have determined the ability of plant cell wall extract (wheat coleoptiles) as well as chemically purified p-coumaric acid to protect against different types of mutations in Salmonella typhimurium strains TA98 and TA102 induced by H2O2 and bleomycin by Ames mutagenicity test. The authors point out that the inhibitory effect of p-coumaric acid on mutagenicity is

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because that it reduces the concentration of mutagenic oxidation products by free radical scavenging. Further these authors have correlated this study to mammalian systems and hypothesize that since hydroxycinnamic acids (that includes $p$-coumaric acid) are likely to be released in the human colon, their antmutagenic properties could have significance for dietary fibre protection against cancer. The antmutagenic activity of $p$-coumaric acid has also been reported in the salmonella-microsome assay (Eaton & Bammer, 1999).