Introduction
INTRODUCTION

Polyphenolic nutraceuticals in chemoprevention

A nutraceutical is defined as a food or part of a food that provides medical or health benefits, including the prevention or treatment of a disease. Epidemiological studies have consistently shown that diet plays a crucial role in the protection against chronic diseases (Willett, 1994; Temple, 2000). Consumption of fruits and vegetables as well as grains, has been strongly associated with reduced risk of cardiovascular diseases, cancer, diabetes, Alzheimers disease, cataract and age related functional decline (Willett, 1994; Willett, 1995; Temple, 2000). It is believed that dietary constituents derived from plant sources have the ability to modify the disease process thus relating the food stuffs, beyond their basic nutritional benefits, to disease prevention [Roger 1993; Thomasset, 2007]. 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). Thus convincing evidence suggests that a change in dietary behaviour such as increasing the consumption of fruits and vegetables is a practical strategy for significantly reducing the incidence of chronic diseases.

Foods and beverages from plant sources are rich in their content of a class of nutraceuticals known as polyphenols. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives (Geissman, 1963). Most are secondary metabolites, of which at least 12000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases these substances serve as plant defense mechanism against predation by microorganisms, insects and herbivores. Polyphenols are widely distributed plant derived dietary constituents and have been implicated as the active components in a number of
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herbal and traditional medicines (Wollenweber, 1988). Several of them are known to possess a wide spectrum of pharmacological properties (Beretz et al., 1977). Polyphenols exhibit several biological effects such as antiinflammatory, anti-microbial, anti-carcinogenic, anti-HIV, cardioprotective and neuroprotective influences. However, more and more evidence suggests that the benefits of antioxidant 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 diseases (Ames et al., 1991). Oxidative stress can cause damage to large biomolecules such as proteins, lipids and DNA resulting in an increased risk of cancer and cardiovascular diseases (Ames et al., 1991; Ames et al., 1993; Liu et al., 1995). To prevent or slow down the oxidative stress induced by free radicals, antioxidants in sufficient amounts 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 evidences (epidemiological and experimental) accumulated over 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 Giavanucci, 1997). Of particular relevance is the consistent cancer protective effect reported for individuals consuming high quantities of fruits and vegetables compared to those with low intakes. The cancer inhibitory action of a variety of human nutrients derived from plants as well as non-nutritive plant derived constituents (phytochemicals) has been confirmed in different animal tumor models (Dragsted et al., 1993; Pezzuto, 1996) and has led to an increased emphasis on cancer prevention strategies in which these dietary factors are utilized. There are two major diet related prevention strategies that have been involved in combating cancer, i.e. cancer chemoprevention and dietary
prevention, with an 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 et al., 1997; Mayne and Lipman, 1997). On the other hand dietary prevention is recognized as the changes in food consumption pattern necessary to reduce the risk of cancer development (Goodman, 1997). Plant derived polyphenolic compounds are important constituents of human diet which include quercetin, delphinidin and resveratrol from red grapes and red wines, curcumin from spice turmeric, epogalocatechin-3-gallate from green tea and isoflavone genistein from soybean. These are known to possess a wide range of pharmacological properties including anti inflammatory, cardioprotective, neuroprotective and anticancer (Szewczuk et al., 2004; Dai et al., 2006; Thomasset et al., 2007; Ullah and Khan, 2008).

Chemical structure and basic classification of flavonoids

Flavonoids are the major polyphenols derived from a wide variety of plant sources. The basic structure of flavonoids contains a heterocyclic skeleton of flavan (2-phenylbenzopyrane). The structure is represented by a benzene ring (A), condensed with a heterocyclic six membered pyran or pyrone ring (C), which in the 2 or 3 position carries a phenyl ring (B) as a substituent. The constituent polyphenolic units are derived from the secondary plant metabolism of the shikimate pathway (Dewick, 1995). Flavonoids are often hydroxylated at positions 3, 5, 7, 2', 3', 4', 5'. Usually in the plant system, these flavonoids exist in conjugated forms, the most common being the glycosides. When glycosides are formed, the glycosidic linkage is normally located at position 3 or 7 and the carbohydrate moiety can be L-rhamnose, D-glucose, gluco-rhamnose, galactose or arabinose (Middleson, 1984).
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Table I gives a classification of flavonoid subclasses along with their important members that are known to carry pharmacological properties.

<table>
<thead>
<tr>
<th>Subclass</th>
<th>General Chemical structure</th>
<th>Bioactive constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonols</td>
<td><img src="image" alt="Flavonols Structure" /></td>
<td>Quercetin, Rutin</td>
</tr>
<tr>
<td>Flavanones</td>
<td><img src="image" alt="Flavanones Structure" /></td>
<td>Hesperidin, Naringenin</td>
</tr>
<tr>
<td>Flavones</td>
<td><img src="image" alt="Flavones Structure" /></td>
<td>Apigenin, Luteolin</td>
</tr>
<tr>
<td>Flavanols</td>
<td><img src="image" alt="Flavanols Structure" /></td>
<td>Catechins, Epicatechins, Epigallocatechin-3-gallate (EGCG)</td>
</tr>
<tr>
<td>Isoflavones</td>
<td><img src="image" alt="Isoflavones Structure" /></td>
<td>Genistein, Biochanin A</td>
</tr>
<tr>
<td>Anthocyanidins</td>
<td><img src="image" alt="Anthocyanidins Structure" /></td>
<td>Delphinidin, Malvidin</td>
</tr>
</tbody>
</table>
Biosynthesis of plant polyphenols

Polyphenolic compounds are produced as secondary metabolites in higher plants. These compounds execute a vast array of important functions in plants (Croteu et al., 2000). For example, stilbenes and coumarins serve to defend pathogen attacks, flavonoids act as UV irradiation protectents while isoflavone and anthocyanins 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 (Phenyl-ammonia lyase). 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, siapic 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. Tetrahydroxychalcone provides the precursor for all classes of flavonoids, which include the flavones, flavonols, flavan-diols, flavan-4-ols, isoflavonoids and anthocyaninidins.

Figure 1 gives the biosynthetic pathway of tetrahydroxychalcone, the basic structural skeleton for the biosynthesis of flavonoids.
Figure 1: Biosynthesis of plant polyphenols

PAL (Phenyl-ammonia lyase); C4H (Cinnamate-4-hydroxylase); TAL (Tyrosine- ammonia lyase); 4CL (4-coumaryl lyase)
Bioavailability and Sources of dietary polyphenols

For any chemical moiety to exert a biological effect, it should be bioavailable i.e. it must be readily absorbed into the bloodstream and reach concentrations that have the potential to exert effects *in vivo*. Most of the polyphenols are known to be readily absorbed (Scalbert and Williamson, 2000; Rowland, 2003) but are prone to be modified into other forms inside biological systems, one such common chemical modification being conjugation (Lambert et al., 2005). Curcumin undergoes metabolic O-conjugation to curcumin glucuronide and curcumin sulfate and bioreduction to tetrahydrocurcumin, hexahydrocurcumin, and hexahydrocurcuminol in rats and mice *in vivo* and in suspensions of human and rat hepatocytes (Ireson et al., 2001). Certain curcumin metabolites, such as tetrahydrocurcumin, possess anti-inflammatory (Mukhopadhyay et al., 1992) and antioxidant activities (Sugiyama et al, 1996) similar to those of their metabolic progenitor. Dietary resveratrol is rapidly absorbed and predominantly present in plasma as glucuronide and sulphate conjugates. When administered in food, such as wine or grape juice, resveratrol metabolism is significantly inhibited by other polyphenols due to competitive reactions with metabolizing phase II enzymes resulting in an increased concentration of the free form (Wenzel and Somoza, 2005). Isoflavones such as genistein are also known to undergo conjugation with glycosides and is metabolized in human intestine to dihydrogenistein and 6'-hydroxy-O-desmethylangolensin. Concentration of genistein has been shown to be higher in individuals consuming soy rich diet (Adlercreutz et al., 1993) and consequently genistein and its metabolites have been detected in plasma, breast aspirate and prostatic fluid (Mills et al., 1989). Similarly, other polyphenols are also known to be absorbed and metabolized into various end products which may or may not possess the biological effects of the parent compound.

*Table II* and *Fig 2* summarize the major sources and bioavailable forms of various popular dietary polyphenols.
Table II: Major dietary polyphenols, their bioavailable forms in plasma and their major food sources

<table>
<thead>
<tr>
<th>Polyphenols</th>
<th>Major dietary forms</th>
<th>Bioavailable forms in plasma</th>
<th>Common food sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanidins</td>
<td>Cyanidin Delphinidin Malvidin</td>
<td>Glucosides</td>
<td>Berries, red and purple grapes, red wine</td>
</tr>
<tr>
<td>Flavanols</td>
<td><strong>Monomers</strong> Catechin Epicatechin EGCG <strong>Polymers</strong> Proanthocyanidins</td>
<td>Methyl, sulphate or glucuronic acid conjugates. EGCG occurs in the unconjugated form</td>
<td><strong>Dimers</strong> Tea (particularly green tea), Apples pears, raspberries, chocolate</td>
</tr>
<tr>
<td>Flavonols</td>
<td>Quercetin, Rutin</td>
<td>Methyl, sulphate or glucuronic acid conjugates</td>
<td>Onions, apples, broccoli, tea, berries</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Genistein, Daidzein, Biochanin A</td>
<td>Sulphates or glucuronides conjugates. Also occur as glycosides and aglycones</td>
<td>Soybeans, soy foods, legumes</td>
</tr>
<tr>
<td>Stilbenes</td>
<td>Resveratrol</td>
<td>Glucuronides, Sulphate conjugates. Unconjugates are also present as product of fermentation</td>
<td>Purple grapes, red wine, peanuts, berries</td>
</tr>
</tbody>
</table>
Grapes

Resveratrol

Green tea

EGCG

Soybean

Genistein

Turmeric

Curcumin

Figure 2: Sources of Dietary Polyphenols
Therapeutic potentials of plant derived polyphenolic compounds

An insight into the investigations, both in vitro and in vivo, reveals the properties of plant polyphenols that can form the basis of their use in the prevention and cure of several disorders. Some of the important therapeutic properties of plant-derived polyphenols with strong evidence from the existing literature have been discussed below.

*Cardioprotective properties*

A longstanding tenet of nutrition holds that people with diets rich in fruits and vegetables enjoy better health than those eating few. Much of current research shows that free radicals are linked to various chronic diseases. As a result dietary antioxidants hold promise in at least delaying the onset/progression of these diseases.

The “French Paradox” – the observation that mortality from coronary heart disease is relatively low in France despite relatively high levels of dietary saturated fat, led to the idea that regular consumption of red wine (rich source of polyphenols) might provide additional protection from cardiovascular diseases (Criqui and Ringel, 1994). Regular, moderate consumption of red wine is linked to a reduced risk of coronary heart disease. Resveratrol, a red wine polyphenol has been linked to a number of potentially cardioprotective effects (Szewczuk et al., 2004). Anthocyanidins have also been found to have antioxidant potential (Falchi et al., 2006) Studies suggest that EGCG can suppress reactive oxygen species and thereby prevent the development of cardiac hypertrophy (Li et al., 2006).

Endothelial dysfunction is involved in the initiation and progression of arteriosclerosis. Some polyphenols have been shown to relax endothelium-denuded arteries. There have been reports that extracts from grape and wine induce endothelium-dependent relaxation via enhanced and/ or increased biological activity of nitric oxide (NO) which leads to the elevation of cGMP.
levels (Andriambeloson, 1997). Resveratrol has been found to promote vasodilation by enhancing the production of NO (Wallerath et al., 2002). Genistein, one of the major isoflavones in soy protein, binds to estrogen receptor β with much higher affinity than to ERα (Kuiper et al., 1998) and can elicit endothelium dependant vasorelaxation in vitro (Figtree et al., 2000) and in vivo (Walker et al., 2001). Other isoflavones such as dihydrodaidzeins have also been reported to enhance endothelial function (Shen et al., 2006). Flavonoids have also been found to be good hypochlorite scavenger in vitro and could have favorable effects in diseases such as atherosclerosis, in which hypochlorite is known to play a significant role (Firuzi, 2004). Increase in LDL is taken as a parameter for the occurrence and susceptibility to cardiovascular diseases. Polyphenols such as dicvertin have been reported to produce a 12% decrease in LDL along with a 14% increase in HDL in coronary heart disease patients (Belaia et al, 2006). Lipid-lowering activity has also been reported in tea flavonoids (Li et al, 2006).

In the prevention of cardiovascular diseases, many of the observed effects of polyphenols, can therefore, be attributed to their recognized antioxidant and radical scavenging properties, which may delay the onset of atherogenesis by reducing chemically and enzymatically mediated peroxidative reaction (German and Walzem, 2000).

Neuroprotective properties

Neurodegenerative disorders are a heterogeneous group of diseases of the nervous system, including the brain, spinal cord and peripheral nerves, which have different aetiologies. The multifactorial etiology of these diseases suggests that interventions having multiple targets such as polyphenols could have therapeutic potential for them. Moreover, epidemiological studies indicate that dietary habits and antioxidants from diet can influence the incidence of neurodegenerative disorders such as Alzheimer and Parkinson's diseases (Morris et al., 2002). The nervous system is rich in fatty acids and iron. High
levels of iron can lead to oxidative stress via the iron-catalyzed formation of ROS (Bauer and Bauer, 1999). In addition brain regions that are rich in catecholamines are vulnerable to free radical generation. One such region of the brain is the substantia nigra, where a connection between antioxidant depletion and tissue degeneration has been established (Perry et al., 2002).

There is substantial evidence that oxidative stress is a causative or at least an ancillary factor in the pathogenesis of many neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS) (Ghadge et al., 1997), Huntington's disease (HD) and Schizophrenia (Philips et al., 1993) Flavonoids exhibit biological effects such as anti-inflammatory, antioxidant and metal chelating properties, which augment their role in neuroprotection. Reports also suggest that red wine that contains high levels of antioxidant polyphenols reduces the incidence of AD (Wang J et al., 2006). Polyphenols such as EGCG, curcumin, extracts of blue berries and Scutellaria are also known to help in AD (Dai et al., 2006). In vitro studies show that green tea extract rich in catechins could protect neurons from the amyloid beta-induced damages in AD (Bastianetto et al., 2006). EGCG is also found to be of use in ALS (Xu et al., 2006) and PD (Ramassamy. 2006). Extract of Scutellaria stem and polyphenols such as curcumin and naringenin also exhibit neuroprotection in PD (Shang et al., 2006). Alzheimer's disease is characterized by chronic inflammation and oxidative damages in the brain. Curcumin posses antioxidative and anti inflammatory properties and has thus been shown to exert a protective effect against oxidative damages initiated by divalent metals or suppress inflammatory damage by preventing metal induction of NF-kB and also inhibits amyloid beta fibril formation (Kim et al., 2005). Dietary polyphenols have potential as protective agents against neuronal apoptosis, through selective actions within stress activated cellular responses including protein kinase signaling cascade (Schroeter et al., 2006). Several dietary supplements with blueberries extracts have been reported to reduce some neurological deficits in aged animal models. Blueberries are a rich source
of polyphenols such as catechins, epicatechins and anthocyanins. Recent studies investigating the effect of polyphenols in cognitive performance have demonstrated that dietary supplementation with blueberries extracts reversed cognitive deficits in Morris water maze performance test and Y-maze test in aged mice models (Joseph et al., 1999; Joseph et al., 2003).

**Anticancer properties**

Many polyphenols from plant sources are recognized as naturally occurring antioxidants and have been implicated as anticancer compounds (Mukhtar et al., 1998). 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 materials 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).

Numerous studies have reported flavonoid mediated antiproliferative effects against human and rodent ovarian, leukemic, intestinal, lung, breast, bladder and prostate 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
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(IC50 10 μM) and growth (IC50 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 IC50 of 0.17 μM (Hirano et al., 1995). Curcumin, a natural phenolic compound found in spice turmeric, has been shown to have antiproliferative action against colon cancer, breast cancer and myeloid leukemia (Tsvetkov et al., 2005; Maheshwari et al., 2006). Antitumor activity of curcumin is believed to be in part due to its ability to block the NF kappa B pathway (Singh et al., 1995). Other studies have shown that curcumin inhibits cell growth and induces apoptosis in MCF-7, a human breast carcinoma cell line through modulation of insulin-like growth factor-1 (IGF-1) system, including IGFs (IGF-1 and IGF-2), IGF-1R (IGF-1 receptor) and IGFBPs (IGF binding proteins), which have been implicated to play a critical role in the development of breast cancer (Xia et al., 2007). Resveratrol, the phenol antioxidant found in berries and grapes has been reported to possess anticancer properties (Aggarwal et al., 2004) and is able to inhibit the growth of prostate tumors by acting on the regulatory genes such as p53 (Narayanan, 2006). Androgen independent DU145 human prostate cancer cells manifest resistance to radiation-induced apoptotic death (Yacoub et al., 2001). Scarlatti et al (2007) have reported that pre-treatment with resveratrol significantly enhances radiation induced cell death in DU145 cells.

Further the capacity of certain dietary polyphenols to protect against either chemically induced or 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 (NMU) reduced the incidence and multiplicity of carcinogen induced mammary tumor by 30 % and 50 % respectively (Verma et al., 1988). Quercetin and luteolin (10 g/Kg diet) decreased 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/Kgb.w) also increases the survival and reduces the tumor burden of mice transplanted intrasplenically with ML-3 hepatoma cells (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 a protective effect of tea polyphenols against carcinogenesis. Rats fed on a diet containing 10 g green tea catechins/Kgb.w 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-nitrosobis(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 mice model.

In addition to their potential as anticancer agents, an important role of plant polyphenols as natural modulators of cancer multidrug resistance (MDR) has been documented (Ullah, 2008). Resistance of recurrent disease to cytotoxic drugs is the principal factor limiting long-term treatment success against cancer. Flavonoids have been found to inhibit breast cancer resistance protein (BCRP), an ABC transporter, which plays an important role in drug disposition leading to chemoresistance in breast cancer (Shuzhong et al., 2005). Isoflavones such as biochanin A, daidzein (Chung et al., 2005) and green tea polyphenol EGCG (Feng et al, 2005) have also been shown to exhibit anti MDR activities in various drug resistant cancer cell lines, such as doxorubicin resistant KB-A1 cells through the inhibition of P-glycoprotein transporters. Curcumin has been reported to induce apoptosis in chemoresistant ovarian cancer cell lines SKOV3 and ES-2 (Wahl et al., 2007).
Isoflavones

Dietary isoflavones, a subclass of flavonoids, are the focus of much of the recent interest in the nutritional benefits of soy foods as these phytoestrogens occur in relatively high concentrations in soybeans (Coward et al., 1993; Setchell and Cole, 2003) and have been implicated as protective agents in a number of diseases (Fig 3). There are multiple lines of compelling evidence from several epidemiological studies supporting a positive association between dietary soy consumption and the risk of cancer. A cross-national study involving 50 countries identified soy products as functional foods with substantial protective effects against prostate cancer (Herbert et al., 1998). It is well documented that Asian women consuming relatively large amounts of soy-derived foods have a low incidence of breast cancer (Adlercreutz et al., 1991; Lee et al., 1991), which is less evident among the second generations of Asian immigrants to the USA who have started adopting a Western-style diet (Ziegler et al., 1993). Furthermore, urinary levels of soy-derived isoflavones including genistein were lower in breast cancer patients compared with case-controls (Ingram et al., 1997; Zheng et al., 1999). Moreover, soy isoflavones have been found not only to decrease the risk of breast and prostate cancers, but also to inhibit the growth of other types of cancers, including leukemia, lymphoma, lung, and head and neck cancer cells (Spinozzi et al., 1994; Constantinou and Huberman 1995; Davis et al., 1998; Lian et al., 1998; Alhasan et al., 1998; Li et al., 1999; Upadhyay et al., 2001). An association between dietary soy intake and the lower incidence of endometrium cancer has also been documented (Goodman et al., 1997).
Figure 3: Proposed targets for beneficial effects of dietary genistein or a high soy diet on human health (from Dixon RA and Ferreira D. Genistein. *Phytochemistry* 2002; 60:205–211)

**Biosynthesis, metabolism and bioavailability of isoflavones**

**Biosynthesis**

Isoflavones are a subclass of the more ubiquitous flavonoids. The basic structural feature of flavonoid compounds is the flavone nucleus, which comprises two benzene rings (A and B) linked through a heterocyclic pyrane C ring. The position of the benzenoid B ring differentiates the isoflavonoids (3-position) from the rest of the subclasses of flavonoids (2-position). The popular isoflavones are genistein (4',5,7-trihydroxyisoflavone) and daidzein (4',7-dihydroxyisoflavone), their respective beta-glycosides, genistin and daidzin (sugars being attached at the 7 position of the A ring) present predominantly in soybean and 4'-methylether derivative of genistein, biochanin A (5,7-dihydroxy-4'-methoxyisoflavone) which is present in legumes most notably red clover (*Trifolium pratense*) (Fig 4)
Isoflavones have a basic skeleton of 3-phenylchroman biogenetically derived by an aryl migration mechanism from the 2-phenylchroman skeleton of flavones [Harborne, 1988]. For entry into the isoflavonoid pathway, the flavone first undergoes abstraction of hydrogen radical at C-3 followed by B-ring migration from C-2 to C-3 and subsequent hydroxylation of the resulting C-2 radical. This reaction requires NADPH and molecular oxygen, and is catalyzed by a microsomal cytochrome P450 enzyme (2-hydroxyisoflavanone synthase). The resulting 2-hydroxyisoflavanone is unstable and undergoes dehydration to yield genistein which is further methylated at 4' position by isoflavone 4'-O-methyltransferase to yield biochanin A.

Figure 5 gives the scheme for the biosynthetic pathway of isoflavones.
EC 1.14.13.86 2-hydroxyisoflavanone synthase; EC 4.2.1.105 2-hydroxyisoflavanone dehydratase; EC 2.1.1.46 isoflavone 4'-O-methyltransferase; EC 2.4.1.170 isoflavone 7-O-glucosyltransferase; EC 1.3.1.46 biochanin-A reductase; EC 2.3.1.115 isoflavone-7-O-β-glucoside 6"-O-malonyltransferase; EC 2.1.1.150 isoflavone 7-O-methyltransferase

Figure 5: Biosynthesis of isoflavones
Metabolism

In plants isoflavonoids exist in conjugation with sugars, mainly with glucose, but also with 6'-O-malonylglucose or 6'-O-acetylglucose. The glycoside conjugates remain unmodified during various food preparation procedures. Thus, in general, soy food when consumed has low levels of aglycones compared to glycosides. However, fermented soy products may contain higher amounts of aglycones (Nakamura et al., 2000).

After ingestion of isoflavone-rich foods, the isoflavone glycosides, which are considered biologically inactive, undergo deglycosylation. For many years it was assumed that only the β-glucosidases of gut microflora were responsible for deglycosylation reactions. However, recent studies have demonstrated that deglycosylation of genistin (genistein 7-O-glucoside) to genistein already begins in the mouth (Allred et al., 2001) and then continues in the small intestine (Day et al., 1998). It has been shown that genistein, but not genistin, can be readily absorbed through the wall of the stomach (Piskula et al., 1999). This may explain the faster absorption rates of aglycones compared to that of glycosides (Izumi et al., 2000). It has been suggested that isoflavones and their metabolites occur mainly as glucuronide conjugates, but sulfates and sulfoglucuronides have also been reported (Adlercreutz et al., 1995).

A comprehensive study on soy isoflavone metabolism was carried out by Kelly et al. (1993). They studied the metabolism of genistein in a feeding study in which 12 human subjects included 40 g of soy flour into their normal western diet for 2 consecutive days. Ingested amount of genistein per day was 39 mg. Urine samples (24 h) were collected before and on three consecutive days after the soy consumption. Along with the ingested genistein two novel metabolites of genistein, dihydrogenistein and 6'-OH-O-desmethylangolensin were reported to be found in the urine.
Kulling et al. (2000) further studied the oxidative metabolism of daidzein and genistein in vitro with rat and human microsomes. Isoflavones are good substrates for cytochrome P450 enzymes and are extensively metabolized. Ten new metabolites were reported for daidzein and six for genistein. Most of the metabolites were formed by hydroxylation at an ortho position of existing hydroxyl group in the phenolic rings yielding mono-, di-, tri-, tetra- and pentahydroxylated metabolites. One monohydroxylated metabolite of daidzein and one monohydroxylated metabolite of genistein were suggested to be hydroxylated at 2-position of the C-ring. Most of these newly identified oxidative metabolites were found in human urine collected after soy supplementation (Kulling et al., 2001). Methylation of isoflavones that have two vicinal hydroxyl groups seems to be a minor metabolic reaction of isoflavones. Kulling et al. have tentatively identified four methylated metabolites with an isoflavone structure in human urine after soy supplementation. One of the metabolites was suggested to be 3'- or 4'-O-methyl-7, 3', 4'-trihydroxyisoflavone, and three others, one dimethylated and two monomethylated metabolites, were proposed to be formed by methylation of 6, 7, 3, 4'-tetrahydroxyisoflavone. The presence of hydroxylated and methylated genistein metabolites correlated positively with inhibition of cancer cell proliferation, but genistein sulfates were not associated with antiproliferative effects of genistein, suggesting that only some types of metabolism of the isoflavones may be crucial for their action (Peterson et al., 1998).

**Bioavailability**

Genistein and their metabolites have been detected in plasma, prostatic fluid, breast aspirate and cyst fluid, urine, and feces [Mills et al., 1989; Knight and Eden 1996; Zava et al., 1998; Adlercreutz et al., 1993]. Adlercreutz et al. have found that the plasma level of genistein in people having a soy rich diet was 1–5 µM after metabolism and excretion. Another study targeting phase 1 pharmacokinetic and pharmacodynamic analysis following administration of
unconjugated soy isoflavones (containing 43% and 90% genistein, respectively), to individuals with cancer, found plasma concentration of genistein supposedly associated with antimetastatic activity in vitro (Takimoto et al., 2003). Genistein is relatively hydrophobic and expected to be taken up by cells without previous cleavage and does not need to be biologically activated to exert its inhibitory effects on cancer cell growth (Russo et al., 2006).

Anticancer Properties of Genistein

Genistein (5,7,4'-trihydroxyisoflavone) is an isoflavone that is present in soybeans in high concentrations and shows diverse biological activities (Dixon and Ferreira, 2002). In recent years, increasing evidence has accumulated indicating that this natural ingredient of soy shows preventive and therapeutic effects for cancer and cardiovascular diseases in animals and humans. It displays many anticancer properties which includes suppression of the proliferation of a variety of human gastrointestinal cancer cell lines, induction of differentiation of leukemia cells, and inhibition of endothelial cell angiogenesis relevant to tumor metastasis (Farina et al., 2006). Experiments have shown that genistein inhibits the growth of several cancer cells including leukemia, lymphoma, ovarian, cervical, leiomyoma, melanoma, neuroblastoma, gastric, pancreatic, breast, and prostate cancer cells (Peterson and Barnes, 1993, 1996; Constantinou et al., 1990; Buckley et al., 1993; Matsukawa et al., 1993; Pagliacci et al., 1994). The growth inhibition of cancer cells could be due to cell cycle arrest, which ultimately results in cessation of cell proliferation. It has been demonstrated that genistein induces a G2/M cell cycle arrest in breast cancer, gastric adenocarcinoma and melanoma cells (Pagliacci et al., 1994; Casagrande and Darbon, 2000). It was also shown that genistein induces a G2/M cell cycle arrest in PC3 and LNCaP prostate cancer cells; H460 and H322 non-small cell lung cancer cells; MDA-MB-231 and MCF-10CA1a breast cancer cells (Davis et al., 1998; Lian et al., 1998). In addition to cell cycle arrest, another specialized event of genistein action involves the induction of programmed cell death known as 'apoptosis'. It has been shown
that genistein could induce apoptosis in MDA-MB-231, MDA-MB-435, and MCF-7 breast cancer cells; PC3 and LNCaP prostate cancer cells; H460 and H322 non-small cell lung cancer cells; HN4 head and neck squamous carcinoma cells, and pancreatic cancer cells (Davis et al., 1998; Lian et al., 1998; Li et al., 1999; Alhasan et al., 1999; Banerjee et al., 2005, 2007). Flow cytometry revealed that the number of apoptotic cells increased by 43-57%, with longer genistein treatment (Banerjee et al., 2005). Moiseeva et al. (2007) reported that physiological concentrations of a dietary phytochemical including genistein results in reduced growth and induction of apoptosis in cancer cells.

NF-κB plays important roles in the control of cell growth, differentiation, apoptosis and stress response. Under non-stimulating conditions, NF-κB is sequestered in the cytoplasm through tight association with the impeding IκB proteins. Following stimulation, IκB protein is phosphorylated and degraded, allowing the NF-κB to translocate to the nucleus, bind to the NF-κB-specific DNA-binding sites or interact with other transcription factors, and thus regulate gene transcription. It has been reported that genistein treatment could modulate NF-κB DNA binding activity in prostate, breast, head and neck, and pancreatic cancer cells (Li et al., 1999; Davis et al., 1999; Alhasan et al., 2000, Natarajan et al., 1998). One of the studies investigated the effects of isoflavone supplementation on NF-κB activation in vivo in human volunteers. The lymphocytes from healthy male subjects were harvested from peripheral blood and cultured for 24 h in the absence and presence of genistein. Electrophoretic mobility shift assay (EMSA) revealed that genistein treatment inhibited basal levels of NF-κB DNA binding activity by 56% and abrogated TNF-α induced NF-κB activity by 50% (Karin and Delhase 2000). The results indicate that genistein inhibits the translocation of NF-κB to the nucleus preventing NF-κB from binding to its target DNA and thereby inhibiting the transcription of NF-κB downstream genes. This process ultimately inhibits cell growth and also induces apoptotic cell death. Akt signaling is another important transduction pathway that plays a critical role in controlling the balance between cell survival and apoptosis (Tanaka et al., 2002). Evidence suggests that Akt also
regulates the NF-kB pathway via phosphorylation and activation of molecules in the NF-kB pathway (Romashkova and Makarov, 1999; Ozes et al., 1999). Thus, strategies to block the activity of Akt would ideally lead to the inhibition of cell proliferation and the induction of apoptosis. By immunoprecipitation, Western blot and kinase assays it was found that genistein treatment reduced the level of the phosphorylated Akt protein at Ser473 compared to control cells, resulting in a dose dependent induction of apoptosis after genistein treatment of cells that display constitutively active Akt (Banerjee et al., 2007). These data demonstrate that genistein inhibits the activation of Akt, which may result in the inhibition of survival signals ultimately leading to induction of apoptotic signals. Consistent with the in vitro findings, there is growing in vivo evidence demonstrating the inhibitory effects of genistein on carcinogenesis. Prepubertal exposure to soy or genistein reduced mammary carcinogenesis in rats treated with carcinogens (Cabanes et al., 2004). Soy isoflavone supplemented diets also prevented the development of adenocarcinomas in the prostate and seminal vesicles in a rat carcinogenesis model (Onozawa et al., 1999). It has also been reported to be effective against chemical carcinogen- induced rat ovarian carcinogenesis (Tanaka et al., 2002). The soy diet reduced growth of transplantable prostate adenocarcinomas and inhibited tumor cell proliferation and angiogenesis of transplantable prostate cancer in immunodeficient mice (Landstrom et al., 1998; Zhou et al., 1999). A diet rich in soy also inhibited pulmonary metastasis of melanoma cells in C57Bl/6 mice (Li et al., 1999). Genistein inhibited the growth of carcinogen-induced cancers in rats and human leukemia cells transplanted into mice (Hawrylewicz et al., 1995; Ravindranath et al., 2004; Lamartiniere et al., 1995; Uckun et al., 1995). Singh et al. (2006) evaluated the natural form of genistein, and the isoflavone-rich soy phytochemical concentrate (SPC) on the growth and metastasis of human bladder cancer cells 253J BV induced tumors in an orthotopic site. Both treatment regimes were effective in reducing tumor weight by more than 50%, accompanied by induction of tumor cell apoptosis and inhibition of tumor angiogenesis in vivo. Isoflavones, including genistein, are known antioxidants.
Genistein has been shown to protect cells against reactive oxygen species (ROS) by scavenging free radicals and reducing the expression of stress-response related genes (Larrea et al., 1997; Zhou et al., 1998). It has been demonstrated that genistein inhibits tumor-promoter 12-O-tetradecanoylphorbol-13-acetate-induced hydrogen peroxide production in human polymorphonuclear leukocytes, and HL-60 cells (Wei et al., 1993; Rotondo et al., 2007). However, genistein has also been shown to induce DNA damage in cancer cells (Bianco et al., 2005; Boos et al., 2000; Lutz et al., 2005). An association of phytoestrogen and ROS mediated DNA breakage has also been demonstrated. Such oxidative injury was shown to be inhibited by both enzymatic and non enzymatic antioxidants such as catalase, superoxide dismutase and ascorbic acid respectively indicating the involvement of a possible prooxidant anticancer mechanism (Anderson et al., 2003; Cemeli et al., 2004).

Anticancer Properties of Biochanin A

Although the chemopreventive properties of biochanin A are less documented than genistein this compound has also been demonstrated to cause a dose dependent inhibition of growth through induction of apoptosis in human bladder cancer cell lines and human hepatoma cell lines (Su et al., 2000; 2003). Red clover derived dietary isoflavones of which biochanin A is a major constituent have been shown to induce apoptosis in low to moderate-grade Human Prostate Carcinoma (Jarred et al., 2000). It has also been shown to inhibit chemical-induced tumor carcinogenesis and prevent tumor growth after implantation in animal models (Lee et al., 1991; Rice et al., 2002). Studies of the dietary phytoestrogen biochanin A on cell proliferation of the cultured estrogen responsive cells human breast carcinoma MCF-7 showed that biochanin A exhibits biphasic regulation on MCF-7 cells. At lower concentrations less than 30 μM, cells respond to biochanin A by increasing cell growth and de novo DNA synthesis. The addition of biochanin A at higher concentrations significantly inhibited cell growth and DNA synthesis in a dose-dependent
fashion, resulting in an IC₅₀ value of 140 μM. The reversibility of these inhibitory effects by biochanin A appears to be concentration dependent (Hsu et al., 1999). Effect of biochanin A on the growth and differentiation of myeloid leukemia has been reported with biochanin A inhibiting the growth of cells in dose dependant manner and also inducing the morphological differentiation of cells (Fung, 1997). Biochanin A also inhibited growth of human pancreatic tumor cells in vitro. The inhibition was observed in both male (HPAF-11) and female (Su 86.86) adenocarcinoma cell lines (Lyn Cook et al., 1999). Biochanin A induced a dose dependant inhibition of proliferation in prostate LNCaP cell lines and [³H] thymidine incorporation that correlated with increased DNA fragmentation indicative of apoptosis (Rice et al., 2002).