FLAVONOIDS AND THEIR THERAPEUTIC POTENTIAL AS ANTI CANCER AGENTS: BIOSYNTHESIS, METABOLISM AND REGULATION.

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INTRODUCTION

Flavonoids are a class of plant secondary metabolites and are referred to as Vitamin P. They are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. Flavonoids are widely distributed in plants, fulfilling many functions. They have been shown to possess a variety of biological activities at nontoxic concentrations in organisms. The role of dietary flavonoids in cancer prevention indicate that flavonoids have important effects on cancer chemoprevention and chemotherapy (Ren W, 2003). Many mechanisms of action have been identified, including carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis, differentiation and inhibition of angiogenesis.

Cancer is a major public health concern in both developed and developing countries and use of flavonoids to prevent or suppress the carcinogenic progression is gaining importance these days. These compounds possess a common phenylbenzopyrone moiety (C6-C3-C6) and they are categorized according to the saturation level and opening of the central pyran ring, mainly into flavones, flavanols, isoflavones, flavonols, flavanones, and flavanonols. The most extensively studied among flavonoids are polyphenolic terpenoid (PPT), which are characterized by a C6-C3-C6 structure (Robards K, 1997). Usually 110 to 121 mg/day of flavonoids has been recommended as a healthy diet for an adult. Even in very high amounts (140 grams per day) flavonoids do not appear to cause unwanted side effects. Even when
raised to the level of 10% of total caloric intake flavonoid supplementation has been shown non-toxic (Hertog et.al, 1992, 1993).

**Subclasses and Dietary Sources of Flavonoids : (Wang HK, 2000)**

<table>
<thead>
<tr>
<th>Flavonoid subgroup</th>
<th>Representative flavonoids</th>
<th>Major food sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonols</td>
<td>Kaempferol, myricetin, Quercetin, rutin.</td>
<td>Onions, cherries, apples, broccoli, kale, tomato, Berries, tea, redwine, tartary buckwheat.</td>
</tr>
<tr>
<td>Flavones</td>
<td>Apigenin, chrysin, luteolin.</td>
<td>Parsley, thyme, celery, capsicum, pepper, broccoli.</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Daidzein, genistein, glycitein, Formononetin.</td>
<td>Soya beans, legumes.</td>
</tr>
<tr>
<td>Flavanols</td>
<td>Catechin, gallocatechin.</td>
<td>Apples, tea.</td>
</tr>
<tr>
<td>Flavanones</td>
<td>Eriodictyol, hesperitin, Naringenin.</td>
<td>Oranges, grapefruit.</td>
</tr>
<tr>
<td>Flavanonols</td>
<td>Taxifolin</td>
<td>Limon, aurantium.</td>
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</tbody>
</table>

Flavonoids are one of the common components in the human diet. They are present in foods generally as O-glycosides with sugars bound at C3 position. Phenolic acids, flavonoids, stilbenes and lignans are the most abundantly occurring polyphenols in plants out of which flavonoids and phenolic acids account for 60% and 30% of dietary polyphenols. (Kuhnau J., 1976). Major sources of polyphenols are fruits, vegetables, and seeds. Flavonoids are widely present in the genus Citrus [family Rutaceae], (Obdulio BG, 1997).

Cancer chemoprevention by use of natural or synthetic substances and its prevention through dietary intervention has become an important issue. It may be controlled by various means including suppression, blockage and transformation (Manthey JA et.al;2001) Suppressing agents prevent the formation of new cancers from procarcinogens, blocking agents prevent carcinogenic compounds from reaching critical initiation sites and transformation agents facilitate the metabolism of carcinogenic components into less toxic materials and prevent their biological actions. Flavonoids can act in all the three ways. Many other potential chemopreventive polyphenols may interrupt or reverse the carcinogenesis process (Surh YJ; 2003).

**BIOSYNTHESIS OF FLAVONOIDS**

Flavonoids are synthesized along the general **phenylpropanoid pathway** by the activity of a cytosolic multienzyme complex known as flavonoid metabolon, loosely associated to the
cytoplasmic face of the endoplasmic reticulum (ER). Some of these enzymes belong to the cytochrome-P450 family and possess the ability to bind to membranes. Some of the enzymes involved in the biosynthetic pathway are loosely associated with membranes of different organelles such as vacuole, plastids and nucleus. The metabolic pathway continues through a series of enzymatic modifications to yield flavanones $\rightarrow$ dihydroflavonols $\rightarrow$ anthocyanins. Along this pathway, many products can be formed including the flavonols, flavan-3-ols, proanthocyanidins (tannins) and a host of other various polyphenolics.

Mutants affecting flavonoid synthesis were isolated in a range of plant species. Maize (Zea mays) and petunia (Petunia hybrida) were established as the first major experimental models in this system, leading to the isolation of many structural and regulatory flavonoid genes (Holton et al., 1993; Mol et al., 1998). More recently, Arabidopsis (Arabidopsis thaliana) has facilitated the analysis of the regulation and subcellular localization of the flavonoid pathway. An interesting aspect of using Arabidopsis for studying flavonoid biosynthesis is that single copy genes encode all enzymes of the central flavonoid metabolism, with the exception of flavonol synthase (FLS), which is encoded by six genes. (Owens et al., 2008; Preuss et al., 2009).

![General flavonoid biosynthetic pathway](image_url)
Most of the flavonoid synthesizing enzymes are recovered in soluble cell fractions; immunolocalization experiments suggest that they are loosely bound to the endoplasmic reticulum (ER) possibly in a multi-enzyme complex, whereas the pigments themselves accumulate in the vacuole (anthocyanins and proanthocyanidins) or the cell wall (Winkel-Shirley, 2001).

They exhibit properties beneficial for human health because they interact with number of cellular targets such as anti-oxidant, free-radical scavenger activities, anti-inflammatory, antiviral and especially anti-cancer properties. Flavonoids undergo extensive biotransformation and conjugation that occur during their absorption from the gastrointestinal tract, in the liver and finally in cells (Williamson G, 2005).

Dietary flavonoids are substrates for phase I and phase II enzymes in the small intestine and liver. They are deglycosylated and metabolized into glucuronides, sulfates and O-methylated derivatives (Spencer JPE; 2003). Flavonoid absorption from the intestine occurs by several different pathways. Flavonoid aglycones can be easily absorbed into the intestinal cells because their lipophilicity facilitates their passage across the mucosal phospholipid bilayer of cells. flavonoid monoglycosides can be transported by the sodium glucose transporter (Walgren RAet.al; 2000) on the brush border membrane of intestinal cells. Most flavonoid glycosides entering enterocytes are deglycosylated by β-glucosidases, namely, broad-specificity cytosolic β-glucosidase. The flavonoids appear to be subjected to glucuronidation, sulfation, and methylation in the intestinal epithelial cells before entering circulation (Nemeth K; 2003). These flavonoid conjugates are excreted into the urine and also into bile fluid, thereby returning to the intestinal lumen (Matsukawa N; 2009). Further metabolism occurs in the colon, where enzymes of the gut microflora induce the breakdown of flavonoids to phenolic acids which may undergo absorption and be further metabolized in the liver (Scheline RR. 1999).

**METABOLISM AND REGULATION OF FLAVONOIDS**

Enzymes for metabolism of flavonoids are present in small intestine, liver and kidneys. The polar hydroxyl groups undergo conjugation with glucuronic acid, sulphate or glycine (Hollman PCH and Katan MB; 1999). In addition, deglycosylation as well as O-methylation are other important events in flavonoid metabolism. Conjugation can alter the antioxidant activity and protein interaction of flavonoids and hence plays important role in flavonoid-mediated health effects (King RA; 1998). Flavonoids that are unabsorbable from
the small intestine and that are absorbed and secreted with bile will reach the colon. In the colon microorganisms degrade the flavonoids molecule by splitting the heterocyclic oxygen containing ring and subsequent degradation products are absorbed as they are found in urine and plasma. (Olthof MR; 2003). Flavonols are degraded to phenyl acetic acid and phenyl propionic acids. Catechins produces valerolactones (a benzene ring with a side chain of five C-atoms) and phenyl propionic acids. Flavones and flavanones metabolize to form phenylpropionic acids that further undergo bacterial degradation and enzymatic transformations in body tissues to form benzoic acid (Walle T; 2005).

Reverse phase HPLC coupled with diode array detection (DAD) has been employed for detection of various classes of flavonoid compounds formed from grape extracts and wines (Norbaek R; 2000). Other techniques such as coupling of HPLC with mass spectroscopy (MS) equipped with mild ionization techniques such as electrospray ionization (ESI-MS) or atmospheric pressure chemical ionization (APCI-MS) and gas chromatography (GC) analysis have become increasingly popular (Nicoletti et.al; 2007). However, MS does not allow to distinguish between isomers such as glucose and galactose in flavonoid glycosides, or (-) epicatechin and (+) catechin units in procyanidins. Sugars in glycosides can be identified by using specific glycosides or by analyzing the sugar moiety released after acid catalysed cleavage of the glycosidic linkage. This has been achieved by comparison with reference compounds in TLC and more recently by GC analysis (Rodríguez-Medina IC et.al; 2009).

The flavonoid biosynthesis genes are regulated by the interaction of different families of transcription factors. Genes involved in the anthocyanin pathway are differentially regulated in monocot and dicot species by R2R3 MYB transcription factors, basic helix-loop-helix (bHLH), and WD40 proteins (Grotewold, 2005). Thus, combinations of the R2R3-MYB, bHLH, and WD40 transcription factors and their interactions (MYB-bHLH-WD40 complex) determine the activation and spatial and temporal expression of structural genes of anthocyanin biosynthesis (Petroni and Tonelli, 2011). There are differences in anthocyanin regulation between monocot and dicot species like Arabidopsis and maize. In Arabidopsis TT2, TT8 and TTG1 form a ternary complex and activate proanthocyanidin biosynthesis in developing seeds. while, TTG1 a WD40 transcription factor different bHLH (TT8, GL3, and EGL3) and MYB transcription factors (PAP1 and PAP2) interact to activate anthocyanin synthesis in vegetative tissues. In maize, MYB and bHLH proteins are encoded by two multigene families and each member has a tissue and developmental specific pattern, while a
WD40 protein PAC1 is required by both B1 and R1 proteins for full activation of anthocyanin biosynthetic genes in seeds and roots. In Arabidopsis, three R2R3-MYB proteins, MYB12, MYB11 and MYB111 (PFG1-3), which exhibit differential spatial expression patterns, regulate \textit{AtFLS1} expression in a tissue and developmental specific manner (Stracke et al., 2007).

**BIOLOGICAL FUNCTIONS OF FLAVONOIDS**

A variety of derivatives of the initial phenylpropanoid scaffold serve vital roles in plant structural integrity, UV photoprotection, reproduction, internal regulation of plant cell physiology and signaling. Phenylpropanoids also act as key chemical modulators of plant communication with insects and microbes, either as attractants or repellants, as phytoalexins against pathogens and herbivores and as attractants to pollinators. They also induce root nodulation when excreted by symbiotic nitrogen-fixing rhizobia (Mandal et al., 2010). The biological functions of flavonoids are linked to their potential cytotoxicity and their capacity to interact with enzymes through protein complexation. Some flavonoids provide stress protection eg; acting as scavengers of free radicals such as reactive oxygen species (ROS), as well as chelating metals that generate ROS via the Fenton reaction (Williams et al., 2004). Evidence links flavonoids with the control of the polar transport of auxins. This hormone probably has a role in the stress response by controlling stomatal opening and by allocating resources under poor growth conditions (Lewis et al., 2011). Flavonoids such as quercetin, kaempferol, apigenin and other aglycone molecules synthesized in the first steps of the flavonoid biosynthesis pathway inhibit polar auxin transport and enhance consequent localized auxin accumulation. (Lewis et al., 2011).

**MOLECULAR MECHANISM OF ACTION**

Studies in vitro and in vivo have shown that some flavonoids modulate the metabolism and disposition of carcinogens and can contribute to cancer prevention (Wattenberg LW; 1992). One important mechanism by which flavonoids may exert their effects is through their interaction with phase I metabolizing enzymes (cytochrome P450), which metabolically activate a large number of procarcinogens to reactive intermediates that can interact with cellular nucleophiles and ultimately trigger carcinogenesis (Le Marchand; 2000). They are likely to have a protective role against the induction of cellular damage by the activation of carcinogens. Another mechanism of action is the induction of phase II metabolizing enzymes such as glutathione-S-transferase, quinone reductase, and UDP-glucuronyl transferase by
which carcinogens are detoxified and thus more readily eliminated from the body. This would also help explain the chemopreventive effects of flavonoids against carcinogenesis (Bu-Abbas A, Clifford MN; 1998).

**Biomolecular activities of flavonoids:**
- Antioxidative effects: inactivation of oxygen radicals.
- Binding of electrophils.
- Induction of protective enzymes: phase 2 with conjugating activities (GT, GST).
- Apoptosis rate increase.
- Cell proliferation inhibition.
- Lipid peroxidation inhibition.
- Angiogenesis inhibition.
- H-Donation (e.g. GSH-peroxidases).
- DNA oxidation inhibition.

**Preventing carcinogen metabolic activation**
One of the most important mechanism by which flavonoids can exert their effects is through their interaction with phase I metabolizing enzymes (cytochrome P450) which metabolically activate a large number of procarcinogens to reactivate intermediates that can interact with cellular nucleophiles and ultimately trigger carcinogenesis. Flavonoids inhibit the activities of certain P450 isozymes, such as CYP1A1 and CYP1A2 (Marchand LL et.al; 2000), (Tsyrlov IB et.al; 1994). Thus they are likely to have a protective role against the induction of cellular damage by the activation of carcinogens. Another mechanism of action is the induction of phase II metabolizing enzymes (eg, GST, quinone reductase, and UDP-GT) by which carcinogens are detoxified & eliminated from the body. This helps in explaining the chemopreventive effects of flavonoids against carcinogenesis (Bu-Abbas A; 1998).

**Antiproliferation**
The molecular mechanism of antiproliferation may involve the inhibition of the prooxidant process that causes tumor promotion. Growth promoting oxidants and ROS are the major catalysts of the tumor promotion and progression stages. Flavonoids are effective in inhibiting xanthine oxidase (Chang WS et.al; 1993) COX or LOX55 and inhibit tumor cell proliferation (Mutoh M et.al; 2000). The mechanism of inhibition of polyamine biosynthesis can contribute to the antiproliferative activities of flavonoids. Ornithine decarboxylase is a rate-limiting enzyme in polyamine biosynthesis and is correlated with the rate of DNA
synthesis and cell proliferation in several tissues. Several experiments show that flavonoids can inhibit ornithine decarboxylase induced by tumor promoters causing a subsequent decrease in polyamine and inhibition of DNA and protein synthesis (Tanaka T; 1997). Furthermore, flavonoids are also effective at inhibiting signal transduction enzymes, protein tyrosine kinase (PTK), protein kinase C (PKC) and phosphoinositide 3-kinases (PIP3) which are involved in the regulation of cell proliferation (Makita H et.al; 1996).

**Cell cycle arrest**

Perturbations in cell cycle progression may account for the anticarcinogenic effects of flavonoids. Mitogenic signals commit cells to entry into a series of regulated steps allowing traverse of the cell cycle. Synthesis of DNA (S phase) and separation of two daughter cells (M phase) are the main features of cell cycle progression (Senderowicz AM; 1999). The time between the S and M phases is known as G2 phase. This phase is important to allow cells to repair errors that occur during DNA duplication, preventing the propagation of these errors to daughter cells. The G1 phase represents the period of commitment to cell cycle progression that separates M and S phases as cells prepare for DNA duplication upon mitogenic signals (Senderowicz AM; 2001).

CDKs have been recognized as key regulators of cell cycle progression. Alteration and deregulation of CDK activity are pathogenic hallmarks of neoplasia. A number of cancers are associated with hyperactivation of CDKs as a result of mutation of the CDK genes or CDK inhibitor genes. Therefore, inhibitors or modulators would be of interest to explore as novel therapeutic agents in cancer (Choi JA et.al; 2001). Checkpoints at both G1/S and G2/M of the cell cycle in cultured cancer cell lines have been found to be perturbed by flavonoids such as silymarin, genistein, quercetin, daidzein, luteolin, kaempferol, apigenin, and epigallocatechin 3-gallate. Studies from different laboratories revealed that flavopiridol could induce cell cycle arrest during either G1 or G2/M by the inhibition of all CDKs (Wang HK; 2000).

**Induction of apoptosis**

The significant anticancer properties observed in flavonoids may be due to frank apoptosis (Sakagami H et.al; 2000). Apoptosis is an active form of cell death that plays an essential role in the development and survival by eliminating damaged or unwanted cells (Wenzel U et.al; 2000). It is tightly regulated by a set of genes that promote apoptosis cell survival and is mediated through a highly organized network of interacting protease and their inhibitors in response to noxious stimuli from either inside or outside of the cell. Dysregulation of
apoptosis plays a critical role in oncogenesis. Flavonoids have shown to induce apoptosis in some cancer cell lines, while sparing normal cells (Lee WR, Shen SC; 2002). The molecular mechanisms by which flavonoids induce apoptosis have not been clarified. Several mechanisms may be involved, including inhibition of DNA topoisomerase I/II activity, decrease of ROS, regulation of heat shock protein expression, modulation of signaling pathways, down regulation of nuclear transcription factor kappa B (NF-κB), activation of endonuclease, and suppression of Mcl-1 protein (Konig A et.al; 1997).

**FLAVONOIDS AND THEIR HEALTH BENEFITS**

There has been increasing interest in the research of flavonoids from dietary sources, due to growing evidence of the versatile health benefits of flavonoids through epidemiological studies. Whether this effect is by preventing exposure to harmful substances such as oxidized cholesterol, pyrolysis mutagens, salt saturated fatty acids (FA) etc or by increasing the availability of certain useful nutrients like isothiocyanates, mono and poly unsaturated FA, PPT, Polyacetylenes, selenium, terpenes etc (Fang SC; 2010). Flavonoids have been reported to have multiple effects including antibacterial, anticancer, antiviral, antiinflammatory, vasodilatory anti-ischemic function. Many flavonoids are shown to have antioxidative activity, free-radical scavenging capacity, coronary heart disease prevention, and anticancer activity, while some flavonoids exhibit potential for anti-human immunodeficiency virus functions (Procházková D, 2011).

Flavonoids and isoflavonoids are relatively abundant in our diet, partially bioavailable, and possibly involved in still incompletely understood mechanisms related to the prevention of cancers, cardiovascular diseases, and neurodegeneration (Bazzano LA, Ogden LG et al; 2002). Flavonoids have a remarkable reducing ability (antioxidant properties by electron or H-atom donation) and their ability to interact with proteins making those important agents in terms of protection of human health. Biochemical studies devoted to the possible health effects of flavonoids try to assess either their nutritional value in the prevention of degenerative diseases or their therapeutic value as potential drugs (Haslam E; 1996), (McRae JM, 2011).

**Interactions between flavonoids and proteins**

There is increasing evidence that specific proteins or groups of proteins exhibit interactions with flavonoids in vivo. Many of these interactions have been shown to depend upon the B-ring substitution pattern of the interacting flavonoids (Marko et.al;2004). Flavanols are the
most common of these molecules and their high degree of activity is suggested by the tight
developmental and spatial regulation of flavanol synthesis. Proteins are considered as major
targets to know the therapeutic effect of a potential drug hence flavonoid protein interaction
studies are important to know the molecular mechanism of their action and to convert them to
novel medicines (Murphy et al; 2000). Their affinity to bind with plasma protein can be
helpful in assessing pharmacokinetics and pharmacodynamics properties (Peer et al; 2001).

Flavonoid-protein interactions can also be studied by measuring interaction of flavonoids
with specific amino acid residues. (Hernandez et al.; 2007) strengthened the theoretical
affinity order previously suggested for the interactions of flavonol species with 20 aa
residues. It was confirmed that hydrophilic aa residues demonstrated high affinity interacting
with flavonoid molecules. Flavonoids can function as in vitro enzyme inhibitors and as
ligands of various receptors involved in signal transduction (Havsteen BH; 2002). Some of
the health effects of flavonoids can be due to a combination of radical scavenging and
molecular (noncovalent) interaction of phenolic ring with protein. These molecular
interactions can be either vander wall interactions of polarisable aromatic ring with non-polar
aa residues or electrostatic interactions (Balasuriya N and Rupasinghe V; 2011).

**ATP-Binding Proteins**

Regulation of cell growth and proliferation is a complex mechanism using a number of signal
transduction pathways involving phosphorylation of proteins on serine, threonine and
tyrosine residues by a number of different protein kinases (Birt DF and Wang W; 2001).
Similarly, different events in cell cycle, like chromatin remodeling is characterized by
phosphorylation controlled by cyclin-dependent kinases (CDKs). Therefore, an ability of a
molecule to inhibit various PKs can contribute to its potential anti-carcinogenic effects.
Genistein and butein have been shown to compete with ATP for its binding sites on EGF
receptor and tyrosine kinases. Whereas, some of the flavones and flavonols has been found to
inhibit PKC (Bridges AJ; 2001). Phosphoinositide 3-kinase (PI3K) is a signal transducer
catalyzing phophorylation of C-3 hydroxyl group of inositol ring of phosphatidylinositol to
form new intracellular second messengers involved in a number of cellular functions
including cell growth, proliferation, differentiation, motility, survival and intracellular
trafficking. This enzyme is also inhibited by flavonoids (Hou DX and Kumamoto T; 2010).
Flavonoids have also been reported to cause cell cycle arrest by inhibiting CDKs and can
have potential antineuro degenerative action by modulating the activity of MAPKs.
(Casagrande and Darbon;2001) reported that presence of hydroxyl group at the 3’-position of ring B correlated to G1 cell cycle arrest while its absence in Kaemferol and apigenin correlated to G2 block. Genistein with hydroxyl group at 5- position of ring A arrested cells in G2. G1 cell cycle block was reported due to 40-60% inhibition of CDK2.

**Ligand- Receptor Interactions**

1. **GABA-A Receptors**
   GABA-A receptors are the most important inhibitory receptors in the central nervous system (Chebib and Johnston, 2000). They are members of the cys-loop superfamily of ligand-gated ion channels (LGICs) that encompasses both cationic and anion (GABA<sub>A</sub>, GABA<sub>C</sub> and glycine) receptors. These channels are membrane bound, structurally similar and considered to be composed of pentamers formed from distinct subunit combinations (Carter et al., 2010). The chloride flux can be regulated by a variety of neuroactive ligands including flavones that are able to bind to the BDZ binding site at the interface. Flavones typically behave as partial agonists potentiating the GABA activated ion current in a sub-maximal manner. The effects of benzodiazepines on GABA<sub>A</sub> receptors are complex and dependent on receptor subunit composition. In general, the γ2-subunit is required for the most widely observed effects of benzodiazepines on native GABA<sub>A</sub> receptors (Wafford et al., 1993) with the α-subunit determining benzodiazepine sensitivity. Benzodiazepine agonists such as diazepam and flunitrazepam enhance the action of GABA with high affinity. (Walters et al.; 2000) showed that benzodiazepines can act on GABA<sub>A</sub> receptors via two distinct and separable mechanisms. At nanomolar concentrations, benzodiazepines act in a classic flumazenil-sensitive manner to enhance the action of GABA, while at micromolar concentrations, benzodiazepines act in a flumazenil-insensitive manner.

2. **Adenosine Receptors**
   In humans, four adenosine receptors (subtypes A1, A2A, A2B and A3) have been demonstrated playing a role in brain (regulating the release of neurotransmitters), in heart (regulating myocardial oxygen consumption and coronary blood flow) and involved in inflammatory and immune response processes. Interestingly, several flavones and flavonol aglycones have rather high affinities for adenosine receptors. Varying the substitution pattern of flavone nucleus can produce fairly selective A3 ligands and can typically increases both the affinity and selectivity. The most potent ligands were also shown to reverse inhibition of adenyl cyclase, thus demonstrating antagonism.
3. Estrogen Receptors

The isoflavones genistein, bind both estrogen receptors (ER) α and β and act as full agonists. The two receptors have different structures, distinctly different localizations and concentrations within our body allowing for a wide range of diverse and complex processes to take place (Lee GS et.al; 2004). Such binding interactions can not only influence the growth, differentiation and functioning of many reproductive tissues but can also have cardioprotective effects (McCarty MF; 2006). This effect could provide a basis for interpreting the inverse relation between the risk of prostate and breast cancers and intake of isoflavone-rich soy foods as shown by various epidemiological studies. Like 17β-estradiol binding, genistein binding promotes dimerization of the receptor and subsequent binding to DNA at the estrogen receptor element for gene activation (Kostelac D; 2003). Studies using cultured human breast and prostate cancer cells, have not confirmed that genistein has a direct effect on the autophosphorylation of EGF receptor (Peterson TG; 1996),(Galluzzo P and Marino M; 2006).

Redox Enzymes

Lipoxygenases (LOXs) and cycloxygenases (COXs) and Xanthine oxidase (XO) are metalloenzymes whose catalytic cycle involves ROS such as superoxide and hydrogen peroxide. LOXs and COXs catalyze important steps in the biosynthesis of leukotrienes from arachidonic acid, which is an important cascade in the development of inflammatory responses. Flavonoids may exert part of their antioxidant and anti-inflammatory properties Via direct inhibition of LOXs, COXs and XO (Batra P and Sharma AK; 2013).

Lipoxygenases and Cyclooxygenases

Mammalian 15-lipoxygenase 1 is an endogenous pro-oxidant enzyme capable of oxidizing LDLs, an early event in the development of atherosclerosis. Hence its inhibition by flavonoids is a potential mechanism for the prevention of cardiovascular diseases by this antioxidant (Galati G et al; 2001, 2002). Flavone and Flavanol aglycones come up as the most potent inhibitors and effect enzyme activity in three distinct ways: prolongation of the initial lag phase during which the accumulation of lipid hydroperoxides is very slow, lowering of the maximal peroxidation rate during the subsequent phase of hydroperoxide accumulation and inactivation of the enzyme in a third phase due to the combined action of the flavonoids and the intermediates of the catalytic cycle. The inhibition is insensitive to the
presence of Fe(III) and stronger with flavones and flavanols having a catechol group either on the A ring or on the B ring (Moridani MY, Scobie H; 2001).

**LOXs and COXs**

LOXs and COXs are capable of co oxidizing molecules other than their regular substrates and hence can increase oxidative lesion in some tissues. Flavonoids have the ability to inhibit cyclooxygenase (COX-2) and lipoxygenase (Hong J et.al; 2001). Direct scavenging of ROS by flavonoid antioxidants lead to flavonoid oxidation forming aryloxyl radicals, quinones or quinoid compounds that form covalent adducts with enzyme causing enzyme inactivation (Sandhar HK et.al; 2011). Another redox enzyme, 17 β-hydroxysteroid dehydrogenase which is involved in steroid metabolism is strongly inhibited by flavone apigenin (Le Bail JC; 1998).

**Peroxidases and Tyrosinases**

Both these enzymes have been used to oxidize flavonoids to their aryloxyl radicals (one-electron oxidation) and o-quinones (two-electron oxidation). Aryloxyl radicals can oxidize glutathione and NADH with reduction of dioxygen and ROS formation indicating a metal-independent mechanism for the prooxidant activity of flavonoids. O-quinones can then be reduced by NADH or form conjugates with glutathione without dioxygen activation or can react with Cys residues of proteins to form covalent adduct (Galati G et.al; 1999).

**Flavonoids in Angiogenesis**

Flavonoids are known as angiogenesis inhibitors derived from natural sources. The abilities of particular flavonoids to block solid tumor growth may be due to their inhibition of the neoangiogenic process (Paper DH; 1998). Angiogenesis is a strictly controlled process in the healthy adult human body, which is regulated by a variety of endogenous angiogenic and angiostatic factors. When deprived of proper vascularization, the high proliferation rate in the tumor would be balanced by cell death due to the lack of diffusion of nutrients and oxygen. Angiogenesis inhibitors such as flavonoids are able to interfere with various steps of angiogenesis, like basement destruction of blood vessels, proliferation and migration of endothelial cells or the lumen formation (Tosetti F et.al; 2002). Therefore, these compounds may have potential for the treatment of solid tumors (Fotsis T, 1998). Genistein is known for its anti-angiogenesis having implications in the therapeutic intervention of neovascular disorders. Studies on the inhibition of cell proliferation and angiogenesis by flavonoids in six different cancer cell lines had been reported and noted that the IC50 of active flavonoids were
in the low micromolar range, physiologically available concentrations (Kim MH; 2003), (Su SJ, Yeh TM; 2005). Genistein, Luteolin and Delphinidin have been found to inhibit VEGF-induced angiogenesis, inhibition of endothelial cell survival and proliferation by targeting phosphatidylinositol-3- kinase action. Genistein may enhance transforming growth factor- β (TGF-β) playing an important role in atherosclerosis and hereditary hemorrhagic telangiectasia (Schindler R and Mentlein R; 2006).

Modulation of Multidrug Resistance
Multidrug resistance due to P-glycoprotein or multidrug resistance associated protein (MRP) is a serious impediment to successful chemotherapy of cancer. Certain flavonoids have been reported to possess potent inhibitory activity against the drug exporting function of Pgp, a plasma membrane ATP-binding cassette transporter that extrudes cytotoxic drugs at the expense of ATP hydrolysis. Pgp consists of two homologous halves each containing a transmembrane domain (TMD) involved in drug binding and efflux, and a cytosolic nucleotide-binding domain (NBD) involved in ATP binding and hydrolysis, with an overall (TMD-NBD)2 domain topology. Modulation by flavonoids of cell multidrug resistance mediated by Pgp may be through;

(i) Inhibiting the overexpression of multidrug resistance gene-1 (Kioka M et.al; 1992).
(ii) Direct binding to NBDs with high affinity (Di Pietro A, Dayan G; 1999).
(iii) Inhibiting ATPase activity, nucleotide hydrolysis and energy-dependent drug interaction with transporter-enriched membranes (Shapiro AB, Ling V; 1997).

FLAVONOIDS AS A VERSATILE SOURCE OF ANTICANCER DRUGS
In search for anticancer drugs compelling data from laboratories, epidemiologic investigations and human clinical trials showed that flavonoids have important effects on cancer chemoprevention and chemotherapy. In many molecular mechanisms of action for prevention against cancer, flavonoids play a major role by interacting between different types of genes and enzymes. An impressive body of information exists on the antitumor action of plant flavonoids.

In vitro work has concentrated on the direct and indirect actions of flavonoids on tumor cells and has found a variety of anticancer effects such as cell growth and kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metalloproteinases and of tumor invasive behavior (Kamei H, Koide T; 1996). Studies have reported the impairment
of in vivo angiogenesis by dietary flavonoids. Experimental animal studies indicate that
certain dietary flavonoids possess antitumor activity. The hydroxylation pattern of the B ring
of the flavones and flavonols such as luteolin and quercetin, seems to critically influence their
activities, especially the inhibition of protein kinase activity and anti proliferation. Certain
dietary flavonols and flavones targeting cell surface signal transduction enzymes such as
protein tyrosine and focal adhesion kinases and the processes of angiogenesis appear to be
promising candidates as anticancer agents (Caltagirone S et.al; 2000).

**Anticancer Activities of Flavonoids in Various Cancer Cell Lines**

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Cell</th>
<th>Flavonoid</th>
<th>References</th>
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TREATMENT OF DIFFERENT TYPES OF CANCER BY FLAVONOIDS

The weight of the epidemiological evidence for a protective effect of flavonoids against cancer is impressive (Parkin DM, Ferlay J; 1999). A growing number of epidemiological studies suggest that high flavonoid intake may be correlated with a decreased risk of cancer. In Western countries, breast cancer is one of the most common causes of death in women and prostate cancer is the second most common cause of death in men. In China, Japan and other Asian countries, where diets include relatively high concentrations of soy isoflavones, death due to cancer is comparatively rare. Phytoestrogens are plant-derived chemicals that bind to the estrogen receptor (ER) and induce various estrogenic and anti-estrogenic responses. The extensively studied class of phytoestrogens are the isoflavones. High concentrations of the isoflavones genistein and diadzein are present in legumes and ingestion of these substances may reduce the risk of cancer, particularly in the breast and prostate (Peterson G et.al; 1996).

Soy containing isoflavones are among the most versatile biopharmaceuticals known. Genistein, daidzein and glycetine are the main isoflavones found in soy foods. Isoflavones, one of the major class of phytoestrogens are structurally similar to estrogens, binds to ERs and hence have estrogenic and anti-estrogenic activities and their own growth-inhibitory effects are independent of ER (Markovits J, Linassier C;1989). Isoflavones and their metabolites are considered to reduce the risk of cancer and to have potent anticarcinogenic activities by direct inhibition of PTK, inhibition of DNA-topoisomerase II, inhibition of angiogenesis, antiproliferation and cell cycle arrest and induction of apoptosis (Matsukawa Y; 1993).

MAMMARY TUMOR

Estrogens are involved in mammary carcinomas. Researchers have found that in ER-positive and ER-negative mammary cell lines of women affected with breast cancer, the tumor-suppressing gene pRb2/p13 binds to a specific region on the ER gene alpha and forms molecular complexes recruiting and interacting with several proteins (Martin PM, Horwitz KB; 1978). They discovered that ER-negative cells that are able to silent the expression of the ER pRb2/p13 form a specific molecular complex recruiting a different sequence of proteins than in the ER-positive cells (Peterson G, Barnes S; 1996). Investigation of seven metabolites of isoflavones for their growth-inhibitory effects and later compared with the isoflavones genistein, daidzein and glycetine present on human breast cancer MCF-7 and MDA-MB-468 cells. The novel metabolite exhibited a potent growth inhibitory effect on human breast ER-
positive MCF-7 cells and ER-negative MDA-MB-468 cells (Kamath N, Murley JS; 1998). This metabolite was further examined on other human breast cancer SK-BR-3 (ER-negative), human breast noncancer MCF-10A (ER-negative), human prostate cancer LNCaP and DU145 (AR-negative) cell lines. This study shows that the novel metabolite 2-de-O-DMA is still able to inhibit the proliferation of MCF-10A (ER-negative), SK-BR-3 (ER-negative), LNCaP, and DU145 cells. Epidemiologic studies have showed that populations with high isoflavone intake through soy consumption have low rates of breast, prostate, and colon cancer. The isoflavone polyphenol genistein in soybean is considered to be a potent chemopreventive agent against cancer (Ullah MF et.al; 2009).

**PROSTATE CANCER**

Prostate cancer (PCA) is considered as one of the major concerns in the field of cancer therapy. PCA is an aging disease and oxidative stress and is a major factor in the promotion and progression of malignancy (Sohal RS, Weindruch R; 1996). Activation of many kinases involved in NF-κB pathway is dependent on oxidative stress. ROS cause prolonged NF-κB DNA binding activity and antioxidants have shown to diminish this activity (Ripple MO et.al; 1999). One approach to control PCA growth and progression can be inhibition of constitutive NF-κB activation, but limited efforts have been made in this direction. Some flavonoids play an important role in preventing PCA by various modes of action. Silibinin is a flavonolignan present in milk thistle seeds. It is a promising chemopreventive agent against human PCA without showing any apparent toxic side effects (Wellington K; 2001). Silibinin has shown strong anticancer efficacy against both androgen-dependent and -independent advanced human PCA cells. Silibinin inhibits TGF expression, secretion and down-regulates EGFR-Erk1/2 activation in both LNCaP and DU145 cells, which contributes to the growth inhibitory effects in these cell lines (Zi X, Agarwal R; 1999).

Luteolin (5,7-tetrahydroxyflavone) has an antiproliferation property that acts via arresting cell cycle and apoptosis in many human cancer cells including PCA cells. Studies showed that luteolin inhibits the expression of AR and growth in LNCaP human PCA cells and xenografted mice. The reduction in AR levels by luteolin involve a transcriptional or post translational mechanism (Lim DY, Jeong Y; 2007). It also suggests that luteolin suppresses the association between AR and heat-shock protein 9 and induces AR protein degradation through a proteasome-mediated pathway. These results indicate that AR is critical for PCA cell growth and survival and that it is a potential molecular target for luteolin-mediated
anticancer therapy. Identification of biomarkers plays an important role in this effort through the use of new and emerging technologies. Also gene expression profiling and proteomics provide novel insights into cancer-related traits. Early detection is the desired strategy for reducing cancer-related morbidity and mortality and collaborative effort between academic and industry leaders brings expert solutions for cancer (Vayalil PK, Mittal A; 2004).

FLAVONOID SUPPLEMENTS AND ADVERSE EFFECT:
Extensive range of flavonoid supplements is available in the market, inspite of the toxicity as well as nutrient/drug interaction issues (Egert S and Rimbach G; 2011). A scarce data is available about safety, contraindications and effectiveness of these supplements, therefore their unnecessary use must be avoided as following type of interactions have been extensively reported (Espin JC et.al; 2007):

Interactions with Trace Elements
Interactions between flavonoids and copper have been reported. Iron element bioavailability seems to be impaired by purified flavonoids, flavonoid extracts and flavonoid-rich food items (Sandberg AS; 2002),( Korver O et.al; 2000). Nonheme iron absorption was reported to decrease in a dose-dependent manner by epigallocatechin gallate (EGCG). (Ren et al. 2008) studied the complexation mechanisms of several flavonoids (quercetin, luteolin, galangin, kaempferol and chrysin) with iron in a randomized, double-blind, placebo-controlled trial. (Ma et al. 2010) have shown that polyphenols also inhibit heme iron absorption mainly by reducing basolateral iron exit rather than decreasing apical heme iron uptake in intestinal cells.

Interactions with Vitamins
EGCG and other gallated derivatives of green tea polyphenols have been reported to inhibit dihydrofolate reductase activity (in vitro) and folic acid uptake in Caco-3 cells. Green tea extracts significantly decreased serum 5-methyltetrahydrofolate concentrations in rats. Similar study in humans suggests a potential risk of diminished folic acid bioavailability by both green and black tea extracts, while another study revealed no differences in plasma folate Concentrations (Frank J et.al; 2009). Inhibition of sodium dependent vitamin C transporter 1 by quercetin has been reported in cultured cells with significant decrease in ascorbate absorption. Serum thyroid hormone tri-iodothyronine and thyroxine levels were found to be significantly reduced and associated with a significant elevation of serum thyroid-stimulating hormone (Milerova J et.al; 2006). However, a recent long term human
study in almost 400 osteopenic, postmenopausal women suggested that genistein aglycone intake does not significantly increase the risk of clinical or subclinical hypothyroidism at the dose of 54 mg/d (Polito F et.al;2010).

**Interactions Between Drugs and Flavonoids**

Flavonoids may interfere with the absorption, tissue distribution, metabolism and excretion of drugs, altering their pharmacokinetics profiles. Though the ability of flavonoids to interact with cytochrome P450 monooxygenase (CYP) isoforms, Phase II conjugation enzymes and drug transporters enable them to be potential anticancerous agents that can prevent drug efflux during cancer therapy (Cermak R, Wolffram S; 2006). Among the various CYP isoforms, CYP3A4 and 3A5 are the major CYP isoforms in the liver and intestinal tract and are responsible for the metabolism of most of the prescribed drugs. This flavonoid-ABC-transporter interaction could be beneficial for poorly absorbed drugs but could also result in severe drug intoxication (Rendic S; 2002). Therefore, a “healthy diet” rich in fruits and vegetables should be a preferred source to exploit their health benefits.

**CONCLUSION**

Flavonoids are generally nontoxic and manifest a diverse range of beneficial biological activities. Epidemiological studies have provided data that high dietary intake of flavonoids with fruits and vegetables could be associated with a low cancer prevalence in humans. This is supported by a multitude of in vitro and in vivo studies, which show that flavonoids may inhibit various stages in the carcinogenesis process namely tumor initiation, promotion and progression. A strong correlation between flavonoid induced modulation of kinases with apoptosis, cell proliferation and tumor cell invasive behaviour has been established in vitro. Some of the dietary flavonoids have also been known to display in vivo antitumor activity and repress in vivo angiogenesis. All these studies will pave the way for a potential chemotherapeutic strategy against cancer. Flavonoids may interfere with the absorption, tissue distribution, metabolism and excretion of drugs thus can be anticancerous agents by preventing the efflux of drug during cancer therapy. Overall the flavonoids are required substances from plant kingdom through animals to humans but their type and concentrations are very determined for healthy life. In conclusion, considering that many chemotherapeutic agents against tumor cells without sparing normal cells remain a major obstacle and development of multidrug resistance further limits chemotherapy in cancer. The promising
results will stimulate the development of flavonoids for cancer chemoprevention and chemotherapy.

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