Pro-apoptotic Effects of Dietary Flavonoids in Oxidative Stress-Induced Cancer

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Abstract

Flavonoids are bioactives that are seen abundantly in various parts of plants, especially in fruits and vegetables. Flavonoids are classified into different sub-classes, including anthocyanins, flavanols, chalcones, flavanones, flavones, and isoflavones. Many dietary flavonoids exhibit health-promoting effects, mainly disease preventive actions such as antioxidant, antimicrobial, cardioprotective, hepatoprotective, neuroprotective, antidiabetic, anti-inflammatory, anti-proliferative, and anti-cancer. Dietary flavonoids typically help to reduce cellular oxidative stress and modulate dysregulated signaling pathways and thereby gene expression. Oxidative stress regulation is an essential factor in cancer treatment. Normal and cancerous cells mainly differ in their redox status. In cancer cells, there are increased levels of reactive oxygen species; hence, several anti-cancer therapies largely focus on modulating these reactive oxygen species levels. Recent studies suggest that small molecules with bioactive properties support to reduce the side effects of anti-cancer drugs. This chapter will briefly discuss the pro-apoptotic effects of some of the major dietary flavonoids such as quercetin, morin, EGCG, genistein, daidzein, apigenin, luteolin, and myricetin in oxidative stress-induced cancers.

Introduction

Flavonoids are mostly plant secondary metabolites which help in the defense system and to attract pollinating insects. Flavonoids are categorized under the class of polyphenols, which possess a C15 flavone skeleton. Phenolic compounds are known to be synthesized in plants as secondary metabolites and are categorized as phenolic acids, flavonoids, and proanthocyanidins. Flavonoids, which constitute a substantial portion, have a general structure with two aromatic rings with a minimum of one hydroxyl group connected to a six-membered heterocyclic pyran ring by a bridge formed by three carbon atoms. The major subclasses of flavonoids are anthocyanidins, flavan-3-ols, flavonols, flavanones, flavones, and isoflavones (Ibrahim et al. 2012). The different subclasses of flavonoids differ in the connections between the aromatic ring and the heterocyclic pyran rings. Within each flavonoid subclass, the individual compounds have characteristic hydroxylation and conjugation patterns. Flavonoids are synthesized through two biosynthetic pathways such as the shikimic acid pathway and the acetate pathway (Nabavi et al. 2020).

Flavonoids typically originate in plant-based food products such as wine, fruit juices, tea, coffee, chocolate, etc., which are generally termed dietary flavonoids. Berries and drupes are the richest sources of flavonoids among fruits, while in vegetables mainly red onion, shallots, broad bean pods, olives, spinach, etc. Seeds

like beans, soy, and soy-derived food products are also rich sources of flavonoids. Beverages like green tea, black tea, and cocoa are reported to have high flavonoid content. Although the above dietary sources are rich in flavonoids, the type of flavonoid and its amount vary from one source to another. Therefore, a diet rich in fruits, vegetables, nuts, and cereals can ensure sufficient amounts of flavonoids required for the normal well-being of our body (Liu et al. 2018).

In plants, flavonoids are mostly found either in glycoside bound or as free aglycone form. The flavones and flavanols consumed in the diet are commonly in the glycoside bound form. The beneficial effect of flavonoids depends largely on their concentration and bioavailability. Once flavonoids enter the oral cavity, their metabolism and absorption begin in the digestive tract. Gut microbiota also play a vital role in the metabolism and absorption of flavonoids via the production of absorbable aglycone form in the large intestine. However, the absorption and bioavailability of flavonoids are increased or decreased by various factors. Typical high-fat diet increases the absorption of flavonoids due to increased secretion of bile acids, resulting in the formation of flavonoid-rich micelles. However, a protein-rich diet reduces the bioavailability of flavonoids, thereby decreasing its antioxidant activity and the digestion of proteins. Flavonoids are also shown to inhibit glycolytic enzymes and modulate glucose absorption when a diet rich in carbohydrates is consumed. The bioavailability of flavonoids also differs depending on their source and type. For example, quercetin glycoside is one of the most common flavonoid consumed through the diet, but its bioavailability varies from one source to another. Quercetin obtained from apple shows lower availability when compared to quercetin from onion (Rodriguez-Mateos et al. 2014).

Flavonoids possess a wide range of biological and pharmacological activities, thereby altering multiple mechanisms in the cell leading to their beneficial effects. Flavonoids were shown to modify enzymes and signaling pathways related to oxidative stress and inflammation. The antioxidant potential of flavonoids largely attributed to their structure due to the presence of hydroxyl groups. The detoxifying enzymes especially cytochrome p450 (CYC p450) are reported for activating chemical carcinogens, and flavonoids are shown to inhibit the CYC p450, thereby inhibiting carcinogenesis. Tea catechins are also identified to induce detoxifying and antioxidant enzymes present in the cells like superoxide dismutase (SOD), catalase, etc. The anti-inflammatory effects of flavonoids are due to their inhibitory effect on cyclooxygenase (COX) and activation of NADPH oxidase (NOX) enzymes.

Oxidative Stress and Reactive Oxygen Species

Maintaining a balance between generation and elimination of oxidants or reactive chemical species is termed a redox state. Oxidative stress is the destruction of redox equilibrium resulted due to increased levels of reactive oxygen species (ROS) further leading to damage to biomacromolecules (Pizzino et al. 2017). ROS are reactive molecules which contain oxygen either in radical form or non-radical derivatives. For a healthy environment, it is significant to retain the equilibrium of ROS in the cell between its production due to cellular metabolism and control by endogenous

antioxidant enzymes. Oxidative stress leads to various clinical conditions such as inflammatory diseases, diabetes, cardiovascular disorders, cancer, etc.

Regulation of ROS at Enzymatic Level

ROS are unstable, partially reduced forms of specific metabolic products, which are repetitively produced and eliminated from cells. Some of the notable ROS in the biological system includes superoxide anion radical ($O2•^-$), hydroxyl radical ($•OH$), singlet oxygen, hydrogen peroxide (H_2O_2), peroxynitrite, etc. ROS has an unpaired electron and shortened life span. Under stable physiological conditions, the stability among intracellular ROS and intercellular antioxidants is strictly delimited with a capable defense mechanism that wedges unwanted ROS production. In the presence of antioxidant defense mechanism, simple and grouped nucleic ROS levels allow sovereign regulation of mitochondrial, cytoplasmic ROS signals (Jones 2008).

SOD is an endogenous enzyme and is the central key for the defense system against oxidative stress. SOD are a group of metalloprotein enzymes found in all organisms, which can rapidly act as a free radical scavenger of oxygen. Joe McCord and Irwin Fridovich discovered the action of SOD in 1968, which catalyzes the partitioning or dispute the SOD resilient H_2O_2 by enzymatic Haber-Weiss reaction. In mammals, SODs are classified into three types, namely, copper/zinc SOD (SOD 1), manganese SOD (SOD 2), and extracellular SOD (Ec SOD 3). SOD 1 and SOD 2 are present in the cytosol, while SOD 3 is located in the extracellular matrix. In mitochondria, $O2•^-$ is the initiative molecule for the production of oxidant toxicity and ROS. Mitochondrial enzyme Mn-SOD provides essential protection against $O2•^-$, SOD coupled with specific other proteins such as catalase, peroxidase, glutathione peroxidase, and glutathione S-transferase (GST), which finally convert H_2O_2 to water. All these antioxidant enzymes act on ROS to prevent the Fenton reaction and increasing H_2O_2 concentration, as well as $•OH$ formation (Thomas et al. 2009).

Besides SOD, other enzymes also take part in the ROS detoxification. Glutathione (g-L-glutamyl-L-cysteinyl glycine), an intracellular thiol family of Phase II detoxification flavoprotein, is formed with a combination of glycine, cysteine, and glutamic acid. It is expressed more commonly by reduced form as glutathione (GSH) or oxidized form as glutathione disulfide (GSSG); the thiol mechanism gives its core biochemical properties. GSH can expel ROS by solid chemical reaction or by reducing peroxide, as a cofactor of GSH peroxidase, which induced a cycle among the reduced form and dimerized oxidized form of glutathione. GSH reductase, a homodimeric cellular antioxidant, mainly presents NADPH in the reduced form involved in many detoxifying types of ROS compounds. The thioredoxin reductase system has ubiquitous functions like SOD and glutathione. Thioredoxin is a protein dithiol/disulfide active site that turns to aid the reduction of additional proteins by establishing disulfide bridges between cysteine residues. Such interactive basic instincts enable cells to thrive in an antioxidant oxidizing environment, carry out the obligatory biochemical processes, and use ROS as signaling molecule (Arnér and Holmgren 2000).

Regulation of ROS at Molecular Levels

Antioxidant protein expression is regulated by NF-κB, a transcription factor which involves multiple cellular process defense against ROS such as inflammatory response, apoptosis, and cell adhesion. NF-κB associates with Rel (c-Rel), RelB, p50/p105 (NF-κB1), RelA (p65), and p52/p100 (NF-κB2). Also, NF-κB disorders have been confirmed to be correlated with cancer. Mitogen-activated protein kinase (MAPK) plays a central role in complex cellular processes such as replication, cell cycle, development, and apoptosis. It consists of extracellular signal-related kinases (ERK1/2), c-Jun N-terminal kinases (JNK), p38 kinases (p38), and a large MAP kinase 1 (BMK1/ERK5) pathway. Recent studies show that ROS could activate ERK and JNK pathways, which ultimately induces oxidative stress-induced cancer (Wei and Liu 2002).

Keap1-Nrf2-ARE and phosphoinositide-3-kinase(PI3K-)/Akt signaling pathways also have some significant roles related to oxidative stress-induced cancers. Keap1-Nrf2-ARE is involved in redox balance and metabolism and adaptive immune response, and deregulation leads to many disorders like diabetes, inflammatory disease, Alzheimer's disease, and cancer. PI3K/Akt pathway possesses multiple cellular responses, including cell cycle, protein synthesis, proliferation, and apoptosis. It also has a drug resistance response and is involved in stimulating hormones and cytokines (Cahill et al. 2012). Upregulation of PI3K/Akt signaling leads to oxidative stress-induced cancers including lung, liver, ovarian, prostate, and breast cancer.

Apoptosis: Pathways and Proteins Involved

Apoptosis is precisely programmed cell death, controlled by a sequence of cellular proteins and signal transduction cascades. When there is a fault in this orderly cell death, it will lead to uncontrolled growth of cells such as in many disease conditions such as Parkinson's syndrome, cancer, and diabetes. The two major apoptotic pathways are the intrinsic or mitochondrial pathway and the extrinsic or cytoplasmic pathway. The liberation of cytochrome C originates the intrinsic pathway to the mitochondria, which attaches to Apaf1, dATP, and procaspase-9. Extrinsic pathway is initiated by inducing cell surface death receptors by binding death signals to trimeric death ligands Fas, TNF, etc. Proteins involved in apoptosis can be categorized into two classes, pro-apoptotic and anti-apoptotic proteins. Pro-apoptotic proteins are those proteins that induce apoptosis, while anti-apoptotic proteins will try to prevent apoptosis. Pro-apoptotic proteins are further classified into caspases and BCL2 proteins. Caspases are cysteine-dependent aspartate-directed proteases. Based on the function, caspases are of three types of inflammatory, initiator, and effector caspases. Caspases that serve in the extrinsic pathway are caspase-8, caspase-3, caspase-6, and caspase-7, while caspases that help in intrinsic pathways are caspase-2, caspase-3, caspase-7, and caspase-9. BCL2 proteins regulate apoptosis by controlling the

permeability of the mitochondrial outer membrane and thereby releasing cyto-chrome C. Bcl2 proteins that induce apoptosis in various ways, such as by inhibiting apoptotic inhibitors, e.g., Noxa, Puma, Arts, SMAC, etc., belong to BH3 only class. Bid protein takes part in the intermediate pathway of apoptosis. Examples of Bcl2 multidomain proteins are Bax and Bak. Anti-apoptotic proteins mainly are of two types: Bcl2 multidomain proteins and BIR domain proteins. DIVA, Bcl-Xl, Bcl-b, Bcl-w, etc. are examples of Bcl2 multidomain proteins and survivin, livin, bruce, etc. are examples of BIR domain proteins (Abotaleb et al. 2019).

Intrinsic or Mitochondria-Dependent Pathway

The intrinsic pathway of apoptosis depends on the trophic factors, also known as survival factors. Trophic factors such as nerve growth factor (NGF) bind to the receptors present in the cell surface and activate the phosphatidylinositol 3-kinase (PI3K) signaling. PI3K activate protein kinase B, which further induce phosphory-lation of the Bad (BCL-2-associated agonist of cell death), that are present in the outer membrane of mitochondria. When cells fail to receive the trophic factors, all this cascade of reactions will be inhibited and Bad proteins will be free, binding to Bcl-2 or Bcl-xL and releasing Bak or Bax, which stay attached to Bcl-2 or Bcl-xL. Anti-apoptotic proteins. Bak or Bax oligomerizes and forms pores in the surface of mitochondria and releases cytochrome C. Cytochrome C binds to Apaf-1 and activates caspase-9. These executioner caspases induce activation of effector caspases such as caspase-3, caspase-6, and caspase-7 resulting in the cleavage of substrates and finally cell death (Ozben 2007).

Extrinsic or Death Receptor-Induced Pathway

The extrinsic pathway is stimulated by favorably influencing death signals such as tumor necrosis factor-α (TNF-α) and Fas/CD95 ligand. Macrophages generate TNF-α, while Fas ligands were released by cytotoxic T lymphocytes and natural killer cells. Fas ligand and TNF-α are trimeric proteins present in the surface of a cell that attach to the death receptors of another cell surface. These ligands can bring three receptors together and then bind to cytosolic proteins Fas-associated death domain protein known as FADD and TNF receptor-associated death domain protein known as TRADD from the inner cell and form an oligomeric complex. FADD activate caspase-8 which further activate other effector caspases such as caspase-3, caspase-6, and caspase-7. Caspase-8 also connect to the intrinsic pathway of apoptosis by cleaving Bid, bh3 interacting domain death agonist into fragment, namely, tBiD, which bind to Bcl2/Bcl-xL proteins resulting in the release of Bad or Bax leads to the formation of pores on the mitochondrial membrane, which will release cytochrome C and continues to the intrinsic pathway (Matés et al. 2012).

A wide range of proteins participate during apoptosis with significant function. X-linked inhibitors of apoptotic proteins (XIAPs) typically inhibit apoptotic pathways by binding to caspase-3 and caspase-7 or caspase-9. SMAC/DIABLO are proteins released from mitochondria through the pores created by Bak or Bax and bind to XIAPs, preventing them from attaching to caspases and inhibiting apoptotic pathways (Lodish et al. 2004).

Dietary Flavonoids with Pro-apoptotic Effect Against Experimental Cancers

As a preventive approach against carcinogenesis, cell proliferation should be inhibited and apoptosis should be induced. Apoptosis mediates a physiological mechanism, thereby facilitating to eliminate cancerous cells. Dietary flavonoids are shown to exhibit many biological activities especially pro-apoptotic action which is employed in cancer treatments; however, detailed exploration of signaling pathways are yet to be discovered. The pro-apoptotic effects of major dietary flavonoids mediated by increased ROS production in experimental cancers are listed in Table 1.

Quercetin

Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) is a dietary flavonoid commonly found in apples, onions, grapes, berries, cherry, citrus fruits, etc. Quercetin is the most abundant flavonoid present in the human diet (Erlund 2004) and well reported for its antioxidant and pro-apoptotic activity (Lee et al. 2003). In a study conducted by Yi et al. (2014), quercetin induced apoptosis of ovarian cancer cells (SKOV-3, OVCAR-3, TOV-21G, and HOSE) via ROS-mediated ER stress (Yi et al. 2014). In HL-60 cells, quercetin treatment increased intracellular oxidative stress and potentiated apoptosis through activation of ERK-mediated pathway (Lee et al. 2015). In another study, quercetin glycoside (hyperoside) treatment to breast cancer cells (MCF-7) inhibited NF-κB signaling and exhibited ROS-related apoptosis (Qiu et al. 2019). Quercetin treatment in combination with paclitaxel to prostate cancer cells (PC-3) increased ROS generation and induced apoptosis and ER stress (Zhang et al. 2020).

Morin

Morin (2′, 3, 4′, 5, 7-pentahydroxyflavone) is a dietary flavonoid majorly present in guava leaves (Psidium guajava), old fustic (Maclura tinctoria), Osage orange (Maclura pomifera), etc. (MadanKumar et al. 2014). Morin possesses numerous pharmacological activities such as antioxidant, antimicrobial, antibacterial, antiviral, hepatoprotective, neuroprotective, cardioprotective, antifibrotic, anti-cancer, etc. (MadanKumar et al. 2015; Perumal et al. 2017a). The molecular mechanism behind

Table 1 Pro-apoptotic effects of dietary flavonoids mediated by increased ROS production in experimental cancers

Flavonoid name	Source of the flavonoid	Cancer type	Cancer cell line name	Reference
Quercetin	*Malus domestica, Allium cepa, Vitis vinifera, Prunus avium, Citrus* fruits	Ovary	SKOV-3, OVCAR-3, TOV-21G and HOSE	Yi et al. (2014)
		Leukemia	HL-60	Lee et al. (2015)
		Breast	MCF-7	Qiu et al. (2019)
		Prostate	PC-3	Zhang et al. (2020)
Morin	*Psidium guajava, Maclura tinctoria, Maclura pomifera*	Leukemia	U937	Park et al. (2015)
		Liver	HepG2	Perumal et al. (2017b)
		Cervical	HeLa	Zhang et al. (2018)
		Colorectal	SW480	Nie et al. (2019)
Epigallocatechin-3-gallate	*Camellia sinensis, Vaccinium oxycoccos, Actinidia deliciosa*	Lung	A549, H1299, and H460	Yu et al. (2017)
		Liver	HepG2	Khiewkamrop et al. (2018)
		Lung	A549	Velavan et al. (2018)
Genistein	*Glycine max,* Leguminosae	Colon	HT-29, SW-620, and SW-116	Salti et al. (2000)
		Breast	MCF-7	Prietsch et al. (2014)
		Pancreas	Mia-PaCa2 and PANC-1	Bi et al. (2018)
		Head and neck	Primary cells from cancer patients	Hsieh et al. (2020)
Daidzein	*Glycine max,* Leguminosae	Breast	MCF-7	Jin et al. (2010) and Kumar and Chauhan (2021)
		Liver	SK-HEP-1	Park et al. (2013), Liang et al. (2018)
		Colon	HT-29	
Apigenin	*Spinacia oleracea, Allium sativum,*	Cervical	HeLa, SiHa,	Souza et al. (2017)

Table 1 (continued)

Flavonoid name	Source of the flavonoid	Cancer type	Cancer cell line name	Reference
	Apium graveolens, Daucus carota		CaSki, and C33A	
		Mesothelioma	MSTO-211H and H-2452	Lee et al. (2020)
		Glioblastoma	U87	Shendge et al. (2021)
		Multiple myeloma	NCI-H929	Adham et al. (2021)
Luteolin	*Apium graveolens, Daucus carota, Brassica oleracea, Thymus vulgaris, Piper nigrum, Malus domestica*	Liver	H4IIE	Michels et al. (2005)
		Oral squamous		Yang et al. (2008)
		Glioblastoma	U251MG and U87MG	Wang et al. (2017)
Myricetin	*Solanum lycopersicum, Citrus sinensis, Camellia sinensis,* red wine, nuts, berries	Skin	HaCaT	Kim et al. (2010)
		Colon	HCT-15	Kim et al. (2014)
		Lung	A549	Rajendran et al. (2021)
Hesperidin	*Citrus sinensis, Citrus paradise, Citrus limon, Allium fistulosum, Citrus reticulata*	Liver	HepG2	Banjerdpongchai et al. (2016)
		Prostate	DU145	Ning et al. (2020)
		Cervical	HeLa	Pandey et al. (2021)

the anti-cancer potential of morin was studied in several cancer cells such as liver (HepG2), cervical (HeLa), breast (MCF-7), colon (HCT-116), etc. In human leukemic cells (U937), morin-induced cytotoxicity and internucleosomal cleavage associated with apoptosis (Park et al. 2015). Morin treatment to HepG2 cells induced nucleosomal DNA fragmentation and promoted apoptosis via Mst1-JNK-caspase-mediated mechanism (Perumal et al. 2017b). In a study conducted by Zhang et al. (2018), morin treatment led to ROS generation, G2/M cell cycle arrest, and induced apoptosis to HeLa cells (Zhang et al. 2018). In another study, morin suppressed miR-188-5p and thereby induced apoptosis of chronic myeloid leukemia cells (Nie et al. 2019). In colorectal cancer cells (SW480), morin treatment-induced apoptosis was associated with ROS generation and loss of mitochondrial membrane potential (mmp) (Sithara et al. 2017).

Epigallocatechin-3-Gallate

Tea (Camellia sinensis) is the most widely consumed beverage in the world and composed of many bioactive components, such as polyphenols and alkaloids. Major polyphenols found in tea are epigallocatechin-3-gallate (EGCG), epigallocatechin, epicatechin, etc. Green tea polyphenols, including EGCG, are reported for anti-cancer potential mainly due to its antioxidant, antiproliferative, anti-angiogenic, anti-metastatic, and pro-apoptotic activities (Lambert and Elias 2010). A study by Manohar et al. (2013) demonstrated the anti-cancer effect of EGCG in human endometrial cancer cells, where EGCG treatment potentiated apoptosis via increased ROS and activation of p38 MAPK (Manohar et al. 2013). Combinational treatment of EGCG and metformin to non-small cell lung cancer cells (NSCLC) increased ROS production, suppressed Nrf2/HO-1 signaling, and induced apoptosis (Yu et al. 2017). EGCG treatment to HepG2 cells induced oxidative stress via increased ROS and mitochondrial-mediated apoptosis (Khiewkamrop et al. 2018). Velavan et al. (2018) compared the cellular and molecular effects of EGCG and nano-EGCG in lung cancer cells (A549). Both EGCG and nano-EGCG induced cytotoxicity of A549 cells and increased ROS levels leading to loss of mmp and apoptosis (Velavan et al. 2018). In NSCLC, EGCG treatment exerted anti-cancer effect mediated by increased ROS production and downregulation of ERK1/2 expression (Chen et al. 2020).

Genistein

Genistein (5, 7, 4-trihydroxyisoflavone) is an isoflavonoid commonly found in soybeans and legumes. In a study conducted by Salti et al. (2000), genistein treatment to colon cancer cells (HT-29, SW-620, and SW-116) induced G2/M phase arrest, apoptosis, and topoisomerase II-mediated DNA cleavage (Salti et al. 2000). Genistein (100µM) treatment reduced cell proliferation and induced apoptosis mediated by decreased survivin expression in MCF-7 cells (Prietsch et al. 2014). In pancreatic cells (Mia-PaCa2 and PANC-1), genistein treatment increased ROS production and reduced mmp, thereby inducing apoptosis (Bi et al. 2018). Genistein treatment suppressed phosphorylation of PI3K/Akt/NF-κB and improved the effect of centchroman in breast cancer cells by increasing ROS levels and potentiating apoptosis (Kaushik et al. 2019). Recently, Hsieh et al. (2020) studied the anti-cancer effect of genistein in head and neck cancer cells and found genistein treatment induced oxidative stress and apoptosis of tumor cells via the upregulation of miR-34a (Hsieh et al. 2020).

Daidzein

Daidzein (7, 4'-dihydroxyisoflavone) is an isoflavone commonly found in soybeans and legumes. Genistein and daidzein are structurally similar; the only difference is the hydroxyl group in the fifth position of genistein. Daidzein reported for various pharmacological activities such as antidiabetic, antifibrotic, antiproliferative, anti-cancer, etc. Daidzein treatment induced apoptosis in MCF-7 cells by the intrinsic mitochondrial

pathway of cell death. Daidzein treatment caused ROS generation leading to the destruction of mmp, upregulation of Bax, and downregulation of Bcl-2 resulting in the release of cytochrome C, thereby activating caspases and finally apoptosis (Jin et al. 2010). A similar effect was also observed in hepatic cancer cells (SK-HEP-1) upon daidzein treatment, where daidzein induced apoptosis via the upregulation of Bak, downregulation of Bcl-2 and Bcl-xL proteins releasing cytochrome C, and activation of caspases (Park et al. 2013). Daidzein, along with genistein, induced apoptosis in HT-29 colon cancer cells by inhibiting the accumulation of lipid droplets. Daidzein decreased the expression of PI3K and increased the expression of FOXO3a and caspase-8 (Liang et al. 2018). Recently, Kumar and Chauhan (2021) showed daidzein (50μm and 100μm) treatment to MCF-7 cells resulted in ROS-mediated apoptosis and inhibition of cell growth (Kumar and Chauhan 2021).

Apigenin

Apigenin (5, 7, 4-trihydroxyflavone) is a dietary flavone present in spinach, garlic, celery, vine, carrot, etc. The cytotoxic effect of apigenin against hematological cancers was studied using primary effusion lymphoma (PEL) and found that apigenin activated p53, induced catalase, and inhibited STAT3 leading to apoptosis (Granato et al. 2017). Souza et al. (2017) demonstrated the anti-cancer effect of apigenin against human cervical cancer cells, where apigenin treatment affected the cell viability and induced mitochondrial redox impairment and apoptosis of cervical cancer cells (Souza et al. 2017). In another study, the anti-cancer effect of apigenin was studied using two human malignant mesothelioma cell lines (MSTO-211H and H-2452). Treatment of apigenin exerted a cytotoxicity in MSTO-211H and H-2452 cells mediated by ROS-dependent necroptotic cell death (Lee et al. 2020). The pro-apoptotic effect of apigenin was also studied using glioblastoma cells (U87) and found that apigenin treatment potentiated apoptosis via increased ROS generation (Shendge et al. 2021). Recently, Adham et al. (2021) studied the anti-cancer and pro-apoptotic potentials of apigenin against multiple myeloma cells. In this study, apigenin treatment inhibited cell growth and induced apoptosis and cell cycle arrest in NCI-H929 cells (Adham et al. 2021). A combinational treatment of metformin and apigenin to pancreatic cancer cells (AsPC-1) increased ROS levels and thereby decreased MP and potentiated apoptosis. Also, metformin and apigenin markedly reduced the tumor size in AsPC-1-xenografted nude mice when orally administered (Warkad et al. 2021).

Luteolin

Luteolin (3', 4', 5, 7-tetrahydroxyflavone) naturally occurs in various fruits and vegetables such as celery, carrot, cabbage, thyme, and green peppers with potent antioxidant, antimicrobial, anti-inflammatory, anti-cancer, and neuroprotective properties. In rat hepatoma cells (H4IIE), luteolin exhibited cytotoxicity by induction of apoptosis. Luteolin treatment caused nuclear fragmentation and activation of caspases, thereby triggering mitochondrial pathways of apoptosis (Michels et al. 2005). In another study,

luteolin reduced the cell viability of oral squamous cancer cells and downregulated expression of anti-apoptotic proteins, promoted expression of caspases, and increased level of cleaved poly-ADP-ribose polymerase (Yang et al. 2008). Administration of luteolin (1.2 mg/kg body weight/day) to azoxymethane-induced colorectal cancer bearing rats exhibited anti-cancer effects by modulation of GST-α, μ, and Nrf2 expression (Pandurangan et al. 2014). In human glioblastoma cells (U251MG and U87MG), the underlying mechanism of pro-apoptotic effect of luteolin was investigated. Treatment of luteolin increased intracellular ROS and induced apoptosis mediated by activation of ER stress (Wang et al. 2017). Seydi et al. (2018) studied the anti-cancer property of luteolin in diethylnitrosamine-induced hepatocellular carcinoma (HCC) in a rat model. Luteolin treatment resulted in ROS production, thereby inducing mmp loss and apoptosis in the mitochondria from hepatocytes of HCC group (Seydi et al. 2018).

Myricetin

Myricetin (3, 5, 7-trihydroxy-2-(3, 4, 5-trihydroxyphenyl) chromen-4-one) is a dietary flavonoid found majorly in berries, fruits, nuts, vegetables, tea, and wine. Myricetin inhibited the Akt activity, downregulated phosphorylation of Bad protein, and induced loss in mmp and apoptosis in UV-B irradiated skin cancer cells (HaCaT) (Kim et al. 2010). In human colon cancer cells (HCT-15), myricetin caused cytotoxicity and DNA condensation in a dose-dependent manner and promoted apoptosis-inducing mitochondrial factors (Kim et al. 2014). Yang et al. (2017) tested the effect of myricetin in two choriocarcinoma cell lines (JAR and JEG-3) and found that myricetin treatment potentiated apoptosis with enhanced ROS production, glutathione depletion, and loss of mmp (Yang et al. 2017). Treatment of myricetin to triple-negative breast cancer cells exhibited intracellular ROS accumulation, double-strand DNA breaks, ERK1/2 and p38 MAPK activation, mitochondrial membrane destabilization, and apoptosis (Knickle et al. 2018). In another study, myricetin treatment to canine osteosarcoma cells (D-17 and DSN) induced ROS generation, lipid peroxidation, and depolarization of mmp (Park et al. 2018). Recently, Rajendran et al. (2021) demonstrated the anti-cancer effect of myricetin in A549 cells. Myricetin (73μg/ml) treatment prevented A549 cell growth by inducing ROS-mediated apoptosis (Rajendran et al. 2021).

Hesperidin

Hesperidin is a bioflavanone glycoside found majorly in citrus fruits. Hesperidin was shown to possess many pharmacological properties such as antioxidant, antiviral, anti-inflammatory, antiproliferative, anti-cancer, etc. Hesperidin treatment induced apoptosis in HepG2 cells through mitochondrial and death receptor pathways in a dose-dependent manner. Hesperidin-treated cells showed loss of mmp and increased activity of caspases and expression of pro-apoptotic proteins (Banjerdpongchai et al. 2016). A study by Pandey et al. (2019) described the anti-cancer effect of hesperidin using human gall bladder carcinoma cells. Hesperidin treatment increased ROS

production and led to caspase-3 activation and G2/M phase cell cycle arrest in a dose-dependent manner (Pandey et al. 2019). In prostate cancer cells (DU145), hesperidin treatment inhibited the cell growth and triggered apoptosis via ROS pathway (Ning et al. 2020). In a recent study performed with HeLa cells, hesperidin downregulated Jab1 and upregulated p27, thereby potentiating apoptosis via increased ROS production (Pandey et al. 2021).

Conclusion

Dietary flavonoids are potent anticarcinogenic bioactive principles that inhibit cancer development by interfering with cell proliferation, apoptosis, and metastasis. Dietary flavonoids have come out as a safe and effective alternative for cancer treatment by inducing apoptosis. Flavonoids possess various characteristics contributing to their pro-apoptotic effect, such as upregulation of caspases, release of cytochrome C, induction of cell cycle arrest, regulation of Bcl-2 family proteins, suppression of survival signals, etc. To replace anti-cancer drugs with dietary flavonoids, we have to explore more about the mechanism of their anti-cancer activity. Combination therapy of flavonoids with present anti-cancer drugs could significantly reduce the concentration of drugs administered, improve bioavailability, extend resistance to medication, etc.
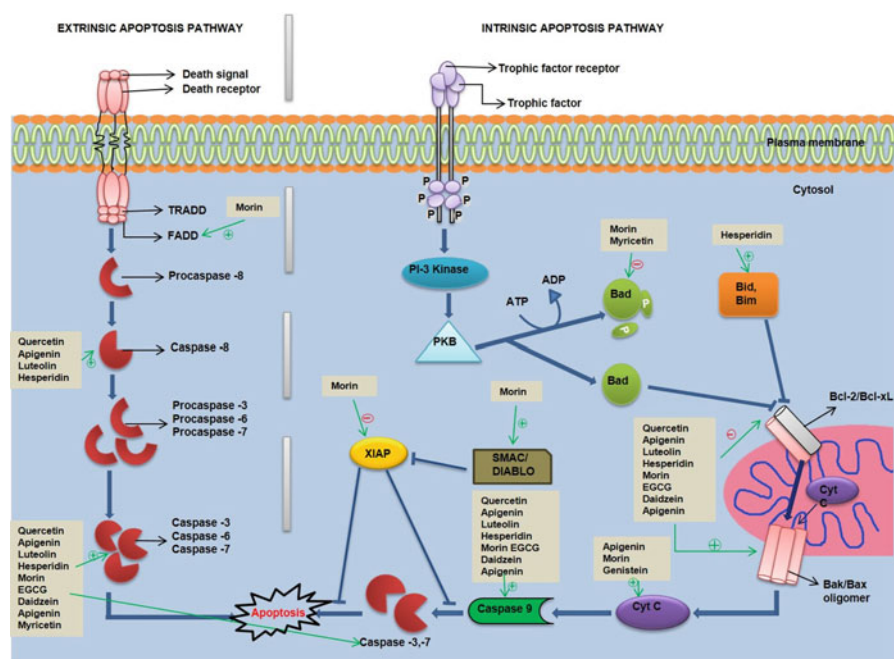
Scheme representation of the mechanism of dietary flavonoid intervention in extrinsic and intrinsic apoptosis pathways

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