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Inhibition of Akt/mTOR Signaling by the Dietary Flavonoid Fisetin

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Abstract

Plants have long been providing mankind with remedies of different ailments. Flavonoids, a family of naturally occurring polyphenolic compounds are ubiquitous in plants. Development of polyphenol-based drugs has not attracted much attention by researchers and drug companies. Therefore, despite extensive studies on polyphenols, this vast group of compounds is underrepresented in clinical medicine. Fisetin (3,7,3',4'-tetrahydroxyflavone) belongs to the flavonol subgroup of flavonoids together with quercetin, myricetin and kaempferol and is found in several fruits and vegetables including strawberries, apples, persimmons and onions. Fisetin is showing promise as a useful natural agent against cancer and has been evaluated for its potential inhibitory role against cancer in several *in vitro* and *in vivo* studies. The Akt/mTOR pathway is known to play a central role in various cellular processes that contribute to the malignant phenotype. Accordingly, inhibition of this signaling cascade has been a focus of recent therapeutic studies. Novel inhibitors of PI3-K, Akt, and mTOR are now passing through early phase clinical trials. Herein, we review the effect of fisetin on the PI3-K/Akt/mTOR pathway as studied in different cancer cell models.

Keywords

Fisetin; Akt; mTOR; lung cancer; prostate cancer; colon cancer; myeloma; melanoma

1. INTRODUCTION

Carcinogenesis is a multistep process involving the transformation, survival, proliferation, invasion, angiogenesis, and metastasis of the tumor and generally takes years to complete [1]. The majority of cancer related deaths are linked to metastasis of the tumor. Once localized cancer metastasizes to other body sites, it is difficult to treat [2]. Therapies are often complicated, intensive, and costly, and typically produce only modest improvement in survival and symptom palliation. In addition, costs associated with the development of new anticancer regimens continue to escalate with each passing year [2]. Thus, novel strategies

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CONFLICT OF INTEREST
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are needed, that can slow or prevent the process of carcinogenesis. Plants have been used for the treatment of various ailments for millennia. It was centuries ago, that Hippocrates, the father of medicine, had stated “Let food be thy medicine and medicine be thy food”. Exploring the association between diet and health continues today. There is a considerable body of evidence that a plant-based diet can be effective against various diseases including cancer [3].

Flavonoids, a family of naturally occurring polyphenolic compounds are ubiquitous in plants; almost all plant tissues are able to synthesize flavonoids. Studies show that flavonoids can modulate various signaling pathways and thus affect the proliferation, progression and metastasis of the cancer cells [4]. More than 8000 compounds with the flavonoid structure have been identified till date. This large number of compounds arises from the various combinations of multiple hydroxyl and methoxyl group substituents on the basic flavonoid skeleton [5]. Although the intake varies widely it has been estimated that the average total intake of flavonoids in the United States is about 1 g/day per individual [4].

Flavonoids are further subdivided into various sub-classes which include chalcones, flavones, flavonols, flavanones, flavanols, anthocyanins and isoflavones (Fig. 1) [6]. Fisetin (3,7,3’,4’-tetrahydroxyflavone) (Fig. 2) belongs to the flavonol subgroup of flavonoids together with quercetin, myricetin and kaempferol and is found in several fruits and vegetables including strawberries, apples, persimmons and onions [7]. Fisetin was originally identified in a screen for compounds that could prevent oxidative stress-induced nerve cell death [8]. Further studies showed that fisetin also possessed neurotrophic activity, promoting nerve cell differentiation *via* activation of the RAS-ERK MAPKinase (MAPK) cascade [9]. Oral administration of fisetin was shown to enhance learning and memory in mice [10]. In addition, it has anti-inflammatory activity and has been shown to inhibit the activity of 5-lipoxygenase in microglia cells, thereby reducing the production of lipid peroxides and their pro-inflammatory by-products [11].

2. ANTI-PROLIFERATIVE EFFECT OF FISETIN

Emerging data indicate that fisetin possesses potent anti-proliferative activity against various cancer cells [12]. Twenty four flavonoids were compared for their cytotoxicity on cancer cells and their effect on the morphology of endothelial cells so as to predict the antiangiogenic activity of these compounds. Ten flavonoids including fisetin had an IC_{50} below 50 μM (rhamnetin, 3’,4’-dihydroxyflavone, luteolin, 3-hydroxyflavone, acacetin, apigenin, quercetin, baicalein, fisetin, and galangin) [13]. Important structure activity relationship for cytotoxicity included the C2-C3 double bond and 3’,4’-dihydroxylation. Of the several flavonoids screened only fisetin, quercetin, kaempferol, apigenin, and morin could induce the formation of cell extensions and filopodias at noncytotoxic concentrations [13]. The structure activity relationship for morphologic activity differed from cytotoxicity, and involved hydroxylation at C-7 and C-4’. Fisetin, the most active agent, presented cell morphology that was distinct compared to colchicine, combretastatin A-4, docetaxel, and cytochalasin D. In addition, resistance to cold depolymerization and a significant increase in acetylated alpha-tubulin demonstrated that fisetin was a microtubule stabilizer [13].

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Another study reported that fisetin perturbed spindle checkpoint signaling, which may contribute to the antiproliferative effects of the compound [14]. Using a cell-based high-throughput screen for small molecules that could override chemically induced mitotic arrest, fisetin was identified as an antimitotic compound. It was demonstrated that fisetin rapidly compromised microtubule drug-induced mitotic block in a proteasome-dependent manner in several human cell lines. Moreover, in unperturbed human cancer cells, fisetin caused premature initiation of chromosome segregation and exit from mitosis without normal cytokinesis [14]. To understand the molecular mechanism(s) behind these mitotic errors, the consequences of fisetin treatment on the localization and phosphorylation of several mitotic proteins were analyzed. Aurora B, Bub1, BubR1 and Cenp-F rapidly lost their kinetochore/centromere localization upon addition of fisetin to the culture medium. The forced mitotic exit, failure of cytokinesis and decreased viability observed in cells treated with fisetin were linked to the significantly reduced activity of Aurora B kinase, which has been identified as a novel direct target of fisetin [14].

Cyclin-dependent kinases (Cdks) play a central role in cell cycle control, apoptosis, transcription, and neuronal functions [15]. They are important targets for the design of drugs with antimitotic or antineurodegenerative effects. Cdk-4 and Cdk-6 form a subfamily among the Cdks in mammalian cells, as defined by sequence similarities. Fisetin binds to the active form of Cdk-6, forming hydrogen bonds with the side chains of residues in the binding pocket that undergo large conformational changes during Cdk activation through interactions with cyclins. The 4-keto group and the 3-hydroxyl group of fisetin are hydrogen bonded with the backbone in the hinge region between the N-terminal and C-terminal kinase domain [14]. Fisetin has been co-crystallized with Cdk-6 and shown to inhibit its activity, however this inhibition is not sufficient to explain various activities assigned to this flavonoid. A recent study showed that fisetin mediates its anti-proliferative and anti-inflammatory effects, in part, through modulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways [12]. Of the nine different flavones tested, fisetin was the most potent in suppressing tumor necrosis factor (TNF)-induced NF-κB activation. Fisetin also suppressed NF-κB activation induced by various inflammatory agents and carcinogens, and blocked the phosphorylation and degradation of IκBα, which in turn led to suppression of the phosphorylation and nuclear translocation of NF-κB/p65 [16].

3. FISETIN AND OXIDATIVE STRESS

Fisetin not only has direct antioxidant activity but can also increase the intracellular levels of glutathione, the major intracellular antioxidant. Fisetin can maintain mitochondrial function in the presence of oxidative stress [17]. A study performed in human endothelial cells suggested that induction of Heme Oygenase-1 expression *via* NF-E2-related factor 2 activation may contribute to the cytoprotection exerted by fisetin against oxidative stress [18]. Fisetin induces transcription of phase II enzyme NADPH:quinone oxidoreductase through an antioxidant responsive element-involved activation. It is thought that this effect of fisetin can reduce carcinogen-induced mutagenesis and tumor formation [19].

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4. FISETIN AND CANCER

Fisetin has been reported to induce apoptosis in a number of cancer cell lines including promyeloleukemic and hepatocellular carcinoma cells through activation of the caspase 3 cascade [12]. Inhibition of urokinase-type plasminogen activator by fisetin in the advancing capillary vessels surrounding the tumor may be responsible for reducing angiogenesis and consequently tumor growth [20]. Thus, in addition to its neurotrophic activity, it is a potential anti-cancer agent with anti-proliferative, pro-apoptotic activities. Several cell signaling pathways have been identified as targets of fisetin and modulation of these are thought to be responsible for its inhibitory effects on cancer growth and progression. These include the NF-κB, MAPK, Wnt, Akt and mammalian target of rapamycin (mTOR) pathways that are known to influence cell survival and proliferation.

4.1. Akt/ mTOR Signaling in Cancer

Phosphoinositide 3-kinases (PI3-K) are important intracellular signal-transducing enzymes, which phosphorylate the 3- hydroxyl group of the inositol ring of phosphatidylinositol to generate PtdIns(3)P, PtdIns(3,4)P2, and PtdIns(3,4,5)P3 [21]. These phospholipids interact with Akt and cause its translocation to the inner membrane, where Akt is phosphorylated and activated [21]. Active Akt then phosphorylates and regulates multiple target proteins, implicated in the regulation of apoptosis, DNA repair, metabolism, protein synthesis and cell division [22]. Full activation of Akt requires dual phosphorylation at the serine and threonine residues. The mTOR-Rictor complex, and the kinases ILK-1 and DNA-PK phosphorylate Akt on Ser^{473}, while the kinase PDK1 phosphorylates it on Thr^{308} [23]. Furthermore, Akt stimulates angiogenesis and induces epithelial–mesenchymal transition characterized by morphological changes, activation of metallo-proteinases, loss of cell-cell adhesion and increased cell migration and invasion [22].

mTOR, an atypical serine/threonine protein kinase that belongs to the PI3-K-related kinase family, shares an evolutionarily related kinase domain with PI3-K lipid kinase. mTOR interacts with several proteins to form two distinct complexes named mTOR complex (mTORC) 1 and 2 [24]. The mTORC1 has six while the mTORC2 contains seven known protein components and share the catalytic mTOR subunit, mLST8/GβL, DEPTOR, and the Tti1/Tel2 complex. Raptor and PRAS40 are specific to mTORC1, whereas Rictor, mSin1 and protor 1 and 2 are part of mTORC2 [24]. Oncogenic activation of mTOR signaling induces several processes required for cancer cell growth, survival, and proliferation. It is increasingly being appreciated that deregulation of protein synthesis downstream of mTORC1 at the level of eukaryotic translation initiation factor 4E-binding protein-1 (4E-BP-1)/eukaryotic translation initiation factor 4-E (eIF4-E) has a crucial role in tumor formation. Loss of 4E-BP-1/2 and concomitant activation of cap-dependent translation promotes cell-cycle progression and cell proliferation. In addition, the 4E-BP-1/eIF4-E axis mediates the effects of oncogenic Akt signaling on mRNA translation, cell growth, and tumor progression [24]. Thus, the PI3-K/Akt activity is kept under tight control through feedback loops from mTOR and other pathways. One consequence of mTOR inhibition is alleviation of this negative feedback loop resulting in activation of the PI3-K/Akt signaling. As a result of their similar ATP sites, the prototypical PI3-K inhibitors LY294002 and

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wortmannin inhibit both mTOR and PI3-K kinases, although the compounds have been primarily thought of as inhibitors of PI3-Ks [25]. The similarity between the catalytic domains of mTOR and class I PI3-K has also allowed for the development of compounds that simultaneously inhibit both kinases and decrease the phosphorylation of Akt, S6K1, and 4E-BP-1. In this context, phase I clinical trials with the dual PI3-K/mTOR inhibitor NVP-BEZ235 (Novartis) or XL-765 (Exelixis) are being explored in patients with various types of tumors [25].

PTEN plays an important role in multiple cellular functions such as cell metabolism, proliferation and survival. It is one of the most frequently mutated tumor suppressor gene in human sporadic cancers, as reduced PTEN protein expression occurs in approximately half of all tumors [26]. The major substrate of the lipid phosphatase activity of PTEN is PtdIns(3,4,5)P3, an important intracellular second messenger. By dephosphorylating the D3-position of PtdIns(3,4,5)P3, PTEN negatively regulates the PI3-K pathway and Akt activation and thus suppresses tumorigenesis [26].

4.2. Akt/mTOR Signaling and Fisetin

A brief summary of the relevant findings on the effects of fisetin on Akt/mTOR signaling in various cancers is provided in Table 1.

4.2.1. Lung Cancer—Lung cancer is a leading cause of cancer mortality worldwide [27]. A plethora of genetic and molecular alterations have been reported, including autocrine signaling loops, oncogene activation and loss of tumor-suppressor genes. Three important intracellular signaling proteins, the mTOR, Akt and MAPKs have emerged as attractive targets for lung cancer therapy [28]. We examined the effect of fisetin in lung cancer and showed that fisetin was able to inhibit PI3-K/Akt and mTOR signaling in human lung cancer cells. Treatment of A549 and H1792 human lung cancer cells with fisetin caused decrease in cell viability and clonogenecity but had minimal effects on normal bronchial cells [29]. Furthermore, treatment with fisetin resulted in significant increase in the protein levels of PTEN and decreased the expression of regulatory (p85) and catalytic (p110) subunits of PI3-K in A549 lung cancer cells [29]. Fisetin also caused inhibition in the phosphorylation of Akt at both Ser^{473} and Thr^{308} in A549 cells with activation of the tumor suppressor complex (TSC) 1 and 2, suppression of Akt mediated phosphorylation of TSC-2, and decrease in the phosphorylation and activation of the mTOR kinase [29].

Using *in silico* modeling, we showed that fisetin physically interacted with the mTOR complex at two sites [29]. The binding energies were in the -7 to -8 kcal/mol range for the binding constant. The binding in the best site included hydrogen bonding to a glutamate by two hydroxyl groups. The second site was mostly hydrophobic, with the ring of fisetin stacking on rings from the peptide [29]. Fisetin caused upregulation of AMP activated protein kinase (AMPK), a member of the protein kinase family which plays an essential role as a cellular energy sensor in nutrient deprived conditions [29]. Studies show that activation of AMPK suppresses mTOR signaling and is associated with inhibition of cancer cell growth [30]. Fisetin-mediated decrease in mTOR phosphorylation was accompanied with decrease in the expressions of Raptor, Rictor, PRAS40 and GβL and inhibition of mTOR

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complexes in lung cancer cells [29]. In addition downstream targets of mTOR including p70S6K1, eIF4-E and 4E-BP-1 involved in controlling ribosome protein synthesis, cell survival and proliferation were significantly downregulated with fisetin treatment [29]. These observations clearly suggest that fisetin mediated growth inhibition of lung cancer cells is mediated, in part, through inhibition of the Akt/mTOR signaling.

4.2.2. Prostate Cancer—Prostate cancer is one of the most important medical problems facing the male population, especially over the age of 50, with additional factors such as age, race and familial history amplifying the risk of disease [31]. Remarkably, supplementation of various nutrients from natural sources to the diet can provide benefit in the prevention and therapy of this deadly disease [12]. The National Cancer Institute recommends the ingestion of at least five servings daily of fruits and vegetables to protect against the development and progression of prostate cancer [32]. Dietary agents such as silymarin, genistein, and epigallocatechin 3-gallate are being studied for their growth inhibitory effect on prostate cancer cells [12]. In this context, data from our laboratory and others has unequivocally established that fisetin possesses potent anti-proliferative activity against prostate cancer cells. In our initial studies, we demonstrated that fisetin inhibited human prostate cancer cell growth in *in vitro* and *in vivo* mouse model [33, 34]. Treatment of LnCaP, CWR22Rν1 and PC-3 prostate cancer cells with fisetin resulted in decrease in cell viability but had minimal effect on normal prostate epithelial cells [33]. Fisetin induced apoptosis, in a caspase-dependent manner, accompanied by arrest of LnCaP cells in the G0-G1 phase of the cell cycle [33]. Furthermore, administration of fisetin to athymic nude mice implanted with AR-positive CWR22 Rν1 cancer cells resulted in inhibition of tumor growth and reduction in serum prostate-specific antigen (PSA) levels [34].

Cell culture studies showed that fisetin competed with, and had higher affinity than the natural ligand dihydrotestosterone for the androgen receptor and physically interacted with its ligand-binding domain causing reduction in receptor stability. This resulted in decreased interaction between the amino- and carboxyl-terminal ends of the receptor with subsequent blunting of androgen receptor-mediated transactivation of target genes including PSA [34].

We found that treatment of LNCaP prostate cancer cells with fisetin caused decrease in the protein expression of PI3-K (p85) and phosphorylation of Akt at both Thr^{308} and Ser^{473} [33]. Studies show that cell survival is influenced by Akt through a variety of effector proteins including inhibition of the pro-apoptotic Bcl-2 family member Bad and the forkhead family of transcription factors that normally activate apoptosis-related genes [35]. In our study, silencing of Akt caused increase in the protein expressions of pro-apoptotic Bad and Bax and decrease in anti-apoptotic Bcl-2 and Bcl-xL, which was further augmented on treatment with fisetin [33]. These findings were consistent with other studies demonstrating that the growth inhibitory effects of fisetin are mediated in part through suppression of Akt signaling [36]. However, further studies are needed to delineate whether fisetin mediated inhibition of Akt-induced cell survival in prostate cancer cells is androgen dependent or if fisetin modulates these pathways independent of each other.

An early response of the cellular metabolic adjustments to nutrient starvation, stress, or reduced availability of growth factors involves inhibition of growth and induction of

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autophagy [37]. It is now being recognized that autophagy is not only a survival response to growth factor or nutrient deprivation, but also an important mechanism for tumor cell suicide. Inhibition of cytoprotective autophagy by genetic or pharmacological means has been shown to enhance anticancer drug-induced cell death. In contrast, autophagy may protect against tumorigenesis through limiting necrosis and chronic inflammation [38]. Among the numerous components involved in the regulation of autophagy, mTOR is a key component that coordinates the cellular balance between growth and autophagy in response to physiological conditions and environmental stress [37]. Treatment with fisetin for 72 h resulted in induction of cytotoxic autophagy in androgen-independent, PTEN-negative human prostate cancer PC-3 cells [39]. We showed that fisetin inhibited mTOR signaling pathway in PC-3 prostate cancer cells and downregulated Raptor, Rictor, PRAS40 and GβL with subsequent decrease in the formation of mTORC1 and mTORC2. Fisetin mediated inhibition of the mTOR signaling pathway was associated with decrease in the phosphorylation and activation of downstream kinase p70-S6K and inhibition of the ribosomal protein S6 and eukaryotic translation initiation factor eIF4-B. Furthermore, fisetin converted 4E-BP-1 from its hyperphosphorylated γ form to the hypo- or non-phosphorylated α form, which permits 4E-BP-1 to sequester eIF4-E resulting in reduced levels of Cap-dependent translation [39]. A potential mechanism of resistance to mTORC1 inhibitors, observed in clinical trials, occurs through a negative feedback loop in which mTORC2 mediated activation of Akt stimulates mTORC1 activity. We found that fisetin mediated inhibition of both mTORC1 and mTORC2 kept the feedback loop in check with subsequent suppression of Akt signaling [39].

In addition to its growth inhibitory, pro-apototic effects, inhibition of Akt signaling has been associated with decreased migration, invasion and metastasis observed in fisetin treated cells. Chien *et al* have linked the anti-invasive effect of fisetin to the suppression of Akt/ c-Jun N-terminal kinase (JNK) signaling in PC-3 cells [36]. Fisetin suppressed the phosphorylation of JNK1/2 and Akt and decreased the nuclear translocation and activation of NF-κB and AP-1 transcription factors. The study showed that fisetin inhibited the metastatic ability of PC-3 by reducing matrix metalloproteinase (-2 and -9) expressions through suppressing PI3-K/Akt and JNK signaling pathways [36].

4.2.3. Myeloma—Multiple myeloma, a neoplasm of plasma cells, accounts for approximately 15% of lymphatohematopoietic cancers. A limited number of studies have examined the effect of polyphenols such as EGCG, genistein, butein in myeloma cells [40]. Jang *et al* showed that fisetin elicited cytotoxicity in multiple myeloma U266 cells, manifested by an increased fraction of the cells with sub-G1 content. Fisetin treatment resulted in caspase-3 activation, associated with downregulation of anti-apoptotic Bcl-2 and Mcl-1(L), and upregulation of pro-apoptotic Bax, Bim and Bad proteins [41]. Fisetin activated AMPK as well as its substrate acetyl-CoA carboxylase in these cells, and decreased the phosphorylation of AKT and mTOR. The study demonstrated that activation of AMPK, inhibition of AKT/mTOR pathways in conjunction with generation of reactive oxygen species was responsible for fisetin-induced apoptosis observed in U266 cells [41].

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4.2.4. Colon Cancer—Fisetin was investigated for its potential radiosensitizing effect in human colorectal cancer cells [42]. It was shown that fisetin pretreatment enhanced the radiosensitivity of chemo-resistant p53-mutant HT-29 human colorectal cancer cells with subsequent increase in radiation-induced cell growth arrest in the G2-M phase, and augmentation of radiation-induced caspase-dependent apoptosis [42]. As a read-out of repressed proliferation, phosphorylation of Akt and ERK1/2 MAPK, involved in cell proliferation and anti-apoptotic pathways, were inhibited in cells treated with fisetin in combination with irradiation. It was suggested that the suppression of fisetin-induced phosphorylation of AKT and ERK1/2 after irradiation may be the consequence of the shutdown of survival signals caused by apoptosis in HT-29 cells with serious DNA damage [42].

4.2.5. Melanoma—We have examined the effect of fisetin on melanoma growth and progression [43]. In our ongoing studies we employed a three dimensional human skin equivalent melanoma model comprising of A375 melanoma cells, cultured with epidermal keratinocytes and dermal fibroblasts to study the role of Akt/mTOR inhibition on melanoma progression from radial to vertical growth phase [44]. The melanoma reconstructs were treated with fisetin every alternate day for 16 days and cross-sections were taken at four days interval. We found that fisetin-treated reconstructs exhibited significantly less melanocytic lesions when compared to untreated control. This was associated with decreased phosphorylation of Akt and mTOR kinases in fisetin-treated tissue sections [44]. These observations further validated our previous data that shows that fisetin mediated growth in cancer cells inhibition is associated with suppression of Akt/mTOR signaling axis.

5. CONCLUSION

The interactions between signaling pathways endows a cell with greater capabilities for signal processing and decoding, and enables it to adapt easily to a number of adverse conditions, thereby propagating growth and survival. Anti-cancer agents that modulate multiple pathways are preferred as they can better target cross-talks leading to activation of compensatory signaling, which allow cancer cells to evade apoptosis. Thus, combined inhibition of PI3-K/Akt and mTOR pathways seems to be a more efficient way of suppressing cancer cell growth and viability than targeting the components of each pathway alone. In this context, the emergence of data that establishes fisetin as an effective dual inhibitor of mTOR and PI3-K/Akt signaling pathways is very exciting. The development of a naturally occurring agent that can selectively modulate the activity of two key signaling pathways associated with growth and progression of cancer represents a possible avenue to inhibit the process of carcinogenesis in a more specific fashion. Synergistic targeting with specific inhibitors may have a therapeutic potential in malignancies with constitutive activation of these pathways. Studies done so far have not shown any toxicity associated with the administration of the compound. However, detailed research in *in vitro*, and preclinical models, are needed to understand the precise mechanism(s) through which fisetin functions to inhibit the growth and viability of cancer cells through modulation of these pathways. It remains to be determined how broadly useful these targets will be in the clinical setting.

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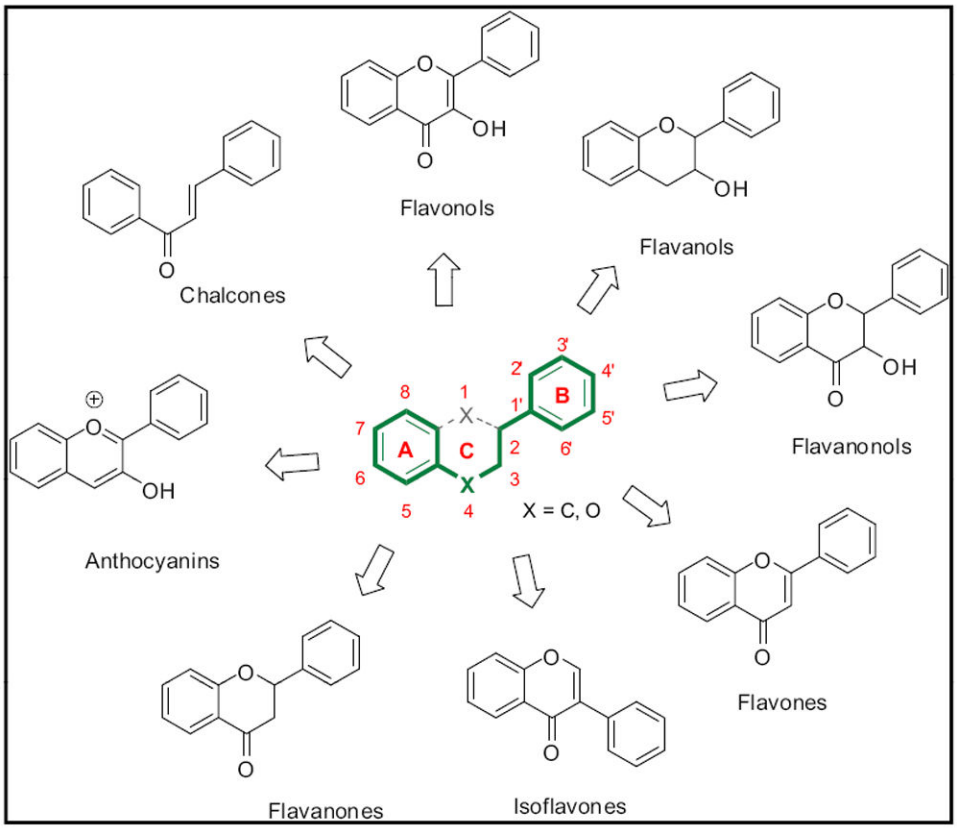

Fig. (1).
Sub-classes of flavonoids: Center depicts the basic structure of flavonoid.

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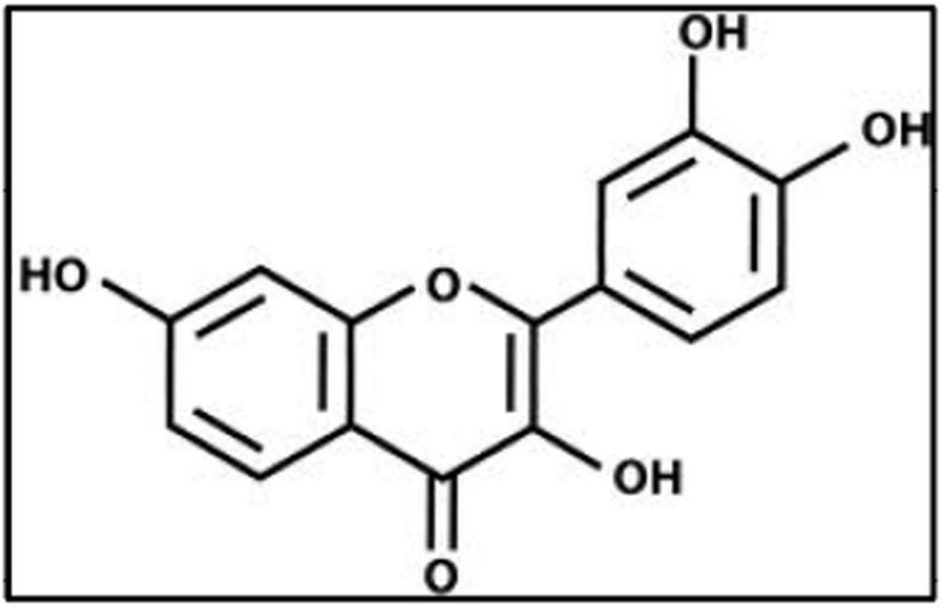

Fig. (2).
Structure of fisetin.

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Table 1

Summary of the Inhibitory Effects of Fisetin on Akt/mTOR Signaling

Organ	Study Model	Target/Mechanism(s)	Reference
Lung			
	Monolayer cell cultures	↓ cancer cell viability & clonogenecity	[29]
		↑ PTEN protein levels	
		↓ PI3-K (p85 & p110) expression	
		↓ Akt phosphorylation at Ser^{473} & Thr^{308}	
		Activates TSC	
		↓ phosphorylation & activation of mTOR	
		↓ phosphorylation of p70S6K1, eIF-4E and 4E-BP1	
		Inhibits constituents of mTORC such as Rictor, Raptor, GβL and PRAS40	
		Activates AMPK	
Prostate			
	Monolayer cell cultures	↓ cancer cell viability	[33]
		Induces apoptosis; ↑ Bax/Bcl-2 ratio	
		Induces cell cycle arrest in G1 phase	
		↓ cyclins D1, D2 and E	
		↓ cdks 2, 4	
		↑ WAF1/p21 and KIP1/p27	
		Competes with DHT and interacts with AR	[34]
		Blunts transactivation of target gene PSA	
		↓ PI3-K (p85) expression	[33]
		↓ Akt phosphorylation at Ser^{473} & Thr^{308}	
		Induces autophagy in cancer cells	[39]
		↓ phosphorylation & activation of mTOR	
		Inhibits mTORC formation	
		↓ phosphorylation of p70S6K1, eIF-4E and 4E-BP1	
		↓ Rictor, Raptor, GβL and PRAS40	
		↓ Akt phosphorylation	
		Inhibits cancer cell growth	[36]
		Induces apoptosis	
		Inhibits NFκB signaling	
		Inhibits PI3-K/JNK signaling	
		↓ migration, invasion through MMP suppression	
	Athymic nude mice	Inhibits tumor growth & multiplicity	[34]
		↓ serum PSA levels	
Blood			

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Organ	Study Model	Target/Mechanism(s)	Reference
	Monolayer cell cultures	Induces apoptosis ↓ Bcl-2 and Mcl-1(L) ↑ Bax, Bim and Bad Activates AMPK Inhibits AKT/mTOR signaling	[41]
Colon			
	Monolayer cell cultures	↑ radiosensitivity of p53-mutant cancer cells ↑ growth arrest ↑ apoptosis ↓ phosphorylation of Akt & ERK1/2	[42]
Skin			
	Monolayer cell cultures	Inhibits melanoma cell growth and viability ↓ phosphorylation of Akt, mTOR & p70S6K	[43][37]
	3-D constructs	↓ phosphorylation of Akt, mTOR & p70S6K	[44]