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Review article:

Fisetin: A bioactive phytochemical with potential for cancer prevention and pharmacotherapy

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Running Title: Anticancer properties of fisetin

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Abstract:

A wide variety of chronic diseases, such as neurodegenerative and cardiovascular disorders, diabetes mellitus, osteoarthtitis, obesity and various cancers, are now being treated with cost effective phytomedicines. Since synthetic medicines are very expensive, concerted efforts are being made in developing and poor countries to discover cost effective medicines for the treatment of non-communicable diseases (NCDs). Understanding the underlying mechanisms of bioactive medicines from natural sources would not only open incipient avenues for the scientific community and pharmaceutical industry to discover new drug molecules for the therapy of NCDs, but also help to garner knowledge for alternative therapeutic approaches for the management of chronic diseases. Fisetin is a polyphenolic molecule of flavonoids class, and belongs to the bioactive phytochemicals that have potential to block multiple signaling pathways associated with NCDs such as cell division, angiogenesis, metastasis, oxidative stress, and inflammation. The emerging evidence suggests that fisetin may be useful for the prevention and management of several types of human malignancies. Efforts are being made to enhance the bioavailability of fisetin after oral administration to prevent and/or treat cancer of the liver, breast, ovary and other organs. The intent of this review is to highlight the *in vitro* and *in vivo* activities of fisetin and to provide up-to-date information about the molecular interactions of fisetin with its cellular targets involved in cancer initiation, promotion and progression as well as to focus on strategies underway to increase the bioavailability and reduce the risk of deleterious effects, if any, associated with fisetin administration.

Keywords: Fisetin, biosynthesis, anticancer properties, bioavailability, adverse effects.

1. Introduction

Despite longstanding efforts in the development of anticancer chemotherapeutic drugs, some cancers still remain incurable and kill millions of people each year all over the world [1]. Prevention of this devastating disorder is certainly more convenient and prudent approach than elusive cancer cure. Therefore, strategies to reduce the incidence and risk of cancer through education will be even more important for primary health care in the future. Unfortunately, according to the International Agency for Research on Cancer, the incidence of cancer cases is projected to increase with the number of new cases expected to rise to 25 million over the next two decades, compared to 14.1 million in 2012 [1]. Consumption antioxidant-containing fruits and vegetables has been associated with decreased susceptibility to different NCDs, including malignant neoplasms [2]. However, the exact mechanisms of action of anticancer bioactive compounds derived from plants still remain to be established [3–5]. Fisetin (3,7,3′,4′-tetrahydroxyflavone) is one of the major polyphenolic flavonoids found in various fruits and vegetables such as apples, grapes, persimmons, strawberries, cucumbers, and onions [6-11]. The levels of this natural flavonol products range from 2 μg/g to 160 μg/g in different fruits and vegetables and the average daily intake of fisetin is estimated to be around 0.4 mg in humans [2]. It has been reported that fisetin can exert numerous beneficial biological activities, including antioxidant, anti-inflammatory, antiangiogenic, hypolipidemic, neuroprotective, and antitumor effects [12-17]. Fisetin can block multiple signaling pathways such as the phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) and p38, mitogen-activated protein kinases (MAPK)-dependent nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling which playing a central role in various cellular processes contributing to the malignancy [16-19]. In this review article, we have focused on the biochemical activities

of fisetin in various cancer cell lines and a few available *in-vivo* model systems used for investigating its action at the molecular level. We have also pondered to highlight the potential anticancer properties of this promising molecule as a possible novel compound useful either alone as anticancer substance or as an adjuvant agent in combination with radiation therapy and conventional chemotherapeutic drugs used in treating cancer patients.

2. Biosynthesis and chemistry of fisetin

Fisetin is a bioactive flavonol and has diphenylpropane structure (table 1) which contains two aromatic rings linked through a three carbons-oxygenated heterocyclic ring, which is supplemented with four hydroxyl group substitutions and one oxo group [20,21] (Fig. 1). It is naturally synthesized in strawberries, apples, persimmons, onions, and cucumbers, and also serves as a coloring agent in plants [22]. Fisetin also found in various acacia trees and shrubs (table 2) belonging to *Fabaceae* family, such as *Acacia greggii*, *Acacia berlandieri* and *Gleditschia triacanthow*; *Anacardiaceae* family members, such as the parrot tree (*Butea fronds*) and the honey locust (*Gleditsia triacanthos*); *Quebracho colorado*, *Rhus cotinus*, lac tree (*Rhus vemiciflua* Stokes), smoke tree (*Cotinus coggygria*) and *Pinopyta* species, such as *Callitropsis nootkatensis* (yellow cypress) [21].

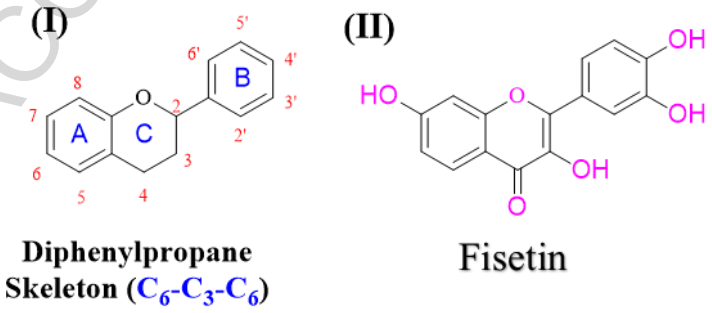

Fig. 1. Chemical structures of diphenylpropane skeleton (I) and fisetin aglycone (II). The basic diphenylpropane skeleton is common in both chemical structures.

Table 1. Physicochemical properties of fisetin.

IUPAC Name	2-(3,4-dihydroxyphenyl)-3,7-dihydroychromen-4-one
Chemical Formula	$C_{15}H_{10}O_6$
CAS Number	528-48-3
Melting Point	330℃
Molar Mass	286.239 g/mol
λ_{max} (ethanol)	252, 320, 360 nm
Solubility	Alcohol, acetone, acetic acid. Solution of fixed alkali hydroxide, DMSO; Practical insoluble in water, ether, benzene, chloroform and petroleum ether

Table 2. Various dietary sources of fisetin and its concentrations measured by dry weight basis method after acidic hydrolysis of parent glycosides into the respective aglycone [22].

Food source	Fisetin conc. (µg/g)
Strawberry (*Fragaria* sp.)	160
Apple (*Malus* sp.)	26.9
Persimmon (*Diospyros* sp.)	10.6
Lotus root (*Nelumbo* sp.)	5.8
Onion (*Allium* sp.)	4.8
Grape (*Vitis* sp.)	3.9
Kiwi (*Actinidia* sp.)	2.0
Peach (*Prunus* sp.)	0.6
Cucumber (*Cucumis* sp.)	0.1
Tomato (*Solanum* sp.)	0.1

The biosynthesis of fisetin in plants remains unknown. Stahlhut et al. [23, 24] have proposed the biosynthetic pathway for fisetin production by using *E. coli* as microbial strain. This procedure as illustrated in Fig. 2 involves conversion of *p*-coumaroyl-CoA (i) and 3-malonyl-CoA (ii) into isoliquiritigenin (iii) using chalcone synthase (CHS), chalcone reductase (CHR) and nicotinamide adenine dinucleotide phosphate (NADPH). The isoliquiritigenin (iii) is converted to liquiritigenin (iv) by chalcone isomerase (CHI) followed by the formation of garbanzol (v) from liquiritigenin (iv) using flavanone 3-hydroxylase (F3H), *a*-ketoglutarate (*aKG*) and O_2. The garbanzol (v) is converted to resokaempferol (vi) by the action of flavonol synthase (FLS), *a*-ketoglutarate (*aKG*) and O_2 followed by the conversion of resokaempferol (vi) into desired fisetin compound by action of flavonoid 3'-monooxygenase (FMO), cytochrome P450 reductase (CPR), NADPH and O_2 [23,24].

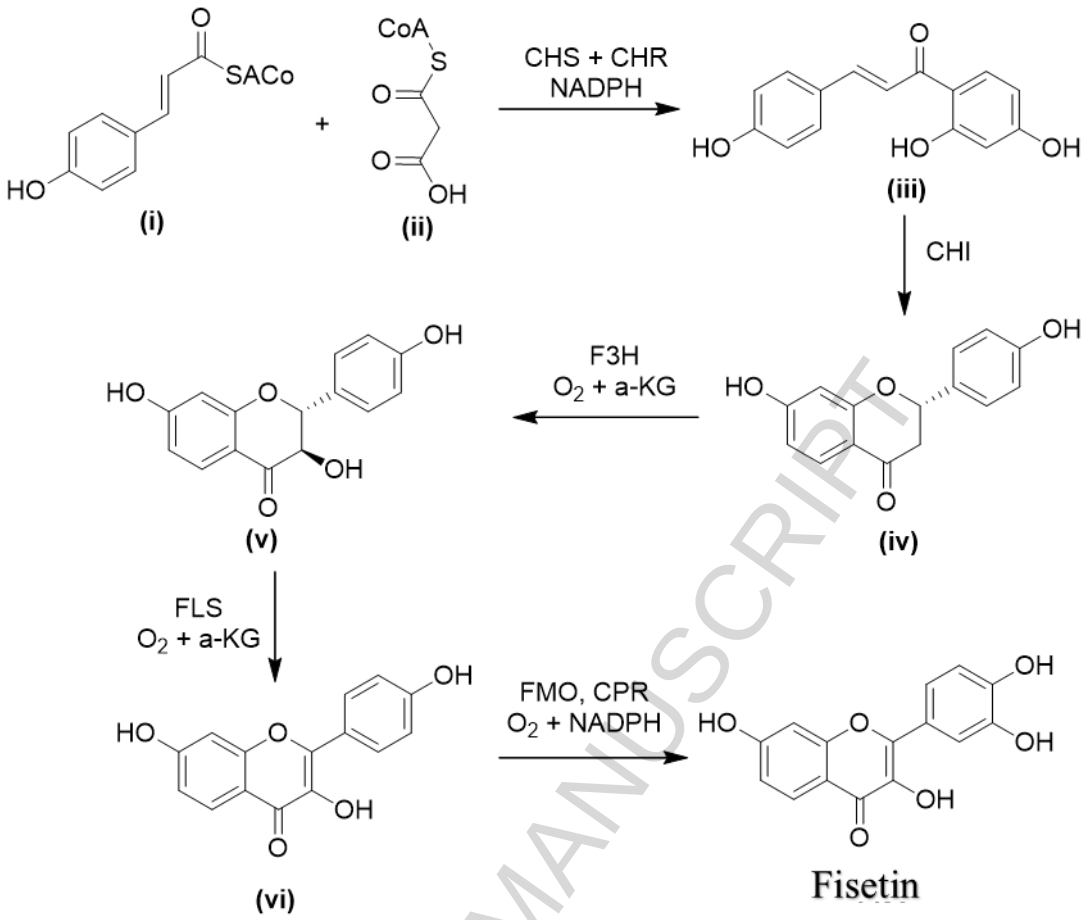

Fig. 2. Proposed biosynthetic pathways for fisetin production using *E. coli* from *p*-coumaroyl-CoA and 3-malonyl-CoA through isoliquiritigenin, liquiritigenin, garbanzol and resokaempferol intermediary stages [ref. 23, 24].

Chemically, fisetin can be synthesized by two methods, namely Kostanecki's method and Allan & Robinson's method. Kostanecki's method involves the formation of chalcone by reaction of 2-hydroxy-4-ethoxyacetophenone and 3,4-dimethoxybenzaldehyde in the presence of NaOH. The cyclization of chalcone into the respective substituted chroman-4-one was carried out by H_2SO_4 and C_2H_5OH. The treatment of substituted croman-4-one with *n*-amylnitrite and H_2SO_4 followed by hydroiodic acid results in the hydroxylation at C_3 with generation of hydroxyl groups at 7, 3', 4' and double bond between C_2-C_3 [25] (Fig 3). Allan & Robinson's method involves the reaction of *ω*-methoxyresacetophenone, veratric anhydride

and potassium veratrate for 4.5 h in an oil-bath at 175-180^{o}C followed by the treatment with hydroiodic acid [ref. 26] (Fig. 4).

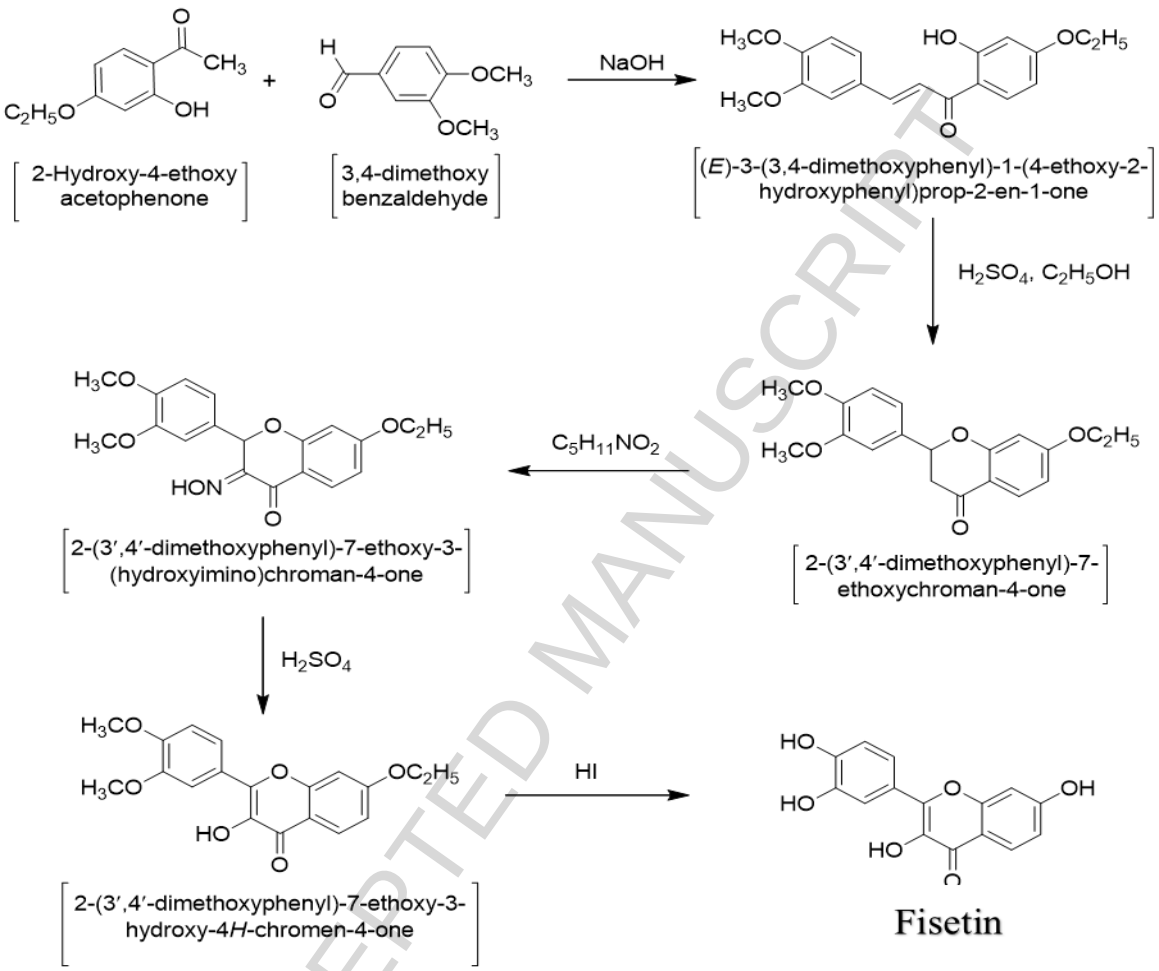

Fig. 3. Kostanecki's chemical method for synthesis of fisetin from 2-hydroxy-4-ethoxyacetophenone and 3, 4-dimethoxybenzaldehyde through chalcone intermediate pathway.

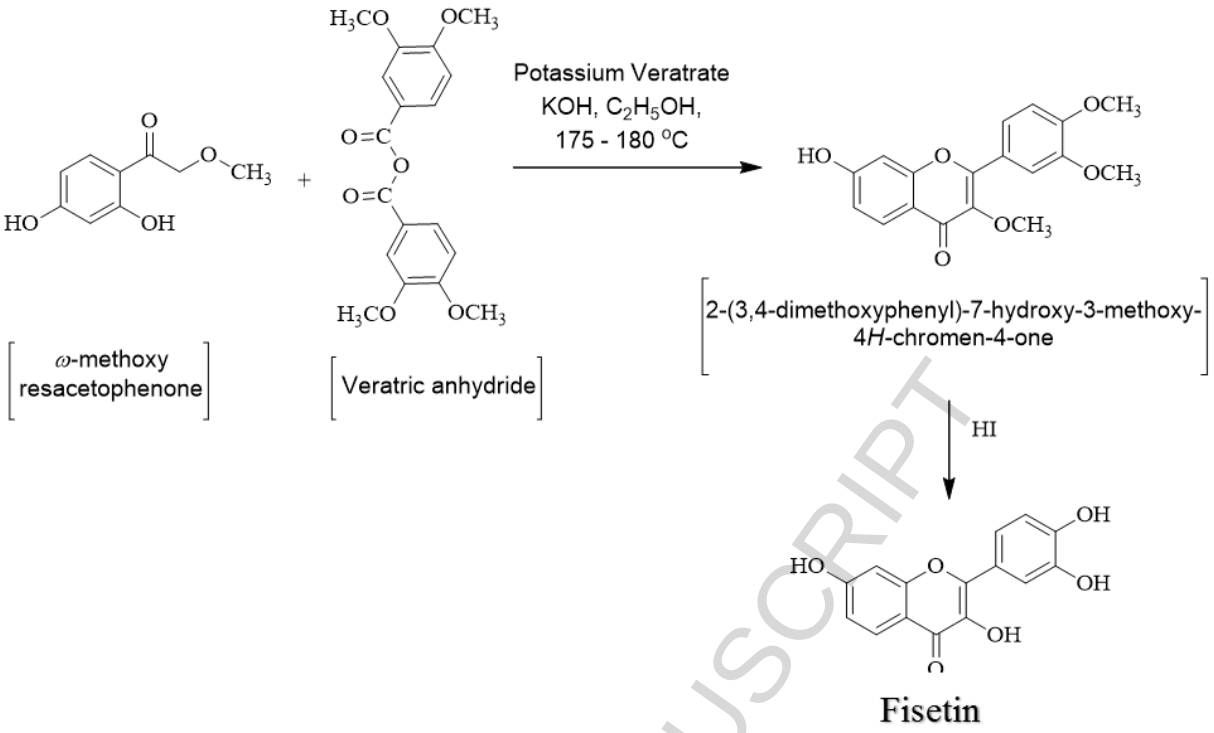

Fig. 4. Allan & Robinson's method for fisetin synthesis from *ω*-methoxyresacetophenone, veratric anhydride and potassium veratrate.

Although fisetin is a potent bioactive phytoconstituent, whereas it exhibits low aqueous solubility and poor absorption from the gut and hence small bioavailability. A number of investigators have reported that the solubility and bioavailability of fisetin can be improved by co-crystallization with caffeine, isonicotinamide and nicotinamide [27,28], complexation with cyclodextrins and encapsulation with nanoparticles [29-31]. These modifications result in the enhancement of solubility, stability and biological activities of fisetin. The biological activity of fisetin depends up on the presence of hydroxyl groups at 3, 7, 3', 4' positions and oxo group at 4 position with double bond between C2 and C3. The hydroxyl group at C-7 and the double bond between C2 and C3 are essential for its antioxidant activity. Additionally, the presence of a hydroxyl group at C3′ in the ring B and at C3 is associated with its high antioxidant activity [32,33]. Awad et al. proposed the possible quinone/quinine methide structures (vii-ix)

of fisetin (Fig. 5). The 2'-glutathionylfisetin formed by addition of glutathione to fisetin quinone occurs in the C ring at position 2 as a major product [34]. Fisetin is o-hydroquinone electrophilic compound form and acts as the neuroprotectant and antioxidant by activating the nuclear factor erythroid 2–related factor 2/antioxidant response element (Nrf2/ARE) pathway [25-37].

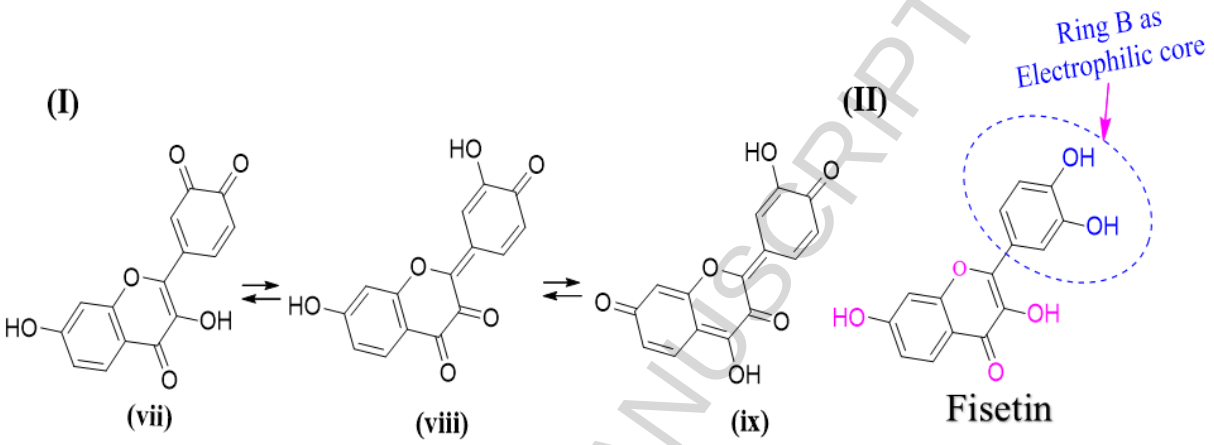

Fig. 5. The quinone/quinine methide isomerism in fisetin and electrophilic nature of ring B. (I) The possible quinone/quinine methide structures (vii-ix) of fisetin; (II) Ring B represents the electrophilic core present in fisetin.

3. Anticancer effects of fisetin

3.1. Apoptosis activation

Induction of physiological or programmed cell death in tumor cells is a common mechanism of anticancer medications [38]. Numerous studies in the last few decades have determined the apoptotic activity of various plant-derived molecules, such as ursolic acid [39], quercetin [3], kaempferol [40] and cordycepin [41]. It has been observed that fisetin invokes antitumor activity through the activation of both intrinsic and extrinsic pathways of apoptosis determined in multiple *in vitro* and *in vivo* studies (Fig. 6). For instance, proapoptotic and antiapoptotic proteins, such as cyt. c, caspase-9, caspase-3 and Bcl-2 and Bax were found to

be modulated in fisetin-treated mice (25 mg/kg) [42]. In another study, Li et al. [43] reported the apoptosis-inducing effect of fisetin in human T24 and EJ bladder cancer cells via upregulation of p53, Bax, Bak and downregulation of NF-κB, Bcl-2 and Bcl-xL. Similarly, apoptosis induction was noticed in U266 cancer cell line through activation of caspase-3, downregulation of Bcl-2 and Mcl-1L, upregulation of Bax, Bim and Bad. Additionally, activation of 5'adenosine monophosphate-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC) and decreased phosphorylation of AKT and mTOR were also observed [44]. Fisetin was also found to exhibit cytotoxic activity in NCI-H460 cells through induction of DNA fragmentation, mitochondrial membrane depolarization, modulation of the expression of Bcl2, Bcl-xL and activation of caspase-9 and caspase-3 [45]. The results of another study showed that fisetin in combination with a citrus flavanone, hesperetin mediated apoptosis by mitochondrial membrane depolarization and caspase-3 activation along with Janus kinase/ signal transducer and activator of transcription (JAK/STAT) pathways, KIT, and growth hormone receptor signaling alteration in human K562 chronic myeloid leukemia cells [46]. Similarly, in NCI-H460 human non-small cell lung cancer line, fisetin generated reactive oxygen species (ROS), endoplasmic reticulum (ER) stress and phosphorylation of extracellular signal–regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and p38 MAPK apoptotic markers [47]. Sabarwal et al. [48] also investigated the fisetin-mediated tumor growth inhibition in human gastric carcinoma cells (AGS and SNU-1) through ROS production, occurring most likely via mitochondrial complex 1. Further, Kim et al. [49] reported antiproliferative effect in Huh-7 human liver carcinoma cells by DNA damage, intracellular accumulation of ROS and downregulation of BIRC8 and the Bcl2L2 in the presence of fisetin. At 20-100 μM concentration, this flavonol significantly reduced the viability of U-2 osteosarcoma cells by inducing the expression of caspase-3, caspase-8 and caspase-9, Bax and Bad, downregulation of Bcl-xL and Bcl-2 and generation of ROS and

modulation of MAPK and PI3K/Akt signaling cascades [50]. Moreover, activation of a dose- and time-dependent apoptosis was analyzed in HeLa human cervical cancer cells after the treatment with fisetin through activation of caspase-3 and caspase-8, the cleavage of poly (ADP-ribose) polymerase (PARP) and activation of the phosphorylation of Erk 1/2 [51]. Similarly, fisetin treatment resulted in PARP cleavage, modulation of the expression of Bcl-2 family proteins, inhibition of PI3K and phosphorylation of Akt at Ser473 and Thr308, release of cyt. *c* and significant activation of caspase-3, caspase-8 and caspase-9 [52]. Studies using A431 human epidermoid carcinoma cells revealed that fisetin treatment (5–80 μM) results in a significant decrease of cell viability by decreasing the expression of Bcl2, Bcl-xL, Mcl-1 and by increasing the expression of Bax, Bak, and Bad, release of cyt. *c* and Smac/DIABLO from mitochondria, caspases and cleavage of PARP protein [53]. In HCT-116 human colon cancer cells, fisetin-induce apoptosis involved DNA condensation, cleavage of PARP, activation of caspase-9 and caspase-7, reduction of Bcl-xL and Bcl-2, increased levels of Bak and Bim, enhancement of the permeability of mitochondrial membrane, release of cyt. *c* and Smac/DIABLO, increase in the protein levels of cleaved caspase-8, Fas ligand, death receptor 5, and tumor necrotic factor (TNF)-related and increased p53 protein levels [54]. Fisetin inhibited triple-negative breast cancer (TNBC) cell (MDA-MB-468 and MDA-MB-231) division and induced apoptosis, associated with mitochondrial membrane permeabilization and the activation of caspase-9 and caspase-8, as well as the cleavage of PARP 1 [55]. Fisetin inhibited the survival of cholangiocarcinoma (CCA) cells by means of phosphorylating Erk, reduced phospho-p65 and Myc oncogene expression [56]. Further, utilizing human Burkitt's lymphoma Raji cells, fisetin was found to have apoptotic effect accomplished by inhibiting both PI3Ks and mTOR signaling molecules [57]. Kim et al. demonstrated that fisetin causes inhibition of proliferation by the modulation of heat shock protein 70 (HSP70), HSP27 and Bcl-2-associated athanogene domain 3 (BAG3) which protect cancer cells from apoptosis by

stabilizing Bcl-2 family proteins in the HCT-116 cancer cells [58]. Pal et al. [8] analyzed the inhibition of PI3K signaling pathway, activation of caspase-3 and cleavage of PARP, increase expression of Bax and Bak, inhibition of Bcl2 and Mcl-1, and phosphorylation of MEK1/2, Erk 1/2, AKT and mTOR in melanoma cells (A375 and SK-MEL-28) in response to fisetin. Further, treatment of COLO205 human colon cancer cells with fisetin in combination with geldanamycin (GA) and radicicol (RAD) resulted in significantly enhanced cytotoxicity, increased expression of caspase-3, PAPR protein, and DNA degradation [59]. Syed et al. [60] also determined an ER stress and phosphorylation and activation of AMPK in highly aggressive A375 and 451Lu human melanoma cells. Similarly, in combination with N-acetylcysteine (NAC) treatment, fisetin increased the expression of cleaved caspase-3 and PAPR protein, and produced greater density of DNA ladders in COLO205 cells with induction of caspase-9 protein and inhibition on Erk protein phosphorylation [11]. Moreover, fisetin-mediated apoptosis induction in HCT-116 cells was revealed by Yu et al. through the Ser15-phosphorylation of p53, and cleavages of procaspase-3 and PARP [61]. Also, the treatment of human HL60 acute promyelocytic leukemia cells with fisetin resulted in the MAPK modulation and inhibition of DNA binding (ID) signaling pathways [62]. Fisetin showed concentration-dependent cytotoxic effects on SK-HEP-1 human hepatic adenocarcinoma cells which were accompanied by DNA fragmentation, induction of caspase-3 and CPP32 activity, cleavage of caspase-3 substrates including PARP and D4-GDI protein, and decrease of procaspase-3 protein [63]. Studies using colon cancer cell line demonstrated the anti-proliferative effects of fisetin through the activation of apoptosis via inhibition of cyclooxygenase-2 (COX-2) and Wnt/EGFR/NF-κB signaling pathways [64]. Fisetin-induced intrinsic apoptotic activation was observed in androgen-dependent LNCaP and androgen-independent DU145 and PC3 prostate cancer cells, suggesting the antitumor effect of fisetin, which was confirmed by increased expression of TNF-related apoptosis-inducing ligand-

receptor 1 **(**TRAIL-R1**)** and decreased activity of NF-κB and activation of caspase-8, caspase-3 and caspase-9 [65]. A bird eye view of various fisetin governed apoptotic signaling pathways have been presented in table 3.

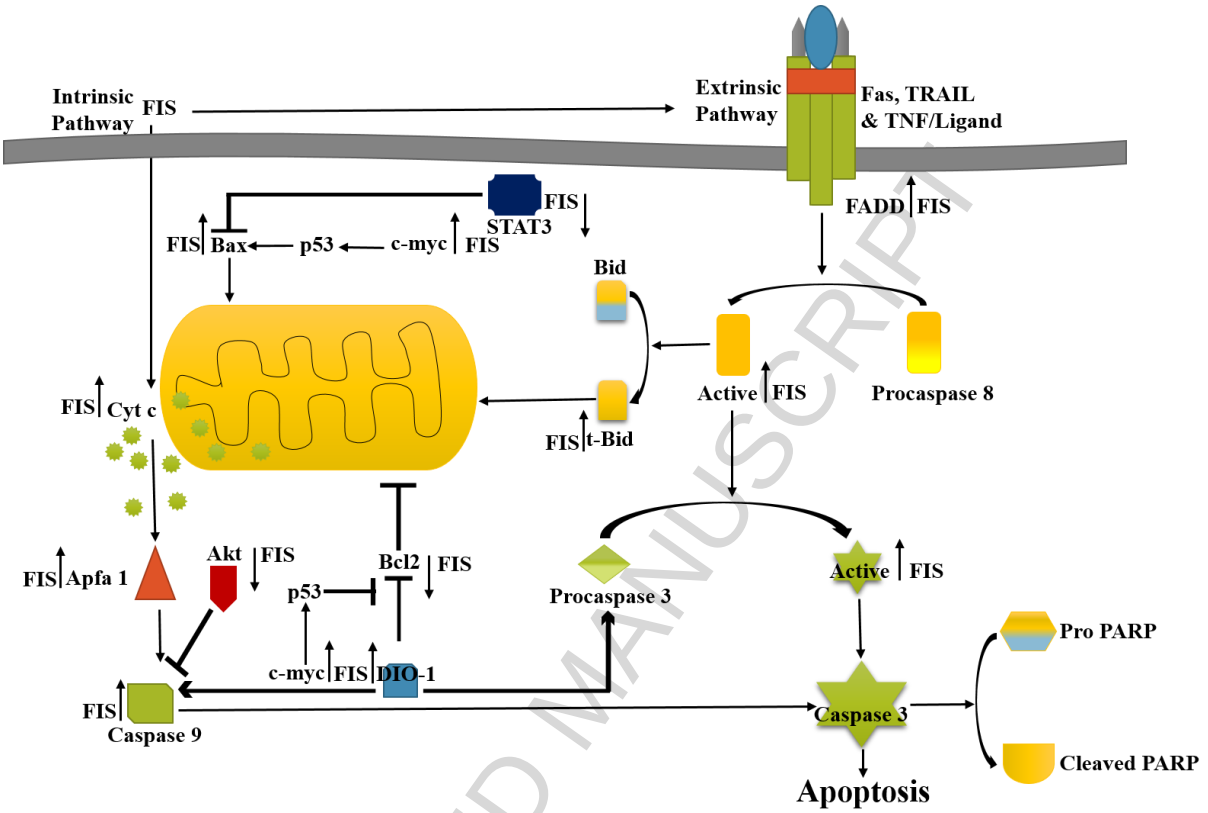

Fig. 6. Schematic illustration of fisetin (FIS) induced apoptosis in cancer cells. FIS modulates various pro-apoptotic and anti-apoptotic proteins involved in mitochondrial and Fas-mediated programmed cell death.

3.2. Cell cycle arrest

Malignancies alter all the components of the cell cycle machinery. Cyclin-dependent kinases (cdks) are central players and regulate the initiation, progression, and completion of cell cycle [66–69]. The well organized and controlled association of cyclins and cdk inhibitors maintains the homeostasis of cell division. During malignant event, both genetic and epigenetic dysregulations affect the expression of cell cycle regulatory proteins, causing overexpression of cyclins and loss of expression of cdk inhibitors [68]. The crucial role of cell

cycle regulatory proteins has prompted great interest in the development of specific strategies to block cell cycle progression and induce growth arrest (table 3). In this context, fisetin was found to have potential to reduce the expression of different overexpressed malignancy promoting factors (Fig. 7). The anti-proliferative effects of fisetin and hesperetin were shown to be occurred through S, G2/M, and G0/G1 phase arrest in K562 cell progression [46,70]. In human gastric carcinoma cells (AGS and SNU-1), Sabarwal et al. [48] observed fisetin-mediated growth inhibition at 25–100 μM concentration and a significant decrease in the levels of G1 phase cyclins and CDKs, and increased levels of p53 and its S15 phosphorylation [48]. Similarly, treatment of A431 cells with fisetin also resulted in G2/M phase arrest [53]. In bladder cancer cells (T24 and EJ), fisetin caused blockage of cell cycle progression in G0⁄G1 phase through a significant increase in expression of p53 and p21 proteins, and decrease in levels of cyclin D1, cyclin A, Cdk-4 and Cdk-2 [43]. Treatment of prostate cancer cells (LNCaP, CWR22Ry1 and PC-3) with fisetin at concentrations of 10–60 μM for 48 h decreased cellular viability through arrest in G1-phase via a marked decrease in the protein expression of cyclins D1, D2 and E and their activating partner cyclin-dependent kinases 2, 4 and 6 with concomitant induction of WAF1/p21 and KIP1/p27 [52]. The cell cycle progression was arrested at G2/M phase in TNBC breast cancer cells (MDA-MB-468 and MDA-MB-231) in response to fisetin [55]. Also, a reduction of the phosphorylation state of the Rb proteins was observed by Lu et al. [71] with 40 μM fisetin along with decreased activities of Cdk-2 and Cdk-4 through decreases in the levels of cyclin E and D1 and an increase in $p21^{CIP1/WAF1}$ levels in HT-29 human colon cancer cells [71].

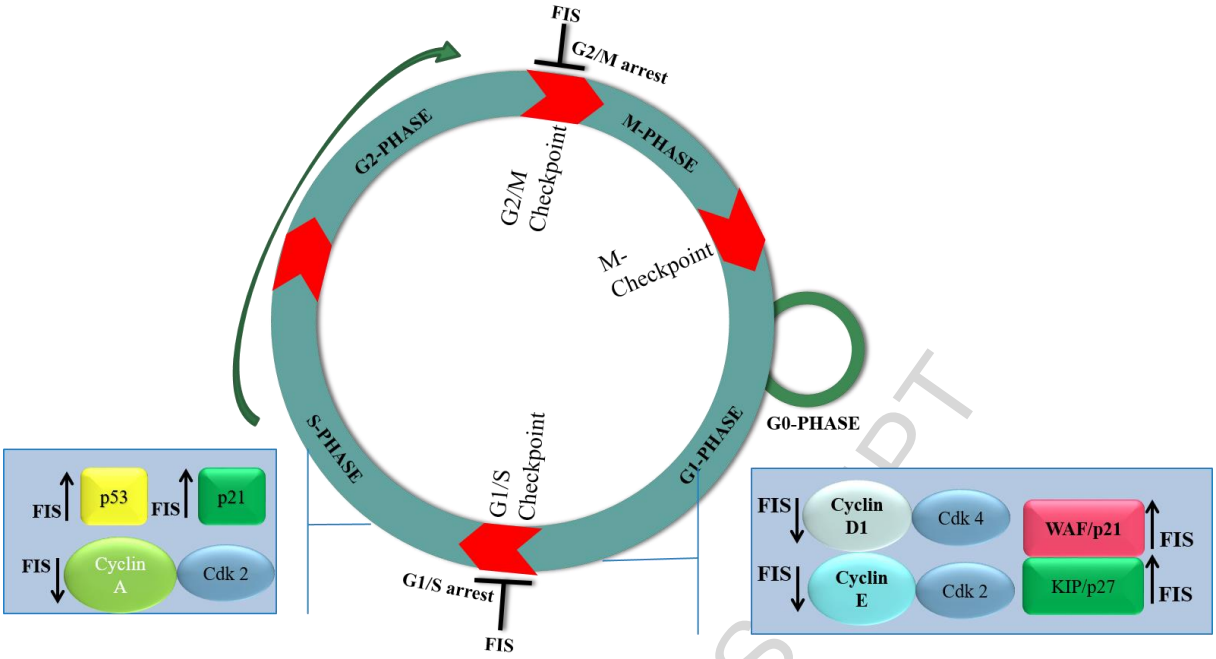

Fig. 7. Cell cycle arrest caused by FIS at various check points by controlling the expressions of cyclin/Cdk complexes.

3.3. Antimetastasis

Major cause of cancer mortality and morbidity is associated with the spread or invasion of cancer cells throughout the body. Malignant cells can spread locally and regionally and can move into nearby lymph nodes as well as to distant tissues or organs such as bone, liver, and lungs using circulation and called metastasize [72, 73]. The main aim of anticancer treatments is to inhibit or reduce the growth of cancer cells and to increase the patient's survival. Various preclinical studies using *in-vivo* and *in-vitro* models have been designed to determine the antimetastatic role of various phytochemicals, including fisetin. In several studies (table 3), fisetin has exhibited tumor inhibitory effects by blocking matrix metalloproteinase-2 (MMP-2) and MMP-9 at mRNA and protein levels, phosphorylation of JNK1/2 and Akt and significantly decreased NF-κB, c-Fos, and c-Jun, and the binding abilities of NF-κB and activator protein-1 (AP-1) in PC-3 cells [74]. In addition, fisetin also inhibited the MMP-14, MMP-1, MMP-3, MMP-7, and MMP-9 gene expression those involved in ECM remodeling

found in HT-1080 fibrosarcoma cells and human umbilical vascular endothelial cells (HUVECs) [75]. The metastasis-inhibitory effects of fisetin (5–20 μM) were also revealed through a decrease in the phosphorylation of MEK1/2 and Erk1/2, reduction of the activation of the NF-κB signaling pathway, promotion of mesenchymal to epithelial transition associated with a decrease in mesenchymal markers i.e. N-cadherin, vimentin, snail and fibronectin and an increase in epithelial markers i.e. E-cadherin and desmoglein in melanoma cells [76]. The results of another study showed that fisetin suppressed the expression and activity of urokinase plasminogen activator (uPA) via interruption of p38 MAPK-dependent NF-κB signaling pathway in cervical cancer cells [77]. Furthermore, 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cell invasion in MCF-7 human breast cancer cells was found to be reduced by fisetin through a reduced NF-κB activation, reduced TPA activation of PKCα/ROS/Erk1/2 and p38 MAPK signaling pathways which ultimately led to the downregulation of MMP-9 expression (fig 8) [78]. In human glioma GBM8401 cells fisetin produced antitumor effect via the modulation of Erk1/2 and ADAM9 expression [79]. Pal et al. [9] found that combination treatment of fisetin and sorafenib reduced the migration and invasion of BRAF-mutated melanoma cells both in *in-vitro* and in raft cultures and inhibited epithelial to mesenchymal transition (EMT) as observed by a decrease in N-cadherin, vimentin and fibronectin and an increase in E-cadherin. In addition, this combination therapy also effectively inhibited expression of Snail1, Twist1, Slug, ZEB1 and MMP-2 and MMP-9 metastatic biomarkers [9]. In another study, the antimetastatic effect of fisetin in A549 human lung adenocarcinoma cells was observed through the inhibition of the phosphorylation of Erk1/2 and downregulation of expressions of MMP-2 and urokinase-type uPA at both the protein and mRNA levels, followed by a significant decrease in NF-κB, c-Fos, and c-Jun levels [80]. Fisetin inhibited epidermal growth factor (EGF) induced YB-1 phosphorylation and markers of EMT both in vitro and in vivo [81].

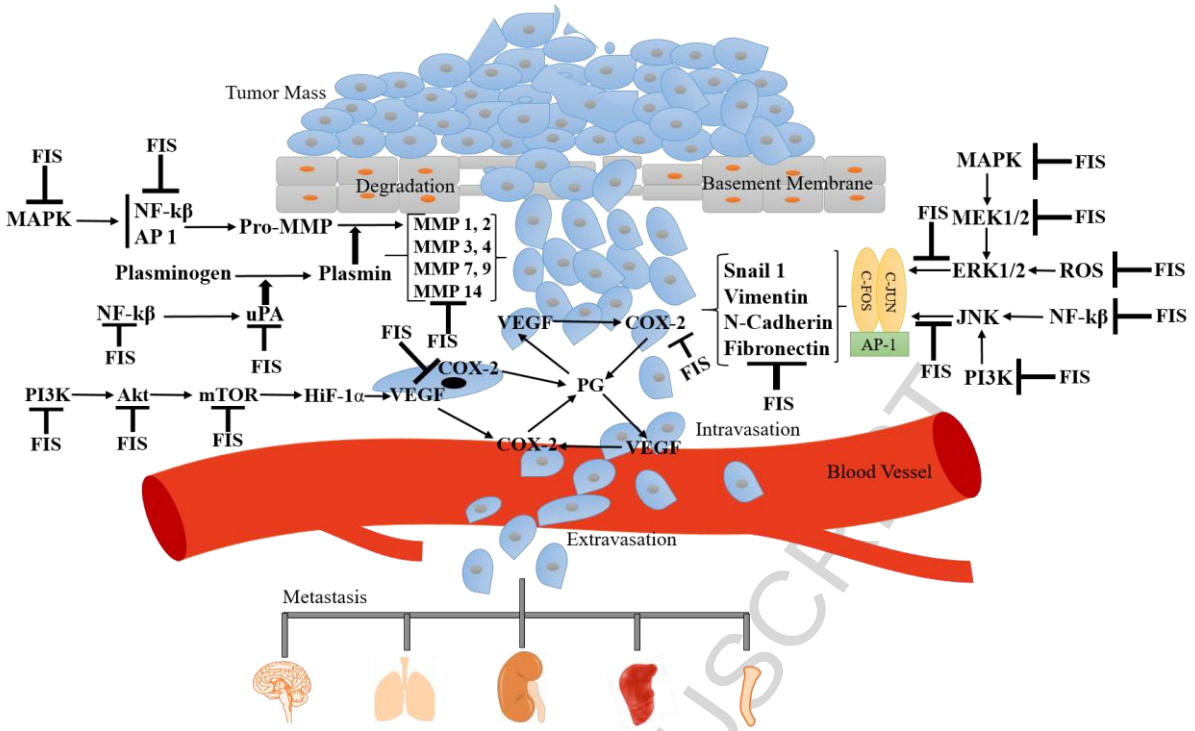

Fig. 8. FIS mediated modulations of multiple signaling pathways involved in tumor metastasis and angiogenesis.

3.4. Antiangiogenesis

Proliferation and metastatic spread of cancer cells depends on the new vascular network to adequately supply oxygen and nutrients and to remove waste products or gas exchange [82]. The process angiogenesis is controlled by more than a dozen different activator and inhibitor molecules [83]. Tumor growth is known to promote the imbalance between proangiogenic factors such as vascular endothelial growth factor/receptor (VEGF/R), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and antiangiogenic factors, including angiostatin and endostatin [84,85]. The discovery of angiogenic inhibitors would help to reduce both morbidity and mortality from carcinomas. However, despite their theoretical efficacy, antiangiogenic treatments have still not proven beneficial for long-term survival of cancer patients. Therefore, there is an urgent need for comprehensive treatment strategies combining antiangiogenic agents with conventional cytoreductive treatments to control the

spread and treatment of cancer. In the present scenario, the inhibition of neovascularization in developing tumor is being targeted by anticancer therapies. There have been a variety of natural metabolites, including fisetin, that hinder the neovascularization and even disrupt the functioning of existing blood vessels [86]. Thus, the present evidence suggests that fisetin-mediated antiangiogenesis approaches (table 3) may be utilized with the combination of existing VEGF antibody-based therapy (Fig. 8). In a study using Lewis lung carcinoma mouse model as well as matrigel plug assay, Touli et al. [86] investigated the antiangiogenic effects of fisetin in combination with cyclophosphamide. Mechanistic insight of fisetin-mediated anti-angiogenesis revealed the decreased expression of endothelial nitric oxide synthase (eNOS) and VEGF, EGFR, COX-2 and wingless and Wnt-signaling [64,87]. Iftikhar and Rashid [82] also investigated the role of polyphenolic compounds (isorhamnetin, fisetin, genistein and silibinin) as potent inhibitors of angiogenesis and tumor progression via Wnt signaling inhibition. The binding energies of these inhibitors were found to be in the range of −5.68 to −4.98 kcal/mol [88]. Recently the findings of Lall et al., 2016 investigated the role of fisetin as hyaluronan synthesis inhibitor which consequently increases the concentration of an antiangiogenic protein such as high-molecular-mass (HMM)-HA [89]. Therefore, the evidence suggests a promising role of fisetin in designing antiangiogenic drug therapy for cancer treatment. Fisetin in combination with a chemotherapeutic agent sorafenib was also reported to inhibit Snail1, Twist1, Slug and ZEB1 gene expressions and inhibit tumor growth by downregulating PI3K/AKT and mTOR signaling and expressing PTEN protein levels in A549 lung carcinoma cells [90]. In addition, fisetin decreased phosphorylation of AKT, mTOR, mitf and p70S6K proteins in 451Lu human melanoma cells [91].

3.5. Antioxidant

Recent findings have suggested that the dietary components can modulate the free radical scavenging ability of our body via up regulation of Nrf2. It has been found that fisetin treatment results in significantly increased nuclear translocation of Nrf2 and antioxidant response element (ARE) luciferase activity, leading to upregulation of HO-1 expression. In addition, fisetin pretreatment reduced hydrogen peroxide (H_2O_2)-induced cell death, and this effect was reversed by ZnPP, an inhibitor of HO-1, and had protective effect against H_2O2-induced oxidative stress in HUVECs [92]. Fisetin inhibited the formation of osteoclasts (OCLs) in a dose-dependent or concentration-dependent manner, suppressed the bone-resorbing activity of OCLs, decreased phosphorylation of Erk, Akt, and JNK, and upregulated mRNA expression of phase II antioxidant enzymes, including HO-1. Fisetin also triggered the suppression of Nrf2 and significantly decreased RANKL-induced nuclear translocation of cFos and nuclear factor of activated T cells cytoplasmic-1 (NFATc1) [93]. Fisetin attenuated oxidative stress-inflammatory pathway of aflatoin B1 (AFB1)-induced hepato-carcinogenesis by normalizing the enhanced expression of TNF-α and IL-1α, two proinflammatory cytokines, and declined placental type glutathione S-transferase (GST-p) level in the liver of the fisetin-treated rats with hepatocellular carcinoma (HCC) [94]. Fisetin affected electron flow as indicated by the inhibition of NADH oxidation and ATPase activity in male Wistar rats [95]. Fisetin also rapidly increased the levels of both Nrf2 and ATF4 as well as Nrf2- and ATF4-dependent gene transcription via distinct mechanisms [96]. Overview of fisetin mediated mechanistic insight in antioxidant potential have been given in table 3.

3.6. Anti-inflammation

Inflammation has long been a well-known symptom of many infectious diseases, but molecular and epidemiological research increasingly suggests that inflammation is also

intimately linked with a broad range of non-infectious diseases, e.g., atherosclerosis and osteoarthritis. Chronic inflammation is also implicated at every level of tumorigenesis, including initiation, promotion, malignant conversion, invasion and finally metastasis [97]. Advances in molecular biology have established a fundamental role of inflammation in mediating different stages of chronic and non-communicable diseases from initiation to progression and, ultimately to complications states, including cancer, cardiovascular disorders, and atherosclerosis [98]. Over the last few decades, overwhelming evidence has emerged demonstrating a close link between metabolism and immunity. For instance, it is now considered that obesity is associated with a state of chronic low-level inflammation [99, 100]. This crucial role played by the inflammatory processes in causing cancer and other chronic diseases warrants the development of a new generation of drugs to treat different conditions, including cancers, autoimmune disorders and infectious diseases. Therefore, there is an urgent need to explore the usefulness of bioactive agents, such as fisetin, to develop anti-inflammatory and anticancer drugs. Fisetin exhibits the anti-inflammatory potential (table 3) through cooperation with NF-κB signaling pathway (Fig. 9). Previously, some research groups have suggested the potential therapeutic role of fisetin for its inhibitory actions on nitric oxide, pro-inflammatory cytokines e.g. TNF-R, IL-1β, IL-6 and IL-8 and basophil-induced production of TH2-type cytokines in human mast cells (HMC-1) [101,102]. The key players associated with allergic airway inflammation, such as eotaxin-1, TSLP and Th2-associated cytokines (IL-4, IL-5, and IL-13) in lungs and transcription factor (GATA-3) and cytokines in thoracic lymph node cells and splenocytes, showed lower expression after fisetin treatment [103]. Proinflammatory genes are controlled by the transcription factor NF-κB, a central molecule of many effective pathways in immune system. Effects of fisetin on the activity of NF-κB were demonstrated in RAW 264.7 macrophages, where this flavonoid inhibited the NF-κB activity and reduced LPS-induced production of TNF-R and inducible

nitric oxide synthase (iNOS). Fisetin was also suggested to block the MAPK pathways, COX-2, and PARP-1 activities and thereby diminished the secretion of LPS-induced interleukin (IL)-8 in pulmonary epithelial cells and human gingival fibroblasts (HGFs). Synthesis and expression of prostaglandin E2, extracellular signal-regulated kinase, JNK and p38 MAPK were also reduced in response to fisetin [104]. In addition, fisetin reduced the molecular components, such as Src, Syk and IκBα, through blockage of nuclear translocation of p65/nuclear factor NF-κB [105]. The combinatorial effect of fisetin and luteolin is known to prevent the blindness disease, age-related macular degeneration (AMD), in the Western world by inhibting the activation of MAPKs and CREB in the ARPE-19 cells [106]. In another study, fisetin treatment of UVB-exposed mice resulted in decreased hyperplasia and reduced infiltration of inflammatory cells, reduced inflammatory mediators such as COX-2, PGE_2 as well as its receptors (EP1–EP4) and MPO activity. Moreover, fisetin reduced the level of inflammatory cytokines, namely TNFα, IL-1β and IL-6, reduced cell proliferation markers as well as DNA damage as evidenced by increased expression of p53 and p21 proteins and also inhibited expression of PI3K, phosphorylation of AKT and activation of the NF-κB signaling pathway [107]. A study using human immortalized keratinocytes (HaCaT cells), fisetin at 1–20 μM concentrations revealed an increased expression of HO-1, cell viability, Akt phosphorylation and reduced ROS production. Fisetin treatment also inhibited the production of NO, PGE2, IL-1β, and IL-6, expression of iNOS and COX-2, and activation of NF-κB in HaCaT cells [93]. Fisetin inhibited IL-1β-induced expression of NO, PGE2, TNF-α, IL-6, COX-2, iNOS, MMP-3, MMP-13, and ADAMTS-5, remarkably decreased IL-1β-induced degradation of Sox-9, aggrecan and collagen-II and significantly inhibited IL-1β-induced SIRT1 decrease and inactivation. Based on the foregoing preclinical findings regarding the anti-angiogenesis, anti-oxidant and anti-inflammatory properties of fisetin, this plant-derived substance appear to be a potential candidate for treating cancer in humans [115].

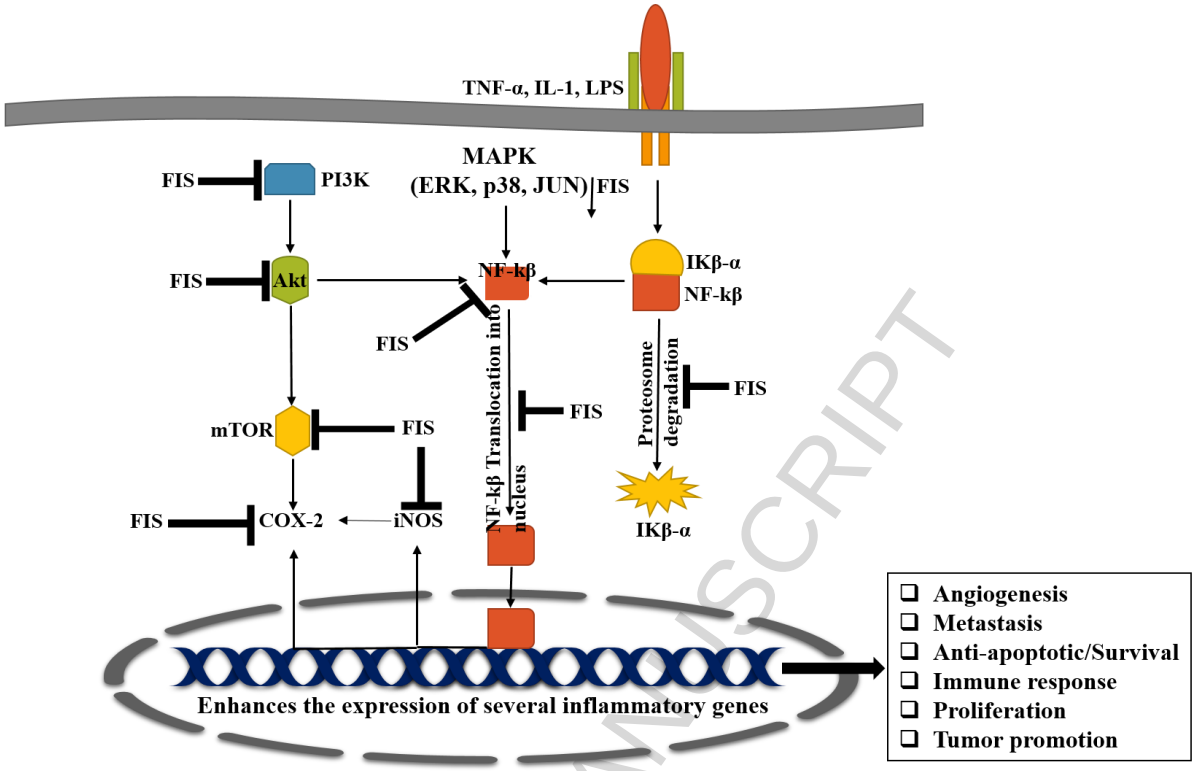

Fig. 9. Anti-inflammatory mechanism of actions of FIS via Akt/mTOR and NF-κβ pathways.

Table-3: Summary of various Fisetin mediated effects using *in-vivo* and *in-vitro* system

Targets	Effects of fisetin	Model	Ref
Apoptosis	- Modulate cyt. c, caspase-9, caspase-3 and Bcl-2 and Bax, DNA fragmentation, mitochondrial membrane depolarization.	Mice, NCI-H460 cell line	42 45
	- Up regulate p53, Bax, Bak and down regulate NF-κB, Bcl-2 and Bcl-xL.	T24 and EJ cell lines	43
	- Activate caspase-3, down regulate Bcl-2 and Mcl-1L, and up regulate Bax, Bim and Bad, activate AMPK, ACC and decrease phosphorylation of AKT and mTOR.	U266, A431 cell lines	44 53
	- Regulate JAK/STAT pathways, KIT, and growth hormone receptor signaling alteration.	K562 cell line	46
	- Generate ROS, ER stress and phosphorylation of ERK,	NCI-H460 cell line	47

	JNK, and p38 MAPK apoptotic markers.		
	- ROS production, via mitochondrial complex 1.	AGS and SNU-1 cell lines	48
	- Activate caspase-3 and caspase-8, cleavage of PARP and phosphorylation of Erk ½.	HeLa, MDA-MB-468, MDA-MB-231, COLO205, and HCT-116 cell lines	51 55 11
	- Release cyt. *c* and Smac/DIABLO from mitochondria, activate caspases and cleavage of PARP protein.	HCT-116 cell line	54
	- Phosphorylate Erk, reduce phospho-p65 and Myc.	CCA cell line	56
	- Inhibit both PI3Ks and mTOR signaling molecules.	Human Burkitt's lymphoma Raji cells	57
	- Modulate HSP70, HSP27 and BAG3 molecules.	HCT-116 cell line	58
	- Inhibit PI3K signaling pathway, activate cleavage of caspase-3 and PARP, expression of Bax and Bak, and inhibit Bcl_2, Mcl-1, and phosphorylation of MEK1/2, Erk 1/2, Akt and mTOR.	A375 and SK-MEL-28 cell lines	59
	- Modulate MAPK and inhibit DNA binding (ID) signaling pathways	HL60 cell line	62
	- Increase DNA fragmentation, activate caspase-3 and CPP32 activity, cleavage of PARP and D4-GDI protein, and decrease procaspase-3 protein.	SK-HEP-1 cell line	63
	- Inhibit COX-2 and Wnt/EGFR/NF-κB signaling.	HT29 cell line	64
	- Increase expression of TRAIL-R1 and decrease NF-κB and activate caspase-3, 8, & 9.	LNCaP, DU145 PC3 cell lines	65
Cell cycle	- Induce S, G2/M, and G0/G1 phase arrest.	K562, A43, MDA-MB-468, MDA-MB-231 cell lines	46 53 55

			70
	- Decrease G1 phase cyclins and CDKs, and increase levels of p53 and its S15 phosphorylation.	AGS and SNU-1 cell lines	48
	- G0⁄G1 phase arrest through increase in p53 and p21 proteins, and decrease cyclin D1, cyclin A, Cdk-4 and Cdk-2.	T24 and EJ cell lines	43
	- G1-phase arrest via decrease in cyclins D1, D2 and E and their activating partner cyclin-dependent kinases 2, 4 and 6 with concomitant induction of WAF1/p21 and KIP1/p27.	LNCaP, CWR22Ry1, PC-3, HT29 cell lines	52 71
Metastasis	- Block MMP-2 and MMP-9 at mRNA and protein levels, phosphorylation of JNK1/2 and Akt and decrease NF-κB, c-Fos, and c-Jun, and the binding abilities of NF-κB and AP-1.	PC-3, A549 cell lines	74 80
	- Inhibit MMP-14, MMP-1, MMP-3, MMP-7, and MMP-9 gene expression.	HT-1080 and HUVECs cell lines	75
	- Decrease phosphorylation of MEK1/2 and Erk1/2, reduce activation of NF-κB signaling pathway, EMT associated markers i.e. N-cadherin, vimentin, snail, and fibronectin and increase in epithelial markers i.e. E-cadherin and desmoglein, also inhibite Snail1, Twist1, Slug, and ZEB1 metastatic biomarkers.	MLNM-FT-A375, A375 cell lines	76 9
	- Suppress uPA via interruption of p38 MAPK-dependent NF-κB signaling pathway.	SiHa and CaSki cell lines	77
	- Reduce NF-κB activation and PKCα/ROS/Erk1/2 and p38 MAPK signaling pathways which ultimately led to down regulation of MMP-9 expression.	MCF-7 cell line	78
	- Modulate Erk1/2 and ADAM9 expression.	GBM8401 cell line	79
	- Inhibit EGF induced YB-1 phosphorylation and EMT.	Pca cell line	81
Angiogenesis	- Decrease expression of eNOS, VEGF, EGFR, COX-2	Swiss Albino	64

	and wingless and Wnt-signaling.	Mouse	87
	- Wnt signaling inhibition.		88
	- Inhibit Snail1, Twist1, Slug and ZEB1 protein expressions and down regulate PI3K/AKT and mTOR signaling and expression of PTEN protein levels.	A549 cell line	90
	- Decrease phosphorylation of Akt, mTOR, Mitf and p70S6K proteins.	451Lu cell line	91
Anti-oxidation	- Suppress bone-resorbing activity of OCLs, decrease phosphorylation of Erk, Akt, and JNK, up regulate mRNA expression of phase II antioxidant enzymes i.e. HO-1, suppress Nrf2 and significantly decrease RANKL-induced nuclear translocation of cFos and NFATc1.	OCL cells	93
	- Attenuate AFB1-induced hepato-carcinogenesis by normalizing the enhanced expression of TNF-α and IL-1α, proinflammatory cytokines, and declined GST-p.	HCC	94
	- Inhibit NADH oxidation and ATPase activity.	Male Wistar rats	95
	- Increase levels of Nrf2 and ATF4 as well as Nrf2- and ATF4-dependent gene transcription.	HT22 cell line	96
Anti-inflammation	- Inhibit nitric oxide, pro-inflammatory cytokines e.g. TNF-R, IL-1β, IL-6 and IL-8 and basophil-induced production of TH2-type cytokines.	HMC-1	101 102
	- Decrease eotaxin-1, TSLP and Th2-associated cytokines i.e. IL-4, IL-5, and IL-13 and transcription factor (GATA-3).	NL 20, Murine Asthma Model	103
	- Inhibit NF-κB activity and reduce LPS-induced production of TNF-R and iNOS, block MAPK pathways, COX-2, and PARP-1 activities and IL-8, reduce synthesis and expression of prostaglandin E2, Erk, JNK and p38 MAPK signaling molecules.	RAW 264.7 macrophages, pulmonary epithelial cells and HGFs	104

- Reduce Src, Syk, and IκBα through blockage of nuclear translocation of p65/nuclear factor NF-κB.	RAW26.4 cell line	105
- Reduce COX-2, PGE_2 as well as its receptors (EP1–EP4) and MPO activity, reduce the levels of inflammatory cytokines i.e. TNFα, IL-1β, and IL-6, reduce cell proliferation markers as well as DNA damage and also inhibit expression of PI3K, phosphorylation of AKT and activation of NF-κB signaling pathway.	Mice	107
- Inhibit production of NO, PGE2, IL-1β, and IL-6, expression of iNOS and COX-2, and activation of NF-κB.	HaCaT cell	93
- Inhibit IL-1β-induced expression of NO, PGE2, TNF-α, IL-6, COX-2, iNOS, MMP-3, MMP-13, and ADAMTS-5, decrease IL-1β-induced degradation of Sox-9, aggrecan and collagen-II and significantly inhibit IL-1β-induced SIRT1.	Mice	115

4. Synergigstic effects of fisetin in conjunction with other anticancer agents

Besides anticancer properties of fisetin itself, several investigators have reported that this flavonol can potentiate the cytotoxic effects of standard chemotherapeutic drugs and other natural compounds. Indeed, fisetin was shown to act synergistically with paclitaxel to reduce the viability of A549 human non-small cell lung cancer cells, whereas the induction of mitotic catastrophe and autophagic cell death were involved in this effect [108]. Also, combined exposure of NT2/D1 human embryonal carcinoma cells with fisetin and cisplatin led to a synergistic increase in cytotoxic responses as compared with either agent alone by activating both the mitochondrial mediated apoptosis and cell death receptor pathways [109]. Moreover, this combination effectively reduced the tumor size in NT2/D1 mouse xenograft model and

allowing administration of cisplatin at decreased doses where the extent of adverse side effects was lowered [109]. Adding the similar combination (fisetin and cisplatin) to A549-CR cisplatin-resistant human non-small cell lung cancer cells, fisetin effectively enhanced sensitivity of cells to cisplatin and reversed cisplatin-resistance, possibly via inhibition of MAPK signaling pathway and downregulation of surviving proteins [110]. Similarly, fisetin not only potentiated cytotoxicity of cisplatin, but also doxorubicin, in H1299 human non-small cell lung cancer cells [2]. In addition, using a rat model, fisetin pretreatment led to a remarkable amelioration of cisplatin-evoked renal damage and histopathological changes revealing protective properties against cisplatin-induced acute nephrotoxicity, thereby showing that fisetin could be applied as a beneficial adjunct in cisplatin therapy [10]. Remarkable augmentation of cytotoxic responses has also been shown in combining fisetin along etoposide in Saos-2 human osteosarcoma cells [111] and with cyclophosphamide in mice xenografted with Lewis lung carcinoma cells [86]. In this study, a significant inhibition of tumor growth with a dramatic decrease in microvessel density was observed suggesting the involvement of antiangiogenic effects in antitumor activity [86]. In BRAF-mutated human melanoma cells, co-treatment of fisetin with sorafenib resulted in synergistic growth inhibition and induction of apoptosis by simultaneous downregulation of both MAPK and PI3K pathways [8]. These *in-vitro* effects were translated under *in-vivo* conditions as in athymic nude mice subcutaneously implanted with A375 and SK-MEL-28 melanoma cells, adding the combination of fisetin with sorafenib led to a significantly greater suppression of tumor growth and more efficient reduction in angiogenesis as compared to individual agent alone [8]. Moreover, fisetin also potentiated anti-invasive and antimetastatic properties of sorafenib in BRAF-mutated melanoma models leading to fewer lung metastases, thus representing a potential adjuvant compound for chemotherapeutic management of metastatic melanoma [9]. Besides melanoma cells, the combination of fisetin along sorafenib potentiated

cytotoxicity in HeLa human cervical cancer cells and HeLa xenograft model by decreasing tumor volume and weight [7]. This synergistic action occurred via induction of both mitochondrial as well as death receptor 5-dependent apoptotic pathways [7]. In addition, treating COLO205 human colon cancer cells with combinations of fisetin and HSP90 inhibitors geldanamycin or radicicol, a significant enhancement of cytotoxicity through induction of intrinsic mitochondria-mediated apoptotic signaling was described [11]. Although, the above described synergistic actions between fisetin and classical chemotherapeutic drugs certainly need further clinical studies and optimizations of regimens by improving the dosages and pharmaceutical combinations. Overall, it seems that such combinations may have potential clinical applications in treating different carcinoma cells. In addition to potentiation of antitumor activity of conventional chemotherapeutic drugs, fisetin pretreatment was also found to potentiate radiosensitivity in p53-mutant HT-29 human colorectal cancer cells [6]. This natural flavonol prolonged radiation-evoked arrest of malignant cells in radiosensitive G2/M phase and increased apoptotic cell death that revealed fisetin's potential as a possible novel radiosensitizer for further clinical applications [6]. Furthermore, fisetin can influence the action of several other natural compounds, such as hormonal substance melatonin or other flavonoids found in plant-based foodstuffs. In this way, treatment of the MeWo and SK-MEL-28 human melanoma cells with combination of fisetin and melatonin led to a significant inhibition of cellular viability, migration and enhancement of caspase-dependent apoptotic cell death [112]. Cotreating of HG-3 and EHEB human chronic lymphocytic leukemia cells simultaneously with fisetin and flavone luteolin also resulted in synergistic cytotoxic effects, probably possessing a potential physiological significance in chemoprevention of leukemia [113]. Altogether, fisetin can significantly potentiate the cytotoxic action of conventional chemotherapeutic drugs, act as a radiosensitizing substance and augment the antitumor effects of other natural compounds.

Therefore, this natural flavonol has potential for combination in future chemotherapeutic regimens as well as in chemopreventive carsinogenic approaches.

5. In *in-vivo* Metabolism of fisetin

In some ways, it is rather premature to conclude whether or not the results obtained under *in-vitro* conditions can be extrapolated to *in-vivo* settings. After ingestion, flavonoids undergo biotransformation in the gastrointestinal tract and liver, and are mostly converted to different metabolites which can be detected in various body fluids. Shia et al. [114] found that following intravenous injection in male Sprague-Dawley rats (10 mg/kg), blood levels of fisetin rapidly declined with appearance of derivatives containing sulfate and glucuronide conjugates. After oral administration (50 mg/kg), fisetin was only transiently present in its intact form in rat serum, but was predominantly present in the blood in the form of conjugated sulfates and glucuronides. Pharmacokinetic studies of fisetin in male/female rats showed that in comparison with rats, elimination rate of this flavonol in female C57BL/6J mice was considerably slower with a half-life of 3.12 h compared to male mice. Following intraperitoneal administration of fisetin to mice (223 mg/kg), its blood concentration reached 2.5 μg/ml after 15 min, with formation of glucuronides and methoxylated metabolites, including 3,4´,7-trihydroxy-3´-methoxyflavone or geraldol [86]. Due to commercial non availability of most conjugates, only few studies have been performed to date on the biological effects of fisetin derivatives, showing on the one hand significantly lower activity against 2, 2'-azobis (2-amidinopropane) dihydrochlorode (AAPH)-induced erythrocyte hemolysis for sulfates/glucuronides [114], but on the other hand, somewhat stronger cytotoxic activity on Lewis lung carcinoma cells for geraldol as compared to intact fisetin [86]. However, as several target tissues express sulfatase or glucuronidase enzymes, deconjugation might also occur in various tissues, leading to local re-formation of parent fisetin. It is clear

that metabolic pathways of fisetin are different in rodent species. To understand the in-depth biological activities of fisetin and its various conjugates, further studies are needed to evaluate the safety and efficacy of this phytochemical in suitable animal models, as well as its short and long-term safety profile.

6. Conclusion and future perspectives

Despite the availability of effective approaches, the safe and cost effective cancer therapy still needs to be improved or discovered. In the current scenario of using phytochemicals as anti-cancer substances, fisetin holds a promising potential to be utilized as a chemopreventive agent. It has been confirmed that Patients diagnosed with cancer have exhausted antioxidant concentrations in blood and augmented quantity of lipid peroxidation [116, 117]. Dietary polyphenols such as fisetin in plasma was found to influence risks of various cancers by modulating inflammatory genes and lowering down oxidative stress. In addition, fisetin is known to induce natural cell death and inhibits the vasculature network and metastasis in tumour [52, 60, 64 and 81]. However, the fact that fisetin is poorly absorbed after oral administration restricts to increase plasma concentrations required for therapeutic effects and constrains its bioclinical utility against cancer. Alternative routes like intravenous, intraperitoneal or intramuscular may be used for its clinical application. Some of the strategies to enhance the oral bioavailability of fisetin have been mentioned in this review. Therefore, low bioavailability of fisetin as well as rather poor knowledge about the potential biological activity of its various metabolites still limit the possible clinical application of this attractive natural molecule and suggest the necessity for further in vitro and in vivo studies. However, discerning the involution of fisetin in a variety of molecular pathways, there is a more preponderant need to understand the anticancer properties of this molecule as future therapeutic agent. Studies such as quantitative structure–activity relationship (QSAR) should be carried out to decipher and delineate unknown targets of fisetin. The clinical effectiveness

of fisetin can be further enhanced by utilizing synergistic drug treatments. Further, the synthesis of fisetin derivatives could be utilized as a consequential strategy not only to modulate its therapeutic potential but also to resolve bioavailability and solubility issues. The results of preclinical studies on pharmacological properties of fisetin both in vitro conditions as well as with animal models clearly suggest the necessity to conduct well-designed clinical trials in humans. Only these works could give us conclusive answers about the anticancer efficacy of this attractive natural compound and open new perspectives on potential clinical use of fisetin in the future.

Conflict of interest

Authors declare no conflict of interest with any content of this review article.

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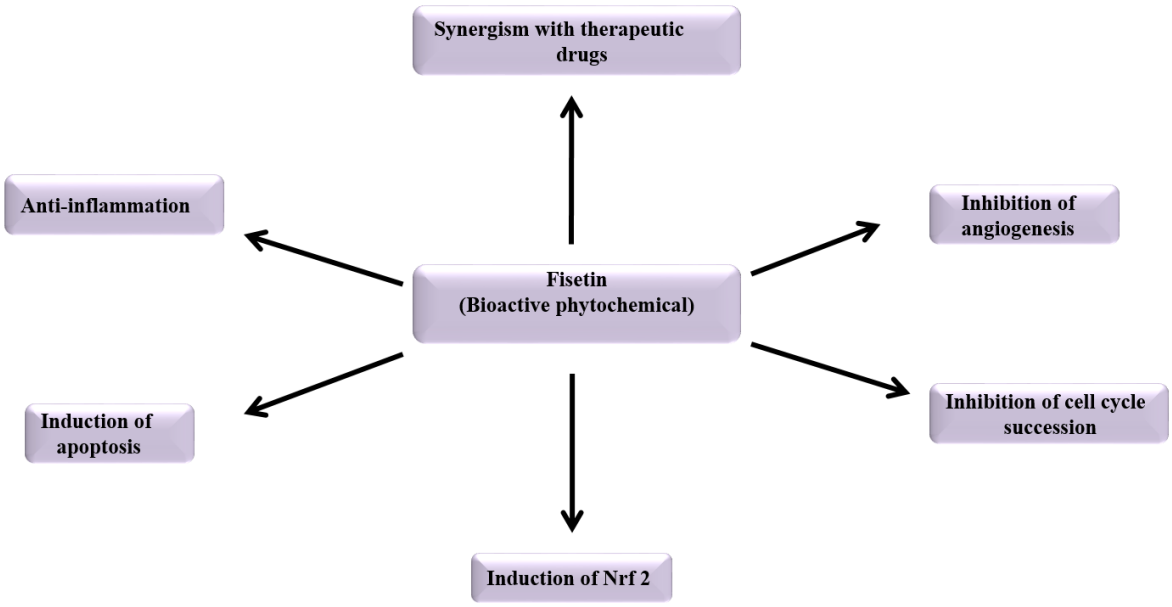

Graphical abstract