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REVIEW

A comprehensive view on the fisetin impact on colorectal cancer in animal models: Focusing on cellular and molecular mechanisms

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Abstract

Flavonoids, including fisetin, have been linked to a reduced risk of colorectal cancer (CRC) and have potential therapeutic applications for the condition. Fisetin, a natural flavonoid found in various fruits and vegetables, has shown promise in managing CRC due to its diverse biological activities. It has been found to influence key cell signaling pathways related to inflammation, angiogenesis, apoptosis, and transcription factors. The results of this study demonstrate that fisetin induces colon cancer cell apoptosis through multiple mechanisms. It impacts the p53 pathway, leading to increased levels of p53 and decreased levels of murine double minute 2, contributing to apoptosis induction. Fisetin also triggers the release of important components in the apoptotic process, such as second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI and cytochrome c. Furthermore, fisetin inhibits the cyclooxygenase-2 and wingless-related integration site (Wnt)/epidermal growth factor receptor/nuclear factor kappa B signaling pathways, reducing Wnt target gene expression and hindering colony formation. It achieves this by regulating the activities

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of cyclin-dependent kinase 2 and cyclin-dependent kinase 4, reducing retinoblastoma protein phosphorylation, decreasing cyclin E levels, and increasing p21 levels, ultimately influencing E2 promoter binding factor 1 and cell division cycle 2 (CDC2) protein levels. Additionally, fisetin exhibits various effects on CRC cells, including inhibiting the phosphorylation of Y-box binding protein 1 and ribosomal S6 kinase, promoting the phosphorylation of extracellular signal-regulated kinase 1/2, and disrupting the repair process of DNA double-strand breaks. Moreover, fisetin serves as an adjunct therapy for the prevention and treatment of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA)-mutant CRC, resulting in a reduction in phosphatidylinositol-3 kinase (PI3K) expression, Ak strain transforming phosphorylation, mTOR activity, and downstream target proteins in CRC cells with a PIK3CA mutation. These findings highlight the multifaceted potential of fisetin in managing CRC and position it as a promising candidate for future therapy development.

KEYWORDS
apoptosis, colorectal cancer, fisetin, inflammation, p53 pathway

1 | INTRODUCTION

Colorectal cancer (CRC) is ranked as the third most prevalent cancer globally and is a significant contributor to cancer-related mortality.[1,2] It represents 10% of all cancer cases worldwide and is responsible for 9.4% of cancer-related deaths. Projections indicate an alarming increase in CRC cases, with an estimated increase of 3.2 million new cases globally by 2040.[3] Despite notable progress in cancer treatment and early detection strategies, finding a definitive cure for CRC remains a challenge, particularly in cases involving liver metastasis, a common and life-threatening aspect of CRC progression.[4,5] Although therapeutic approaches and early detection have decreased the incidence of CRC and improved prognosis, about 50% of CRC cases progress to metastatic stages, leading to 40% of patients succumbing to CRC metastasis.[6] After surgery, 5-fluorouracil (5-FU) is the primary chemotherapy drug for colon cancer treatment, but it comes with significant concerns about toxicity.[7–9]

The increase in drug resistance has generated interest in natural substances for disease management, particularly phytocompounds. These compounds are known for their accessibility and effectiveness.[10]

Flavonoids, which are polyphenolic secondary metabolites, are abundantly found in fruits, vegetables, and tea, and offer a wide range of health benefits. They are used in nutraceuticals, pharmaceuticals, and cosmetics.[11–16]

Flavonoids have been found to possess neuroprotective and cardioprotective effects, as well as the potential to reduce the risk of colon and rectal cancer.[17–19] Furthermore, flavonoids have shown promise in treating CRC, as they exhibit antiproliferative and palliative effects.[20,21]

Fisetin (Figure 1) is a natural flavonoid with the chemical name 3,7,3′,4′-tetrahydroxyflavone. It can be found abundantly in various fruits, vegetables, nuts, and plant sources such as strawberries, grapes, apples, cucumbers, persimmons, and onions.[22–24]

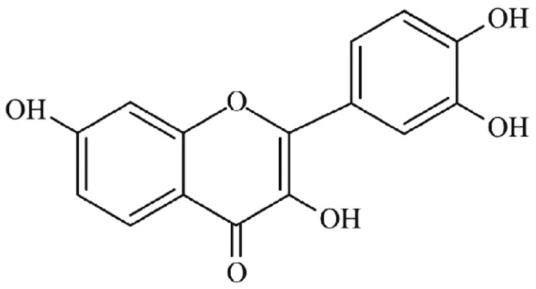

FIGURE 1 Chemical structure of fisetin.

Researchers have demonstrated that fisetin plays a role in managing diseases by affecting various biological activities. Previous studies have highlighted its antioxidant and anti-inflammatory properties, as well as its ability to reduce oxidative stress, inflammation, and apoptosis induced by hyperglycemia and hyperlipidemia in diabetic cardiomyopathy. Fisetin has also exhibited potential in fighting different types of cancers.[25,26] Fisetin has been extensively studied both in vitro and in vivo, highlighting its therapeutic benefits across various diseases.[22,27]

Particularly, fisetin's potential as an anticancer agent stems from its capability to influence critical cell signaling pathways linked to inflammation, angiogenesis, apoptosis, growth factors, and transcription factors.[26,28] Apoptosis, which is a key mechanism in CRC cells that influences cell survival and death, is an important target for fisetin's actions.[25,29–31] Jeng et al. demonstrated that fisetin induces apoptosis in both parental and chemoresistant colon cancer cells. It reduces cell viability and promotes cell death by affecting caspase activation and cytochrome C release, and inhibiting the insulin-like growth factor 1 receptor (IGF1R)/Ak strain transforming (AKT) pathway, which is associated with apoptosis regulation.[32]

Additionally, fisetin has the ability to suppress autophagy, downregulate nuclear erythroid-related factor 2 (Nrf2), and inhibit specific signaling pathways like cyclooxygenase-2 (COX-2) and wingless-related integration site (Wnt)/epidermal growth factor receptor (EGFR)/nuclear factor kappa B (NF-κB). This highlights its potential as a promising agent for CRC prevention and treatment.[33,34]

The methodology used in narrative review articles involves a thorough examination of existing literature on a specific topic. In this study, relevant keywords were selected based on their frequency in the text and their relevance to the subject. To ensure a comprehensive literature search, electronic databases such as PubMed, ScienceDirect, Google Scholar, Web of Science, and Scopus were utilized. The main objective was to understand the molecular processes that contribute to the beneficial effects of fisetin on CRC, with a focus on its antiapoptotic and anti-inflammatory properties. This investigation extensively examined the potential chemopreventive effects of fisetin and its derivatives on CRC, with a specific exploration of their cellular and molecular mechanisms of action.

2 | OVERVIEW OF FISETIN

Fisetin, a natural flavonoid commonly used in traditional Chinese medicine, is derived from plants like mulberry leaves and *Cotinus coggygria*.[35]

It is abundant in fruits and vegetables such as strawberries, onions, apples, persimmons, kiwifruit, and grapes.[36,37]

Strawberries have the highest fisetin concentration at 160 μg/g, whereas apples and persimmons have lower levels at 26.9 and 10.5 μg/g, respectively.[38]

Despite being rare in plants, fisetin offers significant health benefits with minimal negative effects.[39]

Fisetin possesses distinctive physicochemical properties. Its chemical formula is $C_{15}H_{10}O_6$ and molecular weight is286.2363 g/mol, and it exhibits low lipophilicity with a *Clogp* value of 1.24. The topological polar surface area of fisetin is measured at 107 Å, its melting point is 330°C, its density is 1.688 g/mL. Notably, fisetin is highly soluble in dimethyl sulfoxide (~30 mg/mL at 25°C, resulting in a yellow color) and moderately soluble in ethanol (~5 mg/mL), but exhibits near-insolubility in water. Biochemically, fisetin features one rotatable bond, six hydrogen bond acceptors, and four hydrogen bond donors.[22]

Regarding its structural configuration, fisetin is distinguished by its diphenyl propane arrangement, characterized by two aromatic rings linked by oxidized heterocycles through three carbon atoms. This compound is known for its four hydroxyl substituents and one oxygen group, deviating from the typical 5-hydroxy substitutions often observed in its chemical class.[22,40]

Furthermore, fisetin's bioactivity is intricately tied to its structural components, particularly the carbonyl group located at four positions, the double bond linking C2 and C3, and the presence of hydroxyl groups at positions 3, 7, 3′, and 4′. These specific structural features are pivotal in defining fisetin's antioxidant properties, underlining its potential benefits in various biological contexts.[23,41] Furthermore, the presence of 7-OH groups and 4-ketones in its core structure contributes to its anti-inflammatory effects.[42]

A mouse study showed that 223 mg/kg of fisetin administered intraperitoneally led to peak plasma concentration at 2.5 μg/mL after 15 min. Then, fisetin levels showed a biphasic decrease with a half-life of 0.09 h. Three fisetin metabolites were identified, comprising two glucuronides and one methoxylated metabolite known as geraldol (3,4′,7-trihydroxy-3′-methoxyflavone).[43]

Geraldol, which is the primary metabolite of fisetin in mice, undergoes rapid conversion from fisetin after the administration of 2 mg/kg (intravenously) and 100 and 200 mg/kg (orally).[44]

Studies on oral bioavailability show that fisetin's oral bioavailability was reported to be 7.8% for 100 mg/kg and 31.7% for 200 mg/kg. Furthermore, the C_{max} and area under the curve (AUC) values of geraldol were found to be higher than those of fisetin, indicating the dynamics of metabolic transformation.[44]

It has also been observed that fisetin and its phase II conjugated derivatives are partially excreted through biliary excretion facilitated by P-glycoprotein in rats. This excretion pattern is associated with fisetin's 3-hydroxyl group and a 2,3-double bond, which results in a notable binding affinity to P-glycoprotein.[45] Fisetin exhibits rapid absorption and extensive conjugation through sulfation and glucuronidation in mice, with the liver, kidney, and intestine exhibiting the highest concentrations of fisetin among mouse tissues.[46]

Various strategies have been explored to enhance the bioavailability of fisetin, ranging from macro- to nano-modifications. Polymeric nanoparticles have been found to improve the bioavailability of fisetin by providing a sustained release profile and protecting the compound from rapid metabolism. For example, fisetin encapsulated in polymer nanoparticles exhibited prolonged release over a period of 4 days and improved pharmacokinetic parameters such as half-life and maximum concentration.[47] Polymeric micelles enhance the solubility and stability of fisetin and have been shown to increase its bioavailability by 1.8- to 6.3-fold, depending on the specific formulation. Additionally, these micelles enhance the anticancer activity of fisetin by providing sustained release and targeting the acidic environment of cancer cells.[48] Nanoemulsions are also an effective strategy for improving the oral bioavailability of fisetin. They enhance the absorption of fisetin in the gastrointestinal tract, resulting in higher plasma concentrations and prolonged circulation time. For instance, a fisetin nanoemulsion demonstrated a higher maximum plasma concentration (C_{max}) and longer time to reach maximum concentration (T_{max}) compared to free fisetin.[49] Liposomes, which are vesicular structures capable of encapsulating fisetin, have been shown to improve its solubility and bioavailability. Liposomal formulations have increased the bioavailability of fisetin 1.64- to 4.43-fold, depending on the route of administration.[50] These strategies collectively aim to overcome the inherent limitations of fisetin, such as poor solubility, rapid metabolism, and low absorption, thereby enhancing its therapeutic potential.

Flavonoids, such as fisetin, are present in various dietary plants. This ubiquitous distribution implies a favorable safety profile, with minimal to no notable concerns regarding toxicity.[51]

In animal studies, oral doses of fisetin typically range from 5 to 25 mg/kg, whereas intraperitoneal doses vary between 0.3 and 3 mg/kg, and they are usually dissolved in dimethyl sulfoxide.[41]

Clinical trials often involve the oral administration of 20 mg/kg of fisetin daily. This dosage is typically administered for two consecutive days or can be administered continuously for a duration of 2 months.[52]

Acute toxicity assessments of fisetin have shown no signs of adverse effects, including decreased body weight, diarrhea, respiratory distress, contractions, or coma, indicating its safety profile.[43,53]

In acute toxicity studies, fisetin demonstrated no signs of toxicity at doses up to 2 g/kg. Histopathological analysis showed no adverse effects on vital organs including the heart, lungs, spleen, intestines, kidneys, liver, and reproductive organs.[54]

The LD50 values for fisetin in mice vary depending on the route of administration, with reported values of 1700 mg/kg (oral), 400 mg/kg (subcutaneous), 250 mg/kg (intraperitoneal), and 200 mg/kg (intravenous).[55] The unique physicochemical properties of fisetin contribute to its wide range of biological activities.[56]

Most notably, research has primarily investigated fisetin's potential as an anticancer agent. Its antitumor efficacy is linked to the modulation of key pathways such as phosphatidylinositol-3 kinase (PI3K), AKT, mammalian target of rapamycin (mTOR), and Wnt/β-catenin, as well as the activation of the TNF-related apoptosis-inducing ligand (TRAIL), which triggers apoptosis, inhibits angiogenesis by downregulating vascular endothelial growth factor (VEGF) expression, and induces autophagic and apoptotic cell death, ultimately suppressing tumor growth.[26,50]

Fisetin exhibits anti-inflammatory properties by influencing the NF-κB signaling pathway; inhibiting nitric oxide production; and reducing the release of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6).[57]

Furthermore, fisetin demonstrates antioxidant properties by engaging with redox-related signaling pathways such as NF-κB, mitogen-activated protein kinases (MAPK), Nrf2, and PI3K/AKT.[41] It scavenges free radicals and induces the expression of antioxidant enzymes to protect cellular components from oxidative damage.[41,58,59]

3 | MOLECULAR AND CELLULAR MECHANISMS OF CRC

CRC development involves a complex interplay between cellular and molecular mechanisms. Genomic instability is a critical factor, leading to mutations and epigenetic changes that drive CRC progression.[60] One key player is the poly(ADP-ribose) polymerase (PARP), which is involved in DNA repair and genomic stability maintenance. PARP inhibitors have shown promise in CRC treatment by targeting these repair mechanisms.[60] Additionally, the tumor microenvironment (TME) significantly influences CRC development. The TME consists of various cell types, including immune cells, fibroblasts, and endothelial cells, which interact with cancer cells to promote tumor growth and metastasis.[61,62] Angiogenesis, the formation of new blood vessels, is another crucial process in CRC progression. VEGF and other pro-angiogenic factors are upregulated in CRC, facilitating tumor growth and metastasis.[61,62] Hypoxia within the TME upregulates hypoxia-inducible factor-1α, which in turn increases VEGF expression, further promoting angiogenesis.[63,64]

Antiangiogenic therapies, such as bevacizumab, target the VEGF pathway and have been shown to improve outcomes in metastatic CRC by inhibiting tumor vascularization and growth.[65,66]

Other antiangiogenic agents, including ramucirumab, ziv-aflibercept, and regorafenib, have also been approved for use in CRC, particularly after the failure of first-line therapies.[67,68]

Despite the benefits of antiangiogenic therapy, resistance remains a significant challenge. Tumors can adapt by activating alternative angiogenic pathways or increasing the expression of pro-angiogenic factors.[69,70]

The TME, including immune cells and fibroblasts, also plays a role in modulating angiogenesis and contributing to therapy resistance.[71,72] Combining antiangiogenic agents with other treatments, such as chemotherapy or immunotherapy, has shown promise in overcoming resistance and improving patient outcomes.[73,74]

Microvessel density and the expression of angiogenic markers like VEGF, CD31, and CD105 are used to assess angiogenic activity in CRC and can provide prognostic information.[69] High levels of these markers are often associated with larger tumor size, higher grade, and advanced stage, indicating a poor prognosis.[69,75,76]

The development of reliable biomarkers to predict response to antiangiogenic therapy is crucial for personalizing treatment and improving outcomes. Research is ongoing to identify such biomarkers and to understand the molecular mechanisms underlying angiogenesis in CRC. Emerging therapies targeting multiple angiogenic pathways or combining antiangiogenic agents with novel treatments hold promise for the future management of CRC.

Cancer stem cells (CSC) play a pivotal role in CRC development and resistance to therapy. These cells possess self-renewal capabilities and can differentiate into various cell types within the tumor, contributing to tumor heterogeneity and recurrence.[62]

The FBXW7-ZEB2 axis has been identified as a key regulator of epithelial–mesenchymal transition and CSC properties in CRC, linking these processes to chemoresistance. Additionally, the tumor suppressor gene *FBXW7* is frequently mutated in CRC, leading to aberrant cell cycle regulation and increased tumorigenicity.[77]

Environmental factors, such as exposure to bisphenol A (BPA), have also been implicated in CRC development. BPA can induce cytotoxic effects and enhance the invasiveness of colon epithelial cells by altering gene expression and activating carcinogenic pathways.[78]

Furthermore, dietary factors, including high intake of ultra-processed foods, have been associated with an increased risk of CRC, highlighting the role of lifestyle in cancer development.[78]

Oxidative stress and chronic inflammation play crucial roles in the development and progression of CRC.[79] Chronic intestinal inflammation leads to excessive tissue regeneration, triggering the proliferation and clonal expansion of initiated tumor cells.[80] Inflammatory bowel diseases like ulcerative colitis and Crohn's disease significantly increase the risk of developing CRC.[81] Chronic inflammation promotes DNA damage, genomic instability, and epigenetic modifications that can lead to oncogenic mutations.[82] Reactive oxygen species (ROS) produced during inflammation can directly damage DNA, proteins, and lipids.[83,84]

Elevated levels of oxidative stress markers, such as malondialdehyde and 8-hydroxy-2′-deoxyguanosine, have been observed in CRC patients, indicating increased DNA damage and lipid peroxidation.[85] The oxidative balance score (OBS), which assesses an individual's antioxidant status by accounting for both prooxidant and antioxidant components of dietary and lifestyle factors, has been linked to CRC risk, with higher OBS associated with lower CRC risk, particularly in women.[84] Additionally, recent research has highlighted the potential of targeting oxidative stress pathways as a therapeutic strategy for CRC, with studies exploring the use of antioxidants and compounds that modulate cellular redox balance to inhibit cancer cell growth and promote apoptosis.[86] ROS can promote chronic inflammation in the colon by activating inflammatory mediators and the nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3) inflammasome. This creates a pro-tumorigenic microenvironment conducive to cancer initiation.[87,88]

The inflammatory microenvironment supports tumor growth by providing growth factors, pro-angiogenic factors, and matrix-degrading enzymes.[89]

Pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β activate oncogenic signaling pathways such as NF-κB and STAT3.[90–92]

These pathways promote cell survival, proliferation, and resistance to apoptosis in tumor cells.[93]

COX-2 catalyzes the conversion of arachidonic acid to prostaglandins (PG), particularly prostaglandin E2 (PGE2), which promotes tumorigenesis by stimulating cell proliferation, inhibiting apoptosis, and enhancing angiogenesis.[94,95] COX-2 is upregulated in response to inflammatory stimuli, such as cytokines (e.g., IL-1β and TNF-α) and growth factors. Chronic inflammation in the colon, often seen in conditions like inflammatory bowel disease, leads to persistent COX-2 expression, creating a pro-tumorigenic environment.[94,96]

The interplay between oxidative stress and inflammation results in a self-perpetuating cycle that drives CRC progression and metastasis. Targeting inflammatory pathways and oxidative stress has shown promise as a therapeutic strategy in CRC prevention and treatment. Understanding the complex interactions between oxidative stress, inflammation, and the TME is crucial for developing more effective strategies to prevent and treat CRC.

4 | EFFECTS OF FISETIN ON COLON AND CRC

4.1 | Fisetin suppresses the growth of cancer cells by inducing apoptosis

Apoptosis plays a crucial role in regulating cell growth and proliferation, thereby preventing the development of cancer.[97] In CRC, apoptosis serves as a natural cellular mechanism to control excessive cell division and maintain tissue homeostasis.[98] The induction of apoptosis in CRC cells can occur through various pathways that involve natural agents. These mechanisms include the death receptor pathway; mitochondrial pathway; endoplasmic reticulum stress pathway; and related signaling pathways such as PI3K/AKT, MAPK, and p53 signaling.[99,100] Understanding these mechanisms is crucial for developing effective treatments that target specific pathways to trigger apoptosis in CRC cells.

HCT116, a human colon cancer cell line commonly utilized in colon cancer and anticancer research, exists in various forms, including p53-wild type, p53-mutant, and p53-null, enabling investigations into different genetic and molecular influences on colon cancer cell behavior.[101,102]

The *p53* gene, recognized as the tumor protein 53, serves as a critical tumor suppressor gene governing cell proliferation, DNA repair, and apoptosis.[103] At the forefront, p53 functions as a sentinel by impeding cell proliferation in response to cellular stressors, such as DNA damage and oncogene activation.[104] Mutations in the *p53* gene are prevalent in approximately 50% of human cancers. These mutations drive cell proliferation, enhance survival, contribute to genomic instability, and confer resistance to specific cancer therapies like chemotherapy and radiotherapy.[103] Mutated p53 correlates with heightened resistance to apoptosis-triggering therapies, underscoring the importance of overcoming this resistance to enhance cancer therapy efficacy.[105]

Yu et al. demonstrated that fisetin exhibits potent cytotoxic effects on securin-null HCT116 human colon cancer cells. The study utilized clonogenic assays and cell growth assessments to reveal a dose-dependent reduction in cell viability and growth, particularly significantly in securin-deficient HCT116 cells. Additionally, fisetin treatment induced heightened levels of DNA fragmentation and apoptosis in securin-null HCT116 cells, as evidenced by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay results. Interestingly, the study found that reestablishing securin expression rescued HCT116 cells from fisetin-induced cell death. Furthermore, research investigated the influence of p53 on fisetin-induced cytotoxicity, underscoring that p53-deficient colon cancer cells exhibited resistance to apoptosis induced by fisetin. These insights elucidate the roles of securin and p53 in determining the sensitivity of human colon cancer cells to fisetin, providing significant implications for strategies in colon cancer treatment.[106]

Second mitochondria-derived activator of caspase (Smac)/direct inhibitor of apoptosis-binding protein with low pI (DIABLO), also

recognized as the Smacs and a direct inhibitor of apoptosis protein–binding protein with low pI, plays a critical role in regulating apoptosis.[107] In the context of apoptosis triggered by changes in Bcl-2 family proteins, Smac/DIABLO, operating within the intrinsic pathway, enhances mitochondrial membrane permeability. This action leads to the release of cytochrome c and Smac/DIABLO, initiating the activation of caspase-9.[108,109] By inhibiting IAPs, Smac/DIABLO facilitates caspase activation, initiating apoptosis.[110]

Murine double minute 2 (MDM2) is an E3 ubiquitin ligase that primarily functions as a negative regulator of the tumor suppressor protein p53. It promotes the degradation of p53 by ubiquitination, thereby controlling the levels and activity of p53 within the cell. This regulation is crucial because p53 is involved in cell cycle arrest, DNA repair, and apoptosis in response to cellular stress and DNA damage.[111,112] MDM2 is often overexpressed in various cancers, leading to the inactivation of p53 and allowing cancer cells to proliferate unchecked. The overexpression of MDM2 can occur due to gene amplification or increased transcriptional activity. This makes MDM2 a potential target for cancer therapy, as inhibiting MDM2 can restore p53 function and induce tumor cell death.[113–115]

Lim and Park conducted a study on the ability of fisetin, a compound found in fruits and vegetables, to induce apoptosis in HCT116 human colon cancer cells. The study showed that fisetin treatment resulted in the upregulation of various molecular pathways, including cleaved caspase-8, Fas ligand, TRAIL, and DR5 levels, in the cancer cells. Fisetin also caused mitochondrial membrane depolarization, leading to the release of Smac/DIABLO and cytochrome c. Additionally, fisetin increased p53 levels and decreased MDM2, its negative regulator. These findings confirmed fisetin's potential as a preventive and therapeutic agent for CRC, as it was able to inhibit the growth of HCT116 cells with the wild-type p53 gene in a dose-dependent manner.[116]

Fisetin micelles, biodegradable nanoassemblies formed from a copolymer of monomethyl poly(ethylene glycol)-poly(ε-caprolactone) (MPEG-PCL) and fisetin, possess exceptional attributes.[117] The micelles exhibit a small and consistent particle size, averaging 22 ± 3 nm. They have a low polydisperse index of 0.163 ± 0.032, a high drug loading capacity of 9.88 ± 0.14%, and an exceptional encapsulation efficiency of 98.53 ± 0.02%.[117] Notably, fisetin micelles demonstrate sustained release behavior, heightened cytotoxicity, enhanced cellular uptake, and induction of apoptosis in cancer cells.[50]

Chen et al. highlighted the enhanced therapeutic efficacy of fisetin micelles, formulated with the MPEG-PCL copolymer, in colon cancer treatment. In vitro investigations revealed that fisetin micelles exhibited sustained release characteristics, heightened cytotoxicity, improved cellular uptake, and apoptosis induction in CT26 cells compared with free fisetin. Furthermore, in a CT26 subcutaneous tumor model, fisetin micelles proved more adept at suppressing tumor growth and extending survival rates compared to free fisetin. Notably, fisetin micelles demonstrated superior effectiveness in inducing apoptosis in late-stage CT26 cells than free fisetin. These results suggest that polymeric micelles encapsulating fisetin hold promise for delivering enhanced antitumor activity with reduced systemic toxicity, positioning them as a compelling candidate for future colon cancer therapy.[117]

Nrf2 serves as a pivotal transcription factor orchestrating the expression of endogenous antioxidant enzymes in response to oxidative stress. It functions as a detector of ROS levels within cells, crucial for triggering the cellular antioxidant response.[118] Nrf2 plays a crucial role in preserving the intricate equilibrium between the production of ROS and the availability of inherent antioxidant elements, such as classical antioxidant enzymes.[119] Although its activation is associated with protecting cancer cells from oxidative stress and enhancing cell survival, excessive Nrf2 activation has been linked to chemoresistance and the upregulation of antiapoptotic proteins, demonstrating a complex influence on cancer development and regression.[120,121]

In their study, Pandey and Trigun revealed that fisetin induces apoptosis in CRC cells by inhibiting autophagy and suppressing Nrf2. Fisetin treatment resulted in reduced autophagy flux, increased apoptotic cells marked by heightened cleaved caspase-3 and nuclear poly(ADP-ribose) polymerase-1 signals, compromised mitochondrial membrane potential, and cytochrome c release. Furthermore, fisetin elevated ROS levels and downregulated Nrf2 expression, indicating Nrf2 suppression in fisetin-induced apoptosis in CRC cells. The study revealed fisetin's ability to diminish CRC cell viability in a dose- and time-dependent manner, with an IC50 value of 30 μM after 24 h and 14 μM after 48 h. These insights shed light on fisetin's potential anticancer attributes and its capacity to trigger apoptosis in CRC cells.[33]

Jeng et al. demonstrated that fisetin treatment induced apoptotic cell demise not only in parental LoVo cells but also in oxaliplatin-resistant (OR) and irinotecan-resistant (CPT11) LoVo cells. Examination of cell death post-fisetin treatment revealed a marked decline in cell viability, particularly observed in CPT11-LoVo cells. Fisetin administration elevated apoptotic cell levels across all three cancer cell variants, with a notably higher increase in TUNEL-positive cells in CPT11-LoVo cells compared to parental or OR-LoVo cells. Moreover, fisetin treatment curbed IGF1R and AKT phosphorylation, triggered cytochrome C release, and activated the caspase-8 cascade in the resistant cancer cells. Utilizing a xenograft mouse model, in vivo studies showed that fisetin treatment led to diminished tumor volumes in a dose-dependent fashion. These findings suggest that fisetin may be a promising preventive intervention for colon cancer, demonstrating potential effectiveness in treating both hereditary and chemotherapy-resistant forms of the disease. Further preclinical and clinical trials are necessary to validate and translate these promising results.[32]

Conversely, securin, a versatile protein, regulates various biological functions such as cellular transformation, gene transcription, sister chromatid separation, and DNA damage repair.[122] This protein's interaction with p53 can disrupt p53-mediated transcription and apoptosis in tumor cells, earning it the classification of an oncoprotein.[123]

Leu et al. conducted a study to assess the effects of fisetin on colorectal tumor growth using mouse xenograft models, both alone

and in combination with radiation. The findings revealed that fisetin treatment alone reduced the growth of murine CT26 colon tumors but did not impact human HCT116 colorectal tumors. However, fisetin in conjunction with radiation exhibited augmented tumor-suppressive effects on both CT26 and HCT116 xenograft tumors. Notably, securin-null HCT116 tumors exhibited heightened sensitivity to fisetin treatment, suggesting the potential of fisetin as an adjunct therapy in CRC radiotherapy. The combination of fisetin and radiation proved markedly effective in suppressing CRC growth while circumventing systemic toxicity. Furthermore, the study showed that fisetin induced p53 expression and suppressed securin expression, irrespective of the p53/securin status, contributing to tumor growth inhibition. These findings provide valuable preclinical insights for evaluating fisetin's potential in adjunct cancer radiotherapy.[123]

In their study, Youns and Hegazy explored the inhibitory effects of fisetin, a natural flavonoid, on the proliferation of hepatic, colorectal, and pancreatic cancer cells. Their findings demonstrated that fisetin effectively suppressed cellular growth and induced apoptosis in all tested cell lines. Particularly, HepG-2 cells exhibited notable inhibition of cellular growth, whereas Caco-2 cells exhibited significant apoptosis. Fisetin also affected the activities of cytochrome P450 (CYP450 3A4) and glutathione-*S*-transferase. The study identified key signaling pathways such as NRF2-mediated oxidative stress response, glucocorticoid signaling, cyclin-dependent kinase 5, and extracellular signal-regulated kinase (ERK)/MAPK pathways as crucial contributors to fisetin's growth inhibitory effects on pancreatic and hepatic cancer cells. Gene expression analysis using *reverse transcription polymerase chain reaction* (RT-PCR) and identification of commonly upregulated biomarkers post-fisetin treatment highlighted the potential of fisetin as a safe and valuable antitumor agent.[124]

p38 MAPK serves as a vital signaling protein crucial for cellular responses to various stressors, notably radiation-induced DNA damage, impacting cell survival and apoptosis regulation.[125,126]

Extracellular signal-regulated kinase 1/2 (ERK1/2) belongs to the MAPK family, playing essential roles in regulating cell proliferation, survival, and differentiation.[127,128]

Chen et al. explored the potential of the flavonoid fisetin as a radiosensitizer for human CRC cells, specifically targeting those harboring a p53 mutation associated with radiotherapy resistance. The study outcomes demonstrated that fisetin pretreatment heightened the radiosensitivity of p53-mutant HT29 human CRC cells, resulting in prolonged radiation-induced G2/M arrest and increased apoptosis. Fisetin also impeded radiation-triggered DNA repair mechanisms and cell survival pathways, bolstering radiation-induced cell death. Moreover, fisetin intensified radiation-induced p38 MAPK activation while suppressing AKT and ERK1/2 phosphorylation, curtailing cell survival signals, and fostering apoptosis. These results indicate fisetin's potential as an innovative radiosensitizer against radioresistant human cancer cells, particularly those carrying a p53 mutation.[129]

Capecitabine is an orally administered prodrug of fluorouracil used primarily for the treatment of metastatic breast cancer and CRC.[130,131] It undergoes a three-step enzymatic conversion to its active form, fluorouracil, predominantly within tumor cells, which helps to minimize systemic toxicity.[132]

Zehra et al.[133] successfully developed capecitabine-resistant HT29 cells (CR/HT29) from wild-type HT29 CRC cells, with increased P-gp expression observed in the resistant cells. Capecitabine was found to be less effective on CR/HT29 cells compared to wild-type HT29 cells, demonstrating the acquired resistance. The combination of fisetin and capecitabine led to greater reductions in cell proliferation in wild-type HT29 cells than in capecitabine-resistant cells. Fisetin demonstrated additive effects on the apoptotic pathway in CR/HT29 cells, suggesting its potential in overcoming capecitabine resistance. The study also revealed that CR/HT29 cells appeared to be partially protected from apoptosis compared to their wild-type counterparts. These findings were supported by various experimental methods, including wound healing, colony formation, and cell proliferation assays, which consistently exhibited the additive effects of fisetin on the apoptotic pathway of capecitabine in both wild-type HT29 cells and CR/HT29 cells.

4.2 | Fisetin suppresses inflammation in the colon and CRC

Inflammation plays a significant role in the development of CRC.[134] Pro-inflammatory mediators such as COX-2 and PGE2 contribute to cancer progression.[134,135] Inflammatory mediators contribute to the TME and impact the response to chemotherapy and overall patient survival rates.[135] Understanding the role of inflammatory mediators in CRC is crucial for developing targeted therapies and personalized treatment strategies to improve patient outcomes.

COX, also known as prostaglandin H2 synthase, is the key enzyme responsible for converting arachidonic acid into PGs.[136] Two distinct forms of COX exist: COX-1 and COX-2.[137] Notably, the upregulation of COX-2 is frequently noted in colon tumors, significantly influencing colon carcinogenesis.[138] Research underscores the role of the COX-2 enzyme metabolite, PGE2, as a potent mitogen contributing to colon cancer progression.[139] Although COX-2 stands out as a promising target for CRC therapy and prevention, the extended use of COX-2 inhibitors is constrained by associated cardiovascular side effects.[94] Furthermore, COX-2 exerts downstream effects on the EGFR pathway, a critical player in colon carcinogenesis, suggesting that dual targeting of COX-2 and EGFR may enhance cancer suppression.[140,141]

Suh et al. investigated the therapeutic potential of fisetin in treating colon cancer, revealing its capability to effectively downregulate β-catenin and COX-2, providing a strong foundation for further exploration. Fisetin exhibited inhibitory effects on growth and induced apoptosis in HCT116 and HT29 colon cancer cells, regardless of COX-2 expression. Moreover, fisetin suppressed the COX-2 and Wnt/EGFR/NF-κB signaling pathways, reduced Wnt target gene expression, and hindered colony formation. Additionally, fisetin decreased EGFR and NF-κB activation in HT29 cells, highlighting its

ability to comprehensively target various signaling pathways to combat the progression of colon cancer.[34]

Interleukin-8 (IL-8) acts as a pro-inflammatory cytokine pivotal in immune responses, renowned for its chemoattractant attributes for leukocytes and involvement in pro-tumoral functions like angiogenesis and local growth factor induction.[142] Elevated IL-8 levels have been correlated with various cancers, including CRC, impacting tumor characteristics such as size, stage, infiltration depth, and metastasis.[143]

High-sensitivity C-reactive protein (hs-CRP) serves as a systemic inflammation marker, frequently employed to assess the risk of cardiovascular disease and other inflammatory conditions. Elevated levels of hs-CRP in the bloodstream are linked to an increased likelihood of cardiovascular events, indicating an underlying state of bodily inflammation.[144]

Farsad-Naeimi et al. investigated the impact of fisetin supplementation on inflammatory and metastatic factors in CRC patients receiving chemotherapy. The findings revealed notable reductions in plasma levels of IL-8, hs-CRP, and matrix metalloproteinase-7 in the fisetin group, with IL-8 concentrations showing the most significant changes compared to the placebo group. This suggests that fisetin has the potential to improve the inflammatory status in CRC patients, offering promise as a complementary antitumor therapy. Further studies are needed to fully explore and validate this potential.[145]

4.3 | Modulation of cell cycle by fisetin and other mechanisms

Cell cycle arrest is an important mechanism in the prevention and treatment of CRC.[146] Cell cycle regulation is abnormal in CRC cells, which is a notable characteristic of cancer cells.[146] Induction of cell cycle arrest can inhibit the growth and proliferation of CRC cells.[146]

Cyclin-dependent kinase 2 (CDK2) is a pivotal protein kinase essential for cell cycle regulation.[147] Together with cyclins, CDK2 guides cells from the G1 phase to the S phase, a critical step in DNA synthesis and cell division.[148,149]

Retinoblastoma protein (Rb), an essential tumor suppressor for regulating cell cycle, undergoes phosphorylation by the addition of phosphate groups. This process plays a critical role in halting cell progression from the G1 phase to the S phase.[150,151] Phosphorylation of Rb by cyclin-dependent kinases like CDK2 and cyclin-dependent kinase 4 (CDK4) deactivates Rb, enabling cell cycle progression. The hyperphosphorylated Rb releases E2 promoter binding factor (E2F) transcription factors, stimulating gene expression necessary for cell cycle progression.[147,152] Conversely, hypo-phosphorylated Rb binds to E2F, suppressing its transcriptional activity and hindering cell cycle progression.[153]

Lu et al.[154] investigated the impact of fisetin on cell cycle progression in human colon cancer. The study revealed that fisetin inhibits cell growth and DNA synthesis in HT29 cells in a dose-dependent manner. It induces cell cycle arrest, disrupting the transition from the G1 to the S phase, as well as causing G2/M phase arrest. Fisetin reduces the activities of CDK2 and CDK4, which leads to decreased phosphorylation of Rb. Additionally, fisetin decreases the levels of cyclin E, increases the levels of p21, and is associated with inhibition of CDK2 activity. The study also observed decreased levels of E2 promoter binding factor 1 and cell division cycle 2 (CDC2) proteins. These results suggest that fisetin exerts potential anticarcinogenic effects by directly affecting key regulators of the cell cycle, thereby restraining the growth and progression of colon cancer cells. The study highlights that fisetin is a promising natural anticancer agent and opens doors for further exploration of natural compounds in the development of cancer therapies.[154]

DNA double-strand break (DSB) represents a severe form of DNA damage where both strands of the DNA molecule rupture, often caused by factors like ionizing radiation, ROS, or errors during DNA replication.[155,156] These breaks are critical as they can lead to genetic mutations, cell demise, or genomic instability if not appropriately repaired.[157]

Kirsten rat sarcoma viral oncogene homolog (KRAS) encodes a protein pivotal in cell signaling pathways that regulate cell growth and division.[158] Frequently mutated in cancers like CRC, mutant KRAS is linked to radiotherapy resistance and the activation of downstream signaling cascades such as MAPK/ribosomal S6 kinase (RSK) and PI3K/AKT, which trigger Y-box binding protein 1 (YB-1) activation.[159,160] This YB-1 activation fosters replication stress, heightening downstream signaling and perpetuating constitutive activation of DNA repair pathways.[161]

YB-1 plays crucial roles in CRC progression, metastasis, and treatment resistance. High-grade nuclear expression of YB-1 is detected in a significant percentage of CRC cases and serves as an independent predictor of poor prognosis.[162,163] YB-1 promotes cell proliferation and migration in CRC cell lines, regardless of their RAS/RAF mutation status, and its overexpression is associated with lymph node metastasis and invasion.[162] Furthermore, YB-1 contributes to radioresistance in CRC by activating the CSC marker CD44 and the PI3K/AKT/mTOR signaling pathway, making it a potential therapeutic target for improving treatment outcomes.[162]

The p90 RSK family plays a significant role in CRC progression and metastasis.[161,164] Furthermore, RSK has been implicated in the regulation of cell cycle progression and migration in CRC cells stimulated by parathyroid hormone–related peptide, suggesting its involvement in multiple signaling pathways that contribute to CRC progression.[165] Targeting RSK is therefore being explored as a potential therapeutic strategy for CRC.[166,167]

Khozooei and colleagues revealed significant findings regarding the effects of fisetin on CRC cells. They observed that fisetin exerts a variety of effects on these cells, including inhibiting the phosphorylation of YB-1 and RSK, promoting the phosphorylation of ERK1/2, and disrupting the repair process of DSBs. This interference in DSB repair leads to increased sensitivity of the cells to radiation, regardless of their KRAS mutation status. When combined with ionizing radiation, fisetin not only increases the number of unrepaired DSBs beyond what would occur from the individual effects alone but also enhances the radiosensitivity of CRC cells. The researchers also

demonstrated that inhibiting RSK, AKT, and PAK with FRAX486 impedes the DSB repair induced by ionizing radiation (IR) in CRC cells. However, impaired DSB repair alone is not sufficient to trigger clonogenic activity in response to IR. These findings suggest that fisetin holds promise in enhancing the outcomes of chemoradiotherapy for CRC, regardless of the presence of KRAS mutations.[161]

Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA) is a pivotal kinase within the PI3K/AKT/mTOR pathway, crucial for cellular growth, proliferation, and survival in various solid tumors, particularly CRC.[168,169] Activating mutations in the *PIK3CA* gene are prevalent in about 15%–20% of advanced CRC, correlating with heightened mortality rates.[170] These mutations are recognized as significant oncogenic drivers in multiple cancers, presenting a promising target for novel agents in chemoprevention and therapy endeavors.[171]

5-FU is a standard chemotherapeutic utilized in CRC treatment postsurgery.[172] Despite its common usage, 5-FU's efficacy is limited, and it can induce severe toxicities in patients.[173] This drug functions by inhibiting the growth of cancer cells, leading to their demise. It is commonly administered in conjunction with other treatments to enhance its anticancer efficacy.[173]

Khan et al. investigated the potential of fisetin as an adjunct therapy for the prevention and treatment of PIK3CA-mutant CRC. The study revealed that the combined treatment of fisetin and 5-FU

TABLE 1 Studies consistent with the purpose of this study.

Authors	Dosage	Model	Mechanisms
Yu et al.[106]	100 μM	HCT116 cells	Fisetin induced apoptosis in these cells, as shown by various indicators such as TUNEL assay, annexin V-FITC/PI double staining, Ser15-phosphorylation of p53, and cleavages of procaspase-3 and PARP. These effects were more pronounced in HCT116 cells lacking securin or in cells where securin was suppressed using siRNA
Lim and Park[116]	5–20 micromol/L	HCT116 cells	Fisetin treatment led to DNA condensation; cleavage of PARP; and cleavage of caspases 9, 7, and 3 in HCT116 cells. It also resulted in decreased levels of antiapoptotic proteins, Bcl-xL and Bcl-2, and increased proapoptotic proteins, Bak and Bim
Pandey and Trigun[33]	30 μM	SW-480 cells	Fisetin treatment led to a decrease in the number of autophagic vacuoles, a decline in autophagy flux markers, and an accumulation of p62 in cells. This correlated with an increase in apoptotic cells, as indicated by various markers like annexin-V/propidium iodide and cleaved caspase-3, along with mitochondrial membrane potential collapse and cytochrome c release
Jeng et al.[32]	80 μM	CPT11-LoVo cells	Fisetin treatment effectively inhibited cell viability and induced apoptosis of CPT11-LoVo cells more than oxaliplatin and parental LoVo cancer cells. Western blot assays suggested that fisetin induced apoptosis by promoting the expressions of caspase-8 and cytochrome-C, possibly through inhibiting the abnormal activation of IGF1R and AKT proteins
Leu et al.[123]	5 mg/kg	Mouse	In the murine xenograft tumor model, fisetin treatment alone effectively suppressed the growth of CT26 colon cancer tumors, with limited impact on human HCT116 colorectal tumors. Notably, fisetin downregulated securin protein expression in a p53-independent manner
Chen et al.[129]	100 μM	HT29 cells	Fisetin pretreatment enhanced the radiosensitivity of p53-mutant HT29 human colorectal cancer cells by prolonging radiation-induced G(2)/M arrest, enhancing cell growth arrest, and suppressing radiation-induced phospho-H2AX and phospho-Chk2 in these cells. Fisetin also increased radiation-induced caspase-dependent apoptosis and augmented the phosphorylation of p38 MAPK involved in apoptosis
Suh et al.[34]	240 μM	HT29 cells	Fisetin induced apoptosis in HT29 colon cancer cells, as evidenced by dose-dependent increases in apoptotic markers such as cleaved PARP and caspase-3. Additionally, fisetin decreased the expression of COX-2, a key player in colon carcinogenesis, through multiple mechanisms, including inhibiting COX-2 promoter activity and reducing PGE2 secretion
Farsad-Naeimi et al.[145]	100 mg/kg	Randomized placebo-controlled clinical trial	Fisetin supplementation demonstrated notable effects on reducing inflammatory markers such as IL-8 and hs-CRP and suppressing MMP-7 levels in CRC patients undergoing chemotherapy
Lu et al.[154]	60 μM	HT29cells	Fisetin inhibited cell growth and DNA synthesis dose dependently, resulting in a 79% decrease in cell number with 60 micromol/L fisetin after 72 h. Moreover, treatment with fisetin caused a disturbance in cell cycle progression from G(1) to S phase at 8 h and induced a G2/M phase arrest after 24 h

Abbreviations: AKT, Ak strain transforming; COX-2, cyclooxygenase-2; CRC, colorectal cancer; hs-CRP, high-sensitivity C-reactive protein; IGF1R, insulin-like growth factor 1 receptor; IL-8, interleukin-8; MAPK, mitogen-activated protein kinase; MMP-7, matrix metalloproteinase-7; PARP, poly(ADP-ribose) polymerase; PGE2, prostaglandin E2; siRNA, small interfering RNA; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

resulted in a reduction in PI3K expression, AKT phosphorylation, mTOR activity, and downstream target proteins in CRC cells with a PIK3CA mutation. This combination not only lowered tumor multiplicity in experimental mice but also significantly reduced tumor numbers throughout the intestinal tract. Moreover, the intervention with fisetin and 5-FU appeared to extend the lifespan of the experimental animals. The research suggests that fisetin could serve as both a preventive measure and an additional therapy in conjunction with 5-FU for PIK3CA-mutant CRC. Although these findings highlight the potential of fisetin as a dietary adjunct in preventing and treating PIK3CA-mutant CRC, the necessity for larger-scale studies to confirm its impact on mouse survival is emphasized. The overall results underscore the promise of fisetin as a complementary approach for managing PIK3CA-mutant CRC.[168] Table 1 and Figure 2 summarize the results of the studies.

5 | CONCLUSION

This study provides a comprehensive examination of the effects of fisetin on CRC in animal models, providing valuable insights into the compound's potential as a therapeutic agent. Fisetin, a natural flavonoid found in various fruits and vegetables, exhibits a wide range of biological activities that are beneficial in combating CRC. The compound has been shown to induce apoptosis in CRC cells through multiple mechanisms, including the activation of the p53 pathway, release of apoptotic components, and inhibition of key signaling pathways such as COX-2 and Wnt/EGFR/NF-κB. Additionally, fisetin demonstrates anti-inflammatory properties by reducing the levels of pro-inflammatory cytokines and inhibiting the NF-κB signaling pathway. The antioxidant properties of fisetin further contribute to its anticancer effects by scavenging ROS and enhancing the cellular antioxidant defense system.

Moreover, fisetin has been found to enhance the efficacy of conventional chemotherapeutic agents like 5-FU in treating PIK3CA-mutant CRC, suggesting its potential as an adjunct therapy. The compound's ability to inhibit angiogenesis, a critical process in tumor growth and metastasis, adds another layer to its therapeutic potential. Fisetin's impact on cell cycle regulation, particularly its ability to induce cell cycle arrest, further underscores its role in inhibiting cancer cell proliferation. The findings from various in vitro and in vivo studies provide a strong foundation for the potential use of fisetin in clinical settings.

Despite the promising results, further research is needed to fully understand the molecular mechanisms underlying fisetin's anticancer effects and to optimize its bioavailability and

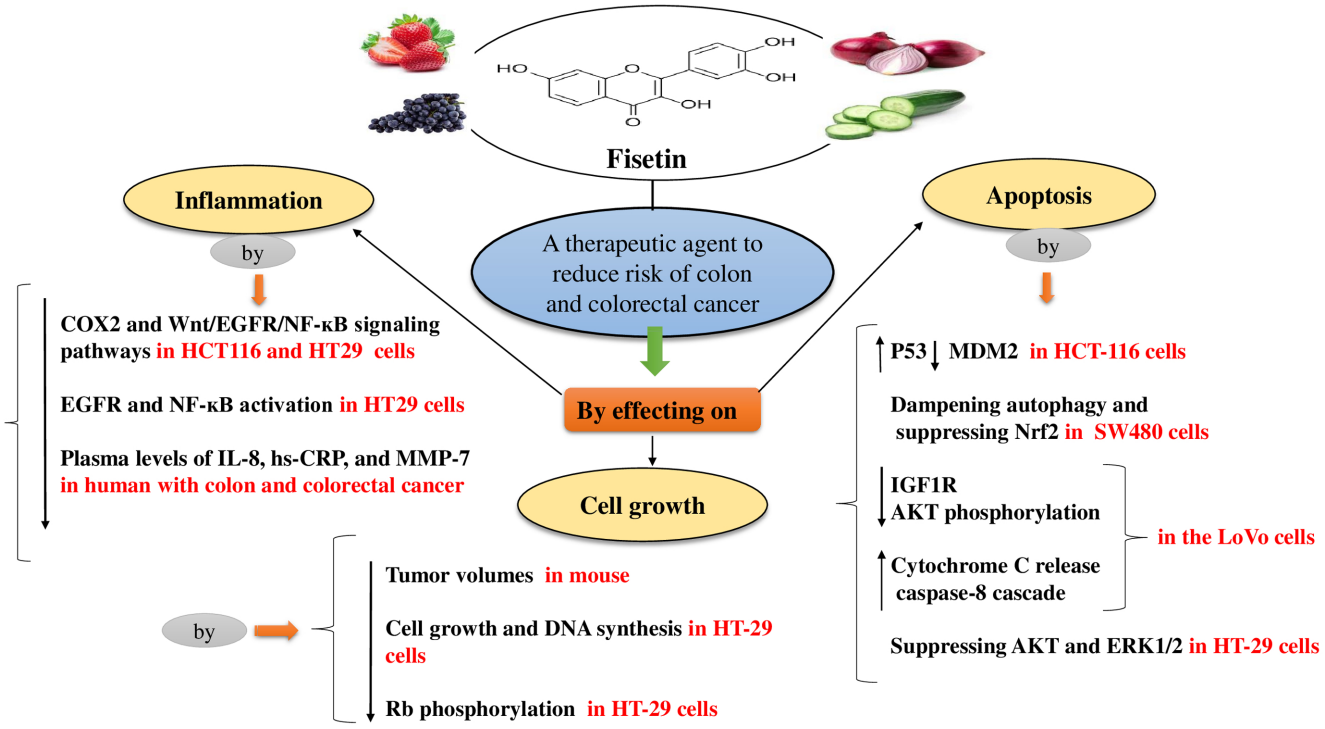

FIGURE 2 Effects of fisetin on colon and colorectal cancer (CRC). Fisetin exerts significant effects on inflammation, apoptosis, and cell growth in colon and CRC. It suppresses inflammation in the colon and CRC, and promotes apoptosis to suppress the growth of cancer cells. Fisetin induces colon cancer cell apoptosis through multiple mechanisms. It impacts the p53 pathway, releases crucial apoptotic components, and inhibits the cyclooxygenase-2 (COX-2) and Wnt/epidermal growth factor receptor (EGFR)/nuclear factor kappa B (NF-κB) signaling pathways. Fisetin reduces cell viability and promotes cell death by affecting caspase activation and cytochrome C release. It also inhibits the insulin-like growth factor 1 receptor (IGF1R)/protein kinase B (AKT [Ak strain transforming]) pathway, which is associated with apoptosis regulation. Additionally, fisetin has been found to induce cell cycle arrest and disrupt G1- to S-phase transition, as well as cause a G2/M phase arrest, ultimately influencing key cell cycle regulators.

therapeutic efficacy. Clinical trials are essential to validate the preclinical findings and to determine the safety and efficacy of fisetin in human subjects. The development of novel delivery systems, such as polymeric nanoparticles and micelles, could enhance the bioavailability and therapeutic potential of fisetin. Additionally, exploring the synergistic effects of fisetin with other anticancer agents could open new avenues for combination therapies in CRC treatment.

Overall, fisetin represents a promising natural compound with multifaceted anticancer properties that could significantly improve the management of CRC. The integration of fisetin into current therapeutic regimens could potentially reduce the toxicity and resistance associated with conventional chemotherapies. As research progresses, fisetin may emerge as a valuable component in the fight against CRC, offering hope for better patient outcomes and improved quality of life.

AUTHOR CONTRIBUTIONS

Mohammad Yasin Zamanian: Conceptualization; investigation; project administration; supervision; writing – review and editing. **Niloofar Taheri:** Writing – original draft. **Montather F. Ramadan:** Resources. **Yasser Fakri Mustafa:** Data curation; methodology. **Safa Alkhayyat:** Resources. **Klunko Nataliya Sergeevna:** Writing – original draft. **Hashem O. Alsaab:** Data curation; resources. **Farnoosh Molavi Vasei:** Writing – original draft. **Ahmed Hjazi:** Resources; validation. **Siamak Daneshvar:** Conceptualization; project administration; writing – review and editing.

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REFERENCES

1. Sharma I, Kim S, Sridhar S, Basha R. Colorectal cancer: an emphasis on factors influencing racial/ethnic disparities. *Crit Rev Oncog*. 2020;25(2):151-160.
2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249.
3. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol*. 2021;14(10):101174.
4. Pita-Fernandez S, Alhayek-Ai M, Gonzalez-Martin C, Lopez-Calvino B, Seoane-Pillado T, Pértega-Díaz S. Intensive follow-up strategies improve outcomes in nonmetastatic colorectal cancer patients after curative surgery: a systematic review and meta-analysis. *Ann Oncol*. 2015;26(4):644-656.
5. Mitchell D, Puckett Y, Nguyen QN. Literature review of current management of colorectal liver metastasis. *Cureus*. 2019;11(1):39-49.
6. Zhu W, Oteiza PI. NADPH oxidase 1: a target in the capacity of dimeric ECG and EGCG procyanidins to inhibit colorectal cancer cell invasion. *Redox Biol*. 2023;65:102827.
7. Vodenkova S, Buchler T, Cervena K, Veskrnova V, Vodicka P, Vymetalkova V. 5-fluorouracil and other fluoropyrimidines in colorectal cancer: past, present and future. *Pharmacol Ther*. 2020;206:107447.
8. Lee Y-J, Cho JM, Sai S, et al. 5-fluorouracil as a tumor-treating field-sensitizer in colon cancer therapy. *Cancer*. 2019;11(12):1999.
9. Lee JJ, Beumer JH, Chu E. Therapeutic drug monitoring of 5-fluorouracil. *Cancer Chemother Pharmacol*. 2016;78:447-464.
10. Khorrami S, Zarepour A, Zarrabi A. Green synthesis of silver nanoparticles at low temperature in a fast pace with unique DPPH radical scavenging and selective cytotoxicity against MCF-7 and BT-20 tumor cell lines. *Biotech Rep*. 2019;24:e00393.
11. Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. Plant flavonoids: classification, distribution, biosynthesis, and antioxidant activity. *Food Chem*. 2022;383:132531.
12. Swallah MS, Sun H, Affoh R, Fu H, Yu H. Antioxidant potential overviews of secondary metabolites (polyphenols) in fruits. *Int J Food Sci*. 2020;2020:1-8.
13. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *J Nutr Sci*. 2016;5:e47.
14. Karak P. Biological activities of flavonoids: An overview. *Int J Pharm Sci Res*. 2019;10(4):1567-1574.
15. Mehra A, Chatterjee A, Kumar N. Biochemistry and applications of flavonoids. In: Saini D, Kesharwani RK, Keservani RK, eds. *The Flavonoids*. Apple Academic Press; 2024:79-98.
16. Gervasi T, Calderaro A, Barreca D, et al. Biotechnological applications and health-promoting properties of flavonols: An updated view. *Int J Mol Sci*. 2022;23(3):1710.
17. Kesharwani V, Kabra S, Semwal BC, Saini D. Neuroprotective effects of flavonoids. In: Sharma N, Saini D, Kesharwani RK, Gupta PC, Keservani RK, eds. *Advances in Flavonoids for Human Health and Prevention of Diseases*. Apple Academic Press; 2024:95-123.
18. Chang H, Lei L, Zhou Y, Ye F, Zhao G. Dietary flavonoids and the risk of colorectal cancer: An updated meta-analysis of epidemiological studies. *Nutrients*. 2018;10(7):950.
19. Li Y, Zhang T, Chen GY. Flavonoids and colorectal cancer prevention. *Antioxidants*. 2018;7(12):187.
20. Fernández J, Silván B, Entrialgo-Cadierno R, et al. Antiproliferative and palliative activity of flavonoids in colorectal cancer. *Biomed Pharmacother*. 2021;143:112241.
21. Hassani S, Maghsoudi H, Fattahi F, et al. Flavonoids nanostructures promising therapeutic efficiencies in colorectal cancer. *Int J Biol Macromol*. 2023;241:124508.
22. Pal HC, Pearlman RL, Afaq F. Fisetin and its role in chronic diseases. *Anti-Inflamm Nutraceut Chron Dis*. 2016;928:213-244.
23. Jash SK, Mondal S. Bioactive flavonoid fisetin—a molecule of pharmacological interest. *Cardiovasc Dis*. 2014;5(6):89-128.
24. Kubina R, Iriti M, Kabała-Dzik A. Anticancer potential of selected flavonols: Fisetin, kaempferol, and quercetin on head and neck cancers. *Nutrients*. 2021;13(3):845.
25. Rahmani AH, Almatroudi A, Allemailem KS, Khan AA, Almatroodi SA. The potential role of fisetin, a flavonoid in cancer prevention and treatment. *Molecules*. 2022;27(24):9009.
26. Farooqi AA, Naureen H, Zahid R, Youssef L, Attar R, Xu B. Cancer chemopreventive role of fisetin: regulation of cell signaling pathways in different cancers. *Pharmacol Res*. 2021;172:105784.

27. Ravula AR, Teegala SB, Kalakotla S, Pasangulapati JP, Perumal V, Boyina HK. Fisetin, potential flavonoid with multifarious targets for treating neurological disorders: An updated review. *Eur J Pharmacol*. 2021;910:174492.
28. Rengarajan T, Yaacob NS. The flavonoid fisetin as an anticancer agent targeting the growth signaling pathways. *Eur J Pharmacol*. 2016;789:8-16.
29. Wang H. MicroRNAs and apoptosis in colorectal cancer. *Int J Mol Sci*. 2020;21(15):5353.
30. Ismail NI, Othman I, Abas F, H. Lajis N, Naidu R. Mechanism of apoptosis induced by curcumin in colorectal cancer. *Int J Mol Sci*. 2019;20(10):2454.
31. Sundarraj K, Raghunath A, Perumal E. A review on the chemotherapeutic potential of fisetin: in vitro evidences. *Biomed Pharmacother*. 2018;97:928-940.
32. Jeng LB, Kumar Velmurugan B, Chen MC, et al. Fisetin mediated apoptotic cell death in parental and oxaliplatin/irinotecan resistant colorectal cancer cells in vitro and in vivo. *J Cell Physiol*. 2018;233(9):7134-7142.
33. Pandey A, Trigun SK. Fisetin induces apoptosis in colorectal cancer cells by suppressing autophagy and down-regulating nuclear factor erythroid 2-related factor 2 (Nrf2). *J Cell Biochem*. 2023;124(9):1289-1308.
34. Suh Y, Afaq F, Johnson JJ, Mukhtar H. A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-κB-signaling pathways. *Carcinogenesis*. 2009;30(2):300-307.
35. Ding H, Li Y, Chen S, et al. Fisetin ameliorates cognitive impairment by activating mitophagy and suppressing neuroinflammation in rats with sepsis-associated encephalopathy. *CNS Neurosci Ther*. 2022;28(2):247-258.
36. Markowska A, Antoszczak M, Kacprzak K, Markowska J, Huczyński A. Role of Fisetin in selected malignant neoplasms in women. *Nutrients*. 2023;15(21):4686.
37. Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J Nutr*. 2000;130(9):2243-2250.
38. Kimira M, Arai Y, Shimoi K, Watanabe S. Japanese intake of flavonoids and isoflavonoids from foods. *J Epidemiol*. 1998;8(3):168-175.
39. Prabhu K, Bhute AS. Plant based natural dyes and mordants: a review. *J Nat Prod Plant Resour*. 2012;2(6):649-664.
40. Kashyap D, Sharma A, Sak K, Tuli HS, Buttar HS, Bishayee A. Fisetin: a bioactive phytochemical with potential for cancer prevention and pharmacotherapy. *Life Sci*. 2018;194:75-87.
41. Hassan SS, Samanta S, Dash R, et al. The neuroprotective effects of fisetin, a natural flavonoid in neurodegenerative diseases: focus on the role of oxidative stress. *Front Pharmacol*. 2022;13:1015835.
42. Tordera M, Ferrándiz ML, Alcaraz MJ. Influence of anti-inflammatory flavonoids on degranulation and arachidonic acid release in rat neutrophils. *Zeitschrift für Naturforschung C*. 1994;49(3–4):235-240.
43. Touil YS, Auzeil N, Boulinguez F, et al. Fisetin disposition and metabolism in mice: identification of geraldol as an active metabolite. *Biochem Pharmacol*. 2011;82(11):1731-1739.
44. Jo JH, Jo JJ, Lee JM, Lee S. Identification of absolute conversion to geraldol from fisetin and pharmacokinetics in mouse. *J Chromatogr B*. 2016;1038:95-100.
45. Huang M-C, Hsueh TY, Cheng YY, Lin LC, Tsai TH. Pharmacokinetics and biliary excretion of fisetin in rats. *J Agric Food Chem*. 2018;66(25):6300-6307.
46. Shia C-S, Tsai SY, Kuo SC, Hou YC, Chao PDL. Metabolism and pharmacokinetics of 3, 3′, 4′, 7-tetrahydroxyflavone (fisetin), 5-hydroxyflavone, and 7-hydroxyflavone and antihemolysis effects of fisetin and its serum metabolites. *J Agric Food Chem*. 2009;57(1):83-89.
47. Feng C, Yuan X, Chu K, Zhang H, Ji W, Rui M. Preparation and optimization of poly (lactic acid) nanoparticles loaded with fisetin to improve anti-cancer therapy. *Int J Biol Macromol*. 2019;125:700-710.
48. Pawar A, Singh S, Rajalakshmi S, Shaikh K, Bothiraja C. Development of fisetin-loaded folate functionalized pluronic micelles for breast cancer targeting. *Artif Cells, Nanomed Biotech*. 2018;46(sup1):347-361.
49. Kumar R, Kumar R, Khurana N, et al. Enhanced oral bioavailability and neuroprotective effect of fisetin through its SNEDDS against rotenone-induced Parkinson's disease rat model. *Food Chem Toxicol*. 2020;144:111590.
50. Szymczak J, Cielecka-Piontek J. Fisetin—in search of better bioavailability—from macro to nano modifications: a review. *Int J Mol Sci*. 2023;24(18):14158.
51. Syed DN, Adhami VM, Khan N, Khan MI, Mukhtar H. Exploring the molecular targets of dietary flavonoid fisetin in cancer. In: Vincent T, ed. *Seminars in Cancer Biology*. Vol 40–41. Elsevier; 2016:130-140.
52. Sari EN, Soysal Y. Molecular and therapeutic effects of fisetin flavonoid in diseases. *J Basic Clin Health Sci*. 2020;4(3):190-196.
53. Prasath GS, Subramanian SP. Modulatory effects of fisetin, a bioflavonoid, on hyperglycemia by attenuating the key enzymes of carbohydrate metabolism in hepatic and renal tissues in streptozotocin-induced diabetic rats. *Eur J Pharmacol*. 2011;668(3):492-496.
54. Currais A, Prior M, Dargusch R, et al. Modulation of p25 and inflammatory pathways by fisetin maintains cognitive function in a lzheimer's disease transgenic mice. *Aging Cell*. 2014;13(2):379-390.
55. Guo P, Feng Y-Y. Anti-inflammatory effects of kaempferol, myricetin, fisetin and ibuprofen in neonatal rats. *Trop J Pharmaceut Res*. 2017;16(8):1819-1826.
56. Kumar RM, Kumar H, Bhatt T, et al. Fisetin in cancer: attributes, developmental aspects, and nanotherapeutics. *Pharmaceuticals*. 2023;16(2):196.
57. Park H-H, Lee S, Son HY, et al. Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. *Arch Pharm Res*. 2008;31:1303-1311.
58. Kang KA, Piao MJ, Kim KC, et al. Fisetin attenuates hydrogen peroxide-induced cell damage by scavenging reactive oxygen species and activating protective functions of cellular glutathione system. *In Vitro Cell Develop Biol-Animal*. 2014;50:66-74.
59. Naeimi AF, Alizadeh M. Antioxidant properties of the flavonoid fisetin: An updated review of in vivo and in vitro studies. *Trends Food Sci Technol*. 2017;70:34-44.
60. Wu F-H, Wei HZ, Deng HY, Xiao GH, Zhang YC. PARP in colorectal cancer: molecular mechanisms, immunity, clinical trials, and drug combinations. *Neoplasma*. 2022;70(1):220724N745.
61. Rudmik L, Magliocco A. Molecular mechanisms of hepatic metastasis in colorectal cancer. *J Surg Oncol*. 2005;92(4):347-359.
62. Ni Y, Liang Y, Li M, et al. The updates on metastatic mechanism and treatment of colorectal cancer. *Pathol-Res Pract*. 2023;251:154837.
63. Dakowicz D, Zajkowska M, Mroczko B. Relationship between VEGF family members, their receptors and cell death in the neoplastic transformation of colorectal cancer. *Int J Mol Sci*. 2022;23(6):3375.
64. Yang Z, Zhang X, Bai X, Xi X, Liu W, Zhong W. Anti-angiogenesis in colorectal cancer therapy. *Cancer Sci*. 2024;115:734-751.
65. Yin L, Li J, Ma D, Li D, Sun Y. Angiogenesis in primary colorectal cancer and matched metastatic tissues: biological and clinical implications for anti-angiogenic therapies. *Oncol Lett*. 2020;19(5):3558-3566.
66. Sun W. Angiogenesis in metastatic colorectal cancer and the benefits of targeted therapy. *J Hematol Oncol*. 2012;5:1-9.

67. Rasheed S, McDonald PJ, Northover JM, Guenther T. Angiogenesis and hypoxic factors in colorectal cancer. *Pathol-Res Pract*. 2008;204(7):501-510.
68. Bendardaf R, Buhmeida A, Hilska M, et al. VEGF-1 expression in colorectal cancer is associated with disease localization, stage, and long-term disease-specific survival. *Anticancer Res*. 2008;28(6B):3865-3870.
69. Mohamed SY, Mohammed HL, Ibrahim HM, Mohamed EM, Salah M. Role of VEGF, CD105, and CD31 in the prognosis of colorectal cancer cases. *J Gastrointest Cancer*. 2019;50:23-34.
70. Mazeda I, Martins SF, Garcia EA, Rodrigues M, Longatto A. VEGF expression in colorectal cancer metastatic lymph nodes: clinicopathological correlation and prognostic significance. *Gastrointest Disorders*. 2020;2(3):25-280.
71. Jitawatanarat P, Ma WW. Update on antiangiogenic therapy in colorectal cancer: aflibercept and regorafenib. *J Gastrointest Oncol*. 2013;4(2):231.
72. Lopez A, Harada K, Vasilakopoulou M, Shanbhag N, Ajani JA. Targeting angiogenesis in colorectal carcinoma. *Drugs*. 2019;79:63-74.
73. Oguntade AS, al-Amodi F, Alrumayh A, Alobaida M, Bwalya M. Anti-angiogenesis in cancer therapeutics: the magic bullet. *J Egypt Natl Canc Inst*. 2021;33(1):1-11.
74. Hansen TF, Qvortrup C, Pfeiffer P. Angiogenesis inhibitors for colorectal cancer. A review of the clinical data. *Cancer*. 2021;13(5):1031.
75. Saclarides TJ. Angiogenesis in colorectal cancer. *Surg Clin North America*. 1997;77(1):253-260.
76. Baker L, Robinson N, Wilson D, Tabaqchali M, Leaper D. Vascular endothelial growth factor in colorectal cancer pathology, survival and treatment. *Ann Oncol*. 2019;30:v12.
77. Li N, Babaei-Jadidi R, Lorenzi F, et al. An FBXW7-ZEB2 axis links EMT and tumour microenvironment to promote colorectal cancer stem cells and chemoresistance. *Oncogenesis*. 2019;8(3):13.
78. Nair VA, Malhab LJB, Abdel-Rahman WM. Characterization of the molecular alterations induced by the prolonged exposure of Normal Colon mucosa and Colon Cancer cells to low-dose Bisphenol a. *Int J Mol Sci*. 2022;23(19):11620.
79. Bardelčíková A, Šoltys J, Mojžiš J. Oxidative stress, inflammation and colorectal cancer: an overview. *Antioxidants*. 2023;12(4):901.
80. Carini F, Mazzola M, Rappa F, et al. Colorectal carcinogenesis: role of oxidative stress and antioxidants. *Anticancer Res*. 2017;37(9):4759-4766.
81. Basak D, Uddin MN, Hancock J. The role of oxidative stress and its counteractive utility in colorectal cancer (CRC). *Cancer*. 2020;12(11):3336.
82. Acevedo-León D, Gómez-Abril SÁ, Sanz-García P, Estañ-Capell N, Bañuls C, Sáez G. The role of oxidative stress, tumor and inflammatory markers in colorectal cancer patients: a one-year follow-up study. *Redox Biol*. 2023;62:102662.
83. Zamanian M, Hajizadeh M, Shamsizadeh A, et al. Effects of naringin on physical fatigue and serum MMP-9 concentration in female rats. *Pharm Biol*. 2017;55(1):423-427.
84. Gu H, Li B, Xiang L, et al. Association between oxidative stress exposure and colorectal cancer risk in 98,395 participants: results from a prospective study. *Front Nutr*. 2023;10(1):1284066.
85. Qadir VA, Abdoulrahman KK. Oxidative stress assessment in colorectal cancer patients. *ARO-Sci J KOYA Univ*. 2024;12(1):115-123.
86. Abdul-Aziz Ahmed K, Jabbar AAJ, Abdulla MA, et al. Mangiferin (mango) attenuates AOM-induced colorectal cancer in rat's colon by augmentation of apoptotic proteins and antioxidant mechanisms. *Sci Rep*. 2024;14(1):813.
87. Liu H, Liu X, Zhang C, et al. Redox imbalance in the development of colorectal cancer. *J Cancer*. 2017;8(9):1586-1597.
88. Wu H, Zhong M, Wang Y. The interdependence of inflammation and ROS in cancer: focus on tumor microenvironment. *Handb Oxid Stress Cancer: Mech Aspects*. 2021;3:1-17.
89. Muthusami S, Ramachandran IK, Babu KN, et al. Role of inflammation in the development of colorectal cancer. *Endocr Metab Immune Disord-Drug Targ*. 2021;21(1):77-90.
90. Chen X-W, Zhou S-F. *Inflammation, Cytokines, the IL-17/IL-6/STAT3/NF-κB Axis, and Tumorigenesis*. Taylor & Francis; 2015:2941-2946.
91. Janakiram NB, Rao CV. The role of inflammation in colon cancer. *Inflamm Cancer*. 2014;816:25-52.
92. Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-κB collaboration and crosstalk in cancer. *Cytokine Growth Factor Rev*. 2010;21(1):11-19.
93. Lee D-Y, Song M-Y, Kim E-H. Role of oxidative stress and Nrf2/KEAP1 signaling in colorectal cancer: mechanisms and therapeutic perspectives with phytochemicals. *Antioxidants*. 2021;10(5):743.
94. Wang D, DuBois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene*. 2010;29(6):781-788.
95. Sheng J, Sun H, Yu FB, Li B, Zhang Y, Zhu YT. The role of cyclooxygenase-2 in colorectal cancer. *Int J Med Sci*. 2020;17(8):1095-1101.
96. Liu Y, Sun H, Hu M, et al. The role of cyclooxygenase-2 in colorectal carcinogenesis. *Clin Colorectal Cancer*. 2017;16(3):165-172.
97. Orlandi G, Roncucci L, Carnevale G, Sena P. Different roles of apoptosis and autophagy in the development of human colorectal cancer. *Int J Mol Sci*. 2023;24(12):10201.
98. Tanzadehpanah H, Avan A, Ghayour-Mobarhan M, et al. MicroRNAs regulate inhibitor of apoptosis proteins (IAPs) in colorectal cancer. *MicroRNA*. 2023;12(3):210-220.
99. Liu Z-B, Zhang T, Ye X, et al. Natural substances derived from herbs or plants are promising sources of anticancer agents against colorectal cancer via triggering apoptosis. *J Pharm Pharmacol*. 2022;74(2):162-178.
100. Phang C-W, Karsani SA, Sethi G, Abd Malek SN. Flavokawain C inhibits cell cycle and promotes apoptosis, associated with endoplasmic reticulum stress and regulation of MAPKs and Akt signaling pathways in HCT 116 human colon carcinoma cells. *PLoS ONE*. 2016;11(2):e0148775.
101. Ganash MA. Anticancer potential of ascorbic acid and inorganic selenium on human breast cancer cell line MCF-7 and colon carcinoma HCT-116. *J Cancer Res Ther*. 2021;17(1):122-129.
102. Li X-L, Zhou J, Chen ZR, Chng WJ. P53 mutations in colorectal cancer-molecular pathogenesis and pharmacological reactivation. *World J Gastroenterol: WJG*. 2015;21(1):84-93.
103. Marei HE, Althani A, Afifi N, et al. p53 signaling in cancer progression and therapy. *Cancer Cell Int*. 2021;21(1):1-15.
104. Cui D, Qu R, Liu D, Xiong X, Liang T, Zhao Y. The cross talk between p53 and mTOR pathways in response to physiological and genotoxic stresses. *Front Cell Develop Biol*. 2021;9:775507.
105. Hientz K, Mohr A, Bhakta-Guha D, Efferth T. The role of p53 in cancer drug resistance and targeted chemotherapy. *Oncotarget*. 2017;8(5):8921-8946.
106. Yu S-H, Yang PM, Peng CW, Yu YC, Chiu SJ. Securin depletion sensitizes human colon cancer cells to fisetin-induced apoptosis. *Cancer Lett*. 2011;300(1):96-104.
107. Berthelet J, Dubrez L. Regulation of apoptosis by inhibitors of apoptosis (IAPs). *Cells*. 2013;2(1):163-187.
108. Ola MS, Nawaz M, Ahsan H. Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. *Mol Cell Biochem*. 2011;351:41-58.
109. Shi M, Yan SG, Xie ST, Wang HN. Tip30-induced apoptosis requires translocation of Bax and involves mitochondrial release of cytochrome c and Smac/DIABLO in hepatocellular carcinoma cells. *Biochim Biophys Acta Mol Cell Res*. 2008;1783(2):263-274.
110. Shiozaki EN, Shi Y. Caspases, IAPs and Smac/DIABLO: mechanisms from structural biology. *Trends Biochem Sci*. 2004;29(9):486-494.
111. Shi D, Gu W. Dual roles of MDM2 in the regulation of p53: ubiquitination dependent and ubiquitination independent

mechanisms of MDM2 repression of p53 activity. *Genes Cancer*. 2012;3(3–4):240-248.
112. Giansanti C, Manzini V, Dickmanns A, et al. MDM2 binds and ubiquitinates PARP1 to enhance DNA replication fork progression. *Cell Rep*. 2022;39(9):110879.
113. Jiang H, Luo J, Lei H. The roles of mouse double minute 2 (MDM2) oncoprotein in ocular diseases: a review. *Exp Eye Res*. 2022;217:108910.
114. Lu X, Yan C, Huang Y, et al. Mouse double minute 2 (MDM2) upregulates snail expression and induces epithelial-to-mesenchymal transition in breast cancer cells in vitro and in vivo. *Oncotarget*. 2016;7(24):37177-37191.
115. Li W, Peng X, Lang J, Xu C. Targeting mouse double minute 2: current concepts in DNA damage repair and therapeutic approaches in cancer. *Front Pharmacol*. 2020;11:537486.
116. Lim DY, Park JHY. Induction of p53 contributes to apoptosis of HCT-116 human colon cancer cells induced by the dietary compound fisetin. *Am J Physiol Gastrointest Liver Phys Ther*. 2009;296(5):G1060-G1068.
117. Chen Y, Wu Q, Song L, et al. Polymeric micelles encapsulating fisetin improve the therapeutic effect in colon cancer. *ACS Appl Mater Interfaces*. 2015;7(1):534-542.
118. Kasai S, Shimizu S, Tatara Y, Mimura J, Itoh K. Regulation of Nrf2 by mitochondrial reactive oxygen species in physiology and pathology. *Biomolecules*. 2020;10(2):320.
119. Hegazy AM, el-Sayed EM, Ibrahim KS, Abdel-Azeem AS. Dietary antioxidant for disease prevention corroborated by the Nrf2 pathway. *J Complement Integr Med*. 2019;16(3):20180161.
120. Sajadimajd S, Khazaei M. Oxidative stress and cancer: the role of Nrf2. *Curr Cancer Drug Targets*. 2018;18(6):538-557.
121. Panieri E, Saso L. Inhibition of the NRF2/KEAP1 axis: a promising therapeutic strategy to alter redox balance of cancer cells. *Antioxid Redox Signal*. 2021;34(18):1428-1483.
122. Chen T, Sun Y, Ji P, Kopetz S, Zhang W. Topoisomerase IIα in chromosome instability and personalized cancer therapy. *Oncogene*. 2015;34(31):4019-4031.
123. Leu JD, Wang BS, Chiu SJ, et al. Combining fisetin and ionizing radiation suppresses the growth of mammalian colorectal cancers in xenograft tumor models. *Oncol Lett*. 2016;12(6):4975-4982.
124. Youns M, Abdel Halim Hegazy W. The natural flavonoid fisetin inhibits cellular proliferation of hepatic, colorectal, and pancreatic cancer cells through modulation of multiple signaling pathways. *PLoS ONE*. 2017;12(1):e0169335.
125. Roy S, Roy S, Rana A, Akhter Y, Hande MP, Banerjee B. The role of p38 MAPK pathway in p53 compromised state and telomere mediated DNA damage response. *Mut Res/Genetic Toxicol Environ Mutagen*. 2018;836:89-97.
126. Yue J, López JM. Understanding MAPK signaling pathways in apoptosis. *Int J Mol Sci*. 2020;21(7):2346.
127. Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y, Hu LL. ERK/MAPK signalling pathway and tumorigenesis. *Exp Ther Med*. 2020;19(3):1997-2007.
128. Cheng P, Alberts I, Li X. The role of ERK1/2 in the regulation of proliferation and differentiation of astrocytes in developing brain. *Int J Dev Neurosci*. 2013;31(8):783-789.
129. Chen W-S, Lee YJ, Yu YC, et al. Enhancement of p53-mutant human colorectal cancer cells radiosensitivity by flavonoid fisetin. *Int J Radiat Oncol Biol Phys Ther*. 2010;77(5):1527-1535.
130. Lang T, Zhu R, Zhu X, et al. Combining gut microbiota modulation and chemotherapy by capecitabine-loaded prebiotic nanoparticle improves colorectal cancer therapy. *Nat Commun*. 2023;14(1):4746.
131. Knudsen A-DM, Modvig MW, Vogsen M, Kodahl AR. Effect of capecitabine as monotherapy for HER2 normal metastatic breast cancer. *Med Oncol*. 2024;41(5):99.
132. Saif MW, Katirtzoglou NA, Syrigos KN. Capecitabine: an overview of the side effects and their management. *Anti-Cancer Drugs*. 2008;19(5):447-464.
133. Zehra K, Banu A, Can E, Hülya C. Fisetin and/or capecitabine causes changes in apoptosis pathways in capecitabine-resistant colorectal cancer cell lines. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2024;397:1-14.
134. Aoyama T, Yukawa N, Saito A. Clinical impact of nutrition and inflammation assessment tools in colorectal cancer treatment. *Anticancer Res*. 2024;44(4):1335-1351.
135. Borowczak J, Szczerbowski K, Maniewski M, et al. The role of inflammatory cytokines in the pathogenesis of colorectal carcinoma—recent findings and review. *Biomedicine*. 2022;10(7):1670.
136. Smith WL, Urade Y, Jakobsson P-J. Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis. *Chem Rev*. 2011;111(10):5821-5865.
137. Morita I. Distinct functions of COX-1 and COX-2. *Prostaglandins Other Lipid Mediat*. 2002;68:165-175.
138. Negi RR, Rana SV, Gupta V, et al. Over-expression of cyclooxygenase-2 in colorectal cancer patients. *Asian Pac J Cancer Prevent*. 2019;20(6):1675-1681.
139. Karpisheh V, Nikkhoo A, Hojjat-Farsangi M, et al. Prostaglandin E2 as a potent therapeutic target for treatment of colon cancer. *Prostaglandins Other Lipid Mediat*. 2019;144:106338.
140. Wang D, Xia D, DuBois RN. The crosstalk of PTGS2 and EGF signaling pathways in colorectal cancer. *Cancer*. 2011;3(4):3894-3908.
141. Musa A, Mostafa EM, Bukhari SNA, et al. EGFR and COX-2 dual inhibitor: the design, synthesis, and biological evaluation of novel chalcones. *Molecules*. 2022;27(4):1158.
142. Mir MA, Rashid M, Jan N. The interleukin-8 pathway in cancer. In: In: Mir MA, ed. *Cytokine and Chemokine Networks in Cancer*. Springer; 2023;14(21):165-190.
143. Rubie C, Frick VO, Pfeil S, et al. Correlation of IL-8 with induction, progression and metastatic potential of colorectal cancer. *World J Gastroenterol: WJG*. 2007;13(37):4996-5002.
144. Denegri A, Boriani G. High sensitivity C-reactive protein (hsCRP) and its implications in cardiovascular outcomes. *Curr Pharm Des*. 2021;27(2):263-275.
145. Farsad-Naeimi A, Alizadeh M, Esfahani A, Darvish Aminabad E. Effect of fisetin supplementation on inflammatory factors and matrix metalloproteinase enzymes in colorectal cancer patients. *Food Funct*. 2018;9(4):2025-2031.
146. Kouroshnia A, Zeinali S, Irani S, Sadeghi A. Induction of apoptosis and cell cycle arrest in colorectal cancer cells by novel anticancer metabolites of Streptomyces sp. 801. *Cancer Cell Int*. 2022;22(1):235.
147. Ding L, Cao J, Lin W, et al. The roles of cyclin-dependent kinases in cell-cycle progression and therapeutic strategies in human breast cancer. *Int J Mol Sci*. 2020;21(6):1960.
148. Golsteyn RM. Cdk1 and Cdk2 complexes (cyclin dependent kinases) in apoptosis: a role beyond the cell cycle. *Cancer Lett*. 2005;217(2):129-138.
149. Satyanarayana A, Kaldis P. A dual role of Cdk2 in DNA damage response. *Cell Div*. 2009;4:1-4.
150. Burke JR, Hura GL, Rubin SM. Structures of inactive retinoblastoma protein reveal multiple mechanisms for cell cycle control. *Genes Dev*. 2012;26:1156-1166.
151. Giacinti C, Giordano A. RB and cell cycle progression. *Oncogene*. 2006;25(38):5220-5227.
152. Engeland K. Cell cycle regulation: p53-p21-RB signaling. *Cell Death Differ*. 2022;29(5):946-960.
153. Burkhart DL, Morel KL, Sheahan AV, Richards ZA, Ellis L. The role of RB in prostate cancer progression. *Prost Cancer: Cell Genet Mech Dis Develop Progr*. 2019;12:301-318.

154. Lu X, Jung Ji, Cho HJ, et al. Fisetin inhibits the activities of cyclin-dependent kinases leading to cell cycle arrest in HT-29 human colon cancer cells. *J Nutr*. 2005;135(12):2884-2890.
155. Cannan WJ, Pederson DS. Mechanisms and consequences of double-strand DNA break formation in chromatin. *J Cell Physiol*. 2016;231(1):3-14.
156. Santivasi WL, Xia F. Ionizing radiation-induced DNA damage, response, and repair. *Antioxid Redox Signal*. 2014;21(2):251-259.
157. Terabayashi T, Hanada K. Genome instability syndromes caused by impaired DNA repair and aberrant DNA damage responses. *Cell Biol Toxicol*. 2018;34:337-350.
158. Khan AQ, Kuttikrishnan S, Siveen KS, et al. RAS-mediated oncogenic signaling pathways in human malignancies. In: Vincent T, ed. *Seminars in Cancer Biology*. Vol 54. Elsevier; 2019:1-13.
159. Zhu G, Pei L, Xia H, Tang Q, Bi F. Role of oncogenic KRAS in the prognosis, diagnosis and treatment of colorectal cancer. *Mol Cancer*. 2021;20(1):1-17.
160. Khozooei S, Veerappan S, Toulany M. YB-1 activating cascades as potential targets in KRAS-mutated tumors. *Strahlenther Onkol*. 2023;199:1-18.
161. Khozooei S, Veerappan S, Bonzheim I, Singer S, Gani C, Toulany M. Fisetin overcomes non-targetability of mutated KRAS induced YB-1 signaling in colorectal cancer cells and improves radiosensitivity by blocking repair of radiation-induced DNA double-strand breaks. *Radiother Oncol*. 2023;188:109867.
162. Kim A, Shim S, Kim YH, Kim MJ, Park S, Myung JK. Inhibition of Y box binding protein 1 suppresses cell growth and motility in colorectal cancer. *Mol Cancer Ther*. 2020;19(2):479-489.
163. Alkrekshi A, Wang W, Rana PS, Markovic V, Sossey-Alaoui K. A comprehensive review of the functions of YB-1 in cancer stemness, metastasis and drug resistance. *Cell Signal*. 2021;85:110073.
164. Ye Q, Wang X, Jin M, et al. Effect of RSK4 on biological characteristics of colorectal cancer. *World J Surg Oncol*. 2018;16:1-9.
165. Calvo N, Carriere P, Martin MJ, Gentili C. RSK activation via ERK modulates human colon cancer cells response to PTHrP. *J Mol Endocrinol*. 2017;59(1):13-27.
166. Jiang X, du W, Yang C, et al. TBX21 attenuates colorectal cancer progression via an ARHGAP29/RSK/GSK3β dependent manner. *Cell Oncol*. 2023;46(5):1269-1283.
167. Shen W, du W, Li Y, et al. TIFA promotes colorectal cancer cell proliferation in an RSK-and PRAS40-dependent manner. *Cancer Sci*. 2022;113(9):3018-3031.
168. Khan N, Jajeh F, Eberhardt EL, et al. Fisetin and 5-fluorouracil: effective combination for PIK3CA-mutant colorectal cancer. *Int J Cancer*. 2019;145(11):3022-3032.
169. Arafeh R, Samuels Y. PIK3CA in cancer: the past 30 years. In: Vincent T, ed. *Seminars in Cancer Biology*. Vol 59. Elsevier; 2019:36-49.
170. Mei Z, Duan CY, Li CB, Cui L, Ogino S. Prognostic role of tumor PIK3CA mutation in colorectal cancer: a systematic review and meta-analysis. *Ann Oncol*. 2016;27(10):1836-1848.
171. Polivka J Jr, Janku F. Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. *Pharmacol Ther*. 2014;142(2):164-175.
172. Yuan L, Zhang S, Li H, et al. The influence of gut microbiota dysbiosis to the efficacy of 5-Fluorouracil treatment on colorectal cancer. *Biomed Pharmacother*. 2018;108:184-193.
173. Sethy C, Kundu CN. 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: implication of DNA repair inhibition. *Biomed Pharmacother*. 2021;137:111285.

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