Received: 10 September 2021 | Revised: 7 December 2021 | Accepted: 10 December 2021

DOI: 10.1111/jfbc.14060

Journal of Food Biochemistry WILEY

REVIEW

Reactive oxygen species: Key players in the anticancer effects of apigenin?

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Funding information
ORO and ABO are grateful for the Postdoctoral and Innovation fellowships respectively awarded by the Cape Peninsula University of Technology, South Africa.

Abstract

Reactive oxygen species (ROS) exhibit a double-edged sword in cancer—hence their modulation has been an attractive strategy in cancer prevention and therapy. The abundance of scientific information on the pro-oxidant effects of apigenin in cancer cells suggests the crucial role of ROS in its mechanisms of action. Although apigenin is known to enhance the cellular ROS levels to cytotoxic degrees in cancer cells in vitro, it remains to be determined if these pro-oxidant effects prevail or are relevant in experimental tumor models and clinical trials. Here, we critically examine the pro-oxidant and antioxidant effects of apigenin in cancer to provide insightful perspectives on the association between its ROS-modulating action and anticancer potential. We also discussed these effects in a cell/tissue type-specific context to highlight the factors influencing the switch between antioxidant and pro-oxidant effects. Finally, we raised some questions that need addressing for the potential translation of these studies into clinical applications. Further research into this duality in oxidant actions of apigenin, especially in vivo, may enable better exploitation of its anticancer potential.

Practical application: Apigenin is a naturally occurring compound found in chamomile flowers, parsley, celery, peppermint, and citrus fruits. Many human trials of dietary interventions with apigenin-containing herbs and flavonoid mixture on oxidative stress markers, for instance, point to their antioxidant effects and health benefits in many diseases. Preclinical studies suggest that apigenin alone or its combination with chemotherapeutics has a strong anti-neoplastic effect and can induce ROS-mediated cytotoxicity at concentrations in the micromolar (μM) range, which may not be feasible with dietary interventions. Enhancing the in vivo pharmacokinetic properties of apigenin may be indispensable for its potential cancer-specific pro-oxidant therapy and may provide relevant information for clinical studies of apigenin either as a single agent or an adjuvant to chemotherapeutics.

KEYWORDS
antioxidant, apigenin, cancer, clinical translation, free radicals, pro-oxidant

1 | INTRODUCTION

Polyphenols have been proposed to be instrumental to the medicinal activities of many anticancer plant extracts and phytomedicines (Oyenihi & Smith, 2019). The sheer importance of the polyphenol sub-class, flavonoids in cancer chemoprevention, is evidenced by the amounts of reports from many laboratories and study groups worldwide. In the early 20th century, plant pigments were identified as flavonoids, a group of plants secondary metabolites found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine (Nijveldt et al., 2001). Also, the research of Albert Szent-Györgyi mostly in the 1930s advanced the knowledge on the potential health benefits of flavonoids (Szent-Györgyi (1937). Subsequently, the global interest in healthy foods led to a research impulse from the 1960s to date on the health-promoting potential of flavonoids. Epidemiological studies, short-term randomized controlled clinical trials, and preclinical studies showed a relationship between intake of foods rich in fruits and vegetables and the incidence of some cancers, suggesting that certain dietary constituents may thus be effective cancer chemopreventives (Bode & Dong, 2009; Bondonno et al., 2019; Carocho & CFR Ferreira, 2013; Den Hartog et al., 1965; Kromhout et al., 1982; Perez-Vizcaino & Fraga, 2018).

In the late 1970, Oberley and colleagues proposed that the increased flux of reactive oxygen species (ROS) from cancer cell mitochondria could contribute to uncontrolled growth, the inability of cancer cells to differentiate, genomic instability, and disease progression. These hypotheses formed the bases for the free radical theory of cancer (Oberley & Buettner, 1979; Oberley et al., 1980, 1981). Studies in the 1990s set the stage for the concept that ROS drives tumorigenesis (Safford et al., 1994; St. Clair et al., 1992). Flavonoids have long been recognized as antioxidants (Kelley & Watts, 1957). The conventional wisdom holds that flavonoids act as antioxidants and, this action is primarily involved in their role as cancer preventive agents. While the antioxidant property of flavonoids is unquestionable, this may only partly explain their anticancer effect. Over the last three decades, research has also convincingly presented evidence to support their pro-oxidant (oxygen radical generating) behavior. Research data from the mid-1980s supported the concept that flavonoids act as pro-oxidants under certain conditions (Hodnick et al., 1986; Laughton et al., 1989; Rahman et al., 1989). The relevance of the dual antioxidant and pro-oxidant characteristics of flavonoids in cancer therapy is demonstrated by mounting evidence regarding the two sides of the ROS coin in cancer: the tumor-promoting and tumor-killing effects of ROS (Perillo et al., 2020). The differential redox states between normal and cancer cells for instance is one of the important determinants of the pro- or antioxidant activities of flavonoids (Chen et al., 2009; Kerimi & Williamson, 2018; Yang et al., 2018). Higher levels of endogenous ROS production and free-form metal ions in cancer cells compared to normal cells, sensitize them to phytochemical-mediated pro-oxidant cytotoxicity (Kocyigit et al., 2018). Interestingly, both the pro-and antioxidant effects of many flavonoids have been reported in cancer (Kopustinskiene et al., 2020) indicating that other factors such as the number and positions of hydroxyl groups, capacity to chelate redox-active metal ions, concentration-and cell-dependent effects, etc., play roles in their ROS-modulating action (Cao et al., 1997; Eghbaliferiz & Iranshahi, 2016; Kumar & Pandey, 2013; Simunkova et al., 2021). The antioxidant properties of flavonoids are potentially useful in cancer prevention because they can protect healthy cells from oxidative DNA damage. Conversely, their pro-oxidant behavior is particularly effective in treating aggressive tumors with abnormally radical-reactive cellular environments. Pro-oxidant flavonoids act by tipping the threshold of oxidative stress that can be tolerated by tumor cells over a limit that triggers death. The significance of the pro-oxidant action of flavonoids in cancer chemoprevention is also supported by the direct or indirect ROS generating effects of many anticancer regimens; ionizing radiation, chemotherapeutics, and targeted therapeutics (Sahoo et al., 2021). Figure 1 shows the key research timelines in the evolution of flavonoids particularly, apigenin in cancer research in relation to their antioxidant and pro-oxidant actions.

Apigenin (4′,5,7-trihydroxy-flavone) structurally belongs to the flavone subclass of flavonoids with three hydroxyl substituents on the backbone of two benzene rings that are linked *via* a heterocyclic pyrane ring (Figure 2). Apigenin is commonly found in varying concentrations in many herbs, grains, fruits, and vegetables such as parsley, onions, grapefruit, oranges, and chamomile tea, rice, maize, lettuce, cabbage, chicory leaves (Awika, 2017; Hostetler et al., 2017). It occurs naturally in the form of apigenin *O*-glycosides in dry chamomile flowers (*Matricaria chamomilla*) and parsley (*Petroselinum crispum*) at concentrations up to 5.3 and 1.3 mg/100 g; about 5% and 1% of the dried plant material, respectively. Apigenin is also present in many plants, vegetables, and spices such as celery, peppermint, thyme green peppers, *Aspalathus linearis* (Rooibos/redbush tea), and *Citrus bergamia* (Bergamot juice) (Hostetler et al., 2017).

The pharmacological versatility of apigenin, for example, antioxidant, neuroprotective, anti-inflammatory, antidiabetic, blood pressure-lowering, and antimicrobial properties have been well-reported (Ali et al., 2017; Nabavi et al., 2018). The phyto-compound has also demonstrated tissue-protective actions against toxicities induced by many xenobiotics and chemotherapeutic drugs (He et al., 2016; Liu et al., 2018; Wu et al., 2021; Zhong et al., 2018). Its high selectivity in terms of toxicity against cancerous cells versus normal cells in comparison to other structurally related flavonoids is valuable in chemoprevention (Gupta et al., 2001; Madunić et al., 2018). The relatively tolerable safety profile coupled with the little scientific evidence of adverse metabolic reactions following apigenin supplementation has further increased its therapeutic utility. The cytotoxic and antitumor effects of apigenin have been reviewed in many recent articles. (Ahmed et al., 2020; Ashrafizadeh et al., 2020; Imran et al., 2020; Javed et al., 2021; Khan et al., 2021; Yan et al., 2017). These studies suggest the potential of apigenin to modulate different signaling pathways implicated in cancer progression by inhibiting cell growth and migration and mediating cell death. Some of the cytotoxic mechanisms of apigenin include blockage of the Wnt/β-catenin signaling pathway, inhibition of cell cycle

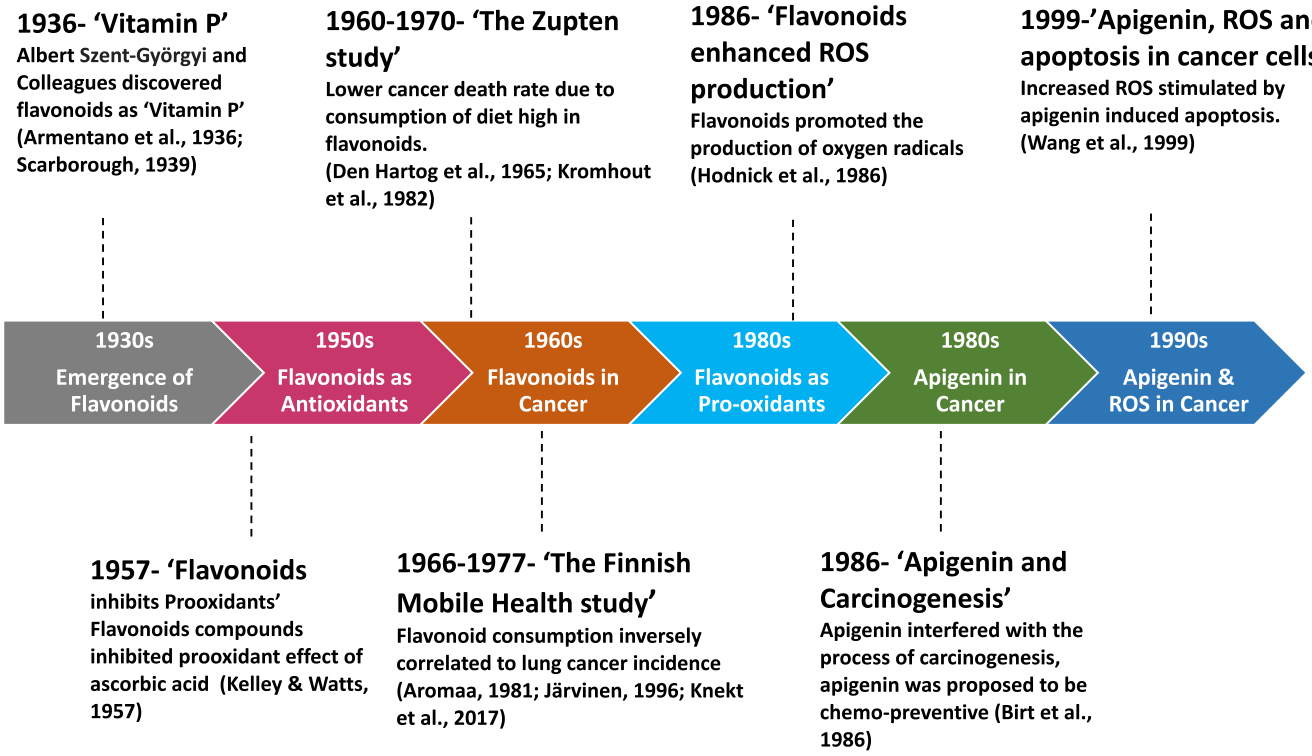

FIGURE 1 Research timelines on the anticancer effects of flavonoids/apigenin

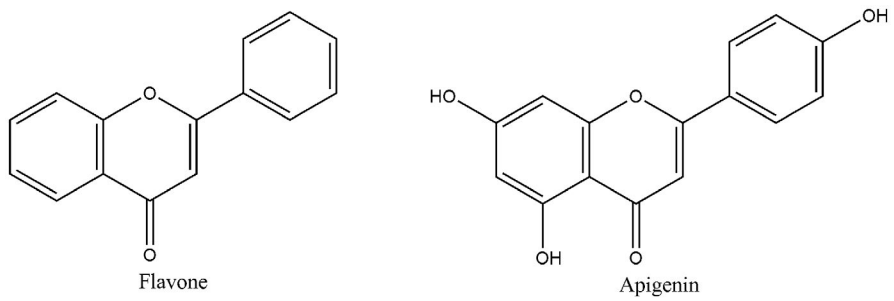

FIGURE 2 Chemical structures of the flavone backbone and apigenin

proteins, activation of p21WAF1/CIP1, modulation of Akt pathway and inhibition of NF-κB/p65 pathway. Others include the down-regulation of proteins involved in tumor invasion and metastasis, modulation of pro- and anti-apoptotic proteins expression, inhibition of the Hedgehog/Gli1 signaling pathway, modulation of ATM signaling, deactivation of Wnt/β-catenin pathway and blockage of cellular nutrient transport *via* inhibition of transporter activity (Bao et al., 2015; Cao et al., 2016; Erdogan et al., 2016; Lin et al., 2017; Meng et al., 2017; Tang et al., 2015).

Besides the efficacy of apigenin as a monotherapy, it is reported to enhance the efficacy of many chemotherapeutic drugs (Erdogan et al., 2017; Hu et al., 2018; Strouch et al., 2009). As a result of these reported benefits and low toxicity in normal cells, it has been described as a potential dietary supplement and adjuvant chemotherapeutics for cancer therapy. However, these purported anticancer potentials have been primarily based on the positive results obtained from preclinical studies but have not been confirmed by any clinical data yet. There are two clinical trials on apigenin in cancer patients recorded to date (ClinicalTrials.gov Identifier; NCT00609310 and NCT03139227). These trials were designed to investigate a flavonoid supplement containing apigenin and epigallocatechin gallate in colonic neoplasia and a dietary supplement of apigenin-rich celery-banana bread in high-risk breast cancer patients. However, these studies have been suspended or withdrawn. Hence, there is a need to further clarify the cellular and molecular effects of apigenin in cancer chemoprevention and therapy.

One of the main vulnerabilities targeted by apigenin in cancer cells is redox imbalance. The abundance of scientific information on the pro-oxidant effects of apigenin suggests that an elevation of ROS/reactive nitrogen species (RNS) to cytotoxic levels is one of the most important mechanisms associated with its anticancer properties. However, data from in vitro studies conflicts with the scanty

in vivo experiments. This review focuses on the ROS-modulatory effect of apigenin in mediating cancer cell death. The available data on the antioxidant versus pro-oxidant effects of apigenin in cancer is presented in a cell/tissue type-specific context to highlight the factors influencing the switch between antioxidant and pro-oxidant effects. The insights provided here may direct future research to further understand the relevance of ROS modulation by apigenin in cancer therapy for potential clinical application.

2 | ROS MODULATION IN CANCER

Moderate amounts of ROS have positive effects including the killing of invading pathogens, wound healing, and repairing processes (He et al., 2017). Cellular oxidative stress results from the abnormal production of ROS/RNS that is beyond cellular needs and thus overwhelms the cellular detoxification mechanisms (Snezhkina et al., 2019). The ROS produced mainly in the mitochondria and cytoplasm as by-products of the normal numerous oxidative metabolisms are usually expressed in greater amounts in the cancer cells than their normal counterparts due to the increase in metabolic processes (Zhang et al., 2019). Excess ROS cause cellular injury by abstracting electrons from macromolecular components rendering them incapable of performing essential functions such as cellular signaling, gene regulation, and cell growth. Notable ROS/RNS include superoxide anion ($^{\bullet}O_2^-$), hydroxyl ($^{\bullet}OH$), nitric oxide ($^{\bullet}NO^-$), peroxyl ($^{\bullet}RO_2^-$), hydrogen peroxide (H_2O_2), peroxynitrite (ONOO), etc. (Valko et al., 2007). The onset of oxidative stress has been postulated to have dual roles in carcinogenesis and tumor progression. As schematically summarized in Figure 3, on one hand, it triggers inflammatory responses and activates a variety of molecular signaling cascades that promote cell proliferation, angiogenesis, and metastasis. Cells can become tumorigenic when the attacks by excess ROS on DNA (adducts), proteins (cross-links), lipids, and carbohydrates (advanced glycation end-products) lead to genomic instability and induction of various tumor-promoting signaling cascades (Prasad et al., 2017). ROS-mediated DNA damage may also result in the mutations of proto-oncogenes leading to activation or mutations in tumor suppressor genes leading to inactivation. Once initiated, tumorigenic cells continue to multiply through angiogenesis, invasion, and metastasis—cellular processes that are also primarily triggered by excess ROS (Galadari et al., 2017). A sustained increase in cellular ROS generation in malignant cells amplifies genomic instability and drives tumor progression and survival. On the other hand, at toxic levels, oxidative stress can disrupt redox homeostasis and induce cancer cell death through the upregulation of apoptotic, necrotic, necroptotic, and autophagic pathways (Kim, Kim, et al., 2019). Therefore, the optimal modulation of oxidative stress mechanisms has become a common strategy for chemoprevention. The dependence of cancer cells on their antioxidant capacity to protect against ROS-mediated toxicity makes them vulnerable to pro-oxidant agents that dampen or abrogate this adaptation mechanism. These ROS-based therapeutic strategies (e.g., radiotherapy, photodynamic theory, and drugs) kill cancer cells by elevating the ROS within the tumor microenvironment to toxic levels or/and reducing the local antioxidant response to ROS (Kim, Kim, et al., 2019).

Since cancer cells exhibit higher ROS levels, they have also been shown to display a comprehensive antioxidant network to counteract the effects of ROS, maintaining redox homeostasis to perpetuate cancer progression. This inherent cellular defense machinery involves the use of different antioxidants that act through a myriad of actions and at various oxidative stages to halt the activities of ROS. Any compromise in the amount/actions of the antioxidants allows the uncontrolled overproduction of ROS leading to cellular

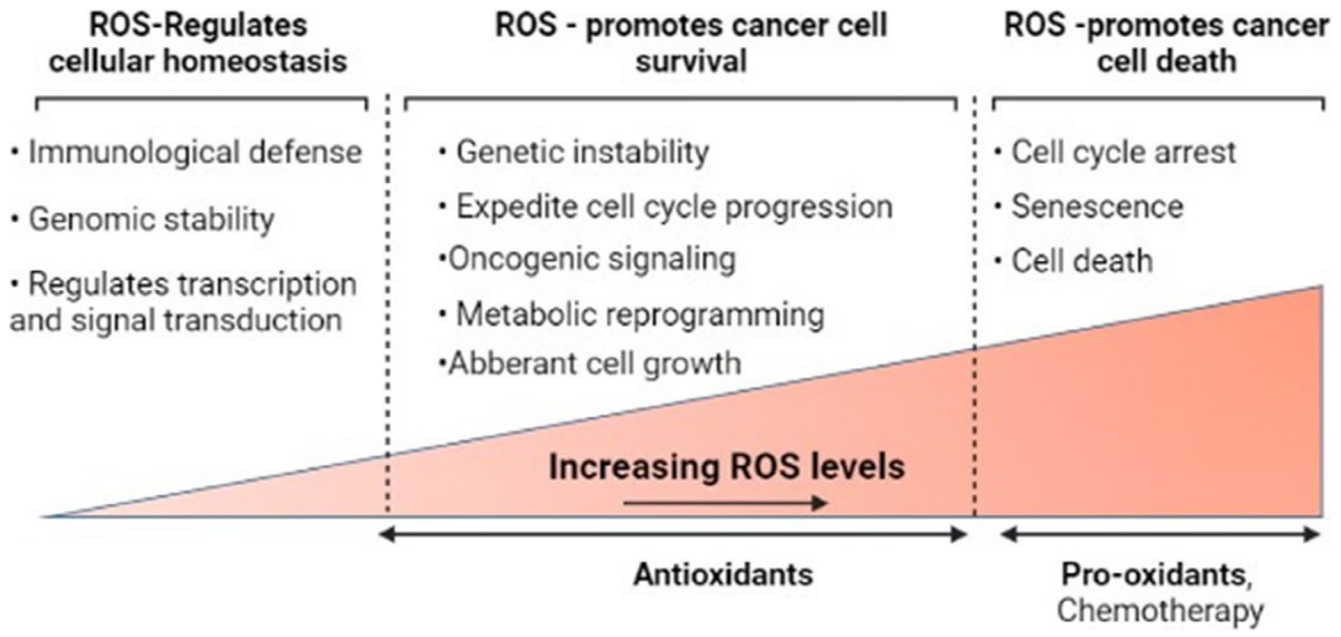

FIGURE 3 Dual role of ROS as a tumor promotor and suppressor

injury (Halliwell, 2011; Zahra et al., 2021). Notable antioxidants include the enzymes e.g. superoxide dismutase (SOD), catalase (CAT), glutathione-*S*-transferases, and glutathione peroxidase (GPx) or non-enzymes, for example, reduced glutathione (GSH), vitamins A, C, and E. One major goal of the antioxidant mechanism is to significantly amplify the cancer cell antioxidant capacity to scavenge ROS eventually denying the cell its ability to become tumorigenic and reducing its ability to proliferate uncontrollably. For example, in achieving chemoprevention, the use of exogenous antioxidant supplements has been widely favored. The *N*-acetyl cysteine (NAC) and alpha-tocopherol antioxidants exemplified this mechanism in many pre-clinical trials on different types of cancer, although the successes achieved in these trials could not be translated for human benefits in clinical trials (Harris & DeNicola, 2020). Exogenous antioxidants were found to exhibit tumor-promoting qualities in the clinical trials, a phenomenon that has been attributed to their capacity to induce the transcription factor, nuclear factor-erythroid factor 2-related factor 2 (Nrf2)—the same factor that is employed by the *ras* and *myc* oncogenes to evade death (DeNicola et al., 2011; Menegon et al., 2016). The exogenous administration of antioxidants has also been implicated in chemoresistance through their inhibition of the ROS-dependent anticancer agents such as DNA topoisomerase II inhibitors (Chen et al., 2017). However, despite these conflicts, a recent systematic review and meta-analysis on the impact of antioxidants in cancer concluded that high-antioxidant diets may be beneficial against the chronic disease (Parohan et al., 2019). Therefore, research in the identification of the anticancer therapeutic threshold of antioxidants must continue, to be successful in clinical trials. Some pro-oxidant- and antioxidant-based anticancer agents have already received approval from the US Food and Drug Administration. Examples of ROS-inducing chemotherapeutic drugs are elesclomol, OSU-03012, imexon, and doxorubicin whereas histamine and minodronate are known for their ROS suppressing effects (Arfin et al., 2021; Blackman et al., 2012; Gao et al., 2008; Gupta et al., 2012; Kim, Kim, et al., 2019).

3 | PRO-OXIDANT EFFECTS OF APIGENIN IN CANCER THERAPY

Several studies have demonstrated the antioxidant effects of apigenin through direct free radical scavenging action and upregulation of intracellular antioxidant defense (Ali et al., 2014; Salehi et al., 2019; Sharma et al., 2014). Interestingly, apigenin is also regarded as a prominent pro-oxidant flavonoid (Chen et al., 2003). The presence of catechol hydroxyl groups, in the B ring strongly determines the antioxidant capacity of flavonoids (Kumar & Pandey, 2013). This catechol hydroxyl group, which is absent in apigenin, lowers its antioxidant capacity (Tavsan & Kayali, 2019). Furthermore, structure-activity relationship data suggest that a 4′-mono hydroxyl group, which can be oxidized to phenoxyl radical is essential for the pro-oxidant effect of apigenin. This oxidizing ability of the phenol B-ring provides a rationale for the cytotoxicity of apigenin and other phenol B-ring-containing flavonoids *via* oxidative stress (Galati et al., 2001; Miyoshi et al., 2007). The antioxidant effect of apigenin is displayed primarily in normal cells while it exhibits pro-oxidation activity in cancer cells. The higher metabolic activity and basal oxidative stress of tumor cells relative to normal cells may have promoted the pro-oxidant effect of apigenin in the former (Aboelella et al., 2021).

The evidence for the pro-oxidant effects of apigenin has emanated majorly from in vitro data using cancer cell lines. For example, in prostate cancer, apigenin induces cell cycle arrest and apoptosis in LNCaP cells and has been suggested to inhibit NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and cause a mitochondrial-mediated cell death pathway in DU145 cells (Gupta et al., 2002; Shukla & Gupta, 2008). Using a panel of prostate cancer cell lines, Morrissey and colleagues (Morrissey et al., 2005) also examined the effects of apigenin on the cell cycle and apoptosis. Apigenin (25 and 50 mM) altered the redox status and increased ROS levels in LNCaP, PC-3, PWR1E, and DU145 prostate epithelial cancer cell lines. Apigenin induced mitochondrial permeabilization in LNCaP, PC-3, and DU145 causing a time-dependent release of cytochrome c into the cytosol and a caspase-dependent apoptotic death. Differential response of apigenin was observed in PWR1E cells where it mediated apoptosis independent of cytochrome c release despite the transient change in mitochondrial permeability. This differential response of apigenin on PWR1E cells could be explained by a high level of ROS in this cell line and a possible bypass of the mitochondrial-mediated pathway (Morrissey et al., 2005). Additionally, using the transgenic adenocarcinoma of the mouse prostate model, apigenin treatment (20 and 50 μg/day, for 20 weeks) was shown to inhibit the prostate tumor growth by suppressing the PI3K/Akt/FoxO signaling pathway (Shukla et al., 2014).

Another study (Wang et al., 1999) examined the mechanism of apoptosis induction by apigenin and other structurally related flavonoids like quercetin, myricetin, and kaempferol, in human HL-60 leukemic cells. Treatment with the flavonoids at a dose of 60 μM, induced a loss of membrane potential, increased ROS generation and caused a release of mitochondrial cytochrome c into the cytosol. Subsequent induction of caspase-3 activation and proteolytic cleavage of poly-(ADP-ribose) polymerase (PARP) were observed. The apoptosis-induction potency of these flavonoids was in the order of apigenin >quercetin > myricetin >kaempferol in the HL-60 cells. The number of hydroxyl groups on the 2-phenyl group and the existence of the 3-hydroxyl group were suggested as contributing factors to the potency of the flavonoids—that is, the greater the number of hydroxyl groups in the 2-phenyl group, the greater the potency in recovering the apoptosis-inducing ability which was inhibited by the existing 3-hydroxyl group (Wang et al., 1999).

In the human colon carcinoma line (HCT-116), the apoptotic mechanisms of apigenin have been shown to involve intracellular ROS generation and endoplasmic reticulum (ER) stress (Wang & Zhao, 2017). Apigenin treatment (40–160 μM) caused a dose-dependent cell cycle arrest at the G0/G1 phase. With increasing doses, a lower cell population was observed at G2/M and S phases and the proportion of cells at the G0/G1 phase increased.

Apigenin-induced death of HCT-116 cells *via* the intrinsic and extrinsic apoptotic pathway by triggering ROS generation and release of Ca^{2+} from the ER lumen to the cytosol, thereby causing ER stress. A cytoplasmic Ca^{2+} overload and an accelerated influx of Ca^{2+} into the mitochondria potentially promoted mitochondria ROS generation and stimulated the Ca^{2+} release channels on the ER in a feedback mechanism.

Furthermore, the pro-oxidative effect of apigenin was evaluated in HT-29 and HCT-15 colon cancer cell lines (Banerjee & Mandal, 2015). Apigenin at all tested doses (1.5625 μM to 100 μM) increased intracellular ROS/RNS generation in HCT-15 at 24 hr time point whereas, in HT-29, a burst of ROS/RNS production was observed at 6.25–100 μM doses. At the 24-hr time point, superoxide levels were however elevated from 1.56 μM dose to 100 μM in both cells. The ROS generated by apigenin in HCT-15 cells (from 1.56 μM) was accompanied by a significant increase in apoptosis which was only observed from 3.12 μM in the case of HT-29 cells, suggesting greater susceptibility of HCT-15 cells to apigenin in comparison to HT-29 cells. Chronic oxidative stress-induced senescence in both HCT-15 and HT-29 cells was noted following treatment with low-dose apigenin for an extended period (6 days; 1.56–25 μM). Furthermore, apigenin-induced ROS generation promoted the dephosphorylation of retinoblastoma (Rb) protein—a tumor suppressor, by up-regulating p16Ink4a and p21 protein expression, which suppresses the activities of cyclins D1 and E, respectively. This subsequently leads to cell cycle arrest senescence in the p53 mutant cell lines.

Apigenin exhibited cell-type-dependent ROS modulating effects on ovarian cancer cells—A2780 and SKOV-3. Apigenin, increased ROS generation in A2780 cells at a dose of 1 and 20 μM but reduced ROS levels in SKOV-3 cells at the same concentrations. On the other hand, luteolin, also belonging to the flavone class of flavonoid, exhibited the opposite effect on ROS modulation, as observed by a decrease in ROS levels in A2780 (at the 20 μM dose) and SKOV-3 cells (at the 1 μM dose). The anticancer activities of these flavones were attributed to the alterations of ROS signaling, induction of apoptosis, and cell cycle arrest (Tavsan & Kayali, 2019).

Souza et al. (2017) also reported the cytotoxic effects of apigenin (10–100 μM) on a panel of human cervical cancer-derived cell lines HeLa, SiHa, CaSki, and C33A. These effects were also attributed to ROS-induced mitochondrial dysfunction, induction of apoptosis, and the inhibition of cancer cell migration and invasion. In addition, Liu et al. (2020) demonstrated the ROS upregulating effect of apigenin on cervical cancer HeLa cells. Apigenin (20–60 μM; 24–48 hr) induced apoptosis through the death receptor pathway and caused G0/G1 phase arrest. Apigenin treatment reduced mitochondrial membrane potential (MMP), upgraded intracellular ROS production, and inhibited PTEN/PI3K/Akt pathway and cell migration. Zhang, Cheng, Qiu, et al. (2015) in their study, indicated that autophagy induction by apigenin is in part due to cellular ROS accumulation. The apigenin-induced autophagic cell death in papillary thyroid cancer cell line—BCPAP was associated with intracellular ROS accumulation, DNA damage, and G2/M cell cycle arrest *via* the downregulation of Cdc25C expression (Zhang, Cheng, Qiu, et al., 2015).

Apigenin (6–50 μM) inhibited the growth of PaCa44 and Panc1 pancreatic cell lines but exerted a stronger cytotoxic effect on Panc1 cell line (IC_{50} = 9.8 ± 0.6 μM) compared to PaCa44 (IC_{50} = 24.8 ± 0.9 μM) (Gilardini Montani et al., 2019). Intriguingly, mechanistic studies revealed that the higher cytotoxic effect of apigenin on the Panc1 cell line was due to induction of a higher level of intracellular ROS, reduction of mutant (mut) p53, expression of heat shock protein 90 (HSP90), and the inhibition of the mammalian target of rapamycin complex 1 (mTORC1). The reduced cytotoxic response of apigenin was due to the stabilization of mut p53 by its interplay with HSP90, the activation of Nrf2, and the increase of p62. The reduced susceptibility of PaCa44 cells to apigenin correlated with the up-regulation of antioxidant response by Nrf2. Therefore, the mTOR-HSP90-mutp53-p62-Nrf2-antioxidant response axis could help to overcome the chemoresistance of pancreatic cancer to apigenin (Gilardini Montani et al., 2019). Other studies have also reported that apigenin treatment reduced cell proliferation, triggered the spontaneous release of ROS, and stimulated death of different cancer cell lines such as; lung adenocarcinoma A549, cells, prostate cancer 22Rv1 cells, and human breast cancer MCF-7 cells lines (Bai et al., 2014; Bruno et al., 2011; Shukla & Gupta, 2008).

Exposure of glioblastoma cells (U87MG and T98G) to a dose of 50 μM apigenin, caused cellular ROS accumulation which was accompanied by phosphorylation and activation of p38 mitogen-activated protein kinase (p38 MAPK) and the initiation of the redox-sensitive c-Jun N-terminal kinase 1 (c-JNK 1) pathway (Das et al., 2010). Increases in intracellular free Ca^{2+} and activation of caspase-4 indicated the involvement of endoplasmic reticulum stress in apigenin-induced apoptosis. Apigenin-mediated mitochondria-dependent apoptosis was evident by the mitochondrial apoptotic events such as; increased Bax and decreased Bcl-2 protein and mRNA expression; increased Bax: Bcl-2 ratio, loss of mitochondrial membrane potential ($\Delta\Psi_m$), and the activation of caspase-9, and -3 (Das et al., 2010). Apigenin induced apoptosis in U87MG and T98G cells but did not affect normal astrocytes, indicating its selective cytotoxicity on glioblastoma cells.

Masuelli et al. (2017) analyzed the anticancer mechanisms of apigenin in human (MM-F1, H-Meso-1, and MM-B1) and mouse (MM #40a) malignant mesothelioma (MM) cells. Apigenin treatment elicited a dose- and time-dependent cytotoxicity on MM cells—a dose range of 12.5–100 μM was cytotoxic in all human cells after 48 and 72 hr whereas 25, 50, and 100 μM concentrations were cytotoxic to #40a cells after 24–72 hr of exposure. Apigenin 20 mg/kg administered daily for 2 weeks also reduced tumor growth in C57BL/6 mice transplanted with #40a cells and increased the median survival (Masuelli et al., 2017). A significant ROS generation was noted at 6.25–100 μM in MM-F1 and MM-B1 cell lines. However, in H-Meso-1 and #40a cells, ROS levels were increased only between 12.5 to 100 μM doses. Apigenin-induced ROS generation resulted in DNA damage, up-regulation of p53, and an increase in Bax/Bcl-2 ratio leading to the induction of extrinsic apoptotic pathways. Other studies have also shown that apigenin increased ROS generation which triggered p53 activation

in hepatoma and prostate cancer cells (Shukla & Gupta, 2008; Q. Zhang, Cheng, Qiu, et al., 2015). Furthermore, apigenin treatment affected the extracellular signal-regulated kinases (ERK 1/2), JNK, and p38 MAPKs in a cell-type dependent manner. Specifically, apigenin reduced the expression of JNK p54 in all malignant mesothelioma (MM) cells except MM-F1 cells and decreased JNK p46 in MM-B1 and #40a cell lines. Apigenin treatment also induced p38 MAPK activation in all MM cells except H-Meso-1 cells and increased ERK1/2 phosphorylation in all MM cells except in MM-F1 cells. Furthermore, apigenin inhibited Akt phosphorylation, decreased the expression and phosphorylation of c-Jun, and inhibited NF-κB nuclear translocation. Depending on cancer cell type, apigenin has been reported to activate or inhibit MAPKs, p38, and JNK pathways (Kim et al., 2013; Lim et al., 2016; Sui et al., 2014; Sung et al., 2016; Wu et al., 2015). In addition to these studies, further reports of the pro-oxidant effects of apigenin in cancer therapy are presented in Table 1. Most of these in vitro studies employed the 2′,7′-dichlorofluorescein diacetate (DCFHDA) assay to evaluate the pro-oxidant effects of apigenin. DCFHDA is a commonly used probe for detecting intracellular ROS. After penetration of cellular membranes, DCFH-DA is deacetylated by cellular esterases to DCFH, a non-fluorescent compound, which is then oxidized by ROS into the fluorescent compound-2',7'-dichlorofluorescein (DCF). Furthermore, some of the studies assessed enzymatic and non-enzymatic antioxidants to confirm the pro-oxidant actions of apigenin.

4 | EFFECTS OF ROS SCAVENGERS ON APIGENIN-INDUCED ROS GENERATION AND CELL DEATH

Validation experiments to determine the involvement of ROS in the cytotoxic activity of apigenin have generated conflicting results. While some studies found a correlation between cellular ROS upregulation by apigenin and apigenin-mediated cell death, others reported no association. For instance, Im Choi et al. (2007) indicated that ROS generated through the activation of the NADPH oxidase may play an essential role in the apoptosis induced by apigenin in HepG2 cells. Treatment with various inhibitors of the NADPH oxidase (diphenylene iodonium, apocynin, and neopterin) significantly blunted both the generation of ROS and induction of apoptosis by apigenin. An increase in the intracellular Ca^{2+} concentration by apigenin which may, in turn, activate Ca^{2+}/calmodulin-dependent protein kinase-II was suggested as a mechanism by which apigenin activates NADPH oxidase (Im Choi et al., 2007). In human prostate 22Rv1 cancer cells, apigenin (10–80 μM) caused p14ARF-mediated downregulation of Mouse double minute 2 homolog (MDM2) protein, resulting in the stabilization and activation of p53, and inhibition of NF-κB/p65 transcriptional activity (Shukla & Gupta, 2008). The increased p53 protein expression correlated with an increase in the levels of its transcriptional target p21/WAF-1 in a dose/time-dependent manner. The attenuation of apigenin-induced apoptosis by p53-specific antisense oligonucleotide also indicates that p53 is a potential target for apigenin action. Furthermore, exposure of 22Rv1 cells to apigenin (10–80 μM for 3 hr) significantly increased ROS generation which positively correlated with apoptosis. Interestingly, within 3 hr of cell exposure to apigenin, mitochondria accumulation of a p53 fraction was observed which suggests stress-induced p53-dependent apoptosis. In further validation experiments, NAC pre-treatment before cell exposure to apigenin inhibited p53 translocation to the mitochondria and the release of cytochrome c into the cytosol (Shukla & Gupta, 2008).

Induction of ROS production has also been reported to be important in apigenin-induced apoptosis of gastric cancer cells (Sun et al., 2018). Apigenin suppresses proliferation and induces apoptosis in gastric cancer cells by reducing anti-apoptotic protein expression, Bcl-2, and increasing cleavage of apoptotic proteins; Bax, Caspase-3 and 9, and PARP. Apigenin-induced apoptosis was significantly abrogated by NAC pre-treatment suggesting that the generation of intracellular reactive oxygen species (ROS) in cells is crucial for apigenin-induced apoptosis. Apigenin also suppressed constitutive and interleukin-6 (IL-6)-stimulated activation of signal transducer and activator of transcription 3 (STAT3), Janus kinase 2 gene (JAK2), and Src. Interestingly, apigenin-mediated inactivation of STAT3/JAK2 was abrogated by NAC pre-treatment (Sun et al., 2018). Also, in another recent report (Lee et al., 2020), apigenin induced apoptosis and necroptosis in MSTO-211H and H-2452 human malignant mesothelioma cell lines *via* excessive generation of mitochondria ROS and ATP depletion. The critical role of ROS as an upstream molecule in apigenin-induced necroptosis was supported by the recovery of cell viability and necroptosis-related protein levels by NAC pretreatment.

Another study (Kang et al., 2018), however, did not find a correlation between cellular ROS upregulation by apigenin (10–25 μM) and apigenin/TRAIL (TNF-related apoptosis-inducing ligand)-induced apoptosis in human hepatocarcinoma cells, Hep3B and HepG2. Intriguingly, the ROS inhibitors; glutathione (GSH) or NAC, moderately increased apigenin/TRAIL-induced apoptosis. Apigenin amplified TRAIL-induced apoptosis by upregulating DR5 expression and activating apoptotic caspases regardless of ROS generation. Interestingly, apigenin-induced ROS generation stimulates autophagy formation in Hep3B cells, and treatment with GSH and NAC inhibited apigenin-induced autophagic cell death. Also, in human leukemia cells (THP-1, U937, and HL60), apigenin at concentrations above 50 μM reduced cell viability and increased apoptosis of leukemia cells in addition to increasing intracellular ROS level in U937 cells (Jayasooriya et al., 2012). However, pre-treatment of U937 cells with antioxidants; NAC or GSH, completely attenuated ROS production but did not affect cell cycle progression and apigenin-induced cell death. Furthermore, apigenin decreased c-Myc mRNA and protein levels and downregulated telomerase activity. These effects of apigenin in U937 leukemia cells were sustained even in the presence of NAC or GSH which further indicates that ROS generation and suppression of c-Myc-dependent telomerase activity by apigenin are two independent events.

TABLE 1 Other in vitro evidence on the pro-oxidant effects of apigenin in cancer therapy

Cancer cell line	Dose/treatment duration	ROS assays	Major outcomes after apigenin treatment	References
Human pancreatic MIAPaCa–2, PANC–1, AsPc1, and BxPC–3	10–90 μM 24–72 hr	DCFHDA	• Blocked Hsp90/Cdc37 interaction and induced degradation of downstream proteins; Akt, CDK4, and MMP-9 • Induced ROS accumulation and inhibited pancreatic cancer cell growth	He et al. (2015)
Human hepatoma HepG2	10–100 μM 24–144 hr	DCFHDA, GSH, and GSSG	• Induced apoptosis may be mediated by an H_2O_2-dependent pathway *via* a decrease in antioxidant defenses • Lowered GSH level and GSH: GSSG ratio • The addition of catalase reduced apigenin-induced cell death	Valdameri et al. (2011)
Human breast MDA-MB468	10–30 μM 24–72 hr	DCFHDA	• Induced ROS generation, caused G2/M phase cell cycle arrest and reduced phosphorylation of Akt serine/threonine that promoted tumor growth • GSH did not abrogate the apigenin-mediated inhibition of Akt phosphorylation and tumor regression	Harrison et al. (2014)
Human breast MCF–7	20–100 μM 24 and 48 hr	DCFH-DA, DHE	• Induced ROS overproduction which resulted in cell apoptosis and morphological changes	Bai et al. (2014)
Human breast MCF–7 and MDA MB–231	20–100 μM 24 and 72 hr	Lipid peroxidation	• Induced cell morphological changes and apoptotic cell death • Induced oxidative lipid damage contributes to its overall cytotoxic potential	Madunić, Madunić, Antunović, et al. (2018)
Lung H1299, H460, and H2030	5–20 μM 24 and 48 hr	DCFH-DA, GSH and GSSG	• Displayed cell growth inhibitory effect and induced apoptotic death through metabolic and oxidative stress • Co-treatment with antioxidant- N-acetyl-L-cysteine (NAC) rescued cells from cell apoptosis	Lee et al. (2016)
Human melanoma A375 Human lung A549	20–90 μg/mL 24 and 48 hr	DCFH-DA, GSH, and SOD	• Induced apoptosis mainly by triggering ROS production, mitochondrial dysfunction, and caspase activation • Depleted GSH level and reduced SOD activity	Das et al. (2012)
Lung adenocarcinoma A549	50 μM 24–72 hr	DCFH-DA	• Inhibited cell growth and elevated cellular ROS generation, which augmented cell death and apoptosis	Bruno et al. (2011)
Human papillary thyroid carcinoma BCPAP	12.5–50 μM 24 hr	DCFH-DA	• Induced autophagic cell death is associated with intracellular ROS accumulation, oxidative DNA damage, and subsequent arrest of the G2/M cell cycle phase	Zhang, Cheng, Gao, et al. (2015)
Human bladder T–24	1–50 μg/mL 1–48 hr	DCFH-DA, GSH	• Induced apoptosis and blockage of cell cycle progression involving an increase in intracellular ROS, depletion of GSH, p53 upregulation, and modulation of cell cycle proteins expression	Shi et al. (2015)
Human colon SW620	10–40 μM 24–72 hr	DCFH-DA	• Mediated antiproliferative effects by inducing polyamine catabolism through the upregulation of SSAT expression and increasing intracellular ROS levels to induce cell apoptosis	Wang et al. (2017)

Abbreviations: Akt, protein kinase B; Cdc37, cell division cycle protein 37; CDK4, cyclin-dependent-kinase 4; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; GSH, reduced glutathione; GSSG, oxidized glutathione; Hsp90, heat shock protein 90; MMP-9, matrix metalloproteinase-9; ROS, reactive oxygen species; SOD, superoxide dismutase; SSAT, spermidine/spermine-N1-Acetyl transferase.

5 | APIGENIN AMPLIFIES CHEMOTHERAPY-INDUCED ROS GENERATION

Many commonly used chemotherapeutic drugs generate different levels of ROS in cancer cells. It is hypothesized that chemotherapeutic amplification of ROS levels pushes the already high basal oxidative stress in cancer cells over a certain limit that causes cell death (Yang et al., 2018). Existing data have established that apigenin enhances the anticancer abilities of some chemotherapeutic agents and may reduce cancer resistance to chemotherapeutics. The anticancer-enhancing effects of apigenin when conjugated with other chemotherapeutic agents such as; cisplatin, cetuximab, gemcitabine, 5-fluorouracil, among others have been reported. For instance, it promoted the cytotoxic effects of cisplatin in $CD44^+$ prostate cancer stem cells—apigenin cotreatment lowered the IC_{50} dose of cisplatin from 7.5 to 2.2 mM (Erdogan et al., 2017). Also, treatment with apigenin plus gemcitabine repressed growth in the pancreatic cell lines (MiaPaca-2, APC-1) and increased apoptosis in tumors of male BALB/c nude mice through the downregulation of NF-kB activity and suppression of Akt activation (Lee et al., 2008). Apigenin in combination with gemcitabine also reduced the proliferation of resistant pancreatic cancer cells and abrogated gemcitabine resistance (Strouch et al., 2009). Furthermore, apigenin amplified the apoptotic effects of 5-fluorouracil in 5-fluorouracil resistant human breast cancer MDA-MB-453 cells overexpressing ErbB2 (Choi & Kim, 2009). In addition, apigenin enhanced the anticancer action of cetuximab in human nasopharyngeal carcinoma cell lines (HONE1 and CNE2) and specific pathogen-free (SPF) mice (Hu et al., 2018). Apigenin also increases the cytotoxic effects of sorafenib—a multikinase inhibitor by 100% in HepG2 cells (Şirin et al., 2020).

The enhanced cytotoxic effects of apigenin on some of the aforementioned chemotherapeutic are related to increased ROS generation. For instance, the role of ROS in the synergistic pro-apoptotic effects of apigenin and paclitaxel was studied on a cervical carcinoma cell line—HeLa (Xu et al., 2011). The induction of apoptosis in HeLa cells treated with a combination of apigenin and paclitaxel was related to the accumulation of superoxide and cleavage of caspase-2. Decreased activity of SOD1 (CuZnSOD) and SOD2 (MnSOD) antioxidant enzymes by apigenin in HeLa cells suggests that apigenin promotes the accumulation of ROS *via* direct inhibitory effects on SOD activity. Apigenin may be forming a stable complexation with $Cu^{2+,}$ $Zn^{2+,}$ or Mn^{2+} in cells to suppress SOD activity (Zhang et al., 2005). Previous studies in caspase-2 knocked-out mice have shown that caspase-2 activation was related to ROS accumulation (Zhang et al., 2007). Also, combined, but not separate treatment of human pancreatic AsPC-1 cancer cells with metformin (5 mM) and apigenin (1 or 20 μM) significantly inhibited cell viability (Warkad et al., 2021). The combined treatment with metformin (0.005, 0.05, and 0.5 mM) and apigenin (20 μM) amplified ROS production and triggered cell killing via apoptosis, necroptosis, and autophagy. Simultaneous blockade of ROS production and cell death by NAC further confirmed the key role of ROS in apigenin/metformin-induced cell death (Warkad et al., 2021). The anti-tumorigenic effect of metformin and apigenin combination was further demonstrated in the mice xenograft cancer model. Metformin (125 mg/kg) combined with apigenin (40 mg/kg) for 2 weeks inhibited tumor growth. Tumor growth inhibition was not observed at lower doses, 75 and 5 mg/kg, of metformin and apigenin respectively. While the exact mechanisms by which metformin and apigenin amplify ROS production still require further investigations, it may involve an interaction with the mitochondrial electron transport chain (Warkad et al., 2021). AMP-activated protein kinase (AMPK)-dependent inhibition of the mTOR pathway and other AMPK-independent mechanisms have been suggested to be involved in metformin-mediated cancer cell death (Vancura et al., 2018). The inhibition of mTOR by metformin may be triggered by increased ROS production mediated by the interaction of metformin with complex I of the mitochondria electron transport chain (Bridges et al., 2014). Elevated ROS and a decrease in ATP levels, signal for the activation of AMPK and subsequent inhibition of the mTOR pathway. Co-treatment of AsPC-1 cells with apigenin and metformin however did not activate AMPK suggesting AMPK-independent mechanism(s).

Another study (Hu et al., 2015) investigated the synergistic effect of apigenin and 5-fluorouracil (FU) against hepatocellular carcinoma in SK-Hep-1 cells and SPF nude mice. Apigenin at sub-toxic concentrations (4 μM), enhanced the cytotoxicity, growth-inhibitory, and apoptotic effects of 5-fluorouracil (100 μg/mL) in SK-Hep-1 cells. Apigenin potentiated the reduced expression of Bcl-2 protein and induced the overproduction of ROS which decreased the mitochondria membrane potential, thus, activating the mitochondrial pathway of apoptosis. It was further shown that a combination of apigenin (20 mg/kg; 3-weeks treatment) and 5-FU (20 mg/kg; 5 days) suppressed the growth of hepatocellular carcinoma in vivo (Hu et al., 2015).

6 | ANTIOXIDANT EFFECTS OF APIGENIN IN CANCER THERAPY AND PREVENTION

Although apigenin induced cancers cell death *via* increasing cellular ROS generation and depleting antioxidants defense, there are a few reports on its antioxidant action in animal models of hepatocellular carcinoma and buccal pouch carcinogenesis (Figure 4). The anticancer efficacy of the apigenin was evaluated in N-nitrosodiethylamine-induced and phenobarbital-promoted hepatocellular carcinoma in rats (Singh et al., 2004). N-nitrosodiethylamine is a potent hepatotoxin, carcinogen, and mutagen, and its toxicities are mediated by reactive metabolic intermediates produced by bioactivation of the parent compound by cytochrome P450 in the hepatocytes (George et al., 2019). Apigenin administered for 2 weeks at a dose of 25 mg/kg body weight/day demonstrated antioxidant effects. These were evident by reduced levels of lipid peroxidation product—malondialdehyde, in the liver and an increase in the activities of SOD, CAT, and GPX. Levels of non-enzymatic antioxidants; GSH, vitamin C, and vitamin

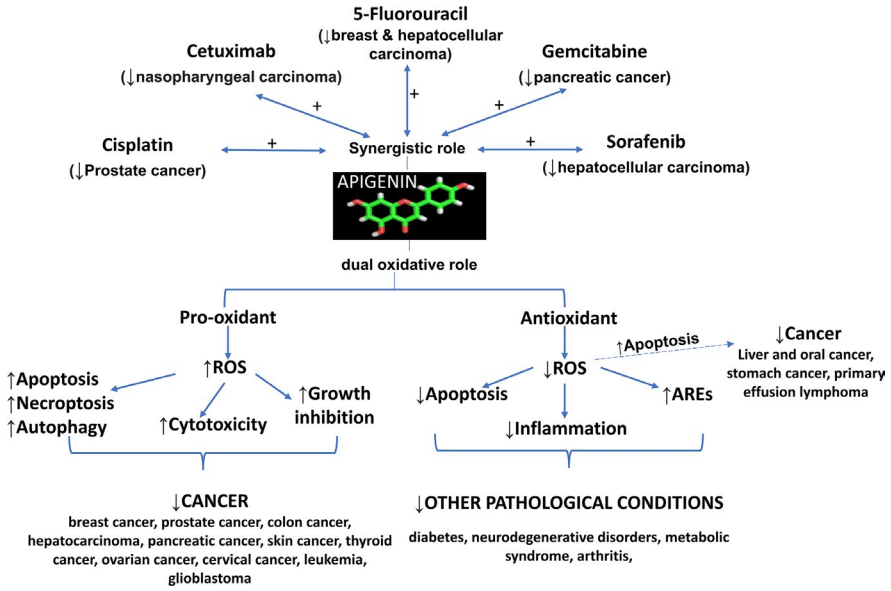

FIGURE 4 Redox modulation of apigenin in cancer therapy and other pathological conditions

E were also increased suggesting alleviation of oxidative stress. Similarly, another study reported the protective action of apigenin (10–40 mg/mL for 21 days) against N-nitrosodiethylamine-induced hepatotoxicity and oxidative damage, evident by a reduction of protein carbonyl and lipid peroxidation products along with reduced serum markers of liver injury; aspartate aminotransferase (AST), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) (Ali et al., 2014). There are extensive reports on the key involvement of free radicals in diethylnitrosamine-induced hepatocarcinogenesis (Bansal et al., 2000; Hussein et al., 2015; Janani et al., 2010; Kujawska et al., 2016).

The study of Silvan and colleagues (Silvan et al., 2011) further supports the chemopreventive and antioxidant effects of apigenin in DMBA (7,12-dimethylbenz[*a*]anthracene) animal model of carcinogenesis. The DMBA chemical carcinogen model is particularly useful for investigating blocking agents that interfere with the early stages of carcinogenesis (initiation and promotion). Apigenin (2.5 mg/kg bwt/day) administered before exposure to the carcinogen inhibited the progression of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis by improving antioxidant defense and modulating the activities of phase I and phase II detoxification agents, therefore, facilitating the detoxification of active metabolite of DMBA. Further studies by the same research group showed that apigenin at the same dose modulates the expression patterns of molecular markers of apoptosis, cell proliferation, angiogenesis, and inflammation in DMBA-induced hamster buccal pouch carcinogenesis. Apigenin downregulated the overexpression of p53, proliferating cell nuclear antigen (PCNA), bcl-2, and vascular endothelial growth factor (VEGF), and decreased cFOS, cyclin D1, NF-kB, and COX-2. The expression of apoptotic proteins (Bax, caspase-3, and 9) were increased following apigenin treatment (Silvan & Manoharan, 2013).

A few in vitro studies have also reported the ROS-suppressing effects of apigenin in cancer. Granato et al. (2017) assessed the effect of apigenin on primary effusion lymphoma (PEL)—a Kaposi sarcoma-associated herpesvirus-associated B cell lymphoma with constitutive activation of STAT3 and wild-type p53 (wtp53). Apigenin caused cell cycle arrest and induced apoptotic cell death, which was confirmed by the cleavage of PARP. Interestingly, apigenin-induced cell death correlates with a dose-dependent reduction in ROS levels in BC3 and BCBL-1 cells. The decrease in ROS levels and reduction in cell survival caused by apigenin treatment (12.5 and 25 μM; 24 hr) was similar to the effect achieved by the ROS scavenger—NAC (25 and 50 mM; 24 hr).

Wang & Huang (2013) also demonstrated the ROS-suppressing effects of apigenin in human stomach cancer cells (MKN45 cells) infected with *Helicobacter pylori*. The infected MKN45 cells were exposed to varying doses (9.3, 18.5, 37, and 74 μM) of apigenin. Treatment of the cells with apigenin significantly lowered inflammation by inhibiting NF-kB activation, the expression of other inflammatory markers; COX-2, ICAM-1, ROS, IL-6, and IL-8, and simultaneously scavenged ROS levels. The anti-inflammatory properties of apigenin in adenocarcinoma cells were dose-dependent, while the lowest dose of 9.3 μM mitigated ROS as much as the highest dose of 74 μM, which shows the antioxidant effect of apigenin in cancer therapy.

7 | PRO-OXIDANT VERSUS ANTIOXIDANT EFFECTS OF APIGENIN IN CANCER THERAPY

Apigenin displayed dose-dependent cytotoxic effects against different cancer cell lines which is strongly associated with its ROS generating effects. Apigenin also enhances ROS-dependent

cancer cell killing mediated by chemotherapeutic drugs. However, no animal tumor model study has reported this pro-oxidant effect. Intriguingly, and as illustrated in Figures 4 and 5, the antioxidant effects of apigenin rather than its pro-oxidative effect have been reported in the few available in vivo tumor studies and other pathological conditions (Ali et al., 2017; Kim, Jung, et al., 2019; Zhou et al., 2019). It is noteworthy that very limited studies have evaluated the antioxidant/pro-oxidant effects of apigenin in tumor models. The scanty data on redox modulation by apigenin in tumor models in vivo is therefore insufficient to infer its antioxidant/pro-oxidant action in cancer therapy. Andueza et al. (2015) reported that irrespective of ROS levels, apigenin-derived phenoxyl radical directly oxidizes H_2DCFDA—a commonly used probe for measuring ROS, by acting as an electron shuttle. Thus, oxidation of H_2DCFDA derivatives in apigenin-treated cells may be an unreliable measure of ROS formation. However, the pro-oxidant effects displayed by apigenin in cancer cell lines have also been assessed using other probes and biochemical assays (Table 1) which excludes methodological issues as the cause of the observed ROS accumulation in vitro. To confirm if ROS indeed plays a primary role in the anticancer effects of apigenin, a few studies included antioxidants such as catalase, GSH, or NAC to distinguish the effects of oxidation products from "real" anticancer effects of apigenin. In most of these studies, the abrogation of the cytotoxic effects of apigenin by the addition of ROS scavengers unambiguously indicates the critical role of ROS in apigenin-mediated cancer cell death. Interestingly, a few other studies found no association between ROS generation and the cell-killing effect of apigenin. This suggests that the pro-oxidant effect of apigenin may not be the most important cytotoxic mechanism of action, although it may be responsible for the selective anticancer activity of apigenin as observed for other antioxidant phytochemicals and anticancer drugs such as paclitaxel, metformin, vitamin C, resveratrol, which selectively mediate cancer-killing via ROS accumulation (Alexandre et al., 2006; Martin-Cordero et al., 2012; Shin et al., 2020; Warkad et al., 2021).

The cellular response generated by apigenin may also be affected by dose, duration of exposure, level of ROS generated, and antioxidant status of tissue/cells. The concentrations of apigenin required to cause a significant cellular ROS accumulation varies substantially between different cell lines but are all within micromolar concentrations (ranging from 1 to 100 μM) for an exposure time of 24–72 hr. The pharmacological concentrations required to generate a similar response in vivo have not been investigated. Unlike in cell culture conditions, animal-based cancer models have a more adapted endogenous antioxidant network to scavenge excessive ROS. The

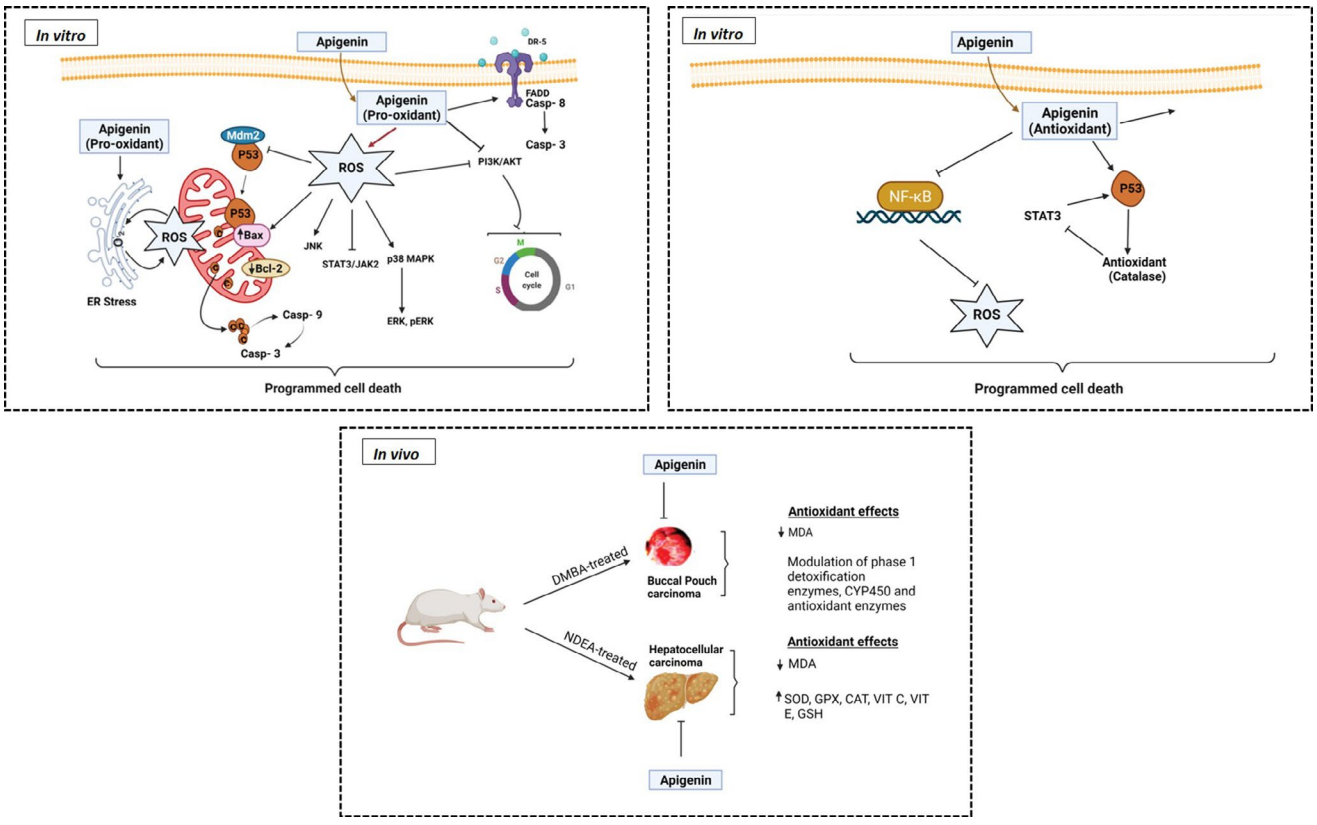

FIGURE 5 Pro-oxidant versus antioxidant action of apigenin and molecular targets in cancer therapy. Abbreviations: DMBA, 7,12-dimethylbenz[*a*]anthracene; ERK, extracellular signal-regulated kinases; JAK, The Janus kinase; JNK, c-Jun N-terminal kinase; MAPK, Mitogen-Activated Protein Kinase; NDEA, N-Nitrosodiethylamine; NF-Kb, Nuclear Factor kappa-light-chain-enhancer of activated B cells; $_p$53, Tumor protein; STAT, signal transducer and activator of transcription

differences in the antioxidant status between these models could affect how a flavonoid is metabolized (Halliwell, 2008). It, therefore, remains to be determined if the pro-oxidant activity of apigenin can be translated in tumors in vivo.

Arguably, due to the possible accumulation of apigenin in the gastrointestinal tract and the presence of unabsorbed transition metal ions, apigenin may exert a protective pro-oxidant effect in gastrointestinal tumors. However, only a few studies have investigated the effect of apigenin on gastrointestinal tumors and the antioxidant status was not investigated in such studies. For instance, apigenin increases apoptosis in azoxymethane-induced colon cancer in rats, although the mechanism is still unknown (Leonardi et al., 2010). Another study showed that apigenin inhibited colon carcinogenesis induced by azoxymethane/dextran sodium sulfate by influencing the gut microbiota but the precise mechanism is also yet to be determined (Bian et al., 2020). In a different study, no protective effect was observed in a model of human gastrointestinal malignancies using ApcMin mice after treatment with dietary doses of 0.2% apigenin, which equate to approximately 300 mg/kg mice body weight (Cai et al., 2009). In contrast, apigenin (10–30 mg/kg bwt/day for 30 days) significantly induced tumor apoptosis and inhibited STAT3/JAK2 pathways in a gastric cancer cell xenograft tumorigenesis (Sun et al., 2018). In the same study, apoptosis mediated by apigenin on gastric cancer cell lines was observed to be regulated by inhibition of the STAT3/JAK2 pathway and enhanced ROS generation (Sun et al., 2018).

The anticancer activity of flavonoids is related not only to the parent flavonoid ingested but also to its metabolites, and metabolism may either decrease or enhance the antioxidant and pro-oxidant abilities. Free apigenin can be directly absorbed systemically or undergo extensive phase I and II metabolism in the intestines and liver to generate hydroxylated metabolites such as luteolin or metabolites of glucuronidated and sulfated conjugates. Luteolin is the primary metabolite of phase I hydroxylation by cytochrome P450 (CYP) enzyme in the liver (Gradolatto et al., 2004; Hostetler et al., 2017). Possibly, ROS generated by apigenin could be neutralized to a large extent in the intestine, where it is extensively metabolized (Chen et al., 2003). Plasma apigenin concentrations in vivo may likely be insufficient to exert systemic pro-oxidant effects. A mild degree of oxidative stress generated in this case may raise the levels of antioxidant defenses and xenobiotic-metabolizing enzymes, leading to net antioxidant rather than pro-oxidant effect. Also, some of the products of apigenin metabolism, such as luteolin and glucuronidated conjugates have decreased pro-oxidant abilities. Unlike apigenin, luteolin has demonstrated antioxidant properties in both cancer cell lines and animal tumor models (Ashokkumar & Sudhandiran, 2008; Balamurugan & Karthikeyan, 2012; Kang et al., 2018; Kasala et al., 2016; Kitakaze et al., 2019; Leung et al., 2006; Son et al., 2017). The interaction between apigenin and its metabolites may dictate a different effect on redox homeostasis in vivo compared to that obtained in cell lines. However, more in vivo studies are needed to clarify this aspect.

8 | BIOAVAILABILITY OF APIGENIN IN THE TUMOR AND BIOLOGICAL FLUIDS

Pharmacokinetics data indicates that the plasma concentration of apigenin is lower than that used in in vitro experiments. Shukla et al. (2005) investigated the serum and tumor apigenin concentration in a prostate carcinoma tumor xenograft model. The oral administration of apigenin at doses of 20 or 50 μg/mouse/day for 2 weeks before tumor implantation and for 8 weeks thereafter resulted in about 0.84 and 1.26 μM serum apigenin concentration respectively whereas the tumor concentration of apigenin was ~0.42 and 0.68 μm/g tumor tissue respectively. Treatment resulted in the inhibition of tumor volume by 44% and 59%, respectively. In another study, following intravenous administration of apigenin 20 mg/kg to animals, apigenin reached a tolerable peak plasma concentration (C_{max}) of approximately 11,000 ng/mL immediately after administration. This concentration however decreased to 100 ng/mL (a 100-fold decrease) within an hour. This rapid phase of decline was followed by a slower disappearance of apigenin from the circulation as apigenin was detected at 50 ng/mL after 8 hr of initial apigenin concentration (Wan et al., 2007).

Although the pharmacokinetic profile of pure apigenin is not available in humans, the relationship between dietary intake of apigenin and circulating levels has been studied in healthy humans. Systemic levels of apigenin following oral intake of apigenin-rich parsley (149.5 ± 35.2 g) resulted in peak serum apigenin concentration of 127.0 ± 24.3 nM (34.3 ± 6.57 ng/mL), 7 hr after intake and declined significantly to 2.3 nM after 28 hr of ingestion (Meyer et al., 2006). Another clinical trial showed that the consumption of 2 g/kg body weight of celery leaf, resulted in a mean apigenin plasma concentration of 190.4 ± 98.5 nM which also dropped below the detection limit (2.3 nM) by 28 hr after administration (Cao et al., 2010).

9 | CONCLUSION AND PERSPECTIVES

The ROS-inducing ability of apigenin as observed in cell-based assays appears to be related to its cytotoxic and antitumor effects. However, the significance of ROS generation in the anticancer actions of apigenin requires further detailed in vivo tests-based investigations. Furthermore, there remain some unanswered questions that need to be addressed. Available evidence in vitro indicates that apigenin can induce ROS-mediated cytotoxicity at concentrations in the micromolar (μM) range (1–100 μM). These concentrations are not feasible with dietary interventions to achieve these effects. It remains to be determined if these concentrations are achievable in vivo. Also, what are the pharmacological concentrations of apigenin that would be sufficient to exert systemic pro-oxidant effects? Can the pro-oxidant effects of apigenin prevail in the tumor cells? Are these pro-oxidant effects relevant for apigenin therapeutic benefits in vivo?

There are no pharmacokinetic studies on pure apigenin in humans to date. Also, data on the tumor concentration of apigenin are scanty

in pre-clinical studies. Clinical pharmacokinetic studies of apigenin are necessary to provide relevant information on whether pharmacologic apigenin concentrations similar to those showing efficacy in tumor-bearing mice can be attained in humans. Enhancing the in vivo pharmacokinetic properties of apigenin may be indispensable for its potential cancer-specific pro-oxidant therapy. To improve the bioavailability of apigenin in vivo, novel drug delivery systems that have shown minimal off-target toxicity on normal cells may be used for targeted delivery of apigenin to the tumor. Different drug delivery approaches of apigenin such as apigenin–phospholipid phytosome, apigenin polymeric micelles, apigenin nano-formulation, for example, aptamer-conjugated apigenin loaded nanoparticles , apigenin-loaded bovine serum albumin nanoparticles, and mesoporous silica nanoparticles drug carrier of apigenin are being investigated as drug delivery systems (Dutta et al., 2018; Huang et al., 2019; Kazi et al., 2020; Pápay et al., 2015, 2017; Telange et al., 2017). The optimal dosing frequency required to achieve sufficient tumor apigenin concentrations needs to be determined. Higher apigenin concentrations within the tumor tissue may clarify the effect of apigenin on cancer redox homeostasis.

The available studies indicate that redox regulation is an indisputable anticancer mechanism of apigenin. Apigenin either singly or as an adjunct to chemo-/radiotherapy modulate cellular or mitochondrial ROS levels. It is also noteworthy that apigenin showed protective effects against toxicities mediated by chemotherapeutic agents in normal tissues while simultaneously enhancing the sensitivity of tumor cells to chemotherapeutic drugs. Redox-related signaling pathways such as Nrf2/Keap1, MAPKs, JNK p53, NF-κB, and STAT3 are involved in the anticancer mechanism of action of apigenin in multiple cells and animal models. Considering that ROS is highly reactive, small-molecule probes for cellular ROS and real-time monitoring of the kinetic changes in the redox state in vivo, will improve the understanding of the cellular stress response following different apigenin doses. These would shed more light on the implication of the pro-oxidant effects of apigenin in cancer therapy in vivo. In addition, appropriate preclinical models should be selected to study the ROS modulatory effects of apigenin at different stages of the disease (e.g., late-stage cancer cells or early tumor development). Modeling of patient tumor heterogeneity and its complexity using appropriate preclinical models such as the patient-derived xenograft could provide better predictive clinical efficacy of apigenin. Further research on the pro-oxidant-antioxidant effects of apigenin in vivo may enable its manipulation for therapeutic benefits.

ACKNOWLEDGMENTS

ORO and ABO are grateful for the Postdoctoral and Innovation fellowships respectively awarded by the Cape Peninsula University of Technology, South Africa.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Omolola Oyenihi: Conceptualization; Resources; Writing—original draft; Writing—review & editing. **Ayodeji Oyenihi:** Writing—original draft; Writing—review & editing. **Toyin D Alabi:** Writing—original draft; Writing—review & editing. **Oluwatosin Tade:** Writing—original draft; Writing—review & editing. **Anne Adeyanju:** Writing—original draft; Writing—review & editing. **Oluwafemi O. Oguntibeju:** Resources; Writing—original draft; Writing—review & editing.

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How to cite this article: Oyenihi, O. R., Oyenihi, A. B., Alabi, T. D., Tade, O. G., Adeyanju, A. A., & Oguntibeju, O. O. (2022). Reactive oxygen species: Key players in the anticancer effects of apigenin? *Journal of Food Biochemistry*, 46, e14060. https://doi.org/10.1111/jfbc.14060