Minireview

Molecular mechanisms of action of hesperidin in cancer: Recent trends and advancements

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Impact statement

Experimental findings from numerous studies have demonstrated the anticancer effects of hesperidin (Hesp) to be associated with anti-oxidant and anti-inflammatory activities along with its potential role in inhibiting the tumor cell metastasis and angiogenesis. Additionally, Hesp can also reverse drug resistance of cancer cells, which make it a promising candidate to be used in combination with existing anti-cancer drugs. This review will be helpful for upcoming researchers and scientific community to find out complete capsular package about cancer drug targets of Hesp and its role in modulating various important hallmarks of cancer.

Abstract

Hesperidin belongs to flavanones class of flavonoids and is known to possess broad-spectrum applicability to prevent dreadful diseases such as cardiovascular disease, neurodegeneration, and cancer. The reported anticancer effects of hesperidin have been found to be associated with its anti-oxidant and anti-inflammatory activities. Hesperidin interacts with numerous recognized cellular targets and inhibits cancer cell proliferation by inducing apoptosis and cell cycle arrest. In addition, evidence has suggested its promising role in inhibiting tumor cell metastasis, angiogenesis, and chemoresistance. The present mini-review highlights the ongoing development to identify hesperidin targets in cancer. Furthermore, the potential of nano technology-based hesperidin combinations and delivery systems will also be discussed. Overall, this review highlights all the possible molecular targets affected by hesperidin in tumor cells on a single platform.

Keywords: Hesperidin, anticancer, synergistic effect, nanotechnology

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Introduction

Citrus fruits, such as oranges, tangerines, lemons, and limes, are well known for their health-promoting and chemopreventive properties.[1] One of the most important bioactive components which elicits promising anti-cancer potential is a flavonoid hesperidin (Hesp), also known as hesperetin 7-rutinoside.[2] Hesp was first isolated from orange peels in 1828 and was one of the two compounds formerly erroneously named as "vitamin P."[3] This polyphenolic glycoside can be abundantly found in various citrus fruits. For instance, sweet oranges contain about 200–600 mg/l of Hesp, whereas the total amount of this molecule varies within 50–850 mg/l in clementines, 8.1–460 mg/l in mandarins, 38–410 mg/l in lime and lemon juice, and 20–170 mg/l in grapefruit juice.[1] Although the bioavailability of dietary flavonoids is known to be limited, the low micromolar levels of Hesp (around 1 μM range) have been detected in the blood serum for 5–7 h after the intake of citrus juice,[3,4] probably high enough to exert its health-promoting activities in the human body.

Hesp belongs to the flavanone family of bioflavonoids. Differently from some other common groups of flavonoids, such as flavones, flavonols or isoflavones, flavanones lack the double bond between the positions 2 and 3 in the C ring of general flavonoid structure.[1] Hesp has been shown to exert a broad range of pharmacological activities, being a potent anti-oxidant, anti-inflammatory, anti-atherosclerotic, cardioprotective, neuroprotective, anti-allergic, anti-viral, anti-microbial, and anti-cancer compound.[1,2,4,5] Its role in protection against malignant transformation and progression has been described in multiple preclinical studies, acting through diverse cellular signalling pathways.[5,6] Indeed, Hesp can affect diverse molecular targets involved in survival, division, and death mechanisms of tumor cells.[5,6]

Cancer is the second leading cause of death worldwide, just behind cardiovascular diseases.[7] In fact, there was an estimated 18.1 million new cancer cases and 9.6 million cancer deaths in 2018 around the world.[8] Due to the continuously increasing global prevalence of malignancies, novel efficient therapeutics and treatment strategies are highly needed. Application of safe natural compounds with strong anticancer properties, such as Hesp, may open new avenues in cancer treatment.[9–11] Therefore, different anti-tumor mechanisms of this attractive dietary bioflavonoid, i.e. antioxidant, anti-inflammatory, anti-proliferative, anti-angiogenic, anti-invasive, anti-metastatic and, pro-apoptotic properties, are summarized in this review. The dataset presented in this article may be valuable for initiating clinical trials in patients suffering from different cancers, either alone or in combination with traditional therapies.

Chemistry and synthetic preview of hesperidin

On the chemical perspective, hesperidin structure harbors an aglycone known as methyl eriodictyol (hesperetin) bonded to rutinose.[12] Hesp contains a glycoside moiety which is a disaccharide (glucose and rhamnose) which is present in two isomeric forms, i.e. neohesperidose and rutinose.[12] Neohesperidose is chemically known as 2-O-alpha-l-Rhamnopyranosyl-d-glucopyranose. It is reported to be present in citrus fruits in the form of hesperetin 7-O-neohesperidoside.[13] Rutinose on the other hand, a disaccharide, usually obtained from herbal sources is chemically 6-O-(-l-Rhamnosyl)-d-glucose or 6-O-(-l-Rhamnopyranosyl)-d-glucopyranose.[12,14] These disaccharide moieties are responsible for the bitterness of citrus bioflavonoids. Of the disaccharides, the taste of citrus fruits is tasteless because of the presence of rutinosides moiety, while the neohesperidosides moiety is responsible for the bitter taste. Hesp is commonly found in neohesperidosides in the form of grapefruit (bitter) and in rutinoside in the form of orange (non-bitter).[12,14,15] In the chemical Hesp skeleton, hesperetin (aglycone structure) is bonded to glucose and rhamnose is further bonded through glucose moiety to this structure.[12]

In hesperetin, a bioflavonoid, aglycone structure is (S)-2,3-dihydro-5,7-dihydroxy-2–(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one (Figure 1).[12] Hesp is produced from the alkaline hydrolysis of hesperetenic acid and phloroglucinol, which during acid hydrolysis gets converted into hesperetin, l-rhamnose, and d-glucose.[12,16] The biological activity of Hesp is due to the presence of hydroxyl moieties in both the heterocyclic and aromatic rings.[12,16] Further, there is a close relationship between the presence and number of hydroxyl moieties with the potential antioxidant capacity of hesperidin.[12,16,17] Additionally, Hesp also harbors neuro-protective actions and has the capability of penetrating the blood–brain barrier. [12,16,17]

Hesperidin: Molecular mechanisms of action

Apoptotic and cell cycle arrest

Apoptosis induction and cell cycle arrest are among the most important mechanisms of Hesp action against cancer cells. Studies revealed that the pro-apoptotic action of Hesp is related to different kinase pathways. For example, suppression of phosphatidylinositol-4,5-bisphosphate 3-kinase subunit/ AKT Serine/threonine kinase/ inhibitor of kappa light polypeptide gene enhancer in B-cells (PI3K/Akt/IKK) signalling pathway in NALM-6 human pre-B cells by Hesp was reported to induce apoptosis (Figure 2).[18] In breast cancer cell line, MCF-7, Hesp induced apoptotic events like phosphatidyl-serine externalization, DNA fragmentation, caspase-7 activation, and PARP (Poly (ADP-ribose)polymerase) cleavage, which are associated with the activation of caspase-9, loss of mitochondrial membrane potential, release of cytochrome c, and an increase Bax:Bcl-2 ratio. Further experiments revealed that Hesp induced apoptosis by accumulating reactive oxygen species (ROS) and activation of apoptosis signal regulating kinase 1/ Jun N-terminal kinase (ASK1/JNK) pathway.[19] Besides these, the most important mechanism of apoptotic effect of Hesp is via generation of ROS. As reported by Zhang *et al.*,[20] high ROS levels, along with adenosine triphosphate (ATP) and calcium are responsible for the induction of apoptosis by Hesp in hepatocellular carcinoma cells via the activation of mitochondrial pathway. Similarly, in gastric cancer cells and esophageal cancer cells, Hesp induces apoptosis by increasing ROS and activating mitochondrial pathway.[21,22]

Caspase-dependent apoptosis induction by Hesp in NCI-H358 and A549 non-small cell lung cancer (NSCLC) cells was also observed by Birsu *et al.*[23] and Xia *et al.*[24], while Hesp was also found to arrest cell cycle at G_0/G_1 phase via the downregulation of cyclinD1, and increasing the expression of p21 and p53. In addition, the endoplasmic reticulum stress pathway was also found to be involved in apoptosis induction in HeLa (immortal cervical cancer cells) by Hesp, along with arresting cell cycle at G_0/G_1 phase via the downregulation of cyclin D1, cyclin E1, and cyclin-dependent kinase 2 (Cdk2) at protein level[25]. The ROS-mediated apoptosis induction by hesp in human gall bladder carcinoma was also reported by Pandey *et al.*,[26] but they found cell cycle arrest at G_2/M phase. A recent[25] evidence showed that Hesp is a potent inhibitor of calcium/calmodulin-dependent protein kinase IV

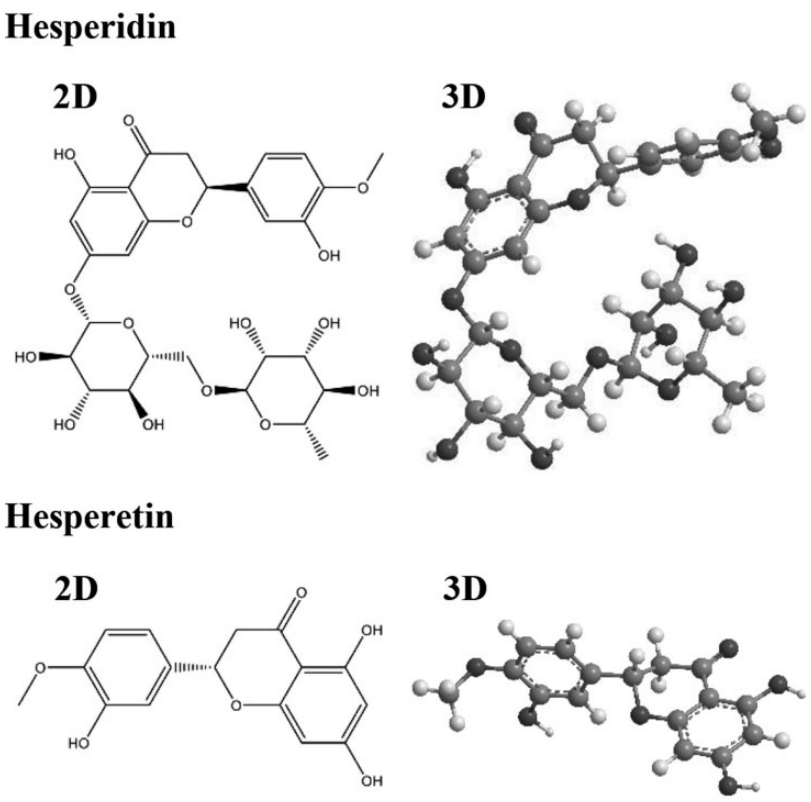

Figure 1. The chemical structures and three-dimensional (3D) conformers of Hesperidin and Hesperetin.

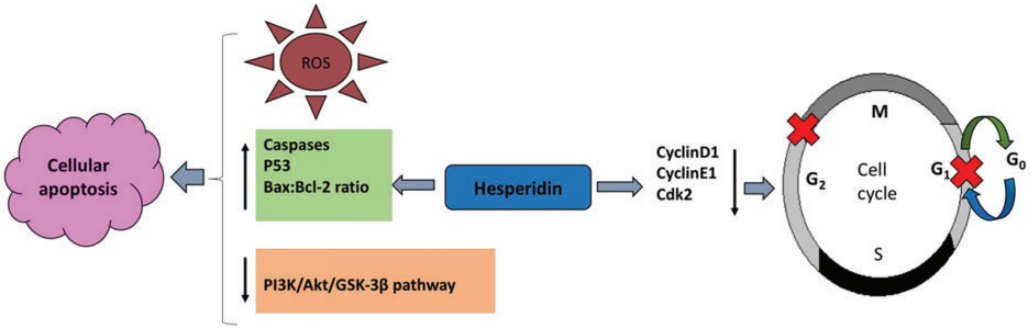

Figure 2. Role of hesperidin on apoptosis and cell cycle. Hesperidin can generate reactive oxygen species (ROS) in cancer cells and activate mitochondrial pathways (by upregulating caspases) and inhibit kinases, which can induce apoptosis. Also by regulating cell cycle-related proteins, hesperidin can arrest cancer cell cycle in G_0/G_1 phase and G_2/M phase. (A color version of this figure is available in the online journal.)

(CAMKIV), and by inhibiting CAMKIV along with activating the caspase-3-dependent intrinsic pathway through the upregulation of pro-apoptotic protein, Bax (BCL2 associated X, apoptosis regulator), Hesp exerts its anti-apoptotic and anticancer activities.[27]

The *in vitro* activity of Hesp in inducing apoptosis and arresting cell cycle is also proved *in vivo* (Table 1). For example, in azoxymethane-induced mouse model of colon cancer, Hesp was found to alter the anti-apoptotic scenario by modulating Bax/Bcl-2 ratio, together with enhanced release of cytochrome-c and activation of caspase-3/9. Experimental studies revealed that Hesp initiates apoptosis by inhibiting constitutively activated Aurora-A-mediated PI3K/Akt/GSK-3β pathway, and mTOR (mammalian target of rapamycin) pathway coupled with the stimulation of autophagy in this colon cancer model.[28] Hesp in combination with fistein has been reported to inhibit cellular proliferation via triggering programmed cell death in human K562 chronic myeloid leukemia (CML) cells through activation of caspase-3 and JAK/STAT (Janus Kinase/Signal transducer and activator of transcription 3) pathway and genes of JAK/STAT pathway have also been identified as candidates of CML therapy.[29] In ferric nitrilotriacetate (Fe-NTA)-induced renal cancer model of Wistar rats, Hesp was found to induce apoptosis-related proteins caspase-3, caspase-9, Bax expression and downregulation of Bcl-2 (BCL2 apoptosis regulator), NF-κB (nuclear factor kappa B subunit), iNOS (inducible nitric oxide synthase), TNF-α

Table 1. A brief overview of the in vivo studies carried out using hesperidin.

Compound of interest	Model of study	Type of cancer	Treatment dose	Underlying mechanisms of observed results	Refs
Hesperidin	Diethylnitrosamine/ CCl4-induced rats	Hepatocellular carcinoma	11 mg/kg	↓oxidative stress, inflammation, cell proliferation, TGF-β1/Smad3 signaling, and collagen deposition by activating Nrf2/ ARE/HO-1 and PPARγ pathways	32
	Diethyl nitrosamine hepatocarcinogenesis-induced rat	Hepatocellular carcinoma	1000, 500, and 250 ppm	Hypomethylating effect on the LINE-1 sequence (up to 47% hypomethylation at 12.5 mM) and on the ALU-M2 repetitive sequences (up to 32% at 6 mM)	33
	Rats	Hepatocellular carcinoma	150 mg/kg/day	↓Wnt3a, β-catenin, Cyclin D1, and Wnt5a gene expressions	34
	Female Sprague-Dawley rats	Breast cancer	30 mg/kg/body weight	Elevation in glycoproteins, nucleic acids, lysosomal enzymes and also significant alterations in macromolecules in renal tissues of cancer bearing animals	35
	Rats	Hepatocellular carcinoma	50, 100, and 200 mg/kg/d	↓Exosomal RAB11A messenger RNA and long noncoding RNA-RP11-583F2.2 along with the increase in exosomal miR-1298	36
	Wistar rats	Renal cancer	100 and 200 mg/kg body weight	↓PGE2, COX-2, VEGF, and improved renal function by ↓BUN, creatinine, and KIM-1	37
	Wistar rats	Renal cancer	100 and 200 mg/kg b wt	Induce caspase-3, caspase-9, bax expression, and downregulate bcl-2, NFκB, iNOS, TNF-α, PCNA expression	30
	Wistar rats	Hepatocellular carcinoma	200 mg/kg body weight	↓PI3K, Akt, CDK-2 protein expression	38
	Mice	Lung cancer	25 mg/kg body weight	↓COX-2, MMP-2, and MMP-9	39
	Colon carcinoma (CT-26)–bearing mice	Colon cancer	200 mg/kg	↓WBC count caused by cyclophosphamide to reduce antitumor effect	40
	7, 12-Dimethybenz (a) anthracene-induced rats	Breast cancer	30 mg/kg	Decline in lipid peroxidation and membrane bound marker enzyme AST, ALT, ALP, ACP, 5′ND, γ-GT	41
	Benzo(a)pyrene-induced Swiss albino mice	Lung cancer	50 mg/kg	↓lipid peroxides, aryl hydrocarbon hydroxylase (AHH), gamma glutamyl transpeptidase (γ-GT), 5′-nucleotidase (5′-ND) and lactate dehydrogenase (LDH)	42

(tumor necrosis factor-alpha), PCNA (proliferating cell nuclear antigen) expression, which were associated with Hesp anti-cancer activities *in vivo*.[30] In xenograft model of colon cancerous mice, Hesp also showed mitochondrial pathway-mediated apoptosis induction, and cell cycle arrest at G_2/M phase.[31]

Anti-angiogenesis and anti-metastasis

In view of the limitations of presently marketed monoclonal antibodies and synthetic compounds as anti-angiogenic agents pertaining to toxicity and high cost incurred, natural compounds are being extensively explored as potential anti-angiogenic agents.[43,44] These natural products such as flavonoids (e.g. hesp) modulate tumor angiogenesis via targeting vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), basic fibroblast growth factor (bFGF), endothelial cell proliferation, migration, and metastasis owing to their anti-proliferative potential.[5] The first excerpts of the anti-metastatic and anti-proliferative effect of hesperetin were documented in 2007 by Lentini *et al.*[45] in B16-F10 metastatic murine melanoma cells *in vitro* and C57BL6/N mice *in vivo*. Thereafter, in 2009, Yeh *et al.*[46] illustrated anti-metastatic potential of Hesp *in vitro* in HepG2 human hepatocellular carcinoma cells where hesperidin suppressed secreted cytosolic MMP-9 expression via inhibition of activator protein-1 (AP-1, JNK signaling pathway) and NF-κB (NF-κB signaling pathway). This was further supported by findings from other studies in 2010 which reported Hesp inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cytosolic MMP-2 and MMP-9 and cyclooxygenase-2 (COX-2) expression via modulating NF-κB and AP-1 induced tumor cell invasion and metastasis in lung cancer and hepatocellular carcinoma (Figure 3).[39,47] Later, in 2012, hesperetin was documented to inhibit TGF-β1 (transforming growth factor-β signaling pathway)-induced tumor migration and metastasis via phosphorylation of Smad3 (SMAD family member 3).[48]

In 2015, Kim [49] reported the inhibitory effect of Hesp on vascular formation in human umbilical vascular endothelial cells (HUVECS) and mouse embryonic stem cell (mES)-derived endothelial like cells via blocking AKT and mTOR signaling pathway which lead to inhibition of cell migration, suppression of micro-vessel sprouting, and tube

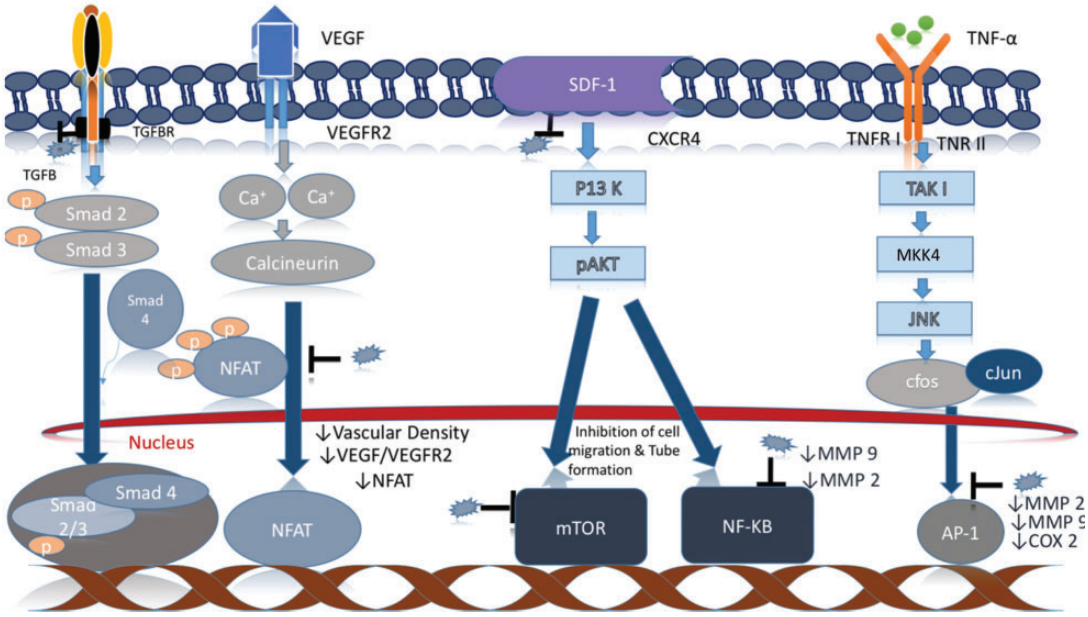

Figure 3. Cellular targets of hesperidin while acting as anti-angiogenic and anti-metastatic agent. (A color version of this figure is available in the online journal.)

formation in HUVECS. Zhao *et al*.[50] documented the anti-angiogenic effect of Hesp (a component of Qingdu granule) in MCF-7 and HUVECs cells where Hesp inhibited migration and tube formation in human breast cancer cells via downregulation of NFATc3 (nuclear factor of activated T-cells) expression. In female BALB/c nude mice, a xenograft tumor model *in vivo* Hesp inhibited tumor growth, decreased vascular density, inhibited VEGF, and down-regulated NFATc3, VEGF, and VEGFR2 expression via NFAT signaling pathway.[50] Further research in the domain of anti-metastatic potential of Hesp in pancreatic cancer has shown to target MKK3/6 and p38 intracellular signaling pathways.[51] In A549 non-small cell lung cancer cells, Hesp was described to significantly inhibit tumor migration capability via targeting SDF-1α (stromal cell-derived factor 1) leading to downregulation of CXCR-4 (C-X-C chemokine receptor type 4), p-Akt, p-IκB (phosphorylated-I kappa B), and p-p65 expression (SDF-1/CXCR-4 signaling cascade).[52] Hesp was also documented to inhibit cell migration and invasion in human osteosarcoma MG-63 cells via wound healing and matrigel assay and *in vivo* in male BALB/c xenograft mice model.[31]

Hesperetin administered in combination with platinum drugs *in vitro* in A549 lung adenocarcinoma cells and in *in vivo* in C57BL/6 mice inhibited tumor proliferation and migration via targeting UDP-glucuronosyltransferse (UGT) family 1 member A3 (UGT1A3) more significantly in comparison to single drug treatment regime[53]. In another study, combined administration of naringenin with hesperetin was shown to maximize anti-metastatic effect in Panc-1 human pancreatic cancer cells via downregulation of FAK (focal adhesion kinase) and p38 signaling pathway[54]. These *in vitro* (Table 2) and *in vivo* studies (Table 1) on the cumulative front document the potential anti-angiogenic and anti-metastatic potential of hesperidin which may be used as a promising anti-cancer strategy for the management of human cancers without eliciting toxic effects on surrounding normal cells.

Anti-oxidant and anti-inflammatory effects

Inflammation is a complex physiological and biological process in which body fights toward the harmful stimuli such as undigested particles, chemical irritants, damaged cells, moreover viral, bacterial and parasitic infections by overexpression of various cytokines, chemokines, and pro-inflammatory mediators, including TNF-α, COX-2, IL-1β (interleukin-1β), IL-6, IL-8, iNOS, NO (nitric oxide), prostaglandins, and eicosanoids.[60–65] Apart from the vigorous dependence of the expression level of pro-inflammatory mediators on the activation of different signaling pathways that can be regulated by various factors such as MAPKs (mitogen-activated protein kinases), NF-κB, ICAM-1 (intercellular adhesion molecule-1), and VCAM-1 (vascular cell adhesion molecule-1), there is a tight relationship between inflammation and production of ROS and RNS (reactive nitrogen species).[66–71] Moreover, the mounting evidences indicate that both inflammation and oxidative stress drive carcinogenesis by inactivation of tumor suppressor genes, activation of oncogenes, and disruption of various cellular signaling pathways.[72,73] On the other hand, it is a wide thought that Hesp and its derivatives can be considered as the substantial and effective traditional flavonoids on oxidative stress and inflammation along with proliferation, apoptosis, DNA damage, free radicals, carcinogenesis, hypertension, hyperglycemia, and hypolipidemia.[74,75] The studies on anti-inflammatory mechanisms of Hesp indicate that Hesp can reduce the level of inflammatory factors such as VCAM-1, COX-2, MMP-2, MMP-9, PGE2 (prostaglandin E2), IL-4, IL-6, iNOS, and NO_2.[74,76] The anti-oxidative, chelating, and strong reducing properties along with the hydroxyl, hydrogen peroxide, superoxide, and free radical scavenging activities of Hesp occurred depending on the

Table 2. A brief overview of the in vitro studies carried out using hesperidin to study effect on cellular processes.

Effect	Underlying mechanism	Concentration	Cell line	Cancer type	Refs
Apoptosis	↑Caspase-9, -8, and -3 activities, Bax, Bak, and tBid protein levels ↓Bcl-xL protein	150.43 ± 12.32 μM	HepG2	Hepatic cancer	55
	↑caspase-3 and ↓mitochondrial membrane potential	50 μM	A549 and NCI-H358	Lung cancer	23
	cleavages of Bid, caspase-3, and PARP, upregulation of Bax, and down-regulation of Bcl-xl	152.3 μM	MSTO-211H	Human malignant pleural mesothelioma	56
	↑caspase-3 cleavage expression through ADD153'CHOP'GRP78 and cytochrome c signaling pathways	10 μM	A2780	Ovarian Cancer	57
	↑expression pf PPARγ and p53 accumulation, ↓NF-κB	10 μM	NALM-6	Lymphoblastic leukemia	58
	↑ROS, nuclear condensation, and activation of Caspase-3	143.39 mM	Primary GBC	Gall bladder carcinoma	26
Cell cycle arrest	G2/M phase cell cycle arrest	33.5, 23.8 and 17.6 μM, respectively, at 24, 48 and 72 h	MG-63	Cervical cancer	31
	G0/G1 arrest by ↓cyclinD1, cyclinE1, and cyclin-dependent kinase 2 at the protein level.	0, 40, 80, and 160 μM	HeLa, HT-29	Cervical cancer and Colon cancer	
	G0/G1 arrest by ↓cyclin D1 and ↑p21 , p53	75–125 μg/mL	A549	Lung cancer	24
Anti-metastatic	↓acetaldehyde-activated NF-κB and activator protein 1 (AP-1) activity results in ↓MMP-9 expression	50 μM	HepG2	Hepatocellular carcinoma	46
	↓MMP-9 via NF-kappaB an AP-1 signaling pathway	50 μM	HepG2	Hepatocellular carcinoma	47
	14-fold increase in loss of MMP via FGF and NF-κB signal transduction pathways	50 μM	A549	Lung cancer	23
	inhibit migratory and invasive capability by SDF-1/CXCR-4 signaling cascade	25–65.5 μg/mL	A549	Lung cancer	52
Anti-angiogenic	inhibits vascular formation by blocking the AKT/mTOR signaling pathways	100 μM	HUVECs	Nil	49
Anti-oxidant	Cytotoxicity mediated through antioxidant properties	IC50 recorded at b10 μg/mL	MCF-7, HEp-2, HeLa and HepG-2	Breast, larynx, cervix and liver carcinoma	59

concentration and originated from the chemical structure by acting as a hydrogen donor to the radical molecules, and played a radical target role to form new complexes between the antioxidant radicals and the lipid radicals thanks to the presence of 300-hydroxy, 400-o-methoxy system in the B ring.[2]

Moreover, the radical scavenging activity is increased due to the entity of 300-OH and 5-OH groups, in combination with a 4-carbonyl function and C40–C80 double bond though the anti-oxidant activity of Hesp can be considered as moderate according to the other flavonoids due to the lack of OH at C4.[2,9,68,77] On the other hand, the non-glycosylated form of Hesp named hesperetin displays a stronger anti-oxidant activity than hesp because it has an additional hydroxyl group in its molecular structure and O-glycosylation reduces the antioxidant activity along with the electronic delocalization capacity (Figure 1).[12,78] The anti-oxidant activity of Hesp and hesperetin, which can be achieved by boosting cellular anti-oxidant defense and radical scavenging, has been verified by various *in vivo* and *in vitro* experimental models.[2,74] For example, it was reported that Hesp could protect the cellular components such as DNA and proteins by radical scavenging and ROS neutralizing activity.[12,76,79] Moreover, it has been proved in the experimental models that Hesp counteracts the harmful activities of hydrogen peroxide, peroxynitrite, carbon tetrachloride, cadmium, acetaminophen, nicotine, cyclophosphamide, acrylonitrile, dimethylbenz[a]anthracene, tert-butyl hydroperoxide, lipopolysaccharide, benzo[α] pyrene, technetium, gamma radiation and many others by increasing the level and activity of antioxidant enzymes and compounds such as GSH (reduced glutathione), GPx (glutathione peroxidase), GST (glutathione S-transferase), GR (glutathione reductase), SOD (superoxide dismutase), CAT (catalase), vitamin C and vitamin A, and decreasing the level of cellular damage markers such as LDH (lactate dehydrogenase), LPO (lipid peroxides), GGT (gamma glutamyl transpeptidase), AHH (aryl hydrocarbon hydroxylase), 5 ND (5 nucleotidase), ALP (alkaline phosphatase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), and bilirubin.[76,80,81]

The Hesp and hesperetin induced increase in the level and activity of the anti-oxidant enzymes can be achieved via Keap1-Nrf2 (nuclear factor erythroid 2-related factor 2) pathway, which is known as the major regulator of oxidative and electrophilic stress.[75] Briefly, Hesp and hesperetin increase the expression of Nrf2, separate Keap1-Nrf2 complex, and increase the nuclear translocation of Nrf2, and the

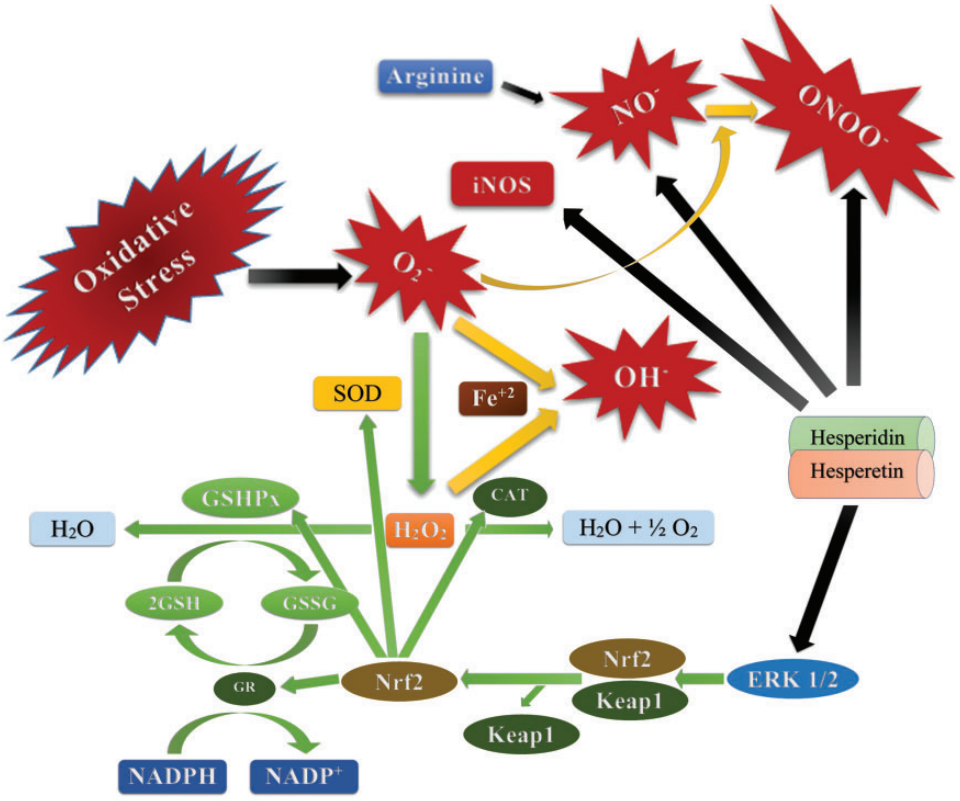

Figure 4. The anti-oxidant activity mechanisms of hesperidin. (A color version of this figure is available in the online journal.)

production of anti-oxidant enzymes is increased by the activation of gene transcription thanks to the binding of Nrf2 to the antioxidant response element (ARE) within gene promoter region.[75,82] Further, Hesperetin derivative-14 (HD-14) has also been reported to have anti-inflammatory potential and has been shown to inhibit p-JAK1/p-STAT2 via PPAR-γ upregulation in LPS-treated RAW264.7 cells.[83] Similar results were reported using another Hesperetin derivative-12 (HDND-12) in RAW264.7 cells and were reported to down-regulate p-JAK2/p-STAT3 expression.[84] Consequently, the derived perception from the published studies about hesperidin and its derivatives indicates that the anticancer property of Hesp seems to be largely originated from its anti-oxidant and anti-inflammatory activity (Figure 4).

Synergistic effect of hesperidin with other anti-cancer agents

Hesp, a flavanone, is present in different citrus fruits and possesses a number of biological activities.[85] Hesp is identified to possess potent anti-inflammatory, anti-carcinogenic, and anti-oxidant activities in different studies.[5] The chemical structure of Hesp consists of hesperetin (methyl eriodictyol) bound to rutinose.[12] The glycoside entity of Hesp is a disaccharide that comprises rhamnose and glucose.[12] The research in present times on anticancer activities of natural compounds has been targeted on induction of cancer cell death. These natural compound induces cancer cell death initiated by apoptosis (type I programmed cell death), autophagic cell death (type II programmed cell death), and necroptosis (programmed necrosis).[85–88] Hesp and Hesperetin cause cell proliferation delay in different cancer models. Different effects of these compounds have been reported depending on the factors including dose, type of compound, and cell line under study (Table 3). Cell cycle arrest related to cytostatic effects has been reported in cells that have elevated p53 and cyclin-dependent kinase inhibitor levels, along with lowered levels of cyclins and cyclin-dependent kinases.[55]

Synergistic interaction of hesperidin with different compounds

Doxorubicin

Doxorubicin (DOX) is widely employed in anti-tumour therapies.[94,95] In spite of the systematic chemotherapy with doxorubicin, it provides only peripheral improvements in a survival of the hepatocellular carcinoma patients.[96,97] The main mode of action of doxorubicin includes intercalation within DNA base pairs, thus resulting in DNA strand breakage and an inhibition of DNA and RNA synthesis by inhibition of topoisomerase II, resulting in DNA damage and apoptosis induction.[98] The doxorubicin also initiates the ROS generation thus causing cell death. The application of apigenin and Hesp alongside doxorubicin revealed the effect on doxorubicin-induced

Table 3. Synergistic relationship of hesperidin with other natural compounds against cancer.

Molecules in synergism	Effects	Refs
Doxorubicin – hesperidin	Alterations in the expression levels of HK2 and LDHA	89
Doxorubicin – hesperidin	PgP expression inhibition	90
Cytarabine – hesperidin	Lowering in IC50 values of Cytarabine	91
Tamoxifen – hesperidin	Apoptosis induction, cell cycle arrest and downregulation of EGFR (epidermal growth factor receptor) and ERα (estrogen receptor alpha).	92
Quercetin – hesperidin	H_2O_2 scavenging	99

toxicity. These compounds altered the expression levels of glycolytic pathway genes – HK2 (hexokinase 2) and LDHA (lactate dehydrogenase A), which possess a major role in the Warburg effect. The simultaneous administration of doxorubicin and apigenin or Hesp eradicated the damage with the simultaneous increase in the doxorubicin toxicity. In another study, effect of Hesp has been investigated in combination with doxorubicin to check its effect on doxorubicin-resistant MCF7 breast cancer cell lines. The cytotoxic effects were studied using MTT assay. Hesp combined with doxorubicin was unable to increase the apoptotic initiation but inhibited the PgP (P-glycoprotein) expression[90] which is mainly responsible for developing the multidrug resistance after administration of doxorubicin.

Cytarabine

In another study, the use of Silibinin and Hesp showed 50% cell inhibition at 16.2 μM and 50.12 μM, respectively,[91] whereas the fixed doses of Hesp and Silibinin in conjunction with Cytarabine at different concentrations lowered the IC 50 value of Cytarabine by 5.9 and 4.5 folds, respectively. Drug interaction analysis exhibited that Silibinin and Hesp showed synergistic effect with Cytarabine in 1:50 to 1:250 ratios.[91] Hence, these compounds can be employed as chemotherapeutic agents either alone or in combination.

Tamoxifen

Tamoxifen (Tam) is a commonly used anticancer drug for estrogen receptor (ER)-positive breast cancer treatment. In different studies, it was shown that the some natural compounds, like Hesp, piperine (Pip) and bee venom (BV) have inhibitory effect on the breast cancer cells growth when used individually. The combined effect of these natural compounds and Tam was investigated in a study with a hypothesis that these compounds can increase the potential efficacy of growth inhibitory activity of Tam. The cytotoxic activity of Hesp, BV, and Pip was examined on MCF7 and T47D breast cancer cell lines via MTT assay and achieved equitable IC50 comparable to Tam results.[92] The effect of different combinations was investigated and enhanced anti-proliferative result was obtained on MCF7 and T47D cell lines due to the synergistic effect. These natural compounds can synergistically increase the potential anticancer property of Tam against MCF7 and T47D cells probably by inducing the apoptosis, cell cycle size and EGFR (epidermal growth factor receptor), and ERα (estrogen receptor alpha) downregulation.

Quercetin

Etoposide [40-demethylepipodo- phyllotoxin- 9–(4,6-O-ethylidene) -b-d glucopyranoside] is a derivative of natural product, podophyllotoxin. Etoposide acts as therapeutic agent in different types of cancer due to its ability to inhibit the topoisomerase II enzyme and induction of DNA breaks. In a study, the synergistic effect of Hesp and quercetin was studied to improve oxidative damage caused by Etoposide on reproductive system in male rats. In this study, it was observed that administration of quercetin at concentration levels of 20 mg/kg body weight and Hesp at 25 mg/kg body weight for two months significantly enhanced sperm motility and count in experimental groups in comparison to etoposide-treated group. This improvement in sperm motility and count can be attributed to the H_2O_2 scavenging by quercetin and Hesp, resulting in inhibition of cellular DNA damage[93,99].

Role of nano-technology in hesperidin delivery

Natural anti-oxidants such as hesperidin has shown promising impact for the treatment of malignant growth and other alike diseases due to high efficacy and lower side effects as compared to synthetic drugs.[100,101] But Hesp's clinical use was extremely restricted due to lower aqueous solubility and poor bioavailability.[102] So there is need to overcome these issues for optimal use of this compound.[103] Nanotechnology is an interdisciplinary area of research having broad applications like molecular imaging, molecular diagnosis, and particularly targeted drug delivery.[104] Further, this technology can also overcome the solubility and bioavailability issues of drugs[105] as these parameters have tremendous impact on treatment of cancer.[106] Therefore, several studies were initiated on developing Hesp-based nanoparticles to improve the bioavailability, absorption, and bio-distribution of this flavonoids.[103,1062525–109] Gu *et al.*[106] have developed two nano-based formulations, i.e. hesperetin-TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate) micelles and hesperetin-phosphatidylcholine (PC) complexes to improve the water solubility, antioxidant activity, and oral absorption of hesperetin and these formulation led to the increase of 16.2- and 18.0-fold in *in-vitro* antioxidant potential and *in vivo* oral absorption of hesperetin.

Similarly, Duranolu *et al.*[103] developed hesperetin-loaded nanoparticles with high encapsulation efficiency and low particle size and various process parameters were optimized for the optimal use of hesperetin. Further to develop new targeting strategies Ferrari *et al.*[108] have developed Hesp-coated solid lipid nanoparticles and evaluated there various physiochemical properties for optimum-targeted delivery for the treatment of several disorders. Praveen Kumar *et al.*[109] evaluated cerebroprotective potential of hesperidin nanoparticles for the effective treatment of cerebral ischemia in rats. To increase the drug delivery potential for topical applications, Menezes *et al.*[110] reported to fabricate textile-based Hesp-loaded nanocapsules. These fabric-based nanocapsules were found to be suitable for sustained release of drug. Although efforts were initiated for the fabrication of Hesp-coated nanoparticles for the effective treatment of various disorders, but there is lacunae of reports where Hesp-coated nanoparticles were clinically tried for cancer treatment. Therefore, more progressive endeavors are required to integrate hesperidin-based nanoparticles for the effective treatment of cancer and other diseases.

Conclusion and future perspectives

The importance of citrus bioflavonoids is attributed to the pharmacological activity of Hesp. Despite extensive treatment protocols and regimes for cancer patients ranging from surgery to chemotherapy to immunotherapy, cancer is not completely curable and the conventional treatment regime is associated with short-term and long-term adverse events. Recently, lot of research is ongoing to explore bioflavonoids, e.g. Hesp in cancer treatment in view of their potential antioxidant property. Recent literature from different research groups have emphasized on the potent antioxidant capacity of Hesp and its potential role as anticancer agent. This review demonstrates the anti-tumor effects of Hesp in different malignancies with special emphasis on its molecular mechanism of action. The importance of Hesp as anti-cancer agents is evident from the experimental evidences emphasizing on how Hesp modulates oxidative stress, inflammation, and cancer cell death (hallmarks of cancer). Further, Hesp has been reported to augment apoptosis in malignant cells via NF-κB B, mTOR, PI3K/AKT pathways.[111] Hesp has also been documented to down-regulate pro-inflammatory mediators and enzymes (IL-1/6, TNF, COX-2) in tumorigenesis and improve anti-oxidant defense mechanism. In addition, Hesp significantly improves pharmacological symptoms of other diseases such as arthritis, myocardial, and infertility.[112–116] Therefore, main benefit of using flavonoids in contrast to chemotherapeutic agents is attributed to their low toxicity and tolerability, and it is worthwhile to mention that even at highest dose, Hesp/hesperetin do not cause cytotoxic effects or acute oxidative damage. However, further studies are required to unravel the therapeutic effects of Hesp/hesperetin in cancer treatment. Though, Hesp is presently in pre-clinical trials, promising data from clinical trials are warranted to increase the translation applicability of Hesp in cancer treatment. From the future perspective, future *in vitro* and *in vivo* studies focusing on the following dimensions of Hesp need to be studied to translate the practical applicability of Hesp as anticancer agent:

1. To increase the bioavailability and absorption of Hesp/hesperetin (aglycone form).
2. Defining the precise molecular mechanisms of action of hesp through meticulously designed and executed experimental studies for anticancer effects Hesp.
3. Standardizing the optimum effective dose of Hesp for translation in clinical trials in combination with conventional chemotherapeutic agents and targeted therapies.
4. Evaluation of safety and efficacy findings in cancer patients undergoing treatment with Hesp.

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