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Review Article

Exploring the Potential of Hesperidin and Synergistic Formulations in Breast Cancer Management: A Comprehensive Review

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ABSTRACT

Hesperidin, a natural compound abundant in citrus fruits, shows promise in breast cancer management due to its safety and diverse mechanisms of action. This review explores hesperidin's origin, chemical properties, and its ability to inhibit cell proliferation through various pathways. While hesperidin alone has limited efficacy against breast cancer, it demonstrates synergistic effects when combined with other compounds. Formulation strategies to enhance hesperidin's efficacy include improving solubility, stability, and absorption for targeted delivery. Combisomes, liposomal delivery systems encapsulating hesperidin and anticancer compounds, offer a potentially efficacious and safe platform. They target cancer cells specifically, minimizing adverse effects on healthy cells. Hesperidin's ability to induce cell cycle arrest, apoptosis, and modulate PD-L1 expression contributes to its anticancer effects. Combining hesperidin with other agents enhances its therapeutic potential. Overall, hesperidin emerges as a promising candidate for breast cancer treatment, with formulation enhancements and innovative delivery systems like combisomes offering significant advancements in therapeutic strategies. Further research into hesperidin's efficacy and safety, especially in combination therapies, is warranted for its potential translation into clinical practice.

Keywords: Breast cancer, Hesperidin, Synergistic effect, Combisomes, Citrus fruits.

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INTRODUCTION

Breast cancer is a pervasive and devastating disease that accounts for 31% of the cancer cases in females, with 2,97,790 estimated new cases and the estimated death of 43,170.[1] The prevalence of breast cancer has been on the rise since the 1970s, posing a significant public health challenge.[2] Conventional treatment strategies have focused on a comprehensive approach, including surgery, radiotherapy, chemotherapy, and immunotherapy. Recent advancements in molecular targeted therapy have provided promising avenues for the management of breast cancer. Modern cancer treatment involves targeted agents that selectively inhibit specific molecular pathways or signalling cascades in tumor growth and proliferation. These targeted therapies aim to enhance the efficacy of cancer treatment while minimizing the unfavourable side effects associated with traditional chemotherapeutic agents.[3]

However, the existing treatment approaches still face significant challenges, such as developing drug resistance and the inability to eliminate residual cancer cells. To overcome these problems, researchers are exploring alternative treatment modalities, including herbal remedies and photothermal/photodynamic therapy.

Hesperidin, a flavonoid that is found abundantly in citrus fruits has gained significant importance for its remarkable therapeutic properties. Hesperidin has been reported to cause apoptosis and cell cycle arrest through various mechanisms against breast cancer. Additionally, hesperidin showcases significant promise, with favourable safety profiles observed across short and long-term evaluations. Notably, its toxicity parameters highlight its low potential for harm to humans. The maximum tolerated dose in humans (0.525 log mg/kg/day) indicates minimal risk at higher doses. Furthermore, its oral rat acute toxicity (LD_{50}) of 2.506 mol/kg suggests a low acute toxicity level. In chronic toxicity assessments, the oral rat chronic toxicity (LOAEL) of

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3.167 log mg/kg_bw/day indicates a higher threshold for adverse effects over prolonged exposure.[4] The median Lethal Dose (LD_{50}) of hesperidin administered intraperitoneally in mice is 1 g/kg body weight.[5] Oral acute toxicity is observed at an LD_{50} of hesperidin exceeding 2000 mg/kg, while subacute and chronic toxicity occurs at doses exceeding 2000 mg/kg.[6] This underscores Hesperidin's promising status as a flavonoid with favourable attributes and negligible toxicity concerns.

Hesperidin exhibits low bioavailability mainly due to solubility issues and is overcome by various formulation approaches and by structural modifications of hesperidin. Further, hesperidin has shown to demonstrate synergistic effect against breast cancer when combined with anticancer agents or another phytomolecule. This paper aims to provide a comprehensive overview of the existing research on the anticancer potential of hesperidin in breast cancer.

Hesperidin source and chemistry

Hesperidin was initially isolated in 1828 by Leverton from the spongy inner portion of orange peel.[7] Subsequently, it was identified in lemons by Pheffer in 1874 and has also been isolated with neohesperidin, an isomer of hesperidin, from citrus fruits.[8] The name "hesperidin" is derived from "Hesperidium," which signifies fruits of citrus.[9] Additionally, it is known as hesperetin-7-rutinoside. Hesperidin serves as the food-bound form of hesperitin and was previously termed Vitamin P due to its vitamin-like attributes, particularly in wound healing.[8]

Hesperidin, as discussed, is primarily present in orange peels and various citrus fruits (family Rutaceae) such as unripe, sour, and sweet oranges; *Citrus sinensis*,*Citrus aurantium, Citrus unshiu, Citrus reticulata* (Tangerines) and*Citrus mitis*, clementine, lemons, lime, and grapefruits. It is a polyphenolic bioflavonoid flavanone glycoside.[10] It has also been discovered in abundance within the peel and membranous parts of orange peel and in various organic citrus products.[11] A recent review[12] shows that the Hesperidin content in 100 mL of juice varies: oranges contain 20-60 mg, tangerines 8-46 mg, lemons 4-41 mg, and grapefruits 2-17 mg. The concentration of Hesperidin varies even within different parts of the fruits. For example, citrus flavedo (the outer colored layer of the peel), albedo (soft white middle part), membrane, and pith contain higher amounts of hesperidin compared to other parts such as juice vesicles and seeds.[12,13] In addition to citrus fruits, hesperidin has also been discovered in mint plants (Mentha)[14,15] and honeybush (*Cyclopia maculata*).[16] It is noteworthy that hesperetin, the aglycone form of hesperidin, is less prevalent than its glycosides.

Hesperidin is a complex glycoside composed of aglycone flavanone moiety-hesperitin and the glycon sugar molecule-rutinose.[17,18] The structure of Hesperidin and Hesperitin had been represented in Figure 1. Hence, hesperidin is chemically also known as hesperitin-7-O-rutinoside. Hesperidin serves as a prodrug for hesperitin, the primary active molecule within the structure, akin to how G-hesperidin functions as a prodrug of hesperidin.[8] Both hesperitin and hesperidin can exist in S and R isomeric forms.[19] Pure hesperidin is a white needle-like powder with a melting point between 252-254ºC, a molecular weight 610.6, and a chemical formula of $C_{28}H_{34}O_{15}$.[20]

Pharmacokinetics and bioavailability enhancement

Pharmacokinetics is a process that describes how the body processes and interacts with drugs, which includes absorption, distribution, metabolism, and excretion of drugs. Hesperidin is deglycosylated by the colonic microbiota to a more readily absorbed form, hesperetin.[21] Hesperidin can be converted to hesperetin through either a one-step or a two-step process. In the one-step process, α-rhamnosyl-β-glucosidase catalyzes the deglycosylation directly. Conversely, in the two-step process, α-rhamnosidase and β-glucosidase catalyze sequential deglycosylation steps, with hesperetin-7-O-glucoside serving as an intermediate.[22] In the colon, hesperetin is absorbed by the colonocytes via proton-coupled active transport and transcellular passive diffusion.[23]

On the other hand, hesperetin and hesperetin-7-O-glucoside are directly absorbed by the enterocytes in the intestine.[24,25] After absorption, hesperetin undergoes significant phase II metabolism mediated by Uridine Diphosphate (UDP) glucuronosyl transferases and sulfotransferases. This metabolism results in the formation of glucuronide and sulfate metabolites, which are highly hydrophilic, indicating that they have a strong affinity for water and can easily dissolve in bodily fluids like plasma[26] and subsequently, hesperidin gets distributed in various tissues and organs of the body post-absorption. The extensive metabolism of hesperetin within intestinal cells, coupled with the subsequent transport of hesperetin conjugates back into the gut lumen by apical Adenosine Triphosphate (ATP)-Binding Cassette (ABC) transporters like ABC transporter G2 (ABCG2), ultimately reduces hesperetin's bioavailability.[27] Hesperidin taken orally exhibits a delayed half-life of 5-8 hr, primarily due to the activity of gut microbes. It is mainly eliminated through urine within 24 hr after ingestion, primarily in monoglucuronide.[25,28]

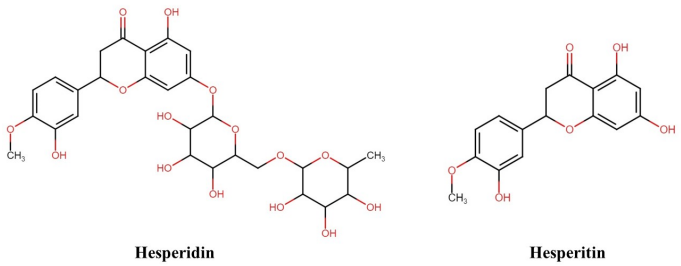

Figure 1: Chemical structure of Hesperidin and Hesperetin.

The absolute bioavailability of hesperetin was reported to be twice as high as that of hesperidin, with values of 61.5 and 30.1 nmol.h/mL, respectively.[29] The reduced bioavailability of hesperidin in research studies is attributed to its poor water solubility, which significantly hampers its absorption.[29,30] Various strategies have been employed to improve the bioavailability of hesperidin, including nano- or micro-encapsulation, to protect it from harsh environmental conditions and enhance its stability during storage and digestion. Another approach is to improve the solubility of hesperidin by utilizing techniques such as complexation, emulsification, or solid dispersion.[31]

Additionally, nanotechnology techniques such as nanosuspensions or self-nanoemulsified drug delivery systems can further increase the dissolution rates and *in vivo* bioavailability of hesperidin. These strategies can improve the bioavailability of hesperidin by increasing its stability, solubility, and absorption in the body,[32] which are summarized in Table 1. Additionally, various forms of hesperidin have been developed to enhance its solubility in water and thus its absorption in the gut. Glucosyl-hesperidin (G-Hesperidin), also known as alpha glycosyl hesperidin, is a modified version where the diglycoside group is altered into a triglycoside, while maintaining the hesperitin structure unchanged.[33] G-hesperidin exhibits significant water solubility.[34] Once inside the body, G-hesperidin is converted into hesperidin, releasing free hesperitin responsible for its biological effects. Studies on rats indicate that G-hesperidin leads to faster appearance in blood plasma (3.7 fold) and higher bioavailability (4 μM) of hesperitin compared to hesperidin alone.[34] G-hesperidin is commonly used as a skin tonic, enhancing blood circulation when topically applied, alleviating stress, fatigue, and cold sensations.[8] Another synthetic variant is Hesperidin-7,3'-O-Dimethylether (HDME), a lipid-soluble form with one modified hydroxyl group converted into a methoxyl group on the B ring. HDME demonstrates more significant phosphodiesterase inhibitory potential compared to hesperitin.[35] Hesperidin methyl chalcone, another water-soluble variant, is often used in medicinal formulations. This semi-synthetic form features an open ring with multiple methyl group substitutions, enhancing water solubility and metabolic stability.[36,37]

Mechanism of action against breast cancer

The mechanism of action of hesperidin against breast cancer involves several pathways, as shown in Figure 2. One of the fundamental mechanisms is inhibiting cancer cell proliferation by inducing cell cycle arrest at the G0/G1 phase.[46] This is achieved by downregulating the expression of cyclin D1 and cyclin-dependent kinase 4, which are essential for cell cycle progression.[47] Additionally, hesperidin has been found to induce apoptosis in breast cancer cells, which is mediated by the upregulation of pro-apoptotic proteins such as Bax and the down regulation of anti-apoptotic proteins like Bcl-2. Hesperidin was found to induce apoptosis in both MCF-7 and MDA-MB-231 breast cancer cell lines but through distinct mechanisms. In MCF-7 cells, apoptosis is triggered by downregulating Bcl-2 and upregulating Bax proteins, leading to cellular death. Conversely, in MDA-MB-231 cells, hesperidin induces apoptosis without activating the Bax/Bcl-2 pathway. Specifically, it can modulate the expression balance of Bax and Bcl-2 proteins in ER/PR (+) MCF-7 cells, while inducing apoptosis in ER/PR (-) MDA-MB-231 cells through different mechanisms.[48] Another study investigated hesperidin's impact on PD-L1 expression and cancer progression in breast cancer cells. MDA-MB231, a triple-negative breast adenocarcinoma cell line known for high aggressiveness, exhibited elevated PD-L1 expression compared to MCF-7 cells, which have lower aggressiveness. Hesperidin inhibited cell proliferation in MDA-MB231 cells, effectively suppressing PD-L1 expression at both mRNA and protein levels through Akt and NF-κB signaling inhibition. Furthermore, hesperidin treatment decreased the activation of matrix metalloproteinases like MMP-9 and MMP-2, thereby impeding the metastatic phenotype and cell migration in PD-L1 high-expressing MDA-MB231 cells.[49] Hesperidin-induced apoptosis in breast cancer cells primarily occurs through upregulation of caspase-3 protein expression and accumulation of p53 protein. Additionally, apoptosis is facilitated by perturbations in redox balance, evidenced by decreased Glutathione (GSH) levels and increased Lactate Dehydrogenase (LDH) levels within breast cancer cells.[50] Hesperidin exhibits constrained efficacy against breast cancer when employed as a monotherapy. Although it has potent antioxidant and anti-inflammatory properties, theoretically suggesting potential anticancer effects, empirical evidence reveals its limitations in yielding substantial therapeutic benefits when utilized as a standalone treatment.

Bax- pro-apoptotic protein; Bcl-2-antiapoptotic protein; p53-tumor suppressor protein; PD-L1-programmed cell death ligand 1; Akt- Protein Kinase B (PKB), plays a key role in cellular processes; NF-κB-Nuclear Factor-kappa B, a protein complex that controls DNA transcription; MMP-9 -Matrix Metalloproteinase-9 and MMP-2-Matrix Metalloproteinase-2, both facilitate tumor invasion and metastasis by breaking down the extracellular matrix barriers.

Synergistic combinations with Hesperidin

Combination therapy involving hesperidin, along with anticancer agents or phytochemicals, presents a promising strategy for enhancing therapeutic outcomes in breast cancer management. While hesperidin alone may exhibit limited potency against breast cancer, its synergistic interactions with other compounds offer multifaceted advantages. In a study, hesperidin and doxorubicin showed a synergistic effect on MCF-7 cells resistant to doxorubicin by inhibiting Pgp expression.[51] Combining tamoxifen with hesperidin, piperine, and bee venom led to increased apoptosis, downregulation of EGFR and ERα, and G2/M phase cell cycle arrest in breast cancer cells. Synergistic effects suggest potential adjuvant use of these compounds

with tamoxifen pending *in vivo* validation.[52] Hesperidin and chlorogenic acid, commonly found in plants and foods, exhibit synergistic inhibition of breast cancer cell growth, particularly in MCF-7 cells, without affecting normal breast cells (MCF-10A). This combination modulates mitochondrial function and ATP production via the estrogen receptor pathway.[53] Apigenin and hesperidin enhance doxorubicin's cytotoxicity on MCF-7 breast cancer cells. Despite reducing doxorubicin-induced oxidative damage, they increase double-strand breaks and downregulate DNA repair genes, suggesting an augmented cytotoxic effect irrespective of oxidative stress.[54]

Through such combinatory approaches, synergistic effects can be harnessed to potentiate the anticancer activity of hesperidin, enhancing its efficacy in inhibiting tumor progression and metastasis. Moreover, incorporating hesperidin into combination regimens can reduce toxicity profiles, thereby mitigating adverse effects commonly associated with conventional chemotherapeutic agents. *In vitro*, hesperidin enhances doxorubicin's cytotoxicity in highly metastatic breast cancer cells, 4T1 cells, leading to increased apoptosis, G2/M cell cycle arrest, and inhibited migration. Combined treatment reduces the expression of migration-related proteins MMP-9 and Rac-1, suggesting a potential strategy to enhance doxorubicin's anticancer efficacy while minimizing chemotherapy risks in metastatic breast cancer.[30] Combined therapy with tamoxifen, hesperidin, and camel milk-derived exosomes synergistically inhibits MCF7 breast cancer cell proliferation, migration, and invasion while reducing tamoxifen's adverse effects. This cotreatment upregulates pro-apoptotic markers, downregulates antiapoptotic markers and breast cancer-related genes, and modulates MMP9/TIMP1 expression, suggesting a promising adjuvant strategy for breast cancer chemotherapy.[31] Additionally, the diverse pharmacological properties of phytochemicals like hesperidin, including antioxidant, anti-inflammatory, and immunomodulatory effects, complement the mechanisms of action of other anticancer agents, thereby diversifying the treatments against breast cancer. Hence, the application of combination therapy stands as a captivating approach to optimize treatment efficacy while circumventing the inherent limitations of individual phytochemicals such as hesperidin in combating breast cancer.

In addition to *in vitro* studies, preclinical and clinical evidence supports the enhanced therapeutic effects of hesperidin and its combination in breast cancer management. Preclinical studies, typically conducted in animal models, demonstrate the ability of hesperidin alone and in combination with other agents to inhibit tumor growth, suppress metastasis, and improve overall survival outcomes. Combining camel milk-derived exosomes, tamoxifen, and hesperidin synergistically inhibits breast cancer *in vivo*, reducing tumor size and modulating the expression of apoptotic and angiogenic markers. This cotreatment also attenuates tamoxifen-associated side effects, as demonstrated by decreased serum levels of liver and kidney markers, lipid peroxidation, and enhanced antioxidant enzyme activities. Exosomes and hesperidin are potential safe adjuncts to tamoxifen in breast cancer chemotherapy.[55] Hesperidin showed potential as an anticancer agent in breast cancer treatment, especially when combined with doxorubicin. This combination led to higher survival rates, increased levels of IFNγ, reduced expression of cancer-related genes, and improved pathological complete response scores compared to doxorubicin alone. Immunohistochemical analysis revealed decreased Ki-67 and VEGF levels and increased E-cadherin expression with the combined treatment. Hesperidin exhibits synergistic effects with other chemotherapeutics, suggesting its suitability as a therapeutic agent for breast cancer.[56] Another study investigated the protective effects of hesperidin, alone and in combination with doxorubicin, against 7,12-dimethylbenz(a)anthracene (DMBA) induced breast cancer in female rats. Hesperidin pretreatment reduced tumor occurrence volume and improved survival rates. Combination with doxorubicin further enhanced these effects and mitigated doxorubicin-induced toxicity, as evidenced by reduced oxidative stress and inflammatory markers, improved histopathology, and decreased Ki67 expression, suggesting the potential of hesperidin as a protective agent against breast cancer.[57] Nanoencapsulated Imatinib (IM) and/or Hesperidin (HES) were evaluated for efficacy in breast cancer treatment and reduction of cardiotoxicity. Solid Ehrlich carcinoma-bearing mice were treated with various formulations. Nano IM and/or Nano HES significantly reduced tumor volume and weight, downregulated MDR-1 gene expression, and decreased cardiac toxicity markers compared to conventional treatments. Hesperidin as an adjuvant improved IM's cytotoxic effects, while nanoencapsulation enhanced anticancer activity, suggesting potential therapeutic strategies.[58] The combined effects of hesperidin, piperine, bee venom, and tamoxifen were investigated on oxidative stress and hepato-nephrotoxicity in rats with breast cancer. Combined with or without tamoxifen, these natural compounds demonstrated low hepato-nephrotoxicity and protective effects against tamoxifen-induced toxicity in MCF-7 breast cancer xenografts. They improved liver and kidney function serum markers, reduced oxidative stress, and enhanced antioxidant activity, suggesting their potential as modulatory agents against breast cancer.[58] Another study investigated the synergistic effects of bee venom, hesperidin, and piperine with tamoxifen on apoptotic and angiogenesis biomarkers in MCF-7-injected rats. Results showed upregulation of apoptotic genes and downregulation of antiapoptotic and angiogenesis genes. Tamoxifen and hesperidin induced G0/G1 phase arrest, while piperine and bee venom-induced G2/M phase arrest. The combination enhanced tamoxifen's efficacy in breast cancer management.[59] The therapeutic potential of hesperidin and tiger nut was investigated to mitigate the carcinogenic effects of DMBA in female rats. Groups treated with hesperidin showed more significant antioxidant and chemoprotective effects compared

Table 1: Formulation strategies to improve absorption of Hesperidin.

Sl. No.	Strategy	Outcome
1.	Micronized 2S-Hesperidin.	1.27-fold increase in bioavailability.[38]
2.	Nanophytosomes of Hesperidin and Hesperetin.	4-fold increase in bioavailability.[39]
3.	Hesperidin-zein-2-Hydroxypropyl-β-cyclodextrin.	Solubility increased by 6 times.[40]
4.	Electrospun Orange-Peel-Extract-Loaded Nanofibers.	8-fold increase in solubility.[41]
5.	Hesperidin-loaded sulfobutylether-β-cyclodextrin/chitosan nanoparticles.	Improved solubility and 2.21-fold increased intestinal permeability.[42]
6.	Amorphous Solid Dispersion of Hesperidin.	300-fold improvement in solubility.[43]
7.	Hesperidin-Phospholipid Complex.	1.66 times increased dissolution.[44]
8.	Hesperidin solid lipid nanoparticles.	20-fold increase in aqueous solubility with 4.5-fold higher bioavailability.[45]

to those treated with tiger nut, as demonstrated by changes in serum markers and tissue parameters in breast, liver, and kidney tissues.[60]

Combining findings from observational studies revealed a negative correlation between the consumption of citrus fruits and the incidence of breast cancer. Given that citrus fruits serve as the primary reservoir of hesperidin, this suggests that the intake of citrus fruits is associated with a decreased risk of breast cancer.[61] The convergence of preclinical and clinical evidence emphasizes the significance of hesperidin and its combination therapies as promising candidates for further investigation and potential integration into the clinical management of breast cancer.

Formulations of Hesperidin for breast cancer management

Due to several factors, incorporating hesperidin into formulations, either as a standalone drug or in combination with other agents, enhances its efficacy in breast cancer treatment. Firstly, hesperidin has limited bioavailability and metabolic instability, which necessitates hesperidin to be incorporated into the formulation. Formulations can enhance the solubility of hesperidin and improve stability and absorption, thereby facilitating its delivery to target tissues and improving its pharmacokinetic profile. Hesperidin-loaded Nanoemulsions (HP-NEM) were developed to enhance solubility and efficacy in breast cancer treatment using MCF-7 cells. The optimized HP-NEM exhibited favorable characteristics and selectively induced cytotoxicity and apoptosis in MCF-7 cells without affecting normal cells.

Additionally, HP-NEM treatment led to cell cycle arrest and downregulation of miR-21 and miR-155 expression in MCF-7 cells, suggesting its potential as a therapeutic agent for breast cancer.[62] Hesperidin nanoparticles loaded with Poly Lactic-co-Glycolic Acid (PLGA) and Poloxamer 407 were developed to improve therapeutic efficiency and cytotoxicity. The nano-hesperidin exhibited enhanced stability and antioxidant effects, confirmed through *in vitro* studies and structural analysis. It demonstrated blood compatibility and exerted anticancer activity against MCF-7 cells, inducing cell growth arrest, DNA fragmentation, and apoptotic cell death via caspase-3 and p53-dependent pathways. These results suggest nano-hesperidin as a potential candidate for clinical trials and novel chemotherapeutic agents.[63] Hesperidin loaded onto gold Nanoparticles (Hsp-AuNPs) was synthesized to overcome its poor solubility and bioavailability. Hsp-AuNPs exhibited significant cytotoxicity against MDA-MB-231 breast cancer cells while sparing normal breast epithelial cells. *In vivo* studies showed no significant toxicity in mice, with histological analysis revealing no abnormalities. Hsp-AuNPs enhanced macrophage activity against tumor cells and inhibited pro-inflammatory cytokine secretion. These findings suggest that Hsp-AuNPs are promising biocompatible, anticancer, anti-inflammatory, and phagocytosis inducers for potential therapeutic applications.[64] Another study developed chitosan-functionalized Fe_3O_4 magnetic nanoparticles (Cs-f-Fe_3O_4 MNPs) to deliver Hesperidin (HD) for enhanced therapeutic effects efficiently. The synthesized HD-Cs-f-Fe_3O_4 MNPs exhibited potent antioxidant activity, with increased scavenging activity at higher concentrations. Additionally, they demonstrated more significant cytotoxicity against MCF-7 cancer cells than Cs-f-Fe_3O_4 MNPs alone, highlighting their potential as promising candidates for further investigation in cancer treatment.[65]

Moreover, combination formulations aid synergistic interactions between hesperidin and other anticancer agents or adjuvants, increasing their collective efficacy against breast cancer, summarized in Table 2. A magnetic nanohybrid carrier conjugated with progesterone was developed for targeted delivery of hesperidin extracted from citrus peel to breast cancer cells. The carrier, composed of casein-calcium ferrite, demonstrated high hesperidin encapsulation efficiency and magnetic-induced drug delivery. Stimuli-responsive drug release was observed, favoring anticancer applications. The formulation showed biocompatibility and significantly enhanced cytotoxicity against MDA-MB-231 breast cancer cells, suggesting its potential for targeted chemotherapy.[66] Another study aimed to produce nanoparticles of hesperidin and essential oil extracted from sweet orange peels, evaluating their biological activities compared to native products. Nanoparticles were characterized and assessed for antioxidant,

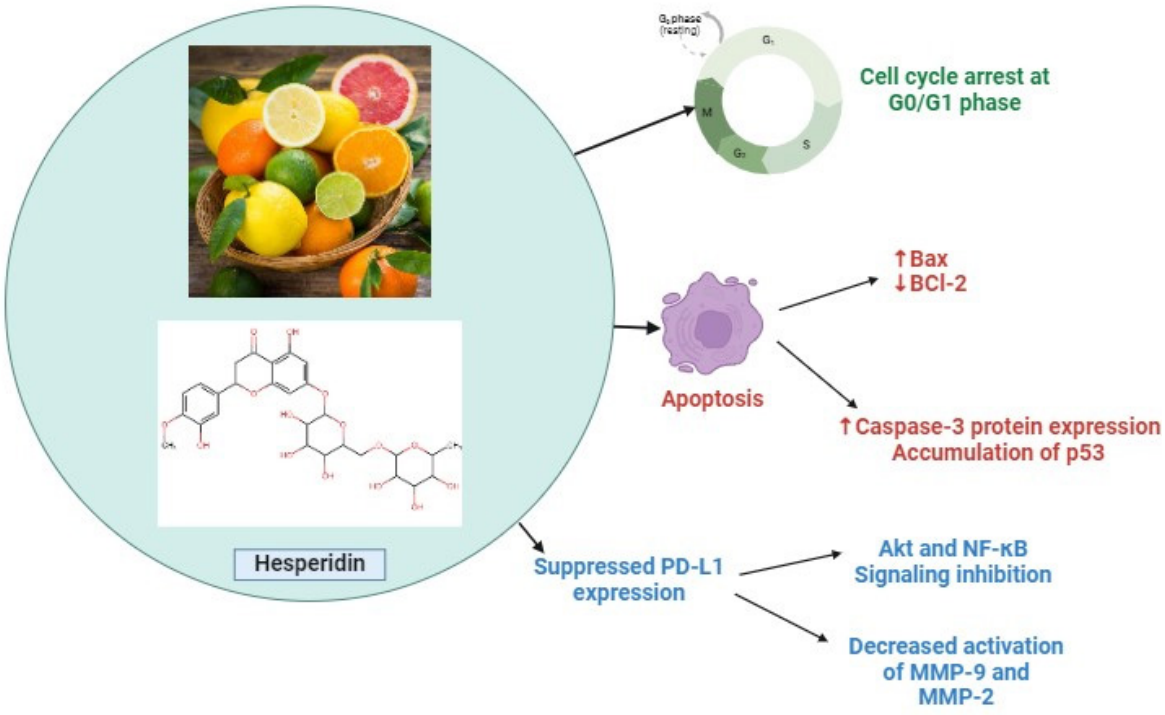

Figure 2: Mechanism of action of Hesperidin.

antimicrobial, and cytotoxic effects. Nano-formulated hesperidin and essential oil demonstrated enhanced antioxidant activity and DNA damage prevention compared to native products. *In vitro* cytotoxicity assays showed inhibition of cancer cell proliferation, suggesting potential therapeutic applications. These findings indicate the promising role of nano-hesperidin and nano-essential oils as novel chemotherapeutic agents and food additives, warranting further investigation for clinical use and industrial applications.[67] The efficacy of nano-encapsulated Imatinib (IM) and Hesperidin (HES) was evaluated alone or in combination in reducing tumor growth and cardiotoxicity in Solid Ehrlich Carcinoma (SEC)-bearing mice. IM and HES were loaded into PLGA polymer nanoparticles. Results demonstrated significant reductions in tumor volume, weight, hematological markers, cardiac markers, and MDR-1 gene expression in nano IM- and/or nano HES-treated groups compared to conventional treatments. Nanoencapsulation enhanced anticancer activity and mitigated cardiac toxicity, suggesting the potential of HES as an adjuvant therapy with IM.[58] Chitosan nanocarriers were loaded with 5-Fluorouracil (5Fu) and Hesperidin (Hesp) to enhance therapeutic efficacy and mitigate toxicity in breast cancer treatment. The nanocarriers exhibited high encapsulation efficiency and sustained drug release, especially in acidic environments. Dual-loaded nanocarriers showed enhanced toxicity against MCF-7 cells, inhibiting migration and increasing ROS levels compared to single-loaded carriers. This approach holds promise for improving breast cancer therapy by synergistically combining chemotherapeutic and natural bioflavonoid agents in a biodegradable nanocarrier system.[68] Folic acid-conjugated bovine serum albumin-calcium ferrite nanohybrid carrier was developed for targeted delivery of natural cytotoxic drugs, hesperidin, and eugenol, to breast cancer cells overexpressing folate receptors. The carrier exhibited pH-responsive drug release and magnetic field sensitivity, enabling site-specific delivery. Encapsulation efficiencies of 62.94% for hesperidin and 85.58% for eugenol were achieved. *In vitro* studies demonstrated enhanced cytotoxicity against MCF-7 cells, with a significant reduction in IC_{50} values, indicating potential for targeted cancer therapy.[69] The cytotoxic effects of chitosan-Citrus aurantium peels extract Nanoparticles (NPs-CA) was investigated against MDA-MB-231 breast cancer cells. The nanoparticles were synthesized using ionic gelation methods, and their size and stability were confirmed through particle size analysis and zeta potential measurements. The cytotoxicity of the nanoparticles was evaluated using the MTT assay. The results showed that NPs-CA exhibited better cytotoxic effects than Citrus aurantium peels extract alone, with an IC_{50} value of 83 µg/mL for NPs-CA and 320 µg/mL for the extract. These findings highlight the potential of NPs-CA in cancer therapy and warrant further research in this area.[70] Additionally, formulation approaches offer prospects for controlled release and targeted delivery of hesperidin to tumor sites, minimizing off-target effects and enhancing therapeutic selectivity. Consequently, the development of optimized formulations signifies a critical avenue for harnessing the therapeutic benefits of hesperidin in breast cancer management.

Table 2: Nanocarriers loaded with combinations of hesperidin with potential applications in breast cancer.

Type of nanocarrier	Components of delivery system	Combination	Purpose	Major results or conclusion
Magnetic nanohybrid carrier	Casein-calcium ferrite	Hesperidin+Progesterone	Targeted delivery	Stimuli responsive drug release with significantly enhanced cytotoxicity against MDA-MB-231 cells.[66]
Nanoparticles	PEG 8000	Hesperidin+essential oil extracted from sweet orange peels.	Study the biological impact as cytotoxic agent compared to native products.	Enhanced antioxidant activity and DNA damage prevention compared to native products.[67]
PLGA nanoparticles	PLGA (polylactic-co-Glycolic Acid) polymer and Polyvinyl Alcohol (PVA).	Hesperidin+Imatinib.	Enhance anticancer activity and ameliorate cardiotoxicity.	Significant reduction in tumor parameters and cardiac markers, and MDR-1 gene expression.[58]
Chitosan nanocarriers	Chitosan and Sodium Tripolyphosphate (STPP).	Hesperidin+5-Fluorouracil.	Enhance therapeutic efficacy and mitigate toxicity in breast cancer treatment.	Enhanced toxicity against MCF-7 cells, inhibiting migration and increasing ROS levels compared to single-loaded carriers.[68]
Folic acid-conjugated bovine serum albumin-calcium ferrite nanohybrid carrier.	Bovine serum albumin, calcium ferrite, and folic acid.	Hesperidin+Eugenol	Targeted delivery of hesperidin and eugenol to breast cancer cells overexpressing folate receptors.	Enhanced cytotoxicity against MCF-7 cells, with a significant reduction in IC_{50} values.[69]

Future directions and challenges

Hesperidin, when employed as a monotherapy, has demonstrated noteworthy efficacy in breast cancer treatment. However, synergistic benefits arise when hesperidin is administered in combination with either an anticancer agent or another phytomolecule. The investigation of these combinations presents a promising avenue for enhancing therapeutic outcomes. Particularly, the integration of the concept of combisomes stands as a potential development in this domain.

Combisomes are liposomes encapsulating both an anticancer agent and a bioactive compound in optimized ratios, offer a compelling strategy for enhancing treatment efficacy.[71] By maximizing the dosage of the bioactive compound while minimizing the dosage of the anticancer agent, combisomes present a viable approach towards attaining enhanced therapeutic effects. Moreover, their selective targeting of cancer cells while preserving normal non-cancerous cells underscores their potential as a safe and effective platform for breast cancer management.

Nevertheless, several challenges persist in the utilization of hesperidin for breast cancer treatment. One notable hurdle is the need for comprehensive elucidation of the precise mechanisms underlying the synergistic interactions between hesperidin and other therapeutic agents. Additionally, the development of combisomes necessitates meticulous optimization to ensure optimal efficacy and safety profiles. Furthermore, the translation of these findings from preclinical studies to clinical settings warrants thorough evaluation to ascertain their clinical applicability and efficacy.

Addressing these challenges necessitates interdisciplinary efforts encompassing pharmacology, nanotechnology, and oncology. Furthermore, rigorous preclinical and clinical investigations are imperative to validate the efficacy and safety of hesperidin-based combination therapies, particularly those utilizing combisomes. Ultimately, overcoming these challenges holds the potential to significantly advance the landscape of breast cancer management, offering novel and efficacious treatment modalities for patients.

CONCLUSION

In conclusion, the utilization of hesperidin in breast cancer management presents a promising platform for therapeutic intervention. As a flavonoid abundantly present in citrus fruits, hesperidin exhibits noteworthy therapeutic properties, including the induction of apoptosis and cell cycle arrest through diverse mechanisms against breast cancer cells. Moreover, hesperidin demonstrates favourable safety profiles, enhancing its potential for clinical application. Despite challenges such as low bioavailability, innovative formulation approaches and structural modifications

have been explored to overcome these limitations effectively. Furthermore, the synergistic effects of hesperidin in combination with anticancer agents or other phytomolecules emphasize its potential as a complementary therapeutic strategy. Continued research efforts in elucidating hesperidin's mechanisms of action and optimizing its delivery hold promise for enhancing its efficacy in breast cancer treatment. Thus, hesperidin emerges as a valuable candidate warranting further exploration as a component of comprehensive breast cancer management strategies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PD-L1: Programmed Death Ligand 1; **LOAEL:** Low Acute Toxicity Level; **LD_{50}:** Median Lethal Dose; **UDP:** Uridine Diphosphate; **MMP-9:** Matrix Metalloproteinase-9; **MMP-2:** Matrix Metalloproteinase-2; **GSH:** Glutathione; **LDH:** Lactate Dehydrogenase; **Pgp:** P-glycoprotein; **EGFR:** Epidermal Growth Factor Receptor; **Erα:** Estrogen Receptor alpha; **IFNγ:** Interferon-gamma; **VEGF:** Vascular Endothelial Growth Factor; **DMBA:** 7,12-dimethylbenz(a)anthracene; **PLGA:** Poly lactic-co-glycolic acid; **SEC:** Solid Ehrlich Carcinoma; **IC_{50}:** Half maximal inhibitory concentration; **MTT:** 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide.

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