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EXPLORING HESPERIDIN'S THERAPEUTIC POTENTIAL IN CANCER THERAPY: A REVIEW

BY

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Abstract. Hesperidin, a flavonoid abundant in citrus fruits, has gathered attention for its promising anti-cancer properties. This review explores the complex mechanisms through which hesperidin influences oncogenesis and tumour progression. Studies reveal hesperidin's ability to target key pathways involved in cancer development, including hypoxia-induced HIF-1α activation, oxidative stress-mediated signalling, and epithelial-mesenchymal transition (EMT). *In vitro* and *in vivo* experiments showed hesperidin's capacity to inhibit angiogenesis, to suppress cell proliferation, and to induce apoptosis in various cancer models. Furthermore, hesperidin has been demonstrated to exhibit synergistic effects with conventional chemotherapy, thereby enhancing its anti-cancer efficacy, while simultaneously reducing its associated risks. Aside from the promising preclinical findings, further clinical studies are needed in order to elucidate hesperidin's therapeutic potential and to optimize its use in cancer treatment strategies. Understanding the complex interaction between hesperidin and cancer-related pathways could accelerate the development of novel therapeutic approaches, with the goal of preventing cancer progression and improving patient outcomes.

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1. Introduction

Hesperidin, 3,5,7-trihydroxy flavanone 7-rhamnoglucoside, is found in various citrus fruits, including mandarins, grapefruits, oranges, limes, lemons, sweet oranges, and clementines. Its concentration in these fruits vary from 0.15 mg to 39.9 mg per 100 mL, following an ascending order (ur Rehman *et al*., 2021).

In our prior research, we highlighted the promising effects of hesperidin on immunity, cardiovascular health, dyslipidemia, and inflammation, along with its favourable *in vivo* safety profile (Cernatescu *et al*., 2023). But our main focus was to integrate *in silico*, *in vitro*, and *in vivo* study results indicating Hesperidin's potential in combating SARS-CoV-2 infection.

COVID-19, characterised as a respiratory disorder, is associated with hypoxia and enhanced production of reactive oxygen species (ROS). Thereby it is responsible for activating pathways involving hypoxia-inducible factor-1α (HIF-1α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Serebrovska *et al*., 2020; Amini *et al*., 2022). Apart from exacerbating oxidative cellular stress, HIF-1α is a known promoter of angiogenesis, accelerating cancer progression (Shi *et al*., 2004). Considering the benefits of hesperidin against SARS-CoV-2 infection, we speculate on its potential to slow down or even prevent cancer development and are interested in exploring the underlying mechanisms.

2. Hesperidin - generalities

Hesperidin stands out as one of the most recognized bioactive flavonoids found abundantly in citrus fruits, particularly in their peel. Notably, citrus peel exhibits a higher flavonoid content than the pulp, thereby showcasing stronger anti-inflammatory properties (Lee *et al*., 2022).

In the food processing industry, following juice extraction, citrus fruits generate substantial by-products such as seeds and peels. Often overlooked, these residues serve as rich reservoirs of hesperidin, making them economically appealing sources (Pyrzynska, 2022; Wdowiak *et al*., 2022).

Hesperetin, a metabolite derived from hesperidin, represents the aglycone form of the compound. It possesses a higher bioavailability profile compared to hesperidin, due to better absorption and slower clearance. However, the industrial process of obtaining hesperetin is more complex, involving the modification of hesperidin using bacterial enzymes, which leads to elevated production costs (Wdowiak, 2022, Choi *et al*, 2022) (Fig. 1).

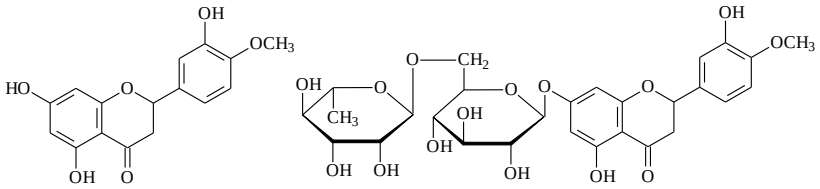

Fig. 1 – Structures of hesperetin (A) and hesperidin (B).

The anticancer properties of hesperidin are mentioned in several studies, focusing on three key mechanisms: hypoxia, oxidative stress, and the epithelial-mesenchymal transition.

3. Hypoxia Induced Oncogenesis

In conditions of hypoxia, growth factors and oncogenes become stimulated, leading to the activation and overexpression of Hypoxia-Inducible Factor 1α (HIF-1α). This protein, along with other signalling pathways, promotes angiogenesis and the metabolic adaptation to low oxygen levels, therefore enhancing tumour cell survival (Shi *et al*., 2004).

TP53, acknowledged as one of the most extensively studied tumour-suppressor genes, encodes the p53 protein, known as the "guardian of the genome." The p53 pathway is crucial for maintaining genomic integrity and its inactivation is widespread in many tumours (Sermeus *and* Michiels, 2011). Interestingly, within the tumour microenvironment, regions deprived of oxygen often exhibit heightened expression of mutated p53 protein. The immunohistological colocalization of hypoxia and p53 mutations in tumour samples is associated with a more aggressive phenotype and an unfavourable prognosis (Soussi *et al*., 2001; Sermeus *and* Michiels, 2011). This highlights the intricate relationship between TP53 mutations, hypoxia, and tumour aggressiveness, highlighting the importance of understanding these interactions in cancer progression.

Hesperidin was observed to inhibit the activation of hypoxia-inducible factor-1α (HIF-1α) and the generation of inflammatory cytokines, such as vascular endothelial growth factor (VEGF), interleukin-8, interleukin-1β and tumour necrosis factor-alpha (TNF-α). This *in vitro* experiment was performed on the human mast cell line HMC-1, induced by phorbol myristate acetate (PMA) and calcium ionophore A23187 (Choi *et al*., 2007). The results indicate the suppressing effect Hesperidin has on angiogenesis and inflammation.

In an *in vivo* study involving HR-1 hairless mice, the effect of hesperidin on Ultraviolet B radiation (UVB)-induced angiogenesis was investigated, demonstrating its capacity to inhibit neovascularization. Immunohistochemistry results further supported this finding, by indicating lower levels of VEGF, matrix

metalloproteinase 13 (MMP-13) and MMP-9, coupled with the absence of HIF-1α expression (Kim *et al*., 2021).

In conclusion, HIF-1 production and VEGF expression are critical drivers of angiogenesis. Hypoxia also promotes TP53 mutations, compromising its role as a tumour suppressor gene. In this context, research findings on hesperidin, both *in vitro* on mast cell lines and *in vivo* on hairless mice, demonstrate its ability to inhibit HIF-1 production and counteract hypoxia-induced angiogenesis. These promising results suggest that hesperidin holds potential for slowing down cancer progression by targeting fundamental mechanisms associated with angiogenesis.

4. The Role of Oxidative Stress in Oncogenesis

It is worth mentioning that ROS have a dual effect in oncogenesis. Moderate levels of ROS contribute to cancer promotion, while excessive ROS levels exert a pro-apoptotic effect (Zhao *et al*., 2023).

In oncological therapy, strategies for regulating ROS levels can involve either reducing ROS to prevent oncogene activation (antioxidant-based therapy) or elevating ROS above a critical threshold to selectively target cancer cells (pro-oxidative therapy). Pro-oxidative approaches are widely preferred and more frequently used in clinical practice. Many pro-oxidative agents have been identified. They can raise ROS levels either indirectly by impairing the cell's antioxidant defence mechanisms or directly by generating more ROS (Somu *et al*., 2022). Attacking tumour cells by ROS generation offers advantages over traditional drugs like 5-fluorouracil and cisplatin, which are prone to resistance-related issues (Zhao, 2023).

Another *in vitro* study showed that hesperidin reduces cell proliferation and activates ROS-related signalling pathways in hepatocarcinoma cells (HePG-2 cells). Firstly, hesperidin effectively inhibits HePG-2 cell proliferation by inducing apoptosis through Bax, Bcl-2, and p53 pathways. This hesperidin-induced apoptosis relies on the overproduction of ROS, resulting in DNA damage. These findings suggest that ROS-mediated signalling pathways play a crucial role in hesperidin-induced apoptosis of hepatocarcinoma cells (Pang *et al*., 2023).

In another study, gastric tumour xenograft models were created using nude mice to assess the impact of hesperetin on cancer progression *in vivo*. The findings revealed a significant inhibition of gastric cancer cell proliferation in the hesperetin-treated groups compared to the control group showing time- and dose-dependent effects. Moreover, pre-treatment with N-acetyl-L-cysteine (NAC) or H_2O_2 either attenuated or enhanced the inhibitory effects of hesperetin on cell viability. It's noteworthy that H_2O_2 induces oxidative stress, while NAC, a precursor of glutathione, serves as a potent antioxidant, adding context to their effects in this experimental setup. These variations together with an increase in Apoptotic peptidase activating factor 1 (Apaf-1), Bax, Cytochrome C, Caspase-3

and Caspase-9 support the claim that hesperetin may suppress the growth and prompt the apoptosis of gastric cancer cells by stimulating the mitochondrial pathway through ROS elevation (Zhang *et al*., 2015).

5. The Epithelial-Mesenchymal Transition (EMT)

During the early 1980s, researchers reported a link between EMT and cancer. As tumours progress, cells can acquire invasive and metastatic abilities through EMT. Activation of EMT leads to loss of cell polarity and cell adhesion in tumour epithelial cells, while also promoting migratory and invasive properties, transforming them into mesenchymal cells. Studies have shown that the transforming growth factor beta (TGF-β)/Smads pathway is one of the most potent inducers of EMT, as it upregulates EMT-related transcription factors (Ribatti *et al*., 2020).

An in vitro study demonstrated that hesperidin exerted dose-dependent anti-proliferative effects on A549 alveolar epithelial cells. TGF-β1 was utilised to activate the Smad signalling pathway and induce EMT artificially. Hesperidin treatment aimed to counteract Smad pathway activation, successfully suppressing the expression of mesenchymal markers (α-SMA and Col1α-1) while maintaining the expression of epithelial markers (E-cadherin). These findings suggest that hesperidin can inhibit EMT in human alveolar epithelial cells (Ren *et al*., 2019).

Furthermore, another *in vitro* study demonstrated that hesperidin amplified the cytotoxic effects of doxorubicin (DOX), leading to apoptotic cell death, G2/M cell cycle arrest, and suppressed migration of highly metastatic breast cancer cells (4T1 cells). It is noteworthy that treatment with DOX alone induced lamellipodia formation and upregulated Rac-1 and MMP-9 expression, potentially accelerating cell migration *in vitro*. However, the combination of DOX and hesperidin decreased MMP-9 and Rac-1 levels, thus reducing the chemotherapy-related risk of cell migration in highly metastatic breast cancer (Amalina *et al*., 2023)

Moreover, in an *in vivo* study, researchers found that tobacco smoke (TS) increased the expression of mesenchymal markers like proliferating cell nuclear antigen, vimentin, and N-cadherin, while reducing the expression of epithelial markers like E-cadherin. Additionally, TS was observed to activate the p38 pathway, leading to enhanced proliferation and EMT. However, treatment with hesperidin prevented the activation of the p38 pathway by TS, thereby inhibiting EMT and cell proliferation in the lungs of mice. (Liang *et al*., 2023).

Overall, these studies highlight the complex mechanisms underlying epithelial-mesenchymal transition and its implications in cancer progression. Hesperidin demonstrates promising effects in inhibiting EMT-related pathways and enhancing the efficacy of existing cancer treatments, as shown by its ability to suppress mesenchymal markers while maintaining the epithelial ones. These findings suggest a potential therapeutic role for hesperidin in combating

chemotherapy resistance and the metastatic process in cancer, paving the way for further exploration of its clinical utility

6. Conclusions

In conclusion, the far-reaching potential of hesperidin in cancer therapy has been highlighted through our exploration of its mechanisms. By targeting key pathways such as hypoxia-induced oncogenesis, oxidative stress-mediated signalling, and the mesenchymal-epithelial transition, hesperidin exhibits promising anti-cancer properties both in vitro and in vivo.

Firstly, hesperidin has demonstrated its ability to inhibit HIF-1 production, thereby suppressing angiogenesis, a critical process in tumour progression. Additionally, it promotes apoptosis in cancer cells through pro-oxidative pathways, while minimizing resistance issues commonly associated with traditional chemotherapeutic agents. Moreover, hesperidin impedes the epithelial-mesenchymal transition, a pivotal step in tumour metastasis, and enhances the cytotoxic effects of chemotherapy, providing a synergistic approach to cancer treatment.

While our findings offer valuable insights into hesperidin's anti-cancer properties, further clinical studies are necessary to validate its efficacy and safety in human patients. Future research should focus on elucidating hesperidin's precise mechanisms of action and exploring its potential alongside existing cancer treatments.

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INVESTIGAREA POTENȚIALULUI TERAPEUTIC AL HESPERIDINEI ÎN ONCOLOGIE: STUDIU DE LITERATURĂ

(Rezumat)

Hesperidina, un flavonoid prezent în cantități semnificative în citrice, a captat atenția cercetătorilor datorită potențialului său în lupta împotriva celulelor canceroase. În literatura de specialitate este explorată capacitatea hesperidinei de a influența căile de semnalizare specifice implicate în dezvoltarea cancerului. Acest studiu investighează mecanismele complexe prin care hesperidina influențează oncogeneza și progresia tumorală. Trei dintre aceste mecanisme includ expresia factorului-indus de hipoxie (HIF-1α), stresul oxidativ și tranziția epitelial-mesenchimală. Rezultate *in vitro* și *in vivo* demonstrează capacitatea hesperidinei de a inhiba angiogeneza, de a suprima proliferarea celulară și de a induce apoptoza în diverse modele tumorale. În plus, s-a constatat că hesperidina prezintă efecte sinergice cu chimioterapia convențională, îmbunătățind eficacitatea sa anti-tumorală și reducând simultan riscurile asociate acesteia. Deși rezultatele preliminare din studiile preclinice sunt promițătoare, studiile clinice sunt necesare pentru a înțelege cum poate fi integrată hesperidina în strategiile de tratament oncologic. Aprecierea modului în care hesperidina interacționează cu căile de semnalizare implicate în oncogeneză este esențială pentru dezvoltarea unor terapii inovatoare, menite să încetinească progresia bolii și să îmbunătățească perspectivele terapeutice pentru pacienți.