International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Hesperidin: Unveiling its Chemotherapeutic Potential and Mechanism of Action in Cancer

Shahrukh Iqbal[1], Anshuman Kumar[2], Priyetesh[3]

[1,2]Msc Microbiology, Department of Biosciences, Jamia Millia Islamia
[3]Msc Biochemistry, Department of Biosciences, Jamia Millia Islamia

Abstract

Cancer ranks among the top causes of death globally, and current treatments like radiation and chemotherapy often come with notable side effects. Natural compounds particularly those found in citrus fruits like hesperidin, offer potential advantages and benefits in dealing with the illness by impacting the biological processes such as oxidative stress and inflammation. Natural products are not a replacement for medicine, but they can complement standard traditional therapies. Hesperidin, known for its antioxidant, anti-inflammatory, and liver-protecting properties, also shows promise in cancer treatment by affecting key cellular pathways involved in cell growth, apoptosis, and angiogenesis. Additionally, it may boost the effectiveness of existing cancer treatments when used in combination with other drugs or natural substances.

Keywords: Cancer, Inflammation, Phytochemical, Angiogenesis

Introduction

Cancer is a disease characterized by abnormal cell growth and proliferation throughout the body. With over 200 types, symptoms and signs vary depending on factors like size, location, and stage, which can be identified through biopsy results. [1] Globally, approximately 11 million individuals receive cancer diagnosis annually, resulting in 6.7 million deaths. Breast, lung, and colorectal cancers are the most frequently diagnosed types worldwide, while lung, stomach, and liver cancers have the highest mortality rates among all cancer types. [2] The drawbacks of contemporary cancer therapies, such as disease severity, elevated mortality rates, and diminished quality of life for patients during treatment, underscore the necessity for exploring herbal therapeutic approaches for disease management and prevention. And hence a significant amount of scientific effort has been dedicated to the quest for novel chemicals or therapeutic pathways that may prove advantageous in the management of various cancer forms. For example, it was seen that oxidative stress plays a key factor in the pathophysiology of various types of cancer. And that's why much heed was given to antioxidants as novel therapeutic agents. [3] Among the many bioactive substances are polyphenol phytochemicals, which are an intriguing class to research since they have strong antibacterial, anticarcinogenic, antioxidant, and anti-inflammatory properties. [4] various malignant tumor tissues. [5] Numerous epidemiological studies show a negative correlation between the risk of cancer and the amount of flavonoids consumed through diet. [6] Hesperidin is a prominent flavanone in the diet, predominantly present in various citrus fruits such as oranges, and it displays numerous biological attributes. Furthermore, hesperidin is considered one of the safest and most significant bioflavonoids found in the Citrus genus. [7] Hesperidin is identified as a powerful agent with

anti-inflammatory, anti-carcinogenic, and antioxidant properties, as evidenced by data from various in vitro and in vivo studies. [8]

Cancer

Cancer progresses through a series of genetic and epigenetic changes that enable cells to evade natural checks that typically prevent excessive growth and promote the survival of cells growing abnormally outside their usual environments. Most cancers originate from epithelial cells, leading to carcinomas that affect organs like the lung, skin, breast, liver, and pancreas. Sarcomas, on the other hand, originate from mesenchymal tissues such as fibroblasts, myocytes, adipocytes, and osteoblasts. Additionally, tumors can arise in non-epithelial cells of the nervous system (e.g., gliomas, neuroblastomas, and medulloblastomas) and hematopoietic tissues (leukemia and lymphoma) [9]

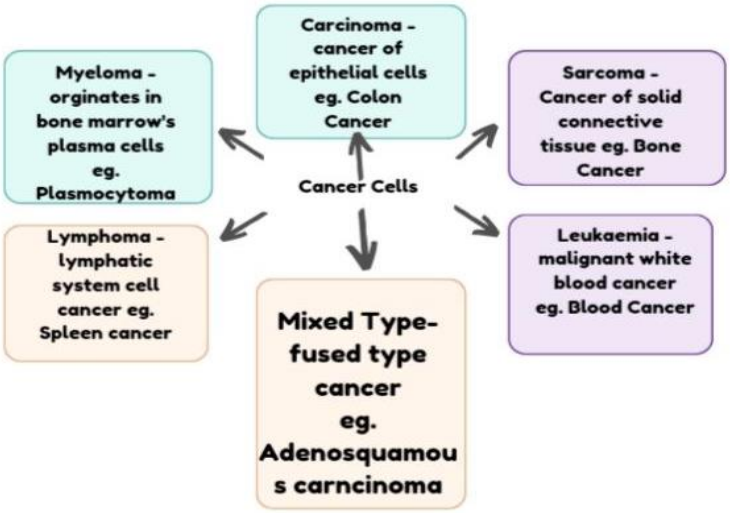

Fig Cancer Cell and its Progression

In solid tumors, these changes usually drive the transition from a somewhat harmless cluster of growing cells (hyperplasias) to a cluster of cells with irregular shape, structure, and organization. As the tumor grows, the inner core lacks oxygen and nutrients, prompting the formation of new blood vessels (angiogenesis) to restore the supply of oxygen and nutrients. This allows the tumor cells to acquire the capability to invade surrounding tissues, breach their normal limits, enter the bloodstream, and form new tumors at distant sites (metastasis), which is a key characteristic of malignancy. [10] The organization and strength of a cell's cytoskeleton have been recognized as significant factors in cancer advancement for a long time. The cytoskeleton consists of actin microfilaments made up of globular actin subunits that combine with non-muscle myosin II, forming actomyosin stress fibers, intermediate filaments (like vimentin and keratin), and microtubules composed of α and β-tubulin. In cancer, this network typically transitions from a structured and firm state to a disorganized and flexible one, aiding enhanced cell growth and movement. [11]

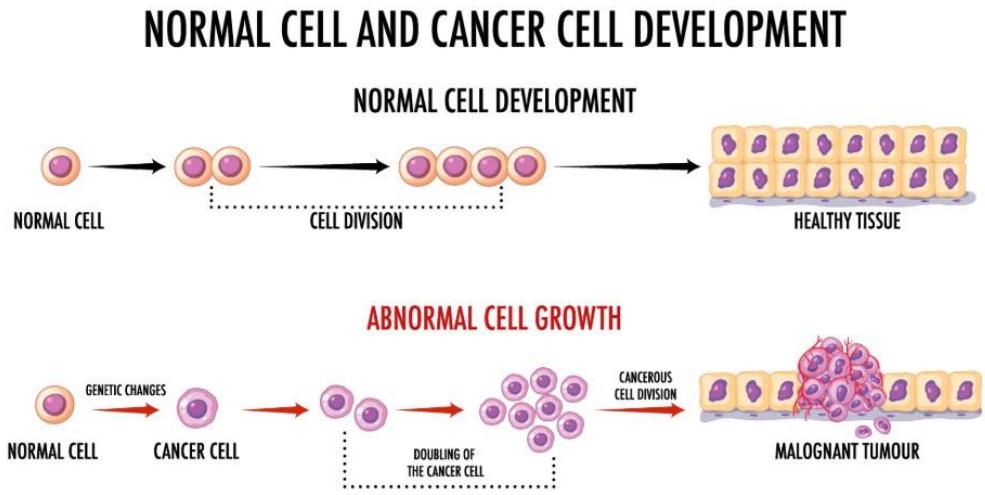

Fig. Cancer Progression

Hallmarks of Cancer

The defining characteristics of cancer encompass six biological abilities that develop as human tumors progress through multiple stages. These characteristics serve as a framework for understanding the complexities of cancer. They involve maintaining signals for cell growth, avoiding mechanisms that suppress growth, resisting cell death, enabling unlimited cell replication, triggering the growth of blood vessels, and initiating the spread and invasion of cancer cells to other parts of the body. [12]

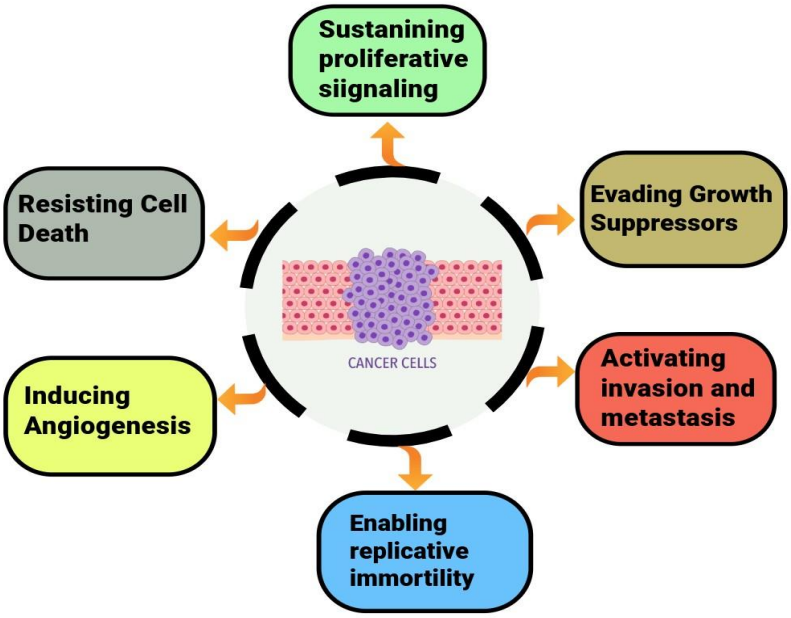

Fig. Depiction of 6 Hallmarks of Cancer

Sustaining Proliferative Signaling

One of the key characteristics of cancer cells is arguably their capacity for continuous and uncontrolled growth. Healthy tissues regulate the production and release of signals that promote cell growth and

division, maintaining a balance in cell numbers and preserving normal tissue structure and function. In contrast, cancer cells disrupt these regulatory signals, taking control of their own growth and destiny. [13]

Evading Growth Suppressors

Cancer cells not only need to promote growth but also evade mechanisms that restrain cell proliferation, often mediated by tumor suppressor genes. Many of these genes have been identified through their inactivation in cancer, and their role as suppressors has been confirmed through experiments in mice. Two main tumor suppressors, RB and TP53, play pivotal roles in regulating cell proliferation, senescence, and apoptosis, forming essential parts of cellular control circuits. The RB protein receives signals from both external and internal sources and then determines whether a cell should continue with its cycle of growth and division based on these inputs. [14]

Activating Invasion and Metastasis

Understanding the processes of invasion and metastasis has long been a mystery. As carcinomas progress to more malignant stages, characterized by local invasion and distant metastasis, cancer cells typically undergo changes in shape and adhesion to other cells and the extracellular matrix (ECM). One well-studied change involves the loss of E-cadherin, a crucial molecule for cell-to-cell adhesion in epithelial tissues. E-cadherin helps assemble epithelial cell layers and maintain cell quiescence within these layers. Increased E-cadherin expression inhibits invasion and metastasis, while reduced expression enhances these processes. The frequent downregulation and occasional mutation of E-cadherin in human carcinomas strongly support its role as a key suppressor of invasive behavior in cancer cells. [15]

Induce Angiogenesis

Tumors, like healthy tissues, require nutrients, oxygen, and mechanisms to remove waste. This is facilitated by the formation of new blood vessels, known as neovascularization, which occurs through angiogenesis. While in normal development, the formation of blood vessels involves the birth of new endothelial cells and their organization into tubes (vasculogenesis) as well as the sprouting of new vessels from existing ones (angiogenesis), in adulthood, this process is typically quiescent, except during certain physiological events like wound healing or reproductive cycling in females. However, in tumor progression, there is a constant activation of the "angiogenic switch," leading to continual sprouting of new blood vessels from previously dormant ones. This sustained angiogenesis provides the necessary nourishment for the growing tumor mass. [16]

Enabling Replicative Immortality

It is widely acknowledged that cancer cells require unlimited replicative capacity to form visible tumors. This contrasts sharply with the behavior of most normal cells, which have a limited ability to divide and grow before entering a non-dividing, but still viable state called senescence, or experiencing cell death known as crisis. When cultured, normal cells typically undergo cycles of division, first reaching senescence and then, for the rare cells bypassing this barrier, entering a crisis phase where most cells die. However, occasionally, cells emerge from this crisis phase with the ability for unlimited replication, termed immortalization, a trait seen in established cell lines which can proliferate continuously in culture. Numerous studies indicate that telomeres, protective caps at the ends of chromosomes, play a significant

role in enabling cells to divide indefinitely. [17]

Resisting Cell Death

Functional studies conducted over the past years have convincingly demonstrated that apoptosis, a programmed cell death process, acts as a natural defense mechanism against the development of cancer. [18] Understanding the signaling pathways controlling apoptosis has shed light on how cells initiate programmed cell death in response to physiological stresses encountered during cancer development or treatment. Notably, imbalances in signaling pathways due to increased oncogene activity and DNA damage from rapid cell division are significant triggers for apoptosis. Additionally, research has unveiled mechanisms through which apoptosis is suppressed in tumors that advance to highly malignant stages and become resistant to treatment. [19]

Phytochemicals – An Overview

Phytochemicals are plant-derived compounds that are non-nutritive and play a significant role in the flavor and color of plants and beverages made from them. They are also under scrutiny for their potential positive effects on health. [20] Population and laboratory investigations are increasingly providing evidence of the significant anticarcinogenic and antimutagenic properties of phytochemicals. Numerous studies conducted on cell lines and animal models have shown that phytochemicals extracted from medicinal plants are effective in both treating and preventing cancer, with promising results indicating their ability to reduce cell proliferation, induce apoptosis, slow metastasis, and inhibit angiogenesis. [21] Phytochemicals modify proteins within various signal transduction pathways and interact with specific molecular signals, ultimately playing a crucial role in both chemoprevention and/or chemotherapy. [22] A standard procedure for developing a side-effect-free anticancer therapy using phytochemicals involves several stages: assessing plant extracts for anticancer properties, isolating active compounds through bioassay-guided fractionation, characterizing the fractions or compounds with activity, conducting preclinical evaluations of the identified phytochemical compounds through in silico, in vitro, and/or in vivo studies to determine their potential antitumor effects, and eventually progressing to clinical trials. [23]

Phytochemicals are typically known as the active secondary metabolites found in plants and fruits such as citrus fruits which have an abundance of them including the likes of polyphenols and terpenoids. In citrus, a hundred types of polyphenols have been identified, among which flavonoids are the most significant bioactive elements. These flavonoids have a broad range and are distributed in nearly all parts of citrus fruits across various species. [24]

Flavonoids are naturally occurring antioxidants with strong anticancer properties. It is believed that these compounds are produced by plants in response to various conditions such as UV radiation, extreme temperatures, tissue damage, infections, and the actions of reactive oxygen species. (ROS) [25] While the composition and varieties of flavonoids differ among Citrus species and parts of the fruit, a subclass flavanones are considered the most significant in Citrus species which is largely represented by HESPERIDIN and its aglycone hesperetin, can be found in all parts of the plant, including stems, branches, bark, flowers, leaves, roots, peels and seeds. [26] These flavones are well-known for their positive impacts on human health. Besides citrus fruits, they can also be found in other natural sources like honey, mint, and tomatoes. [27]

Hesperidin: Source and Chemistry

The term "hesperidin" is derived from the Greek mythology of the Hesperides, nymphs tasked with protecting an orchard where trees bearing golden apples flourished. [28] Hesperidin is a member of the flavanones, with a molecular structure of C28H34O15 and a specific molecular weight of 610.57 Da. Hesperidin (3′,5,7-trihydroxy-4′-methoxy flavanone) is the aglycon component of hesperidin (hesperetin-7-rutinose), which is glycosylated by Rutinose, Rutinose is a disaccharide consisting of one d-rhamnose molecule linked via a glycosidic bond to a d-glucose unit as illustrated in the figure. [29]

Hesperidin and its related compounds are distinctive elements found in citrus fruits belonging to the Rutaceae family. These fruits include orange (Citrus sinensis), grapefruit (Citrus paradise), tangerine (Citrus reticulata), lime (Citrus aurantifolia), and lemon (Citrus limon). [30]

Hesperidin, due to its biological properties, is frequently utilized in the food, cosmetic, and pharmaceutical sectors. These applications necessitate optimal extraction methods from plant materials that ensure high quality and purity. Numerous methods for extracting flavonoids, including hesperidin, have been investigated, with a focus on environmentally friendly approaches. [31]

Typical extraction methods encompass techniques such as dipping, percolation, and reflux or continuous reflux. The effectiveness of the procedure and the quality of the extract are influenced by various factors, including the type of solvent, temperature, duration of extraction, and the ratio of liquid to solid. Commonly used solvents include methanol and ethanol, either alone or mixed with water in varying ratios, as well as dimethyl sulfoxide

(DMSO). Traditional methods like maceration and Soxhlet extraction are gradually being superseded by more advanced techniques that enhance efficiency and selectivity. These modern methods are generally quicker, more eco-friendly, and offer higher levels of automation. [32]

The advantages of hesperidin, whether obtained from fruits, beverages, or pharmaceuticals, largely hinge on its bioavailability. Bioavailability is typically described as the amount of a specific substance that is released from food, absorbed through the intestinal barrier, enters the bloodstream, and reaches systemic circulation. This substance is then distributed to various organs and tissues, where it is converted into a biochemically active form that the body can effectively utilize. [33] The biological and pharmacological characteristics of hesperidin have been thoroughly researched, uncovering its antioxidant, anti-inflammatory, anticancer, and antiviral properties, as well as its ability to inhibit other types of pathogenesis. [34] This review discusses how hesperidin influences a variety of cell signalling pathways that affect various forms of cancer. Additionally, it details the cooperative effects of hesperidin when combined with other natural or anticancer substances.

Potential Mechanisms of Hesperidin in Cancer Prevention and Treatment

Hesperidin's anticancer properties stem from its capacity to regulate a multitude of cell signaling molecules. Its effectiveness in combating cancer has been evidenced by its modulation of inflammation, cell cycle regulation, apoptosis, angiogenesis, and various cell signaling pathways.

Inflammation

Germline mutations account for around 10% of cancer occurrences; the bulk of malignancies are caused by acquired variables, such as environmental causes, which are intimately related to chronic inflammation. Because inflammation is the first line of defense against different carcinogens, a decrease in the inflammatory response may lead to an increased risk of cancer growth and development. Moreover,

Chronic Inflammation often leads to an increase in pro-inflammatory agents such as cyclooxygenase-2 and nitric oxide synthase.
These agents are responsible for the heightened levels of prostaglandins and nitric oxide. [35]
Hesperidin has been demonstrated to have strong anti-inflammatory properties, as confirmed by numerous investigations. Hesperidin has been shown in a mouse model of asthma with allergies to decrease the production of inflammatory mediators. Hesperidin administration was observed to significantly lower Th2 cytokines and the proportion of infiltrating inflammatory cells in the bronchoalveolar lavage fluid when compared to animals treated to ovalbumin. Hesperidin lowered the serum levels of ovalbumin specific IgE. [36] Hesperidin therapy reduced the generation of nitrogen dioxide, prostaglandin E2 (PGE2), and the expression of the nitric oxide synthase (iNOS) protein. As a result, hesperidin is now recognized to be a cyclooxygenase-2 and iNOS inhibitor, which could explain its antitumorigenic and anti-inflammatory characteristics. [37] Hesperidin's effectiveness was studied on renal carcinogenesis, and it was discovered that Hesperidin reduces PGE2 levels and downregulates the expression of cyclooxygenase-2 (COX-2) and vascular endothelial growth factor. These findings show that hesperidin could serve as an effective inhibitor of kidney cancer because of its ability to lower oxidative stress and interfere with the COX-2/PGE2 pathway. [38]

Oxidative Stress

Oxidative stress is the uneven generation and depletion of reactive oxygen species (ROS) and other free radicals caused by a defective antioxidant defense mechanism. [39] Oxidative stress is known to play a role in the onset of numerous diseases, including cancer. Furthermore, cancer cells exhibit a disruption in redox balance. Reactive oxygen species (ROS) are generally considered to promote tumor growth. However, when present in high concentrations, ROS can actually be toxic to cells. This implies that while ROS can contribute to cancer progression, they can also potentially be leveraged for cancer therapy under certain conditions. Natural products or bioactive chemicals are known to limit pathogenesis by reducing free radical generation and oxidative stress. [40] Research has shown that hesperidin can inhibit the growth of Michigan Cancer Foundation-7 (MCF-7) cells in a manner that is dependent on the concentration of hesperidin. When MCF-7 cells were treated with hesperidin, there was a reported decrease in glutathione (GSH), an antioxidant, while the level of lactate dehydrogenase (LDH), an enzyme involved in energy production, increased. This reduction in GSH may trigger apoptosis, or programmed cell death, in MCF-7 cells. [41]
The therapeutic potential of hesperidin was further explored in a study involving rats with breast cancer induced by 7,12-dimethybenz(a)anthracene. This study examined the effects of hesperidin on lipid peroxidation and various membrane-bound marker enzymes. The rats were given hesperidin orally each day, which resulted in a significant decrease in lipid peroxidation and membrane-bound marker enzyme levels. Moreover, hesperidin administration notably restored the normal function of a key membrane-bound marker in the serum of both control and breast cancer trial rats. This suggests that hesperidin could potentially be beneficial in the treatment of breast cancer.[42] Hesperidin administration was found to reduce oxidative stress in the liver and kidneys of rats, which was induced by carbon tetrachloride. This study underscored the positive effects of hesperidin. [43]

Apoptosis

Apoptosis, or programmed cell death, is understood to be a vital process for development and maintaining

cellular balance. [44] Natural substances or bioactive compounds, including hesperidin, have been implicated in managing cancer by inducing apoptosis. The effects of hesperidin on apoptosis in individuals with cervical cancer were studied. DNA fragmentation and increased nuclear condensation in cervical cancer HeLa cells were suggested as signs of apoptosis initiated by hesperidin. Moreover, hesperidin-induced apoptosis in cancer cells occurred through a caspase-dependent mechanism, which seemed to be downstream of the endoplasmic reticulum stress pathway, as indicated by increased levels of GADD153/CHOP and GRP78. Hesperidin also facilitated the production of reactive oxygen species (ROS) and a decrease in mitochondrial membrane potential. Additionally, it triggered the release of intracellular calcium, increased release of cytochrome C, and an apoptosis-inducing factor from mitochondria. It was also reported to promote the activation of caspase 3. Hesperidin was found to inhibit cell cycle progression and apoptosis by linking endoplasmic reticulum stress pathways. [45]

Furthermore, hesperidin was found to trigger apoptotic signaling, which led to the degradation of several key proteins including the BH3 interacting domain death agonist (Bid), caspase-3 (CASP3), and poly (ADP-ribose) polymerase (PARP). This process was also associated with an increase in the levels of Bcl-2-associated X protein (Bax). Additionally, hesperidin was observed to cause a decrease in Bcl-xl (B-cell lymphoma-extra-large), a protein known for its role in preventing apoptosis. [46] The impact of hesperidin on the apoptosis and survival of lung cancer H460 and A549 cells was examined. The findings revealed that the administration of hesperidin inhibited the growth of both H460 and A549 cells and triggered apoptosis in a manner that was dependent on the dosage. A flow cytometry apoptosis assay was conducted to determine the molecular mechanisms associated with hesperidin's effect on reducing the viability of the A549 lung cancer cell line. The results showed that the rate of apoptosis in the control group was less than 5%. However, when comparing the apoptosis rates in groups given varying doses of hesperidin over different periods of time to those in the control group, it was observed that the effect was both time- and dose-dependent. [47] Research on the gene expression controlling apoptosis has shown that treatment with hesperidin can reduce the expression of B-cell CLL/lymphoma 2 (BCL2) mRNA and enhance the expression of BCL2-associated X protein. The administration of hesperidin significantly increased both the expression and functions of the primary apoptotic factor, CASP3. Hesperidin was found to decrease the protein expression of pro-CASP3 and increase the level of active CASP3. These findings suggest that hesperidin might activate CASP3, thereby promoting apoptosis in human colon cancer cells. [48]

Angiogenesis

Bioactive substances, including hesperidin, contribute to cancer prevention by inhibiting angiogenesis, the formation of new blood vessels. [49] According to a study on renal carcinogenesis, hesperidin was observed to reduce PGE2 levels and suppress the expression of vascular endothelial growth factor (VEGF) and COX-2. In an immunohistochemical analysis of VEGF, a small amount of VEGF immunostaining was detected in the renal cells of control rats. However, renal cells in both DEN-initiated and Fe-NTA conditions exhibited significant VEGF immunostaining. Treatment with a low dose of hesperidin partially reduced VEGF immunostaining. When rats were given a higher dose of hesperidin, a decrease in VEGF expression was observed. This suggests that hesperidin may have potential therapeutic applications in cancer treatment. [50]

Cell Cycle

Eukaryotic cells possess mechanisms known as cell cycle checkpoints that allow for the examination and

repair of cellular damage induced by stress. The regulation of the cell cycle is a crucial step in cancer prevention. Hesperidin, along with other bioactive substances, plays a significant role in cancer prevention. Flow cytometry-based results have shown that hesperidin can halt the progression of the normal cell cycle in MG-63 cell lines of human osteosarcoma. The data revealed that hesperidin led to a G2/M phase cell cycle arrest at increasing doses. [51]

Hesperidin might influence the distribution of lung cancer A549 cells throughout the cell cycle. Compared to the control group, the groups exposed to varying doses of hesperidin at different times exhibited a significant increase in the percentage of cells in the G0/G1 phase, indicating a halt or slowdown in cell cycle progression at this stage. Furthermore, hesperidin reduced the expression levels of cyclin D1, a protein that regulates the cell cycle, while it increased the relative expression levels of p53 and p21. A comparative quantitative analysis showed notable differences from the control. These results suggest that hesperidin induced a pause or stoppage at the G0/G1 phase in A549 cells by adjusting the proportional protein expression associated with the cell cycle. [52] At an equivalent dosage, hesperidin treatment led to an increase in nuclear condensation and ROS generation. There was also a dose-dependent rise in the activation of CASP3 and a halt in the cell cycle at the G2/M phase. These observations suggest that hesperidin could potentially serve as an anticancer agent for treating gallbladder carcinoma. [53]

ERK1/2 MAPK Pathway

The expression of ERK1/2 was examined to determine whether mitogen-activated protein kinases (MAPKs) may be involved in HepG2 cell death brought on by hesperidin. It was found that hesperidin significantly increased the levels of phosphorylated ERK1/2 protein while notably decreasing the levels of total ERK1/2 protein. This indicates that hesperidin induces paraptosis, a type of cell death, by activating ERK1/2. To further assess the functional significance of MAPK, changes in ERK protein levels were studied in the presence or absence of U0126, a specific ERK inhibitor. It was observed that U0126 significantly blocked ERK1/2 phosphorylation and also reversed the effects induced by pERK1/2. The study showed that U0126, when used, prevents HepG2 cells that have been treated with hesperidin alone from showing extensive cytoplasmic vacuolation and swelling of the endoplasmic reticulum and/or mitochondria. These findings suggest that HepG2 cells exposed to hesperidin undergo a type of cell death known as paraptosis. [54]

Hesperidin's Efficacy as a Cancer Preventive and Therapeutic Agent

Lung Cancer

Non-small-cell lung cancer (NSCLC), which accounts for around 85% of cases, has become the most prominent cancer worldwide. [55] Radiotherapy, surgical resection, and chemotherapy are the primary treatment methods for non-small-cell lung cancer, yet the chances of survival for individuals who have these surgeries are relatively poor. [56] A study exploiting natural chemicals found that they can decrease cancer initiation and growth. Hesperidin administration significantly lowered the expression of zinc finger E-box binding homeobox 3 mRNA (ZEB2 mRNA) and protein in a concentration-dependent manner. These findings indicate that hesperidin inhibits ZEB2 expression. Furthermore, the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay and flow cytometry analysis revealed that hesperidin treatment significantly reduced the proliferation of A549 and H460 cells. Furthermore, it considerably promoted apoptosis in a dose-dependent manner. [57] Lung cancer cells (A549 and NCI-H358) treated with hesperidin demonstrated a concentration- and time-dependent decrease in cell

proliferation. Furthermore, these evaluated cells exhibited enhanced CASP3 and additional apoptotic activities, as well as decreased mitochondrial membrane potential activity. These findings show that hesperidin suppresses NSCLC cell growth in vitro via modifying immune response-related pathways that regulate apoptosis. [58]

Hesperidin's anticancer potential in the context of cell proliferation and apoptosis in human lung cancer was examined, as well as the mechanisms behind these effects. Hesperidin treatment lowered cell viability in a concentration- and time-dependent manner. Hesperidin treatment dramatically reduced the expression of c-myc, PCNA, and β-catenin proteins, inhibiting cell growth. Hesperidin greatly boosted the levels of tumor suppressor proteins p53 and p21, preventing cyclin D and cyclin-dependent kinase 4 from being produced. Further study has revealed that hesperidin therapy directly affects the Bcl-2/Bax ratio by reestablishing tumor suppressor proteins. Overall, these results indicate hesperidin's anticancer potential among human lung cancer cells. [59] Hesperidin inhibited lung cancer cell proliferation and invasion in a dose-dependent manner. Furthermore, hesperidin stimulated the death of fatigued cancer cells. Hesperidin elevated p53 expression, inhibited p53's interaction with MDMX (murine double minute X) and had a cancer-prevention impact. [60]

Breast Cancer

Breast cancer is the most common cancer to be diagnosed in developed nations and the leading cause of death for women globally. [61] There are 500,000 breast cancer-related deaths and 2 million new cases reported globally each year. [62] Hesperidin's anticancer effects in breast cancer patients were studied both alone and in conjunction with the chemotherapy drug doxorubicin. The animal groups treated with hesperidin had lower tumor volume, lower tumor incidence, and higher survival rates than the animals exposed to 7,12-dimethylbenz(a)anthracene (DMBA). When doxorubicin-treated mice were compared to those that had received hesperidin pretreatment, the latter group displayed increased levels of glutathione and inflammatory markers along with a significant drop in malondialdehyde (MDA). Histopathology and Ki67 expression demonstrated that pretreatment with hesperidin facilitated tumor control when compared to DMBA-induced tumors. [63] Hesperidin's potential cytotoxicity against MDA-MB231 cells was assessed using the MTT test. The results showed that, as compared to the control group, hesperidin significantly decreased cell viability. Furthermore, in comparison to the control group, hesperidin administration (10 to 50 µM) significantly decreased the expression of PD-L1 as well as the signaling pathway proteins p-p65, p-Akt, and p-ER. According to the research, hesperidin prevents breast cancer from progressing by blocking the NF-κB and Akt signaling pathways, while PD-L1 encourages the disease's advancement. [64] Antioxidant status significantly declined and drug-metabolizing enzymes were changed in animals exposed to the genotoxin DMBA. Remarkably, hesperidin-administered mice showed significant restoration of their altered levels to nearly normal via elevated levels of internal antioxidants, phase II enzyme activation, and phase I enzyme modulation. [65] Hesperidin and luteolin both markedly elevated the proportion of cells exhibiting apoptosis in the sub-G1 and G0/G1 cell cycle stages. Furthermore, both medications induced apoptosis through intrinsic and extrinsic mechanisms. Cell viability was reduced in a manner that varied with dosage and duration. Furthermore, a downregulation of the antiapoptotic gene Bcl-2 occurred. Pro-apoptotic Bax, however, was increased. Furthermore, there was a notable increase in the turning on (expression) of microRNAs, such as miR-34a and 16. Conversely, hesperidin and luteolin dramatically decreased the expression of miR-21 in MCF-7. [66]

Leukaemia

Hesperidin was shown to induce a higher rate of apoptosis in NALM-6 cells; thus an investigation was conducted to see if this apoptosis was caspase-dependent. Hesperidin at several dosages was applied to NALM-6 cells for a full day. The findings demonstrated that the treatment of the cells with hesperidin increased the concentration-dependent cleavage of caspase-9 and caspase-3. Hesperidin also increased the expression of the Bax protein, while it significantly and dose-dependently decreased the expression of the Bcl-2 protein. The findings also demonstrated that hesperidin for 48 hours significantly reduced the NALM-6 cells' occurrence of XIAP. [67] The pro-apoptotic properties of hesperidin were investigated in KG1a cells. Treatment with hesperidin resulted in altered apoptotic cell shape and elevated CASP3 activity. The results showed that, in comparison to the control group, hesperidin treatment greatly boosted the expression of the pro-apoptotic genes Bax and the cell cycle regulator p21 and significantly lowered the expression of the antiapoptotic genes, such as Bcl2 and survivin. These findings suggested that hesperidin might be a useful apoptosis initiator and a desirable treatment choice for acute myeloid leukemia. [68] Hesperidin was found to be the most harmful to K562 cells based on the results of another study. [69]

Renal Cancer

A study was carried out to ascertain the hesperidin therapeutic interventions on rat renal cancer, which were prompted by DEN injection and supported by Fe-NTA. Hesperidin's anticancer potential was evaluated in connection to PGE2 levels, antioxidant activity, renal
function, and the expression of COX-2 and VEGF. Renal antioxidant enzymes were restored, and renal function was enhanced by hesperidin. It is also shown to be useful in reducing lipid peroxidation brought on by Fe-NTA and DEN. The potential of hesperidin to reduce PGE2 levels and to inhibit the production of COX-2 and VEGF was also studied. Hesperidin was found to be able to guard against kidney damage caused by both DEN and Fe-NTA in histological investigations. [70] On top of that, it was shown that hesperidin decreased the expression of PCNA, NF-κB, bcl-2, inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF)-α, and caspase-9, caspase-3, and bax. With regard to kidney carcinogenesis, these results offer strong evidence that hesperidin is a useful chemopreventive drug. [71]

Liver Cancer

HepG2 cells were treated with acetaldehyde and hesperidin in an invasion chamber to assess hesperidin's impact on acetaldehyde-induced cell invasion. The migration of HepG2 cells through Matrigel-coated filters was enhanced six-fold by acetaldehyde. Significantly, hesperidin reduced the proportion of HepG2 cells. Furthermore, hesperidin successfully suppressed MMP-9 activation in HepG2 cells, which had been markedly enhanced by acetaldehyde. After being treated with acetaldehyde, MMP-9 expression rose. On the other hand, hesperidin inhibited its expression in a concentration-dependent way. Furthermore, hesperidin inhibited acetaldehyde-induced IB phosphorylation in a manner that was related to hesperidin concentration. [72] Hesperidin inhibited cell invasion induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) and both the released and cytoplasmic forms of matrix metalloproteinase (MMP)-9 within HepG2 cells. Hesperidin dramatically lowered the matrix metalloproteinase-9 mRNA level that TPA had produced. It has been observed that hesperidin inhibits NF-kB and AP-1 activity, hence preventing the transcription of matrix metalloproteinase-9. Moreover, hesperidin blocked c-Jun N-terminal kinase signaling pathways and p38 kinase phosphorylation, which in turn reduced AP-1 activity brought on by

TPA. NF-kB inhibitory signaling pathways also prevented TPA-stimulated NF-kB translocation into the nucleus. [73] The gene expressions and associated protein levels of Cyclin D1, β-catenin, Wnt3a, and Wnt5a (wingless-related integration sites 3a and 5a) were significantly elevated in the hepatocellular carcinoma group [74]. Hesperidin significantly suppressed the Wnt5a and Wnt3a/β-catenin pathways that are triggered by thioacetamide. Furthermore, by significantly lowering inflammation, oxidative imbalance, serum ALT and AST levels, liver function indicators, and albumin levels, hesperidin demonstrated a hepatoprotective effect. In summary, hesperidin has been shown to limit the induction of both canonical and non-canonical Wnt pathways, hence exerting a preventive effect against hepato-carcinogenesis. [75]

Endometrial Cancer

Hesperidin was incubated at different concentrations for varying lengths of time in order to determine its proliferative potential. Hesperidin-treated endometrial cancer cells exhibited dose- and time-dependent decreases compared to untreated cells. Furthermore, the growth inhibition ratio was evaluated following a 24-72-hour incubation period, and it was found that

Hesperidin exhibited dose- and time-dependent growth inhibition effects on endometrial cancer cells. This study looked at changes in CASP3 activation to see if hesperidin had any potential apoptotic effects on these cancer cells. After treating ECC-1 cells with 50 µM hesperidin for 48 hours, the proportion of CASP 3 increased 1.5 times compared to untreated cells. [76]

Cervix Cancer

Hesperidin treatment caused the c-Jun activation domain-binding protein-1 (Jab1) gene to be down-regulated and p27 to be up-regulated in a dose-dependent manner. These gene intonations may arise from an excess of ROS production and CASP3 activation, which in turn causes an induction of apoptosis. Apoptotic cells count increased, as demonstrated by Hoechst staining and cell cycle analysis. Our data clearly imply that Jab1 is the therapeutic target of hesperidin, which inhibits cell proliferation and induces death in HeLa cells. [77] HeLa cell proliferation was suppressed by hesperidin in a time- and concentration-dependent manner. Hesperidin-induced apoptosis in this cancer cell population was typified by increased nuclear condensation and DNA fragmentation. Furthermore, hesperidin-induced apoptosis in HeLa cells was associated with elevated GRP78 and elevated GADD153/CHOP levels. This apoptosis was mediated by a caspase-dependent pathway, which seems to be a downstream element of the endoplasmic reticulum's stress mechanism. These two proteins serve as markers for stress within the body's endoplasmic reticulum. [78] Hesperidin also promoted the activation of CASP3, increased the release of cytochrome c and a factor that causes apoptosis from the mitochondria, decreased the potential of the mitochondrial membrane, mobilized intracellular Ca2+, and produced reactive oxygen species. Additionally, the cell cycle stopped at the G0/G1 phase in HeLa cells due to a decrease in the protein expression of cyclinE1, cyclin-dependent kinase 2, and cyclinD1. [79]

Prostate Cancer

The growth inhibitory potential of hesperidin was evaluated by using a flow cytometry approach. According to the results, the percentage of G2/M phase DU145 prostate cancer cells rose in direct proportion to the increase in hesperidin concentration. Moreover, a noteworthy rise was observed in the proportion of late apoptotic prostate cancer cells, which was correlated in a linear fashion with increasing

hesperidin dosages. LDH release concentrations were shown to rise significantly in a concentration-related manner when hesperidin was administered. All things considered, the data are consistent with the theory that hesperidin causes G2/M phase cell cycle arrest in addition to necrosis-like prostate cancer cell death, which is defined as a reduction in the cells' growth and proliferation. Furthermore, it was discovered that the ROS levels were noticeably increased with every hesperidin dosage that was used. The percentage of ROS in DU145 cells was found to be roughly doubled when they were exposed to 20 µM hesperidin. The mitochondrial membrane potential (MMP) of cancer cells from the DU145 strain significantly decreased when the concentration of hesperidin rose, according to analysis of the MMP. [80]

Gastric Cancer

Hesperidin exhibited a suppressive effect on the ability of GC cells to proliferate, as evidenced by the concentration-dependent and gradual reduction in GC cell viability. Furthermore, the results demonstrated that as the duration of exposure to hesperidin increased, the detected green fluorescence intensity slowly enhanced, indicating that the degree of apoptosis in AGS cells increased slowly. This allowed researchers to investigate whether hesperidin prevented the proliferation of gastric cancer AGS cells by encouraging apoptosis. Additionally, morphological alterations in apoptosis were observed, such as cell shrinkage and a decrease in cell density. The quantity of apoptotic cells increased gradually as the length of the hesperidin treatment was prolonged, reaching its maximum degree of apoptosis during the 24-hour treatment. Furthermore, the administration of hesperidin was intended to decelerate and cause a time-dependent increase in ROS. In addition, compared to the group treated with hesperidin alone, apoptosis was much lower in the group treated with hesperidin plus NAC. [81]

Gallbladder

Despite its small size—less than 2 cm—gallbladder cancer can kill 170,000 people yearly, making up 1.7% of all cancer-related fatalities, according to current figures.[82] There were notable changes observed in the appearance of the cancer cells following their exposure to hesperidin in gallbladder carcinoma cells. The outcomes unequivocally demonstrated that hesperidin, in a dose-dependent manner, stimulated nuclear condensation in these cancer cells. Condensed and broken nuclei are characteristics of apoptotic cells. The hesperidin-induced apoptosis in treated cells was demonstrated by condensed nuclei staining in Hoechst 33342 and bright-blue fluorescence. These findings clearly demonstrate that hesperidin induces dose-dependent apoptosis in gallbladder cancer cells. After hesperidin therapy, annexin V-FITC/PI double-labelled flow cytometry was also used to assess apoplastic induction in gallbladder cancer cells. The same results were obtained because the proportion of cells going through apoptosis increased significantly in a concentration-dependent manner. Furthermore, it was shown that CASP3 activation and cell cycle arrest at the G2/M phase increased in a dose-dependent manner. These findings all point to the possibility that hesperidin may have anticancer properties and be utilised to treat gallbladder carcinoma. [83]

Colon Cancer

The chemopreventive effect of hesperidin in 1, 2-dimethylhydrazine (DMH)-induced colorectal cancer (CRC) was examined in a recent study. Significant increases in MDA and NO levels, as well as the expression of the activin A gene and protein, were seen in the 2-dimethylhydrazine (DMH) group. Superoxide dismutase (SOD), GSH, and SMA- and MAD-related protein 4 (Smad4) levels in tissues all

sharply declined, as did the expression of the respective genes and proteins. The activin A and Smad4 gene expression was considerably elevated by hesperidin treatment in both the 1,2-dimethylhydrazine (DMH) plus hesperidin and the DMH after hesperidin groups. This study validated hesperidin's capacity to prevent colorectal cancer via regulating Smad4 and activin A signalling in vivo. [84]

According to a different study, hesperidin treatments significantly reduced the incidence of tumor development as well as the quantity and multiplicities of azoxymethane-induced ACF. It has been shown that hesperidin improves antioxidant status and lowers oxidative stress markers. Furthermore, following hesperidin treatment, the PCNA index decreased. Hesperidin treatments effectively decreased inflammation by downregulating NF-kB and its target molecules, COX-2 and iNOS. [85] The impact of hesperidin on the anticancer activity of cyclophosphamide was investigated in tumor-bearing mice. Before cyclophosphamide was given, white blood cell counts were elevated by 200 mg/kg of hesperidin. On the fourth- and seventh-days following cyclophosphamide injections, this remarkable protective capacity was documented. It was discovered that co-administering hesperidin and cyclophosphamide considerably lessened or prevented the tumor development delay that cyclophosphamide generated in colon cancer-bearing animals. These findings showed that hesperidin obstructs cyclophosphamide's anticancer action. The CT-26 tumor did not grow because of the estrogen receptor. These results suggest that meals high in hesperidin, such as citrus fruits, may lessen the effectiveness of cyclophosphamide when used as a treatment for colon cancer patients. [86]

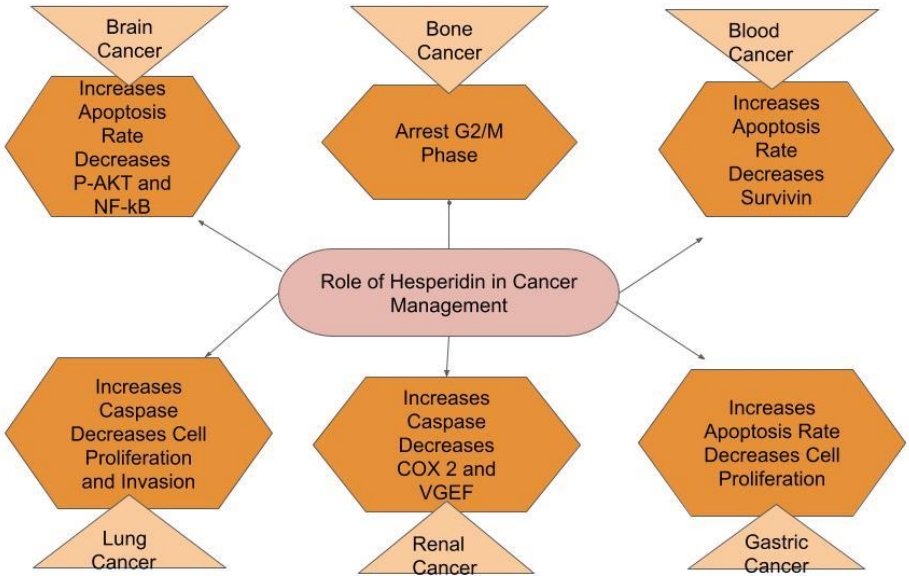

Fig. Hesperidin action in various Cancers

Enhancing Cancer Treatment: Synergistic Effects of Hesperidin with anti-cancer Drugs or Natural Compounds

Combining various natural substances or their biomolecules with anticancer medications has demonstrated its role in cancer prevention by increasing the effectiveness of these treatments and lowering unfavorable side effects. Accordingly, when used in conjunction with other natural substances or anticancer medications, hesperidin exhibits synergistic effects in the treatment of cancer. A study on breast cancer found that hesperidin plus chlorogenic acid affected the estrogen receptor pathway, which in turn controlled the mitochondrial activity and ATP synthesis in breast tumor (MCF-7) cells. Lastly, studies

showed that hesperidin and chlorogenic acid effectively treated breast cancer patients when taken in addition to chemotherapy.[87] The cytotoxic activity of hesperidin and silibinin was indicated by their respective IC50 values, which were 50.12 µM and 16.2 µM, based on in vitro research. A Combination Index study found that silibinin and hesperidin both had synergistic potential and reduced the IC50 value of cytarabine by nearly 4.5 and 5.9 times, respectively. As part of acute myeloid leukemia (AML) therapy, both natural substances have the potential to have antileukemic effects and can be used either alone or in combination with other chemotherapeutic drugs. [88]

A549 and NCI-H460 cells showed a positive interaction with the medications and induced the highest level of apoptosis in the hesperidin plus carboplatin treatment group. The findings demonstrated that hesperidin and carboplatin inhibited cell growth. The A549 and NCI-H460 cell proliferation rates were lowest in the hesperidin plus carboplatin therapy group. The combination had a synergistic effect on the drugs. The examination of cell invasion data has shown that hesperidin and carboplatin are particularly effective in blocking the invasion of NCI-H460 and A549 cells. The combination of hesperidin and carboplatin had the best regarded inhibitory effect. Furthermore, hesperidin and carboplatin boosted p53 expression in NCI-H460 and A549 cells. The group that was treated with hesperidin plus carboplatin exhibited the highest expression of p53. When treated to either hesperidin or carboplatin, the expression levels of the apoptosis-related genes p21, Bax, and PUMA increased. The group that got both hesperidin and carboplatin had the greatest expression levels of p21, PUMA, and Bax. [89] Tamoxifen's anticancer activity was enhanced by hesperidin, piperine, and bee venom when combined; these substances could be a safe adjuvant or vehicle for tamoxifen in the treatment of breast cancer. Another experiment looked at the potential of hesperidin to overcome doxorubicin resistance in MCF-7-resistant doxorubicin cells (MCF-7/Dox) with respect to cytotoxicity, apoptosis, and P-glycoprotein (Pgp) expression [90]

Another study examined how effectively hesperidin worked in combination with doxorubicin to overcome doxorubicin resistance in MCF-7-resistant doxorubicin cells (MCF-7/Dox) in terms of cytotoxicity, apoptosis, and P-glycoprotein (Pgp) expression. With an IC50 value of 11 µmol/L, hesperidin alone showed cytotoxic capability on MCF-7/Dox cells. The combined therapeutic effects of hesperidin and doxorubicin were hostile and addicting. Hesperidin did not increase apoptotic induction, but when paired with doxorubicin at a low dose, it did decrease the level of P-glycoprotein expression.[91] When coupled with doxorubicin, hesperidin and apigenin both affect MCF-7 breast cancer cells. Although flavonoids really reduced the ability of doxorubicin to generate oxidative stress-related damage, it was found that they increased the drug's potential for cytotoxicity. Ultimately, doxorubicin's cytotoxic effect on breast cancer cells is enhanced by both apigenin and hesperidin. [92]

Strategies to Enhance the Effectiveness of Hesperidin

Several experimental investigations have demonstrated the efficacy of hesperidin in treating a wide range of illnesses. Hesperidin's extremely low water solubility, which may severely limit its absorption, has been demonstrated in multiple studies to have a decreased bioavailability.[93] The issues of poor solubility, rapid elimination, and metabolism have been addressed by a plethora of techniques based on nano-formulations, and their potential to prevent cancer has been explored. A large study was conducted to determine whether hesperidin nanoparticles and/or imatinib mesylate increased the cancer-protective potential independently or combined. Additionally, it investigated the possibility of using nanoencapsulation to lessen imatinib mesylate's cardiotoxicity in mice that had solid Ehrlich carcinoma. Imatinib mesylate and hesperidin were added to poly (lactic-co-glycolic acid) polymer.[94]

Mice bearing solid Ehrlich carcinoma have been used as a model to study experimentally induced breast cancer. When these mice were treated with nano-imatinib mesylate and/or nano-hesperidin, there was a significant reduction in weight, tumor size, hematological markers, and cardiac markers compared to control groups that received only standard treatment. Additionally, there was a noticeable downregulation of the MDR-1 gene in the tumor, which is often associated with drug resistance. [95] A tailored nanohybrid carrier system for hesperidin delivery was studied. To produce a casein-calcium-ferrite nanohybrid carrier, the ionic-gelation and desolvation processes were combined. After hesperidin was first encapsulated in a carrier, the guiding ligand, progesterone, was conjugated via an active ester process. Taguchi optimization produced a maximum hesperidin encapsulation in the carrier of 89.54%. Superparamagnetic calcium ferrite nanoparticles merged to create improved drug encapsulation and magnetic drug delivery. With improved stability and a higher release of medication applications that were helpful in treating cancer, the carrier's drug release behavior was stimuli responsive. Using L929 fibroblast cells in a cell viability assay, the formulation's biocompatibility was confirmed. The specific detection and targeted chemotherapy of the progesterone-conjugated carrier enhanced the cytotoxic effect of CHD against MDA-MB-231 breast cancer cells and SKOV-3 ovarian cells, resulting in a notable 30-fold reduction in the half-maximal inhibitory concentration (IC50) values. [96]

Hesperidin loaded on gold nanoparticles (Hsp-AuNPs) was tested for its ability to kill MDA-MB-231 (human breast cancer) cells. When treated cells were compared to normal human breast epithelial cells, the results revealed a significant decrease in the repression of growth and proliferation. No histological alterations or damage was observed in the kidney, spleen, liver, or lung following the administration of Hsp-AuNPs. In mice with Ehrlich ascites tumors, these nanoparticles improved the functions of macrophages. [97]

Research Trials Involving Hesperidin

There aren't many human research studies assessing hesperidin's potential in managing diseases, such cancer. A clinical trial randomized to nutritional intervention was carried out in accordance with the ethical guidelines provided in the 1975 Helsinki Declaration and its amendments. [98] This experiment set out to investigate the metabolic profile of dietary polyphenols in glandular breast tissues from patients who had just been diagnosed with breast cancer, both malignant and benign. This study's ClinicalTrials.gov Identifier is NCT03482401. Out of the total of 43 patients, 28 patients gave written agreement, and these patients were randomly split into two groups. From the time of diagnosis until the time of surgery, the patients in the polyphenol group received capsules. The control group, however, did not take any pills. The capsule was a dietary supplement rich in polyphenols, including polyphenols such as procyanidins, hesperidin, eriocitrin, curcumin, resveratrol, punicalagin,
and ellagic acid, as well as simple phenolics such hydroxytyrosol. Theobromine, caffeine, and methylxanthines were also found in the capsules containing cocoa extract. A total of 101 metabolites were discovered in urine, 69 in plasma, 39 in healthy (NT) tissues, and 33 in malignant (MT) tissues using UPLC-ESI-QTOF-MS. As controls, eight patients did not get any extracts. Phenolic-derived metabolites are predominantly glucuronidated and/or sulfated in MT and NT. Metabolites detected in breast tissues do not exhibit any antiproliferative or estrogenic/antiestrogenic properties in MCF-7 breast cancer cells. [99]

Conclusion

Our lifestyle choices and environment can significantly influence our risk of developing cancer. Making conscious decisions such as reducing carbohydrate intake, quitting smoking, and incorporating bioactive compounds like hesperidin into our diet can indeed help mitigate some of these risks. Hesperidin, known for its antioxidant and anti-inflammatory properties, has shown promise in various pharmacological applications, including its potential in fighting cancer by modulating important cellular pathways involved in cancer progression such as cell cycle, apoptosis, and angiogenesis. This highlights the importance of both dietary and environmental interventions in cancer prevention and management. The combined use of hesperidin with other cancer treatments or natural substances has been shown to have a synergistic effect, indicating its potential in developing effective anticancer strategies. Research suggests that hesperidin could be highly advantageous in the prevention and treatment of various cancer types such as lung, colon, and prostate cancer, among others.

References:

1. De Flora, S., & Ferguson, L. R. (2005). Overview of mechanisms of cancer chemopreventive agents. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 591(1-2), 8-15.
2. Bonovas, S., Tsantes, A., Drosos, T., & Sitaras, N. M. (2008). Cancer chemoprevention: a summary of the current evidence. Anticancer research, 28(3B), 1857-1866.
3. Roohbakhsh, A., Parhiz, H., Soltani, F., Rezaee, R., & Iranshahi, M. (2015). Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases. Life sciences, 124, 64-74.
4. Reuter, S., Gupta, S. C., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: how are they linked?. Free radical biology and medicine, 49(11), 1603-1616.
5. 6, Devi, K. P., Rajavel, T., Nabavi, S. F., Setzer, W. N., Ahmadi, A., Mansouri, K., & Nabavi, S. M. (2015). Hesperidin: A promising anticancer agent from nature. Industrial crops and products, 76, 582-589.
6. 8 Liskova, A., Samec, M., Koklesova, L., Brockmueller, A., Zhai, K., Abdellatif, B., ... & Kubatka, P. (2021). Flavonoids as an effective sensitizer for anti-cancer therapy: Insights into multi-faceted mechanisms and applicability towards individualized patient profiles. Epma Journal, 12(2), 155-176.
7. 10, 11, 12 Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. cell, 144(5), 646-674.
8. Lemmon, M. A., & Schlessinger, J. (2010). Cell signaling by receptor tyrosine kinases. Cell, 141(7), 1117–1134.
9. Burkhart, D. L., & Sage, J. (2008). Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nature Reviews Cancer, 8(9), 671-682.
10. Van Roy, F., & Berx, G. (2008). The cell-cell adhesion molecule E-cadherin. Cellular and molecular life sciences, 65, 3756-3788.
11. Hanahan, D., & Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. cell, 86(3), 353-364.
12. Blasco, M. A. (2005). Telomeres and human disease: ageing, cancer and beyond. Nature Reviews Genetics, 6(8), 611-622.
13. 19. Adams, J. M., & Cory, S. (2007). The Bcl-2 apoptotic switch in cancer development and therapy.

Oncogene, 26(9), 1324-1337.
14. Winston, C., & Beck, L. (1999). Phytochemicals: health protective effects. Canadian Journal of Dietetic Practice and Research, 60(2), 78.
15. Surh, Y. J. (2003). Cancer chemoprevention with dietary phytochemicals. Nature Reviews Cancer, 3(10)
16. Ramos, S. (2008). Cancer chemoprevention and chemotherapy: dietary polyphenols and signalling pathways. Molecular nutrition & food research, 52(5), 507-526.
17. Nguyen, T. H. P., Kumar, V. B., Ponnusamy, V. K., Mai, T. T. T., Nhat, P. T., Brindhadevi, K., & Pugazhendhi, A. (2021). Phytochemicals intended for anticancer effects at preclinical levels to clinical practice: Assessment of formulations at nanoscale for non-small cell lung cancer (NSCLC) therapy. Process Biochemistry, 104, 55-75.
18. ur Rehman, M. F., Batool, A. I., Qadir, R., & Aslam, M. (2021). Hesperidin and naringenin. In A centum of valuable plant bioactives (pp. 403-444). Academic Press.
19. Stanisic, D., Costa, A. F., Cruz, G., Durán, N., & Tasic, L. (2018). Applications of flavonoids, with an emphasis on hesperidin, as anticancer prodrugs: phytotherapy as an alternative to chemotherapy. Studies in Natural Products Chemistry, 58, 161-212.
20. Lucas-Abellán, C., Pérez-Abril, M., Castillo, J., Serrano, A., Mercader, M. T., Fortea, M. I., ... & Núñez-Delicado, E. (2019). Effect of temperature, pH, β-and HP-β-cds on the solubility and stability of flavanones: Naringenin and hesperetin. LWT, 108, 233-239.
21. Addi, M., Elbouzidi, A., Abid, M., Tungmunnithum, D., Elamrani, A., & Hano, C. (2021). An overview of bioactive flavonoids from citrus fruits. Applied Sciences, 12(1), 29.
22. Devi, K. P., Rajavel, T., Nabavi, S. F., Setzer, W. N., Ahmadi, A., Mansouri, K., & Nabavi, S. M. (2015). Hesperidin: A promising anticancer agent from nature. Industrial crops and products, 76, 582-589.
23. Pyrzynska, K. (2022). Hesperidin: a review on extraction methods, stability and biological activities. Nutrients, 14(12), 2387.
24. Stanisic, D., Costa, A. F., Cruz, G., Durán, N., & Tasic, L. (2018). Applications of flavonoids, with an emphasis on hesperidin, as anticancer prodrugs: phytotherapy as an alternative to chemotherapy. Studies in Natural Products Chemistry, 58, 161-212.
25. Chaves, J. O., De Souza, M. C., Da Silva, L. C., Lachos-Perez, D., Torres-Mayanga, P. C., Machado, A. P. D. F., ... & Rostagno, M. A. (2020). Extraction of flavonoids from natural sources using modern techniques. Frontiers in chemistry, 8, 507887.
26. Pyrzynska, K. (2022). Hesperidin: a review on extraction methods, stability and biological activities. Nutrients, 14(12), 2387.
27. Srinivasan, V. S. (2001). Bioavailability of nutrients: a practical approach to in vitro demonstration of the availability of nutrients in multivitamin-mineral combination products. The Journal of nutrition, 131(4), 1349S-1350S.
28. Fraga, C. G., Croft, K. D., Kennedy, D. O., & Tomás-Barberán, F. A. (2019). The effects of polyphenols and other bioactives on human health. Food & function, 10(2), 514-528.
29. Pandya, P. H., Murray, M. E., Pollok, K. E., & Renbarger, J. L. (2016). The immune system in cancer pathogenesis: potential therapeutic approaches. Journal of immunology research, 2016.
30. Wei, D., Ci, X., Chu, X., Wei, M., Hua, S., & Deng, X. (2012). Hesperidin suppresses ovalbumin-induced airway inflammation in a mouse allergic asthma model. Inflammation, 35, 114-121

31. Sakata, K., Hirose, Y., Qiao, Z., Tanaka, T., & Mori, H. (2003). Inhibition of inducible isoforms of cyclooxygenase and nitric oxide synthase by flavonoid hesperidin in mouse macrophage cell line. Cancer letters, 199(2), 139-145.
32. Siddiqi, A., Saidullah, B., & Sultana, S. (2018). Anti-carcinogenic effect of hesperidin against renal cell carcinoma by targeting COX-2/PGE2 pathway in Wistar rats. Environmental toxicology, 33(10), 1069-1077.
33. Forcados, G. E., Chinyere, C. N., & Shu, M. L. (2016). Acalypha wilkesiana: Therapeutic and toxic potential. J. Med. Surg. Pathol, 1, 122.
34. Reczek, C. R., Birsoy, K., Kong, H., Martínez-Reyes, I., Wang, T., Gao, P., ... & Chandel, N. S. (2017). A CRISPR screen identifies a pathway required for paraquat-induced cell death. Nature chemical biology, 13(12), 1274-1279.
35. Natarajan N, Thamaraiselvan R, Lingaiah H, Srinivasan P, and Periyasamy BM: Effect of flavonone hesperidin on the apoptosis of human mammary carcinoma cell line MCF-7. Biomed. Prev. Nutr. **2011**, 1, 207–215
36. Nandakumar N, Jayaprakash R, Rengarajan T, Ramesh V, and Balasubramanian MP: Hesperidin, a natural citrus flavonoglycoside, normalizes lipid peroxidation and membrane bound marker enzymes in 7, 12-dimethylbenz(a) anthracene induced experimental breast cancer rats. Biomed. Prev. Nutr. **2011**, 1, 255–262.
37. Tirkey, N., Pilkhwal, S., Kuhad, A., & Chopra, K. (2005). Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. BMC pharmacology, 5, 1-8.
38. Peng, W., Wu, J. G., Jiang, Y. B., Liu, Y. J., Sun, T., Wu, N., & Wu, C. J. (2015). Antitumor activity of 4-O-(2 ″-O-acetyl-6 ″-Op-coumaroyl-β-D-glucopyranosyl)-p-coumaric acid against lung cancers via mitochondrial-mediated apoptosis. Chemico-biological interactions, 233, 8-13.
39. Wang, Y., Yu, H., Zhang, J., Gao, J., Ge, X., & Lou, G. (2015). Hesperidin inhibits HeLa cell proliferation through apoptosis mediated by endoplasmic reticulum stress pathways and cell cycle arrest. BMC cancer, 15, 1-11.
40. Lee, K. A., Lee, S. H., Lee, Y. J., Baeg, S. M., & Shim, J. H. (2012). Hesperidin induces apoptosis by inhibiting Sp1 and its regulatory protein in MSTO-211H cells. Biomolecules & therapeutics, 20(3), 273.
41. Xia, R., Sheng, X., Xu, X., Yu, C., & Lu, H. (2018). Hesperidin induces apoptosis and G0/G1 arrest in human non-small cell lung cancer A549 cells. International journal of molecular medicine, 41(1), 464-472.
42. Park, H. J., Kim, M. J., Ha, E., & Chung, J. H. (2008). Apoptotic effect of hesperidin through caspase3 activation in human colon cancer cells, SNU-C4. Phytomedicine, 15(1-2), 147-151.
43. Carmeliet, P., & Jain, R. K. (2011). Molecular mechanisms and clinical applications of angiogenesis. Nature, 473(7347), 298-307.
44. Siddiqi, A., Saidullah, B., & Sultana, S. (2018). Anti-carcinogenic effect of hesperidin against renal cell carcinoma by targeting COX-2/PGE2 pathway in Wistar rats. Environmental toxicology, 33(10), 1069-1077.
45. Du, G. Y., He, S. W., Zhang, L., Sun, C. X., Mi, L. D., & Sun, Z. G. (2018).
46. Xia, R., Sheng, X., Xu, X., Yu, C., & Lu, H. (2018). Hesperidin induces apoptosis and G0/G1 arrest in human non-small cell lung cancer A549 cells. International journal of molecular medicine, 41(1),

464-472.
47. Du, G. Y., He, S. W., Zhang, L., Sun, C. X., Mi, L. D., & Sun, Z. G. (2018). Hesperidin exhibits in vitro and in vivo antitumor effects in human osteosarcoma MG-63 cells and xenograft mice models via inhibition of cell migration and invasion, cell cycle arrest and induction of mitochondrial-mediated apoptosis Retraction in/10.3892/ol. 2023.13703. Oncology letters, 16(5), 6299-6306.
48. Yumnam, S., Park, H. S., Kim, M. K., Nagappan, A., Hong, G. E., Lee, H. J., ... & Kim, G. S. (2014). Hesperidin induces paraptosis like cell death in hepatoblatoma, HepG2 cells: Involvement of ERK1/2 MAPK. PloS one, 9(6), e101321.
49. Tan, S., Dai, L., Tan, P., Liu, W., Mu, Y., Wang, J., ... & Hou, A. (2020). Hesperidin administration suppresses the proliferation of lung cancer cells by promoting apoptosis via targeting the miR-132/ZEB2 signalling pathway. International Journal of Molecular Medicine, 46(6), 2069-2077.
50. Birsu Cincin, Z., Unlu, M., Kiran, B., Sinem Bireller, E., Baran, Y., & Cakmakoglu, B. (2015). Anti-proliferative, apoptotic and signal transduction effects of hesperidin in non-small cell lung cancer cells. Cellular Oncology, 38, 195-204.
51. Pandey, P., & Khan, F. (2021). A mechanistic review of the anticancer potential of hesperidin, a natural flavonoid from citrus fruits. Nutrition Research, 92, 21-31.
52. Bansal, K., Bhati, H., & Bajpai, M. (2024). New insights into therapeutic applications and nanoformulation approaches of Hesperidin: An updated review. Pharmacological Research-Modern Chinese Medicine, 100363.
53. 60. Feng, Y., Huo, H., & Tang, Q. (2022). Hesperidin Inhibits the p53-MDMXInteraction-Induced Apoptosis of Non-Small-Cell Lung Cancer and Enhances the Antitumor Effect of Carboplatin. Journal of Oncology, 2022.
54. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 68(6), 394-424.
55. Shakiba, E., Bazi, A., Ghasemi, H., Eshaghi-Gorji, R., Mehdipour, S. A., Nikfar, B., ... & Mirzaei, S. (2023). Hesperidin suppressed metastasis, angiogenesis and tumour growth in Balb/c mice model of breast cancer. Journal of Cellular and Molecular Medicine, 27(18), 2756-2769.
56. Patel, P., & Shah, J. (2021). Protective effects of hesperidin through attenuation of Ki67 expression against DMBA-induced breast cancer in female rats. Life Sciences, 285, 119957.
57. Aggarwal, V., Tuli, H. S., Thakral, F., Singhal, P., Aggarwal, D., Srivastava, S., ... & Sethi, G. (2020). Molecular mechanisms of action of hesperidin in cancer: Recent trends and advancements. Experimental Biology and Medicine, 245(5), 486-497.
58. Magura, J., Moodley, R., & Mackraj, I. (2022). The effect of hesperidin and luteolin isolated from Eriocephalus africanus on apoptosis, cell cycle and miRNA expression in MCF-7. Journal of Biomolecular Structure and Dynamics, 40(4), 1791-1800.
59. Ghorbani, A., Nazari, M., Jeddi-Tehrani, M., & Zand, H. (2012). The citrus flavonoid hesperidin induces p53 and inhibits NF-κB activation in order to trigger apoptosis in NALM-6 cells: involvement of PPARγ-dependent mechanism. European journal of nutrition, 51, 39-46.
60. Novinbahador, T., Araj-Khodaei, M., & Mahdavi, M. (2023). Evidence for Hesperidin as an Effective Factor in Initiating the Intrinsic Pathway of Apoptosis in KG1a Leukemia Cells. International Journal of Toxicology, 42(2), 165-171.
61. Anjum, J., Mitra, S., Das, R., Alam, R., Mojumder, A., Emran, T. B., ... & Khan, H. (2022). A renewed

concept on the MAPK signaling pathway in cancers: Polyphenols as a choice of therapeutics. Pharmacological research, 184, 106398.

62. Siddiqi, A., Saidullah, B., & Sultana, S. (2018). Anti-carcinogenic effect of hesperidin against renal cell carcinoma by targeting COX-2/PGE2 pathway in Wistar rats. Environmental toxicology, 33(10), 1069-1077.
63. Siddiqi, A., Hasan, S. K., Nafees, S., Rashid, S., Saidullah, B., & Sultana, S. (2015). Chemopreventive efficacy of hesperidin against chemically induced nephrotoxicity and renal carcinogenesis via amelioration of oxidative stress and modulation of multiple molecular pathways. Experimental and molecular pathology, 99(3), 641-653.
64. Yeh, M. H., Kao, S. T., Hung, C. M., Liu, C. J., Lee, K. H., & Yeh, C. C. (2009). Hesperidin inhibited acetaldehyde-induced matrix metalloproteinase-9 gene expression in human hepatocellular carcinoma cells. Toxicology letters, 184(3), 204-210.
65. Zaghloul, R. A., Elsherbiny, N. M., Kenawy, H. I., El-Karef, A., Eissa, L. A., & El-Shishtawy, M. M. (2017). Hepatoprotective effect of hesperidin in hepatocellular carcinoma: Involvement of Wnt signaling pathways. Life sciences, 185, 114-125.
66. Cincin, Z. B., Kiran, B., Baran, Y., & Cakmakoglu, B. (2018). Hesperidin promotes programmed cell death by downregulation of nongenomic estrogen receptor signalling pathway in endometrial cancer cells. Biomedicine & Pharmacotherapy, 103, 336-345.
67. Pandey, P., Khan, F., & Maurya, P. (2021). Targeting Jab1 using hesperidin (dietary phytocompound) for inducing apoptosis in HeLa cervical cancer cells. Journal of food biochemistry, 45(7), e13800.
68. 78, 79. Wang, Y., Yu, H., Zhang, J., Gao, J., Ge, X., & Lou, G. (2015). Hesperidin inhibits HeLa cell proliferation through apoptosis mediated by endoplasmic reticulum stress pathways and cell cycle arrest. BMC cancer, 15, 1-11.
69. Ning, L., Zhao, W., Gao, H., & Wu, Y. (2020). Hesperidin induces anticancer effects on human prostate cancer cells via ROS-mediated necrosis like cell death. J. buon, 25(6), 2629-2634.
70. Yu, W., Xie, X., Yu, Z., Jin, Q., & Wu, H. (2019). Mechanism of hesperidin-induced apoptosis in human gastric cancer AGS cells. Tropical Journal of Pharmaceutical Research, 18(11), 2363-2369
71. Rawla, P., Sunkara, T., Thandra, K. C., & Barsouk, A. (2019). Epidemiology of gallbladder cancer. Clinical and experimental hepatology, 5(2), 93-102.
72. Pandey, P., Sayyed, U., Tiwari, R. K., Siddiqui, M. H., Pathak, N., & Bajpai, P. (2019). Hesperidin induces ROS-mediated apoptosis along with cell cycle arrest at G2/M phase in human gall bladder carcinoma. Nutrition and cancer, 71(4), 676-687.
73. El-Deek, S. E., Abd-Elghaffar, S. K., Hna, R. S., Mohamed, H. G., & El-Deek, H. E. (2022). Effect of hesperidin against induced colon cancer in rats: impact of Smad4 and activin a signaling pathway. Nutrition and Cancer, 74(2), 697-714.
74. Saiprasad, G., Chitra, P., Manikandan, R., & Sudhandiran, G. (2013). Hesperidin alleviates oxidative stress and downregulates the expressions of proliferative and inflammatory markers in azoxymethane-induced experimental colon carcinogenesis in mice. Inflammation Research, 62, 425-440.
75. Hosseinimehr, S. J., Jalayer, Z., Naghshvar, F., & Mahmoudzadeh, A. (2012). Hesperidin inhibits cyclophosphamide-induced tumor growth delay in mice. Integrative cancer therapies, 11(3), 251-256.
76. Hsu, P. H., Chen, W. H., Juan-Lu, C., Hsieh, S. C., Lin, S. C., Mai, R. T., & Chen, S. Y. (2021). Hesperidin and chlorogenic acid synergistically inhibit the growth of breast cancer cells via estrogen receptor/mitochondrial pathway. Life, 11(9),

77. Khamis, A. A., Ali, E. M., Abd El-Moneim, M. A., Abd-Alhaseeb, M. M., El-Magd, M. A., & Salim, E. I. (2018). Hesperidin, piperine and bee venom synergistically potentiate the anticancer effect of tamoxifen against breast cancer cells. Biomedicine & pharmacotherapy, 105, 1335-1343.
78. Feng, Y., Huo, H., & Tang, Q. (2022). Hesperidin Inhibits the p53-MDMXInteraction-Induced Apoptosis of Non-Small-Cell Lung Cancer and Enhances the Antitumor Effect of Carboplatin. Journal of Oncology,
79. Febriansah, R., Dyaningtyas, D. P., Nurulita, N. A., Meiyanto, E., & Nugroho, A. E. (2014). Hesperidin as a preventive resistance agent in MCF–7 breast cancer cells line resistance to doxorubicin. Asian Pacific journal of tropical biomedicine, 4(3), 228-233
80. Hermawan, A., Meiyanto, E., & Susidarti, R. A. (2010). Hesperidin increase cytotoxic effect of doxorubicin in MCF-7 cells. Indonesian Journal of Pharmacy, 8-17.
81. Korga-Plewko, A., Michalczyk, M., Adamczuk, G., Humeniuk, E., Ostrowska-Lesko, M., Jozefczyk, A., ... & Dudka, J. (2020). Apigenin and hesperidin downregulate DNA repair genes in MCF-7 breast cancer cells and augment doxorubicin toxicity. Molecules, 25(19), 442178
82. Purushothaman, B. K., & KM, M. S. B. (2020). Magnetic casein-CaFe2O4 nanohybrid carrier conjugated with progesterone for enhanced cytotoxicity of citrus peel derived hesperidin drug towards breast and ovarian cancer. International journal of biological macromolecules, 151, 293-304.
83. El-Sisi, A. E., Sokkar, S. S., Ibrahim, H. A., Hamed, M. F., & Abu-Risha, S. E. (2020). Targeting MDR-1 gene expression, BAX/BCL2, caspase-3, and Ki-67 by nanoencapsulated imatinib and hesperidin to enhance anticancer activity and ameliorate cardiotoxicity. Fundamental & Clinical Pharmacology, 34(4), 458-475
84. Purushothaman, B. K., & KM, M. S. B. (2020). Magnetic casein-CaFe2O4 nanohybrid carrier conjugated with progesterone for enhanced cytotoxicity of citrus peel derived hesperidin drug towards breast and ovarian cancer. International journal of biological macromolecules, 151, 293-304.
85. Sulaiman, G. M., Waheeb, H. M., Jabir, M. S., Khazaal, S. H., Dewir, Y. H., & Naidoo, Y. (2020). Hesperidin loaded on gold nanoparticles as a drug delivery system for a successful biocompatible, anti-cancer, anti-inflammatory and phagocytosis inducer model. Scientific reports, 10(1), 9362.
86. Ávila-Gálvez, M. Á., García-Villalba, R., Martínez-Díaz, F., Ocaña-Castillo, B., Monedero-Saiz, T., Torrecillas-Sánchez, A., ... & Espín, J. C. (2019). Metabolic profiling of dietary polyphenols and methylxanthines in normal and malignant mammary tissues from breast cancer patients. Molecular nutrition & food research, 63(9), 1801239.
87. Ávila-Gálvez, M. Á., García-Villalba, R., Martínez-Díaz, F., Ocaña-Castillo, B., Monedero-Saiz, T., Torrecillas-Sánchez, A., ... & Espín, J. C. (2019). Metabolic profiling of dietary polyphenols and methylxanthines in normal and malignant mammary tissues from breast cancer patients. Molecular nutrition & food research, 63(9), 1801239.