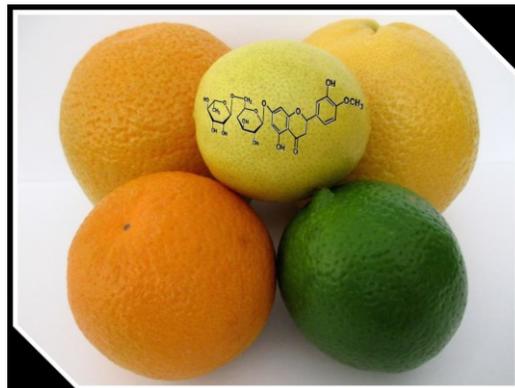


Hesperidin

Hesperidin is a plant flavanone (subclass of flavonoids) predominantly and abundantly found in citrus fruits [USDA]. In nature, most flavonoids are bound to a sugar moiety and are called the glycosides. Hesperidin is also a glycoside composed of the flavanone hesperetin (aglycone) and the disaccharide rutinose (rhamnose linked to glucose).



Hesperidin's name was derived from the word "hesperidium" which refers to fruit produced by citrus trees – lemons, limes, oranges, tangerines, the main source of hesperidin. Its highest concentrations are found in citrus fruit peels (see Fig.2). For instance, peels from tangerines contain hesperidin the equivalent of 5-10 % of their dry mass ¹⁰⁹.

Hesperidin plays a protective role against fungal and other microbial infections in plants ^{39,116}. Apart from its physiological antimicrobial activity, decades of research revealed its many therapeutic applications in prevention and treatment of many human disorders. Most of these benefits are attributed to its antioxidant and anti-inflammatory properties. Interestingly, a French study involving

human volunteers clearly demonstrated that consumption of orange juice or hesperidin alone for 4 weeks may induce changes in the expression of 3422 and 1819 genes, respectively ¹²⁵. This study provided an explanation of the molecular mechanisms behind hesperidin's cardiovascular protective effects. Also, neuroprotective properties of hesperidin have gained attention of scientists during the last decade as this compound demonstrated a wide range of benefits in a variety of neuronal conditions from anxiety

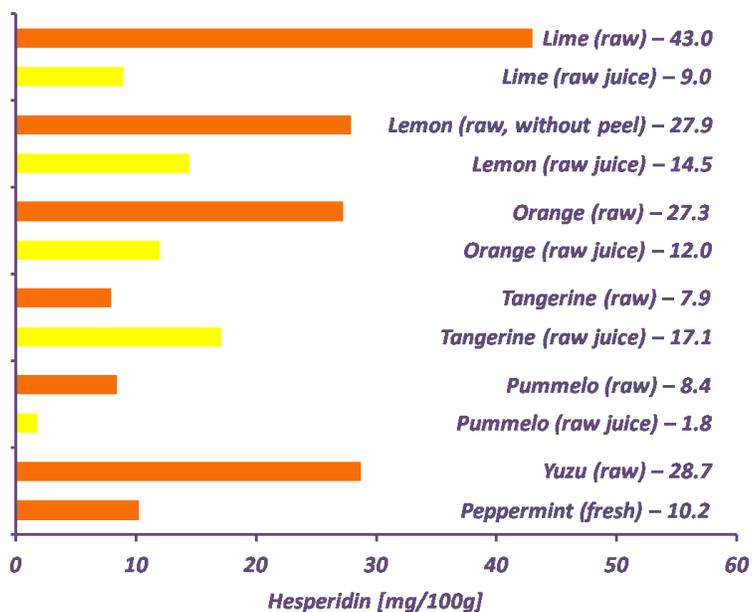


Fig. 1. Hesperidin content in selected foods [179].

and depression to Alzheimer's and Parkinson's diseases ¹⁵³. Additional benefits that come from

hesperidin consumption include radio- and UV-protection, anti-diabetic, anti-allergic, anti-osteoporotic, and anti-cancerous effects.

Due to poor water solubility and the presence of the sugar rutinose moiety the ingested hesperidin must proceed to the colon where gut microorganisms liberate it as the aglycone hesperetin which can be either absorbed or further degraded^{77,192}. This explains the delay in plasma (blood) peak concentration of hesperetin up to 7-7.4 hours after consumption of orange juice. Its modified form called hesperetin-7-glucoside (with glucose moiety instead of rutinose) has good absorption and bioavailability reaching the maximum plasma concentration within 36 minutes of its ingestion. This indicates a shift in the absorption site from colon to small intestine¹³³. Yet, before entering the systemic blood circulation, hesperetin absorbed in the intestine or colon undergoes further metabolic transformation in these organs as well as in the liver. These metabolic changes include mainly glucuronidation (addition of glucuronic acid) and sulfation (addition of sulfate group) since the circulating forms of hesperetin metabolites found in plasma were glucuronides (87%) and sulfoglucuronides (13%)¹¹³. These metabolites were almost completely eliminated from the system within 24 hours after orange juice ingestion via urine excretion accounted for 4.1 - 6.4% of the ingested amount, further pointing to limited bioavailability of hesperidin.

Health benefits



A number of studies conducted on cells, in animals, and human subjects consistently show strong antioxidant activity of hesperidin and its aglycone, hesperetin ¹⁴⁰.

Anti-oxidant effects: The ability to scavenge free radicals is essential and beneficial for human health, since excess of free radicals leads to oxidative stress that adversely affects cell structure and function. Oxidative stress triggers inflammation, which further potentiates oxidative stress in a vicious cycle, triggering various life threatening diseases ranging from cardiovascular and neurodegenerative disorders to diabetes and cancer. Hesperidin not only scavenges free radicals but can also stimulate the endogenous antioxidant defense mechanisms. These mechanisms include enhanced activity and production of cellular antioxidant enzymes such as superoxide dismutase (SOD), heme oxygenase-1 (HO-1), catalase, etc., and elevation of the predominant cellular antioxidant called glutathione ^{48,80,88,99,140,152}. Hence, hesperidin provides valuable support in conditions associated with oxidative stress and provides protection against stress inducing treatments such as chemotherapy and radiation therapy. Animal studies have demonstrated a reduction in

Oxidative stress

Free radicals such as reactive oxygen species (ROS) are generated by the body in various physicochemical reactions. Due to their high reactivity ROS adversely alter lipids, proteins, and DNA triggering a number of diseases. Excessive production and/or inability to eliminate ROS leads to the condition known as oxidative stress

toxic side effects associated with irradiation and chemotherapy (when doxorubicin and cisplatin were used as agents) ^{16,146,156,173} upon treatment with hesperidin. In addition, human peripheral blood samples collected before and after ingestion of a single oral dose of 1000 mg of Daflon (a dietary supplement containing hesperidin), when exposed *in vitro* to gamma rays, demonstrated marked protection against cellular irradiation damage ^{65,66,81}. These findings suggest a possible application of

Dafnon/citrus bioflavonoids as effective protection against planned (radiotherapy) or unplanned (nuclear accidents) radiation exposure.

Inflammation: Often oxidative stress in the body is accompanied by systemic inflammation characteristic of many chronic conditions. Numerous studies indicate that hesperidin and hesperetin are able to reduce various pathologically elevated inflammatory markers ^{3,49,99,140,153}. This inhibitory effect has been predominantly associated with their antioxidant activity and ability to inactivate the pro-inflammatory cascade initiated by free radicals. These compounds were also effective in decreasing the synthesis of pro-inflammatory cytokines e.g. tumor necrosis factor - alpha (TNF- α) as well as pro-inflammatory enzymes such as inducible nitric oxide synthase (iNOS, that yields nitric oxide - NO) and cyclooxygenase-2 (COX-2, involved in the production of inflammatory mediators such as prostaglandins) ^{99,140,190}. Interestingly, unlike classical NSAIDs that block only the activity of cyclooxygenase enzymes, hesperidin was found to suppress the synthesis of the aforementioned inflammatory molecules through the inhibition of a special protein complex called nuclear factor kappa B (NFkB) ¹²⁵. NFkB is a transcription factor that upon activation translocates from cell cytoplasm to the nucleus and triggers pro-inflammatory genes expression (e.g. TNF- α , COX-2, iNOS). Analysis of gene expression after ingestion of hesperidin revealed the increased expression/synthesis of the inhibitor of NFkB that consequently blocked transcriptional activity of NFkB and the expression of all genes regulated by this transcription factor. Therefore, hesperidin appears to be a valuable compound in controlling inflammatory conditions and autoimmune disorders, where inflammation plays a key role.

Rheumatoid arthritis: One of the conditions associated with elevated inflammatory response is rheumatoid arthritis. Though this autoimmune disorder mainly affects joints, other organs such as lung and heart may also be affected which often results in death. The anti-inflammatory properties of hesperidin have been confirmed in animal model of rheumatoid arthritis and also in clinical trials in patients suffering from this disease ^{96,104,105,177}. These study results demonstrated a down-regulation of overactive macrophages (type of white blood cells) and up-regulation of the activity of T lymphocytes in the presence of hesperidin. Moreover, reduction in pro-inflammatory cytokines and oxidative stress and augmentation of anti-inflammatory cytokines and antioxidant enzymes has been observed. In conclusion, it is suggested that hesperidin may be beneficial in rheumatoid arthritis and

should be added to standard anti-rheumatoid therapy since no adverse effects have been seen during concomitant treatment ⁹⁶.

Allergy: Allergy is an overreaction of the immune system to a substance (e.g., pollen, certain food, dust, fur, etc.) that normally causes little or no problem. Hay fever, asthma and food and skin allergies are well known examples of allergy. The symptoms of allergy include a runny nose, itchy rash, shortness of breath and/or swelling. The underlying mechanism of allergy involves immunoglobulin E (IgE) antibodies that bind to the allergen and trigger the release of inflammatory chemicals such as histamine which in turn increases the permeability of capillaries and exacerbates the symptoms. Hesperidin has been shown to inhibit both synthesis and release (degranulation) of histamine from mast cells and basophils (white blood cells) ^{60,94}. Moreover, it can reduce another permeability promoting substance called vascular endothelial growth factor (VEGF) as well as the level of IgE ^{30,72,92,134,187}. The results from a study in the animal model of allergic asthma show that hesperidin is effective in suppressing airway inflammation and sensitivity to bronchoconstrictors (narrowing the airway passages) and also in decreasing infiltration and accumulation of leukocytes in the lungs ^{92,187}. In addition, oral administration of hesperidin is effective in ameliorating allergic rhinitis (hay fever) symptoms in laboratory mice (134). *In vitro* study on human basophils, collected from patients suffering from seasonal allergic rhinitis, also confirmed anti-histamine effects of hesperetin ⁹⁴. Hence, taking into account the above facts hesperidin can be useful in the management of allergy symptoms.

Cardiovascular health: Hesperidin is a well-known cardiovascular protective and strengthening agent. It demonstrates several benefits to the cardiovascular system due to its ability to affect various cellular mechanisms. For instance, due to its anti-oxidant properties hesperidin can prevent LDL oxidation and protect the cell membrane of erythrocytes (red blood cells) from oxidative damage ⁸⁰. It also acts as an inhibitor of two main enzymes in cholesterol metabolism – HMGCoA reductase and ACAT that regulate total (so called “bad” cholesterol”) and “good cholesterol” (HDL) levels. . While HMG-CoA reductase is a regulatory enzyme in cholesterol biosynthesis and a primary target for statin drugs (cholesterol lowering medication), ACAT catalyzes the intracellular esterification of cholesterol and is also engaged in cholesterol absorption, hepatic secretion of very low density lipoprotein (VLDL) and cholesterol accumulation in the vascular wall ¹⁹. Thus, by inhibiting the activity of these two enzymes hesperidin decreases the total "bad" cholesterol (LDL) and increases the "good" cholesterol (HDL)³⁸. A study on rats fed a high cholesterol diet supplemented with flavonoids (hesperidin and naringin)

demonstrated inhibition of liver cholesterol biosynthesis (28.3 %) and the esterification of hepatic cholesterol (23.7 %) by hesperidin¹⁹. In the same study tangerine peel extract was even more potent by decreasing liver cholesterol synthesis by 37 % and its esterification by 32%. These results are in agreement with others, including a human study that demonstrated a marked decrease in triglyceride level after 4 weeks of hesperidin supplementation (G-Hesperidin, 500 mg/day)^{90,126}.

High Blood Pressure: Another health benefit of hesperidin has been associated with its antihypertensive effect. It is believed that hesperidin is responsible for blood pressure lowering effect of orange juice since it promotes nitric oxide production resulting in vasodilation (widening of blood vessels)^{151,193}. Moreover, hesperidin can enhance relaxation of the endothelial cells (cells of the inner blood vessel wall) induced by acetylcholine (a neurotransmitter) and can inhibit secretion of endothelium-derived vasoconstricting factor endothelin-1 (ET-1)^{31,127}. All aforementioned mechanisms aid in blood pressure normalization.

Atherosclerosis: Several *in vitro* studies have shown the inhibitory effect of hesperidin on the expression of cell adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). VCAM-1 and ICAM-1 are the proteins that participate in monocyte (type of leucocytes) recruitment and accumulation in the arterial intima (innermost layer of an artery) and are associated with the development of atherosclerosis^{35,130}.

Blood viscosity: In addition, hesperidin exerts anti-platelet activity. Both *in vitro* and *in vivo* studies have shown the efficacy of hesperidin in suppression of platelet aggregation induced by different stimuli (e.g. collagen, arachidonic acid, thrombin)^{76,198}.

Ischemia/reperfusion injury: Hesperidin can be valuable in protecting cardiac tissue against age, diabetes and ischemia/reperfusion related increase in oxidative stress and damage^{3,47,54,152}. According to animal studies, hesperidin supplementation up-regulated the antioxidant enzymes and decreased lipid peroxidation products and inflammatory markers. Moreover, in case of myocardial infarction after cardiac ischemia/reperfusion injury (ischemia is a restriction in blood supply that leads to myocardial cell death, whereas reperfusion is the restoration of blood flow that enhances myocardial injury and diminishes cardiac contractile function), hesperidin treatment significantly reduced the percentage of infarction, partially by antioxidant and anti-inflammatory activity and also by blocking apoptosis (cell death)³. Researchers discovered that hesperidin notably augmented anti-apoptotic

(Bcl-2) and decreased pro-apoptotic (Bax) protein expression thereby preventing loss of contractile cells.

Nervous system: Several studies have linked the lower intake of some flavonoids including hesperidin to dementia, Parkinson's and Alzheimer's diseases^{55,57}. Hesperidin and hesperetin have also demonstrated the ability to cross the blood-brain barrier making them ideal candidates in the natural treatment of different central nervous system disorders⁴⁰. The neuroprotective effect of hesperidin has been widely studied in the last decade and mainly attributed to its antioxidant and anti-inflammatory properties as seen by an increased level of antioxidant enzymes, decreased level of oxidative stress, inflammatory markers, and pro-apoptotic proteins in neurons.^{28,68,70,123,184}

Parkinson's Disease: In Parkinson's disease, which is a degenerative disease of the central nervous system manifested by a depletion of dopamine producing cells, the oxidative stress, inflammation, and mitochondrial dysfunction have been implied as major contributors to the brain cell loss. The findings from *in vitro* and *in vivo* studies have demonstrated that hesperidin is able to attenuate reduction in levels of cellular antioxidant enzymes, dopamine, and pro-survival proteins all of which would indicate its usefulness in Parkinson's disease treatment^{10,99,167}.

Huntington's Disease: Results from experiments with animal model of Huntington's disease characterized by cognitive, behavioral, and motor abnormalities showed a significant improvement in symptoms (e.g. locomotor activity) upon hesperidin administration¹²³.

Alzheimer's disease: This disease is caused by aggregation of amyloid- β peptides inside neurons and deposition of extracellular amyloid- β plaques, which are believed to interfere with glucose metabolism¹⁹¹. Researchers have found that hesperidin improved impaired energy/glucose metabolism in neuronal cells and thus may prevent the disease progression⁶⁸.

Anxiety: Animal studies suggest that hesperidin can act as a sedative and anti-anxiety agent^{59,84,115,118,185}. Laboratory tests have shown that animals treated with hesperidin showed less anxiety-like behavior¹⁸². Also, the sedative effect of methanolic extract of *Citrus sinensis* flower and *Valeriana officinalis* has been linked to hesperidin. A synergistic interaction between hesperidin and diazepam (medication used for anxiety disorders) has also been observed⁵¹. However, some authors suggest

that the appropriate response to hesperidin treatment could depend on the route of its administration, i.e., oral or intraperitoneal for anxiolytic and sedative effects, respectively¹⁸⁵.

Epilepsy: Epilepsy is another condition that in various laboratory tests was successfully controlled with hesperidin^{97,98}. In mice with chemically induced convulsions hesperidin attenuated the unfavorable biochemical changes in the brain followed by the reduction in seizure score. Besides, co-administration of low dose of hesperidin with drugs such as diazepam or gabapentine potentiated the neuroprotective effects of these medications.

Pain management: Another interesting finding associated with pain perception is that a diet deficient in hesperidin is linked to pain in the extremities¹¹⁰. Besides, it was observed that Intraperitoneal injection of this flavanone causes antinociception (reduced sensitivity to pain), which was partially mediated by μ -opioid receptors.¹¹⁷

Depression: Another study has revealed that hesperidin can increase the level of brain-derived neurotrophic factor (BDNF)⁴² which plays an important role in survival and maintenance of neurons, synaptic integrity, and synaptic plasticity⁴⁵. Evidence suggests that depressed patients have decreased level of BDNF and antidepressants are able to reverse the decrease. Hesperidin has been found to increase the level of BDNF in hippocampus (structure in the brain thought to be the center of memory, emotion and autonomic nervous system), which is in line with antidepressant-like effects of hesperidin injections observed in laboratory animals^{10,42,164}.

Memory: By the very same mechanism of increasing BDNF levels, hesperidin can reduce memory loss and enhance learning since it was discovered that BDNF plays a central role in this process^{6,10,21,97}. In addition to that, in cortical neurons hesperidin was able to activate cellular pro-survival pathway (ERK1/2) that leads to expression of proteins involved in memory formation^{180,189}.

Multiple sclerosis: Multiple sclerosis is a disease resulting from disruption of the myelin sheath surrounding neurons. Formation of a myelin sheath around a nerve allows nerve impulses to move quickly and efficiently along the nerve cell. It has been observed that Chinpi, a sun-dried tangerine/mandarin peel used in Chinese/Japanese traditional medicine. when added to the mice diet promotes myelination^{158,159}. Hesperidin being an active component of Chinpi, this study highlighted the role of hesperidin in this potentially therapeutic effect mediated through cell signaling¹⁵⁸.

Cachexia/Anorexia: Another Japanese medicine called Rikkunshito used for gastrointestinal disorders also contains hesperidin as one of its active ingredients ^{53,121,136,165}. Although it was well known that Rikkunshito stimulates appetite, it was recently determined that the active component responsible is hesperidin, which increases ghrelin secretion. Ghrelin often referred to as hunger hormone stimulates appetite, gastric motility and gastric acid secretion. This finding is especially important for patients suffering from cancer cachexia (loss of weight) or anorexia syndrome when body nourishment relies on well-balanced nutrition intake.

Diabetes: Impaired glucose metabolism and diabetes type 2 often leads to severe complications such as neuropathy and cardiovascular diseases. Animal studies demonstrate that hesperidin can normalize glucose metabolism by influencing enzymes regulating glucose metabolism and reducing lipid levels in the blood and liver ^{8,78,79}. This was confirmed in a human study in 36 female patients with type 2 diabetes ¹⁵⁰. Dietary supplementation with Daflon (hesperidin containing supplement) for 45 days resulted in a significant reduction in blood glucose levels accompanied by a decrease in total and LDL cholesterol, triglycerides, oxidative stress, and inflammation.

Another advantage of hesperidin relates to its potential in decreasing protein glycation ¹⁰². Glycation is a non-enzymatic process of binding sugar molecules to proteins or lipids that alters the structural and functional properties of these molecules leading to the formation of advanced glycation end-products (AGEs). AGEs are harmful to the body and are associated with diabetic complications and age-related degenerative diseases. According to a study hesperidin and hesperetin significantly inhibit the formation of AGEs with the highest inhibitory rate of up to 57.4 % ¹⁰².

Metabolic syndrome which leads to type 2 diabetes has been related to obese adipose tissue. This is because fat tissue produces extensive amounts of inflammatory molecules such as TNF- α . TNF- α stimulates free fatty acid (FFA) secretion through adipocyte (fat cell) lipolysis (i.e., breakdown of lipids; involves hydrolysis of stored triglycerides into glycerol and FFAs), which in turn increases plasma level of FFAs and promotes insulin resistance (a condition in which glucose uptake into cells is impaired due to decreased sensitivity of cells to insulin despite of insulin being secreted by the pancreas) ¹⁹⁷. Hesperidin has been demonstrated to block TNF- α induced FFA-release and also prevent TNF- α from down-regulating two anti-lipolytic genes (perilipin and PDE3B) ¹⁹⁷. Moreover, hesperidin has been noted to stimulate adiponectin expression ^{7,108,138} which is a protein hormone

secreted mainly by adipose tissue²³ and plays an essential role in glucose and lipid metabolism. Levels of adiponectin have been found to be lower in obese than lean individuals, which may be explained by feedback inhibition by other adipocytokines including TNF- α . In addition, a decrease in serum adiponectin has been closely related to insulin resistance and hyperinsulinemia in animals and humans²³. There is also a strong inverse correlation between adiponectin and atherosclerosis. All the above point to the metabolic function of adiponectin and the role of hesperidin in ameliorating the potential pathology associated with its decrease.

Bone health: Recent *in vitro*, *in vivo*, and *ex vivo* research shows that hesperidin may affect bone health^{25,26,64,174,175}. In *in vitro* studies hesperidin was found to stimulate the differentiation of osteoblasts, cells involved in bone formation. In another study, there was a significant increase in bone mineral density (BMD) in young rats that were fed hesperidin⁶⁴. Interestingly, in adult rats the improvement in bone strength was not accompanied by an increase in bone mineralization. In animal model of menopausal osteoporosis (animals after ovariectomy) hesperidin treatment partially inhibited bone loss in adult rats, while in younger rats a complete inhibition was observed⁶⁴. In addition to the protection against bone loss a lipid-lowering effect was also observed in both young and adult ovariectomized rats suggesting that hesperidin supplementation may be beneficial in several ways in post-menopausal women⁶⁴.

Prevalence of osteoporosis in women is about 40 % after 50 years of age, which is much higher than in men of the corresponding age (13%). However, mortality in men related to hip fracture has been reported to be 2-3 fold higher³². The presence of estrogen receptors in both osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) may be the reason behind the age-related bone loss in both genders due to estrogen deficiency¹⁴⁹. In men testosterone undergoes enzymatic conversion to estrogen (17- β -estradiol), which is responsible for bone maturation and homeostasis. . Therefore, a decrease in testosterone (androgen) results in lesser 17- β -estradiol. It is no surprise then that male mice after orchidectomy (removal of testicles) have markedly reduced bone volume, trabecular thickness and bone mineral density²⁶. However, all these adverse effects of orchidectomy are seen to be normalized by hesperidin, indicating the inhibition of bone resorption.

Another study investigated the effects of hesperetin in attenuating osteoblast dysfunction induced by diabetic conditions²⁹. The results showed that hesperetin could ameliorate many negative

consequences of high sugar levels by increasing collagen production and decreasing protein and lipid oxidation in osteoblasts.

Cancer: A number of studies have been conducted to elucidate the anticancer effects of hesperidin and its aglycone hesperetin. Unlike conventional chemotherapeutics these flavonoids can inhibit tumor growth by targeting multiple cellular processes at the same time^{122,152}. Most chemo-preventive properties of hesperidin and hesperetin have been associated with their antioxidant and anti-inflammatory effects^{82,122,168}. Anti-inflammatory properties are especially important because this process plays a critical role at every stage of cancer from its initiation to promotion and progression¹⁶⁸. Various *in vitro* and *in vivo* studies have shown that hesperidin can suppress expression of inflammatory cytokines (e.g., TNF α , IL-1 β , IL-6) and enzymes (e.g., COX2, iNOS)^{82,157,168}. Other *in vitro* studies show that hesperidin exerts anti-proliferative and pro-apoptotic (promoting cell death) effects^{73,82,100,122,141,152}.

As the tumor grows bigger, for a sustained supply of oxygen and other nutrients to all its regions, cancer cells stimulate the formation of new blood vessels (angiogenesis) by secreting pro-angiogenic factors. From the therapeutic perspective it is critical to impede this process since it is essential not only to support tumor growth but also invasion and spread of cancer cells to other tissues (metastasis). It has been shown that hesperetin fed to rats with colon cancer down-regulated expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast factor (bFGF), epidermal growth factor (EGF), and cyclooxygenase-2 (COX-2)¹²⁹.

Many other studies showed a significant reduction in the development of chemically induced cancer in animals treated with hesperidin. For example, frequency of oral (tongue), esophageal (food pipe/gullet) and colon carcinoma was reduced by 75 %, 70 % and 61 %, respectively^{169, 170, 171}. Only 13 % mice fed a diet containing hesperidin developed bladder carcinoma compared to 62 % animals on the same diet without hesperidin¹⁹⁵. It has been also demonstrated that this flavanone was effective against chemically induced lung cancer by protecting against oxidative stress and inhibiting cell proliferation⁸².

Hormone dependent cancers: Occurrence of some cancers including breast and uterine cancers are strongly correlated with estrogen levels¹⁷. However, different estrogens vary in their carcinogenic potential despite having similar hormonal potencies^{18,142}. It is believed that not only proliferation

enhancing properties but also induction of oxidative stress plays a key role in estrogen related carcinogenesis. This is because metabolic breakdown of the most carcinogenic 17- β -estradiol yields free radicals that can damage cellular proteins, fats, and DNA. It is worth noting that isolated estrogens such as 17- α -estradiol or 17- α -ethinylestradiol used in hormone replacement therapy (HRT) do not produce free radicals. However, in the presence of oxidative stress they may also trigger development of cancer¹⁷. This again points to the importance of antioxidant supplementation.

In addition to its antioxidant properties, hesperetin has been identified as a potent inhibitor of an enzyme involved in estrogen synthesis⁷⁵. This enzyme called aromatase (CYP19) is a prime target in therapy of estrogen-responsive breast cancer^{103,196}. Dietary administration of hesperetin to ovariectomized mice transplanted with human breast cancer cells over-expressing aromatase significantly deterred the tumor growth¹⁹⁶. Moreover, unlike synthetic aromatase inhibitors (e.g., letrozole) with associated side effects such as bone deterioration, hesperetin has been shown to inhibit tumor growth without negatively affecting bone health¹⁰³. Hesperetin has thus been suggested to be a potential co-therapeutic agent to aromatase inhibitors.

Hesperidin added to standard chemotherapy was also found to increase sensitivity and decrease resistance of breast cancer cells to doxorubicin⁵⁰. In addition, hesperidin may even ameliorate many adverse histopathological changes and cardiotoxicity caused by doxorubicin resulting in normalization of cardiac biochemical parameters¹.

Skin: Hesperidin can be found in many skin care products mostly in the form of hesperidin methyl chalcone, since its antioxidant and anti-inflammatory properties can provide protection against oxidative stress and UVB radiation, the primary causes of premature skin aging¹¹⁹. A recent study has shown that topical co-application of 2 % hesperidin and glucocorticoids prevented the glucocorticoid-induced impairment of skin barrier permeability¹¹². By strengthening capillaries hesperidin can also improve the appearance of bluish-black circles under the eyes. In addition, skin redness, which may be a symptom of rosacea or skin hypersensitivity, can be calmed by hesperidin. Some *in vitro* studies indicate that hesperidin may reduce skin pigmentation by decreasing the synthesis of melanin (pigment responsible for the color of human skin and hair) by inhibiting tyrosinase, an enzyme involved in melanin synthesis⁶². In contrast, hesperetin applied to the same cell line induced melanin synthesis and tyrosinase activity¹⁷⁸. If these opposite *in vitro* activities of hesperidin and hesperetin

could be reproduced *in vivo* on human skin, both would be useful in the treatment of different skin disorders.

Infections: Hesperidin in citrus fruits acts mainly as an antifungal agent, however, research studies support its efficacy also against viral and bacterial infections^{116,155,194}. One study examined the antibacterial and antifungal activity of ethanolic extract of grapefruit seed and pulp against 20 bacterial and 10 yeast strains³⁴. The strongest antibacterial effect was seen against *Salmonella enteritidis* with minimum inhibitory concentration (MIC) of 2.06% (extract concentration), while MIC for other tested bacteria and yeasts ranged from 4.13% to 16.5%. A recent study with *Aeromonas hydrophila*, a human pathogen that causes intestinal and extra-intestinal infections, demonstrated that hesperidin had bactericidal and immunomodulating effects². Moreover, hesperidin has been found to have protective effects in mice infected with encephalomyocarditis virus (EMCV) and *Staphylococcus aureus* when given before single or combined viral-bacterial infections¹³⁹. Another study showed hesperidin to be effective against human rotavirus, which is the causative agent of diarrhea in infants and young children¹². Also, hesperidin inhibited replication of influenza (flu) virus *in vitro* and decreased the number of infected cells¹⁵⁵.

Both *in vitro* and *in vivo* experiments indicate that hesperidin displays anti parasitic activity against adult worms of *Schistosoma mansoni* that are responsible for the tropical disease schistosomiasis which affects millions of people especially children worldwide⁹. In addition, hesperidin has been proposed as a potential contraceptive microbicide for sexually transmitted diseases⁵⁶ based on its hyaluronidase inhibiting activity (an enzyme that increases tissue permeability). Since hyaluronidase plays a essential role in sperm and microbe penetration into the substrate/target, the ability of hesperidin to inhibit hyaluronidase gave an indication toward its contraceptive effects. (Not required)

Hesperidin was also found to inhibit various sexually transmitted pathogens including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, herpes simplex virus-2 and human immunodeficiency virus (HIV). Moreover hesperidin had no toxic effects on the host cells as well as on the growth of normal vaginal lactobacilli⁵⁶.

Hesperetin (aglycone) displays marked inhibitory activity against *Helicobacter pylori* a bacterium linked to the development of stomach ulcers¹¹. Also replication of several diverse viruses including severe acute respiratory syndrome coronavirus (SARS-CoV), herpes simplex virus type-1, influenza A

virus, parainfluenza virus type-3, respiratory syncytial virus, and poliovirus type-1 have been inhibited by hesperetin in *in vitro* conditions^{37,86,89,106}.

An interesting finding was noticed regarding the influence of hesperidin on human intestinal microbiota⁴⁴. While hesperidin had no impact on the tested bacteria (*Bacteroides galacturonicus*, *Lactobacillus sp.*, *Enterococcus coccae*, *Bifidobacterium catenulatum*, *Ruminococcus gauvreauii*, *Escherichia coli*), its aglycone, hesperetin inhibited growth of almost all of them. This means that hesperetin can modulate the intestinal flora and indirectly interfere with its own bioavailability.

Human studies

The intestinal mucus layer is made up of more than 98 % water. However, since the hesperidin molecule has poorly water solubility, the intestinal absorption of hesperidin is hindered (Fig.3)^{61,192}. Thus in order to improve hesperidin bioavailability and fully exploit its beneficial properties through oral administration, it is important to enhance its water solubility. It has been found that the enzymatic modification of hesperidin by the removal of rhamnose moiety to yield hesperetin-7-glucoside (hesperetin with glucose molecule only; Fig.4) resulted in significant improvement in bioavailability due to increased absorption thereby increasing the total amount of hesperetin in blood¹³³. Maximal plasma concentration of hesperetin was reached within 36 minutes (instead of 7+ hours) and it was 4-fold higher than that from hesperidin ingestion.

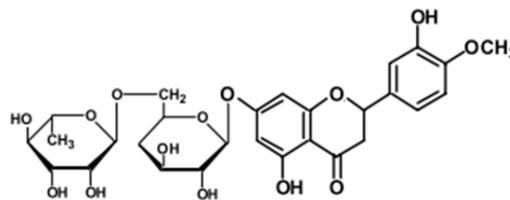


Fig. 3. Hesperidin (Hesperetin-7-Rutinoside)

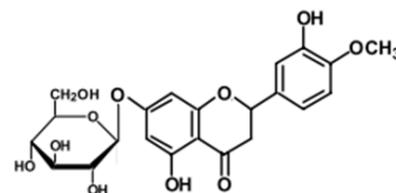


Fig. 4. Hesperetin-7-glucoside

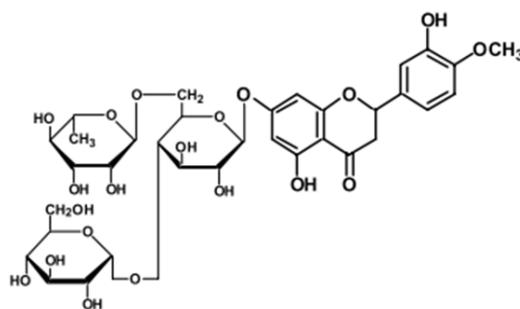


Fig. 5. Glucosyl Hesperidin

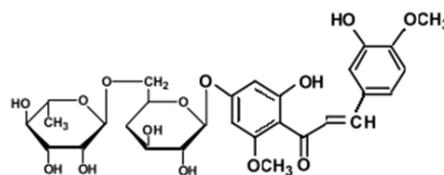


Fig. 6. Hesperidin Methyl Chalcone

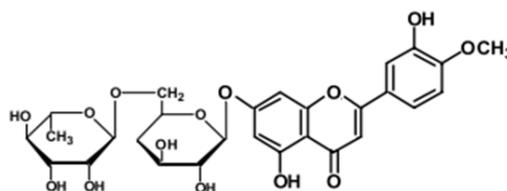


Fig. 7. Diosmin

Glucosyl hesperidin (G-hesperidin, with extra glucose added to hesperidin; Fig.5) is another form with enhanced water solubility (10000 times) and bioavailability¹⁹². In animals fed G-hesperidin, hesperetin appeared more rapidly in plasma and its total amount in the bloodstream was 3.7-fold higher compared to the amount found in rats given hesperidin. Another semi-synthetic form frequently found in medicinal preparations is hesperidin methyl chalcone (Fig. 6) which is characterized by an open ring that contributes to better water solubility, and several methyl group substitutions that contribute to increased metabolic stability as the metabolism is shifted to less efficient enzymes^{22,183}.

Diosmin (Fig.7) is another flavonoid used in combination with hesperidin (Daflon 500 mg), which shares structural similarity to hesperidin but differs in the presence of a double bond between two carbon atoms in diosmin's central ring. Despite its natural occurrence it is often derived from hesperidin. Also, some preparations contain other additional substances such as vitamin C that synergistically enhance the therapeutic effects. Table 1 presents a summary of the human studies involving hesperidin and its derivatives.

Abbreviations used in Table 1. apoA-1-apolipoprotein A-1, BP - blood pressure, CD - control drink, CV- cardiovascular, CVI - chronic venous insufficiency, Daflon - supplement containing 90% diosmin and 10% hesperidin, G-Hsd - glucosyl hesperidin, Hsd - Hesperidin, Hst -hesperetin, OJ - orange juice, ROS - reactive oxygen species, TG - triglyceride, Venarus - supplement containing 90% diosmin and 10% hesperidin.

Table 1.

Objective	Subjects	Form and dose of hesperidin	Primary results
To investigate the effect of OJ and its major flavonoid hesperidin on microvascular reactivity, blood pressure and CV risk biomarkers through both postprandial and chronic intervention studies [127]	24 healthy, overweight men (age 50-65)	Three 4-week periods of: 500 ml of OJ daily; 500 ml CD plus Hsd daily; 500 ml CD plus placebo daily	Diastolic BP and postprandial microvascular reactivity were significantly improved after consumption of OJ or Hsd for 4 weeks.
To investigate the effects of blond OJ on antioxidant markers, CV risk factors and endothelial function [33]	25 men with two CV risk factors i.e. age over 50 years and LDL cholesterol 130-190 mg/L	200 ml of blond OJ or CD 3 times daily for 4 weeks	Marked decrease in ROS, tendency towards reduction of endothelial dysfunction and modest increase in plasma apoA-1 concentration – after blond OJ consumption

To investigate efficacy of Venarus in comprehensive treatment of patients with post-thrombotic syndrome [163]	110 patients with post-thrombotic syndrome	Conservative treatment either with Venarus (51 patients) or without it (59 patients) for 3 months	Adding Venarus to the treatment significantly improved psychological and social activity and quality of life and alleviated symptoms of post-thrombotic syndrome
To determine whether oral pretreatment with Daflon has beneficial effects on cardiac function and outcome after cardiac operations [161]	43 patients with impaired preoperative left ventricular ejection fraction; all scheduled for elective coronary bypass grafting	Before operation patients (n=21) received Daflon (6 tablet daily for 4 days, followed by 2 tablets for 3 days) or placebo (n=22)	Daflon group had significantly lower levels of cardiac markers and improved New York Heart Association status/score
To evaluate the effectiveness of flavonoids diosmin and Hsd in patients with liver cirrhosis with vary degrees of portal hypertension severity [143]	71 men and 54 women at the age of 33 to 74 years with cirrhosis caused by alcohol abuse (82 patients) and viral infection (43 patients)	All patients received diosmin with Hsd for 12 weeks	Patients with alcoholic cirrhosis showed significant improvement in varicose esophageal veins and portal hypertension
To evaluate an association of Ruscus aculeatus, Hsd methyl chalcone and ascorbic acid in CVI [4]	124 patients (109 women, 15 men) with CVI	Patients received 2 capsules per day of <i>Ruscus aculeatus</i> 150 mg/Hsd methylchalcone 150 mg/ascorbic acid 100 mg for 8 weeks	Initial symptoms of 79% pain, 85% heaviness, 74% cramps, 82% edema, decreased to 20%, 12%, 8% and 14% respectively within two weeks and there were no symptoms at the end of treatment.
To evaluate whether G-Hsd is effective in treating arthritis [96]	19 patients with rheumatoid arthritis	All patients received standard therapy. Additionally patients received 3g of G-Hsd (n=9) or placebo (n=10) every morning for 12 weeks	3 of 9 patients in G-Hsd group show significant improvement in accordance with the American College of Rheumatology criteria
To examine the serum TG-lowering effect of G-Hsd [126]	Subjects with high (>150 mg/dL), borderline (110-150 mg/dL) and normal (<110 mg/dL) TG levels	All subjects received 500 mg of G-Hsd daily for 24 weeks	Upon G-Hsd treatment TG levels significantly decreased in subjects with high-TG levels
To examine the efficacy of Daflon in the treatment of acute and chronic symptoms of hemorrhoids [58]	54 men and 66 women suffering from an acute episodes of hemorrhoidal disease	Patients received placebo or 2 tablets of Daflon 500 mg daily for 2 months	In Daflon group only 40% patients had an attack during the trial (vs 70% for placebo) with the mean duration of 2.6 days (vs 4.6 days for placebo) and a mean severity of 1.1 (vs 1.6 for placebo). The overall symptom score decreased from 6.6 to 1.1 in Daflon group and from 6.1 to 4.0 in placebo group
To evaluate the efficacy of	94 menopausal	Women were given a	Symptoms of hot flushes were

formula containing hesperidin, hesperidin methyl chalcone and vitamin C on hot flushes in menopausal women [162]	women with hot flashes	daily formula containing 900 mg hesperidin, 300 mg hesperidin methyl chalcone and 1200 mg vitamin C for 1 month	completely relieved in 53% and lessened in 34% of women
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Synergy

Biochemical reactions in the body are orderly and complex and require the participation of many compounds (substrates, cofactors) to achieve the required biological effects. Cell signaling pathways are themselves comprised of several steps and components that require the presence of various compounds. Several cellular pathways coexist to provide alternate routes and mechanisms to accomplish the same tasks. Thus substances that are required for the same pathway or in alternate pathways can work in synergy when present together. Various compounds also act synergistically by supporting or enhancing the action of other compounds by a variety of mechanisms e.g. by increasing absorption or bioavailability of molecules involved in this process. Thus, synergy allows doses to be lower, non-toxic and still effective.

Hesperidin has been found to work in synergy with both natural compounds (Table 2) and certain drugs (Table 3). It can also protect cells and organs in the body against toxic effects of chemicals such as arsenic and side effects of medications used in clinical practice such as chemotherapeutics (Table 4).

Table 2.

Synergistic effects of hesperidin/hesperetin with selected natural compounds.	
Natural Compounds	Effects
Vitamin C [14,15]	↓ hyaluronidase activity, ↑ capillary resistance
Caffeine [135]	↓ triglycerides, ↓ hepatic lipogenesis, ↓ obesity
Diosmin [144]	↓ capillary permeability
Naringenin and Eriodictyol [114]	↑ antimicrobial activity

Table 3.

Synergistic effects of hesperidin/hesperetin with selected drugs	
Benzodiazepines (diazepam, alprazolam, bromazepam, midazolam, flunitrazepam) [51,98,111]	↑ benzodiazepines effects (↑neuroprotection, ↓ seizures, ↓ anxiety, ↓ pain)
Ketorolac (NSAID) [117]	↓ arthritic gout-type pain
Prednisolone (steroid drug) [52]	↓ inflammation, ↓allergy
Doxorubicin [50]	↓ tumor cells resistance to doxorubicin
Methotrexate [154]	↓ inflammatory and arthritis markers

Table 4.

Protective effects of hesperidin/hesperetin against toxicity of selected compounds and drugs.	
Compounds/Drugs	Effects
Cyclophosphamide (other names: Endoxan, Cytoxan, Neosar, Procytox, Revimmune, Cycloblastin) [5]	↑ chemoprevention, ↓ oxidative stress, ↓ genotoxicity
Sodium arsenite [36]	↓ liver and kidneys toxicity
Carbon tetrachloride [172]	↓ liver and kidneys toxicity

Safety

A multitude of animal and human studies show that hesperidin is safe and well tolerated¹²⁴. Doses of administration in clinical practice vary according to the condition to be treated and form of hesperidin used (see Human studies). One of the most popular hesperidin formulation is Daflon 500 mg (containing 450 mg of diosmin and 50 mg of hesperidin) which is also distributed as Detralex, Arvenum 500, Alvenor, Capiven, Elatec and Venitol. Safety and security of Daflon 500 mg has been examined in animals and large human trials¹²⁴.

The lethal dose at which 50 % of animals died (LD₅₀) for hesperidin was was found to be 3000 mg per kilogram of body weight, which is 180 times the daily therapeutic dose. Doses up to 35 times above those suggested, even if given continuously for 26 weeks, showed absence of any toxic effect. Also, patients who were administered 2 tablets of Daflon 500 mg daily for one year did not experience serious side effects and no changes were seen in their liver and renal function, metabolism, hematological parameters and systolic and diastolic blood pressure. Even treatment of internal

hemorrhoids for the last 8 weeks of pregnancy and 4 weeks after delivery was safe and did not affect pregnancy, fetal development, birth weight, infant growth and feeding ²⁰. Furthermore, studies demonstrating safety include one on 94 menopausal women who had a daily intake of 900 mg of hesperidin with 300 mg of hesperidin methyl chalcone (in addition to 1200 mg of vitamin C) for a period of 1 month ¹⁶² and on patients with rheumatoid arthritis administered 3000 mg of glucosyl hesperidin (G-Hsd) every morning for a three-month treatment ⁹⁶.

Nevertheless, due to the possibility of interfering with drug absorption hesperidin supplementation should be consulted with a physician. In animal studies concomitant administration of hesperidin with calcium channel blockers, β -blockers or statins significantly changed the maximal plasma concentration and the total absorption of these medications suggesting a necessity of adjusting a drug dose and in many cases decreasing it (Table 5)^{27,145,176}.

Table 5.

Influence of hesperidin on bioavailability of selected drugs	
Medications	Effects
Celiprolol (β -blocker; other names: Cardem, Selectol, Celipro, Celol, Cordiax, Dilanorm) [176]	↓ bioavailability of celiprolol (underdosing)
Diltiazem (calcium channel blocker; other names: Cardizem, Cartia XT, Dilacor XR, Dilt-CD, Diltia XT, Diltzac, Matzim LA, Taztia XT, Tiazac) [27]	↑ bioavailability of diltiazem (overdosing)
Verapamil (calcium channel blocker; other names: Isoptin, Verelan, Verelan PM, Calan, Bosoptin, Calaptin, Verogalid ER, Covera-HS) [145]	↑ bioavailability of verapamil (overdosing)
Pravastatin (statin drug; other names: Pravachol, Selektine) [186]	↑ bioavailability of pravastatin (overdosing)

Hesperetin and to an even greater degree diosmetin (aglycone of diosmin, which is present in some hesperidin preparations e.g., Daflon) have been found to inhibit drug metabolizing enzymes such as cytochrome P450 (CYP); specifically CYP2C8 and CYP2C9^{147,148}. Inhibition of these enzymes may affect the metabolism and clearance of drugs that are substrates for these particular CYPs and lead to their accumulation in the body up to the toxic levels (Table 6). It is therefore imperative that patients on

medications get professional advice from qualified health care provider before introducing any additional treatment.

Table 6.

Selected medications/substrates metabolized by CYP2C8 and CYP2C9 [67].	
CYP2C8 substrates	amodiaquine, cerivastatin, paclitaxel, repaglinide, torsemide
CYP2C9 substrates	angiotensin II blockers (losartan, irbesartan); oral hypoglycemic agents (tolbutamide, glipizide); NSAIDs (diclofenac, ibuprofen, S-naproxen, lornoxicam, meloxicam, piroxicam, suprofen); sulfonylureas (glyburide, glibenclamide, glipizide, glimepiride, tolbutamide); amitriptyline, celecoxib, fluoxetine, fluvastatin, glyburide, nateglinide, phenytoin-4-OH2, rosiglitazone, tamoxifen, torsemide, S-warfarin

Bioavailability and absorption

The therapeutic efficacy of orally administered hesperidin depends on its bioavailability which in turn is dependent on its ability to dissolve in water and be absorbed through the intestinal mucosa. While hesperidin on its own is sparingly water soluble, its derivatives hesperidin-7-glucoside and G-hesperidin demonstrate increased water solubility that allows them to pass the intestinal water based mucosal layer and get to the apical side of enterocytes. The entry into the enterocytes may also be facilitated by the glucose moiety allowing the molecules to be transported by sodium-glucose linked transporter 1 (SGLT1)¹⁹².

Another possible mechanism of absorption may be that before entering enterocytes G-Hesperidin is hydrolyzed to hesperidin and glucose by the enzyme α -glucosidase present on the brush border of the small intestine. Then, a portion of the released hesperidin may be further deglycosylated to hesperetin by β -glucosidase found on the brush border or synthesized by intestinal bacteria^{132,192}. Besides, another portion of the released hesperidin is probably transported into the enterocytes without deglycosylation. An *in vitro* study on Caco-2 cell monolayer suggests that G-hesperidin but not hesperidin uses the energy independent paracellular pathway to cross the monolayer⁹¹. However, the *in vivo* study did not detect the intact G-hesperidin in serum, while hesperidin and hesperidin metabolites were found in urine after G-hesperidin administration¹⁹².

The exact mechanism of absorption still needs to be elucidated but from a therapeutic point of view, hesperidin forms with higher water solubility are more useful.

Cellular mechanisms of action

Select hesperidin/hesperetin properties and molecular mechanisms of action

Mechanisms involved in antioxidant protection: Hesperidin has been shown to augment the cellular antioxidant defense mechanism via the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway. Activation of the ERK pathway results in nuclear translocation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and subsequent up-regulation of the hemoxygenase-1 (HO-1) enzyme²⁴. HO-1 catalyzes the breakdown of heme into the antioxidant compound biliverdin, anti-inflammatory agent carbon monoxide, and iron⁷⁴. Nrf2 also induces a group of cytoprotective genes including other antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase as well as phase 2 enzymes¹⁹⁹.

Hesperidin also acts as an anti-inflammatory agent by decreasing the activity of nuclear factor kappa B (NFkB). It was found that hesperidin even increases the expression of the inhibitor of NFkB (NFkBIB) thereby inhibiting the expression of pro-inflammatory genes regulated by NFkB¹²⁵.

Atherosclerosis-related mechanisms: Analysis of gene expression changes in human leukocytes after hesperidin (or orange juice) consumption revealed several beneficial changes promoting cardiovascular health¹²⁵. For instance, hesperidin down-regulated the expression of chemokines and their receptors (e.g. CCL26, CXCL17, CX3CR1) that regulate leukocyte activation and trafficking and their recruitment to atherosclerotic arteries. Other NFkB regulated chemokine genes known to be highly expressed in human atheromas through all stages of plaque development were also down-regulated since hesperidin up-regulated the expression of the inhibitor of NFkB. Moreover, hesperidin decreased expression of the CD80 molecule (upregulated in cardiovascular disease⁴³) by inducing expression of the transcriptional repressor BLC6 that prevents the expression of CD80 by binding to its promoter region¹²⁵. Studies also show that hesperidin causes down-regulation of genes encoding integrins (e.g., ITGBL1) and gap junction adhesion molecules such as connexins¹²⁵. Apart from its effects on the recruitment and infiltration of circulating cells to the vascular wall, hesperidin significantly down-regulated LDL macrophage receptor, responsible for LDL binding and internalization. In addition it up-regulated the ABCA2 membrane transporters involved in reverse

cholesterol transport from macrophages indicating a protective effect against foam cell formation, the hallmark of atherosclerosis.

Cardioprotective mechanisms: Studies have demonstrated the involvement of the PPAR γ (peroxisome proliferator-activated receptor gamma) receptor in the protective effect of hesperidin on the heart tissue³. PPAR γ is a ligand-dependent nuclear receptor that regulates glucose, lipid, and energy homeostasis and also cellular proliferation and differentiation. Hesperidin via PPAR γ activation significantly decreases lipid peroxidation, cardiac injury marker enzymes (CK-MB, LDH) and inflammation factor TNF α . In contrast, the level of cellular antioxidants (SOD, catalase, GSH) increased in the hesperidin treated group. Moreover, via PPAR γ signaling hesperidin augmented Bcl-2 (antiapoptotic) protein and down-regulated Bax (proapoptotic) protein expression. Using all the above mechanisms hesperidin causes a marked decrease in myocardial injury (infarct size)³.

Mechanisms involved in bone health: Hesperidin exerts its inhibitory effects on bone resorption possibly due to its antioxidant activity, activation of estrogen receptors (ERs), and inhibition of HMG-CoA reductase²⁵. It has been shown that superoxide radicals degrade osteocalcin (protein found in the bone) into numerous peptide fragments leading to bone matrix degradation⁸⁷. In addition, increased oxidative stress results in osteoblast (bone forming cell) dysfunction²⁹. The antioxidant activity of hesperidin may therefore inhibit osteocalcin degradation and osteoblast dysfunction. Administration of hesperetin has also been seen to cause a significant elevation of alkaline phosphatase (ALP) activity, collagen content and a decrease in oxidative stress products (MDA, AOPP)²⁹.

The activation of estrogen receptors by hesperetin is considered to contribute to prevention of bone loss^{71,107,120,174,175}. Hesperetin treatment significantly enhanced mRNA expression of the enzyme alkaline phosphatase and transcription factors Runx2 and Osterix that are essential for osteoblast differentiation and bone formation. Additionally, hesperetin inhibits gene expression of receptor activator of nuclear factor kB ligand (RANKL). RANKL activates the osteoclast's surface-bound RANK⁶³ which by binding to TNF receptor associated factor 6 (TRAF6) activates c-jun N-terminal kinase (JNK) and NFkB pathways. The latter in turn trigger differentiation and activation of osteoclasts. Hesperetin thus inhibits osteoclast activation.

Finally, the third mechanism that contributes to inhibition of osteoporosis is caused by inhibition of HMG-CoA reductase^{25,64} an enzyme involved in cholesterol synthesis. It has been found that the

breakdown product of cholesterol called 27-hydroxycholesterol (27HC) can bind to and modulate the transcriptional activity of the estrogen receptors (ERs), preventing the positive actions of estrogens on bone¹³¹. The 27HC additionally activates liver X receptors (LXRs) in osteoblasts thereby decreasing osteoblast proliferation, differentiation, and activity, and increased the production of osteoclastogenic factors such as RANKL and TNF α . Also, 27 HC due to its action on ER α reduced the expression of LXR inhibitor called small heterodimeric partner (SHP) in osteoblasts allowing for unlimited LXR activity. Thus, by blocking cholesterol synthesis hesperetin decreases the total amount of 27 HC and its dual actions on ER and LXR that results in uncoupled osteoblastogenesis and osteoclastogenesis leading to enhanced bone density.

Drug metabolism: It is worth noting that hesperidin/hesperetin may interfere with absorption and clearance of many drugs. This is because of its ability to change the expression and/or activity of proteins involved in drug absorption/excretion and metabolism.

In a cancer study on MCF-7 cells (breast cancer cells) hesperidin has been found to decrease the expression of multi-drug resistance protein 1 (MDR1)⁵⁰. Since MDR1 is engaged in efflux of various drugs from cells, in this case doxorubicin, its down-regulation increased MCF-7 cell sensitivity to doxorubicin and cytotoxicity with hesperidin resulting in an IC50 value of only 11 μ M.

Another study examining the effect of parallel administration of hesperidin and pravastatin showed a decrease in the expression of multidrug resistance-associated protein 2 (MRP2) in intestine and liver, caused by hesperidin¹⁸⁶. MRP2 is also involved in the excretion of various xenobiotics thus its down-regulation significantly increased the peak plasma concentration and the total amount of pravastatin in bloodstream.

Organic acid transporting polypeptide 2B1 (OATP2B1) is a ubiquitously expressed (intestine, liver, heart, brain, lung) influx/uptake transporter with broad substrate specificity for drugs such as statins, rifampicin, bosentan, glyburide or fexofenadine. In a study that aimed to explore the active components in orange juice, hesperidin was found to be the major inhibitor of OATP2B1 with an IC50 of 1.92 μ M¹⁶⁰.

Also specific cytochrome P450 enzymes (CYPs) such as CYP2C8 and CYP2C9 have been shown to be moderately inhibited by hesperetin with IC50 values of 68.5 μ M and 21.5 μ M, respectively^{147,148}.

However, owing to the presence of diosmin in some hesperidin preparations (e.g. Daflon) it is worth mentioning that although hesperetin is a moderate inhibitor of enzyme CYP2C8, diosmetin (aglycone of diosmin) is 16 times stronger with $IC_{50}=4.25\mu M^{147}$. With regard to CYP2C9, the inhibitory potency of diosmetin is even higher, i.e., $IC_{50}=1.71\mu M^{148}$.

Hesperetin has also been shown to be an inhibitor of sulfotransferases (SULTs). SULTs are phase II drug-metabolizing enzymes that catalyze the sulfation of many hormones, neurotransmitters, drugs, and xenobiotic compounds⁶⁹. Hesperetin inhibited the recombinants of human SULTs (hSULT1A1, hSULT1A3, hSULT1E1, hSULT2A1) with varying potency. However, the most potent inhibition was seen against sulfation of estradiol by SULTs (IC_{50} of 3.6 μM).

In a study investigating the inhibitory effects of flavonoids on phosphodiesterase (PDE) isozymes found hesperetin to be selective and a moderate ($IC_{50}=28.2\ \mu M$) inhibitor of phosphodiesterase 4 (PDE4) isozymes⁹³. PDE4 inhibitors block the degradative action of PDE4 on cyclic adenosine monophosphate (cAMP), which may be useful in treatment of inflammatory and neuronal disorders including alcohol dependence^{46,85,188}. In the same study, diosmetin selectively and potently ($IC_{50}=4.8\ \mu M$) inhibited PDE2 isozymes.

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