GENERAL REVIEW

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Evaluating the bioactive effects of flavonoid hesperidin – A new literature data survey

Ocena bioaktivnih efekata flavonoida hesperidina – pregled podataka iz novije literature

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Introduction

A growing number of epidemiologic studies consistently shows a protective effect of polyphenol-rich foods (fruit, tea, wine, cocoa or chocolate…) against many diseases [1–4]. This evidence is supported by the results of numerous studies conducted in animal models, with nutritionally realistic levels of isolated flavonoids [5, 6], and in humans with flavonoid-rich foods [1–4]. The most famous so-called French paradox, the lack of a positive correlation between a high intake of saturated fat and the occurrence of coronary heart disease is related at least partly to the consumption of red wine [7], which is rich in flavonoids. Due to the variety of pharmacological activities in the human body, flavonoids are referred as "nutraceuticals" [8].

In spite of such extensive number of articles about health benefits of flavonoids, further researches in this field are broadly conducted. In this very moment, on PubMed more than 58,000 references about flavonoids can be found. Since flavonoids are the subject of our longtime research [9], our aim was to give a personal account on the development of this field through a retrospective of some basic data about flavonoids, their pharmacological properties and mechanism of action. In this review, special attention was paid to one of the most promising bioactive bioflavonoids, hesperidin (Hesp).

About flavonoids

Flavonoids (*flavus*, the Latin word for yellow) or bioflavonoids, polyphenolic low weight secondary metabolites, are present in great number of higher plant species, principally placed in fruit bark, seeds or flowers. For the time being, more than 8000 different flavonoids have been identified, and that number constantly increases [10]. Starting from 1936, with the first article about flavonoids bioactivity [11], numerous literature data were published about their structure and characteristics primarily connected to antioxidative activity.

Chemically, flavonoid molecules consist of carbon atoms assembled in two aromatic rings that are connected by a three-carbon "bridge", C_6-C_3-C_6, thus forming a diphenylpropane structure with the central unit being a benzo-γ-pyrone (chromone). Multiple hydroxyl groups, sugar, oxygen, or methyl groups are attached to this core structure. A group of flavonoids is differentiated in several classes according to the degrees of oxidation and unsaturation of the heterocyclic C ring. The major flavonoid classes are: catechins, dihydroxychalcones, chalcons, flavanones, flavanols, flavones, isoflavones, anthocyanidols, aurones and flavonols. Depending on the oxidation state of the heterocyclic ring, flavonoids (in constricted sense) are classified as flavones, flavanonols, flavonols and flavanones [12].

A considerable number of plant medicines contain flavonoids, which have been reported by many authors as having antibacterial [13], anti-inflammatory [14], antiallergic [15], antimutagenic [16], antiviral [17], antineoplastic [18, 19], antithrombotic [20, 21], and coronary heart disease actions [22–25]. Overwhelmingly, pharmacological effects are related to antioxidant activity of flavonoids, arising through their ability to scavenge free radicals. When generated in excess, free radi-

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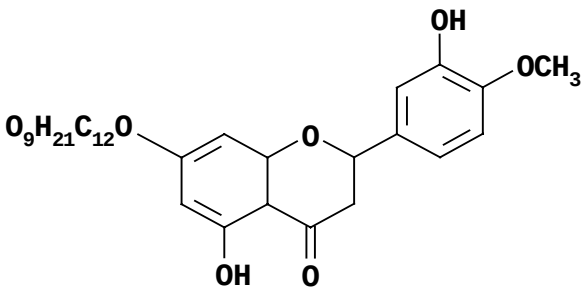
cals can damage biomolecules, and are therefore implicated in the etiology of several diseases and ageing [26]. Radical scavenging by flavonoids occurs *via* electron donation from the free hydroxyls on the flavonoid nucleus, with the formation of a less reactive flavonoid aroxyl radical stabilized by resonance and therefore playing a moderate role in the propagation of the radical-induced damages in biological systems. Antioxidant activity of flavonoids correlates well with their physiological function *in vivo*, because oxidative stress is known to participate in the initial process of different patho-physiological events. Literature data report that flavonoids can prevent injury caused by free radicals by the following mechanisms: direct scavenging of reactive oxygen species (ROS), activation of antioxidant enzymes, metal chelating activity, reduction of α-tocopheryl radicals, inhibition of oxidases, mitigation of oxidative stress caused by nitric oxide, increase in uric acid levels, increase in antioxidant properties of low molecular antioxidants (27 and references therein).

Besides direct free radical scavenging, flavonoids exert antioxidant activity through interactions with the reduced form of transition metals, primarily Cu(I) and Fe(II), as well as Fe(III), which participate in free radical generating reactions [28]. Flavonoids may sequester metal ions by chelation, thereby preventing the metal-mediated generation of free radicals, and accordingly may protect the potential biological targets from oxidative stress. Thus, the overall antioxidant action of flavonoids appears to be a combination of a direct reaction with free radical and chelating metal ions responsible for the production of ROS [29–38]. We also investigated more then 40 metal-flavonoid chelates, as summarized in our review article [9].

Hesperidin

Chemical structure of hesperidin

Hesp, one of the most important flavonoids, is a low molecular weight molecule (molecular weight 610.57 Da), with the bruto formula $C_{28}H_{34}O_{15}$, and belongs to the flavanone class of flavonoids. Chemically, Hesp consists of aglycone (the forms lacking sugar moieties), hesperitin, and sugar rutinoside: hesperetin-7-rutinoside, or IUPAC name: (2*S*)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one. Hesp is represented by the following chemical structure:



Sources of hesperidin

Hesp is the predominant flavonoid in citrus fruits, primarily in sweet orange (in young immature oranges it accounts for up to 14% of the fresh weight of the fruit [39]) and lemon, and consequently in juices made of these citruses. The peel and membranous parts of these fruits have the highest Hesp concentrations, thus hand-squeezed juices contain no detectable traces of Hesp [40]. Commercial juices, on the other hand, are rich in Hesp because the industrial processing of fruits leads to juices being contaminated with the peel constituents. According to the Code of Practice for evaluation of quality and authenticity of fruit and vegetable juices, published by the AIJN – European Fruit Juice Association, special requirements for quality of citrus based juices (oranges, lemon and grapefruit) are that defined the Hesp content be as 250–700 mg/L [41].

Hesperidin pharmacokinetics

Although citrus fruits and juices are widely consumed in the world, little information has been published on flavanone bioavailability in humans. Hesp itself is absorbed from the intestine intact as a glycoside. Its aglycone hesperitin appears in plasma 3 h after ingestion, reaching a peak between 5 and 7 h. The circulating forms of hesperetin are glucuronides (87%) and sulphoglucuronides (13%). For Hesp, urinary excretion is nearly complete 24 h after the orange juice ingestion and does not depend on the dose [42].

Hesperidin bioactivity

Although hesperidin has no usual structural elements to suggest it as a good free radicals capturer and chelator, the ability for chelating metal ions was confirmed in our experiments [43–46]. Several other investigators have examined Hesp antioxidant activity and radical scavenging properties using a variety of assay systems [47, 48]. Jovanovic et al. [49] reports that Hesp reduces superoxide ions in electron transfer plus concerted proton transfer reaction *in vitro*. Further, Hesp was found to be effective in protecting liposomes from UV-irradiation induced peroxidation, probably by scavenging the oxygen free radicals generated by UV-irradiation [50]. Numerous studies confirmed a potent bioactivity of Hesp, such as effects on the vascular system (reduces capillary permeability) [51], anti-inflammatory effects, antioxidant effect, action on enzymes, antimicrobial activity (antibacterial, antifungal, antiviral, ...), anticarcinogenic activity [52], cell aggregation inhibition, antiallergic effects, UV protecting activity, radioprotection, and so on. In the further text, more specific information about Hesp effects on the cardiovascular system are given. Also, the literature describing Hesp as promising protector against ionizing radiation is assembled.

Hesperidin in prevention of (cardio)vascular diseases

In the review article by Garg et al. [53] the pharmacological properties and medicinal uses of Hesp from the available references were widely described. We emphasize some of most essential Hesp activity.

It has been presumed that Hesp could increase capillary resistance thanks to its ability to inhibit hyaluronidase activity. Also, it has been confirmed that Hesp inhibits inflammatory processes in the ischaemia-induced hyperpermeability, characteristic for venous stasis. Hesp capillary antihaemorrhagic activity has also been reported. Thus, Hesp supplementation has been recommended for a wide range of blood vessel disorders, such as fragility and permeability.

Investigations performed on rats showed improved antihypercholesterolaemic activity of Hesp, exhibited as decreasing cholesterol, LDL, total lipids and triglyceride levels, and increased HDL levels. Results of some studies indicate the calcium channel blocker activity of Hesp.

Some studies have demonstrated antihypertensive and diuretic effects of Hesp in rats following oral administration of the drug at the dose of 200 mg/kg body weight, concluding that this hypotensive effect is caused by increased diuresis. On the other hand, it is well established that several flavanones, including Hesp, exhibit influence on enzymes such as protein kinase, lipooxygenase and cyclooxygenase. The antihypertensive activity of Hesp might be due to influences on blood fluidity *via* enzymes. Further, various flavonoids are inhibitors of cyclic-AMP phosphodiesterase, so this activity could be connected to their diuretic effect.

References dating from 2000 to the present time confirm citrus fruit benefits on the vascular system. Thus, the consumption of citrus fruit has been associated with a lower risk of acute coronary events and stroke [54, 55]. From clinical data, citrus juice consumption reduces oxidative DNA damage in blood cells [56] and improves plasma concentrations of inflammation markers and oxidative stress [57–59]. In addition, the consumption of citrus juices improves lipemia in men with previous coronary bypass surgery [60]. Furthermore, in hypertensive subjects, the consumption of flavanone-rich grapefruit juice exerts a significant beneficial effect on blood pressure [61].

The results of a very recent study published in Am J Clin Nutr [62] are in agreement with numerous literature data. In this study, the authors investigated the effect of orange juice and its major flavonoid, Hesp, on microvascular reactivity, blood pressure, and cardiovascular risk biomarkers through both postprandial and chronic intervention studies. During the three 4-week periods, 24 healthy, overweight men (age 50–65) daily consumed 500 mL orange juice, 500 mL control drink plus Hesp (CDH), or 500 mL control drink plus placebo (CDP). All measurements and blood collections were performed in overnight-fasted subjects before and after the 4-week treatment periods. The postprandial study was conducted at the beginning of each experimental period. Diastolic blood pressure was significantly lower after a 4-week consumption of orange juice or CDH than after consumption of CDP, whereas microvascular endothelium-related reactivity was not significantly affected when measured after an overnight fast. However, both orange juice and CDH ingestion significantly improved postprandial microvascular endothelial reactivity compared with CDP when measured at the peak of plasma hesperetin concentration. As a conclusion of this study, Hesp could be linked to the beneficial effect of orange juice.

Although the consumption of Hesp juice rich food has been reported to exert beneficial effects on some intermediate risk factors for CVD, such as LDL cholesterol, blood pressure, and endothelial function [63], only a few clinical trials have dealt with the oral administration of chemically pure Hesp. Thus, the following study with 204 healthy participants evaluated the LDL-C–lowering efficacy of pure Hesp in moderately hypercholesterolemic individuals (serum total cholesterol (TC) concentration of 5.0–8.0 mmol/L) [64]. The study started with a 4 week preintervention period during which the participants had to abstain from consuming citrus fruits and their juices, food supplements containing hesperidin and/or naringin as well as plant sterol/stanol-enriched foods, and other food products or supplements claiming to lower cholesterol. During the 4-week intervention, the participants applied the same dietary restrictions as during the preintervention phase and consumed 4 capsules/d, providing either placebo (cellulose) or the daily dose of 800 mg Hesp or 500 mg naringin. Blood samples to measure serum lipids were taken on 2 consecutive days at the beginning and the end of the intervention phase. The intake of 800 mg/d Hesp administered twice daily with main meals did not lower serum TC and LDL-C in moderately hypercholesterolemic individuals. This outcome suggests that capsule format Hesp exerts no cholesterol-lowering effect in humans.

Hesperidin as radioprotector

During the last several years, some researches were conducted to utilise Hesp as a promising protector against ionizing radiation (IR), since IR, as the part of our environment and in medical treatment, has been established as a strong carcinogen.

IR causes cellular damage which is predominantly mediated through free radicals and resultant ROS [65]. The interaction of IR with water, a major cellular constituent, results in the generation of primary water radical species due to radiolysis of water. These species react with molecules like oxygen-producing secondary radicals (H_2O_2 and O^{2-}), which are highly reactive and could diffuse to vital cellular targets like DNA, proteins, lipids and membrane, ultimately leading to cancer and cell death [66].

Since free radicals play a major role in the initiation and progression of IR induced toxicity, the use of antioxidants either in the diet or as therapeutic agents might offer protection against radiation induced damage. A number of medicinal plants evaluated for their radioprotective efficacy have shown protective effects against the damaging effects of IR. The article of Nambai et al. [67] summeries the effects of various phytochemicals on IR.

The development of effective radioprotectors is of great importance in view of their potential application during both planned radiation exposure (radiotherapy) and unplanned radiation exposure (nuclear accidents, natural background radiation emanating from the earth or other sources). Amifostine, a thiol synthetic compound, is a powerful radioprotective agent (marketed by MedImmune under the trade name Ethyol®). However, due to its side effects and toxicity, the use of this drug should be replaced with less toxic natural compounds [68].

Since much research has been focused on the potential use of flavonoids as free radical scavengers to prevent oxidative damage [69], consequently several researches were conducted to explore Hesp as radioprotector. The experiments were performed either in animals (mouse and rats) or *in vitro* (on human blood), since the study of radioprotective effects is limited due to non-permission to irradiate healthy humans for experimental research. Experiments in animals were performed using different graded doses (mg/kg body weight) of Hesp administered orally *via* intragastric intubations for several days prior to whole body radiation exposure with different doses (1–10 Gy).

Hosseinimehr and Nemati [70] investigated the radioprocective effects of Hesp in mouse bone marrow cells by using the micronucleus test for anticlastogenic and cell proliferation activity. They showed that Hesp has powerful protective effects against DNA damage and on the decline in cell proliferation induced by γ-irradiation in mice by reducing the frequency of the micronuclei.

Pradeep et al. [71] explored the hepatoprotective and antioxidant effects of Hesp against γ-irradiation induced oxidative damage in the liver of male Sprague–Dawley rats. Exposure to γ-irradiation resulted in hepatocellular damage in a dose-dependent manner, featuring a significantly decreased body weight and liver weight and higher levels of serum AST, ALT, ALP, LDH and γ-GT levels and a simultaneous decrease in their levels in the liver tissue. The γ-irradiation induced toxic effects were dramatically and dose-dependently inhibited by Hesp treatment as observed by the restoration in the altered levels of the marker enzymes, lipid peroxidation, enzymatic and non-enzymatic antioxidants.

Kalpana et al. [72] investigated the radioprotective efficacy of Hesp against x-ray radiation induced cellular damage in the liver of Swiss albino mice. The results indicated that radiation-induced decrease in the levels of endogenous antioxidant enzymes and increase in lipid peroxidative index, DNA damage and comet parameters were altered by preadministration with the effective dose of Hesp which restored the antioxidant status to near normal and decreased the levels of lipid peroxidative index, DNA damage and comet parameters, which were confirmed by histopathological examinations.

Besides these studies which have been performed to evaluate natural products for their radioprotective effects in animals, there are a few studies assessing the radioprotective effects of natural origin compounds in human volunteers for reducing the genetic side effects caused by IR. The radioprotective effect of Hesp against genotoxicity induced by γ-irradiation has been investigated *in vivo*/*in vitro* in cultured blood lymphocytes from human volunteers [73, 74]. Peripheral human blood samples were collected predose and after a single oral ingestion of 1,000 mg of Daflon® (a dietary supplement containing 50 mg of Hesp in 500 mg-tablet) corresponding 100 mg of Hesp, then exposed *in vitro* to γ-rays. A significant increase (40%) in the incidence of micronuclei after exposure to γ-irradiation as compared to control unexposed samples was observed. After Daflon® administration, a significant decrease in the incidence of micronuclei was observed in comparison with similarly irradiated lymphocytes collected before administration. These findings suggest a possible application of Daflon® for the protection of human lymphocytes from the genetic damage and side effects induced by γ-irradiation. Similar research was conducted by Kalpana et al. [75] also showing that Hesp offers protection to cultured human peripheral blood lymphocytes against radiation induced cellular damage.

Dosage and administration

In numerous widely spread dietary supplements, Hesp is present as Citrus complex of bioflavonoids, usually in a combination with Vitamin C. In those kinds of products, the content of total bioflavonoids is usually declared by the producer, without the concentration of Hesp alone. For example, in the preparation Vitamin C with citrus bioflavonoids & Rose Hips®, the total citrus flavonoid content, according to the factory declaration, was 751 mg *per* two capsules without declaration for Hesp alone, but we found that each capsule contains 260 mg of Hesp [76]. In combination with a flavone called diosmin, the tablets (trade name Daflon®) for treatment of chronic venous insufficiency and hemorrhoids are broadly available on the European market. A 500-mg dose of this combination product is comprised of 50 mg of Hesp and 450 mg of diosmin. The doses for this type of supplement are usually 500 mg to 2 g daily.

Conclusion

The beneficial effects of flavonoids on human health are universally accepted nowadays. Citrus fruits and juices with Hesp as major flavonoid remain one of the most readily-available dietary sources for their intake. In dietary supplements Hesp is usually present in a combination with vitamin C. Epidemiological studies have shown an inverse association between risk of cardiovascular diseases and intake level of Hesp. Also, some experiments utilise Hesp as a promising protector against IR. Further clinical trials are needed to assess a more precise correlation between the Hesp consumption and human health benefits.

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R E F E R E N C E S

1. *Pękal A,, Dróżdż P, Biesaga M, Pyrzynska K.* Screening of the antioxidant properties and polyphenol composition of aromatised green tea infusions. J Sci Food Agric 2012; 92(11): 2244−9.

2. *Catalgol B, Batirel S, Taga Y, Ozer NK.* Resveratrol: French Paradox Revisited. Front Pharmacol 2012; 3: 141.
3. *Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA*, et al. Flovonoid intake and cardiovascular disease wortality: a prospective study in postmenopausal women. Am J Clin Nutr 2007; 85(3): 895–909.
4. *Buijsse B, Weikert C, Drogan D, Bergmann M, Boeing H.* Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. Eur Heart J 2010; 31(13): 1616–123.
5. *Auclair S, Milenkovic D, Besson C, Chauvet* S, *Gueux* E, *Morand* C, et al. Catechin reduces atherosclerotic lesion development in apo E-deficient mice: a transcriptomic study. Atherosclerosis 2009; 204(2): e21–7.
6. *Norata GD, Marchesi P, Passamonti S, Pirillo A, Violi F, Catapano AL.* Anti-inflammatory and anti-atherogenic effects of cathechin, caffeic acid and trans-resveratrol in apolipoprotein E deficient mice. Atherosclerosis 2007; 191(2): 265–71.
7. *Lippi G, Franchini M, Favaloro EJ, Targher G.* Moderate red wine consumption and cardiovascular disease risk: beyond the "French paradox". Semin Thromb Hemost 2010; 36(1): 59−70.
8. *Tapas AR, Sakarkar DM, Kakde RB.* Flavonoids as Nutraceuticals: A Review. Trop J Pharm Res 2008; 7(3): 1089−99.
9. *Mešev D, Kuntić V.* Investigation of metal–flavonoid chelates and the determination of flavonoids *via* metal–flavonoid complexing reactions. J Serb Chem Soc 2007; 72(10): 921–39.
10. *Andersen OM, Markham KR.* Flavonoids: Chemistry, Biochemistry and Applications. Boca Raton, FL: CRC/Taylor & Francis; 2006.
11. *Bentsath A, Rusznyak S, Szent-Györgyi A.* Vitamin nature of flavones. Nature 1936; 138: 798.
12. *Middleton EJ, Kandaswami C.* The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer. In: *Harborne JB,* editor. The flavonoids: Advances in research since 1986. London: Chapman and Hall; 2005. pp. 619–52.
13. *Cushnie TP, Lamb AJ.* Antimicrobial activity of flavonoids. Int J Antimicrob Agent 2005; 26(5): 343−56.
14. *Dalgård C, Nielsen F, Morrow JD, Enghusen-Poulsen H, Jonung T, Hørder M,* et al. Supplementation with orange and blackcurrant juice, but not vitamin E, improves inflammatory markers in patients with peripheral arterial disease. Br J Nutr 2009; 101(2): 263–9.
15. *Middleton E, Kandaswami C.* Effects of flavonoids on immune and inflammatory cell functions. Biochem Pharmacol 1992; 43(6): 1167–1179.
16. *Tanaka T, Makita H, Kawabata K, Mori H.* Chemoprevention of azoxymethane-induced rat colon carcinogenesis by the naturally occurring flavonoids, diosmin and hesperidin. Carcinogenesis 1997; 18(5): 957−65.
17. *Lee JH, Kim YS, Lee CK, Lee HK, Han SS.* Antiviral activity of some flavonoids on Herpes-simplex virus. Korean Pharmacog 1999; 30: 34−9.
18. *Neuhouser ML.* Dietary flavonoids and cancer risk: evidence from human population studies. Nutr Cancer 2004; 50(1): 1−7.
19. *Ren W, Qiao Z, Wang H, Zhu L, Zhang L.* Flavonoids: promising anticancer agents. Med Res Rev 2003; 23(4): 519−34.
20. *Bouskela E, Donyo KA.* Effects of oral administration of purified micronized flavonoid fraction on increased microvascular permeability induced by various agents and on ischemia/reperfusion in diabetic hamsters. Int J Microcirc Clin Exp 1995; 15(6): 293−300.
21. *McGregor L, Bellangeon M, Chignier E, Lerond L, Rousselle C, McGregor JL.* Effect of a micronized purified flavonoid fraction on in vivo platelet functions in the rat. Thromb Res 1999; 94(4): 235−40.
22. *Huxley RR, Neil HA.* The relation between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. Eur J Clin Nutr 2003; 57(8): 904−8.
23. *Hertog MG, Feskens EJ, Kromhout D.* Antioxidant flavonols and coronary heart disease risk. Lancet 1997; 349(9053): 699.
24. *Grassi D, Desideri G, Croce G, Tiberti S, Aggio A, Ferri C.* Flavonoids, vascular function and cardiovascular protection. Curr Pharm Des 2009; 15(10): 1072–84.
25. *Manach C, Regerat F, Texier O, Agullo G, Demigne C, Remesy C,* et al. Bioavailability, metabolism and physiologic impact of 4-oxo-flavonoids. Nutr Res 1996; 16(3): 517−44.
26. *Ames BN, Shigenaga MK, Hagen TM.* Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci 1993; 90(17): 7915−22.
27. *Procházková D, Boušová I, Wilhelmová N.* Antioxidant and prooxidant properties of flavonoids. Fitoterapia 2011; 82(4): 513–23.
28. *Fenton HJH.* Oxidation of tartaric acid in presence of iron. J Chem Soc Trans 1894; 65: 889−910.
29. *Van Acker VS, van Balen GP, van der Berg DJ, van der Vijgh WJ.* Influence of iron chelation on the antioxidant activity of flavonoids. Biochem Pharmacol 1998; 56(8): 935−43.
30. *Afanas'ev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI.* Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem Pharmacol 1989; 38(11): 1763−9.
31. *Brown JE, Khodr H, Hider RC, Rice–Evans CA.* Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. Biochem J 1998; 330(Pt 3): 1173−8.
32. *Fiorani M, De Sanctis R, De Bellis R, Dacha M.* Intracellular flavonoids as electron donors for extracellular ferricyanide reduction in human erythrocytes. Free Radic Biol Med 2002; 32(1): 64−72.
33. *Tajmir-Riahi HA, Diamantoglou S, Kanakis CD, Tarantilis PA, Polissiou MG.* 2007. Flavonoids interactions with DNA and RNA: binding modes and antioxidative effects Acta Hort. (ISHS); 744: 195−204.
34. *Mira L, Fernandez MT, Santos M, Rocha R, Florêncio MH, Jennings KR.* Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free Radic Res 2002; 36(11): 1199−208.
35. *Kostyuk VA, Potapovich AI, Vladykovskaya EN, Korkina LG, Afanase'ev IB.* Influence of metal ions on flavonoid protection against asbestos-induced cell injury. Arch Biochem Biophys 2001; 385(1): 129−37.
36. *Moridani MY, Pourahmad J, Bui H, Siraki A, O'Brien PJ.* Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers. Free Radic Biol Med 2003; 34(2): 243−53.
37. *De Souza RF, De Giovani WF.* Antioxidant properties of complexes of flavonoids with metal ions. Redox Rep 2004; 9(2): 97−104.
38. *Afanas' IB, Ostrakhovitch EA, Mikhal'chik EV, Ibragimova GA, Korkina LG.* Enhancement of antioxidant and anti-inflammatory activities of bioflavonoid rutin by complexation with transition metals. Biochem Pharmacol 2001; 61(6): 677−84.
39. *Barthe GA, Jourdan PS, McIntosh CA, Mansell RL.* Radioimmunossay for the quantitative determination of hesperidin and analysis of its distribution in Citrus sinensis. Phytochemistry 1988; 27(1): 249−54.
40. *Leuzzi U, Caristi C, Panzera V, Licandro G.* Flavonoids in pigmented orange juice and second-pressure extracts. J Agric Food Chem 2000; 48(11): 5501–6.

41. *AIJN – European Fruit Juice Association*. What is the AIJN Code of Practice? Available from: http://www.aijn.org/pages/cop/cop.html
42. *Manach C, Morand C, Gil-Izquierdo A, Bouteloup-Demange C, Rémésy C.* Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. Eur J Clin Nutr 2003; 57(2): 235−42.
43. *Malešev D, Radović Z, Kuntić V, Kosanić M.* Spectrophotometric determination of hesperidin by Al(III)-hesperidin complex in water-methanol solution. Anal Letters 1997; 30(5): 917−26.
44. *Kuntić V, Kosanić M, Malešev D, Radović Z, Mioč U.* Spectrophotometric investigation of uranil(II)-hesperidin complex in 70% methanol. J Serb Chem Soc 1998; 63(7): 565−72.
45. *Kuntić V, Blagojević S, Malešev D, Radović Z.* Spectrophotometric investigation of Cu(II)-hesperidin complex in 50% ethanol. Pharmazie 1999; 54(7): 548−9.
46. *Kuntić V, Filipović I, Vujić Z.* Effects of rutin and hesperidin and their Al(III) and Cu(II) Complexes on *in Vitro* Plasma Coagulation Assays. Molecules 2011; 16(2): 1378−88.
47. *Deng W, Fang X, Wu J.* Flavonoids function as anti-oxidants: by scavenging reactive oxygen species or by chelating iron? Radiat Phys Chem 1997; 50(3): 271−6.
48. *Suarez J, Herrera MD, Marhuenda E.* In vitro scavenger and antioxidant properties of hesperidin and neohesperidin dihydrochalcone. Phytomedicine 1998; 5(6): 469-473.
49. *Jovanovic SV, Steenken S, Tosic M, Marjanovic B, Simic MG.* Flavonoids as anti-oxidants. J Am Chem Soc 1994; 116(11): 4846−51.
50. *Bonina F, Lanza M, Montenegro L, Puglisi C, Tomaino A, Trombetta D*, et al. Flavonoids as potential protective agents against photooxidative skin damage. Int J Pharm 1996; 145(1−2): 87−94.
51. *Nandakumar N, Balasubramanian MP.* Hesperidin a citrus bioflavonoid modulates hepatic biotransformation enzymes and enhances intrinsic antioxidants in experimental breast cancer rats challenged with 7, 12-dimethylbenz (a) anthracene. J Exp Ther Oncol 2012; 9(4): 321−35.
52. *Hou M, Man M, Man W, Zhu W, Hupe M, Park K,* et al. Topical hesperidin improves epidermal permeability barrier function and epidermal differentiation in normal murine skin. Exp Dermatol 2012; 21(5): 337−40.
53. *Garg A, Garg S, Zaneveld LJD, Singla AK.* Chemistry and pharmacology of the citrus bioflavonoid hesperidin. Phytother Res 2001; 15(8): 655–69.
54. *Johnsen SP, Overvad K, Stripp C, Tjonneland A, Husted SE, Sorensen HT.* Intake of fruit and vegetables and the risk of ischemic stroke in a cohort of Danish men and women. Am J Clin Nutr 2003; 78(1): 57–64.
55. *Dauchet L, Ferrieres J, Arveiler D, Yarnell JW, Gey F, Ducimetiere P*, et al. Frequency of fruit and vegetable consumption and coronary heart disease in France and Northern Ireland: the PRIME study. Br J Nutr 2004; 92(6): 963–72.
56. *Guarnieri S, Riso P, Porrini M.* Orange juice vs vitamin C: effect on hydrogen peroxide-induced DNA damage in mononuclear blood cells. Br J Nutr 2007; 97(4): 639–43.
57. *Johnston CS, Dancho CL, Strong GM.* Orange juice ingestion and supplemental vitamin C are equally effective at reducing plasma lipid peroxidation in healthy adult women. J Am Coll Nutr 2003; 22(6): 519–23.
58. *Ghanim H, Sia CL, Upadhyay M, Korzeniewski K, Viswanathan P, Abuaysheh S,* et al. Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and Toll-like receptor expression. Am J Clin Nutr 2010; 91(4): 940–9.
59. *Sanchez-Moreno C, Cano MP, de Ancos B, Plaza L, Olmedilla B, Granado F*, et al. Effect of orange juice intake on vitamin C concentrations and biomarkers of antioxidant status in humans. Am J Clin Nutr 2003; 78(3): 454–60.
60. *Kurowska EM, Spence JD, Jordan J, Wetmore S, Freeman DJ, Piché LA*, et al. HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. Am J Clin Nutr 2000; 72(5): 1095–100.
61. *Reshef N, Hayari Y, Goren C, Boaz M, Madar Z, Knobler H.* Antihypertensive effect of sweetie fruit in patients with stage I hypertension. Am J Hypertens 2005; 18(10): 1360–3.
62. *Morand C, Dubray C, Milenkovic D, Lioger D, Martin JF, Scalbert A*, et al. Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. Am J Clin Nutr 2011; 93(1): 73–80.
63. *Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA*, et al. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2008; 88(1): 38–50.
64. *Demonty I, Lin Y, Zebregs YE, Vermeer MA, van der Knaap HC, Jakel M*, et al. The citrus flavonoids hesperidin and naringin do not affect serum cholesterol in moderately hypercholesterolemic men and women. J Nutr 2010; 140(9): 1615–20.
65. *Weiss JF, Landauer MR.* Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology 2003; 189(1−2): 1–20.
66. *Mates JM, Sanchez-Jimenez FM.* Role of reactive oxygen species in apoptosis: implications for cancer therapy. Int J Biochem Cell Biol 2000; 32(2): 157–70.
67. *Nambiar D, Rajamani P, Singh RP.* Effects of phytochemicals on ionization radiation-mediated carcinogenesis and cancer therapy. Mutat Res 2011; 728(3): 139–57.
68. *Hosseinimehr SJ.* Trends in the development of radioprotective agents. Drug Discov Today 2007; 12(19−20): 794–805.
69. *Van Acker FA, Schouten O, Haenen RM, van der Vijh WJ, Bast A.* Flavanoid can replace tocopherol as an antioxidant. FEBS Lett 2000; 473(2): 145–8.
70. *Hosseinimehr SJ, Nemati A.* Radioprotective effects of hesperidin against gamma irradiation in mouse bone marrow cells. Br J Radiol 2006; 79(941): 415–8.
71. *Pradeep K, Park SH, Ko KC.* Hesperidin a flavanoglycone protects against γ-irradiation induced hepatocellular damage and oxidative stress in Sprague–Dawley rats. Eur J Pharmacol 2008; 587(1−3): 273–80.
72. *Kalpana KB, Devipriya N, Srinivasan M, Vishwanathan P, Thayalan K, Menon VP.* Evaluating the radioprotective effect of hesperidin in the liver of Swiss albino mice. Eur J Pharmacol 2011; 658(2−3): 206–12.
73. *Hosseinimehr SJ, Ahmadi A, Mahmoudzadeh A, Mohamadifar S.* Radioprotective effects of Daflon against genotoxicity induced by gamma irradiation in human cultured lymphocytes. Environ Mol Mutagen 2009; 50(9): 749–52.
74. *Hosseinimehr SJ, Mahmoudzadeh A, Ahmadi A, Mohamadifar S, Akhlaghpoor S.* Radioprotective effects of hesperidin against genotoxicity induced by γ-irradiation in human lymphocytes. Mutagenesis 2009; 24(3): 233–5.
75. *Kalpana KB, Devipriya N, Srinivasan M, Menon VP.* Investigation of the radioprotective efficacy of hesperidin against gamma-radiation induced cellular damage in cultured human peripheral blood lymphocytes. Mutat Res 2009; 676(1−2): 54–61.
76. *Kuntić V, Pejić N, Mičić S.* Direct spectrophotometric determination of hesperidin in pharmaceutical preparations. Acta Chim Slov 2012; 59(2): 436–41.

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