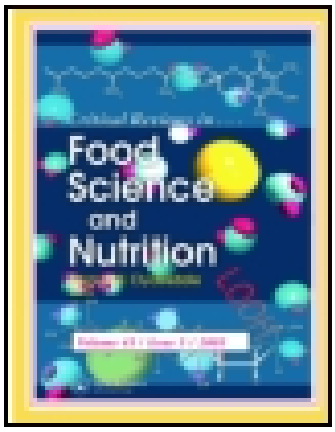

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Health-promoting Effects of the Citrus Flavanone Hesperidin

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Abstract

Hesperidin, a member of the flavanone group of flavonoids, can be isolated in large amounts from the rinds of some citrus species. Considering the wide range of pharmacological activities and widespread application of hesperidin, this paper reviews preclinical and clinical trials of hesperidin and its related compounds, including their occurrence, pharmacokinetics, and some marketed products available. Preclinical studies and clinical trials demonstrated therapeutical effects of hesperidin and its aglycone hesperetin in various diseases, such as neurological disorders, psychiatric disorders and cardiovascular diseases and others, due to its anti-inflammatory, antioxidant, lipid-lowering and insulin-sensitizing properties.

Key words: hesperidin, hesperitin, pharmacokinetics, pharmacology

Introduction

Flavanones constitute a unique subclass of flavonoids, which contain various glycosides of three main aglycones: hesperitin (4'-methoxy-3',5,7-trihydroxyflavanone), naringenin (5,7,4'-trihydroxyflavanone) and eriodictyol (5,7,3',4'-tetrahydroxyflavanone) (Manach et al., 2003). Unlike other flavonoids, such as flavonols and flavones, which could be found in a wide range of foods, flavanones are present in our diet almost exclusively in citrus fruits and, to a lesser extent, in tomatoes and some aromatic herbs (such as mint) (Chanet et al., 2012). Generally, flavanones are glycosylated by a disaccharide at position 7: either a neohesperidose, which imparts a bitter taste, such as naringin in grapefruit, or a flavorless rutinose, such as hesperidin in oranges (Berhow, 1998). Epidemiological studies reported an inverse relationship between their intake and the risk of neurological disorders, cardiovascular events and some chronic diseases (Arts and Hollman, 2005; Devore et al., 2012; Gao et al., 2012; Knekt et al., 2002).

Hesperidin (3,5,7 – trihydroxyflavanone 7- rhamnoglucoside) is the food-bound form of hesperitin (Figure 1) and is one of the two molecules which were erroneously named ‘Vitamin P’ previously (Garg et al., 2001). It was first isolated in 1828 by French chemist Lebreton from the albedo (the spongy inner portion of the peel) of oranges, and has since been found in lemons and other citrus fruits (Manthey and Grohmann, 1998). Hesperidin concentration appears to be high in the Citrus sinensis (15.25±8.21 mg/100g fresh fruit weight) and Citrus reticulate (19.26±11.56 mg/100g fresh fruit weight) (Peterson et al., 2006). In a recent paper on polyphenol intake in Finland, hesperidin was reported to be the most highly consmed flavonoid with an intake of 28.3 mg per day, equivalent to 30% of the total flavonoid intake (Knekt et al., 2002). Also, it is well-

known, that hesperidin is one of the primary constituents of Chenpi, which is made of Satsuma mandarin peel and has traditionally been prescribed as a Traditional Chinese Medicine for inflammation, allergy and hepatopathy (Yamada et al., 2006).

Considering the wide range of pharmacological properties and widespread application of hesperidin, in this review we comprehensively summarize its pharmacokinetics and main pharmacological effects in preclinical and clinical trials.

Pharmacokinetics

Hesperidin has a limited bioavailability due to low water solubility and disposition by phase II enzymes (Li et al., 2008b). Consumption of hesperidin (18.9 mg/kg) by rats resulted into a maximum blood concentration (C_{max}) of 0.35μM at a time-to-maximum (T_{max}) of 6 hours, conferring an area under the curve (AUC) of 2.63 μM/h (Li et al., 2008b). In a double-blind, randomized, crossover study, healthy volunteers consumed orange juice containing 0.93 mg/kg body weight hesperidin (low-dose hesperidin treatment) or 2.92 mg/kg body weight hesperidin (high-dose hesperidin treatment). C_{max} value after subjects consumed high dose hesperidin juice (1.05 μM at 7.4 hours) was 2-fold higher compared with those consuming “low” dose hesperidin juice (0.48 μM at 7.0 hours). The AUC for total plasma hesperitin of subjects consuming high dose hesperidin juice (4.16 μM/h) was even 4-fold higher than that of subjects consuming the low dose hesperidin juice (1.16 μM/h) (Nielsen et al., 2006).

Unlike hesperitin which could be absorbed directly in the small intestine, hesperidin, as a rutinoside, must pass onto the colon and be fermented by intestinal microflora to an alternate

form that is more readily absorbed (Jin et al., 2010). Thus hesperitin is supposed to be absorbed much faster than hesperidin. Gonzalez-Barrio et al. firstly proposed a concept in 2004 that the removal of the rhamnose sugar from hesperidin to yield its flavonoid glucoside would improve its bioavailability (Gonzalez-Barrio et al., 2004). Li et al. proved this hypothesis (Li et al., 2008b). They showed that glucosidase-like BglA protein, an enzyme isoform belonging to the family of glycosyl hydrolases, can be used to hydrolyze hesperidin to hesperitin. The results indicated that after the administration of BglA protein-treated hesperidin (9.4 mg/kg) and a standard preparation of hesperitin (9.4 mg/kg), serum hesperitin were detected as early as 30 min after administration and reached a maximum concentration (0.82 μM and 0.95 μM) at 4 hours in rats. In addition, the AUC values for total hesperitin were 6.82 μM/h after administration of Bg1A-treated hesperidin and 7.91 μM/h after administration of pure hesperitin. Also, the human trial mentioned above also showed a higher C_{max} value (2.60 μM) at 0.6 hour after subjects consumed hesperetin-7-glucoside juice (1.21 mg/kg body weight hesperidin) compared with high or low doses of hesperidin, and a higher AUC (3.45 μM/h) than low dose of hesperidin (Nielsen et al., 2006). Yamada compared the absorption of glucosyl-hesperidin (G-hesperidin, a water-soluble derivative of hesperidin), with hesperidin in rats (Yamada et al., 2006). Serum hesperitin was found more rapidly in blood of rats administered 1 mmol/kg of G-hesperidin (15 min) than in those administrated 1 mmol/kg of hesperidin (6 hours). The AUC for hesperidin in the sera of rats administered G-hesperidin (2.5 μM/h) was approximately 3.7-fold greater than that of rats administered hesperidin (0.7 μM/h). Recently, Kim et al. and Kobayashi et al. reported that hesperitin and G-hesperidin permeated across the Caco-2 cell monolayer, a model of the small intestinal epithelium, via the paracellular pathway, but hesperidin did not (Kim et al., 1999;

Kobayashi et al., 2008). Thus, the difference in permeability among G-hesperidin, hesperitin and hesperidin was considered to be due to the water solubility of those compounds (Kim et al., 1999). Such a passive transport system might explain the high absorbability of hesperitin and G-hesperidin after administration.

The plasma clearance value after administration of 18.9 mg/kg of hesperidin were 56.5 ml/h, which was higher than that after administration of 9.8 mg/kg hesperitin (19.2 ml/h). In addition, hesperitin has a much smaller volume of distribution, perhaps due to its tight binding to plasma protein when compared to hesperidin (Li et al., 2008b). Although hesperidin is poorly absorbed and rapidly eliminated, it has a reasonable half-life (6 hours). This is perhaps due to the prolonged absorption phase, as shown by a long T_{max}.

Pharmacological effects of hesperidin in preclinical and in vitro studies

In the past decade, numerous in vivo and in vitro studies have been performed to evaluate effects of hesperidin. It has been demonstrated that hesperidin might be effective in treating a great variety of diseases, including cardiovascular diseases, neurological disorders, psychiatric disorders, carcinoma and so on. Specific information about model, route, dose, duration and effect in the treatment of hesperidin has been summarized in Table 1-6.

Effects on cardiovascular diseases

Cardiovascular disease represents one of the leading cause of death worldwide. Experimental data showed antihypertensive, lipid-lowing, insulin-sensitizing, antioxidative, and anti-

inflammatory properties of hesperidin, which could explain its cardiovascular protective effects (Table 1).

Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes, 2011). Abnormal regulation of glucose is the key pathological event in diabetes mellitus. Four preclinical trials demonstrated that hesperidin normalized glucose metabolism by altering the activities of glucose-regulating enzymes and reduced the levels of lipids in rodents with type 1 and in type 2 diabetes (Akiyama et al., 2010; Akiyama et al., 2009; Jung et al., 2004; Mahmoud et al., 2012). Moreover, it also potentiated the antioxidant defense system and suppressed pro-inflammatory cytokine production.

As a leading cause of blindness in middle age and elderly people, diabetic retinopathy (DR) is one of the prominent complications of type 1 and type 2 diabetes (Moss et al., 1998). In streptozotocin-induced diabetic rats, treatment of 100 and 200 mg/kg hesperidin for 12 weeks attenuated retina and plasma abnormalities via anti-angiogenic, anti-inflammatory and antioxidative effects, as well as inhibitory effects on the polyol pathway and AGEs accumulation (Shi et al., 2012). Also, an anti-DR effect was shown with 100 mg/kg hesperitin treatment for four weeks (Kumar et al., 2013b).

Hypertension

Oral administration of G-hesperidin with a dosage range from 10-50 mg/kg induced a dose-dependent reduction in systolic blood pressure (SBP) in spontaneously hypertensive rats (SHRs),

reaching peak efficacy at 30 mg/kg (Yamamoto et al., 2008a). A significant hypotensive effect was found 9 to 12 hours after intake of G-hesperidin, correlating with the observation that the major metabolite of G-hesperidin peaks in serum 6 hours after administration and remains elevated until 12 hours. Moreover, intraperitoneal injection of hesperitin (50 mg/kg) into SHRs also caused a significant reduction in SBP (Yamamoto et al., 2008a). The hypotensive effects of G-hesperidin and hesperitin is associated with improved endothelial dysfunction through enhancement of endothelium-dependent relaxation, increase of NO bioavailability and inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression in the vasculature (Ikemura et al., 2012; Yamamoto et al., 2008a; Yamamoto et al., 2008b).

Apart from the rapid antihypertensive effect, long-term effect of hesperidin and G-hesperidin were evaluated by Ohtsuki et al (Ohtsuki et al., 2002). All SHRs were fed with diets containing hesperidin or G-hesperidin (30 mg/kg) for 25 weeks. From 15 weeks onwards, it became obvious that heart rate and blood pressure in treated mice decreased significantly.

Myocardial infarction

Myocardial infarction (MI) is the damage and death of heart muscle from the sudden blockage of a coronary artery by a blood clot. Hypercholesterolemia is deemed to be a crucial risk in the progression of coronary atherosclerosis and associated with an increase in the incidence of myocardial ischemia and cardiac events (Smith et al., 1992). Intragastric hesperidin in isoproterenol (ISO)-induced rats has been found to attenuate the increased level of plasma cholesterol, low density of lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDLC), triglycerides (TG), free fatty acids (FFA) and phospholipids (PL), and decreased level of high density lipoprotein-cholesterol (HDL-C) (Selvaraj and Pugalendi, 2010,

2012). In another MI model, which used rats fed high-cholesterol diet, treatment with hesperidin improved hypercholesterolemia and fatty liver by inhibiting both the synthesis and absorption of cholesterol and by regulating the expression of mRNA for retinol binding protein (RBP), cutaneous fatty acid–binding protein (C-FABP), and heart fatty acid–binding protein (H-FABP) (Wang et al., 2011).

Effects on neurological disorders

Neurological disorders have rapidly become a significant and growing problem, affecting more than 450 million individuals worldwide. The most common forms of neurological disorders include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and ischemic brain injury and are usually associated with oxidative stress, neuroinflammation and impairment of different types of neurons in central nervous system (Table 2).

Alzheimer's disease

Alzheimer's disease is a progressively neurodegenerative disorder, which is pathologically characterized by the accumulation of beta-amyloid (Aβ) (Li et al., 2013a). Excessive Aβ deposition in the brain exerts various cytotoxic effects in neuronal cells by inducing mitochondrial dysfunction. According to Wang et al., 20 μM $A\beta_{25-35}$ increased apoptotic death in PC12 cells, with the total apoptosis reaching approximately 32% of cells by 24 hours (Wang et al., 2013). This effect was significantly inhibited by hesperidin (10-50 μM), reversing $A\beta_{25-35}$-induced mitochondrial dysfunction, including mitochondrial permeability transition pore opening, intracellular free calcium increase and reactive oxygen species (ROS) production.

Moreover, hesperidin increased the level of mitochondrial voltage-dependent anion channel 1 (VDAC1) phosphorylation and inhibited mitochondria-dependent downstream caspase-mediated apoptotic pathway involving caspase-9 and caspase-3.

In addition, downregulation of autophagy may be one of the approaches to control the impairment of energy metabolism leading to neuronal injury in the early development of AD. Treatment with hesperidin or hesperitin (1 and 20 μM) have improved Aβ-impaired glucose uptake partly by inhibiting autophagy (Huang et al., 2012).

Low concentration of hesperitin (0.1 μM) scavenge reactive oxygen species and activate Akt in PC12 cells due to estrogen receptors (ER)- and tyrosine kinase receptor A(TrkA)-mediated induction of antioxidant enzymes, and increase seladin-1 secondary to estrogen signaling. These effects of hesperitin might be mediated by its antioxidant property at a higher concentration (50μM) (Hwang and Yen, 2011).

A recent preclinical trial in our lab using APP/PS1-21 transgenic mouse model of AD showed that after a relatively short-term treatments of 10 days, hesperidin (100 mg/kg) significantly ameliorated deficits in nesting and in social interactive behaviors, and attenuated β-amyloid deposition and microglial activation in brain of these transgenic mice, suggesting that it may be considered a promising therapeutic option of human AD or even further neurodegenerative diseases.

Parkinson’s disease

Parkinson’s disease is the second most common neurodegenerative disease worldwide resulting from progressive loss of dopaminergic neurons within the substantia nigra (Li et al., 2013b).

Rotenone, a used pesticide that inhibits mitochondrial complex I, has been employed to investigate the pathobiology of PD in vivo and vitro (Molina-Jimenez et al., 2004; Radad et al., 2006). In neural Sk-N-SH cells induced by rotenone, treatment of 20 µg hesperidin significantly reduced ROS generation (Tamilselvam et al., 2013), which may be due to direct ROS scavenging and transition metal ion chelation (Gaur and Kumar, 2010). Moreover, other potential mechanisms, such as the ability of hesperidin to increase cellular glutathione content, also contribute to its protective effect on oxidative stress.

Huntington's disease

HD is an autosomal dominantly transmitted disorder characterized by motor, mood and cognitive signs caused by an expansion mutation beyond 36 CAG repeats in the IT15 gene (Kremer et al., 1994). Short term treatment with 3-nitropropionic (NP) acid of rats induced HD like symptoms as indicated by reduced locomotor activity, body weight, grip strength, oxidative defense and mitochondrial complex enzymes (complex-I, -II, and -IV) activities in the striatum. Pretreatment with hesperidin (50 or 100 mg/kg) attenuated all these manifestations through suppression of iNOS induction and reduction of lipid peroxidation (Menze et al., 2012). Nitric oxide based mechanisms are supposed to underlie these effects, as the protective effects can be attenuated with L-arginine administration (Kumar and Kumar, 2010).

Stroke

Stroke is the third leading cause of morbidity and disability globally and overall costs of stroke care will account for 6.2% of the total burden of illness by 2020 (Demaerschalk et al., 2010). Rodent models of stroke provide the experimental backbone for the in-vivo determination of the mechanisms of secondary injury, neuroinflammation, cell death, neural repair and glial scar tissue formation, and are therefore often used in preclinical testing of neuroprotective compounds (Carmichael, 2005). In rodents with cerebral ischemic reperfusion injury or middle cerebral artery occlusion (MCAO)-induced stroke, 50 or 100 mg/kg hesperidin improved neurobehavioral alterations, oxidative defense and histopathological alterations, which also seemed to be dependent on suppressing errant nitric oxide signaling (Gaur et al., 2011; Gaur and Kumar, 2010; Raza et al., 2011).

Epilepsy

As one of the most frequent neurological disorders, epilepsy affects an estimated 50 million people. It is clinically manifested by current episodes of convulsive seizures, loss of consciousness, and sensory disturbances. Pentylenetetrazole (PTZ)-induced kindling primarily represents an animal model of generalized epilepsy, characterized by an increase in convulsive activity, and alteration in antioxidant enzyme levels and mitochondrial complex (I, II, and IV) activities. Treatment with 200 mg/kg hesperidin for 12 days showed therapeutic potential in epileptic conditions, possible involved in the NO–cyclic guanosine monophosphate (cGMP) pathway as these protective effects were modulated by L-arginine or L-NAME (Kumar et al., 2013a).

Neonatal hypoxia–ischemic encephalopathy

Neonatal hypoxia–ischemic encephalopathy (HIE) is associated with high morbidity and mortality rates. About 15% to 25% of affected newborns die during the postnatal period and 25% develop severe and permanent neuropsychological sequelae (Lai and Yang, 2011).

Free radicals play a vital role in neurobehavioral deficit in cerebral ischemia models through oxidative stress. Pretreatment with hesperidin (50 mg/kg) prevented the increase in ROS and lipid peroxide levels, thereby significantly reducing HI-induced brain tissue loss and improving neurological outcomes. Besides, it also activated a key survival signaling kinase, Akt, and suppressed the P-FoxO3 level (Rong et al., 2013).

Effects on psychiatric disorders

Psychiatric disorders are abundant and a major health problem (Baker et al., 2002) and some polyphenols have potential in treatment of these diseases. Up to 300 mg/kg of hesperidin tested 1 hour and 4 hours after oral dosing of mice produced no effects in the holeboard and locomotor activity test (Table 3) (Wasowski et al., 2012). However, a chronic oral intake of hesperidin induced anxiolytic-like effects and inhibited mitochondrial dysfunction caused by acute immobilization stress through the nitrergic pathway (Viswanatha et al., 2012; Wasowski et al., 2012). In turn, acute and chronic administration of hesperidin by the i.p. route produced depressant action on the locomotor and exploratory activities, perhaps depending on an interaction with the serotonergic 5-HT_{1A} receptors (Filho et al., 2013; Souza et al., 2013; Wasowski et al., 2012).

Carcinoma

Cancer is a broad group of diseases involving unregulated cell growth. Generally, cancer cells have an abnormal energy metabolism and glucose is regarded a preferred metabolic substrate (Ortega et al., 2009).

Breast cancer

Concern has grown about early changes of carbohydrate metabolisms as anomalies of glycolytic and gluconeogenic pathways in cancers (Bannasch et al., 1980). In 7,12-dimethylbenz(a)anthracene (DMBA)-induced breast cancer-bearing rats, the activities of carbohydrate-metabolizing enzymes such as hexokinase, phosphoglucoisomerase, and aldolase were dramatically increased, and the glucose-6-phosphatase and fructose-1,6-diphosphatase were decreased. However, administration of hesperidin at a dose of 30 mg/kg for 45 days significantly restored these enzyme to normal levels, thereby interrupting the energy supply of neoplastic tissues. Besides, hesperidin altered the lipid profiles due to its inhibitory effect by free radical quenching, and increased the activities of adenosine triphosphatases (ATPases) because of the protective effects on membrane permeability and stabilizing potential (Table 4) (Nandakumar et al., 2013).

Colon cancer

Aberrant crypt foci (ACF) are putative preneoplastic lesions of colonic neoplasia, which appear in the early stages of colon cancer and subsequently develop into polyps, adenomas and

eventually carcinomas. Azoxymethane (AOM)-induced mice fed with 25 mg/kg hesperidin for 2 weeks showed significantly reduced numbers and multiplicities of ACF. Inflammation and oxidative stress were also ameliorated (Saiprasad et al., 2013). Inhibition of colonic mucosal ornithine decarboxylase (ODC) activity by hesperidin might be another possible mechanism for the decrease of colonic tumors, since ODC has been correlated with the rate of cell proliferation in several tissues (Table 4) (Tanaka et al., 1997).

Radiation protection

Radiation toxicity occurs by either direct attack on the genetic material or by generating free radicals by the radiolysis of water, which generate various ROS that damage cellular macromolecules (Sonntag, 1987). Oxidative stress induced by ultraviolet irradiation might be an initiator in the pathogenesis of skin damage and photoaging (Darr and Fridovich, 1994; Petrova et al., 2011). Petrova et al. investigated the ability of topically applied hesperidin to protect skin via modulation of ultraviolet B (UVB)-induced oxidative damage, inflammation and cell proliferation (Petrova et al., 2011). Treatment of hesperidin by gavage in rats suggested that it might protect against γ-irradiation induced biochemical dysfunction of brain tissue, hepatocellular damage and acute proctitis by scavenging oxygen free radicals (Table 5) (Pradeep et al., 2012; Pradeep et al., 2008).

In vitro, the radioprotective effect of hesperidin against genetic damage and other effects induced by γ-irradiation has been investigated in cultured blood lymphocytes from human volunteers (Hosseinimehr et al., 2009a; Hosseinimehr et al., 2009b; Kalpana et al., 2009). It was also been suggested that hesperidin is a specific inhibitor of the production of inflammatory cytokines by

regulating the NF-kB/IkBa signalling cascade and p38 MAPK in HaCaT cells, which might explain its beneficial effect in treatment of UV radiation-induced inflammatory reaction (Moon and Kim, 2012).

Many other therapeutic efficacy have been also successfully demonstrated in vitro and in vivo (Table 6), such as anti-arthritis, anti-kidney diseases and anti-infection through decreasing oxidative stress, inflammation and some other mechanisms.

Hesperidin in clinical studies

We have searched for clinical trials with hesperidin but only a few were found. All of them focused on evaluating its effects on cardiovascular risk factors and are summarized in Table 7. Endothelial function tightly controls both vascular reactivity and integrity. It has been suggested that the endothelial function is an integrative marker of the net effects of damage from risk factors on the arterial wall and its intrinsic capacity for repair (Deanfield et al., 2007). Endothelial dysfunction refers to an impairment of endothelium-dependent vasorelaxation caused by a loss of nitric oxide (NO) bioactivity in the vessel wall (Cai and Harrison, 2000). In a randomized, controlled, crossover study, a 4-week consumption of hesperidin (292 mg; equivalent to the amount found in 500 mL of orange juice) resulted into a significantly lower diastolic blood pressure (DBP) compared to that measured after consumption of placebo in healthy overweight individuals (Morand et al., 2011). Moreover, hesperidin treatment significantly improved postprandial microvascular endothelial reactivity and these changes were positively correlated with plasma hesperitin concentration. Another double-blind, placebo-

controlled, crossover study involving subjects with metabolic syndrome also showed that hesperidin therapy (500 mg/day) is likely to generate improvements in endothelial function, indicated by improved flow-mediated dilation (FMD) and reduced sE-selectin concentrations. However, blood pressure and vascular smooth muscle function were not affected by 3-week hesperidin treatment.

Some clinical trials studied the effects of hesperidin on the blood lipid profile, but the results were not consistent. In individuals with metabolic syndrome receiving 500 mg once daily for 3 weeks, total cholesterol (TC) and apolipoprotein B (apoB) levels were reduced, and HDL level was increased. In subjects with hypercholesterolemia, consumption of hesperidin (100 mg or 500 mg) for 6 weeks decreased serum TG and LDL levels, while consumption of 750 mL orange juice daily increased HDL, triacylglycerol and folate concentrations (Kurowska et al., 2000; Miwa et al., 2004). However, a more recent study found no effect of hesperidin (800 mg for 4 weeks) on serum cholesterol or triglyceride levels (Demonty et al., 2010). A possible explanation might be related to the high variability in flavanone bioavailability among individuals. Further clinical trials designed to specifically examine effects of hesperidin on lipid metabolism are warranted to confirm the findings.

In terms of inflammation, treatment by 500 mg hesperidin significantly reduced the plasma levels of two inflammatory biomarkers, C-reactive protein (CRP) and serum amyloid A (SAA) in individuals with metabolic syndrome (Rizza et al., 2011). A network analysis showed that hesperidin intake (292 mg daily for 4 weeks) tended to alter gene expression in white blood cells toward an anti-inflammatory and atherogenic profile (Milenkovic et al., 2011).

Combination therapy

Apart from as a monotherapy, hesperidin is also used to be an ingredient of some drug combinations, such as Daflon (Table 8) and Rikkunshito (Table 9).

Daflon

Daflon (Les Laboratory Servier, Oreleans, France) is a flavonoid vasoprotector, a venotonic agent which is prescribed in some European and Asian countries. Each tablet contains 500 mg of micronized purified flavonoid fraction, corresponding to a ratio of 90% of diosmin and 10% of hesperidin. It is a phlebotropic agent that has a proven efficacy in the treatment of venous disorders, such as chronic venous insufficient (CVI) and haemorrhoidal attacks.

Effect of Daflon on chronic venous insufficiency

Various clinical trials have demonstrated that Daflon 500 mg could decrease venous capacitance, distensibility, and emptying time, and resolve the stasis in patients suffering from CVI (Allegra et al., 1995; Geroulakos and Nicolaides, 1994; Le Devehat et al., 1997). Hesperidin provided significant relief by improving disabling symptoms which affect everyday active life, such as heavy legs, pain, heat sensation, cramps, venous leg ulcer and edema (Amato, 1994; Duchene Marullaz et al., 1988; Laurent et al., 1988). This improvement of symptoms contributes to a significant increase in patients' quality of life, indicated by a validated international scale specific to CVI: the CVIIQ questionnaire (Roztocil et al., 2003).

Effect of Daflon on haemorrhoidal attacks

In chronic haemorrhoidal attacks, Daflon 500 mg is highly effective in improving all signs and symptoms, such as bleeding, pain, discharge, tenesmus, and proctitis, thereby reducing the

consumption of oral analgesics (Godeberge, 1994; Meshikhes, 2002, 2004; Thanapongsathorn and Vajrabukka, 1992). Daflon 500 mg also significantly reduces the frequency, severity, and duration of acute hemorrhoidal attacks and relapses (Cospite, 1994).

Rikkunshito

Rikkunshito is a kampo herbal medicine for appetite stimulation and it is thought that hesperidin is a major bioactive compound stimulating ghrelin secretion. It is widely used in Japan for the treatment of the upper gastrointestinal symptoms of patients with functional dyspepsia (FD), gastroesophageal reflux disease (GERD), dyspeptic symptoms of postgastrointestinal surgery patients, and chemotherapy-induced dyspepsia in cancer patients (Arai et al., 2012; Kusunoki et al., 2010; Okuno et al., 2008; Shiratori et al., 2011; Takahashi et al., 2009; Takiguchi et al., 2013).

Discussion

Preclinical and clinical studies demonstrated that hesperidin and its aglycone hesperetin, have antioxidant, anti-inflammatory, lipid-lowering and insulin-sensitizing properties, which could explain their therapeutic effects on a wide range of diseases and disorders.

Oxidative stress refers to the imbalance between the production of ROS and the ability of the cells to eliminate them (Simonian and Coyle, 1996). ROS are derived from many sources including mitochondria, xanthine oxidase, uncoupled nitric oxide synthases and NADPH oxidase (Mueller et al., 2005). Apart from generalized oxidation resulting in cell dysfunction, necrosis or apoptosis, ROS also induce specific post-translational modifications that alter the function of important cellular proteins and signaling pathways (Ho et al., 2013). The harmful effects of ROS

are counteracted by endogenous antioxidant system comprising superoxide e dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), vitamin C and vitamin E (Andriantsitohaina et al., 2012). In several animal models, hesperidin has been demonstrated to increase GSH content and enhance antioxidant enzyme activities (SOD, CAT, and GPx) (El-Sayed el et al., 2008; Kumar et al., 2013b), which might be beneficial in the treatment of most diseases mentioned above, such as cardiovascular diseases, neurological diseases, some cancers and irradiation-induced disorders (Table 1-6). However, clinical data are still insufficient to demonstrate the anti-oxidant effect of hesperidin in human.

Hesperidin exerts anti-inflammatory activity in various animal models and cell types, indicated by a reduction of the production of pro-inflammatory cytokines, such as IL-1β and IL-6, and TNF-α (Li et al., 2010; Yamamoto et al., 2013). Besides, some studies suggested that hesperidin could across the blood-brain barrier (BBB), indicating that it might possess anti-neuroinflammatory activity (Youdim et al., 2003). Indeed, it significantly reduced microglia and astrocyte activation in neurodegenerative diseases (i.e. AD and PD) (Menze et al., 2012). In a double-blind crossover clinical trial, the increased circulating levels of pro-inflammatory markers (TNF-α, high-sensitivity CRP, serum Aβ protein and soluble E-selectin) in patients with metabolic syndrome were attenuated by hesperidin (Rizza et al., 2011).

Hesperidin, hesperitin and G-hesperidin were all reported to improve endothelium-dependent vasorelaxation by increasing the availability of NO, thereby reducing systolic blood pressure in animals and in human (Rizza et al., 2011; Yamamoto et al., 2013). The ability of improving lipid profile and reducing insulin resistance after hesperidin supplementation supported the effects of

hesperidin on diabetes, MI, cancer and hyperlipidemia. These beneficial support future application of hesperidin in cardiovascular diseases.

Daflon is prescribed in some European and Asian countries for treating venous circulation disorders and acute hemorrhoidal attack. Normally, the dosage for treating venous insufficiency is 2 tablets daily (500 mg per tablet), while for acute hemorrhoidal attack, the dosage is 6 tablets daily for 4 days, followed by 4 tablets daily over the next 3 days. Although Rikkunshito is, so far, mainly used in Japan and other East Asian countries, it caused much attention due to its strong ability of appetite stimulation.

In conclusion, hesperidin and its aglycone hesperetin have been widely used in a wide range of diseases and disorders, including neurological disorders, psychiatric disorders, cardiovascular diseases and so on. Hesperidin can be manufactured from readily available, abundant and cheap biological resources and appears to be promising in development of dietary, nutriceutical and therapeutic products.

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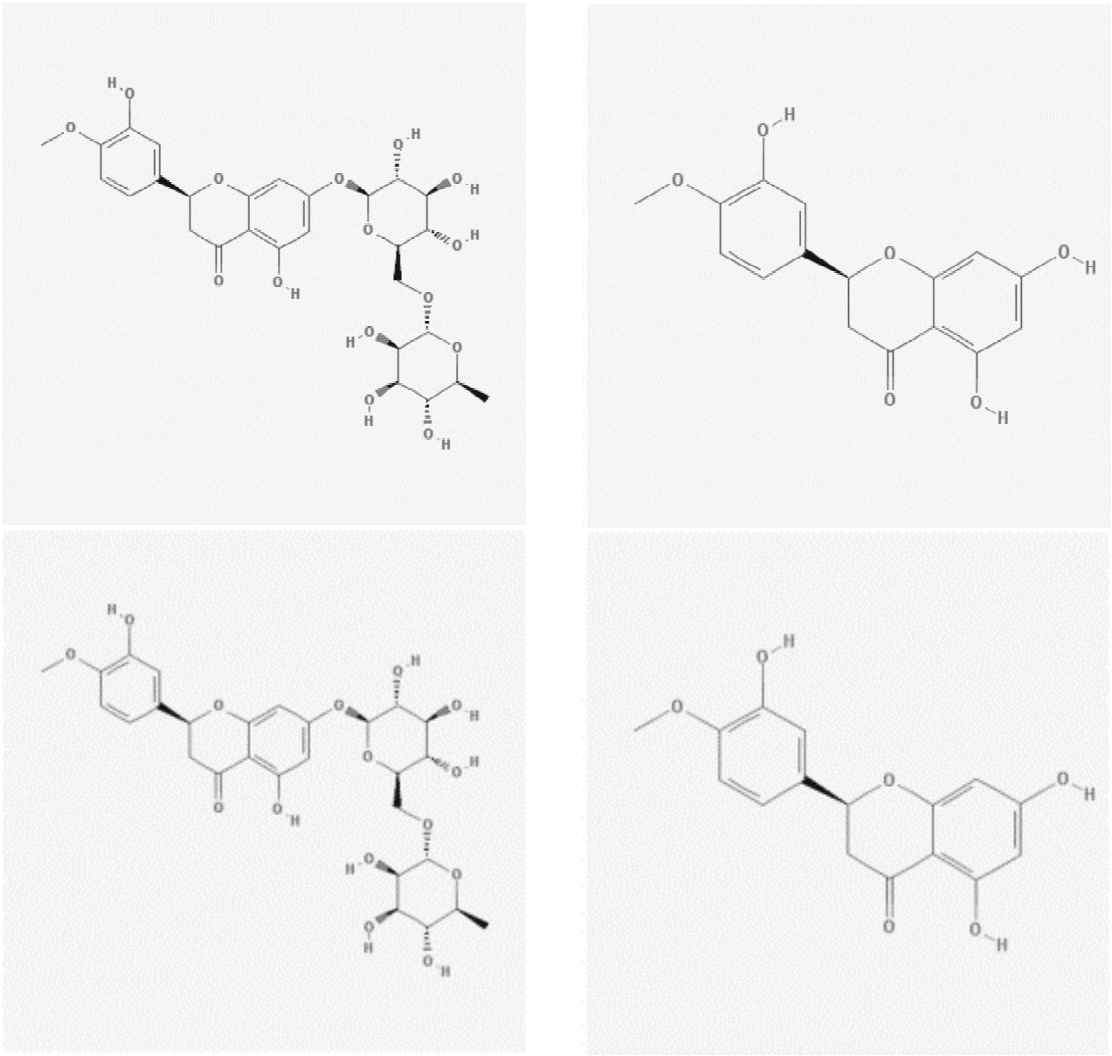

Figure 1 Molecular structure of hesperidin and hesperitin. A. Hesperidin; B. Hesperitin

Table 1 Effects of hesperidin on cardiovascular diseases

	Experimental animals	Route	Dose	Duration	Results	Ref.
Diabetes	High fat fed/streptozotocin (HFD/STZ) treated white male albino rats	p.o.	50 mg/kg body weight	30 days	Attenuates hyperglycemia-mediated oxidative stress and proinflammatory cytokine production	(Mahmoud et al., 2012)
	STZ treated male Wistar rats	diet	10 g/kg diet	4 weeks	Exerts both hypoglycemic and hypolipidemic effects	(Akiyama et al., 2010)
	Goto-Kakizaki (GK) weanling rats	diet		4 weeks	(1) Normalizes glucose metabolism by altering the activities of glucose-regulating enzymes and reducing the levels of lipids in the serum and liver (2) Alters the expression of genes encoding the peroxisome proliferator-activated receptors, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, and the low-density lipoprotein receptor	(Akiyama et al., 2009)

	Male C57BL/KsJ-db/db mice	diet	0.2 g/kg diet	5 weeks	Prevents the progression of hyperglycemia by increasing hepatic glycolysis and glycogen concentration and/or by lowering hepatic gluconeogenesis	(Jung et al., 2004)
Diabetic retinopathy	STZ treated Wistar albino rats	p.o.	100 mg/kg body weight	24 weeks	Rescues retinal oxidative stress, neuroinflammation and apoptosis	(Kumar et al., 2013b)
	STZ treated male Sprague-Dawley rats	p.o.	100, 200 mg/kg body weight	12 weeks	Attenuates retina and plasma abnormalities via anti-angiogenic, anti-inflammatory and antioxidative effects, as well as inhibitory effects on polyol pathway and AGEs accumulation	(Shi et al., 2012)
Hypertension	Male spontaneously hypertensive rats (SHRs)	i.v.	5 mg/kg body weight		Exerts hypotensive, vasodilatory and anti-inflammatory activities	(Yamamoto et al., 2013)
		diet	50 mg/kg body weight	8 weeks	(1) Reduces oxidative stress (2) Ameliorates endothelial dysfunction and hypertension	(Yamamoto et al., 2008b)
		p.o.	10, 30 and 50 mg/kg body weight		Lowers blood pressure	(Yamamoto et al., 2008a)
		p.o.	30 mg/kg body weight	25 weeks	Exerts anti-hypertensive effects	(Ohtsuki et al., 2002)

	Stroke-prone spontaneously hypertensive rats (SHRSP)	diet	14.5 mg/kg body weight	4 weeks	(1) Modulates the inactivation of NO (2) Protects endothelial function from reactive oxygen species (ROS)	(Ikemura et al., 2012)
Myocardial infarction	Isoproterenol treated male albino Wistar rats	p.o.	200 mg/kg body weight	7 days	Exerts hypolipidemic effect	(Selvaraj and Pugalendi, 2012)
			100, 200 and 400 mg/kg body weight		Exerts antilipidperoxidative and antioxidant effect	(Selvaraj and Pugalendi, 2010)
Hypercholesterolemia and fatty liver	Male Wistar rats fed a high-cholesterol diet	diet	20 mg	28, 56 and 84 days	Improves hypercholesterolemia and fatty liver by inhibiting both the synthesis and absorption of cholesterol and regulating the expression of mRNA for RBP, C-FABP, and H-FABP.	(Wang et al., 2011)
Arrhythmias	Ischemia and reperfusion (I/R) treated Sprague-Dawley rats	p.o.	100 mg/kg body weight	15 days	(1) Increases tissue nitrite, antioxidant level (2) Reduces inflammation, arrhythmias and apoptosis	(Gandhi et al., 2009)
Vasculature	SHRs	p.o.	30 mg/kg body weight	25 weeks	(1) Improves serum cholesterol composition (2)Inhibits hypertrophy in vasculature	(Ohtsuki et al., 2003)

Table 2 Effects of hesperidin on neurological disorders

	Experimental animals	Route	Dose	Duration	Results	Ref.
Alzheimer's disease	$A\beta_{25-35}$ treated PC12 rat pheochromocytoma cell line		10, 25 and 50 μM		Protects against Aβ-induced neurotoxicity via VDAC1-regulated mitochondrial apoptotic pathway	(Wang et al., 2013)
	$A\beta_{1-42}$ treated Neuro-2A neuroblastoma cell line		1 and 20 μM		Improves Aβ-impaired glucose uptake partly by inhibiting autophagy	(Huang et al., 2012)
	H_2O_2 treated PC12 cells		0.1, 1 and 50 μM		(1) Inhibits cell viability decreases and reactive oxygen species, intracellular calcium level, and caspase-3 activity increases (2) Stimulats the activation of Akt, ERK, and CREB and induces brain-derived neurotrophic factor, PPARγ coactivator 1α (PGC-1α), and selective Alzheimer's disease indicator-1() via both estrogen receptor (ER) and tyrosine kinase receptor (TrkA)	(Hwang and Yen, 2011)
Parkinson's Disease	Rotenone treated SK-N-SH neuroblastoma cell line		20 μg		Exerts neuroprotective effect against rotenone due to its	(Tamilselvam et al., 2013)

					antioxidant, maintenance of mitochondrial function, and antiapoptotic properties	
Huntington's disease	3-nitropropionic acid (3-NP) treated male Wistar rats	p.o.	100 mg/kg body weight	5 days	Exerts antioxidant and anti-inflammatory effects	(Menze et al., 2012)
			50 and 100 mg/kg body weight	14 days	Attenuates behavioral alterations, oxidative stress and mitochondrial enzyme complex dysfunction	(Kumar and Kumar, 2010)
Stroke	Male Wistar rats with Middle cerebral artery occlusion (MCAO)	p.o.	50 mg/kg body weigh	15 days	(1) Ameliorates functional and histological outcomes by elevated endogenous antioxidants status (2) Reduces induction of proinflammatory cytokines	(Raza et al., 2011)
	Male Wistar rats with I/R		50 and 100 mg/kg body weight	7 days	(1) Improves neurobehavioral alterations (neurological score, locomotor activity, resistance to lateral push and hanging wire latency) (2) Attenuates oxidative damage (3) Restores antioxidant and mitochondrial complex enzyme activities	(Gaur et al., 2011)

					(1) Improves neurobehavioral alterations (delayed fall off time and increased memory retention), oxidative defense and mitochondrial complex enzyme activities in hippocampus (2) Attenuates histopathological alterations	(Gaur and Kumar, 2010)
Epilepsy	Pentylenetetrazole (PTZ) treated male LACA mice	p.o.	200 mg/kg body weight	12 days	Attenuates behavioral, biochemical, and mitochondrial alterations	(Kumar et al., 2013a)
Hypoxia–ischemic encephalopathy (HIE)	Sprague–Dawley rats with HI	p.o.	50 mg/kg body weight	3 days	Protected neonatal HIE by reducing free radicals and activating phosphorylated Akt.	(Rong et al., 2013)
Neurotoxicity	Cisplatin treated adult male Sprague-Dawley rats	p.o.	50 mg/kg body weight	14 days	Protects brain and sciatic nerve tissues against cisplatin-induced oxidative, histological and electromyographical side effects	(Kamisli et al., 2013)
Other neuroprotective effects	Astrocytes		1 nM-100 μM		Increases the number of neural progenitors and post-mitotic neurons	(Nones et al., 2012)
	PC12 cells		1 μM		(1) Triggers both ER- and TrkA-mediated parallel pathways (2) Induces proteins for pro-cellular survival and neuroprotection	(Hwang et al., 2012)

	Neuron		10 μM		(1) Enhances neuronal population as revealed by an 80% increase in the number of β-tubulin III cells (2) Modulates neuronal cell death by activating MAPK and PI3K pathways	(Nones et al., 2011)
	Adult male Swiss mice	i.p.	10 mg/kg body weight		Decreases brain pERK1/2 levels	(Martinez et al., 2009)

Table 3 Effects of hesperidin on psychiatric disorders

	Experimental animals	Route	Dose	Duration	Results	Ref.
Depressive disorders	Male adult Swiss mice	i.p.	0.1, 0.3 and 1 mg/kg body weight		Exerts an antidepressant-like effect which was dependent on an interaction with the serotonergic 5-HT1A receptors.	(Souza et al., 2013)
					Decreases the immobility time in the forced swimming test	(Filho et al., 2013)
		i.p.	4 and 30 mg/kg body weight	10 days	Exerts a decrease in the locomotor and exploratory activities, thus evidencing a depressant activity	(Wasowski et al., 2012)
Anxiety disorders		p.o.	20, 50 and 100 mg/kg body weight	14 days	Induces anxiolytic-like effects	
Stress		p.o.	50, 100 mg/kg body weight	14 days	Ameliorates the behavioral and biochemical alterations and mitochondrial dysfunction by modulating nitrergic pathway	(Viswanatha et al., 2012)

Table 4 Effects of hesperidin in cancer

	Experimental animals	Route	Dose	Duration	Results	Ref.
Breast cancer	7,12-dimethylbenz(a)anthracene (DMBA) treated female Sprague-Dawley rats	p.o.	30 mg/kg body weight	45 days	Ameliorates changes in carbohydrate metabolism, lipid profile, and ATPases through quenching free radicals and thereby suppressing the key enzymes of gluconeogenesis and ATPases	(Nandakumar et al., 2013)
					Decreases the levels of glycoproteins, nucleic acids, lysosomal enzymes	(Nandakumar et al., 2012)
					Modulates hepatic biotransformation enzymes and enhances intrinsic antioxidants	(Nandakumar and Balasubramanian, 2012)

					Attenuates the peroxidation reaction and membrane bound marker enzyme activities as well as upregulation of adenosine triphosphatases, TCA cycle enzymes, and antioxidants	(Nandakumar and Balasubramanian, 2011)
	MCF-7 cells		10, 20, 40, 70 and 100 µM		Inhibits proliferation of MCF-7-GFP-Tubulin cells	(Lee et al., 2010a)
Colon cancer	Azoxymethane (AOM) treated male Swiss albino mice	p.o.	25 mg/kg body weight	1 week	Exerts chemopreventive effect against the deleterious traits of colon carcinogenesis including accelerated proliferation, inflammation and persistent oxidative stress.	(Saiprasad et al., 2013)
	SNU-C4 cells		1, 10, 50 and 100µM		Induces apoptosis in human colon cancer cells through CASP3 activation	(Park et al., 2008)
Genotoxicity	Cyclophosphamide treated male NMRI mice	p.o.	50, 100, 200, and 400 mg/kg body weight	5 days	Reduces the oxidative stress and genotoxicity through antioxidative activity	(Ahmadi et al., 2008)
Prostate cancer	LNCaP cells		10, 20, 40, 70		Inhibits both basal and	(Lee et al., 2010a)

			and 100 µM		testosterone induced proliferation of LNCaP cells	
Liver cancer	HepG2 cells		12.5, 25, 50 and 100 µM		Suppresses MMP-9 enzymatic activity via NF-kappaB and AP-1 signaling pathway	(Lee et al., 2010b)
			5, 25, 50, 100 and 200 µM		(1) Suppresses both acetaldehyde-activated NF-kB and activator protein 1 (AP-1) activity by IkB, JNK, and p38 signaling pathways (2) Reduces MMP-9 expression, secretion, and hepatocarcinoma cellular invasion	(Yeh et al., 2009)
Lung cancer	Benzo(a)pyrene (B(a)P) treated male Swiss albino mice	p.o.	25 mg/kg body weight	16 weeks	(1) Alleviates the mitochondrial dysfunction (2) Restores cellular normalcy	(Kamaraj et al., 2011)
				16 weeks	Exerts antioxidant and anticancer effects	(Kamaraj et al., 2009)
				16 weeks on alternate days	(1) Decreases mast cell density (MCD) (2) Downregulates the expressions of COX-2, MMP-2 and MMP-9	(Kamaraj et al., 2010)

Malignant pleural mesothelioma (MPM)	MSTO-211H cells		40, 80 and 160 μM		Suppresses mesothelioma cell growth through inhibition of specificity protein 1 (Sp1).	(Lee et al., 2012)
Hematopoietic malignancies	NALM-6 cells		10, 25, 50 and 100 μM		Exerts proapoptotic and antiproliferative effects via both PPARγ-dependent and PPARc-independent pathways	(Ghorbani et al., 2012)

Table 5 Effects of hesperidin on radiation toxicity

	Experimental animals	Route	Dose	Duration	Results	Ref.
Biochemical disorders	γ-radiation treated male albino rats	p.o.	50 mg/kg body weight	10 days	Attenuates oxidative stress, monoamines alterations and mitochondrial damage in the cerebral hemispheres	(Said et al., 2012)
Cellular damage	X-ray radiation treated female Balb/C mice	Topically	1 mg	3 days	Promotes DNA photo-damage repair.	(Jin et al., 2011)
	γ-radiation treated human peripheral blood lymphocytes		3.27, 6.55, 9.83, 13.10, 16.38 and 19.65 µM		(1) Modulates the toxic effects through its antioxidant potential (2) Protects human lymphocytes from the genetic damage and side effects induced by γ-irradiation	(Kalpana et al., 2009)
					Protects human lymphocytes from the genetic damage and side effects induced by γ-irradiation	(Hosseinimehr et al., 2009b)
					Protects human lymphocytes from the genetic damage and side effects induced by γ-irradiation	(Hosseinimehr et al., 2009a)
Hepatocellular damage	γ-irradiation treated male Sprague-Dawley rats	p.o.	50 and 100 mg/kg body	7 days	Protects γ-irradiation induced hepatocellular damage and	(Pradeep et al., 2008)

			weight		oxidative stress by exerting a protective effect against hepatocellular necrosis via its free radical scavenging and membrane stabilizing ability	
Inflammation	Radiation treated mice	p.o.	50 and 200 mg/kg body weight	6 weeks	(1) Enhances immunocompetence (2) Exerts beneficial effects on nutritional status (3) Decreases inflammation	(Lee et al., 2011)
Inflammatory skin diseases	UV radiation treated HaCaT cells		20 µg/mL		(1) Inhibits H_2O_2-induced interleukin-8 and tumor necrosis factor-a production as well as its mRNA expression (2) Inhibits activation of the nuclear factor-kB, phosphorylation of IkBa, p38 mitogen-activated protein kinase, and activation of cyclooxygenase-2	(Moon and Kim, 2012)
Skin damage	Ultraviolet B (UVB) treated SKH-1 mice	Topically	0.3 mg	10 days	Protects the skin via modulation of UVB-induced-oxidative damage, inflammation and cell proliferation	(Petrova et al., 2011)
Tissue damage	γ-radiation treated Sprague–Dawley rats	p.o.	50 and 100 mg/kg body weight	7 days	Offers effective protection against γ-radiation-induced cellular damage and oxidative stress	(Pradeep et al., 2012)

Table 6 Effects of hesperidin on other diseases

Disease		Experimental animals	Route	Dose	Duration	Results	Ref.
Acute respiratory distress syndrome (ARDS)		Endotoxin treated Male BALB/c mice	p.o.	200 mg/kg body weight		Exerts a markedly immunomodulatory effect	(Yeh et al., 2007)
Allergic asthma		Ovalbumin (OVA) treated female BALB/c mice	p.o.	10, 30 mg/kg body weight	3 days	Exhibits inhibitory activities not only for allergen-induced airway hyperresponsiveness (AHR) or airway eosinophilic inflammation, but also for airway remodeling due to the downregulation of allergen sensitization and/or Th2 polarizing pathways	(Wei et al., 2012)
				1 and 5 mg/kg body weigh	3 times a week for 5 weeks	Reduces Th2 cytokines (IL-5), eotaxin, OVA-specific IgE production, and eosinophil infiltration via inhibition of GATA-3 transcription factor	(Kim et al., 2011)
Arthritis	Rheumatoid arthritis	Collagen treated male Wistar rats	p.o.	160 mg/kg body weight	3 weeks	Inhibits collagen-induced arthritis through suppression of free radical load and reduction in neutrophil activation and infiltration	(Umar et al., 2013)

	Adjuvant arthritis	Freund's complete adjuvant treated male Sprague-Dawley Rats	p.o.	80 and 160 mg/kg body weight	10 days	Inhibits synoviocyte activity and modulating inflammatory cytokine production of synoviocytes	(Li et al., 2010)
				40, 80 and 160 mg/kg body weight	10 days	Improves Adjuvant arthritis by downregulating the function of over-active macrophages and by upregulating the activities of dysfunctional T lymphocytes	(Li et al., 2008a)
		Freund's complete adjuvant treated female Wistar rats	p.o.	80 mg/kg body weight	21 days	Inhibits both acute and chronic inflammation	(Guardia et al., 2001)
Colitis		Dextran sulphate sodium (DSS) treated male BALB/c mice	p.o.	10, 40 and 80 mg/kg body weight	7 days	(1) Decreases DAI, MPO activity, MDA content and the level of IL-6 in serum (2) Ameliorates DSS-induced experimental colitis	(Xu et al., 2009)
Hepatotoxicity		Lipopolysaccharide (LPS) treated Male Wistar rats	p.o.	50, 100, 200 mg/kg body weight	7 days	Attenuates LPS-induced hepatotoxicity by preventing cytotoxic effects of NO and oxygen free radicals	(Kaur et al., 2006)
Infection		Aeromonas hydrophila treated male MF1-albino mice	p.o.	250 mg/kg/week body weight	4 weeks	Exerts antimicrobial and immunomodulating activities against murine Aeromonas hydrophila infections.	(Abuelsaad et al., 2013)
Influenza		Influenza A virus (IAV) treated madin–Darby canine kidney (MDCK) cells				Prevents IAV replication by inhibition of viral sialidase activity that is involved in	(Saha et al., 2009)

						the entry and release stages on IAV infection	
Kidney diseases	Acute renal injury	Cisplatin treated male Wistar rats	p.o.	100, 200 mg/kg body weight	10 days	Attenuates acute renal injury by decreasing oxidative stress, inflammation and DNA damage	(Sahu et al., 2013)
	Nephrotoxicity	Gentamicin (GEN) treated male Wistar albino rats	p.o.	200 mg/kg body weigh	22 days	Acts as a potent scavenger of free radicals in the kidney to prevent the toxic effects of GEN both at the biochemical and histopathological levels	(Anandan and Subramanian, 2012)
		Acetaminophen treated male Wistar rats	p.o.	100, 200 mg/kg body weigh	2 weeks	Alleviates oxidative stress and toxicity	(Ahmad et al., 2012)
Liver injury		H_2O_2 treated human hepatic L02 Cells		20, 40 and 80 μM		Augments cellular antioxidant defense capacity through the induction of HO-1 via ERK/Nrf2 signaling	(Chen et al., 2010b)
		Tert-butyl hydroperoxide (t-BuOOH) treated human hepatic L02 Cells				(1) Prevents t-BuOOH induced cell damage by augmenting cellular antioxidant defense (2) Exhibits elevated activation of ERK/MAPK	(Chen et al., 2010a)
Nicotine Toxicity		Nicotine treated male albino Wistar rats	p.o.	25 mg/kg body weight	5 days a week for 22 weeks	Exerts protective effects against nicotine-induced toxicity	(Balakrishnan and Menon, 2007c)
						(1) Downregulates expression of matrix metalloproteinases (MMPs) (2) Enhances in antioxidant status	(Balakrishnan and Menon, 2007b)

					(1) Decreases level of marker enzymes such as SOD and CAT (2) Exerts antioxidant effect	(Balakrishnan and Menon, 2007a)
		p.o.	25, 50, 75, 100 and 150 mg/kg body weight	5 days a week for 22 weeks	Modulates the extent of lipid peroxidation	(Balakrishnan and Menon, 2007d)
Osteoporosis	Orchidectomy treated male ddY mice	diet	5 and 7 g/kg diet	4 weeks	Inhibits bone resorption and hyperlipidemia	(Chiba et al., 2013)
	Ovariectomized treated female Wistar rats	diet	5 g/kg diet	90 days	Inhibits ovariectomized-induced osteopenia	(Horcajada et al., 2008)
	Ovariectomized treated female ddY mice	diet	5.0 g/kg diet	4 weeks	(1) Inhibits bone loss (2) Decreases serum and hepatic lipids	(Chiba et al., 2003)
Oxidative stress	Male Wistar rats	p.o.	100 mg/kg body weight	90 days	Protects cardiomyocytes against age-related increase in oxidative stress mediated by nuclear factor erythroid 2-related factor 2 (Nrf2) upregulation	(Elavarasan et al., 2012)
	Male Swiss albino rats	i.p.	200 mg/kg body weight	28 days	Prevents acrylonitrile-induced oxidative stress	(El-Sayed el et al., 2008)

Table 7 Effects of hesperidin in clinical trials

Subjects	Study design	Age	Individual number	Dose	Duration	Results	Ref.
Individuals with metabolic syndrome	Controlled, double-blind, crossover study	21-65 years	Placebo-hesperidin:12 Hesperidin-placebo: 12	500 mg	3 weeks	(1) Improves endothelial dysfunction (2) Reduces circulating markers of inflammation	(Rizza et al., 2011)
Individuals with chronic venous disorders	Observational, multicentre and prospective study		917		12 weeks	Improves the symptoms and QoL	(Guex et al., 2009)
Health overweight men	Controlled, crossover study	50-65 years	Placebo: 24 Hesperidin: 24 Orange juice: 24	292 mg	4 weeks	(1) Decreases DBP (2) Increases endothelium-dependent micro-vascular reactivity	(Morand et al., 2011)
Hypercholesterolemic individuals	Controlled, parallel study	18-75 years	Control: 65 Hesperidin: 59 Naringin: 64	800 mg	4 weeks	Exerts no cholesterol-lowering effect	(Demonty et al., 2010)

Table 8 Effects of Daflon in clinical trials

Target	Study design	Age	Individual number	Dose	Duration	Results	Ref.
Haemorrhoids	Prospective, multi-center, observational study	15-84 years	268	500 mg twice daily	4 weeks	(1) Alleviates haemorrhoidal symptoms (pain, heaviness, bleeding, pruritus and anal discharge) (2) improves the proctoscopic appearance of haemorrhoids	(Meshikhes, 2004)
	Prospective study	19-70 years	105	500 mg twice daily	4 weeks	(1) Improves pain, heaviness, bleeding, pruritus, and mucosal discharge (2) Improves the proctoscopic appearance	(Meshikhes, 2002)
	Double-blind, controlled study		Daflon: 60 Control: 60	500 mg twice daily	2 months	Improves chronic and acute symptoms	(Godeberge, 1994)
	Double-blind controlled study		Daflon and control: 100	1500 mg twice daily the first four days and 1000 mg twice daily the following three days	7 days	Relieves signs and symptoms of acute hemorrhoids	(Cospite, 1994)

	Double-blind, controlled study	Average: 32 year	Daflon and control: 100	1000 mg twice daily	14 days	Improves objective signs including swelling, congestion, bleeding, exudation and prolapse.	(Thanapongsathorn and Vajrabukka, 1992)
Chronic venous insufficiency (CVI)	Controlled study	Average: 65 year	Daflon: 82 Control: 68	500 mg twice daily	6 months	(1) Reduces ulcer surface (2) Accelerate the healing process (3) Increases patients' quality of life	(Roztocil et al., 2003)
	Double-blind, controlled study		Daflon: 39 Control: 38	500 mg twice daily	2 months	Protects against the deleterious influence of stasis on microcirculatory (BF) and hemorheologic (RBC aggregation) parameters	(Le Devehat et al., 1997)
	Open pilot study		24	500 mg daily	28 days	(1) Resolves stasis with an increase in red blood cell velocity (2) Improves flexibility of red blood cells	(Allegra et al., 1995)

	Controlled study		Daflon: 183 Control: 183			(1) Decreases venous capacitance, distensibility, and emptying time (2) Ameliorates severity of symptoms (3) Decreases the supramalleolar circumference	(Geroulakos and Nicolaides, 1994)
	Double-blind, multicenter study		Daflon and control: 90	500 mg twice daily	2 months	Ameliorates severity of symptoms and plethysmographic parameters	(Amato, 1994)
	Double-blind controlled study		Daflon and control: 200	500 mg twice daily	2 months	(1) Reduces CVI signs and symptoms (2) Improves venous hemodynamics according to plethysmographic parameters	(Laurent et al., 1988)
	Controlled study			500 mg twice daily		(1) Improves venous capacitance, distensibility, and emptying times (2) Shows rapid onset and long duration of action (3) exerts hemodynamic effect on the venous system	(Duchene Marullaz et al., 1988)

Lymphedema	Monocenter, double-blind, parallel controlled study		Daflon: 46 Control: 48	500 mg twice daily	6 months	(1) Improves lymphatic migration speed (2) Improves the half-life of the colloidal compound	(Pecking et al., 1997)
	Open pilot study	44-64 years	10	500 mg twice daily	6 months	(1) Ameliorates severity of symptoms and limb volume (2)Decreases volume of the swollen limb (3) Improves functional parameters (half-life, clearance and lymphatic speed of the colloid)	(Pecking, 1995)
Abnormal venous elasticity without varicose veins	Controlled study	18-35 years	Daflon: 12 Control: 13	500 mg twice daily	1 month	Improves venous tone	(Ibegbuna et al., 1997)
Idiopathic cyclic oedema (ICO)	Double-blind, controlled study		Daflon and control: 30	500 mg twice daily	6 weeks	Reveals a decrease in the degree of retention	(Behar et al., 1988)
Pelvic congestion syndrome	Crossover study	28-35 years	Daflon-control: 10 Control-Daflon: 10	500 mg twice daily	6 months	(1) Improves venous tonus (2) Restores pelvic circulation and relieves pelvic symptomatology	(Simsek et al., 2007)
Postoperative thromboembolism	Controlled study	40 years or older	Group A (Enoxoparin 20 mg or fraxiparin 0.3 ml): 591	1000 mg every 8 hours during the day before surgery; 1000	15 days	Prevents symptomatic thromboembolism	(Tsimoyiannis et al., 1996)

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			Group B (Group A+Daflon): 595 Group C (Enoxoparin 40 mg or fraxiparin 0.6 ml): 93 Group D (Group C+Daflon): 93	mg every 6 hours before surgery; 1000 mg once a day on postoperative days 4 to 15			
Venous ulcers	Multicenter, double-blind, controlled study	18-85 years	Daflon: 53 Control: 52	500 mg twice daily	2 months	Accelerates complete healing	(Guilhou et al., 1997)

Table 9 Effects of Rikkunshito in clinical trials

Target	Study design	Age	Individual number	Dose	Duration	Results	Ref.
Gastric cancer	Prospective study	Average: 61.9 years	25	2.5 g ter in die (t.i.d.)	4 weeks	(1) Attenuates gastrointestinal symptoms (2) Increases acylated ghrelin concentration	(Takiguchi et al., 2013)
Functional dyspepsia	Paralleled, controlled trial	Average: 56.5 years	Rikkunshito: 13 Domperidone: 14	2.5 g t.i.d.	4 weeks	(1) Improves upper gastrointestinal symptoms (2) Increases acylated ghrelin concentration	(Arai et al., 2012)
	Crossover trial	19-22 years	9	2.5 g t.i.d.	4 weeks	Improves symptoms and impaired gastric accommodation under distention stimuli of the proximal stomach superimposed by stress.	(Shiratori et al., 2011)
	Prospective study	28-75 years	16	7.5g bis in die (b.d.)	2 weeks	Attenuates impaired gastric accommodation reflex and gastric motility	(Kusunoki et al., 2010)
Gastroesophageal reflux disease	prospective, multicenter, parallel comparative study	Average: 63.6 years	Rikkunshito: 53 Rabeprazole: 51	7.5 g t.i.d.	4 weeks	Decreases the frequency scale for the Gastroesophageal reflux disease score	(Tominaga et al., 2012)
Pylorus-preserving gastrectomy	Crossover design	46-70 years	11	7.5 g quarter in die (q.d.)	8 weeks	(1) Improves gastric emptying (2) Ameliorates postoperative	(Takahashi et al., 2009)

						symptoms	
Postoperative nausea and vomiting (PONV)	Controlled trial		Rikkunshito: 91 Control: 51	2.5 g q.d.	3 days	(1) Reduce the severity of PONV (2) Promotes earlier recovery of oral meal intake after gynecological laparoscopic surgery	(Okuno et al., 2008)

