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REVIEW ARTICLE

Chemistry and Pharmacology of The Citrus Bioflavonoid Hesperidin

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Hesperidin, a bioflavonoid, is an abundant and inexpensive by-product of Citrus cultivation. A deficiency of this substance in the diet has been linked with abnormal capillary leakiness as well as pain in the extremities causing aches, weakness and night leg cramps. No signs of toxicity have been observed with the normal intake of hesperidin or related compounds.

Both hesperidin and its aglycone hesperetin have been reported to possess a wide range of pharmacological properties. This paper reviews various aspects of hesperidin and its related compounds, including their occurrence, physical and chemical properties, analysis, pharmacokinetics, safety and toxicity and the marketed products available. A special emphasis has been laid on the pharmacological properties and medicinal uses of these compounds. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: hesperidin; hesperetin; bioflavonoid; pharmacology; analysis.

INTRODUCTION

Flavonoids comprise a large group of naturally occurring, low molecular weight, polyphenolic compounds widely distributed in the plant kingdom as secondary metabolites. They represent one of the most important and interesting classes of biologically active compounds and occur both in the free state and as glycosides. They are based on the parent compound, flavone (2-phenyl chromone or 2-phenyl benzopyrone), characterized by a C_6-C_3-C_6 carbon skeleton where the C_6 components are aromatic rings (Fig. 1). Flavonoids occur in practically all parts of plants including fruit, vegetables, nuts, seeds, leaves, flowers and bark (Middleton, 1984).

Dr Albert Szent-Gÿorgi, a famed Hungarian researcher, found that Citrus peel flavonoids were effective in preventing capillary bleeding and was the first to report the biological activity of flavonoids on capillary fragility associated with scurvy (Armentano *et al.*, 1936; Szent-Gÿorgi, 1938). The broad spectrum of biological activities within the group and the multiplicity of actions displayed by certain individual members make the flavonoids one of the most intriguing classes of biologically active compounds, and thus these are often termed 'bioflavonoids'.

Hesperidin is an abundant and inexpensive by-product of Citrus cultivation and is the major flavonoid in sweet orange and lemon. In young immature oranges it can account for up to 14% of the fresh weight of the fruit (Barthe *et al.*, 1988). It is usually found in association with vitamin C. Some symptoms originally thought to be due to vitamin C deficiency such as bruising due to capillary fragility were found in early studies to be relieved by crude vitamin C extract but not by purified vitamin C. The bioflavonoids, formerly called 'vitamin P', were found to be the essential components in correcting this bruising tendency and improving the permeability and integrity of the capillary lining. These bioflavonoids include hesperidin, citrin, rutin, flavones, flavonols, catechin and quercetin.

Of historical importance is the observation that 'citrin', a mixture of two flavonoids, eriodictyol and hesperidin, was considered to possess a vitamin-like activity, as early as in 1949 (Scarborough and Bacharach, 1949). This material was later termed 'vitamin P' to indicate that it could decrease capillary permeability and fragility, prolong the life of marginally scorbutic guinea-pigs and reduce the signs of hypovitaminosis C. Although the term vitamin P was subsequently abandoned, there was a strong indication that the material had potent antioxidant-dependent vitamin C sparing activity (Clemetson, 1989). Hesperidin deficiency has since been linked with abnormal capillary leakiness as well as pain in the extremities causing aches, weakness and night leg cramps. Supplemental hesperidin also helps in reducing oedema or excess swelling in the legs due to fluid accumulation. As with other bioflavonoids, hesperidin works best when administered concomitantly with

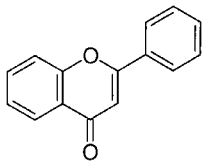

Figure 1. The flavone nucleus (2-phenylbenzopyrone)

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vitamin C. No signs of toxicity have been observed with normal intake of hesperidin.

Hesperidin was first discovered in 1827, by Lebreton, but not in a pure state and has been under continuous investigation since then (Fluckiger and Hanbury, 1986). Though it has been found to possess a wide range of pharmacological properties, no comprehensive report is available on this compound. The following provides a comprehensive review of this particular bioflavonoid.

OCCURRENCE

Hesperidin is isolated in large amounts from the discarded rinds of the ordinary orange *Citrus aurantium* L. (Kanes *et al.*, 1993; Emim *et al.*, 1994), *C. sinensis* (Horowitz and Gentili, 1963), *C. unshiu* (Kawaguchi *et al.*, 1997) and other species of the genus Citrus (family Rutaceae). It has been reported to occur in many plants other than Citrus, such as in genera Fabaceae (Bhalla and Dakwake, 1978), Betulaceae (Pawlowska, 1980), Lamiaceae (Kokkalou and Kapetanidis, 1988) and Papilionaceae. Its presence in the bark of *Zanthoxylum avicennae* and *Z. cuspidatum* (family Rutaceae) has been reported (Arthur *et al.*, 1956). These plants are indigenous to Hong Kong. The presence of hesperetin- 7-β-neohesperidoside (neohesperidin) was recently reported in *C. humilis* and *C. cornigera*, two *Cynara* species growing in Greece (Chinou and Harvala, 1997). Hesperidin has also been isolated from the roots of *Acanthopanax setchuenensis* (family Araliaceae), collected in Sichuan Province, China (Zhao *et al.*, 1999).

Hesperidin occurs in greatest concentration in green fruit and its concentration in the fruit increases during storage (Higby, 1941). Its distribution in the epicarp, mesocarp, endocarp and juice of Citrus fruits has been reported (Kawaguchi *et al.*, 1997). The distribution and concentration of hesperidin within the different tissues of mature fruit of *C. sinensis* has been measured using a radioimmunoassay method and it was found to present in high levels in the albedo, membranes and the pith whereas the concentration was much lower in the juice vesicles and seeds. In seeds, the hesperidin content increases after germination suggesting that there is a net production of this compound in the developing seedling, which is partly stimulated by light (Barthe *et al.*, 1988).

Hesperidin occurs in crystalline, feather-like aggregates or sphaerocrystalline masses in the cells (Evans, 1996). Di Mauro *et al.* (1999) have recently described a novel procedure for obtaining hesperidin from waste orange peel of the Citrus industry based on the adsorption of dilute extracts of hesperidin on a styrene-divinylbenzene resin.

PROPERTIES

Pure hesperidin occurs as long hair-like needles, tan or pale yellow in colour. Its melting point ranges from 258° to 262°C (softens at 250°C). It has a molecular formula $C_{18}H_{34}O_{15}$ and a molecular weight of 610.57 daltons. It is easily soluble in dilute alkali and in pyridine giving a clear yellow solution, slightly soluble in methanol, hot glacial acetic acid and almost insoluble in acetone, benzene and chloroform. The solubility in water is 1 in 50 (Budavari, 1996). It has a property of forming complex crystals with other similar glucosides, which greatly affects its solubility and other physical properties, making it difficult to obtain in a pure state (Higby, 1941). It can, however, be purified by washing with hot water and extraction with 95% methyl alcohol, followed by crystallization (King and Robertson, 1931). It is tasteless and odourless (Kometani *et al.*, 1996).

CHEMISTRY

Hesperidin (Fig. 2) is a flavanone glycoside, comprising of an aglycone, hesperetin (Fig. 3) or methyl eriodictyol (Evans, 1996) and an attached disaccharide, rutinose. Hesperidin is, therefore, a β-7-rutinoside of hesperetin (Preston *et al.*, 1953). The disaccharide unit ($C_{12}H_{22}O_{10}$) is composed of one molecule of rhamnose and one of glucose and may assume one of the two isomeric forms, rutinose or neohesperidose. Rutinose (Fig. 4) is chemically 6-O-(6-deoxy-α-L-mannopyranosyl)-D-glucose or O-α-L-rhamnosyl-(1 $\rightarrow$ 6)glucose. Neohesperidose is chemically O-α-L-rhamnosyl-(1 $\rightarrow$ 2)glucose, differing only in the configuration of the two sugar units (Harborne, 1994). The position of the sugars in hesperidin was determined by partial hydrolysis with dilute acid leading to the formation of L-rhamnose and hesperetin-7-β-D-glucoside which could be cleaved by the enzyme β-D-glucosidase (Fox *et al.*, 1953). Hence, in hesperidin, glucose is attached to hesperetin and rhamnose is attached to the glucose. Hesperetin ($C_{16}H_{14}O_6$) chemically is 3′, 5, 7-trihydroxy-4′-methoxy flavanone. Hesperidin is thus 3′, 5, 7-trihydroxy-4′methoxyflavanone-7-(6-α-L-rhamnopyranosyl-β-D-glucopyranoside or -7-rutinoside (Calomme *et al.*, 1996).

Hesperidin, upon alkaline hydrolysis yields phloroglucinol and hesperetenic acid (Higby, 1941). Upon acid

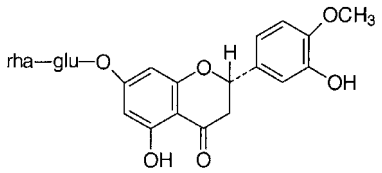

Figure 2. Hesperidin (hesperetin-7-rhamnoglucoside)

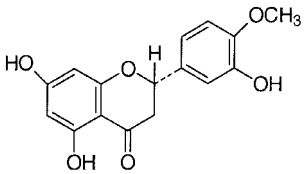

Figure 3. Hesperetin (3, 5,7-trihydroxy-4-methoxyflavanone)

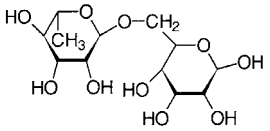

Figure 4. Rutinose [O-α-L-rhamnosyl-(1 $\rightarrow$ 6)glucose]

hydrolysis, hesperidin produces one mole each of the aglycone hesperetin, D-glucose and L-rhamnose (Asahina *et al.*, 1930). Hydrolysis with either dilute sulphuric acid or sulphuric acid in ethylene glycol yields an optically active mixture of (±)- and (−)-hesperetin, which can be separated by fractional recrystallization. Both have been characterized by conversion into derivatives (Arthur *et al.*, 1956). Under carefully controlled conditions of acid hydrolysis, hesperidin yields the optically active laevo-rotatory aglycone, (−)-hesperetin, and this on ozonization, gives L- malic acid, indicating that (−)-hesperetin has the 2S-configuration (Arakawa and Nakazaki, 1960).

There is a known relation between the structure of the disaccharide and the presence or absence of bitterness in the compound (Horowitz and Gentili, 1963). Rutinosides are tasteless, whereas neohesperidosides are intensely bitter. Hesperidin itself is a flavonoid rutinoside and is thereby non-bitter (Kometani *et al.*, 1996). The bitter neohesperidosides mainly accumulate in grapefruit whereas the non-bitter rutinosides predominate in orange and lemon (Horowitz, 1961).

IDENTIFICATION AND ANALYSIS

Both hesperidin and hesperetin show a characteristic flavanone absorption spectrum with UV maxima at 286 and 289 nm, respectively and an inflection of low intensity at 330 nm (Jurd, 1962).

The ^{1}H *NMR spectra* of TMS ether of both hesperidin and hesperetin in CCl_4 have been published (Mabry *et al.*, 1970). The ^{1}H NMR spectra of hesperidin in DMSO-d_6 solution has also been studied and the values for chemical shifts and coupling constants presented (Nieto and Gutierrez, 1986). Aged samples of hesperidin in DMSO show NMR lines from most protons split into two sets of signals, the second being assigned to an isomer of hesperidin with a change of the natural configuration at the C_2 atom. A ^{13}C NMR spectrum of hesperidin has been published (Agarwal, 1989). Markham and Ternai (1976) have described the ^{13}C NMR of hesperidin and compared it with those of other major flavonoid groups including glycosides, relating to the one basic solvent system, DMSO-d_6.

A method for the *fluorimetric determination* of hesperidin has been developed, based on the reaction between rutin and dimethylformamide to form a fluorescent chelate. This chelate shows two excitation maxima at 295 and 370 nm and one emission maximum at 460 nm, indicating a 1:1 molar ratio of ligand to metal ion (Kaito *et al.*, 1979). Manual and flow injection methods are also used along with the spectrofluorimetry (Perez-Ruiz *et al.*, 1999).

Other methods have been developed for the *spectrophotometric determination* of hesperidin by forming its complexes with certain metals. Copper (II)–hesperidin complex (Kuntic *et al.*, 1999), uranil (II)–hesperidin complex (Kuntic *et al.*, 1998), zirconium (IV)–hesperidin complex (Radovic *et al.*, 1996) and aluminium (III)–hesperidin complexes (Malesev *et al.*, 1997) have been formed in water–methanol systems and the determination of the compound is found to be precise (Radovic *et al.*, 1996).

Radioimmunoassay (RIA) methods, which combine sensitivity, specificity, high sample capacity, speed and relative simplicity, are finding wide application for the quantification of plant secondary compounds. A RIA method has been developed for hesperidin utilizing antibodies raised against a hesperidin-4-O-carboxy-methyl-oxime hapten and a tritiated radiotracer prepared by direct reduction of hesperidin with $NaB[^{3}H]_4$. The method possesses a detection limit and measuring range at nanogram levels, a low coefficient of variation and a good correlation with HPLC (Barthe *et al.*, 1988).

Capillary electrophoresis is another method that has been used to simultaneously analyse hesperidin and neohesperidin, along with a range of other compounds. Separation could be easily achieved and the compounds could be subsequently quantified. The method allows rapid monitoring with great specificity (Cancalon, 1999).

A large number of scientists have worked on the development of *chromatographic methods* for the estimation of hesperidin and its analogues. Höerhammer and Wagner (1962), separated and purified Citrus flavonoids by preparative TLC (silica gel) using butanol, acetic acid and water (4:1:5) as the solvent system. The separation of hesperidin, hesperetin, eriodictin, naringin and naringenin was successfully accomplished. Various scientists have worked on the separation and quantification of hesperidin and/or hesperetin from a mixture of a number of flavonoids, using HPLC. Most of these utilize a reversed phase C-18 column and an aqueous mobile phase consisting of water, acetonitrile, methanol and tetrahydrofuran coupled with minor amounts of acids (Fisher, 1978; Casteele *et al.*, 1982; Rouseff, 1988; Rouseff *et al.*, 1992; Mouly *et al.*, 1993; Wang *et al.*, 1994; Mouly *et al.*, 1998; Saija *et al.*, 1998; Ross *et al.*, 2000). The detection is accomplished using a UV detector set at 280 nm. Most of these methods are precise and accurate and can be used for quality control of industrial concentrates and juices. Sheu and Lu (1995) developed a HPLC method for the simultaneous determination of the constituents of a Chinese herbal formula, Hsiao-cheng-chi-tang, containing hesperidin as one of the ingredients. This method utilizes a Cosmosil-5 C-18 column with acetate buffer as the mobile phase and the publication considers the effects of pH, buffer concentration and column selectivity.

A reversed phase HPLC system has been used to separate 141 flavonoids including hesperidin, hesperetin and neohesperidin (Casteele *et al.*, 1982). The column used was Lichrosorb RP-18, the mobile phase a combination of an isocratic and gradient elutions (5% aqueous formic acid and methanol) and a UV detector set at 280 nm. They also discussed the correlations between the structure and retention time values of different flavonoids. Thirty four selected flavonoids including hesperidin and hesperetin were analysed by HPLC, using a μBondapak C-18 column and UV detector at 254 nm (Daigle and Conkerton, 1982). This method employs two pumps, one eluting water–acetic acid (495:5) and the other eluting methanol. The retention times were measured to calculate two chromatographic parameters—the capacity factors and the relative retentions. They emphasized that 'adsorption' and not the size of the molecule is operative in its separation. Another simple and precise method has been established for the simultaneous determination of five selected marker components, including hesperidin, in an oriental pharmaceutical decoction—'Heii-San', by means of HPLC using tetra-N-amylammonium bromide (TAA) as an ion-

pair reagent. An ODS column has been used with multi-step gradient elution with 10 mM TAA (H_3PO_4, pH 4.0)-acetonitrile. The acetonitrile increased linearly from 24% to 90%. This method has also been compared with three other methods, i.e. a water–acetonitrile method, a phosphate buffer method and an ion-suppression method, and was found to give comparable and satisfactory results (Yamauchi *et al.*, 1996). In an attempt to screen the flavonoids from *Larix* species, needles of different larch species were extracted and analysed on HPLC using Dupont 830 chromatograph with a Zorbax ODS column using a gradient of 45% methanol in water to 100% methanol, both with 0.1% acetic acid at 50°C, with double detection at 254 and 360 nm. Hesperidin and hesperetin were eluted at a retention time of 6.5 and 9.9 min, respectively (Niemann and Koerselman-Kooy, 1977).

Hesperidin and neohesperidin have been analysed by liquid chromatography/mass spectrometry (LC-MS) with a turbo-ionspray (TBS) interface. Characterization of isomers differing in the glycosylation was found to be possible on the basis of different mass spectra (Careri *et al.*, 1999). Another LC-MS method using a capillary scale particle beam interface has been developed for the sensitive detection of flavonoid compounds in complex matrices (Capiello *et al.*, 1999).

Other methods used in the analysis of hesperidin include *spectrodensitometry* (El-Bayoumi, 1999), *gas–liquid chromatography* of the trimethylsilyl ether (Drawert *et al.*, 1980) and *micellar electrokinetic capillary chromatography*, MECK (Ferreres *et al.*, 1994). *Voltammetry* has also been utilized (Obendorf and Reichart, 1995; Zoulis and Efstathiou, 1996; Volikakis and Efstathiou, 2000).

COMBINATION PRODUCTS

Hesperidin is commonly used in traditional medicines as a combination product. The most widely used combination product of hesperidin is Daflon- 500 mg® (Servier; Switzerland), which contains hesperidin (50 mg) and diosmin (450 mg). Diosmin (Fig. 5) is another bioflavonoid with a molecular weight of 608.6 daltons and is chemically diosmetin-7-rhamnoglucoside. The aglycone diosmetin can be described by the systematic name 3′, 5,7-trihydroxy-4′-methoxyflavone or 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one. It differs from hesperetin only in having a double bond between C2 and C3, thereby being a flavone, whereas hesperetin is a flavanone.

Treatment with Daflon-500 mg® has demonstrated a wide variety of pharmacological activities including beneficial effects in human subjects with chronic venous insufficiency. Two hundred and fifteen human subjects were administered one tablet of the drug, twice daily for one year. Subjects recorded an overall assessment of efficacy and relief from venous symptoms such as cramps and evening oedema. Clinical laboratory values remained within normal range throughout the study year (Guillot *et al.*, 1989).

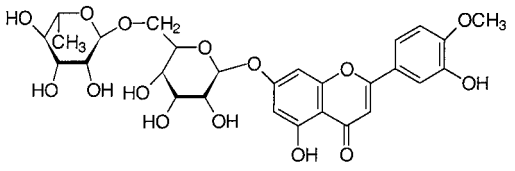

Figure 5. Diosmin (diosmetin-7-rhamnoglucoside)

SAFETY AND TOXICITY

In general, Citrus bioflavonoids including hesperidin appear to be extremely safe and without side effects even during pregnancy (Pizzorno Jr and Murray, 1999). Based on an experiment in male and female mice (Sieve, 1952), it was established that phosphorylated hesperidin (PH) was nontoxic both to the organism and to tissues, easily assimilated, nonaccumulative and caused no allergic reactions. Based on these results Sieve performed a study on humans and reported that phosphorylated hesperidin could be given to humans clinically as an anti fertility agent, along with other substitution factors such as vitamins, endocrines, amphetamine derivatives and decholic acid derivatives. Besides this, trauma, infectious diseases or systemic diseases did not inhibit its antifertility effect.

In a study in rats, when Daflon-500 mg® was administered by gastric intubation for 26 weeks, no deaths, changes in weight or abnormalities of standard functional tests were observed. A mixture of pure compounds in a ratio of 1:9 was used to obtain the reported effect (Damon *et al.*, 1987). Methyl hesperidin, when administered orally to mice at a level as high as 5% in the diet, exerted no mutagenic or carcinogenic effects. No obvious toxic effects in mice of either sex were observed (Kawabe *et al.*, 1993). Moreover, the ingestion of hesperidin did not affect the daily food intake, body weight gain or food efficiency (Kawaguchi *et al.*, 1997).

In a study in humans hesperidin administration was found to result in minor side effects in only 10% of the subjects compared with 13.9% of those treated with placebo (Meyer, 1994). However, there have been some reports of interactions between the aglycone hesperetin (Mitsunaga *et al.*, 2000), hesperidin (Melzig *et al.*, 1997) and conventional drugs.

DRUG–FOOD INTERACTIONS

Hesperidin, as such has not been widely reported to interact with drugs or food substances (Kawaguchi *et al.*, 1997). In an early study conducted on human volunteers, PH was found to have no interaction when given concomitantly with vitamins, endocrines, amphetamine derivatives and decholic acid derivatives (Sieve, 1952).

In a recent study conducted to see whether the grapefruit bioflavonoids alter the permeation of vincristine (an agent used in cancer therapy), across the blood–brain barrier, it was observed that hesperetin increased the [3H] vincristine uptake in the 10–50 μM range, but the glycoside did not have any effect (Mitsunaga *et al.*, 2000). This effect was thought to be brought about by the stimulation of P-glycoprotein-mediated drug efflux through the cells. This indicated that the patients taking drugs that are P-glycoprotein substrates, may need to

restrict their intake of bioflavonoid containing foods and beverages. Hesperidin along with other flavonoids was also found to interact with daunomycin (Melzig *et al.*, 1997).

PHARMACOKINETICS

The pharmacokinetics of hesperidin has not been discussed in great detail in the literature but a few references are available.

Absorption

To evaluate the oral absorption of hesperidin from Citrus products, healthy white males (human volunteers), 25 years of age, were given 500 mg of the drug in water and equivalent amounts of grapefruit and orange juice. It was absorbed from the gastrointestinal tract after oral administration in any form, but cumulative urinary recovery indicated low bioavailability (<25%). The aglycone, hesperetin, was detected in both urine and plasma. Absorbed Citrus flavanones were thought to undergo glucuronidation before urinary excretion (Ameer *et al.*, 1996). Intestinal permeability to hesperidin glycosides was investigated by using a cultured monolayer of Caco-2 cells as a model for the small intestinal epithelium. Whereas hesperidin did not permeate across the Caco-2 monolayer probably owing to its low solubility, its glycosides did permeate, in a time- and dose-dependent manner. This permeation was thought to occur via a paracellular pathway (Kim *et al.*, 1999). The absorption of orally administered hesperidin in rabbits has been found to depend on the diet. It was absorbed when given with a synthetic ration but not with a commercial pelleted ration (Williams, 1964).

Metabolism

The metabolic fate of six flavonoids including hesperidin and hesperetin was studied following oral ingestion in rats. The major metabolic product in the urine was m-hydroxyphenylpropionic acid along with lesser amounts of m-coumaric acid and the aglycones. The aglycones were free as well as conjugated with glucuronic acid. This indicated that absorption had occurred from the intestinal tract followed by dehydroxylation, demethoxylation or demethylation followed by dehydroxylation to yield m- hydroxyphenylpropionic acid. This study also indicated that hesperetin was more readily absorbed than hesperidin in rats, rabbits, as well as in humans (Fig. 6) (Booth *et al.*, 1958). When human volunteers ingested hesperidin, a marked difference was observed in the metabolism. 3-Hydroxy-4-methoxyphenylhydracrylic acid was obtained as the major urinary metabolite, indicating that the pyran ring of hesperetin splits to yield this hydracrylic acid. A small amount of the glucuronide of hesperetin was also detected (Booth *et al.*, 1958; Williams, 1964). Hesperidin was found to be transformed to its aglycone, hesperetin, in the intestine by the bacteria producing alpha-rhamnosidase and beta-glucosidase or endo-beta-glucosidase. It was also found that the antiplatelet activity and cytotoxicity of the metabolite formed in the human intestine was more pronounced than that of the parent compound (Kim *et al.*, 1998).

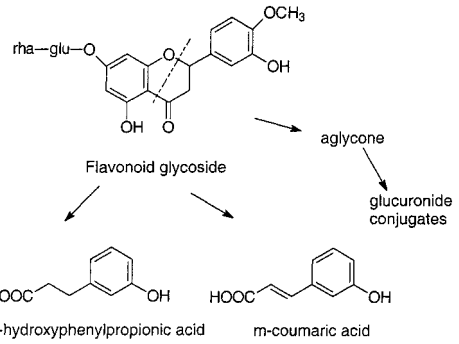

Figure 6. Metabolic fate of a flavonoid glycoside in the rat

Excretion

Recently a study was carried out to study the plasma kinetics and urinary excretion of hesperetin and naringenin from grapefruit and orange juice. Healthy volunteers ingested the juices; blood and urine samples were collected and analysed by HPLC with electrochemical detection. Hesperetin was found to be bioavailable from the study juices with marked inter-individual variations. The urine levels were variable enough to conclude that urinary excretion levels could not be used as biomarkers for dietary intake (Erlund *et al.*, 2001). Animal studies showed virtually complete elimination of Daflon-500 mg®, 96 h after administration, without any untoward accumulation in any particular organ (Meyer, 1994).

A resorption and excretion study was performed on ^{14}C-hesperidin methylchalcone (a hydrosoluble, semi-synthetic derivative of natural hesperidin) in rats and it was observed that, at a dose of 10 mg/kg body weight, it was absorbed 1–2 h after oral administration. The blood kinetic patterns suggested an entero–hepatic cycle, demonstrated by the intravenous administration of the compound at the same dose. The blood profiles demonstrated good bioavailability of the drug. Urinary excretion was lower than faecal excretion after oral ingestion but both were comparable after administration by intravenous route. Moreover, excretion mainly occurred in the first 24 h following administration, by either route (Chanal *et al.*, 1981).

PHARMACOLOGICAL EFFECTS

Hesperidin is effectively used as a supplemental agent in treatment programmes and protocols of broadly complementary settings.

Effects on the vascular system

Hesperidin supplementation has been used in patients suffering from blood vessel disorders including fragility and permeability complaints resulting in easy bruising and varicosities. This effect of hesperidin has been of

considerable interest in the last decade and a large number of publications have addressed this therapeutic application.

Increased permeability of blood capillaries is a feature of several disease states and is manifested in such symptoms as oedema, bleeding and hypertension. Diseases which are usually associated with increased capillary permeability include diabetes, chronic venous insufficiency, haemorrhoids, scurvy, various ulcers and bruising. Early research on flavonoids from plant extracts established that they reduced the permeability and fragility of capillary walls (Bisset *et al.*, 1991). Hesperidin, along with other flavonoid compounds has been widely reported to inhibit an increase of capillary permeability. As early as in 1939, Morii found that daily administration of 30 mg of hesperidin decreased the capillary permeability and increased capillary resistance in various clinical cases suffering from pleurisy, tuberculosis, Grave's disease and beriberi (Morii, 1939). Since then, hesperidin has increasingly been used as a therapeutic agent for increasing capillary resistance.

Scarborough (1940) used hesperidin in the treatment of purpurea, resulting from the use of arsenicals and in the treatment of syphilis. He reported that hesperidin, as well as hesperetin, were active for increasing capillary resistance Higby (1941) reported the successful use of hesperidin in the clinical treatment of haemorrhagic purpurea and disorders arising from abnormal capillary fragility. This role of hesperidin in increasing capillary resistance has been attributed to its inhibitory effect on the action of hyaluronidase enzyme, which in turn has been known to accentuate capillary permeability and fragility (Beiler and Martin, 1947).

Struckmann and Nicolaides (1994) used Daflon-500 mg® to treat chronic venous insufficiency. This effect has been ascribed to an inhibition of inflammatory processes in the ischaemia-induced hyperpermeability that characterizes venous stasis (Jean and Bodinier, 1994). The micronized purified flavonoid fraction containing diosmin and hesperidin (9:1) was found to prevent ischaemia/reperfusion induced leukocyte adhesion in skeletal muscles localized in the ischaemic region (Korthuis and Gute, 1999). An open pilot study, on 24 human patients with third stage chronic venous insufficiency, indicated a beneficial haemorheological effect on oral administration, resolving the stasis with an increase in the blood cell velocity. Furthermore a concomitant increase in relative packed cell volume and red blood cell velocity after therapy suggests an improvement in the flexibility of red blood cells (Allegra *et al.*, 1995). Daflon is also found to possess venotonic and vasculo-protective pharmacological properties and reinforces venous tone by prolonging the activity of parietal norepinephrine (Amiel and Barbe, 1998).

An antihypercholesterolaemic activity of hesperidin in CCl_4-induced hypercholesterolaemic rats has been reported (Son *et al.*, 1991). Intragastric hesperidin has been found to significantly lower cholesterol, low density lipoproteins (LDL), total lipids and triglyceride levels in normo-lipidaemic rats and in diet- or Triton-induced dyslipidaemic rats but increased high density lipoprotein (HDL) levels (Monforte *et al.*, 1995). Tangerine peel extract and a mixture of hesperidin and naringin were also found to significantly lower levels of plasma and hepatic cholesterol, and hepatic triglycerides compared with those of the control. They also reduced the excretion of faecal neutral sterols compared with the control (Bok *et al.*, 1999). These effects are accompanied by a reduction in the plasma and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA (HMGCoA) reductase and acyl CoA: cholesterol transferase (Bok *et al.*, 1999). The hypocholesterolaemic effect of hesperetin in rats may also be mediated via these enzymes (Lee *et al.*, 1999a).

Hesperidin has also been reported to exert antihyperlipidaemic effects and the methanolic extracts of the stems of *Prunus davidiana* Fr. (Rosaceae), containing hesperetin-5-glucoside and other flavonoids, was found to be useful for the treatment of hyperlipidaemia. Intraperitoneal administration of hesperetin-5-glucoside to rats fed on a high fat diet was found to significantly reduce the total cholesterol level compared with the control group but had no effect on the serum triglyceride level (Choi *et al.*, 1991). In another study, the antilipaemic activity of hesperidin in normolipaemic and induced hypercholesterolaemic rats has been demonstrated (Monforte *et al.*, 1995). Hesperidin exerted significant activity, which may be due to increased hepatic cholesterol catabolism. In yet another study, the flavonoids from *C. unshiu* were screened for lipase inhibitory effect and hesperidin was found to inhibit lipase activity from porcine pancreas and that from *Pseudomonas*. Moreover, hesperidin (10%, p.o), decreased plasma triglyceride concentration and increased the amount of faecal lipids excreted in rats (Kawaguchi *et al.*, 1997).

The calcium channel blocker activity of hesperidin has been patented (Morita *et al.*, 1992). It is found to inhibit nitrendipine binding to skeletal muscle membrane protein in rabbits. The capillary antihaemorrhagic activity of hesperidin in mice has also been reported (Jurisson, 1973). Kubo *et al.* (1992a,b) have reported and filed patents relating to the myocardial depressant activity of hesperidin in human adults.

Galati *et al.* (1996) have demonstrated significant antihypertensive and diuretic effects of hesperidin in rats following oral administration of the drug at a dose of 200 mg/kg body weight and ascribed this hypotensive effect to increased diuresis. However, other mechanisms are probably involved. It is well established that various flavanones, including hesperidin, influence various enzymes such as protein kinase, lipooxygenase and cyclooxygenase. This effect on enzymes is responsible for the flavonoids' activity on some haematic parameters, lowering of erythrocytic adhesion, platelet aggregation and blood viscosity. The antihypertensive activity of hesperidin might therefore be due to its activity on enzymatic systems that influence blood rheology. In addition, various flavonoids are potent inhibitors of cyclic-AMP phosphodiesterase, and probably this activity is the basis for the observed diuretic effect (Emim *et al.*, 1994; Galati *et al.*, 1994).

Antiinflammatory effects

Hesperidin has been reported to possess significant antiinflammatory and analgesic effects (Galati *et al.*, 1994). Emim *et al.* (1994) presented pharmacological data favouring the use of hesperidin as an inexpensive antiinflammatory agent or as a lead compound, especially for patients with hypersensitivity to the ordinarily used nonsteroidal antiinflammatory agents. Hesperidin, though ineffective after oral administration, was found to be

active on subcutaneous injection without remarkable changes in rat behaviour or apparent tissue damage at the site of injection even after repeated administration. At high doses, it also reduced dextran induced paw oedema in rats. This effect could be attributed to the inhibition of the release of histamine from basophils by hesperidin or its metabolic products.

Hesperidin in combination with diosmin, shows a marked protective effect against inflammatory disorders, both *in vivo* and *in vitro*, possibly through a mechanism involving an inhibition of eicosanoid synthesis and/or antioxidant free radical scavenger activity (Jean and Bodinier, 1994). Lonchampt *et al.* (1989) studied the scavenging properties of Daflon 500 mg® on active oxygen radicals *in vitro* and *in vivo*, implying its pharmacological action on capillary hyperpermeability as well as antiinflammatory and antioedematous actions.

S-5682 (Daflon) was demonstrated to improve multiple histological aspects of the acute inflammatory reaction (diapedesis of polymorphs, lymphocytes, histocytes and macrophages) and features of the chronic inflammatory reactions (newly formed micro vascularization of the granuloma tissues, perivascular oedema, presence of collagen fibres). Its effects in the chronic treatment of inflammatory granuloma in the rat were studied. It possessed potent antioedematous activity and reduced the synthesis of PGE_2 and $PGF_{2\alpha}$ in inflammatory granuloma (Damon *et al.*, 1987).

The antiinflammatory effect of hesperidin in the acute stage of trinitrobenzene sulphonic acid (TNBS) model of rat colitis was evaluated. Pretreatment with hesperidin reduced colonic damage compared with TNBS control rats. In addition, hesperidin after oral administration reduced the areas of colonic necrosis and hyperaemia, scored according to the severity and extension of involved tissue compared with the control group. A significant reduction in the colonic weight/length ratio was also observed. This intestinal antiinflammatory effect was also shown biochemically since hesperidin was able to reduce colonic myeloperoxidase activity, an enzyme that is considered as a biochemical marker for neutrophilic infiltration in damaged tissue (Crespo *et al.*, 1999). Another possible mechanism that contributes to this effect is its antioxidant property (Jean and Bodinier, 1994). Hesperidin has been reported to ameliorate colonic oxidative stress that occurs in an experimental model of inflammatory bowel disease (Loguercio *et al.*, 1996). When given intragastrically to mice at a dose of 25 mg/kg, it was found to reduce adjuvant-induced arthritis (Kim *et al.*, 1990).

Pretreatment with Daflon-500® prior to the induction of tourniquet ischaemia significantly lowered the number of adherent leukocytes thereby controlling oedema in clinical situations (Friesenecker *et al.*, 1995). This protective effect is associated with the decreased platelet and complement system activation, leading to a lowered release of histamine and decreased leukocyte-dependent endothelial damage.

Action on enzymes

Hyaluronidase is an enzyme which depolymerizes the mucopolysaccharide hyaluronic acid. It has been known to play a part in the typical changes in the morphology of the connective tissue, particularly of the interfibrillator cement substance, which is believed to contain hyaluronic acid. This enzyme plays a role in regulating the permeability of capillary walls and supporting tissues. Hyaluronidase causes a breakdown of hyaluronic acid, thereby increasing tissue permeability. Also, various bacteria produce hyaluronidase, thereby increasing the permeability of the tissue and favouring the invasion of tissue by these and other microorganisms (Hahn, 1952). Various scientists and medical researchers have been working on the hyaluronidase inhibitory effects of different flavonoids. Beiler and Martin, as early as in 1948, prepared various derivatives of hesperidin as hyaluronidase inhibitors. The sulphonated and phosphorylated hesperidins proved to be extremely potent inhibitors, and acetylated hesperidin caused a potentiation in the inhibitory action of ascorbic acid on hyaluronidase. They demonstrated that, although pure hesperidin and hesperetin were active as hyaluronidase inhibitors, this action could be greatly potentiated by the formation of the above mentioned derivatives of the pure compound (Beiler and Martin, 1948). Based on an investigation on the isolated connective membranes from mice, it has been reported that the permeability increasing action of hyaluronidase could be abolished by contact with phosphorylated hesperidin for 15 min or more. It not only abolishes the hyaluronidase action but also restores the original degree of permeability of the membranes. It even reduces the permeability of normal (untreated) membranes (Steincke, 1956).

Besides hyaluronidase, hesperidin has also been reported to inhibit human acrosin, a sperm enzyme, *in vitro* (Jackson, 1959). It also shows an inhibitory effect on aldol reductase (lens) *in vitro* (Varma and Kinoshita, 1976), alkaline phosphatase in rat serum (Son *et al.*, 1991) and a weak activity against alpha-glucosidase *in vitro* (Iio *et al.*, 1984). Inhibition of alkaline phosphatase was, however, found to be absent in a study on rat liver (Son *et al.*, 1991) and another *in vitro* study where the enzyme was derived from calf intestinal mucosa (Iio *et al.*, 1980). Hesperidin, however, did not inhibit xanthine oxidase *in vitro* (Iio *et al.*, 1985). It was also found to be inactive as an inhibitor of the enzyme reverse transcriptase, when tested on virus-raucher murine leukaemia *in vitro* (Ono *et al.*, 1990).

In a study carried out on some flavonoids, on nonenzymatic lipid oxidation and enzymatic oxidation of arachidonic acid *in vitro*, hesperidin was found to stimulate the enzyme cyclooxygenase (Iio *et al.*, 1984). However, inhibition of 1,5-lipoxygenase by Citrus peel flavonoids has been reported (Malterud and Rydland, 2000).

When a range of flavonoids were screened for inhibitory effects against partially purified aromatase prepared from human placenta, hesperetin was found to possess a significant inhibitory effect on the same (Jeong *et al.*, 1999). A series of flavonoids were tested for their effects on low K_m phosphodiesterase with cyclic AMP as the substrate and for their lipolytic activity, employing rat adipocytes. Hesperetin showed inhibition of epinephrine induced lipolysis, in a dose dependent manner but did not inhibit phosphodiesterase significantly (Kuppusamy and Das, 1992).

Hesperidin and diosmin, alone and in combination (1:9), were found to inhibit prostaglandins, when used against sponge-induced granuloma in rats, significantly lowering the prostaglandin levels of the treated animals

compared with those of untreated controls, through 16 days after intragastric sponge implantation (Damon *et al.*, 1987).

Antimicrobial activity

Hesperidin and hesperetin, among other flavonoids, have shown antiinfective and antireplicative activities *in vitro* against several plant and animal microbes.

Antibacterial. In a study involving the investigation of anti-*Helicobacter pylori* (HP) activity, *in vitro*, of some flavonoids and their metabolites, hesperetin and other flavonoids were found to inhibit the growth of HP (Bae *et al.*, 1999). In patients with chronic gastritis, HP promotes the alteration from gastritis to gastric cancer (Correa, 1988). However, Islam and Ahsan (1997), recently showed hesperidin to be inactive *in vitro* on agar plates, against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus hemolyticus*, *Escherichia coli*, *Klebsiella* species, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Shigella flexneri* and *Vibrio cholera*.

Antifungal. The antifungal activity of hesperidin has been reported at a dose ranging from 1 to 10 μg, against *Botrytis cinerea, Trichoderma glaucum* and *Aspergillus fumigatus* (Krolicki and Lamer-Zarawska, 1984). It was, however, found to be inactive against *Aspergillus fumigatus, Aspergillus niger* and *Trichoderma* species (Islam and Ahsan, 1997).

Antiviral. Wacker and Eilmes, in their two studies, reported hesperidin to be active as an antiviral agent, *in vitro*, at different concentrations on cell culture, against virus-vesicular stomatitis. Hyaluronidase was found to reverse its antiviral activity suggesting that this activity of hesperidin was mediated by its antihyaluronidase effect. They also reported hesperidin to be active against influenza virus (Wacker and Eilmes, 1975; Wacker and Eilmes, 1978). In a recent study, hesperidin was found to possess a weak activity against herpes simplex virus (Lee *et al.*, 1999b). It was found that certain flavonoids, including hesperidin, possessed antiviral activity against herpes type-I, para influenza-3, poliovirus type-I and respiratory syncytial virus (RSV) in tissue cell monolayers (Middleton, 1984). Mucsi and Pragai (1985) demonstrated the inhibitory effect of hesperidin among other flavonoids on human herpes simplex virus type-I (HSV-I) and suid (alpha) herpes virus type-I (*Pseudorabies* virus). A direct relationship between the antiviral activity of the flavonoid and its ability to stimulate cyclic AMP synthesis in the cells was exhibited. Quercetin and hesperetin are also known to possess antireplicative activity (inhibition of virus replication after established infection) if introduced at the time of cell activation. Hesperetin was, however, found to be inactive against HIV-virus (Hu *et al.*, 1994), pseudorabies virus, herpes simplex virus (Mucsi and Pragai, 1985) and rhinovirus (Tsuchiya *et al.*, 1985). In a recent study, the inhibitory effects of some flavonoids was tested on infectivity of rotavirus, which predominantly causes sporadic diarrhoea in infants and young children (Bae *et al.*, 2000) and hesperidin was found to have a potent inhibitory effect. Hesperetin, however, did not possess this activity, indicating that the rutinose moiety is essential in protecting against the invasion of rotavirus into the cells. The fruit of *C. aurantium*, having hesperidin and neohesperidin as main constituents, was also found to possess a potent inhibitory effect on rotavirus infectivity (Kim *et al.*, 2000).

When tested for an effect on the infectivity and replication of HSV-1, polio-virus type-1 and parainfluenza virus type-3, hesperetin was found to have no effect on the infectivity but reduced intracellular replication of each of the viruses (Kaul *et al.*, 1985). Hesperidin was also found to have a significant effect against *Staphylococcus aureus* on infected mice (Panasiak *et al.*, 1989).

Anti yeast. Hesperidin was found to have no inhibitory effect on *Candida albicans* and *Saccharomyces cerevisiae*, when tested *in vitro* on agar plates (Islam and Ahsan, 1997).

Anti fertility activity

Hesperidin and its derivatives have been under investigation as anti fertility factors for a very long time. As early as 1948, phosphorylated hesperidin (PH) was reported to act as an effective anti fertility agent mediating this effect by inhibiting the sperm enzyme hyaluronidase (Beiler and Martin, 1948; Martin and Beiler, 1952). In a study on rats, PH was found to have an antifertility effect when given orally or intraperitoneally (Martin and Beiler, 1952). In another attempt to assess the antifertility effect, PH (20 mg/kg) was administered to both male and female mice, intraperitoneally for 8 days. On mating, the percent pregnancies were significantly reduced. The oestrus cycle of the females remained unchanged. Microscopic examination of the seminal fluid from males showed a normal sperm count and sperm motility. No permanent sterility in either sex was observed. Based on these results, a study was further carried out on 300 married human couples. PH tablets (100 mg) were given orally to both the partners, over varying periods, up to 30 months. Contraception occurred in all the cases except for two, where the couples were non cooperative regarding the study parameters. Merely omitting the drug for a period of 48 h could restore fertility. It thereby offers promise as an oral contraceptive without any toxic effects or permanent inhibition of sterility. Trauma, infectious diseases or systemic diseases did not inhibit the antifertility effect (Sieve, 1952).

Later it was reported that the fertilizing capacity of rabbit sperm was inhibited to a certain extent when the sperm was suspended in a 1% solution of PH and inseminated into female rabbits (Chang and Pincus, 1953). PH also exhibited significant contraceptive efficacy on vaginal application in rabbits, even at nonspermicidal concentrations (Joyce and Zaneveld, 1985). In yet another study, PH was proven to inhibit bovine fertilization by mouse spermatozoa by specifically inhibiting the sperm enzyme hyaluronidase. Hyaluronidase is reported to be required for sperm penetration through the follicle cell layer of the egg. PH, by inhibiting sperm hyaluronidase can thereby successfully be used as an antifertility agent (Joyce *et al.*, 1986).

Anticarcinogenic activity

During the past decade, a considerable amount of research has been carried out on the anticancer activities of hesperidin and its aglycone hesperetin. Encouraging results of carcinogenesis inhibition were observed by using a hesperidin/diosmin combination on male mouse urinary bladder. The combination inhibited *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-induced carcinogenesis when treated with the combination for 8 weeks during the tumour initiation phase (Yang *et al.*, 1997). An inhibition of carcinogenesis in rats by hesperidin, at a concentration of 500 ppm/kg body weight has also been reported. The compound was also found to inhibit 4-nitroquinoline-1-oxide-induced oral carcinogenesis and to decrease the number of lesions, polyamine levels in tongue tissue and cell proliferation activities (Tanaka *et al.*, 1994). The same group later reported a similar activity of hesperidin alone, and in combination with diosmin, where it not only inhibited 4-nitroquinoline- 1-oxide initiated tumorigenesis but also showed chemoprevention of azoxymethane-induced rat colon carcinogenesis. These results are thought to be due to increased suppression of cell proliferation (Tanaka *et al.*, 1997a,c).

Hesperidin, when administered subcutaneously to CD-1 mice, did not inhibit 7,12-dimethylbenz(α)anthracene-induced tumour initiation but inhibited 12-O-tetradecanoyl-13-phorbol acetate (TPA)- induced tumour promotion, thus indicating its potential use as a chemopreventive agent (Berkarda *et al.*, 1998). Later they established the protective effect of hesperidin against TPA-stimulated infiltration of neutrophils. It also afforded significant protection against TPA induced hyperplasia in the dorsal skin of CD-1 mouse, through multiple applications prior to TPA (Koyunku *et al.*, 1999).

The mutagenic activity of hesperetin was assessed along with several other flavonoids and their derivatives using *Salmonella typhimurium* mutants that reveal base pair substitutions and frameshift mutagens. Neither hesperetin nor neohesperetin were found to be mutagenic (Bjeldanes and Chang, 1977). Much later when the antimutagenicity of various Citrus flavonoids including naringin, hesperidin, nobiletin and tangeritin was evaluated, hesperidin was found to possess a weak antimutagenic effect on *Salmonella typhimurium* against benz (α) pyrene-induced mutations (Choi *et al.*, 1994; Calomme *et al.*, 1996). An antimutagenic effect has also been reported against *N*-methyl-*N*-amylnitrosamine-induced tumorigenesis, in rats (Tanaka *et al.*, 1997b). When a number of plant flavonoids were tested for mutagenicity in the *Salmonella*/microsomal activation system, both hesperidin and hesperetin were found to require metabolic activation for any mutagenicity (Hardigree and Epler, 1978). Hesperetin has shown an antimutagenic effect against aflatoxin B1 with more than a 70% inhibition rate in *S. typhimurium*, in the presence of a mammalian metabolic activation system (Choi *et al.*, 1994).

When the effects of predominant dietary flavonoids were tested for inhibiting neoplastic transformation induced by 3-methylcolanthrene in C3H 10T1/2 murine fibroblasts, hesperidin and hesperetin were found to be the most potent agents inhibiting this transformation completely (Franke *et al.*, 1998). Structure–activity comparisons were also made and favourable structures discussed.

Platelet and cell aggregation inhibition

Hesperidin was found to increase the survival time of rats by 16–71 days when they were placed on a thrombogenic diet but produced variable results when the rats were on an atherogenic diet (Robbins, 1967). In studies carried out on human adult platelets (Zaragoza *et al.*, 1985; 1986), hesperidin was reported to inhibit epinephrine- and ADP-induced platelet aggregation at a concentration of 0.08 mg/ml. In yet another study carried out on horses, hesperidin was found to effectively reduce aggregation of erythrocytes. This decrease in the blood cell aggregation may explain the beneficial effects of hesperidin on abnormal capillary permeability and fragility, the reduction of disease symptoms and their protection against various traumas and stresses (Robbins, 1971). Hesperidin and diosmin, in combination, were found to decrease leukocyte adhesion to the endothelium in post capillary venules, after ischaemia/reperfusion, as assayed through skin fold window chamber in guinea- pigs following intragastric administration of the compounds (Friesenecker *et al.*, 1994). This antiischaemic effect of hesperidin was again reported in the hamster, at a dose of 20 mg/kg body weight after intragastric administration (Bouskela and Donyo, 1995). In addition a micronized purified flavonoid fraction containing hesperidin and diosmin was found to significantly decrease ADP-induced platelet aggregation and increase platelet disaggregation, in rats. Fibrinogen binding to ADP-induced platelets was also reduced significantly (McGregor *et al.*, 1999).

Ultraviolet protecting activity

Recently much research has been focused on the potential use of flavonoids as free radical scavengers to prevent oxidative skin damage (Schoemaker *et al.*, 1995; Mortimer, 1997). The oxidative stress caused by ultraviolet irradiation might be an initiator in the pathogenesis of skin cancer and photoaging (Dalle Carbonare and Pathak, 1992; Darr and Fridovich, 1994). Bonina *et al.* (1996, 1998) investigated the ability of topically applied hesperetin, alone and in a crude extract of *C. sinensis* along with other flavonoids, to reduce UV-B-induced skin erythema. Employing phosphatidylcholine (PC) vesicles as model membranes they studied the effect of hesperetin on UV irradiation induced peroxidation. *In vitro* human skin permeation of these compounds was also measured. Hesperetin was effectively found to protect PC liposomes from UV-irradiation induced peroxidation probably by scavenging oxygen free radicals generated by UV irradiation. It was also able to permeate through the stratum corneum. It was later concluded from *in vitro* and *in vivo* data, obtained after applying a gel formulation uniformly on the skin sites (human volunteers) and exposing them to UV-B irradiation and monitoring the extent of erythema by means of reflectance spectrophotometry, that hesperetin might be successfully employed as a topical photo-protective agent (Saija *et al.*, 1998).

In another study, transglycosylation of hesperidin by cyclodextrin glucanotransferase was conducted and its mono- and di-glucosides were prepared, both having the same absorption spectra as that of hesperidin. When these were exposed to ultraviolet light, they seemed to stabilize

the colour of the pigments by absorbing the UV rays. In addition they did not have strong spectra in visible light. The authors propose its use as a colour stabilizer in food products (Kometani *et al.*, 1994).

Miscellaneous effects

Analgesic and antipyretic activity. Hesperidin has exhibited analgesic activity in mice on subcutaneous administration. This effect has been explained as being exerted through a peripheral and not a central mechanism (Galati *et al.*, 1994). It also showed analgesic activity in mice following intraperitoneal administration. Also, hesperidin decreased the fever induced by yeast in rats. This effect may be related to the inhibition of yeast-induced prostaglandin biosynthesis. Hesperidin is known to inhibit both histamine and prostaglandin release, thereby acting as a defensive gastric factor and preventing acid secretions and lesions of the gastric mucosa (cited in Emim *et al.*, 1994).

Antioxidant effect. Hesperidin has been reported to possess antioxidant properties. For example, it has been found to reduce superoxide in electron transfer plus concerted proton transfer reaction *in vitro* (Jovanovic *et al.*, 1994). This activity was also exhibited in liver homogenates for hydroperoxide-induced chemiluminescence (Fraga *et al.*, 1987). It has been suggested that the hesperidin/diosmin combination could function as an antioxidant, which may explain its beneficial therapeutic effect in chronic venous insufficiency where oxidative stress is involved in the pathological mechanism (Bouskela *et al.*, 1997). A number of researchers have examined the antioxidant activity and radical scavenging properties of hesperidin using a variety of assay systems (Kroyer, 1986; Brasseur *et al.*, 1986; Ratty and Das, 1988; Yuting *et al.*, 1990; Wang and Zheng, 1992; Miyake *et al.*, 1997; Deng *et al.*, 1997; Miller and Rice-Evans, 1997; Suarez *et al.*, 1998; Malterud and Rydland. 2000). Results from different assays varied considerably, but in most, hesperidin was found to be inactive or only moderately active in comparison with other flavonoids and nonflavonoidal antioxidants. Hesperidin was also found not to inhibit the liberation of reactive oxygen species from stimulated neutrophils (Limasset *et al.*, 1993). Thus hesperidin appears not to be a particularly active antioxidant in comparison with most other flavonoids.

Immuno-modulatory activity. It has been reported that hesperidin possesses an immuno-suppressant activity (Kim and Cho, 1991). It suppresses the bacterial alpha-amylase antibody production in mice on intragastric administration at a dose of 50 mg/kg. In another study, intragastric administration of 50 mg/kg of hesperidin to male mice, significantly increased the development of immunological memory in cellular immune response (Kim and Cho, 1991).

Recently, the colony stimulating factor (CSF) inducing activity of various bioflavones was tested, in order to evaluate their immuno-modulating activities. Samples of different bioflavonoids, suspended in saline were injected intraperitoneally into mice at a dose of 1 mg/kg, 6 h before bleeding. Hesperidin exhibited the strongest CSF inducing activity and the response was dose dependent (Kawaguchi *et al.*, 1999).

Antiallergic effects. Hesperidin's antiallergic and anti-anaphylactic activities have been reported and patented (Kubo and Matsuda, 1992). It was shown to possess antianaphylactic activity where it delayed hypersensitivity against sheep red blood cells, prevented passive cutaneous anaphylaxis, induced mast cell degranulation and also prevented histamine-induced anaphylaxis after intragastric administration to mice at different doses (Kim and Chung, 1990). Later, it was found to inhibit vascular permeability in the homologous passive cutaneous anaphylaxis test conducted on rats after intragastric administration, at a dose of 0.1 mmol/kg (Kubo and Matsuda, 1992). An inhibition of 48 h homologous passive cutaneous anaphylaxis in rats has also been reported with the use of hesperidin (Matsuda *et al.*, 1991).

Activity on haemorrhoids and IUCD-induced bleeding. Oral tablets of a hesperidin/diosmin combination were clinically tested in situations involving intrauterine contraceptive device (IUCD)-induced bleeding. Thirty one women were given the combination over a period of three consecutive cycles. Eighty three percent of the patients showed improvement in the signs and symptoms associated with the bleeding. The acceptability was excellent with minimal side effects (Daftary *et al.*, 1995). Daflon-500 mg® has also been found to reduce inflammation by inhibition of prostaglandin and thromboxane B2 release from macrophages and also to reduce the free radicals. The investigators postulated that inhibition of the inflammatory response in the haemorrhoid surgical wound site reduced defaecation stress and bacterial fibrinolysis, decreasing the risk of secondary bleeding (Ho *et al.*, 1995). The effectiveness and tolerability of a similar hesperidin/diosmin combination was evaluated for the treatment of haemorrhoids. Overall improvement of signs and symptoms was quicker and greater with good acceptability and minimal side effects (Godeberge, 1995). Recently, a similar combination was provided in a micronized form, for a median of 8 weeks before delivery and 4 weeks after delivery, to 50 pregnant women with acute haemorrhoids and was found to be very successful with 66% of the patients reporting relief of symptoms by day 4 of treatment and fewer suffering relapses during the antenatal period. The treatment was well accepted and did not affect pregnancy, fetal development, birth weight, infant growth or feeding (Buckshee *et al.*, 1997).

Hormonal disorders. Hesperidin was reported (Smith, 1964) to help in regulating oestrogen levels and decreasing related pain, inflammation and swelling. In a clinical study, 94 women suffering from hot flashes and other menopausal symptoms were given a formula containing 900 mg of hesperidin, 300 mg of hesperidin methylchalcone and 1200 mg of vitamin C daily. At the end of 1 month, symptoms of hot flashes were relieved in 53% of the patients and reduced in 34%. Improvements were also noted in nocturnal leg cramps, nose bleeds and easy bruising. The only side effect noted was a slightly offensive body odour with a tendency for the perspiration to discolour the clothing.

Antiulcer activity. Hesperidin demonstrated no ulcero-

genic effect in male rats at a dose of 100 mg/kg by subcutaneous administration (Emim *et al.*, 1994). However, when given to rats orally at a dose of 100 mg/kg, the compound exhibited a positive antiulcer effect on cold stress-induced ulcers but had no effect on ethanol-induced ulcers. No increase in gastric mucus was observed. It was found to stimulate hexosamine secretion in cold stress-induced ulcers but inhibited the same in ethanol-induced ulcers (Suarez *et al.*, 1996).

Effect on wound healing. Recently a micronized flavonoid fraction, comprising of 90% diosmin and 10% hesperidin, was tested for its effect on clean wounds and those infected with *S. aureus* on oral and topical administration. The study showed that while there was no significant effect on clean wounds, the combination had a beneficial effect on the infected wounds, when administered both orally and topically (Hasanoglu *et al.*, 2001).

MARKETED THERAPEUTIC SYSTEMS

Hesperidin, though not yet very widely used therapeutically, has been listed by the Martindale Extra Pharmacopoeia to be an ingredient of various formulations internationally used for vascular disorders (Angiopan—Gentili, Italy; Circovenil—Wyeth, Spain; Daflon—therapia, Germany; Varico Sanol Forte—Sanol, Germany), capillary fragility (Cepevit-K—Darcy, France), haemorrhoids (Daflon 500—Servier, Switzerland; Hamamelis complex—Blackmores, Australia), rheumatic and joint disorders (Guaiacum complex—Blackmores, Australia; Ostochort—Adenylchemic, Germany), vitamin C deficiency and dietary supplement (HY-C—Solgar, USA; Min-Detox-C—Eagle, Australia), skin trauma (Proveno—Madaus, Germany; Ondascora—Servier, France), obstetric disorders (Rubus complex—Blackmores, Australia), gingival inflammation (Peridin-C—Hamilton, Australia), fluid retention and gastrointestinal disorders (Hepanephrol—Rosa Phytopharma, France) (Reynolds, 1996).

CONCLUSION

From the above review, it can be concluded that hesperidin, its aglycone hesperetin, and their various derivatives possess significant potential as therapeutic agents for a wide range of diseases and disorders. This promise needs to be effectively studied at the clinical level, so as to firmly establish the usefulness of these compounds in the treatment or prevention of disease in humans.

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