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Review article

Protective effects of flavonoids against microbes and toxins: The cases of hesperidin and hesperetin

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ABSTRACT

Many plants produce flavonoids as secondary metabolites. These organic compounds may be involved in the defense against plant-threatening factors, such as microbes and toxins. Certain flavonoids protect their origin source against plant pathogens, but they also exhibit potential healthy properties in human organisms. Hesperidin (Hsd) and its aglycone, hesperetin (Hst), are two flavonoids from the *Citrus* species that exhibit various biological properties, including antioxidant, antiinflammatory and anticancer effects. Recent studies indicated that Hst and Hsd possess antimicrobial activity. Although the exact mechanisms behind their antimicrobial properties are not fully understood, several mechanisms such as the activation of the host immune system, bacterial membrane disruption, and interference with microbial enzymes, have been proposed.

Hsd and Hst may also have protective effects against toxicity induced by various agents. These natural substances may contribute to the protection of cells and tissues through their antioxidant and radical scavenging activities. This review discusses the protective activities of Hsd and Hst against microbes and several toxicities induced by oxidants, chemicals, toxins, chemotherapy and radiotherapy agents, which were reported *in vitro* and *in vivo*. Furthermore, the probable mechanisms behind these activities are discussed.

Contents

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1. Introduction

Polyphenols, a large class of biologically active substances, are distributed in plants as secondary metabolites. These substances provide color and flavor to different plant parts and they also play an important role in resistance against various microbial pathogens and protect against radiations and toxins. Many recent studies have mainly focused on polyphenols and their diverse biological effects [13,49].

Flavonoids are one of the most common polyphenols, and they exhibit interesting and beneficial medicinal effects on human health. The various biological properties of flavonoids, such as antioxidant, antiinflammatory, anticancer, antibacterial, immune-stimulating and antiviral activities, have been reported extensively [22].

Hesperidin (Hsd) is the major flavonoid in citrus fruits, and it was isolated for the first time from citrus peel by Lebreton in 1827. The Hsd molecule is composed of an aglycone unit, namely hesperetin (Hst), and a disaccharide, rutinose (Fig. 1).

Hsd and Hst possess different activities, such as antioxidant, antiinflammatory, antimicrobial, anticarcinogenic and antiallergic effects. Hsd and Hst substances are also called bioflavonoids because of this wide range of effects. A descriptive review of the different biological activities and physicochemical properties of Hsd was published by Garg et al. in 2001 [21]. In addition, neuropharmacological, antioxidant, and antiinflammatory properties and the pharmacokinetics of Hsd and Hst were published in our recent review papers [42,51]. The protective effects of Hsd and Hst against toxicities induced by certain chemotherapy drugs have been widely investigated [1,58]. However, there has not been a comprehensive report on the protective effects of Hsd and Hst against invading pathogens and various toxicities induced by environmental toxins, occupational hazards, radiotherapy and chemotherapeutic agents.

Accordingly, this review provides a detailed overview of the current state of knowledge of the protective effects of the bioflavonoids, Hsd and Hst, against the aforementioned toxicities. The probable mechanisms governing these particular activities are also discussed.

2. Protective effects of Hsd and Hst against invading pathogens

Plant flavonoids play an important role in the protection against pathogenic microorganisms, such as bacteria, fungi and viruses. However, the high rate of microbial resistance to conventional antibiotics suggested flavonoids as suitable alternatives to antibiotics. Furthermore, flavonoids can be considered natural food preservatives because of their antimicrobial activities [50]. Generally, flavonoids are found in glycosylated forms in plants, and the presence of a sugar moiety is an important factor that determines their bioavailability. However, the antimicrobial efficacy of flavonoids varies depending on their chemical structure and the strain of microorganism.

Flavonoids, such as Hsd and Hst, exhibit anti-infective and antireplicative effects against several microorganisms.

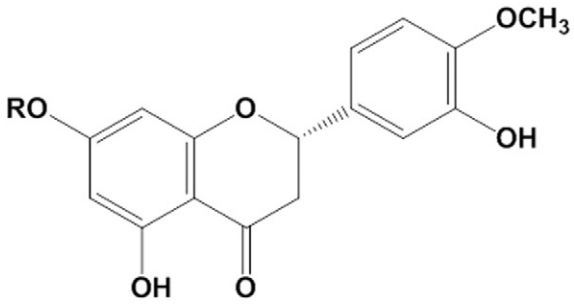

Fig. 1. The chemical structures of hesperetin and hesperidin. Hesperetin: R = H; hesperidin: R = rutinose (glucose + rhamnose).

2.1. Antibacterial activities

The antibacterial activity and bioavailability of flavonoids are affected by various parameters, such as molecular conformation, hydrophobicity, solubility, presence or absence of sugar moiety and the type of sugar in the chemical backbone [35]. However, the exact mechanisms of the antibacterial effects of flavonoids are not clear, but several mechanisms, such as interference with bacterial DNA synthesis, bacterial movement, cytoplasmic membrane permeability and the inhibition of bacterial metalloenzymes, have been proposed [11,23,38]. Different studies evaluated the inhibitory effects of plant flavonoid-rich extracts and pure flavonoids, including Hsd derivatives, against some pathogenic microorganisms (Table 1). For example, an investigation in [14] demonstrated that the ethanolic extract of grapefruit seed and pulp containing flavanones, mainly naringin and Hsd, significantly inhibited only Gram-positive bacteria using an agar diffusion method, however, this extract was effective against Gram-positive and Gram-negative bacteria using a broth dilution susceptibility test [12].

In 2008, Liang evaluated the antimicrobial effects of a flavonoid extract of *Pericarpium citri reticulatae* (FEPCR) and its major constituents, including Hsd, nobiletin and tangeretin, against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Salmonella typhimurium* and *Enterobacter cloacae* using the agar dilution method. This study demonstrated that Hsd and FEPCR exhibited a wide range of antibacterial activity, but the two other FEPCR flavonoids, tangeretin and nobiletin, were nearly inactive against the tested microorganisms. In general, Hsd and FEPCR exerted higher inhibitory activity against Gram-positive bacteria than Gram-negative bacteria. The lowest and highest minimum inhibitory concentrations (MICs) were observed for *S. aureus* (MIC: l00 μg/ml) and *E. cloacae* (MIC: 1600 μg/ml), respectively. The polymethoxylated structure of tangeretin and nobiletin likely produced their low antibacterial activity [62]. The antibacterial activity of Hsd against *Proteus mirabilis* and *S. aureus* was also shown in another study, in which its MIC_{90} was 12 times lower than that of chloramphenicol [15]. Although Hsd was active against *S. aureus* in these studies, it was almost ineffective in an earlier study against some tested pathogenic strains, including *S. aureus*, *Bacillus subtilis*, *Streptococcus betahaemolyticus*, *Enterotoxigenic E. coli*, *Klebsiella* species, *Pseudomonas aeruginosa*, *S. typhimurium*, *Shigella dysenteriae*, *Shigella flexneri* and *Vibrio cholera* [40].

Hsd pretreatment before infection with *S. typhimurium* aroA in a mouse model reduced bacterial numbers in the spleen and liver, and it also reduced plasma lipopolysaccharide (LPS) levels. The decrease in LPS levels also down-regulated early and late endotoxin shock mediators in plasma, namely tumor necrosis factor and high-mobility group protein B1, respectively. Although Hsd reduced the number of bacteria in this study, Hsd did not exhibit direct antibacterial activity against *S. typhimurium* aroA. Hsd increased the influx of immune cells, such as neutrophils, into the peritoneal cavity, which suggests that the antibacterial activity may be due to the activation of host defense systems rather than LPS binding [30].

Narbad's group investigated the antimicrobial properties of flavonoid-rich fractions of bergamot peel against Gram-negative bacteria (*E. coli*, *Pseudomonas putida*, *Salmonella enterica*), Gram-positive bacteria (*Listeria innocua*, *B. subtilis*, *S. aureus*, *Lactococcus lactis*) and the yeast *Saccharomyces cerevisiae* and demonstrated that the analyzed fractions were active only against Gram-negative bacteria. These studies found that treatment of these fractions with Pectinase 62L, which converts flavonoid glycosides into their aglycones, increased the antimicrobial activity of the flavonoids. Additionally, they studied the antimicrobial effects and possible interactions between some pure bergamot flavonoids (neohesperidin, Hst, neoeriocitrin, eriodictyol, naringin and naringenin). The aglycones exhibited MIC values ranging from 250–1000 μg/ml, and a synergistic antimicrobial effect was observed between eriodictyol and Hst against *E. coli* and *S. enterica* and between eriodictyol and naringenin against *S. enterica* and *P. putida*. A slight