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Review article

Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases

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ABSTRACT

Hesperidin (Hsd) and its aglycone, hesperetin (Hst), are two flavonoids from citrus species that have various biological properties, particularly those for the prevention of cancer and cardiovascular diseases. Studies have shown both anti-cancer and cancer chemopreventive effects for Hsd and Hst. Cancer chemopreventive properties of Hsd and Hst are mainly associated with their antioxidant, radical scavenging and anti-inflammatory activities. In addition, Hsd and Hst interfere at different stages of cancer. Unlike conventional anti-cancer drugs, Hsd and Hst inhibit tumor growth by targeting multiple cellular protein targets at the same time, including caspases, Bcl-2 (B-cell lymphoma 2) and Bax (Bcl-2 associated X protein) for the induction of apoptosis, and COX-2 (cyclooxygenase-2), MMP-2 (matrix metalloproteinase-2) and MMP-9 for the inhibition of angiogenesis and metastasis. The results of the recent basic and clinical studies revealed the beneficial effects for Hst, Hsd and their derivatives in the treatment of heart failure and cardiac remodeling, myocardial ischemia and infarction, and hypertension. In addition, the valuable effects of Hst and Hsd in the treatment of diabetes and dyslipidemia with their anti-platelet and anticoagulant effects make them good candidates in the treatment of various cardiovascular diseases. In this review, new findings regarding the molecular targets of Hsd and Hst, animal studies and clinical trials are discussed.

Contents

Introduction

Flavonoids are a large group of phenolic compounds that are widely distributed in plants. To date, a large number of these compounds have

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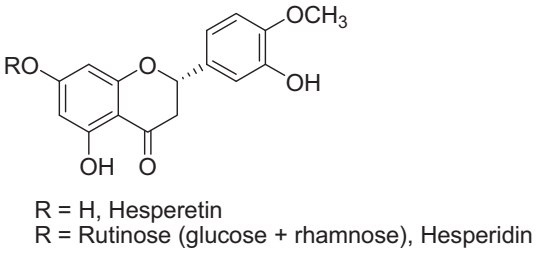

Fig. 1. The chemical structures of hesperidin and hesperetin.

been evaluated both in their free state and as glycosides. In addition to this work, several biological properties have been reported from flavonoids, including antioxidant, anticancer, cancer chemopreventive, and anti-inflammatory properties [29,67]. Hesperidin (Hsd) is a flavanone glycoside (a subclass of flavonoids) that is found abundantly in citrus fruits. Its aglycone form is called hesperetin (Hst). Hsd was first isolated from citrus peel by the French chemist Lebreton. Because of its various biological activities, Hsd is also called a bioflavonoid. Hsd is a β-7-rutinoside of Hst because it consists of an aglycone, Hst, and a disaccharide, rutinose (Fig. 1).

Both Hsd and its aglycone Hst have shown various biological activities [24]. For example, Hsd possesses vitamin-like activity and can decrease capillary permeability (vitamin P), leakiness and fragility. It also showed antioxidant, anti-inflammatory, anticarcinogenic and antiallergic properties [24]. The biological activities of Hsd together with its physicochemical properties were reviewed in a paper published by Garg et al. [24]. However, a large number of studies have been published since then describing its new pharmacological activities, molecular targets and mechanisms of action. For example, the effects of Hsd on the central nervous system have been a topic of research during the past decade, while they have not previously been investigated [21,74]. New findings also revealed that the antioxidant activity of Hsd was not only limited to its radical scavenging activity, but it augmented the antioxidant cellular defenses via the ERK/Nrf2 signaling pathway as well [10,20].

This review addresses the biological and pharmacological properties of Hsd and Hst that have been reported since 2001. Additionally, the current paper provides a deeper insight into the mechanisms of action and molecular targets of Hsd and Hst and shows the gaps in our knowledge about Hsd, which deserve further research.

All of the relevant databases were searched for the terms “hesperidin”, “hesperetin” and “citrus flavonoid” without limitation from 2001 to 30th June 2014. Information on Hsd and Hst was collected via electronic search by using Pubmed, Scopus, Web of Science and ScienceDirect.

Anticancer and cancer chemopreventive properties

There is a noticeable body of evidence that concerns Hsd and Hst actions against tumors. Promising results of these in vivo and in vitro studies have been mostly justified by antioxidant properties of these compounds. Table 1 summarizes the main features of published investigations that focus on the chemopreventive and chemotherapeutic properties of Hsd and Hst.

The two following in vivo studies exhibited promising antineoplastic effects of Hsd.

Kamaraj et al. observed that while the total body weight was decreased in tumor-bearing mice, pre- and post-treatment with Hsd resulted in a significant increase in the body weight and also significantly decreased the lung weight and tumor incidence. In addition, lung tumor caused an increase in lipid peroxides, AHH (aryl hydrocarbon hydroxylase), γ-GT (gamma glutamyl transpeptidase), 5′-ND (5′-nucleotidase) and LDH (lactate dehydrogenase), and decreased enzymatic and non-enzymatic antioxidant activities, which were altered to almost the normal state by Hsd pre- and post-treatment [41].

It was shown that Hsd (30 mg/kg body weight for 45 days) treatment had antineoplastic and antigenotoxic effects due to the modulation of the energy reservoir of the cell and oxidative phosphorylation. In addition, Hsd inhibited enzyme leakage by maintaining the integrity of the lysosomal membrane [60].

One of the main characteristics of Hsd is its radical scavenging property, which results in normalization of the redox profile of treated cells. In this regard, Hsd-treated cells showed less ROS and improved the antioxidant system.

While not being effective at low concentrations (1–10 μM), Hst at higher doses (50 and 100 μM) decreased the development of vessel-like tube structures and PECAM mRNA expression (a vascular marker), thus having anti-angiogenic properties. On the other hand, in the presence of H_2O_2, Hst (50 and 100 μM) scavenged ROS, and at 100 μM concentration, it reduced the lipid peroxidation biomarker and 8-iso-prostaglandin F2α. It is worth mentioning that in contrast to other flavonoids that possess prooxidant properties, Hst even at the highest dose did not cause acute cell damage or cytotoxic effects but instead induced a mild oxidative stress [14].

In the model of rat colon carcinogenesis that is induced by 1,2-dimethylhydrazine (DMH), oral administration of Hst significantly decreased intestinal tumor incidents, which was proposed to be mainly due to the enhancement of the antioxidant defense. During the initiation, post-initiation and entire period phases, Hst brought the liver and colon lipid peroxidation profiles back to normal levels. While DMH treatment decreased the catalase (CAT) and superoxide dismutase (SOD) activities, Hst significantly reversed this trend and potentiated the colon and liver antioxidant system. In addition, Hst caused no toxicity to the main organs [5].

It was shown that benzo(a)pyrene [B(a)P] treatment weakened the antioxidant system in lung mitochondria regarding superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, reduced glutathione, vitamin E, and vitamin C. These factors were back to being almost normal following pre- and post-treatment with Hsd. A similar observation was recorded regarding the levels of mitochondrial ATP levels in the lungs. Furthermore, Hsd preserved mitochondrial integrity and saved it from damage, which suggests its usage as a chemopreventive agent [40].

Cytotoxic effects of Hsd on breast (MCF-7), larynx (HEp-2) (with the least IC_{50}), cervix (HeLa) and liver (HpG-2) carcinoma cell lines were proposed to be related to its antioxidant capacity [4].

Diet supplementation with Hsd (30 mg/kg b.w.) for rats bearing 7,12-dimethybenz(a) anthracene-induced breast cancer resulted in reduced lipid peroxidation and reversing the marker enzymes to normal levels. In addition, cell structure and integrity were recovered via an increase in the total protein and nucleic acid content, which was mainly mediated by the radical scavenging effects of Hsd [61].

There are some studies that concern the pharmacodynamic and pharmacokinetic interactions of Hsd and chemotherapeutic agents.

Cyclophosphamide (CP) administration for the treatment of colon carcinoma resulted in a significant decrease in the WBC (white blood cells) count on days 4, 7, 10 and 14. Hsd treatment increased the WBC count on days 4 and 7, but it had no effect on the counts on days 10 and 14. The co-administration of Hsd and CP reduced the CP effects on tumor growth, which is hypothesized to be due to either its antioxidant effect or its interaction with CP metabolism in the liver [31].

In addition, the effect of Hsd on the multidrug-resistant human leukemia cell line P-gp showed that Hsd increased doxorubicin toxicity toward the cell line due to the decreased P-gp activity [19].

Alteration of inflammatory responses

It was shown that Hsd modifies the production of cytokines and enzymes that are involved in inflammation. This interaction with inflammatory processes could play a crucial role in the anti-cancer effects of Hsd.

Table 1
Anticancer and cancer chemopreventive properties of Hsd and Hst, in vitro and in vivo, and their underlying cellular mechanisms.

Compound of interest	Model of study	Treatment protocol	Underlying mechanisms of the observed results	Ref (s).
Hsd	Swiss albino mice bearing B(a)P-induced lung cancer	25 mg/kg, orally	Decreased lipid peroxides, aryl hydrocarbon hydroxylase (AHH), gamma glutamyl transpeptidase (γ-GT), 5′-nucleotidase (5′-ND) and lactate dehydrogenase (LDH) elevated by B(a)P and improvement of antioxidant defense.	[41]
Hsd	Breast (MCF-7), larynx (HEp-2), cervix (HeLa) and liver (HepG-2) carcinoma cell lines	IC_{50} recorded at <10 μg/ml	Cytotoxic effects mediated by antioxidant properties.	[4]
Hsd	Human colon cancer cells (SNU-C4)	1, 10, 50, and 100 μM	Increase in Bax expression, decrease in Bcl-2 expression, increase in the expression and activity of Casp 3 and down-regulation of pro-Casp3 protein expression.	[66]
β-Cryptoxanthin and Hsd	Lung and colon carcinogenesis in rat and mouse	79, 84, 100 mg or 3.58 g Hsd/100 g compound	Down-regulation of mRNA expression of different cytokines (TNF-α, IL-1β, IL-6) and inflammatory enzymes (COX-2 and iNOS) and up-regulation of mRNA expression of Nrf2 and glutathione *S*-transferase and quinone reductase.	[77]
Hsd and neoHsd	Caco-2, HeLa and COS-7 cell lines	–	Loss of protein flexibility due to the formation of ionic bonds with charged amino acid residues.	[28]
Hsd diosmin	ICR mice bladder carcinogenesis induced by n-butyl-n-(4-hydroxybutyl)nitrosamine	1000 ppm or 100 ppm Hsd with 4900 ppm diosmin	Antioxidant and anti-inflammatory activities.	[85]
Hst	DMH-induced rat colon carcinogenesis	20 mg/kg body weight/day	Decrease in angiogenic growth factors (VEGF, EGF, bFGF), Bcl-2 and COX-2 expression and increase in Bax level.	[59]
Kaempferol, quercetin, rutin, quercetagetin, Hsd, naringin, naringenin and apigenin	Colon cancer cells (SW480)	0, 12.5, 25, 50, 100, and 200 μM for 12, 24, and 48 h.	No changes in cell proliferation, morphological features and no effect on Caspase-3 level but an elevation of Bax:Bcl-2.	[12]
Hsd	Human breast carcinoma cell line MCF-7	20, 40, 60, 80 and 100 μM for 24 h	Anti-proliferative and apoptotic effects featured by an increase in fragmented DNA, p53 accumulation, caspase-3 protein expression and LDH level with a decrease in GSH content.	[63]
Hst	Mouse embryonic stem (mES) cells	1–100 μM for 24 h	Anti-angiogenic properties shown by a decrease in development of vessel-like tube structures and PECAM mRNA expression.	[14]
Hst	1,2-Dimethylhydrazine induced colon carcinogenesis in rats	20 mg/kg body weight/day	Improved anti-oxidant defense resulting in decreased intestinal tumors incidence.	[5]
Hst	DMH-induced colon carcinogenesis in rats	10, 20 and 30 mg/kg body weight/day	Increase in CAT and SOD activities and reduction of lipid peroxide profile which resulted in a decrease in the rate of tumor incidence, multiplicity and average size.	[6]
Hsd	B(a)P-induced lung carcinogenesis in mice	25 mg/kg body weight	Ameliorating antioxidant defense system and increasing ATP level in lung mitochondria.	[40]
Hsd	Human malignant pleural mesothelioma (MSTO-211H)	0, 40, 80 and 160 μM for 24 and 48 h.	Induction of apoptosis via cleavages of Bid, caspase-3, and PARP, upregulation of Bax, and down-regulation of Bcl-xl and augmentation of Sub-G1 population.	[49]
Hsd	HepG-2 cells	0, 5, 25, 50, 100 and 200 μM.	Suppression of acetaldehyde-stimulated NF-κB and activator protein 1 (AP-1) activity via IκB, JNK, and p38 signaling pathways.	[88]
Hsd	Colon carcinoma (CT-26)-bearing Balb/C mice	200 mg/kg orally	Hsd reversed the decrease in WBC count caused by cyclophosphamide while reducing its anti-tumor effects.	[31]
Hsd	UVB-induced cyclobutane pyrimidine dimers (CPD) in Balb/C mice epidermis	1 mg Hsd in 100 μl acetone/cm^2, topically	Reduction of epidermal CPDs and increase in UVB-induced p53 expression.	[36]
Hsd	Breast cancer cells (MCF-7–GFP–Tubulin cells), androgen-independent PC-3 and DU-145, and androgen-dependent LNCaP prostate cancer cells	10, 20, 40, 70 and 100 μM	Inhibition of breast cancer cells proliferation but no effect on PC-3 and DU-145 cells.	[48]
Hsd	Rats bearing breast cancer induced by 7,12-dimethylbenz(a)anthracene	30 mg/kg body weight, orally	Decline in lipid peroxidation and membrane bound marker enzyme (AST, ALT, ALP, ACP, 5'ND, γ-GT) and LDH in serum. Enhancement of macromolecular contents (e.g. total proteins and nucleic acids)	[61]
Hsd	Ramos Burkitt's lymphoma cells	10, 25, 50 and 100 μM	Induction of apoptosis probably related to PPARγ signaling pathway and inhibition of IκB phosphorylation resulting in termination of constitutive and doxorubicin-induced NF-κB activation.	[64]
Hsd	Human leukemia cell line CEM/ADR5000, CCRF-CEM and Caco-2	–	Increased doxorubicin toxicity through a reduction in P-gp activity.	[19]
β-Cryptoxanthin and Hsd	Mice bearing 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced pulmonary tumor	3.9 mg β-cryptoxanthin and 100 mg Hsd/100 g body weight	Decrease in the incidence and the proliferating cell nuclear antigen (PCNA)-positive index of lung tumors.	[45]
Hst	Murine B16-F10 melanoma cells	–	Resulted in melanin synthesis, tyrosinase and microphthalmia-associated transcription factor (MITF) expression, activation of mitogen-activated protein kinases (MAPKs), phosphorylation of cAMP-responsive element binding protein (CREB) and glycogen synthase kinase-3b (GSK3b) and accumulation of β-catenin.	[33]
Hsd	B(a)P-induced lung carcinogenesis in mice	25 mg/kg body weight	Hsd reversed the trend of increasing mast cell density (MCD) and COX-2, MMP-2 and MMP-9 expression induced by B(a)P.	[39]
Hst	HT-29 cell line (colon adenocarcinoma)	5–100 μM for 24, 48 and	Hst induced inhibition of cell growth, DNA damage, decline	[73]

Table 1 (*continued*)

Compound of interest	Model of study	Treatment protocol	Underlying mechanisms of the observed results	Ref (s).
		72 h	in mitochondrial membrane potential, slight decrease in SOD, CAT and glutathione peroxidase levels, and increase in lipid peroxidation profile.	
Hst and Hsd	HL-60 cells	40 and 80 μM	Hst but not Hsd caused DNA laddering, morphological changes, appearance of apoptotic bodies and elevation of caspase-3 activity.	[11]
Hsd-derived flavones	KB (nasopharyngeal epidermoid carcinoma) and HL60 (promyelocytic leukemia)	0.5 nM to 10 μM	More pronounced antiproliferative properties resulted from A-ring substitution pattern and inhibition of tubulin polymerization activities required an OH group at C-5 and OCH_3 groups at C-6, C-7, and C-8.	[51]
β-Cryptoxanthin and Hsd	Colon carcinogenesis induced by azoxymethane (AOM) in rat	–	Inhibition of cyclin D1 overexpression and reduction of index resulting in limiting cell proliferation in colonic neoplasms.	[76]
Hsd	Human pre-B NALM-6 cells	10, 25, 50 and 100 μM	Induction of PPARγ expression and transcriptional activity, accumulation of p53 and reduction of constitutive NF-κB activity.	[27]
Hst	Athymic mice, ovariectomized and transplanted with MCF-7 cells expressing high levels of aromatase	1000 ppm and 5000 ppm, orally.	Decrease in plasma estrogen level, the expression of estrogen-responsive gene pS2 mRNA, cyclin D1, CDK4 and Bcl-xl.	[87]
Hsd	12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cell invasion in HepG2	12.5 μM to 100 μM	Anti-invasive activity by inhibiting MMP-9 enzymatic activity mediated by NF-κB an AP-1 signaling.	[50]
Hsd	Rat bearing 7,12-dimethylbenz(a)anthracene (DMBA)-induced breast cancer	30 mg/kg body weight for 45 days, orally	Antineoplastic and antigenotoxic effects via modulation of energy reservoir and oxidative phosphorylation.	[60]

A mixture of β-cryptoxanthin (0.67 g/100 g) and Hsd (3.58 g/100 g) (CHRP) at 500 ppm for 4 weeks reduced proliferating-cell nuclear-antigen (PCNA) in rat colon, which was induced by azoxymethane (AOM). CHRP significantly diminished the incidence of rat tongue carcinoma that was induced by 4-nitroquinoline-1-oxide (4-NQO). Moreover, CHRP (40, 200, and 400 mg/kg bw) significantly elevated liver glutathione S-transferase (GST) and quinone reductase (QR) activities and GST activity in colonic and tongue mucosa. It was interesting that, in this study, CHRP and mandarin juices reduced the mRNA expression of various cytokines (TNF-α, IL-1β, IL-6) and inflammatory enzymes (COX-2 and iNOS) while augmenting mRNA expression of Nrf2 in the carcinogen-treated rat tongue and colon. It has been shown that the Nrf2 transcription factor modulates the expression of GST and QR, which are crucial in the detoxification process of reactive electrophiles and oxidants that are responsible for the induction of mutations and cancers. On the other hand, the ability of these natural products to reduce cytokine production helps to diminish chronic inflammation that is strictly tied to cancer occurrence [77].

Following an 8-week treatment with Hsd, diosmin or a combination of both, in the initiation phase of bladder carcinogenesis induced by n-butyl-n-(4-hydroxybutyl) nitrosamine, the Hsd inhibitory effect on the cell proliferation of bladder neoplasms was more pronounced compared to diosmin, which exhibited better results in the post-initiation phase (24-week treatment). The mixture of Hsd and diosmin did not show synergetic properties. Because NSAIDS were shown to be able to inhibit n-butyl-n-(4- hydroxybutyl) nitrosamine-induced bladder carcinogenesis [68], it was proposed that Hsd and diosmin anti-inflammatory (inhibition of prostaglandin synthesis) and antioxidant (free radical scavenging) activities might be responsible [85].

In a study by Kohno et al., a tobacco-related carcinogen, NNK was employed to induce lung tumors, and the effects of mandarin juices containing β-cryptoxanthin and Hsd were evaluated. Finally, it was observed that juice-treated mice tumor incidence was reduced, presumably due to the inhibition of cyclooxygenase, the alteration of the immune response and the stimulation of apoptosis [45].

Evaluation of apoptotic features

Apoptosis characteristics that appeared following Hsd treatment have been widely studied in various cell lines.

Hst (20 mg/kg body weight/day for 15 or 32 weeks) effects on 1, 2-dimethylhydrazine (DMH)-induced colon carcinogenesis in male rats were the inhibition of cell proliferation, a decrease in angiogenic growth factor (VEGF, EGF, bFGF) expressions and Bcl-2 levels, an increase in the Bax level, and reduction in the DMH-induced elevation of the COX-2 level in colorectal tissues. The alteration of Bcl-2:Bax that was observed in this study is an observation that furnishes proof for the concept that Hst-induced apoptosis leads to its anti-proliferative properties [59].

In addition to the features mentioned in Table 1, regarding the effect of Hst on the HT-29 human colon adenocarcinoma cell line, apoptotic characteristics, including an increase in cytochrome C, Bax, and cleaved caspase-3 expression and a decrease in the Bcl-2 level, were observed. Altogether, Hst arrests cell proliferation via a Bax-dependent mitochondrial pathway and, at the same time, causes an imbalance in the redox profile, which leads to the proposal that it could be effective for colon cancer treatment [73].

Based on 4,6-diamidino-2-phenylindole (DAPI) staining and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assays, Hsd treatment (10 or 100 μM for 24 h) resulted in apoptotic bodies, nuclear condensation, DNA fragmentation, and perinuclear apoptotic bodies. More in-depth evaluation showed that the apoptotic features caused by Hsd 10 and 100 μM go back during the down-regulation of Bcl-2 and are raised during Bax and caspase-3 mRNA expression. In addition, Hsd boosted caspase-3 activity, dose dependently [66].

During Hsd combat against MCF-7 cells, at two higher concentrations, Hsd provoked apoptosis, as evidenced by the fact that it was shown to result in cell shrinkage and DNA fragmentation. Additionally, the depletion of GSH was observed, which alters the redox balance [63].

Against Human malignant pleural mesothelioma (MSTO-211H), Hsd caused significant time- and dose-dependent inhibition of cell proliferation, nuclear condensation and fragmentation and amplification of the Sub-G1 population. Moreover, Hsd (40, 80, and 160 μM) lessened the Sp1 protein and mRNA expression. SP1 is upregulated in various cancers and is involved in differentiation, cell growth, angiogenesis, metabolism and apoptosis processes. Apoptosis was further confirmed by the activation of PARP and Bid, caspase-3 cleavage, up-regulation of Bax and down-regulation of Bcl-xl as the main apoptotic characteristics. Interestingly, Hsd showed a great impact on Sp1 and modulated Sp1-

downstream target proteins (p27, p21, cyclin D1, Mcl-1 and survivin), proving its chemo-therapeutic and chemo-preventative potentials [49].

Hsd inhibited Ramos Burkitt's lymphoma cell proliferation and induced apoptosis in either PPARγ-dependent or PPARγ-independent fashion. Meanwhile, Hsd treatment showed inhibitory effects on IκB phosphorylation, inhibiting constitutive and doxorubicin-induced NF-κB activation and, hence, sensitizing Ramos cells to chemotherapeutic agent-induced apoptosis [64].

Comparing the effects of Hst and Hsd treatments on HL-60 cells, it was shown that the Hst treatment resulted in DNA laddering, morphological changes, the appearance of apoptotic bodies, an elevation in the caspase-3 activity and a decrease in the anti-apoptotic proteins and Mcl-1 but no alteration in the Bcl-2 family protein levels, while Hsd had no effect on the mentioned factors. Based on the results from the DCHF-DA assay, it was reported that ROS production is not involved in the differential apoptosis-inducing activities. It was proposed that the presence of rutinoside at C7 attenuates the apoptosis-inducing properties of flavonoids [11].

The evaluation of the Hsd (1–100 μM) effects on NALM-6 cells demonstrated that Hsd inhibitory effects on cell proliferation were accompanied by the expression and transcriptional activity of PPARγ. Inhibition of PPARγ partly decreases this anti-proliferative effect. Additionally, PPARγ activation resulted in p53 accumulation, aiding the apoptosis process. Hsd treatment caused inhibitory effects on constitutive phosphorylation of IκB and the activation of NF-κB, which is partly due to PPARγ activation because this effect is reduced when PPARγ is antagonized [27].

Hsd interaction with hormone receptors was shown to have inhibitory effects on cancers in which estrogen or testosterone was involved.

Hst fought against breast cancer via its potency at aromatase inhibition. At the same time, it diminished the expression of cyclin D1, CDK4 and Bcl-xL and increased $p57^{Kip2}$ expression, the majority of these being estrogen responsive [87].

Hsd, being ineffective on the proliferation of PC-3 and DU-145 cells, showed anti-proliferative effects on MCF-7–GFP–Tubulin cells that were not via mitotic inhibition. Hsd had no selective inhibitory effects on testosterone-induced proliferation of LNCaP cells, but a 30% inhibition of LNCaP cells' basal proliferation and an almost flutamide-like potency were observed [48].

From a chemical point of view, possessing more than 6 phenolic hydroxyl groups that enable Hsd and neoHsd to form ionic bonds with charged amino acid residues of proteins results in less flexible structures. Therefore, these compounds exhibited cytotoxic activities against the Caco-2 (highest IC_{50}), HeLa and COS-7 (lowest IC_{50}) cell lines [28].

Unlike the aforementioned studies, some studies showed that Hsd might induce cell proliferation and melanogenesis in epidermal cell. Huang et al. showed that citrus species extract-treated melanoma cells at concentrations below 20 μg/ml had no cytotoxic effects, and acid-hydrolyzed extracts increased the melanin content of the cells while un-hydrolyzed ones did not cause such effects. Hst (50 μM for 2 h) boosted β-catenin expression, stimulated CREB phosphorylation and followed a less than 60 min incubation, while Hst induced quick phosphorylation of p38, MAPK, ERK, and Akt. Taken as a whole, it was concluded that melanogenesis stimulation was induced by Hst via the activations of CREB and MAPKs that are involved in the Wnt/β-catenin pathway [33].

In a unique model that involves topical application of these natural compounds, UVB treatment resulted in an amplified CPD density per nucleus, which was significantly lowered by the topical application of Hsd. This finding could justify that the antioxidant properties of Hsd result in better functioning of the DNA repair enzymes. Additionally, western blotting revealed that 24 and 48 h after UVB exposure, the p53 level was increased, and this increase was more marked following the application of Hsd. However, after 72 h, the p53 level decreased, but it was still higher in the Hsd-treated group. Because p53 plays an important role in DNA repair regulation, an elevation in its expression occurs as a DNA damage response that was stimulated by Hsd. In summary, Hsd enhanced the repair of DNA photo-damage in mice epidermis alongside the amplification of p53 expression [36].

Hsd can also inhibit tumor growth and invasion via the inhibition of angiogenesis and metastasis. While B(a)P lung carcinogenesis caused an increase in the mast cell density (MCD), COX-2, MMP-2 and matrix metalloproteinase-9 (MMP-9) expression, Hsd could decrease MCD, which was most likely due to a reduction in the COX-2 expression, thus reducing the chance of angiogenesis and invasion. MMPs have been shown to be tightly related to angiogenesis, invasion and metastasis, and here, the Hsd treatment significantly reduced the MMP-2 and MMP-9 expression [39].

In a model of acetaldehyde-induced cell invasion, Hsd (50 μM) significantly reduced the number of migrating cells, which was due to the suppression of acetaldehyde-induced activation of MMP-9 and a decrease in its mRNA expression. Regarding the NF-κB or AP-1 promoter, an increase caused by acetaldehyde was dose-dependently reversed by Hsd. In addition, Hsd (50 μM) inhibited acetaldehyde-induced activation of IκB (the principal pathway of NF-κB activation), p38 and JNK (MAP kinase signaling manages the AP-1 activity) phosphorylation, dose-dependently. Overall, Hsd was able to suppress MMP-9 transcription, secretion and activity in HepG2 cells, diminishing cellular invasiveness [88].

To have a better perspective of the cellular targets of Hsd/Hst in cancer, the mechanisms discussed in this part including cancer chemoprevention through increasing the antioxidant defense system, inducing apoptosis in cancerous cells, the inhibition of inflammation via decreasing inflammatory cytokines and enzymes, and the inhibition of angiogenesis and metastasis are summarized in Fig. 2.

Effects on the cardiovascular system

The effects of Hsd/Hst on cardiac functions

Previous studies have shown an association between the dietary intake of flavonoids and the decreased incidence of coronary heart disease [26,30]. Different mechanisms are considered to be involved in broad and nonspecific effects of flavonoids on the cardiovascular system. As discussed previously, both Hst and Hsd have apparent antioxidant properties. This effect of Hst has been evaluated on doxorubicin-induced oxidative stress and DNA damage in rat heart [79]. Doxorubicin is widely used in the chemotherapy of cancer. However, cardiotoxicity has limited its clinical applications. The results revealed that Hst reversed a doxorubicin-induced increase in malondialdehyde (MDA) and a decrease in the GSH levels. It also significantly reduced the DNA damage of cardiomyocytes and the intensity of immunostaining of NF-κB, p38, and caspase-3. It is well known that apoptosis is involved in the pathology of heart failure, myocardial infarction, cardiomyopathy and sepsis. Hst with anti-apoptosis effects is a potential candidate in the treatment of such diseases. The anti-apoptosis effect of Hst in LPS-stimulated H9C2 cardiomyocytes was evaluated recently. The results showed that Hst decreased apoptosis in these cells through the mitochondria-dependent intrinsic apoptotic pathway [86].

Myocardial infarction continues to be the leading cause of mortality world-wide. In this regard, the cardioprotective role of Hsd on isoproterenol-induced myocardial ischemia has been reported [71]. It reduced lipid peroxidation and antioxidant status in experimental animals that were exposed to subcutaneous injections of isoproterenol. PPAR-γ is an important target in the treatment of diabetes. It was discovered that both Hsd and Hst can change the expression of PPAR-γ gene [3]. The beneficial effect of Hsd in the treatment of myocardial toxicity may also be mediated by the activation of PPAR-γ as Hsd is able to decrease streptozotocin–isoproterenol-induced myocardial toxicity [1].

Another target that has an important role in cardiac function is phosphodiesterase 3 (PDE3). PDE3 is able to hydrolyze cyclic adenosine monophosphate. It is inhibited by milrinone, a drug that is used in the

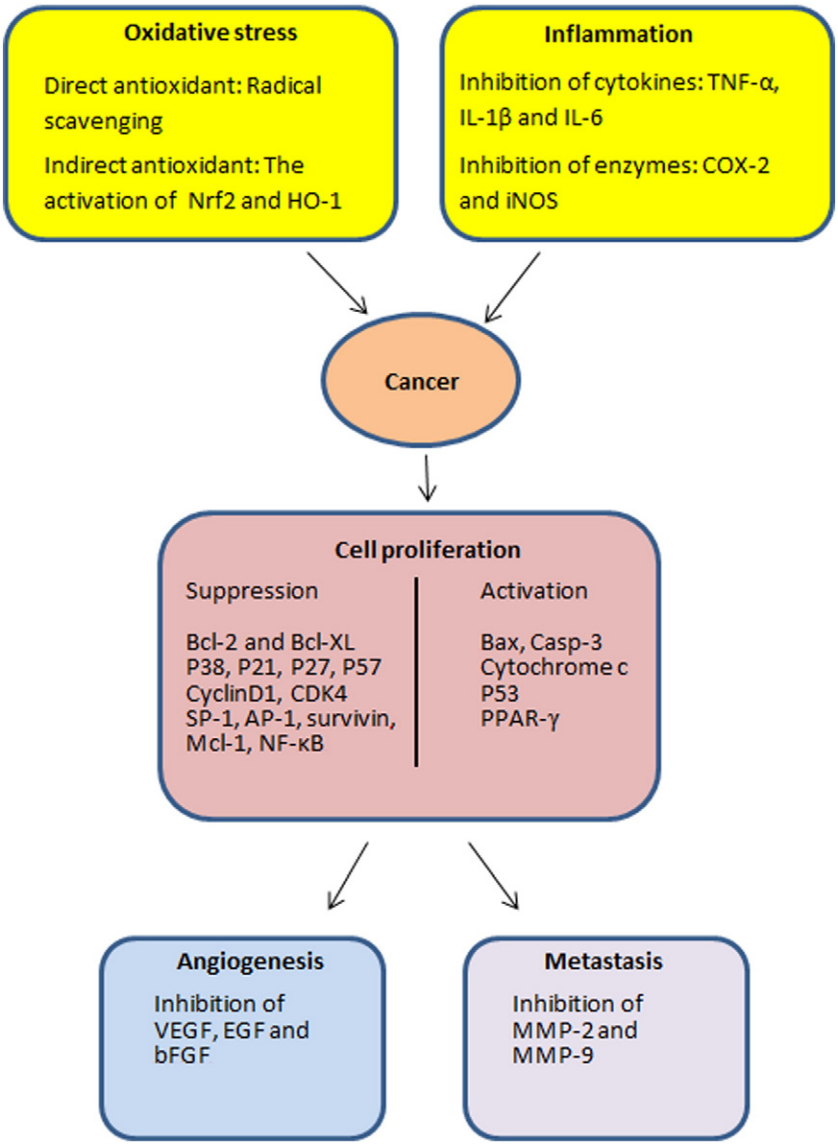

Fig. 2. An overview on cellular targets of Hsd/Hst in each cancer stage.

treatment of heart failure [7]. A recent study showed that PDE3 is also a target for Hst and Hst derivatives and these compounds inhibit PDE3 [32].

In addition, those antioxidant properties of the Hsd plus an increase in the transcription of erythroid 2-related factor 2 have beneficial effects in the hearts of aged rats [20]. The action of Hsd and Hst on the electrical activity of the heart was also investigated. Recent studies showed that Hst acts as a specific low-affinity antagonist of cardiac ether-a-go-go-related gene (hERG) potassium channels [70]. Although this effect of Hst is suggested as being pro-arrhythmic, Gandhi and colleagues showed that Hsd has antiarrhythmic effects by prolonging the QTc interval, the action potential duration and the subsequent refractoriness in an experimental model [23]. They also showed that Hsd could improve both the infarct size and the edema, possibly through a reduction in the oxidative stress and inflammation [23]. Moreover, a significant decrease in serum creatine kinase, aspartate aminotransferase and lactate dehydrogenase activities (which are cardiac function biomarkers) in diabetic rats has been reported following Hsd administration [54]. These findings plus the inhibitory effect of Hst on cardiac remodeling, by blocking PKCα/βII-AKT, JNK and TGFβ1–Smad signaling pathways and inhibiting fibrosis, oxidative stress and myocyte apoptosis, further support the advantages of Hsd consumption for cardiac functions [17].

The effects of Hsd and Hst on the vasculature

Abnormal capillary permeability and pain in the extremities, weakness and night leg cramps have been attributed to the deficiency of Hsd in the diet [24]. On the other hand, the consumption of citrus fruits has been associated with a lower risk of acute coronary events and stroke [15]. Therefore, the effect of Hsd and Hst on the vasculature has been the subject of many studies. For example, it has been documented that a high dose of Hsd (200 mg/kg, oral) has anti-hypertensive and weak hypotensive effects in spontaneously hypertensive (SHRs) and conscious normotensive rats, respectively [22]. A single oral administration of G-Hsd also reduced systolic blood pressure in SHRs but not in normotensive Wistar–Kyoto rats [84]. The effects of Hsd and G-Hsd have also been evaluated by Ikemura and colleagues [34]. They showed that after four weeks of oral administration, both flavonoids suppressed age-related increase in blood pressure and decreased thrombotic tendency in stroke-prone SHRs. In a separate study, the same antihypertensive effects were experienced after short-term administration of Hst [83] and hesperetin-7-O-glucuronide [81].

Angina pectoris is often due to ischemia of heart muscles. Ischemia is a consequence of spasm or obstruction of the coronary arteries. Hst via the inhibition of L-type voltage-gated Ca^{2+} channels and the

enhancement of voltage-gated K^+ channel currents of the myocytes has a direct vasorelaxant effect on coronary arteries [53]. The vasorelaxant effect of Hst on coronary arteries beside its anticoagulant effects (next section) makes it a good candidate in the treatment of angina (both stable and unstable).

Interesting effects of G-Hsd on peripheral vasculature are reported; oral administration of G-Hsd increased the interscapular brown adipose tissue–sympathetic nerve activity and decreased the cutaneous sympathetic nerve activity in rats, resulting in enhanced peripheral body temperature that arises most likely from an increase in thermogenesis and an elevation in the cutaneous blood flow [72]. In addition, the hypotensive effect of Hsd in a randomized clinical study has also been revealed. The results showed a lower diastolic blood pressure after four weeks of supplementation with Hsd [57]. However, the results of another crossover study that included individuals with metabolic syndrome showed that despite an improvement in flow-mediated dilation after a three-week supplementation with Hsd, the blood pressure did not change significantly [69]. Vasculopathy is one of the important complications of diabetic patients. The study of Kumar et al. [46] showed that Hsd can be useful in preventing diabetes-induced vasculopathy via inhibition of the expression of angiogenic parameters (VEGF and PKC-β) and decreasing the vascular permeability and capillary basement membrane thickness.

The hypotensive mechanism of Hst and Hsd has also been explored. Previous studies showed that the underlying mechanism(s) of the hypotensive effects of these flavonoids might be different. Calderone et al. [9] reported that Hst completely relaxed contractions while Hsd produced only a partial vasorelaxant effect in rat aorta. Similarly, Hst relaxed noradrenaline-induced contractions in rat aorta in a concentration-dependent manner, which was not modified by pretreatment with glibenclamide, tetraethylammonium or nifedipine [65]. This finding implies that ATP-sensitive potassium channels, voltage-dependent K^+ channels, or L-type calcium channels do not mediate the vasorelaxant effects of Hst. Further experiments showed that the vasorelaxant effects of Hst are mediated by the inhibition of phosphodiesterase types 1 and 4 [65]. However, the researchers reported that Hsd was inactive throughout the experiments [65]. It is likely that other mechanisms are involved in the hypotensive effects of Hsd; for example, it has been reported that Hsd increases endothelial nitric oxide synthase (eNOS) activity, NO production, and the phosphorylation of Akt and eNOS in human umbilical vein endothelial cells [13]. Furthermore, Hsd, via the NO/protein kinase G pathway, inhibits strain-induced endothelin-1 secretion and increases the formation of reactive oxygen species and extracellular signal-regulated kinase (ERK) phosphorylation [13]. The nitric oxide-mediated effects of Hsd are further supported by the protective effect of Hsd against ischemia–reperfusion injury [25,42]. Interestingly, Hsd also reversed the deficits in sensorimotor and neurological functions following global cerebral ischemia [25]. Measurements of nitrite and nitrate concentrations in urine, as NO metabolites, following ingestion of Hsd and G-Hsd in rats, demonstrated a significant increase in nitrite and nitrate concentrations in the urine, which implies that the production of nitric oxide is increased by these flavonoids [34]. In contrast, the ingestion of orange juice or pure Hsd without significant changes in plasma NO concentration has been reported [57]. Endothelial production of NO has been attributed to antithrombotic, anti-atherogenic, and anti-inflammatory effects [62]. In addition, the impairment of endothelium-dependent vasodilation is reported both in animal models of hypertension [78] and in essential hypertensive patients [75]. Therefore, the beneficial effects of Hsd and Hst on endothelial function have been explored. For example, it has been reported that Hsd supplementation reduced E-selectin, high-sensitivity C-reactive protein and serum amyloid A protein concentrations as biomarkers of endothelial dysfunction [69]. Moreover, Hsd can reduce high glucose-induced intracellular adhesion molecule-1 expression, which is involved in the pathogenesis of atherosclerosis, in human umbilical vein endothelial cells via the p38 MAPK signaling pathway [44]. This effect possibly contributes to the inhibition of monocyte adhesion to endothelial cells. Furthermore, the effect of these flavonoids on oxidative stress in the vasculature has been the subject of some studies. In this regard, it has been reported that G-Hsd reduces mRNA expression of nicotinamide adenine dinucleotide phosphate oxidase subunits (NOX2, $p22^{phox}$ and $p47^{phox}$), which is the main source of superoxide anion in the vasculature, in the aorta of rats [84]. In the same study, the ingestion of G-Hsd significantly reduced the aorta media thickness in SHRs, which suggests that G-Hsd reduced vascular hypertrophy [84].

The effects of Hsd/Hst on coagulant and anticoagulant pathways

Platelet activation and subsequent platelet aggregation are considered to be very important phenomena in the pathogenesis of cardiovascular, cerebral, and peripheral vascular diseases.

It has been demonstrated that collagen and arachidonic acid-induced platelet aggregation is potently inhibited by Hst via the inhibition of PLC-γ2 phosphorylation and cyclooxygenase-1 [37]. Serotonin is also effectively involved in platelet aggregation. Hst, via inhibition of arachidonic acid-induced serotonin secretion, can also have antiplatelet effects (Fig. 3) [37]. Similar to these results, Hsd inhibited the arachidonic acid-, collagen-, ADP- and thrombin-induced rat platelet aggregation dose dependently, in vitro [89]. Using the He–Ne laser technique, Ikemura et al. [34] evaluated the effects of Hsd and G-Hsd on thrombotic tendencies in pial microvessels. The results showed that both flavonoids increased the laser pulses that are required to generate thrombosis. They concluded that an increase in the NO concentration mediates the inhibitory activity of Hsd and G-Hsd on thrombus formation. The effects of Hsd and Hst on coagulation may also be mediated by their inhibition on the gene expression of thromboxane A2 synthase and thromboxane B2 synthase of vascular endothelium, respectively [82].

Stainless steel is often used in cardiovascular implant materials. Regarding the anti-platelet and anticoagulant effects of Hsd and Hst, Li used Hsd on stainless steel surfaces to examine its blood compatibility. This coating prolonged prothrombin time, activated partial thromboplastin time, and thrombin time values compared with stainless steel control suggesting it as a potential natural coating for cardiac implants [52].

Immobilization of hesperidin on stainless steel surfaces and its blood compatibility

The results of a recent study using experimental animals also support the anti-platelet effects of Hsd and Hst. Yu et al. [89] evaluated the effects of orally administered Hsd (1, 3, or 10 mg/kg) or aspirin (50 mg/kg) on the mouse tail bleeding time. They found that Hsd increased the bleeding time more than aspirin. In addition, it has been reported that Daflon® 500 mg "consisting of 90% diosmin and 10% Hsd" significantly inhibited platelet functions, in vivo [55].

The effects of Hsd/Hst on metabolic parameters

Diabetes mellitus is recognized as a major risk factor for cardiovascular diseases (CVD) such as atherosclerosis, heart attack, and stroke. Approximately 75% of the deaths among the men with diabetes and 57% of the deaths among the women with diabetes are attributable to CVD [56]. Furthermore, hyperlipidemia is a proven risk factor for cardiovascular diseases and is one of the major causes of death. On the other hand, the beneficial effect of citrus juice consumption on lipemia in men with previous coronary bypass surgery has been reported [47]. Therefore, there is considerable interest in investigating the hypolipidemic or antidiabetic effects of flavonoids. Previous studies considered the beneficial effects of Hsd and Hst in the treatment of diabetes and dyslipidemia. However, the underlying physiological and molecular

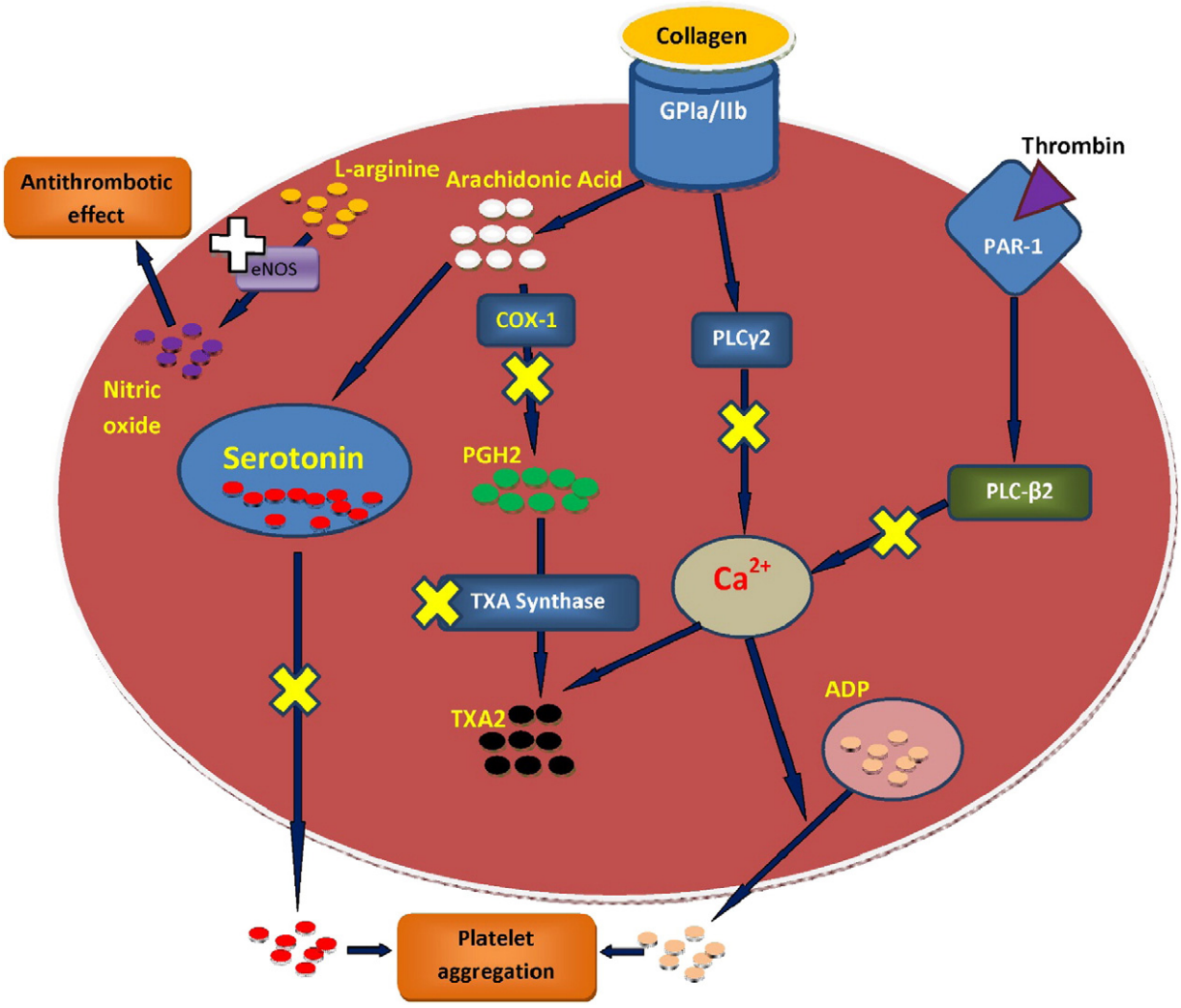

Fig. 3. A schematic illustration of the reported targets that mediate anticoagulant effects of Hsd and Hst. × represents the inhibitory and + represents the induction effects.

mechanisms of the potential cardiovascular health benefits of these flavonoids remain to be identified. In addition, the efficacy of Hsd on the impaired glucose tolerance and insulin resistance of high-fat diet/streptozotocin-induced diabetic rats has been reported [2]; here, oral administration of Hsd significantly decreased blood HbA1c%, blood glucose, and increased serum insulin levels. Additionally, the increases in serum cholesterol, triglycerides, LDL- and VLDL-cholesterol and free fatty acids in type 2 diabetic rats were all ameliorated by Hsd [2]. Administration of Hsd-G (500 mg/day for 24 weeks) to hypertriglyceridemic patients markedly reduced plasma triglyceride and apolipoprotein B concentrations. The effects of oral administration of Hsd (500 mg/day for 3 weeks) on the lipid profile and insulin resistance in a clinical study in patients with metabolic syndrome has also been evaluated [69]. Hsd decreased the total cholesterol and apolipoprotein B concentrations and increased the high-density lipoprotein (HDL). High-density lipoprotein (LDL), lipoprotein, apolipoprotein A-I, triglycerides, fasting plasma glucose, and fasting plasma insulin concentrations did not differ significantly compared to the control group. However, the researchers reported that Hsd treatment caused an improvement toward insulin resistance ($p = 0.06$) [69]. In contrast, a four-week supplementation of one hundred ninety-four hypercholesterolemic individuals with Hsd did not affect the total cholesterol, LDL-C, HDL-cholesterol and triglyceride concentrations significantly [16]. The efficacy of Hst metabolites [3,4-dihydroxyphenylpropionic acid (DHPP) and 3-methoxy-4-hydroxycinnamic acid (ferulic acid)] in the treatment of hypercholesterolemia has been evaluated in hamsters [43]. Supplementation with both metabolites as well as Hst lowered the plasma total cholesterol. Hepatic 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase, the rate-limiting enzyme for cholesterol synthesis, was inhibited by Hst, DHPP, and ferulic acid by 25%, 21% and 21%, respectively. The structure–activity relationship for Hst derivatives in lowering plasma cholesterol has also been assessed. The results showed that the introduction of a lipophilic chain at the 7-hydroxyl position of Hst strongly influences the cholesterol-lowering activity. In this regard, a C-12 lipophilic chain showed the best activity. In a similar way, an effort to optimize the hypoglycemic effects of Hsd derivatives is in progress [35].

Some underlying cellular mechanisms of Hsd hypoglycemic and hypolipidemic effects have been uncovered by previous studies. Hsd could induce its hypoglycemic effects through a reduction in hepatic glucose transporter 2 protein expression and an enhancement of adipocyte glucose transporter 4 expression and hepatic and adipocyte peroxisome proliferator-activated receptor and glucokinase mRNA [38]. Furthermore, Hsd decreases the plasma free fatty acid and the plasma and hepatic triglyceride levels, possibly via the suppression of hepatic fatty acid synthase, glucose-6-phosphate dehydrogenase, and phosphatidate phosphohydrolase activities and by increasing the fecal triglycerides [38]. The hypocholesterolemic effect could be due to decreased hepatic HMG-CoA reductase and cholesterol acyltransferase activities and increased fecal cholesterol [38]. Other suggested mechanisms are the inhibition of cholesterol absorption and the regulation of the expression of the mRNA that is required for lipid metabolism-related proteins,

including cutaneous fatty acid-binding protein, heart fatty acid-binding protein [80], increasing LDL-receptor mRNA [58] and secretion of apolipoprotein B-100 [8].

Conclusion

Hst and Hsd are citrus flavonoids that have various biological activities. Over the past decade, a large number of studies were conducted to determine the molecular targets and underlying mechanisms of Hst and Hsd as well as their metabolites. Some properties of these compounds include anticancer, cancer preventive, anti-inflammatory, neuroprotective and antioxidant properties, which are well-known and promising.

Although, polyphenols are considered as natural compounds with low toxicity but depending on their chemical structure, for instance, in term of presence of sugar moiety in their backbone, they might have some side effects on beneficial microbiota by altering micro-ecology in the gut [18]. On the other hand, since their bioavailability would be increased by commensally intestinal microbiota, more investigations have to be conducted in this regard to determine the suitable doses and structures for use in humans.

Cancer chemopreventive effects of Hsd, Hst and their analogs have been proven in different study models. Considering the cancer chemopreventive effects of Hsd and Hst, a number of targets including NF-κB, TNF-α, IL-1β, IL-6, Nrf2, p38, COX-2 and iNOS have been identified (Table 1). Most of these targets are related to oxidative and inflammatory processes. It is worthwhile mentioning that in contrast to other flavonoids that possess pro-oxidant properties, Hst/Hsd even at the highest dose does not cause acute oxidative damage or cytotoxic effects but Hsd and Hst can induce apoptosis or inhibit proliferation in breast and androgen-dependent prostate cancer cell lines (MCF-7 and LNCaP).

The beneficial effects of the supplementation with Hsd and Hst on the cardiovascular system include antihypertensive, anticoagulant, cardioprotective effects against oxidative stress- and ischemia-induced by drugs, hypolipidemic, and hypoglycemic effects. Although some of these effects are mild to moderate, the total effects of Hst and Hsd lead to the reduction or amelioration of cardiovascular diseases including myocardial infarction and hypertension.

In total, further studies are necessary to unravel more aspects of the therapeutic effects of Hsd and Hst in human diseases. The lack of the clinical data of the therapeutic effects of Hsd and Hst is an important limitation that can be noted regarding most of the previous studies, deserving further research.

It is ultimately recommended that regarding the versatile biological properties of Hsd and Hst, these compounds may even have a broader range of biological applications in the future.

Conflict of interest statement

The authors declare no conflict of interest.

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