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Narrative Review

A Comprehensive Review on the Impact of Hesperidin and Its Aglycone Hesperetin on Metabolic Dysfunction-Associated Steatotic Liver Disease and Other Liver Disorders

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Abstract

The purpose of this review is to examine the effects of hesperidin and hesperetin on liver disorders. Metabolic dysfunction-associated steatotic liver disease is a complicated disorder influenced by many factors such as inflammation, diabetes, and obesity. Currently, the most prominent treatment method is lifestyle changes. If left untreated, it can progress to cirrhosis, liver fibrosis, and liver cancer. Hesperidin, which is a flavanone glycoside polyphenolic plant compound, belongs to the flavanone class and was first isolated from citrus peel. Hesperidin includes aglycone hesperetin and rutinoside sugar. It is the most dominant form of flavonoid in citrus fruits. In our review, we discussed the effects of these phytochemicals on liver diseases, focusing on their relationship with inflammation, blood sugar regulation, and blood lipids. Hesperidin and hesperetin are seen as promising agents for many diseases. Their antioxidant and anti-inflammatory properties support this view. Although their low water solubility limits their potential effects, many studies have demonstrated their benefits. They are thought to play an effective role in inflammatory processes, particularly in liver diseases ore studies are required to find the optimum dosage and to use them as a therapeutic agent for the liver.

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) previously called as non-alcoholic fatty liver disease is a disease whose prevalence is up to 25% in the world and its frequency continues to raise [(1)]. MASLD is caused by the accumulation of triglycerides in hepatocytes over time and is characterized by fatty liver (hepatic steatosis) without any significant signs of inflammation. MASLD is a disorder which is the combination of sedentary lifestyle, high-calorie diet, genetic factors, physiological and metabolic factors [(2)]. Metabolic dysfunction-associated steatohepatitis (MASH) occurs with the progression of MASLD and the onset of an inflammatory process. The basis of the pathogenesis of MASH is the stress response to the excessive supply of nutrients to the liver, which promotes fibrosis and ultimately leads to cirrhosis [(3)].

Hesperidin (HES), a polyphenolic plant compound, belongs to the flavanone class and was first isolated from citrus peel by the French chemist Lebreton in 1828 [(4)]. Hesperidin, also known as hesperetin-7-O-rutinoside, is a flavanone consisting structurally of the aglycone

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form and the disaccharide rutinose [5]. The aglycone form is called hesperetin (HST) [6]. Various pharmacological effects of hesperidin are supported by different studies in the literature. Some of these effects can be exemplified as cardioprotective, anti-inflammatory, anti-diabetic, and antioxidant Welbat *et al.* [7]; [8; 9; 10].

In this review, we will discuss how hesperidin impacts liver health by examining its influence on inflammation, liver enzymes, lipid profile, and blood sugar regulation, all of which are intricately linked to liver function.

2. Methods and materials

This narrative review is based on peer-reviewed articles. Pubmed, Web of Science, and Science Direct databases were used. Only animal studies included in this review, covering the years between 2014 and 2024. The following keywords were used: ‘‘hesperidin’’ OR ‘‘hesperetin 7-O-rutinoside’’ OR ‘‘hesperetin’’ OR ‘‘hesperetin 7-O-glucoside’’ AND ‘‘NAFLD’’ OR ‘‘non-alcoholic fatty liver disease’’ OR ‘‘liver fibrosis’’ OR ‘‘NASH’’ OR ‘‘fatty liver’’ OR ‘‘non-alcoholic steatohepatitis’’. The authors have checked all parts of the article separately. In case of disagreement, the authors have discussed for a common decision. Only animal studies included in this study. As a result of this process, 14 were identified (Table 2). Apart from these, some human studies related to the topic are also presented in Table 3.

3. Definition of Hesperidin and Hesperetin

Flavonoids are a group of polyphenolic structures present in citrus fruits, and they are divided into four groups: flavones, flavanols, flavanones, and flavanols. Flavanones are predominantly present in citrus fruits such as oranges, grapefruits, and lemons. Therefore are sometimes also referred to as citrus flavonoids or bioflavonoids [11].

The origin of the name hesperidin is based on the word "hesperidium" used for citrus tree fruits [12]. Hesperidin is found in different concentrations in various foods [13]. Hesperidin is detected in citrus fruits, particularly in the peel parts such as zest and albedo, in larger amounts compared to seeds and juicy cells [4]. The predominant type of flavanone found in citrus fruits, HES, and its aglycone form HST are particularly abundant in sweet unripe oranges (Citrus Sinensis) at high levels (1-2%) [14]. Hesperetin has similar values to hesperidin in plants because it is a metabolite of hesperidin. It is found in higher proportions in unripe fruits compared to ripe fruits. HES is also a bioflavonoid [4].

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4. Chemical Properties of Hesperidin

Hesperidin (hesperetin-7-rutinoside) is a polyphenolic compound of plant origin. Although it belongs to the flavonoid group, it is more specifically a flavanone [15]. Hesperidin is a flavanone glycoside have an aglycone portion called hesperetin and a sugar portion called rutinoside (shown in Fig.1). In addition, hesperidin can be enzymatically converted to the aglycone hesperetin [14].

Hesperidin is abundant in citrus fruits such as lemon, lime, tangerine, orange and grapefruit, and is the most dominant flavonoid form in citrus fruits (Rutaceae family) [5; 16]. Pure hesperidin is a tasteless and odourless molecule that occurs as long hair-like needles that are skin-coloured or light yellow. Its melting point is between 258° and 262°C. Molecular formula C28H34O15, molecular weight 610.57 Daltons. It is slightly soluble in methanol, and almost insoluble in chloroform, acetone and benzene [17]. However, it may be easily soluble in pyridine. Since it can form complex crystals with other glycosides of similar structure, its solubility and other physical properties are significantly affected. Therefore, it is not easy to obtain in pure form. For purification, it must be washed with hot water, extracted with 95% methyl alcohol, and then crystallized. [18].

Glucosyl hesperidin (G-hes) is a water-soluble form, being 10,000 times more soluble in water than hesperidin alone. Additionally, it's absorbed much faster and more effectively [19]. This form, G-hes, was synthesized by Hijiya [20] and colleagues using regioselective transglucosylation with cyclodextrin glucanotransferase from Bacillus stearothermophilus [19; 21]. G-hes and hesperidin exhibit similar effects [22]. For example, in one study, when HES and G-hes were administered to mice at equal doses, similar improvements were observed in serum cholesterol profiles [23]. In another study, G-hes was found to have anti-obesity and antidiabetic effects. G-hes can easily convert to hesperidin via the α-glucosidase enzyme [19].

Hesperidin can be easily isolated from products of citrus processing, making it economical. Conversely, in industry hesperetin can be produced using bacterial enzymes to modify hesperidin. This results in a more complex aglycone (hesperetin) production process than obtaining hesperidin from the outset, thereby incurring production costs [14].

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5. Sources of Hesperidin

Apart from citrus fruits, hesperidin has been detected in many different plant genera such as Fabaceae and Betulaceae [18]. Hesperidin concentrations in some plant parts are summarized in Table 1.

The Mediterranean Diet is also a high source of hesperidin and hesperetin. It is a diet rich in vegetables and fruits that is frequently recommended for patients with chronic diseases [24]. Therefore, it is very rich in polyphenols, especially citrus bioflavonoids [25]. Following consumption of the Mediterranean diet consumption showed an increase in plasma levels of hesperidin, hesperetin, naringin and routine flavonoids [26].

6. Stability of Hesperidin

Majumdar et al. found that the concentrations of hesperidin aqueous solutions did not change for 2 months in the pH range between 1 and 7.4 in samples other than pH 9 samples [27]. The degradation rate constants, most likely by alkaline hydrolysis, were 0.03 and 23 at pH 9 at 25 and 40 °C, respectively. This indicates hesperidin can undergo alkaline hydrolysis when exposed to elevated pH levels and higher temperatures.

Zhang and colleagues studied how storage conditions and heat processing during pasteurization affect hesperidin levels in orange juice [28]. Although hesperidin levels decreased at both room temperature and 4 °C, the decrease was faster at room temperature. The study concluded that as the storage time of orange juice increases, hesperidin could precipitate, leading to a decrease in its concentration. It is noted that the decomposition of vitamin C may be responsible for the precipitation of hesperidin as the storage time prolongs.

A study on the stability of hesperidin was carried out by Bisega et al. Honey, onion and apple samples were used in the study [29]. The apple matrix largely stabilized these compounds, while the highest degradation occurred in the honey samples. Therefore, it was concluded that hesperidin degradation may also be related to the food matrix.

7. Metabolism of Hesperidin

7.1 Absorption and Transport of Hesperidin

The absorption and metabolism of citrus flavonoids in the intestine vary depending on their chemical structure; This significantly affects their bioactivity [30]. The presence of polyphenols in the form of glycosides, where one or more sugar moieties are attached, contributes to their variable bioavailability [6].

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Hesperidin is a β-glucoside, while hesperetin is its aglycone form [14]. Absorption of glycosides and aglycones is different. The sugar residues markedly influence the absorption because free aglycones can be absorbed in the stomach, whereas the entire glycoside may not be absorbed [11]. Flavonoid aglycones like hesperetin are moderately hydrophobic and are relatively easier and faster to absorb from the lumen compared to flavonoid glycosides (hesperidin) [30]. In studies in which hesperetin and hesperidin were consumed by healthy human subjects, the peak concentration in plasma occurred 4 hours after consuming hesperetin, whereas for hesperidin-rich juices, the peak concentration was achieved after 7 hours. This indicates faster absorption of hesperetin [31; 32].

The main absorption sites for flavanones are the small intestine and colon. In a study where naringenin was injected into different parts of the rat intestines to observe flavanone absorption, the absorption rates of naringenin were found to be similar in the duodenum (47%), jejunum (39%), and ileum (42%). The colon had the highest absorption rate (68%) [33].

The non-detection of hesperidin in human plasma or urine after oral consumption of hesperidin-rich orange juice supports the idea that hesperidin is broken down to hesperetin and related metabolites during absorption [34].

The reason most ingested hesperidin is not hydrolysed by small intestinal β-glucosidases is due to the presence of a routine component that requires its metabolism and absorption in the colon [35]. It has been suggested that the rutinose molecule, hesperetin-7-rutinoside, is hydrolysed by the intestinal microbiota in the large intestine[6]. Hesperidin is absorbed in the colon via the microbiota, where it is converted into the aglycone form, hesperetin. This is then metabolized and hydrolysed [36].

Summary of studies in the literature indicate that hesperidin, also known as hesperidin-7-rutinoside, when orally consumed by humans or animals, is absorbed either by removal of the rutinoside portion to convert it to the aglycone (hesperetin) form, or by conversion of hesperidin to hesperetin 7-glucoside by bacterial enzymes (β-glucosidases) produced by enterobacteria in the intestine [34; 37]. It is believed that after absorption, it is converted into glucuronidated and sulphated metabolites and released into the bloodstream (shown in Fig.2) [5; 38]. Following the cleavage of hesperidin to hesperetin, the passage of aglycone hesperetin into enterocytes occurs via proton-dependent active transport and passive diffusion [11].

There are two hypotheses regarding how flavonoid glycosides are absorbed in the small intestine [39] Firstly, the glycoside may undergo hydrolysis by lactase-phlorizin hydrolase, after which the free aglycone diffuses passively or with facilitation through the

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epithelial cells. Secondly, the glycoside molecule might be transported into enterocytes via SGLT1 carrier and subsequently glycosylated by β-glucosidase enzymes which found in intestinal cells. Both ways lead to intracellular aglycones that are conjugated to glucuronides or sulphates [31].

In a study conducted with human subjects, individuals were given orally low-dose orange juice containing hesperidin, high-dose orange juice containing hesperidin, and orange juice with added hesperidinase, thereby containing hesperetin-7-glucoside [31]. Concentration of hesperetin-7-glucoside was measured in plasma and urine. Subjects consuming orange juices containing low and high doses of hesperidin achieved similar plasma peak concentrations at approximately 7 hours. However, in the group consuming orange juice containing hesperidinase, the peak concentration was achieved after 4 hours. This finding supports the notion that flavonoids with rutinoside groups are absorbed following hydrolysis by colonic microflora [31]. An animal study showed that the disaccharide portion of hesperidin was removed by intestinal bacteria and absorbed as hesperetin in the intestine. This study demonstrated that hesperetin undergoes immediate glucuronidation in the intestinal epithelium, converting it into hesperetin glucuronides [18]. Matsumoto et al. [34] showed that after oral intake of hesperidin, it is converted into hesperetin glucuronides in the intestinal epithelium and then reaches the liver. This molecule undergoes deglucuronidation, demethylation, remethylation, and reglucuronidation in the liver, leading the release of hesperetin conjugates such as hesperetin-7-O-β-D-glucuronide (HPT7G) and hesperetin-3-O-β-D-glucuronide (HPT3G), as well as homoeriodictyol conjugates into the plasma. It was also reported in this study that HPT7G was found in higher amounts than HPT3G. In another animal study, the influence of hesperidin metabolites on endothelial function and blood pressure was investigated, and intravenous administration of HPT7G reduced blood pressure similarly to hesperetin, exhibiting vasodilatory and anti-inflammatory effects. On the other hand, HPT3G had little effect on these parameters [5; 16].

Numerous studies indicate that after oral consumption, hesperidin is hydrolysed to its aglycone form by β-glucosidases, and then converted to glucuronides and sulphates in the colon. Subsequently, absorbed hesperetin undergoes immediate metabolism into glucuronide and sulphate conjugates in the intestinal epithelium and liver [5; 19; 34]. Three hours after intake, hesperetin appears in the plasma primarily as glucuronides, accounting for 87% of the total. The remaining portion consists of sulfoglucuronides. It reaches peak concentration between 5 and 7 hours [40]

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However, hesperidin exhibits low solubility in water (0.01%) and limited bioavailability [37; 41; 42]. Different methods such as micronisation and encapsulation have been proposed especially in pharmaceutical production. With these methods, it is possible to increase the bioavailability of hesperidin. It also improves its stability [42; 43].

7.2 Bioavailability of Hesperidin

The bioavailability of polyphenols varies significantly, with one of the primary factors influencing human absorption is the glycoside structure of polyphenols present in plants. This glycoside structure, where polyphenols are attached to one or more sugar molecules, is considered one of the primary factors determining the absorption level in humans. The general bioavailability of hesperidin, also known as hesperetin-7-O-rutinoside, is comparatively low because of owing a rutinoside molecule in its structure [6]. In a study aimed at increasing the bioavailability of hesperidin, the rutinoside molecule was enzymatically removed from hesperidin under in vitro conditions, resulting in the production of hesperetin-7-O-glucoside. The study reported the bioavailability of hesperetin was approximately tripled [31].

In another study conducted to screen and identify metabolites of hesperidin and hesperetin, and to determine similarities and differences in pharmacodynamics and pharmacokinetics, Sprague-Dawley rats were orally administered equal amounts of hesperidin and hesperetin. It was showed that the bioavailability of hesperetin exceeded that of hesperidin [44].

8. Examining the Factors Associated with Hesperidin and MASLD

8.1. Effects on Inflammation-Possible Mechanisms

The combined causes of MASLD include a sedentary life and a high-calorie diet that also causes obesity, environmental factors, genetic elements, physiological and metabolic factors. Consumption of Western-style diets, particularly elevated intake of simple sugars and saturated fats, obesity, hyperlipemia and insulin resistance increases the prevalence of MASLD. Lifestyle modifications for the management of MASLD and comorbid diseases and elimination of the underlying causes of the disease are the issues that the scientific perspective focuses on [45; 46; 47].

Despite the complex and uncertain pathogenesis of MASH, oxidative stress is widely known as a main reason. In healthy liver tissue, there exists a dynamic balance between

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oxidation and antioxidant systems. Hepatocytes have the ability to effectively neutralize ROS (reactive oxygen species) by using antioxidant mechanisms [48].

In MASH, ROS production in mitochondria increases and the activity of ROS scavenging mechanisms decreases, leading to overproduction ROS and oxidative stress, resulting in the formation of toxic lipid peroxides 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) [49]. Consequently, the accumulation of ROS and lipid peroxides negatively affects protein production following mitochondrial dysfunction. Protein carbonylation and destruction of phospholipid membranes result in hepatocyte damage and apoptosis. In hepatocytes, overaccumulation of ROS can activate the nuclear factor-κB (NF-κB)-mediated inflammatory pathway. NF-κB is a transcription factor that regulates liver inflammation and modulates the inflammation-fibrosis-cancer axis in liver disorders. Chemokines and cytokines increase with NF-κB increase (shown in Fig.3). Activation of Hepatic stellate cells and Kupffer cells have an important part in the development of liver fibrosis. This activation may be mediated by the release of inflammatory cytokines [50; 51]. Chronic inflammation and subsequent continuous increase in NF-κB and cytokines may also trigger hepatic carcinogenesis (shown in Fig.3) [52]. There are studies in the literature showing that the phytochemical hesperidin is effective in stopping cell growth and preventing apoptosis by inhibiting NF-κB [53; 54]. Additionally, in a study, rats with carbon tetrachloride (CCL4) induced liver cirrhosis significantly reduced MDA levels after hesperidin treatment, indicating its antioxidant effect. In this study, the levels of NF-κB increased 11-fold in rats with CCL4-induced liver cirrhosis compared to the control group, but administration of hesperidin treatment inhibited NF-κB and transforming growth factor beta 1 (TGF-β) expression. Interestingly, in this study, administering hesperidin to non-cirrhotic normal rats lowered NF-κB levels to a level below the control values [55].

In another study, rats with liver cancer induced by diethyl nitrosamine and CCL4 were treated with hesperidin, and MDA levels decreased significantly [56]. The levels of TNF-α, TGF-β1, and Smad3 in these rats also decreased significantly. Additionally, very low levels of collagen accumulation were observed in the groups receiving hesperidin treatment. Consistently, in another study, hesperidin treatment showed a protective effect against ethanol-induced damage by reducing MDA levels [57].

In summary, activated Kupffer cells increase liver damage by generating several metabolites that lead to cellular injury. Some of these metabolites are superoxide radicals and proinflammatory cytokines [58]. Eventually, this condition evolves from a simple fatty liver

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disease to NASH. Therefore, controlling oxidative stress and excessive ROS production in the liver is a critical strategy to prevent NASH [48].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a protein which has a vital role in antioxidant system, controls the transcription of various antioxidant genes, providing protection from oxidative damage induced by inflammation [59]. Nrf2 is the main controller of antioxidant stress systems. The Nrf2/ARE signalling pathway has a significant part in combating continuous oxidative stress in cellular defence, regulating the expression of anti-oxidant genes. When oxidative stress increases, Nrf2 moves to the nucleus and attaches to the antioxidant response element (ARE). This starts the downregulation of antioxidant gene transcription, resulting into a decrease in intracellular ROS content and inhibition of oxidative stress [60]. One study showed that Nrf2 deficiency leads to NF-κB activation and increases oxidative stress, thereby worsening liver steatosis, injury, and then leads to fibrosis in mice with MASLD [61]. Other studies have also shown that hesperidin has an anticarcinogenic effect by activating the Nrf2/ARE/HO-1 signalling pathway [56; 62; 63]. Increasing evidence suggests that the activating of Nrf2 may be protecting hepatocytes from oxidative stress and thus lipotoxicity, thereby attenuating MASLD. Considering these aspects, Nrf2 has been considered as a new focus to reduce oxidative stress in the liver and treat the disease [48].

One of the important pathways in NF-κB activation is activated by endoplasmic reticulum (ER) stress [64]. The ER is charged with regulating protein synthesis, proper folding, and post-translational modification. It is susceptible to the build-up of unfolded or misfolded proteins in its lumen, disrupting ER homeostasis. If the accumulation of these unproper protein cargo exceeds the ER's folding capacity, it triggers the unfolded protein response (UPR) [65]. One of the main pathways regulated by ER membrane receptors during UPR is the pancreatic ER kinase-like ER kinase (PERK) pathway. Under ER stress, PERK phosphorylates the α subunit of eukaryotic translation initiation factor-2, which in turn activates the NF-κB pathway. Subsequently, activated NF-κB increases the synthesis of TNF-α and IL-6 [66; 67]. One study showed that hesperidin could attenuate liver ER stress in free fatty acid-stimulated human THP-1 cells [68].

Hepatic fibrosis, which represents a response to the chronicity of liver injury, is marked by the deposition of extracellular matrix particularly α-SMA, collagen I. Permanent fibrosis usually results in cirrhosis or liver failure [69],[70]. Activation of hepatic stellate cell is one of the key factors responsible for the accumulation of extracellular matrix. The persistent activation of hepatic stellate cells results in the continuous production of inflammatory cytokines, such as TGF-β1, and the development of liver fibrosis. Inhibiting this activation

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appears to be an effective therapeutic strategy to prevent liver fibrosis. The adenosine 5-monophosphate activated protein kinase (AMPK) pathway is associated with fibrosis of liver [71]. AMPK inactivation increases hepatic fibrogenesis. Sensitivity to AMPK phosphorylation reduces liver fibrosis. SIRT3, a regulator belonging to the histone deacetylase family, is located in mitochondria. It contributes to homeostasis and stress response, which are some of the physiological activities of the cell. With the upregulation of the AMPK/SIRT3 signalling, mitochondrial function can improve and the progression of the disease slows down [72; 73]. In one study, mice with CCl4-induced liver fibrosis were given hesperetin-16 (HD-16), an increased water-soluble form of hesperetin, and hesperetin-16 reduced inflammation and fibrogenesis in the liver by moderating the AMPK/SIRT3 pathway. In the same study, HD-16 treatment of TGF-β-activated LX-2 cells decreased the expression of genes involved in fibrogenesis, indicating its anti-fibrotic effect [74].

Another study focusing on fibrosis of liver highlights that one of the significant factors leading to fibrosis is the Hedgehog signalling pathway [75]. It is suggested that the reason is that activation of Hedgehog-GLIoma pathway increases the transformation of quiescent stellate cells into fibrogenic myofibroblasts. This pathway is associated with several cellular responses like proliferation, differentiation, and cell viability. Abnormal activation of the this pathway is often shown by the upregulation of Gli-1 and plays a role in liver cancer [76]. In this study, the mRNA levels of fibrotic genes decreased in hepatic stellate cells isolated from mice treated with a derivative of hesperetin compared to hepatic fibrosis mice [77]. These findings indicate that hesperetin interferes with the onset of liver fibrogenesis in mice. Additionally, it has been shown that Gli-1 levels in hepatic stellate cells isolated from mice treated with hesperetin decreased compared to hepatic fibrosis mice [77; 78].

Sarcopenia is a severe condition in the elderly, marked by muscle loss and increased inflammation, including elevated pro-inflammatory cytokines and ROS [79]. One of the main elements of the disease is the increase in inflammation and thus the increase in pro-inflammatory cytokines and ROS. A study investigating the link between sarcopenia and inflammation found that daily administration of hesperidin to animals for 8 weeks improved muscle strength in older mice [80]. At the end of the study, the muscle fibres and strength of elderly mice treated with hesperidin increased. This result was achieved by improving the M1/M2 macrophage imbalance through the anti-inflammatory properties of hesperidin. Additionally, hesperidin reduced muscle protein degradation, increased protein synthesis, and promoted myogenesis, leading to stronger muscle fibres. In addition, there are shared mechanisms involved in developing sarcopenia and MASLD, and the two may coexist in the

elderly. In one study, people with MASLD were found to have consistently lower muscle mass than healthy people over a 12-year period [81].

8.2. The Impact of Hesperidin on Lipid Profile and Liver Enzymes

We mentioned above that there is no definitive treatment method for MASLD. Lifestyle change is still considered the most current treatment approach [82]. In one study, an in vitro model of lipid accumulation related to free fatty acids was created, and this cell line was treated with hesperidin. There was a decrease in lipid accumulation in the cells [83]. In a study with human subjects with MASLD, there was positive effects of dietary intervention and physical activity on the treatment of the disease. In addition to these interventions, the group receiving hesperidin supplementation showed significantly greater reductions in liver enzyme levels, serum TG and total cholesterol levels, and hepatic steatosis and fibrosis amounts compared to the control group [84]. In an animal study, hesperidin treatment improved serum TG levels in a MASLD mouse [85]. In another animal study, high-dose hesperidin lowered serum ALT, AST, total, and direct bilirubin levels, while low-dose hesperidin had no effect on liver enzymes [86]. There are promising studies in the literature regarding the effect of hesperidin on blood lipids. For example, in a randomized controlled trial conducted with obese individuals, hesperidin intervention was added to calorie restriction. The hesperidin intervention didn't affect body weight when combined with calorie restriction, but it did lead to notable decreases in LDL cholesterol and total cholesterol levels. Liver enzymes (ALT, AST, ALP, GGT) decreased in both groups compared to baseline, but there was no significant difference between the two groups. Total antioxidant capacity (TAC) and MDA levels were also evaluated in this study. The TAC and MDA levels of both groups decreased compared to baseline, but hesperidin intervention did not caused a greater decrease than the calorie-restricted group [87]. A randomized control study showed that G-hes decreased serum total cholesterol levels [88].

In an animal study, pre-treatment with hesperidin was protective against the toxic effects of cadmium and ameliorated the impaired lipid profile caused by cadmium. Hesperidin increased HDL cholesterol levels while decreasing LDL cholesterol and total cholesterol levels [89]. However, if the hesperidin treatment impacts lipid profile is controversial. In an animal study, although hesperidin reduced the appearance of steatosis the liver, it did not affect the lipid [68].

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Hesperidin intervention can reduce fat accumulation in the liver. In one study, hesperidin prevented the increase of lipid deposition in the liver. Additionally, due to a selective enrichment of a group of intestinal bacteria like Bacteroidota proven to improve obesity and metabolic syndrome symptoms, and the promotion of arginine synthesis by these bacteria, hesperidin is considered a therapeutic agent for MASLD [90].

8.3.The Effects of Hesperidin on Plasma Glucose

Although its frequency is increasing day by day and it has a significant prevalence in the world, the pathogenesis is not completely understood [91]. Thus, MASLD has become an important public health problem within the scope of metabolism and liver disorders. MASLD, a multisystem metabolic disease, is often accompanied by type 2 diabetes (T2DM) and metabolic syndrome (METs), which together promote disease progression [92]. The liver has a fundamental role in lipogenesis and gluconeogenesis, including cholesterol metabolism. Metabolic dysfunctions in the diseased liver can lead to various pathological conditions. The increasing prevalence of obesity, T2DM, and METs promotes the pathophysiological changes that lead to MASLD. MASLD is thought to be the most common liver disorder in western communities [93]. Changes in insulin response associated with obesity, metabolic syndrome, and diabetes can lead to progression of liver damage due to imbalance in β-oxidation, lipid metabolism, and autophagy. Therefore, these diseases are thought to be interconnected [94]. For instance, it is known that in the presence of diabetes, increased glucose auto-oxidation and protein glycation lead to increased formation of free radicals. The increase in free radicals also leads to an increase in lipid peroxidation [95]. The accumulation of MDA leads to permanent damage in hepatocytes. The contribution of the accumulation of lipid peroxidation products in liver damage is inevitable. Therefore, diabetes and liver diseases can trigger each other's occurrence directly or indirectly [48; 96].

Disruptions exceeding a certain limit in glucose metabolism can lead to MASLD, thus there is an interconnection between the disorder, glucose homeostasis, and lipid metabolism. Some studies in the literature indicated that hesperidin can be positively affect glucose metabolism and insulin activity [87; 97]. In one animal study, elevated blood glucose and insulin levels were reduced with hesperidin treatment. In the same study, a group of mice was given glucose intraperitoneally followed by hesperidin administration. Hesperidin is thought to reduce increased blood sugar levels and thereby reduce glucose tolerance [98; 99].

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Hesperidin is thought to regulate blood glucose levels by modulating enzymes in glucose metabolism like glucose-6-phosphatase. In one study, administration of hesperidin to mice reduced the levels of glucose-6-phosphate dehydrogenase and fatty acid synthase enzymes. Additionally, after hesperidin treatment, significant increases in glucokinase mRNA levels were noted. In this study, hesperidin caused a significant decrease in hepatic GLUT2 expression while increasing adipocyte GLUT4 levels compared to the control group [100]. Other studies suggest that another effect of hesperidin on glucose metabolism may occur via glucose transporters [101; 102].

Conclusion

Hesperidin and hesperetin are being investigated as promising treatments for many diseases. They are thought to play an effective role in inflammatory processes, particularly in liver diseases, but further studies are needed to determine the optimum dosage. Additionally, their low water solubility limits their potential benefits. We believe that deriving water-soluble forms could enhance their beneficial effects. While we consider them as promising agents in preventing and treating liver diseases, especially due to their anti-inflammatory and lipid-lowering effects, it is important to note that more research is required. Based on their effects in in-vivo and in-vitro studies, we must emphasize the necessity for further investigation.

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Table 1. Sources of Hesperidin

Plant	***Plant Part***	***Concentration***	***Reference***
Citrus limonia	Leaves and stems	3,2 g/kg	Soares et al, 2015[103]
Citrus Sinensis	Leaves	13.8 g/kg	Soares et al, 2015[103]
Mentha Piperita	Plant extract	504,2 mg/L	Najafian et al, 2016[104]
Stevia rebaundiana	Plant extract	493,4 mg/L	Najafian et al, 2016[104]
Citrus Limetta	Pulp	0,62 mg/g	Damián-Reyna et al, 2017[105]
	Juice	27,3 mg/L	
	Seed	0,04 mg/g	
Citrus Reticulate	Pulp	0,98 mg/g	Damián-Reyna et al, 2017[105]
	Juice	21,7 mg/L	
	Seed	0,09 mg/g	

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Table 2.Effects of Hesperidin and Hesperetin on Liver Diseases

	Disease	Study group	Treatment	Time	Results
Li et al, 2021[48]	MASLD *induced with HFD	Male Wistar rats	Hesperetin • 100 mg/kg/day • 300 mg/kg/day Orally consumed	16 weeks	• Hesperetin reduced oxidative stress. • Hesperetin reduced TC, TG and LDL levels.
Xie et al, 2022[68]	MASLD * induced with HFD	SD rats	Hesperidin • 200 mg/kg Orally consumed	12 weeks	• While hesperidin suppressed high-fat diet-induced body weight gain and hepatic steatosis, it did not affect serum TG, TC and LDL levels. • Hesperidin reduced endoplasmic reticulum stress in THP-1 cells stimulated with high-fat diet.
Tang et al, 2023[83]	MASLD * induced with HFD	Wistar rats	Shuganzhi Tablet (SGZT) • SGZT (100 mg/kg/day) • SGZT (200 mg/kg/day) • SGZT (400 mg/kg/day) *Main metabolites in SGZT:	12 weeks	• Increased body weights after HFD decreased significantly with SGZT (200 and 400) treatment. No significant change was occurred in the SGZT-100 group. • LDL-c and TG levels, which increased after HFD, decreased significantly with SGZT

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			Hesperidin, polydatin, naringin, emodin, specnuezhenide, saikosaponin A and resveratrol Orally consumed		(200 and 400) treatment. HDL levels increased after treatment. • In the SGZT 100, 200 and 400 treatment groups, hepatic steatosis was moderate or small. The circular vacuole was significantly reduced compared to the HFD group.
Li et al, 2022[90]	MASLD * induced with HFD	C57BL/6J mice	Hesperidin • LFD • HFD • HFD + hesperidin (%2-1.6 g) Orally consumed	16 weeks	• Mice receiving only the high-fat diet gained more weight than the other 2 groups. • There was no significant difference between the final weights of the LFD and HFD +hesperidin groups. • The lowest levels of TG, ALT, AST and LDL were found in LFD mice. The levels of the group receiving hesperidin were found to be lower than the HFD group. • Hesperidin prevented fat accumulation in the liver.
Chen et al, 2019[69]	MASLD * induced with HFD	C57BL/6J mice	Hesperidin • HFD • HFD + Hesperidin (150 mg/kg/day)	12 weeks	• There was no difference in weight between groups. • Hesperidin treatment reduced TG and LDL levels but did not affect total cholesterol

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			• HFD + Hesperidin (300 mg/kg/day) • Hesperidin (300 mg/kg/day) Orally consumed		levels. • Hesperidin treatment reduced liver steatosis. • Hesperidin administration alone, without HFD, prevented fatty liver.
Nie et al, 2024[98]	MASLD * induced with HFD	C57BL/6J mice	Hesperidin • HFD • HFD + Hesperidin (150 mg/kg/day) • HFD + Hesperidin (300 mg/kg/day) • Hesperidin (300 mg/kg/day) Orally consumed	16 weeks	• Increased body weight serum TG levels, TC, LDL, AST, ALT levels decreased and HDL levels increased with hesperidin treatment. • Hesperidin showed a protective effect against MASLD by reducing hepatic fat accumulation.
Prasatthong et al, 2021[106]	Hepatic steatose * induced with HFD	Rats with metabolic syndrome	• Hesperidin ○ 15g/kg ○ 30 mg/kg • Metformin • Metformin + hesperidin	16 weeks	• Less fat accumulation was observed in rats treated with hesperidin or metformin. • Hesperidin and metformin ameliorated high-fat diet-induced dyslipidemia and liver dysfunction in rats.
Li et al, 2022[74]	Liver fibrosis	C57BL/6J mice	Hesperetin Derivative-16	4 weeks	• HD-16 (25, 50, and 100 mg/kg) dose-dependently reduced collagen deposition

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	*induced with CCL4		• 25 mg/kg/day • 50 mg/kg/day • 100 mg/kg/day Orally consumed		caused by CCl4 treatment. • HD-16 treatment increased SIRT3 expression in fibrotic livers.
Chen et al, 2017[61]	Liver fibrosis *induced with CCL4	C57BL/6J mice	Hesperetin Derivative-14 • 25 mg/kg • 50 mg/kg • 100 mg/kg Orally consumed	Once every 2 weeks for 4 weeks	• HD treated mice with hepatic fibrosis dose-dependently inhibited hepatocyte necrosis, inflammatory cell infiltration, and mouse liver fibrogenesis. • ALT, AST, ALP serum levels decreased. • Serum TGF-β1 and IL-1β levels decreased.
Zhu et al, 2022[(107)]	Liver fibrosis *induced with CCl4	C57BL/6J mice	Hesperetin Derivative-14 • 25 mg/kg/day • 50 mg/kg/day • 100 mg/kg/day Orally consumed	6 weeks	• Hesperidin reduced CCL4-induced liver fibrogenesis. • Hesperidin reduced the expression of fibrogenic genes in activated hepatic stellate cells. • 50 and 100 mg/kg/day hesperidin treatments reduced ALT and AST levels.
Sukkasena et al, 2021[(85)]	Fatty liver disease * induced with	ICR mice	Intragastrically administered Hesperidin • 50 mg/kg/day	8 weeks (60 days)	• Hesperidin interventions reduced liver size and improved hepatic, morphological, histological features. • Both doses of intervention reduced TG

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	HFD and ethanol		• 200 mg/kg/day)		levels. TG levels decreased more in the low-dose hesperidin group. • Hesperidin treatment suppressed inflammatory cytokines, improved mRNA expression of metabolic genes, and improved the antioxidative system.
Yi et al, 2023[108]	Alcoholic liver disease *induced with ethanol	C57BL/6J mice	Citrus Honey (CH) *contains 41 chemical components including hesperidin and hesperetin. Orally consumed • Low dose CH (5 mg/kg) • Medium dose CH (10 mg/kg) • High dose CH (20 mg/kg) *Chow diet was applied for 13 weeks, followed by ethanol and CH treatment for 10 days.	10 days	• High and medium dose CH treatment led to improvements in ALT and AST levels. • Alcohol-induced liver damage was ameliorated by honey intake, and levels of cellular edema and steatosis were reduced. • CH treatment (dose-dependent) has shown some significant benefits in maintaining homeostasis of the gut microbiota and manipulating the gut microbiota to control the production of short-chain fatty acids.
Yuan et al, 2023[109]	Alcoholic liver disease *induced with	C57BL/6J mice	Hesperidin • 2 mg/day • 4 mg/day Orally consumed	10 days	• Hesperidin treatment decreased ALT, AST, TG levels. • Hesperidin reduced hepatic steatosis and inflammatory response in ethanol-exposed

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	ethanol				mice.
Ali et al, 2023[110]	Hepatotoxicity *induced with Paclitaxel	Wistar Rats	Hesperidin 10mg/kg/every other day Orally consumed	6 weeks	• ALT, AST, LDH, ALP, GGT levels, which increased after Paclitaxel application, decreased with hesperidin treatment. • Hesperidin treatment reduced the increased lipid peroxidation in the liver. • The combination of Hesperidin with Rutin significantly reduced the damage caused by paclitaxel.

Abbreviations: MASLD, metabolic dysfunction-associated steatotic liver disease; HFD, high-fat diet; LFD, low-fat diet; TC, total cholesterol; TG, Triglyceride; LDL, low density lipoprotein; HDL, high-density lipoprotein; THP-1, The human leukaemia cell line-1; ALT:, alanine aminotransferase; AST, aspartate aminotransferase; HD-16, hesperetin derivative-16; CCL4, carbon tetrachloride-4; LDH, lactate dehydrogenase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transferase; TGF-β1, transforming growth factor beta 1; IL-6, Interleukin-6; SIRT3, Sirtuin 3

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Table 3. Randomized Controlled Studies of Hesperidin and Its Effects on Some Diseases linked with MASLD

	Disease	**Treatment**	**Time**	**Results**
Cheraghpour et al, 2019[84]	MASLD	49 individuals with level 2 and 3 MASLD between the ages of 18-70 • Intervention group (n=25) = two capsules of hesperidin (each contains 500 mg), • Control group (n=25) = placebo (starch) *In addition, individuals were given healthy nutrition recommendations and physical activity recommendations.	12 weeks	• Insulin and blood glucose levels decreased in both groups as a result of the interventions. However, there was no significant difference between the changes in the 2 groups. • There were significant decreases in serum TG, total cholesterol and LDL levels in the hesperidin group. Compared to placebo, the reduction in serum TG and TC was significantly greater. • Hepatic steatosis and fibrosis decreased in both groups, but there was a greater decrease in the hesperidin group.
Yari et al, 2021[111]	METs	98 adult participants • Control group (n=25) *those following the lifestyle intervention program (LIP) • Flaxseed group (n=25) *Those who consume 30 g/day of ground brown flaxseed and follow LIP	12 weeks	• Serum TG levels decreased significantly compared to the baseline value in all groups except the control group. • There was no difference in BMI between the groups. • Flaxseed-Hesperidin group was found to be significantly effective on glucose parameters. • The combination of hesperidin and flaxseed has

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		• Hesperidin group (n=24) *those who consume 2 500 mg hesperidin capsules per day and follow LIP • Flaxseed-Hesperidin group (n=24) * Those consuming 30 g/day ground brown flaxseed + two 500 mg hesperidin capsules and following LIP		been found to have positive effects on METs.
Osama et al, 2023[112]	T2DM	Patients older than 18 years using oral antidiabetic agents * Diabetic patients who have had symptomatic peripheral neuropathy for at least one month and have a Michigan Neuropathy Screening Instrument (MNSI) physical examination score of ≥2.5. • Control group (n=33) • Hesperidin group (n=32) * 500 mg/twice a day • Diosmin group (n=32) * 500 mg/twice a day • Hesperidin +diosmin group (n=32) *	12 weeks	• Fasting plasma glucose decreased in all groups. Hesperidin and diosmin combination group showed the most significant decrease. • The decrease in LDL levels was most clearly observed in the hesperidin and diosmin group. • There was no significant difference between the groups in the increase in HDL levels. • There was a significant decrease in MNSI in the hesperidin group (p=0.003), diosmin group (p=0.015) and hesperidin and diosmin combination group (p<0.001).

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		500 mg/twice a day for each *For 7 days before the start of the study and throughout the study, the subjects continued their normal lifestyle and foods rich in citrus flavonoids were restricted.		
Homayouni et al, 2017[113]	T2DM	64 participants aged 30-65, who had diabetes for at least 3 years, who used oral antidiabetics, and whose BMI was greater than 30. • Control group (n=32) *placebo capsule • Intervention group (n=32) * 500 mg hesperidin/day	6 weeks	• Hesperidin treatment improved antioxidant capacity and glycaemic control in patients with T2DM. • Hesperidin reduced hyperglycaemia-induced oxidative stress. • MDA levels decreased significantly in the hesperidin group.
Yari et al, 2020[114]	Mets	49 participants • Hesperidin group *500 mg capsules twice a day (n=25) • Placebo group (n=24) Patients were also recommended lifestyle intervention.	12 weeks	• Hesperidin reduced serum TNF-α levels. • Hesperidin improved oxidative stress and inflammation. • Hesperidin significantly reduced fasting glucose levels and increased insulin sensitivity.

Abbreviations: MASLD, metabolic dysfunction-associated steatotic liver disease; METs, metabolic syndrome; T2DM, tip 2 diabetes; TC, total cholesterol; TG, Triglyceride; LDL, low density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; MDA, malondialdehyde; TNF-α, tumour necrosis factor- α

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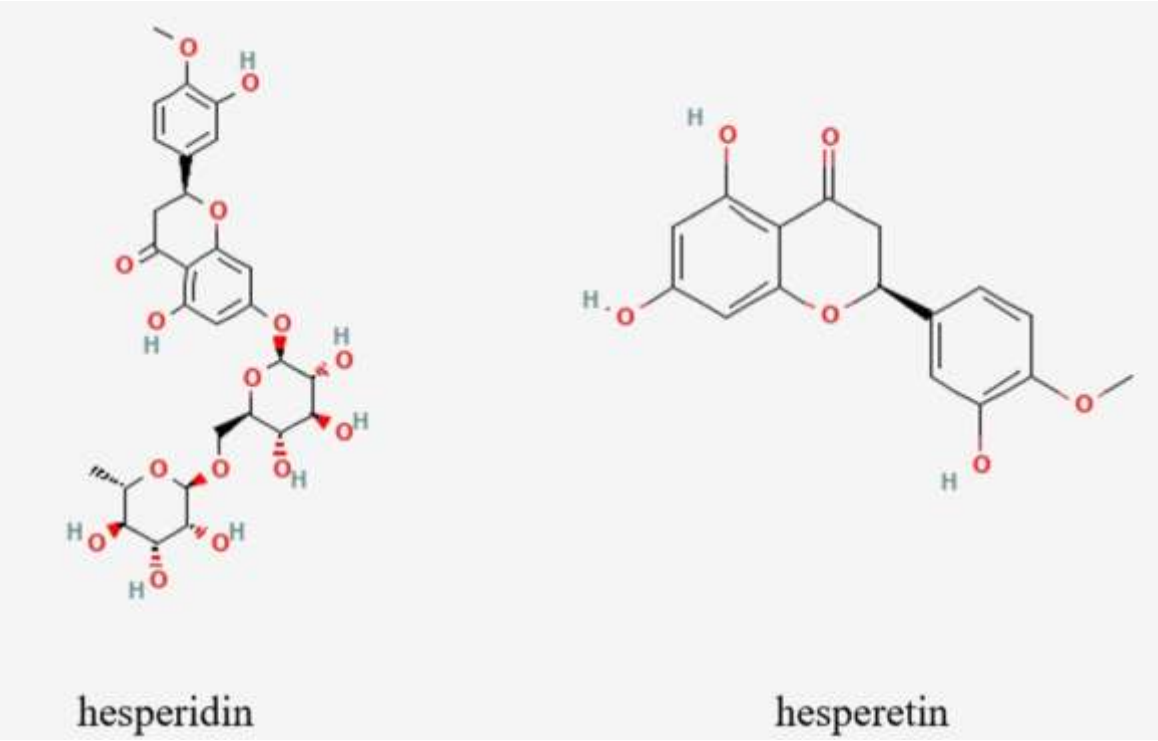

Figure 1: Chemical structures of hesperidin and hesperetin. This figure was created by PubChem.

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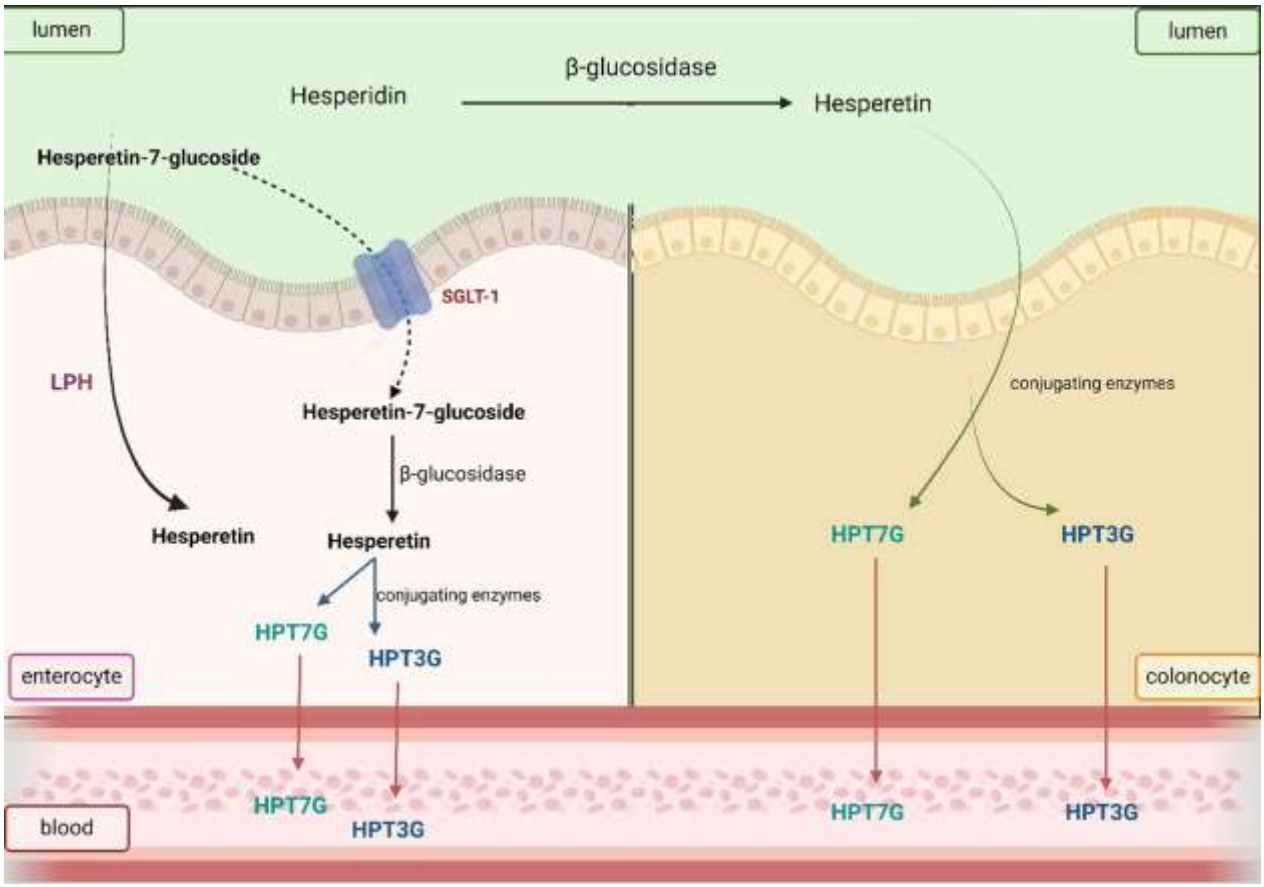

Figure 2: Absorption pathways of hesperidin and hesperetin. This figure was created by BioRender.com. The aglycone form, hesperetin, is absorbed more easily than hesperidin. Hesperidin is first converted to hesperetin with the help of lactase-phlorizin hydrolase or absorbed into enterocytes via SGLT-1. Abbreviations: LPH: Lactase-phlorizin hydrolase; SGLT1: Sodium/glucose cotransporter 1; HPT3G: Hesperetin-3-O-β-D-glucuronide; HPT7G: Hesperetin-7-O-β-D-glucuronide

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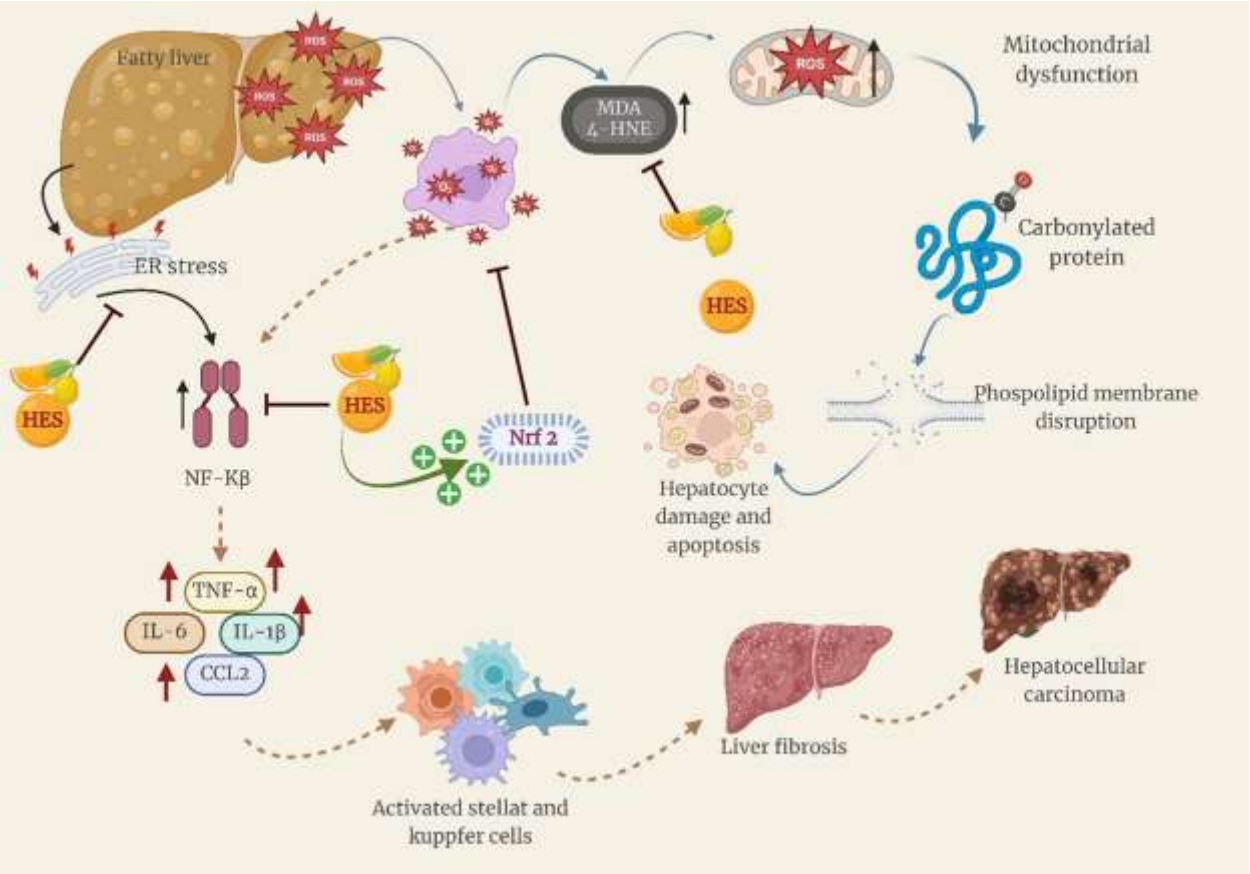

Figure 3: Summary of the effect of hesperidin on inflammatory pathways. This figure was created by BioRender.com. Abbreviations: HES: Hesperidin; ER: Endoplasmic reticulum; ROS: Reactive oxygen species; MDA: malondialdehyde; 4-HNE: 4-hydroxy-2-nonenal; NF-κB: Nuclear factor-κB; TNF-α: Tumour necrosis factor alpha; IL-6: Interleukin-6; IL-1β: Interleukin-1β; CCL2: Chemokine ligand 2; Nrf2: Nuclear factor erythroid 2-related factor

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