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Review Article

Fisetin as a Promising Agent in Non-Alcoholic Fatty Liver Disease: Insights into Pathogenic Mechanisms and Therapeutic Potential

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a prevalent liver condition characterized by fat accumulation in the liver, with its development involving intricate processes such as inflammation, oxidative damage, and lipid metabolism disturbances. Current treatment options are limited, emphasizing the need for multi-targeted approaches that can simultaneously address these pathogenic pathways to improve liver health. This review synthesizes current evidence on how fisetin impacts molecular pathways relevant to NAFLD. It focuses on its effects in reducing inflammation, oxidative stress, and lipid accumulation, based on experimental and clinical studies examining gene expression, enzyme activity, and signaling pathways involved in hepatic steatosis and injury. This review also explores the mechanisms by which fisetin intervention influences NAFLD management, highlighting its role in glycemic control through postprandial glucose reduction, mitigation of insulin resistance, improvements in pancreatic insulin secretion, and suppression of hepatic gluconeogenesis and glycogenolysis. Additionally, fisetin exerts plasma lipid-lowering effects by enhancing hepatic β-oxidation and reducing lipogenesis. Its anti-inflammatory effects are observed both systemically and locally within the liver. Fisetin also strengthens antioxidant defenses by activating antioxidant enzymes, reducing superoxide levels, chelating metal ions, and scavenging free radicals. Furthermore, fisetin modulates endoplasmic reticulum (ER) stress and promotes autophagy, contributing to the amelioration of NAFLD pathology. Taken together, fisetin exhibits a promising hepatoprotective profile and may serve as a beneficial natural supplement for liver health. Its potential benefits in reducing liver steatosis and supporting NAFLD management, combined with its minimal side effects, make it an attractive candidate for further exploration as a complementary therapy.

Keywords: Fisetin, NAFLD, Hepatoprotection, Natural Compound, Mechanism of Action

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver condition characterized by the accumulation of more than 5% fat in liver tissue. It is closely associated with metabolic syndrome and frequently occurs in individuals with Type 2 Diabetes Mellitus (T2DM), insulin resistance and obesity (1).

The incidence of NAFLD has emerged as a significant global public health challenge, necessitating a deeper understanding of its prevalence and impact on affected individuals. Patients with NAFLD are at increased risk of developing cardiovascular disease, Type 2 Diabetes Mellitus (T2DM), and hepatocellular carcinoma (HCC). Furthermore, the progression of NAFLD to advanced stages such as nonalcoholic steatohepatitis (NASH) and cirrhosis can lead to severe liver-related complications and mortality, often requiring costly medical interventions such as liver transplantation. In fact, NAFLD is now the third most common indication for liver transplants (2).

Chronic hyperglycemia and elevated levels of free fatty acids (FFAs) in the bloodstream contribute to fat accumulation in the liver and inflammation, increasing the risk of NASH and advanced fibrosis in diabetic patients. Additionally, genetic polymorphisms involved in lipid metabolism, insulin signaling, and inflammation can predispose individuals to hepatic steatosis and NASH. Lifestyle and environmental factors—including a high-calorie diet, low physical activity, alcohol consumption, and alterations in the gut microbiome—may also influence disease progression and severity in individuals with NAFLD (3).

The first line of treatment for NAFLD typically includes dietary modifications, regular exercise, weight loss, nutritional supplementation, and management of comorbidities such as diabetes (4). The use of vitamin E has been shown to reduce liver inflammation in patients with NASH (5). Furthermore, probiotics and omega-3 supplements may help improve liver function and reduce inflammation. While diabetes medications can assist in managing certain symptoms of fatty liver, they are not specifically prescribed for NAFLD treatment. For example, pioglitazone, a thiazolidinedione, is used in combination therapy to address insulin resistance in NAFLD patients (6).

Pathophysiology of NAFLD

Several factors have been proposed to contribute to the pathogenesis of NAFLD. In the following section, we review these mechanisms.

Insulin Resistance

Hepatic insulin resistance impairs glycogen synthesis and redirects glucose toward lipogenic pathways, leading to atherogenic dyslipidemia and the progression of NAFLD (7). While hepatic insulin resistance hampers the suppression of gluconeogenesis, it also increases hepatic de novo lipogenesis (DNL). Because normal insulin signaling positively regulates hepatic DNL, the condition in which insulin fails to suppress gluconeogenesis but continues to activate DNL is referred to as selective insulin resistance.

Fat accumulation in the liver and NAFLD development result from an imbalance between fatty acid (FA) delivery to the liver and its efflux. In the early stages of NAFLD, both very low-density lipoprotein (VLDL) secretion and β-oxidation increase to compensate for the heightened influx of FAs. However, the continued influx of FAs eventually leads to lipotoxicity, liver damage, and NASH. NASH patients exhibit lower VLDL secretion and reduced FA oxidation compared to individuals with primary fatty liver (8).

Inflammation

The metabolic disorders underlying NAFLD cause liver cell damage and death, leading to the release of signals responsible for the recruitment and activation of immune and fibrogenic cells. These cells amplify disease progression by releasing proinflammatory and profibrogenic factors, thereby perpetuating a vicious cycle (9). Fibrogenesis plays a physiological role in tissue repair, acting as a wound-healing response. However, regardless of the underlying cause, chronic liver injury, inflammation, and subsequent fibrogenesis can lead to progressive fibrosis, which may eventually advance to cirrhosis. Cirrhosis remains a silent condition until complications arise, often resulting in severe consequences and high mortality. Abnormal liver fibrogenesis is a dynamic process characterized by excessive and gradual accumulation of extracellular matrix (ECM) components over time (10).

Activation of hepatic stellate cells (HSCs) occurs in an inflammatory background and depends on interactions with multiple elements, including immune cells that sustain the fibrogenic process by producing various mediators. Among these, profibrogenic cytokines play a crucial role. Transforming Growth Factor-β (TGF-β) is secreted by multiple cell types and is considered the most potent fibrogenic cytokine and activator of HSCs, leading to increased production of type I collagen. Phagocytosis of apoptotic cells by macrophages further stimulates TGF-β release, establishing a direct link between liver cell death and fibrogenesis (11).

Oxidative Stress

The liver is one of the key tissues involved in energy regulation, and its functional failure contributes to metabolic disorders. Maintaining oxidative balance within this network is essential. Oxidative stress results from an imbalance between reactive oxygen species (ROS) levels and antioxidant defenses, leading

to cellular damage. Free radicals directly affect cellular structures, compromising their efficiency and integrity. Additionally, certain oxidative stress products function as signaling molecules, triggering pathways that lead to metabolic dysfunction. This dysregulation exacerbates the condition, creating a vicious cycle of elevated oxidative stress and metabolic disturbances. Consequently, targeting and modulating oxidative stress is considered a promising therapeutic strategy. Enhancing antioxidant defenses may improve components of metabolic syndrome and pave the way for more effective prevention and management of related diseases, including NAFLD (12).

During normal metabolism, free radicals are continuously generated and play essential roles in cell signaling. In addition to mitochondrial production, ROS are synthesized by the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Superoxide anions are rapidly converted to H_2O_2 by superoxide dismutases (SOD). However, if this conversion is insufficient or if ROS production increases due to excessive glucose intake, free radicals inhibit glyceraldehyde-3-phosphate dehydrogenase (GAPDH), causing glucose from glycolysis to enter alternative pathways, such as the hexosamine and polyol pathways. These pathways, in turn, amplify free radical production (13).

Since oxidative stress plays a central role in NAFLD pathogenesis, defense mechanisms against oxidative damage—including the pentose phosphate pathway, which serves as the primary source of NADPH—are considered important (12). However, in NAFLD, which is characterized not only by oxidative stress but also by increased lipogenesis, the NADPH generated via the pentose phosphate pathway is more likely to be utilized for reductive biosynthesis rather than antioxidant defense.

ER Stress

The endoplasmic reticulum (ER) is a vital organelle responsible for protein folding, lipid synthesis, calcium storage, and detoxification processes. When the ER is exposed to excessive metabolic demands or pathological conditions, it accumulates misfolded or unfolded proteins, leading to a state known as ER stress. To restore homeostasis, the cell initiates an adaptive signaling cascade called the unfolded protein response (UPR). The UPR consists of three main branches, mediated by specific ER transmembrane sensors: Inositol-Requiring Enzyme 1 alpha (IRE1α), PKR-like ER kinase (PERK), and Activating Transcription Factor 6 (ATF6). Under mild or acute conditions, the UPR mitigates ER stress by halting protein translation, enhancing protein folding capacity, and promoting the degradation of misfolded proteins. However, chronic or unresolved ER stress shifts the cellular environment toward inflammation, apoptosis, and insulin resistance—all of which are central to the pathogenesis of NAFLD (14).

ER stress disrupts lipid metabolism by altering the expression of key transcription factors such as Sterol Regulatory Element-Binding Protein 1c (SREBP-1c) and Carbohydrate Response Element–Binding Protein (ChREBP), which regulate lipogenesis (15). This

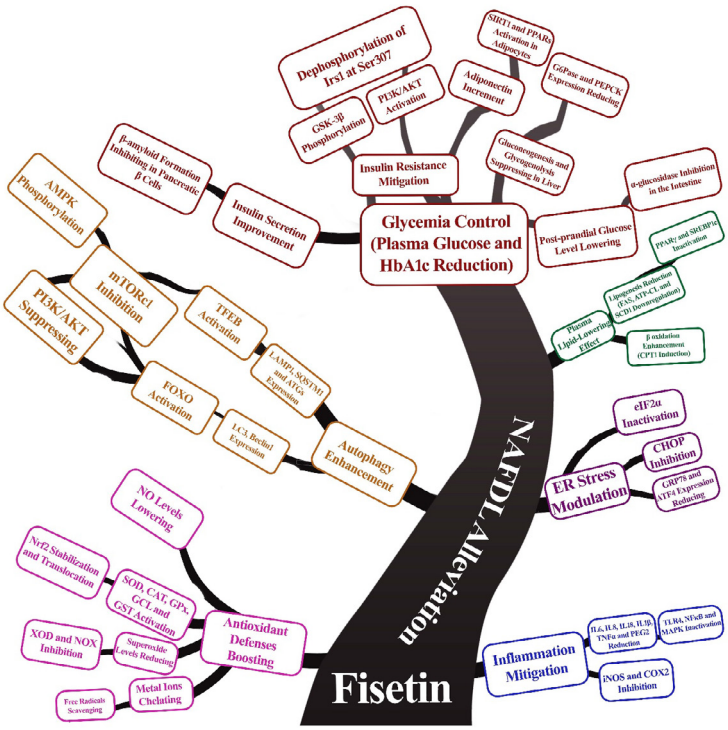

Figure 1. Molecular mechanisms by which fisetin ameliorates NAFLD

leads to increased de novo lipogenesis and triglyceride accumulation in hepatocytes, contributing to hepatic steatosis. ER stress impairs insulin signaling through several mechanisms. One key pathway involves the activation of JNK (c-Jun N-terminal kinase), which phosphorylates IRS-1 (insulin receptor substrate 1), thereby inhibiting downstream insulin signaling. This results in hepatic insulin resistance, a hallmark of NAFLD, and contributes to hyperglycemia and dyslipidemia. Persistent ER stress activates inflammatory pathways, particularly NF-κB, which promotes the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. These cytokines contribute to hepatocyte injury, immune cell infiltration, and progression from simple steatosis to NASH (16). When ER stress cannot be resolved, the UPR triggers apoptotic pathways via the induction of CHOP (C/EBP homologous protein) and activation of caspase-12. Hepatocyte apoptosis exacerbates liver damage and fibrogenesis, accelerating disease progression to advanced stages such as fibrosis and cirrhosis (17). ER stress also contributes to mitochondrial dysfunction and increased reactive oxygen species (ROS) production. Oxidative stress further damages cellular components and amplifies ER stress in a vicious cycle that worsens liver injury (18).

Studies in animal models of NAFLD have shown elevated markers of ER stress in the liver, including increased expression of GRP78 (a chaperone protein indicative of ER stress), spliced XBP-1 (a marker of IRE1 activation), and CHOP (23). Similarly, human liver biopsies from patients with NASH demonstrate upregulated ER stress markers compared to healthy controls (26). Furthermore, genetic or pharmacological inhibition of ER stress has been shown to improve hepatic steatosis, insulin sensitivity, and inflammation in experimental models, underscoring the therapeutic potential of targeting this pathway (27). Given its central role in NAFLD pathogenesis, modulating ER stress represents a promising therapeutic strategy.

Autophagy

Autophagy is a highly regulated process that has evolved to degrade cellular components, such as defective organelles and misfolded protein aggregates, through lysosomes. The process includes three major types: macroautophagy, microautophagy, and chaperone-mediated autophagy, all of which involve the transport of components to lysosomes for degradation (19). Macroautophagy (hereafter referred to as autophagy) was initially considered a non-selective degradation process. However, the discovery of selective autophagy receptors, with p62/SQSTM1 being the first, shifted this perception. Today, autophagy is recognized as a highly selective cellular clearance pathway associated with maintaining cellular and tissue homeostasis. Selective autophagy can be further divided into various subgroups based on the specific structures involved (20). These subgroups include the targeting of macromolecules (glycophagy and lipophagy), mitochondria (mitophagy), the endoplasmic reticulum (reticulophagy or ER-phagy), nuclear segments (nucleophagy), pathogens (xenophagy), and the lysosomes themselves (lysophagy) (21). Autophagy is initiated following the suppression of the mammalian target of rapamycin (mTOR) or the activation of AMP-activated protein kinase (AMPK), both of which regulate autophagy in response to stressors such as starvation, elevated temperatures, and physical activity. AMPK acts as an activator, while mTOR serves as an inhibitor. Additionally, transcription factor EB (TFEB) is an essential positive regulator of autophagy and lysosomal biogenesis, with its translocation to the nucleus being linked to mTOR and AMPK activity. Once autophagy is activated, the process begins with membrane nucleation and phagophore formation. Following elongation and maturation, autophagosomes fuse with lysosomes for cargo degradation and recycling. The regulatory effect of autophagy on lipid metabolism was first demonstrated when researchers showed that autophagy facilitates lipid droplet (LD) degradation in hepatocytes (22). In lipophagy, autophagosomes engulf portions of cytosolic LDs and fuse with lysosomes to release fatty acids into the lysosomal acidic environment. These released fatty acids are subsequently directed toward mitochondrial oxidation. Disruption of autophagy leads to excessive lipid accumulation in hepatocytes, contributing to NAFLD (23). The increased triglyceride and LD accumulation observed in hepatocytes of Atg7 knockout mice underscores the critical role of lipophagy as an in vivo lipolytic system (24).

Dysregulation of autophagy is increasingly recognized as a key contributor to NAFLD pathogenesis and progression. Autophagy inhibition occurs via both short-term and long-term regulatory mechanisms. Short-term inhibition results from increased amino acid concentrations due to overnutrition and/or hyperinsulinemia, which enhances hepatic mTOR activity and suppresses autophagy. Long-term regulation is mediated by transcription factors FoxO and TFEB, which control autophagy-related gene expression. These transcription factors are inhibited by Akt/PKB and mTOR signaling pathways activated by insulin. Additionally, obesity-related elevation of calcium-dependent protease calpain-2 promotes Atg7 degradation, leading to defective autophagy. Impaired lysosomal acidification and reduced expression of cathepsins B, D, and L further disrupt substrate degradation in autolysosomes. Furthermore, defects in organelle fusion—such as autophagosome-lysosome fusion resulting from alterations in membrane lipid composition—further compromise autophagic function. Hepatic autophagy deficiency and reduced lysosomal degradation rates contribute to heightened ER stress and insulin resistance due to nutritional overload (25).

Autophagy also plays a crucial role in mitochondrial quality control via mitophagy, which eliminates damaged mitochondria to prevent excessive reactive oxygen species (ROS) production. In NAFLD, mitochondrial dysfunction and ROS accumulation are prominent. Impaired mitophagy exacerbates oxidative stress, leading to DNA damage, inflammation, and apoptosis (26). For example, reduced Parkin-mediated mitophagy in NASH livers contributes to inflammasome activation and hepatocyte injury. Restoring mitophagy improves metabolic function and reduces liver damage in preclinical models (27).

Autophagy also intersects with ER stress and inflammation, two central pillars of NAFLD progression. By degrading misfolded proteins and attenuating ER stress, autophagy mitigates hepatocyte apoptosis and inflammation. Additionally, autophagy suppresses the NLRP3 inflammasome by clearing damaged mitochondria, thereby reducing IL-1β and IL-18 secretion. Defective autophagy amplifies NF-κB signaling, promoting the production of pro-inflammatory cytokines such as TNF-α and IL-6, which drive NASH progression (28).

Phytochemicals and NAFLD

Phytochemicals, naturally occurring compounds found in plants, hold significant potential for the treatment of NAFLD. These bioactive substances—including polyphenols, flavonoids, and terpenoids—exhibit antioxidant, anti-inflammatory, and insulin-sensitizing properties, which can help mitigate the underlying mechanisms of NAFLD progression (29). By reducing oxidative stress and inflammation, phytochemicals may prevent liver cell damage and fibrosis. Additionally, they can modulate lipid metabolism, promoting fat breakdown and inhibiting its accumulation in the liver (30). Consequently, incorporating phytochemical-rich diets or supplements may serve as a promising natural adjunct to conventional therapies for managing NAFLD.

Phytochemicals Classification

Flavonoids are among the most important phytochemicals. Their primary sources include fruits, soybeans, vegetables, and oils. Flavonoids are generally classified into three groups: bioflavonoids, neoflavonoids, and isoflavonoids (phytoestrogens). Bioflavonoids are further divided into subclasses, including flavanols, flavanones, catechins, chalcones, and anthocyanidins. Among these subclasses, flavanols are the most abundant and widely distributed in plants. The most important flavanols include kaempferol, quercetin, myristicin, morin, galangin, isorhamnetin, and fisetin (18).

General Properties of Fisetin

Fisetin (FSN) (CAS No. [528-48-3]) is a hydrophobic polyphenolic compound with a molecular weight of 286.24 g/mol and a molecular formula of $C_{15}H_{10}O_6$. It was discovered in 1890. FSN is also referred to as 5-deoxyquercetin and 3,3′,4′,7-tetrahydroxyflavan and is found in several fruits (e.g., persimmon, strawberry, apple, mango, peach, grape, and kiwi) and vegetables (e.g., tomato, onion, and cucumber). Numerous studies have demonstrated the wide-ranging medicinal properties of FSN, including antioxidant, anti-inflammatory, antimicrobial, anti-osteoporotic, anti-diabetic, and anti-cancer effects (31).

Fisetin and NAFLD

In the preceding sections, we have explored the intricate mechanisms underlying NAFLD development and progression, highlighting the roles of insulin resistance, lipid metabolism, inflammation, oxidative stress, ER stress, and autophagy in this multifaceted disorder. Each of these pathways plays a significant role in NAFLD pathophysiology, establishing a complex interplay that exacerbates liver injury and disease progression. Moving forward, we aim to investigate the protective effects of FSN within these pathways, as evidenced by various studies across different pathological conditions. FSN has demonstrated considerable promise in modulating inflammation, oxidative stress, metabolic disturbances, ER stress, and autophagy. We will examine how FSN's modulatory effects influence these critical pathways in NAFLD, potentially offering a novel therapeutic avenue for managing this increasingly prevalent condition. By elucidating how FSN interacts with and alters these mechanisms, we can gain valuable insights into its role in the prevention and treatment of NAFLD.

Fisetin and Metabolism

In recent decades, researchers have demonstrated that natural compounds extracted from medicinal plants possess antidiabetic properties. Flavonoid groups, including quercetin and hesperidin, play critical roles in glucose metabolism and gluconeogenesis. FSN, structurally similar to quercetin, has also been investigated as an antidiabetic agent. Various studies have highlighted the beneficial effects of FSN in regulating glucose and lipid metabolism. In an in vitro study incorporating kinetic analyses and molecular docking, FSN was identified as a non-competitive inhibitor of α-glucosidase, indicating its potential for effective blood glucose management (32). In a study involving streptozotocin (STZ)-induced diabetic rats, FSN was administered orally at a dose of 10 mg per kg body weight for 30 days. The results showed a reduction in blood glucose levels and hemoglobin A1c, along with an increase in plasma insulin levels. Improvement in glucose balance was observed via the inhibition of hepatic gluconeogenesis, evidenced by reduced mRNA and protein expression of gluconeogenesis-related genes, including glucose-6-phosphatase (G6Pase) and

phosphoenolpyruvate carboxykinase (PEPCK) (33). Additionally, elevated lipid levels in the kidney, liver tissues, and serum returned to normal, and the imbalance in high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels was corrected (34). In another study on male C57BL/6 mice treated with a high-fat diet (HFD) and concurrently administered FSN at doses of 20, 40, or 80 mg per kg body weight for 16 weeks, significant positive effects were observed in diabetic nephropathy. This treatment contributed to reductions in weight gain, insulin resistance, and glucose intolerance. FSN reduced the phosphorylation of Irs1 at Ser307 while increasing its phosphorylation at Tyr608, as well as activating AKT and glycogen synthase kinase-3 beta (GSK-3β) (35). Treatment with FSN at a dose of 10 mg per kg daily for eight weeks in Sprague-Dawley rats fed a HFD resulted in decreased hepatic expression of SCD-1, SREBP1c, and PPARγ, along with lower levels of ATP citrate lyase (ACLY), G6Pase, and FAS, leading to a reduction in hepatic triglyceride content and serum glucose concentrations (36). In male C57BL/6 mice with HFD-induced NAFLD, intraperitoneal injection of FSN at a dose of 20 mg per kg twice a week for ten weeks led to decreased lipogenesis and increased CPT1 production in liver tissue (37). Additionally, in male C57BL/6 mice treated with FSN at a concentration of 0.02% for 16 weeks, researchers observed a reduction in the expression of lipogenesis-related genes and an increase in genes involved in β-oxidation, contributing to a decreased HOMA-IR index, improved glucose tolerance, and lower plasma insulin and glucose levels (38).

Building on previous studies highlighting the positive effects of FSN on metabolism, further research has focused on elucidating the specific mechanisms through which this compound contributes to metabolic health. FSN exhibits significant binding affinity to peroxisome proliferator-activated receptor gamma (PPARγ) and influences signaling pathways associated with Type 2 Diabetes Mellitus (T2DM), suggesting its potential as a superior therapeutic agent compared to conventional drugs such as metformin (39). Additionally, FSN upregulates adiponectin expression in 3T3-L1 adipocytes by activating SIRT1 and PPARs, thereby enhancing adiponectin secretion and reinforcing its role in treating metabolic disorders (40). Furthermore, FSN promotes energy metabolism through SIRT1 upregulation and the activation of AKT and JNK signaling pathways, which has been linked to reduced follicular atresia in laying chickens (41). Regarding glucose regulation, FSN effectively inhibits hepatic glucose release by suppressing glycogenolysis and glycolysis, thereby helping prevent hyperglycemia (42). Finally, FSN also exhibits protective effects by inhibiting β-amyloid formation and destabilizing amyloid fibrils in pancreatic tissue, potentially mitigating amyloid cytotoxicity to pancreatic β-cells in T2DM (43). Together, these mechanisms underscore FSN's multifaceted role in enhancing metabolic function and highlight its potential as a valuable therapeutic candidate.

Fisetin and Inflammation

Studies have consistently demonstrated that FSN exhibits strong anti-inflammatory properties, and its molecular mechanisms have been well elucidated. Excessive increases in pro-inflammatory cytokines such as IL-6, IL-8, IL-18, IL-1β, TNF-α, and PGE2 are recognized as key pathogenic factors in inflammation, which FSN is capable of suppressing. Research has shown that FSN (100 mg per kg administered as a pre-treatment for three consecutive days) can ameliorate kidney inflammation by reducing the expression of iNOS, COX-2, and HMGB1 in mice suffering from sepsis and acute kidney injury. Additionally, FSN exerts its anti-inflammatory effects by inhibiting TLR4, NFκB, and MAPK signaling pathways. Furthermore, FSN contributes to inflammation regulation through its antioxidant properties and the inhibition of oxidative stress. The anti-inflammatory effects of FSN have also been demonstrated in A549 cells induced by IL-1β, where it suppresses inflammation via NFκB and ERK1/2 signaling pathways (44). A group of researchers reported that the levels of pro-inflammatory cytokines in the plasma of male C57BL/6 mice fed a high-fat diet (HFD) and treated with FSN were reduced, along with decreased expression of the pro-inflammatory genes TLR4 and IL-6 in their livers (38). Additionally, Xu et al. found that oral administration of FSN at doses of 20, 40, and 80 mg/kg for 20 weeks decreased the severity of liver inflammation by suppressing the TNF-α/RIPK3 axis in male C57BL/6 mice fed an HFD (45).

Antioxidant Activity of Fisetin

Naeimi et al. have comprehensively described the antioxidant properties of FSN in a review study (46). FSN is a flavonoid with strong antioxidant capabilities, allowing it to counteract oxidative stress through various mechanisms and demonstrating significant cytoprotective effects. Its unique chemical structure—particularly the hydroxyl groups in ring B and the 4-keto group in ring C—provides enhanced protective effects against oxidation compared to related flavonoids such as morin and myricetin. Due to its lipophilic nature, FSN can penetrate cellular membrane lipid layers, preventing free radicals from accessing the cell membrane. It exhibits free radical scavenging properties and can transfer electrons from its hydroxyl groups. Common assays such as DPPH and ABTS have shown that FSN can inhibit approximately 85% and 90.61%, respectively, of free radicals, with its ABTS scavenging activity even surpassing that of ascorbic acid. Beyond free radical scavenging, FSN regulates their production and influences the activity of oxidizing enzymes such as xanthine oxidase (XOD) and NADPH oxidase (NOX),

reducing superoxide levels. FSN also affects enzymes such as NOS and COX, lowering nitric oxide levels and preventing oxidative damage. Another mechanism through which FSN mitigates oxidative stress is its ability to chelate metal ions such as iron and copper, forming chelated complexes that enhance antioxidant activity. Among flavonoids, FSN is an excellent iron chelator, capable of binding iron (II) across a broad pH range. Additionally, as a biological ligand, FSN can chelate aluminum (III) from acidic to slightly basic pH levels, with increasing pH and aluminum concentration enhancing the stability of these chelated complexes. Moreover, FSN enhances enzymatic and molecular antioxidant levels; studies indicate that FSN supplementation in various animal models increases antioxidant enzyme levels such as SOD, CAT, GPx, and GST while positively influencing antioxidants such as vitamin C and GSH. FSN also regulates intracellular glutathione levels by stimulating GCL activity and promotes detoxifying phase II enzymes and other oxidative stress-responsive enzymes through the activation of Nrf2, a key factor in maintaining redox balance and reducing oxidative damage (46). FSN has demonstrated potent antioxidant effects in metabolic syndrome, where elevated blood glucose levels lead to increased intracellular ROS. The rise in ROS is primarily attributed to mitochondrial oxidative phosphorylation and enhanced expression of NADPH oxidase enzymes. Oxidative stress activates inhibitory kinases that disrupt insulin signaling. Conversely, activation of the PI3K/Akt pathway via tyrosine kinase receptors can enhance Nrf2 activity. FSN contributes to reducing oxidative stress and improving cell survival through both indirect activation of PI3K/Akt and direct stabilization of Nrf2, facilitating its translocation to the nucleus. Oxidative stress exacerbates inflammation associated with metabolic disorders; however, FSN counteracts this effect by modulating pro-inflammatory factors and improving insulin sensitivity (47). In one study, male C57BL/6 mice fed a fructose-rich diet were treated with 5 or 10 mg/kg of FSN daily for 8 weeks. This treatment resulted in improvements in insulin resistance, liver damage, oxidative stress, and dyslipidemia, effects partially attributed to Nrf2 activation (48). Furthermore, FSN exhibited neuroprotective effects in diabetic mice by modulating Nrf2, with doses of 5 and 10 mg/kg administered for two weeks (49). Overall, FSN presents a multifaceted approach for addressing oxidative stress-related pathological components in metabolic syndrome.

Fisetin and ER Stress

Natural products represent a promising avenue for developing effective therapeutic strategies against diseases associated with ER stress (50). Regarding FSN, studies indicate that FSN exerts beneficial effects by reducing ER stress through the suppression of signaling pathway activation. Furthermore, some of its modified derivatives demonstrate even greater therapeutic potential (51). In one study, FSN inhibited the expression of ER stress-related proteins, including GRP78, phosphorylated eIF2-α, ATF4, and CHOP, while also reducing cytosolic calcium levels, suggesting its ability to mitigate pollutant-induced apoptosis by suppressing ER stress and ROS production (52). Additionally, FSN was found to alleviate palmitic acid-induced ER stress in hepatocytes, an effect associated with the suppression of GRP78 and CHOP. In vivo studies using high-fat diet (HFD)-fed mice demonstrated that FSN administration, through GRP78-dependent ER stress inhibition, exhibited preventive and therapeutic potential against NAFLD progression by modulating oxidative stress and mitochondrial dysfunction (53).

Fisetin and Autophagy

FSN has been shown to influence autophagy in various diseases and cellular disorders. A study investigating the effects of FSN on tau protein levels in relation to Alzheimer's disease demonstrated that FSN induces autophagy by activating the transcription factor EB (TFEB) and inhibiting mTORC1, resulting in a significant reduction in phosphorylated tau levels. Additionally, the degradation of phosphorylated tau by FSN is diminished by chemical inhibitors of the autophagy-lysosomal pathway, such as chloroquine and bafilomycin A1 (54). Furthermore, FSN has been shown to induce autophagy and inhibit vascular smooth muscle cell (VSMC) senescence via the FoxO3a signaling pathway. By activating PPARγ and inhibiting mTORC2, FSN enhances autophagy, highlighting its potential as an anti-aging agent in various cellular disorders (55). Researchers have also investigated FSN's protective effects against synaptic dysfunction and neuronal inflammation induced by lead exposure in mice. The results indicated that FSN significantly improves lead-induced behavioral disorders by inactivating pro-inflammatory factors, reducing amyloid-β accumulation, and inhibiting apoptosis. In this study, FSN increased lead-induced autophagy in mouse brains, leading to enhanced phosphorylation of AMPK and SIRT1 (56).

Moreover, FSN was able to mitigate acetaminophen-induced liver injury in laboratory and animal models. FSN increased acetaminophen-induced autophagy and inhibited inflammasome activation, while suppression of the ATG5 gene—an essential autophagy gene—diminished FSN's protective effects. The use of the autophagy inhibitor 3-methyladenine in cell culture further confirmed autophagy's role in FSN's protective effects against acetaminophen toxicity (57). Consistent with these findings, Liou et al. demonstrated that FSN treatment increased p-AMPK expression in the livers of male C57BL/6 mice compared to the high-fat diet (HFD) group (37). Jung et al. also found that FSN inhibits mTORC1 signaling by suppressing Akt activation in preadipocytes (58). Interestingly, some studies suggest

FSN directly inhibits mTORC1 activity (59). In our own study examining FSN's effects on autophagy and related markers in the context of NAFLD, findings indicated that FSN significantly improved autophagy, evidenced by the reversal of the altered LC3II/LC3I ratio and increased expression of autophagy markers such as ATG5 and Beclin1. These effects were linked to mTOR suppression (60). Additionally, in another study, FSN facilitated autophagosome-lysosome fusion and autophagic degradation by inhibiting the PI3K/AKT/mTOR signaling pathway, increasing the expression of LC3B and LAMP1 proteins, and reducing the expression and secretion of inflammatory cytokines in LPS-treated macrophages. This effect was partially reversed by the autophagy inhibitor chloroquine. Thus, the results of this study not only elucidate one of FSN's mechanisms but also highlight the relationship between autophagy and inflammation (61).

Conclusion

In summary, fisetin exhibits promising hepatoprotective effects against NAFLD by targeting multiple mechanisms, including inflammation, oxidative stress, ER stress, autophagy, and lipid metabolism dysregulation. However, the molecular mechanisms discussed here represent only a subset of the numerous pathways FSN may influence, and it is likely that its effects extend beyond these. Further studies are essential to fully elucidate FSN's mechanisms and establish its clinical efficacy and safety in NAFLD management.

Conflict of interests

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