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Food components with anti-fibrotic activity and implications in prevention of liver disease

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Abstract

Increasing prevalence of nonalcoholic fatty liver disease (NAFLD) in parallel with the obesity epidemic has been a major public health concern. NAFLD is the most common chronic liver disease in the U.S., ranging from fatty liver to steatohepatitis, fibrosis and cirrhosis in the liver. In response to chronic liver injury, fibrogenesis in the liver occurs as a protective response; however, prolonged and dysregulated fibrogenesis can lead to liver fibrosis, which can further progress to cirrhosis and eventually hepatocellular carcinoma. Interplay of hepatocytes, macrophages and hepatic stellate cells (HSCs) in the hepatic inflammatory and oxidative milieu is critical for the development of NAFLD. In particular, HSCs play a major role in the production of extracellular matrix proteins. Studies have demonstrated that bioactive food components and natural products, including astaxanthin, curcumin, blueberry, silymarin, coffee, vitamin C, E, and D, resveratrol, quercetin, and epigallocatechin-3-gallate, have anti-fibrotic effects in the liver. This review summarizes current knowledge of the mechanistic insight into the anti-fibrotic actions of the aforementioned bioactive food components.

Keywords

Liver fibrosis; Anti-fibrotic food components; Hepatic stellate cells; Liver disease

1. Introduction

According to the Centers for Disease Control and Prevention, the percentage of adults with obesity was 37.9% and that of adults with overweight including obesity was 70.7% in 2013-2014 [1]. Obesity can cause chronic metabolic diseases, such as type 2 diabetes mellitus, cardiovascular disease, and nonalcoholic fatty liver disease (NAFLD) [2]. In particular, NAFLD is the most common chronic liver disease in the U.S. NAFLD encompasses the entire spectrum of fatty liver diseases in individuals without significant alcohol consumption, ranging from fatty liver to steatohepatitis and cirrhosis [3].

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Nonalcoholic fatty liver (NAFL), also termed simple steatosis, is characterized by excess accumulation of triglycerides in hepatocytes and it is generally considered benign. The major features that differentiate nonalcoholic steatohepatitis (NASH) from NAFL are hepatocyte injury and cell death with inflammation and fibrogenesis [4]. Fibrogenesis in the liver is a protective response to chronic liver injury caused by chronic hepatitis B or hepatitis C infection, excess alcohol consumption, NAFLD, drug toxicity, chemical intoxication, parasitic diseases, congenital abnormalities, and biliary atresia [5-7]. However, progressive deposition of extracellular matrix (ECM) proteins in the liver, namely liver fibrosis, in combination with vascular remodeling may cause cirrhosis and furthermore hepatocellular carcinoma (HCC), the major form of liver cancers [8, 9]. Liver cirrhosis is the major risk factor for HCC with more than 80% of patients with liver cirrhosis developing HCC [10]. Once HCC occurs, the only possible treatment is liver transplantation.

Liver fibrosis afflicts over 100 million people in the world [11]. During the development of liver fibrosis, hepatocytes, macrophages, and hepatic stellate cells (HSCs) interplay with each other. In particular, HSCs are primarily responsible for the production of ECM proteins while inhibiting ECM breakdown by producing tissue inhibitor of metalloproteinases (TIMPs) [12]. Importantly, recent studies have demonstrated that fibrotic liver can be reversed to a normal state when the cause of liver injury is eliminated [8, 13-16]. During the resolution of liver fibrosis, activated HSCs undergo either apoptosis or inactivation to a quiescent phenotype [8]. Therefore, identification of natural products and bioactive food components that can prevent HSC activation and/or facilitate HSC inactivation/apoptosis is important to develop effective preventive/therapeutic strategies against liver fibrosis and advanced liver diseases. This review focuses on current knowledge of food components that can exert an anti-fibrotic action for the prevention of liver fibrosis.

2. Hepatic cells involved in the development of NAFLD

2.1 Role of hepatocytes in NAFLD development

In obesity, lipid-laden myocytes and adipocytes become resistant to insulin signaling, leading to hyperglycemia, hyperlipidemia, and fat accumulation in non-adipose tissues [17]. The normal liver maintains the balance between lipid input and output, by regulating the amount of lipids from dietary fat delivered to the liver, de novo lipogenesis, uptake of free fatty acids from the adipose tissue, and very low-density lipoprotein formation and secretion [18]. Dysregulation of these homeostatic pathways in the liver lead to excess accumulation of triglycerides in hepatocytes, i.e., liver steatosis.

There are several factors affecting fat accumulation in hepatocytes in obesity. In obesity, a limited increase in the number of adipocytes may result in adipocyte hypertrophy, causing adipose tissue dysfunction characterized by hypoxia, insulin resistance, and inflammation [19]. Insulin resistance in the adipose tissue reduces glucose uptake and triglyceride storage in the adipocyte, leading to free fatty acid release into the circulation, which can be stored in the liver [17]. Uptake of free fatty acids in the liver is mediated by fatty acid transport proteins (FATPs) and CD36/fatty acid translocase (FAT) [20]. In a mouse NAFLD model, FATP5 knockdown not only prevented but also reversed hepatic steatosis [21]. In addition, hepatic CD36 is upregulated in the patients with NAFL as well as NASH [22].

In addition to the increased uptake of adipose tissue derived-free fatty acids, enhanced de novo lipogenesis also contributes to triglyceride accumulation in the hepatocyte. The enzymes involved in de novo lipogenesis are primarily regulated by two transcription factors, i.e., sterol regulatory element binding protein 1c (SREBP-1c) and carbohydrate response element binding protein (ChREBP), which are activated by insulin and glucose, respectively [23]. SREBP-1c induces transcription of ATP-citrate lyase, acetyl-CoA synthetase, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD-1), and glycerol-3-phosphate acyltransferase, while transcriptional targets of ChREBP include L-pyruvate kinase, FAS, ACC and SCD-1 [24]. Human liver samples from the patients with NAFL and NASH had significantly higher expression of SREBP-1c [25]. Also, the mRNA levels of ChREBP were positively related to the degree of liver steatosis in NASH patients [26]. In addition to de novo lipogenesis, dietary fat also contributes to fat accumulation in the hepatocyte. Donnelly et al. [27] showed that ~15% of hepatic fatty acids originated from diet, ~59% from adipose tissue, and ~26% from de novo lipogenesis in NAFLD patients.

Excess fat accumulation in the hepatocyte can induce reactive oxygen species (ROS) production, endoplasmic reticulum stress, production of pro-inflammatory cytokines, and apoptosis [18, 28]. Importantly, an increase in hepatocyte apoptosis is present in the patients with NAFLD [29]. Apoptosis of hepatocytes can occur in simple steatosis, however, NASH typically has more hepatocyte injury and apoptosis than NAFL [30]. Apoptotic hepatocytes generate apoptotic bodies, and release ROS and nucleotides into ECM [31, 32]. Apoptotic bodies of hepatocytes can be eliminated by macrophages [32] and HSCs [33], which is the event to perpetuate the progression of NAFLD. In addition to apoptosis of hepatocytes, NASH is also characterized by hepatocyte necrosis and inflammation [34]. Necrosis of hepatocytes induces inflammatory responses in the liver as necrosis results in cellular constituents spill into the extracellular environment by rapid swelling and rupture of cells [17]. Necrotic hepatocytes also release a number of damage-associated molecular pattern molecules, including high-mobility group box-1, heat-shock proteins, hyaluronan, fibronectin, cardiolipin, and DNA fragments [35]. Innate immune cells, such as neutrophils and macrophages, are recruited in the liver and aggravate inflammatory responses [35, 36]. Events occurring in the hepatocyte during steatosis development is summarized in Figure 1.

2.2 Hepatic macrophages in the development of NAFLD

Macrophages are critically involved in the inflammatory response and fibrogenesis in NAFLD development. Upon liver injury, circulating monocytes are recruited to the liver by chemokine receptor C-C motif chemokine receptor 8 (CCR8) [37] and CCR2 [38] present on monocytes in mice. In particular, pro-inflammatory Ly6C^{high} monocytes, not patrolling Ly6C^{low} monocytes, enter the liver, promoting CCl₄-induced liver fibrosis in mice [39]. Other studies have also demonstrated the importance of macrophages in hepatic inflammation and fibrogenesis by using approaches to suppress macrophage infiltration or function in the liver. While dimethylnitrosamine (DMN)-induced liver fibrosis in rats showed macrophage infiltration, mutation in monocyte chemoattractant protein-1 (MCP-1) markedly reduced macrophage infiltration and liver fibrogenesis [40]. Also, gadolinium

chloride, a macrophage-selective inhibitor, decreased the number of macrophages and fibrosis in rat livers treated with thioacetamide (TAA) [41].

Monocyte-derived macrophages as well as liver resident macrophages, i.e., Kupffer cells, produce inflammatory and fibrogenic cytokines such as interleukin-1 β (IL-1 β), IL-4, IL-13, tumor necrosis factor α (TNF α), IL-6, and transforming growth factor β (TGF β) that can trigger inflammatory responses and activate HSCs for the production of ECM substances in the liver [42, 43]. Macrophages also produce ROS and reactive nitrogen species, stimulating the progression of NAFLD [44].

2.3 Hepatic stellate cells in NAFLD development

A critical feature of liver fibrosis is the accumulation of pro-fibrogenic myofibroblasts that have scar-producing, proliferative, migratory, contractile, immunomodulatory, and phagocytic properties [8]. Myofibroblasts are originated from quiescent HSCs, portal fibroblasts, bone marrow-derived mesenchymal cells, and epithelial mesenchymal transition [7]. However, myofibroblasts in the liver are primarily derived from quiescent HSCs upon activation [45], and portal fibroblasts especially in the case of biliary disease [46]. HSCs are present in the subendothelial Space of Disse between endothelial cells and hepatocytes in the liver [47]. In the normal liver, about one-third of the nonparenchymal cells are HSCs [47, 48], accounting for 5-8% of liver cells [49]. Quiescent HSCs are the primary storage site of retinyl esters in their intracellular lipid droplets [50] and ~80% of vitamin A in the whole body is stored in quiescent HSCs [51].

TGF β , a secreted homodimeric cytokine, plays an important role in the development of liver fibrosis particularly by activating quiescent HSCs to myofibroblast-like activated HSCs [52, 53]. Importantly, TGF β is known to activate SMA- and MAD-related protein 2 (SMAD2) or SMAD3, particularly SMAD3 in HSCs [52], for the stimulation of fibrogenesis. When TGF β binds to its type II receptor on the cell surface, type I receptor is phosphorylated [53-55]. Subsequently, the phosphorylated type I receptor phosphorylates SMAD2 or SMAD3, inducing its complex formation with SMAD4, which is then translocated into the nucleus and induces the expression of fibrogenic genes, such as procollagen type 1 α 1 (COL1A1), COL3A1, COL 5A2, COL6A1, COL6A3, and TIMP-1 [56].

Activated HSCs are characterized by a loss of vitamin A-containing intracellular lipid droplets, enhanced proliferation and migration, excessive secretion of ECM proteins, enhanced contractility, and increased production of growth factors and cytokines, such as TGF α , TGF β , hepatocyte growth factor, platelet-derived growth factor (PDGF), and IL-6 [7, 8, 47, 57]. The activation of HSCs is a 2-step process, i.e., initiation and perpetuation [58]. HSC activation is initiated by paracrine stimuli, including apoptotic bodies of hepatocytes, TGF β , inflammatory cytokines, ROS, and lipid peroxides [31, 58]. Subsequently, the perpetuation phase is to maintain the activation state of HSCs, leading to increases in proliferation, fibrogenesis, chemotaxis, contractility, matrix degradation, retinoid loss, and the production of cytokines and chemokines [31]. As a result, ECM accumulation and scar formation occur during the perpetuation phase [12]. Activated HSCs also affect hepatic blood flow as they can increase sinusoidal constriction due to their elevated contractility [59]. Perfusion of endothelin-1, a peptide inducing constriction of blood vessels, into

isolated rat livers showed significant sinusoid constriction at the site of HSCs, but not Kupffer cells or endothelial cells [60], suggesting that activated HSCs can contribute to blood vessel constriction in the liver.

2.4 The role of hepatocytes and macrophages in the activation of HSCs

Apoptotic bodies, ROS, and nucleotides generated from apoptotic hepatocytes can stimulate HSC activation [31, 32]. While apoptotic bodies of hepatocytes are primarily eliminated by macrophages [32], HSCs can also engulf the apoptotic bodies, which can trigger fibrogenesis and promote HSC survival by increasing anti-apoptotic proteins such as myeloid cell leukemia-1 (Mcl-1) and Bcl-2-related protein A1 [33, 61]. Several pathways have been implicated in the activation of HSCs by apoptotic hepatocytes. DNA from apoptotic hepatocytes increased the expression of TGF β 1 and collagen 1 in LX-2 cells, a human HSC cell line, and primary mouse HSCs, which was attenuated by a toll-like receptor 9 (TLR9) antagonist, suggesting that TLR9 pathway may be involved in HSC activation by apoptotic hepatocytes [62]. In addition, apoptotic bodies of hepatocytes induced phosphorylation of Akt and extracellular signal-regulated kinases, increasing nuclear translocation of nuclear factor- κ B (NF- κ B) in LX-2 cells [63]. Phosphorylation and nuclear translocation of Janus kinase 1/signal transducer and activator of transcription 3 (STAT3) were also induced by apoptotic bodies in LX-2 cells, promoting HSC survival by the upregulation of anti-apoptotic Mcl-1 protein [33].

Macrophages facilitate the survival of HSCs by several mechanisms, contributing to the perpetuation of liver fibrosis. NF- κ B pathway was induced in HSCs when they were cocultured with hepatic macrophages isolated from the fibrotic liver [64]. The induction was likely mediated by IL-1 and TNF as knockout of their respective receptors reduced HSC survival and bile duct ligation (BDL)-induced liver fibrosis. In addition to their role in the progression of liver fibrosis, macrophages can also facilitate the resolution of liver fibrosis, which may be attributable to macrophage heterogeneity [65]. Macrophages are classified into pro-inflammatory M1 or immunoregulatory M2 macrophages [66]. M1 macrophages promote liver fibrosis, whereas M2 macrophages enhance the resolution of liver fibrosis [43]. To determine the effect of macrophages on fibrosis progression and resolution, macrophages were depleted in CD11b-human diphtheria toxin (DT) receptor transgenic mice [67]. Macrophage depletion diminished scar formation and myofibroblast number when the mice were treated with CCl₄; however, when macrophages were depleted by DT injection during spontaneous recovery after cessation of CCl₄ treatment, ECM was not degraded unlike control mice [67]. In addition, CCR2 knockout mice showed a decrease in hepatic macrophage infiltration and liver fibrosis when they were injected with CCl₄ [38]. The resolution of liver fibrosis, however, was slower in the CCR2 knockout mice after the end of CCl₄ treatment due to reduced matrix metalloproteinase-2 (MMP-2) and MMP-13 expression in the liver. Therefore, macrophages not only enhance liver fibrosis but also facilitate resolution of liver fibrosis in a different development stage of liver fibrosis. Macrophage polarization from M1 to M2 phenotype may be a potential target to prevent or treat liver fibrosis. Figure 2 depicts the role of hepatocytes and macrophages in the activation of HSCs for ECM accumulation.

2.5 HSCs in the regression of liver fibrosis

Liver fibrosis is a pathological condition that results from excessive accumulation of ECM following chronic liver injury. Fibrosis can aggravate inflammatory damage, parenchymal cell death, and angiogenesis in the liver [7, 8]. When fibrotic insults persist, liver fibrosis can progress to liver cirrhosis, which is characterized by disrupted liver architecture and functions as well as abnormal hepatocyte regeneration [8].

Recent evidence suggests that liver fibrosis and cirrhosis can be reversed in humans and animals. The patients with hepatitis C showed reversal of cirrhosis by treatment with pegylated interferon and ribavirin, an anti-viral drug [13]. Lamivudine, an anti-viral drug for hepatitis B, also decreased fibrogenesis in the patients with hepatitis B [14]. Similarly, another anti-viral drug Entecavir led to the reversal of liver fibrosis and cirrhosis in the patients with chronic hepatitis B [68]. Weight loss also attenuated liver fibrosis in obese patients with NAFLD [15]. In animal models, CCl₄-induced liver fibrosis was regressed after the discontinuation of CCl₄ treatment in male Sprague Dawley (SD) rats [69], and mice [70].

During the resolution, activated HSCs undergo apoptosis [69] or revert to inactivated HSCs [43]. A half of activated HSCs in CCl₄-induced fibrotic mouse livers were shown to undergo apoptosis, while the other half were inactivated and acquired phenotypic characteristics similar to those of quiescent HSCs [43]. Inactivated HSCs share some common features with quiescent HSCs, but they are more rapidly activated by following liver insults than quiescent HSCs [43].

3. Factors for the development of liver fibrosis

3.1 Inflammation

During liver injury, immune cells, including platelets, neutrophils, macrophages, natural killer cells, T cells and B cells, are recruited to the liver and contribute to the development of liver fibrosis [71]. The immune cells release inflammatory and fibrogenic cytokines, including IL-1 β , IL-4, IL-13, TGF β and PDGF, as well as ROS in the liver [72].

Studies have shown that the activation of NF- κ B in hepatocytes, macrophages, and HSCs is critically involved in the development of liver fibrosis. NF- κ B activation in hepatocytes by overexpressing I κ B kinase 2 increased HSC activation while recruiting more macrophages and T lymphocytes into the mouse liver [73]. Also, introduction of a NF- κ B decoy, a synthetic oligodeoxynucleotide that suppresses NF- κ B binding to DNA, into macrophages decreased the expression of TGF β , COL1A1, and α smooth muscle actin (α SMA) in the CCl₄-treated mouse liver [74]. Moreover, TLR4 signaling in HSCs activated NF- κ B, decreasing the expression of BMP and activin membrane-bound inhibitor homolog (BAMBI), a TGF β pseudoreceptor [75] that blocks TGF β signaling as it lacks in an intracellular kinase domain [76]. Therefore, the activation of NF- κ B in HSCs potentiates TGF β signaling, consequently facilitating the development of liver fibrosis.

In addition to macrophages, other immune cells are involved in the pathogenesis of liver fibrosis. Upon liver injury, neutrophils accumulate in the hepatic vasculature and they are

activated by inflammatory mediators, including TNF α , IL-1, CXC chemokines, and platelet activating factor [77]. Neutrophils adhering to distressed hepatocytes produce ROS by NADPH oxidases (NOX) and release proteases, which can induce hepatocyte necrosis [77]. Platelets are also recruited to the site of injury and release PDGF, a potent mitogen for HSCs [78]. The expression of PDGF receptors is increased in activated rat HSCs [79] and in human cirrhotic livers [80].

A variety of inflammatory cytokines secreted from the aforementioned immune cells during liver injury contributes to the development of liver fibrosis. IL-1 β primarily produced by macrophages increases fibrogenic response in mouse HSCs by suppressing BAMBI while elevating TIMP-1 [81]. IL-1 also enhances survival of activated mouse HSCs during fibrosis [64]. TNF α exerts similar effects to those of IL-1 β as it increases TIMP-1 in mouse HSCs [82, 83] and suppresses apoptosis of activated HSCs [83]. IL-17, produced by neutrophils and T lymphocytes [84], increases the expression of collagen type I in HSCs via the activation of STAT3 pathway and stimulates inflammatory cells to produce IL-1, IL-6 and TNF α [85]. The expression of IL-20, a pro-inflammatory cytokine, is elevated in hepatocytes and HSCs from human livers with fibrosis, cirrhosis, and HCC compared to normal livers and in mice with CCl₄-induced liver injury [86]. IL-20 can activate quiescent rat HSCs and it can stimulate proliferation and migration of activated HSCs [86].

3.2 Oxidative stress

Oxidative stress promotes the development of liver fibrosis and cirrhosis, at least partly, by activating HSCs [87-89]. Apoptotic hepatocytes release cellular contents and ROS, which can activate Kupffer cells for the production of inflammatory and fibrogenic cytokines, such as TNF α , IL-1 β , IL-6, and TGF β [43]. ROS and reactive nitrogen species are also produced by neutrophils, monocytes, and Kupffer cells, stimulating HSCs to produce type I collagen [44]. Rat primary HSCs also produce H₂O₂ in response to TGF β [90], which can upregulate the expression of COL1A1 in HSCs [91]. Furthermore, when HSCs engulf apoptotic hepatocytes, their superoxide production was stimulated due to increased NOX, leading to the upregulation of procollagen α 1 expression in LX-1 cells, a human HSC cell line [92].

4. Food components for the prevention of liver fibrosis

4.1 Astaxanthin

Astaxanthin (ASTX), a xanthophyll carotenoid, is abundant in marine animals, such as salmon and shrimp. ASTX is well known for its antioxidant activity [93]. In LX-2 cells, a human HSC cell line, we showed that ASTX attenuated TGF β 1-induced COL1A1 and α SMA expression [94]. The anti-fibrogenic effect of ASTX was attributed to inhibition of TGF β 1-induced phosphorylation and nuclear translocation of SMAD3 as well as reduced mRNA expression of SMAD3, SMAD7, TGF β receptor I, and receptor II [94]. Furthermore, using mouse primary HSCs, we demonstrated that ASTX prevented the activation of quiescent HSCs; and it also reverted the activated HSCs to a quiescent state [95]. The potential molecular mechanism underlying the anti-fibrogenic effect of ASTX is not clear. However, histone deacetylase 9 (HDAC9) may be involved in the inhibitory action of ASTX in HSC fibrogenesis as HDAC9 mRNA and protein levels were significantly higher in

activated mouse primary HSCs than quiescent HSCs, which was inhibited by ASTX; and HDAC9 knockdown in LX-2 cells decreased TGF β 1-induced fibrogenic gene expression [96].

Several animal studies have supported anti-fibrotic actions of ASTX. In mice with CCl₄ or BDL-induced liver fibrosis, ASTX decreased the level of hydroxyproline, a major component of collagen, and mRNA and protein levels of α SMA, β -PDGF receptor, and collagen I in both mRNA and protein level in the liver [97]. In diet-induced obesity and NASH mouse models, ASTX markedly reduced collagen accumulation with a concomitant decrease in the expression of fibrogenic genes in the liver [98].

The protective effect of ASTX on the development of liver fibrosis may be attributed to its capacity to enhance antioxidant defense. ASTX supplementation significantly increased the hepatic mRNA expression of nuclear factor erythroid 2-related factor 2 (NRF2), the major transcription factor for endogenous antioxidant defense, and its target genes including superoxide dismutase 1 and glutathione peroxidase 1 (GPx-1) in apolipoprotein E knockout mice [99, 100]. The anti-fibrotic effect of ASTX may also be associated with its anti-inflammatory capacity. ASTX decreased the expression of pro-inflammatory cytokines, including TNF, IL-6 and IL-1 β in the livers of mice fed a diet high in fat, cholesterol and cholate [101]. The anti-inflammatory effect of ASTX was attributed to attenuated phosphorylation of c-Jun N-terminal protein kinase (JNK), p38 mitogen-activated protein kinase and NF- κ B p65 as well as increased M2 macrophages with decreased M1 macrophages and in the liver [101].

4.2 Curcumin

Curcumin, a polyphenol in turmeric, is known to have anti-inflammatory, antioxidant, anti-viral, and anti-cancer activities [102]. Studies have demonstrated that curcumin ameliorates liver fibrosis by attenuating TGF β 1 signaling. Curcumin decreased mRNA and protein levels of α SMA and collagen deposition in the liver of SD rats with CCl₄-induced liver fibrosis by inhibiting TGF β 1 expression and SMAD2/3 activation while increasing the expression of SMAD7, an inhibitory SMAD [103]. Curcumin also decreased alcohol-induced HSC proliferation and stimulated HSC apoptosis by decreasing protein levels of TGF β 1 and phosphorylated SMAD3 with a concomitant increase in SMAD7 in rat primary HSCs [104]. In addition, curcumin inhibited the proliferation of HSCs by reducing the expression of hypoxia inducible factors 1 α , a transcription factor known to increase the expression of fibrogenic factors [105-108], consequently preventing the development of liver fibrosis in SD rats [109]. Studies have also shown that curcumin promotes HSC senescence in rat fibrotic liver induced by CCl₄ [110], in HSC T6 cells [110], and in LX-2 cells [111].

Anti-inflammatory effect of curcumin contributes to its anti-fibrotic action. Curcumin decreased protein levels of TNF α and MCP-1 in CCl₄-induced mouse fibrotic liver [112]. The mouse liver showed decreased infiltration of immune cells, including inflammatory Gr1^{hi} monocytes and macrophages. Curcumin also significantly reduced the level of inflammatory cytokines, such as TNF α , IL-1 β , IL-6, and cytokine-induced neutrophil chemoattractant-1, a rat homologue to human IL-8, in serum as well as in the liver of CCl₄-treated rats [113].

4.3 Blueberry

Studies have shown that blueberry can prevent the development of liver fibrosis in animal models. Blueberry juice consumption decreased protein levels of α SMA and collagen III in the liver of rats with CCl₄-induced liver fibrosis, which was attributed to its antioxidant properties based on increased SOD activity and metallothionein, a ROS scavenger, with decreased malondialdehyde (MDA), a marker of oxidative stress [114]. Similarly, when blueberry was fed to rats with CCl₄-induced liver fibrosis, liver fibrosis was significantly reduced concomitantly with decreased lipid peroxidation and increased SOD activity in the liver [115]. Also, blueberry supplementation decreased hepatic MDA, diene conjugate, and protein carbonyl contents in rats injected with diethylnitrosamine, a hepatocarcinogenic agent [116]. Therefore, it appears that the anti-fibrotic effect of blueberry is largely due to its antioxidant property.

4.4 Silymarin

Silymarin, an extract of milk thistle, consists of a flavonoid taxifolin and flavonolignans [117]. Silymarin has been shown to exert hepato-protective, anti-inflammatory and antioxidant activities, and to enhance hepatocyte regeneration [118]. Several studies have shown that silymarin prevents the development of liver fibrosis in animal models of liver fibrosis largely due its antioxidant property. In rats with CCl₄-induced liver fibrosis, silymarin decreased protein levels of hydroxyproline, which was attributable to increased activities of SOD and GPx [119]. Silymarin increased glutathione (GSH) but decreased MDA levels in CCl₄-treated rat liver [120, 121]. Furthermore, silymarin reduced HSC activation and fibrogenic gene expression in the liver of obese diabetic Otsuka Long-Evans Tokushima rats fed a methionine and choline-deficient (MCD) diet [122]. This anti-fibrotic effect of silymarin was attributed to its antioxidant capacity as there was an increase in nuclear translocation of NRF2.

Silymarin has been shown to inhibit liver fibrosis induced by *Schistosoma mansoni*. In the liver of mice infected with *Schistosoma mansoni*, silymarin decreased hepatic hydroxyproline content and protein levels of TGF β 1 and MMP-2 [118]. Silymarin also decreased hepatic hydroxyproline contents and serum IL-13 levels of BALB/c mice infected with *Schistosoma mansoni* [123]. The reduction of serum IL-13 by silymarin is important for its anti-fibrotic action as *Il-13* deficient mice showed a marked decrease in liver fibrosis when mice were infected with *Schistosomiasis mansoni* infection [124].

4.5 Coffee

A meta-analysis of 16 studies involving 3,034 coffee drinkers and 132,076 non-coffee drinkers showed that coffee consumption is inversely associated with liver fibrosis [125]. Coffee consumption is also inversely related to hepatic fibrosis based on NAFLD histologic severity in patients with NAFLD [126], and those with hepatitis C [127]. In addition, several animal studies have supported the anti-fibrotic effect of coffee. Coffee consumption reduced liver fibrosis in CCl₄-injected male SD rats by decreasing the expression of TGF- β 1, collagen I, collagen III, bcl-2, and vascular endothelial growth factor [128]. In male SD rats injected with DMN for the induction of liver fibrosis, coffee decreased hydroxyproline

accumulation, MDA production, and HSC activation while increasing total GSH levels, catalase activity, and SOD activity in the liver [129].

Caffeine is likely an important component in coffee that exerts the anti-fibrotic effect. Inverse relationship between caffeine consumption and liver fibrosis existed in the patients with liver fibrosis [130-132]. Several mechanisms have been proposed for possible mechanisms by which caffeine may prevent liver fibrosis. Caffeine enhances apoptosis while inhibiting cell proliferation in LX-2 cells [133] and HSC-T6 cell line [134]. In addition, caffeine attenuated liver fibrosis by inhibiting cAMP/protein kinase A /cAMP response element-binding protein signal pathway in a rat model of alcohol-induced liver fibrosis [135]. The antioxidant activity of caffeine may also contribute to its preventive effect against liver fibrosis as there was decreased MDA levels and increased GPx activity in the liver of TAA-treated male Wistar rats [136]. However, other components in coffee than caffeine may play be critical components that provide anti-fibrotic effects of coffee. Both regular and decaffeinated coffee ameliorated liver fibrosis in TAA-treated male Wistar rats to a similar with increased GPx activity and decreased MDA and collagen content in the liver [137]. Also, both types of coffee decreased TGF β , α SMA, CTGF, IL-10, MMP-13, MMP-2 and MMP-9 protein levels in the rat liver [137]. More studies are needed to determine whether cafestol, kahweol, nicotinic acid and other components in coffee could prevent the development of liver fibrosis.

4.6 Vitamin C and E

Importance of vitamin C in the development of liver fibrosis was investigated by using L-gulonono- γ -lactone oxidase knockout mice that cannot synthesize vitamin C [138]. Vitamin C insufficiency increased TAA-induced liver fibrosis in the knockout mice as evidenced by increases in hepatic collagen deposition, serum TGF β 1, and the number of activated HSCs. This study also demonstrated that oxidative stress in the liver was significantly higher in mice administered with low-dose vitamin C compared to those with high-dose vitamin C. In LX-2 cells, vitamin C suppressed the cell proliferation and H₂O₂-induced COL1A1 expression [138]. Several other studies have suggested that vitamin C exerts its anti-fibrotic action by inhibiting oxidative stress. Vitamin C reduced liver fibrosis by decreasing lipid and protein peroxidation products, e.g., MDA and conjugated dienes, and protein carbonyl content, a protein oxidation product, in the liver of male guinea pigs [139] and rats [140] with alcohol-induced hepatic fibrosis. The activities of antioxidant enzymes, including catalase, SOD and GPx, were concomitantly increased by vitamin C in the liver of rats administered with ethanol [140].

Vitamin E also has been show to exert an anti-fibrotic effect. Vitamin E significantly reduced hepatic collagen content in the liver of Wistar albino rats with BDL [141] and with ethanol-induced hepatic fibrosis [140]. Antioxidant activity of vitamin E is likely to contribute to the anti-fibrotic effect. Vitamin E reduced lipid peroxidation and liver fibrosis in rats with NASH induced by a MCD diet [142], and by ethanol [140]. The activities of antioxidant enzymes, including catalase, SOD and GPx, were increased by vitamin E in the liver of ethanol-administered rats [140]. Furthermore, it was shown that vitamin E decreased liver fibrosis in male Wistar albino mice-treated with nano-sized titanium dioxide (n-TiO₂), a

metallic nanoparticle generating inflammatory responses, apoptosis and necrosis via the stimulation of ROS production [143].

4.7 Vitamin D

An inverse relationship between serum vitamin D levels and the risk of liver fibrosis has been observed. Serum levels of 25(OH)-vitamin D were significantly lower in the patients with chronic hepatitis C [144] and with NAFLD [145] compared with healthy subjects. Low serum vitamin D levels are also associated with severe liver fibrosis in the patients with chronic liver diseases, including hepatitis B, hepatitis C, NAFLD, alcoholic liver disease, autoimmune disease, and primary biliary cirrhosis [146-149]. In particular, the active form of vitamin D, i.e., 1,25(OH)₂D₃, has anti-proliferative and anti-fibrotic effects in the liver, possibly due to its inhibitory effect on the proliferation of HSCs and TIMP-1 mRNA expression [150]. Decreased TIMP-1 protein levels were also observed in TAA-treated rat liver [151], which may inhibit excessive collagen accumulation. In addition, vitamin D decreased TGFβ signaling by reducing phosphorylated SMAD2 in primary human HSCs isolated from NAFLD patients [145].

4.8 Resveratrol

Resveratrol is a flavonoid rich in grapes and berries. It has been demonstrated that resveratrol has antioxidant and anti-inflammatory properties [152], which can contribute to the prevention of liver fibrosis. Resveratrol showed a protective effect against the development of liver fibrosis in rats treated with CCl₄ [153, 154] and with DMN [155]. Resveratrol also reduced fibrotic area and the expression of αSMA and COL1A1 in the liver of high-fat diet fed mice when they were administered with LPS and this effect was attributed to its anti-inflammatory property as hepatic mRNA levels of TNFα and IL-6 were decreased [156]. Resveratrol also inhibited TLR4/NF-κB signaling in T-HSC/Cl-6 cells, an immortalized rat HSC cell line [157] as well as in LX-2 cells [158]. In addition, resveratrol increased GSH levels while decreasing MDA in the liver of male SD rats with DMN-induced liver fibrosis, suggesting that its antioxidant property may play a role in the prevention of liver fibrosis [155]. Promotion of apoptosis by resveratrol may also contribute to its anti-fibrotic effect by activating caspase and inducing autophagosomes as demonstrated in GRX cells, an activated HSC model [159].

4.9 Quercetin

Quercetin is a flavonoid, which has an antioxidant capacity [160]. Quercetin prevented liver fibrosis in CCl₄-treated male Wistar rats with decreases in hepatic collagen deposition and αSMA-positive cells while increasing catalase and SOD activities [161]. In addition, in MCD diet-fed C57BL/6J mice, quercetin consumption inhibited liver fibrosis, which was potentially mediated by attenuating JNK phosphorylation and NF-κB activation in the liver [162]. Furthermore, quercetin decreased αSMA protein levels and the expression of αSMA, COL1A1 and TGFβ1 in activated rat primary HSCs [163].

4.10 Epigallocatechin-3-gallate

Epigallocatechin-3-gallate (EGCG), the most abundant catechin in green tea, has antioxidant, anti-carcinogenic, anti-hypertensive and anti-fibrotic properties [164]. Consumption of EGCG in drinking water decreased hepatic hydroxyproline contents and α SMA protein when fibrosis was induced by CCl₄ in rats [165]. This anti-fibrotic effect of EGCG is potentially mediated by PDGF signaling as EGCG significantly decreased hepatic mRNA and protein levels of PDGF receptor β [165]. Studies also suggest that anti-fibrotic effect of EGCG is mediated by the inhibition of SMAD pathway. In mice fed MCD diet and LX-2 cells, the expression of fibrogenic genes, such as α SMA and COL1A1, were decreased by EGCG with a concomitant decrease in phosphorylation of SMAD2 and SMAD3 [166]. Antioxidant property of EGCG is likely to contribute its anti-fibrotic effect as it significantly decreased MDA level but increased SOD activity in the mouse liver [166].

5. Conclusion

Liver fibrosis is emerging as a major health problem with increasing prevalence of obesity-associated NAFLD. During liver injury, inflammatory and oxidative mediators induced by apoptotic hepatocytes and recruited macrophages activates HSCs, leading to excessive ECM accumulation and liver fibrosis development. Studies have demonstrated that liver fibrosis may be prevented and also reversed by bioactive food components and natural products, including astaxanthin, curcumin, blueberry, silymarin, coffee, vitamin C, D and E, resveratrol, quercetin, and EGCG. The anti-fibrotic effect of these food components is primarily attributed to their anti-oxidant and anti-inflammatory capacity. Potential mechanisms of action by which the aforementioned bioactive food components and natural products exert anti-fibrotic effects are summarized in Table 1 and Figure 3. Therefore, consumption of these bioactive food components may prevent the development of liver fibrosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ACC	Acetyl-CoA carboxylase
ASTX	Astaxanthin
BAMBI	BMP and activin membrane-bound inhibitor homolog
BDL	Bile duct ligation
CCL4	Chemokine (C-C motif) ligand 4

CCR	Chemokine receptor C-C motif chemokine receptor
ChREBP	Carbohydrate response element binding protein
COL1A1	Procollagen type 1 α 1
DMN	Dimethylnitrosamine
DT	Diphtheria toxin
ECM	Extracellular matrix
EGCG	Epigallocatechin-3-gallate
FAS	Fatty acid synthase
FAT	Fatty acid translocase
FATP	Fatty acid transport protein
GPx	Glutathione peroxidase
GSH	Glutathione
HCC	Hepatocellular carcinoma
HDAC9	Histone deacetylase 9
HSC	Hepatic stellate cell
IL	Interleukin
JNK	c-Jun N-terminal protein kinase
MCD	Methionine and choline-deficient
Mcl-11	Myeloid cell leukemia-1
MCP-1	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
MMP	Matrix metalloproteinase
NAFL	Nonalcoholic fatty liver
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NF-κB	Nuclear factor- κ B
NOX	NADPH oxidase
NRF2	Nuclear factor erythroid 2-related factor 2
n-TiO₂	Nano-sized titanium dioxide

PDGF	Platelet-derived growth factor
ROS	Reactive oxygen species
SCD-1	Stearoyl-CoA desaturase-1
SD	Sprague Dawley
SMAD	SMA- and MAD-related protein
SREBP-1c	Sterol regulatory element binding protein 1c
STAT3	Signal transducer and activator of transcription 3
TAA	Thioacetamide
TGFβ	Transforming growth factor β
TIMP	Tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
TNFα	Tumor necrosis factor α
αSMA	α smooth muscle actin

Figure 1.

Major events occurring in the hepatocyte during NAFLD development. Increases in the flux of free fatty acids from adipose tissue and lipogenesis via the activation of SREBP-1c and ChREBP pathways in the liver lead to TG accumulation (steatosis). Also, lipotoxic lipid intermediates are produced, which trigger ER stress, ROS generation, and inflammatory cytokine production, ultimately inducing hepatocyte apoptosis.

Figure 2.

The effect of hepatocytes and macrophages on HSCs for ECM accumulation. Apoptotic bodies of hepatocytes and other substances released from stressed and apoptotic hepatocytes, e.g., nucleotides, ROS, lipid peroxides, and inflammatory cytokines, stimulate macrophages and HSCs. Resident macrophages, i.e., Kupffer cells, and recruited macrophages (from $Ly6C^{high}$ monocytes) are activated by the products of hepatocyte apoptosis, producing inflammatory cytokines and TGF β . The inflammatory and fibrogenic cytokines activate SMAD3 and NF κ B pathways in HSCs, leading to the activation, proliferation, and survival of HSCs. As a result, ECM production is increased while ECM breakdown is decreased.

Figure 3.

Potential mechanisms of action for the anti-fibrotic actions of food components in vivo and in vitro. Food components in blue boxes have been shown to prevent liver fibrosis development in vivo; and those in green boxes have been shown to affect activation, inactivation and/or apoptosis of HSCs in vitro. Red lines indicate “inhibition” and blue arrow lines indicate “increase”.

Table 1

Summary for the anti-fibrotic action of bioactive food components

Bioactive component	Bioactivity	Change	Reference
Astaxanthin	TGF β 1-induced SMAD3 activation in LX-2 cells	↓	[94]
	Antioxidant defense in apolipoprotein E knockout mice	↑	[99, 100]
	Inflammation, and activation of JNK, p38 and NF- κ B p65 in mouse liver with NASH	↓	[101]
	Macrophage phenotypic switching from M1 to M2 macrophages in mouse liver with NASH	↑	[101]
Curcumin	SMAD3 activation in fibrotic rat liver	↓	[103, 104]
	HSC proliferation in rat primary HSC	↓	[104]
	HSC apoptosis in rat primary HSC	↑	[104]
	Hypoxia inducible factors 1 α expression in fibrotic rat liver	↓	[109]
	HSC senescence in CCl ₄ -induced fibrotic rat liver, in HSC T6 cells, and in LX-2 cells	↑	[110, 111]
	Inflammation in rat liver with fibrosis	↓	[112, 113]
Blueberry	Oxidative stress in rat liver with fibrosis/ cirrhosis	↓	[114-116]
Silymarin	Oxidative stress in fibrotic rat liver	↓	[119-122]
	IL-13 level in serum of mice with liver fibrosis	↓	[123]
Coffee	Oxidative stress in fibrotic rat liver	↓	[129, 137]
	ECM degradation in fibrotic rat liver	↑	[137]
Caffeine	HSC apoptosis in LX-2 and HSC-T6 cells	↑	[133, 134]
	HSC proliferation in LX-2 and HSC-T6 cells	↓	[133, 134]
	cAMP/protein kinase A/cAMP response element-binding protein signal pathway in fibrotic rat liver	↓	[135]
	Oxidative stress in fibrotic rat liver	↓	[136, 137]
	ECM degradation in fibrotic rat liver	↑	[137]
Vitamin C	Oxidative stress in fibrotic liver of mouse/guinea pig/rat	↓	[138-140]
	HSC proliferation in LX-2 cells	↓	[138]
Vitamin E	Oxidative stress in fibrotic rat liver	↓	[140, 142]
Vitamin D	HSC proliferation in rat primary HSC	↓	[150]
	Inhibition of ECM degradation in fibrotic rat liver	↓	[150, 151]
	TGF β -mediated SMAD2 activation in primary human HSC from NAFLD patients	↓	[145]
Resveratrol	Inflammation and TLR4/NF- κ B signaling in fibrotic mouse liver, T-HSC/Cl-6 and LX-2 cells	↓	[156-158]
	Oxidative stress in fibrotic rat liver	↓	[155]
	HSC apoptosis in GRX cells	↑	[159]
Quercetin	Antioxidant defense in fibrotic rat liver	↑	[161]
	JNK and NF- κ B activation in fibrotic mouse liver	↓	[162]
EGCG	PDGF signaling in fibrotic rat liver	↓	[165]
	Activation of SMAD2 and SMAD3 in fibrotic mouse liver and LX-2 cells	↓	[166]
	Oxidative stress in fibrotic rat liver	↓	[166]