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Fighting Liver Fibrosis with Naturally Occurring Antioxidants

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ABSTRACT

Liver fibrosis is a wound-healing response characterized by the accumulation of extracellular matrix following various liver injuries, which results in the deformation of the normal liver architecture and the development of liver cirrhosis and even hepatocellular carcinoma. Numerous *in vitro* and *in vivo* studies indicated that oxidative stress mediates the initiation and progression of liver fibrosis. Overaccumulation of reactive oxygen species disrupts macromolecules, induces necrosis and apoptosis of hepatocytes, stimulates the production of pro-fibrogenic mediators, and directly activates hepatic stellate cells, thereby resulting in liver damage and initiating liver fibrosis. Ameliorating oxidative stress is a potential therapeutic strategy for the treatment of liver fibrosis. Natural antioxidants have attracted increasing attention in treating liver fibrosis due to their safety and efficacy. In this review, the pathogenesis of liver fibrosis and the role of oxidative stress in liver fibrosis were discussed. Naturally occurring antioxidants that can treat and prevent liver fibrosis were summarized. Advances in clinical trials were also presented. The main purpose of this review is to provide a comprehensive and up-to-date knowledge from the biological importance of oxidative stress in liver fibrosis to representative antioxidants for treating liver fibrosis. Naturally occurring antioxidants show a potential for further investigations as lead compounds in fighting liver fibrosis.

Introduction

Liver diseases, including chronic hepatitis, steatosis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), have become continuous and increasing threats for public health [1–5]. Liver fibrosis is a wound-healing process during liver injury caused by viral infection, inflammatory response, high lipid diet, drugs, environmental pollutants, excessive alcohol intake, and autoimmune response, which is characterized by the deposition of collagen and accumulation of extracellular matrix (ECM) [6, 7]. Liver fibrosis results in the deformation of the normal liver architecture, HCC, and ultimately liver failure that is accompanied by remarkable morbidity and mortality. Fibrotic diseases are a major cause of mortality in industrialized nations [8, 9]; therefore, anti-fibrotic therapies are urgently needed.

In this review, scientific studies from 1980 to 2018 were searched using Google Scholar, PubMed, Web of Science, and Scopus, with "liver fibrosis", "natural products", and "antioxidant" as keywords. Only English publications were selected. Herbal extracts without clear components and those with inadequate or insufficient data in terms of examination assays with controls are excluded as described in ▶ **Fig. 1**. Review articles are also excluded. Thirty-five naturally occurring antioxidants with anti-liver fibrotic property from 57 studies were summarized in this review.

The Pathogenesis of Liver Fibrosis

Many factors, such as hepatic viral infection, excessive alcohol consumption and drug intake, and high-fat diet (HFD) feeding, can cause temporary (acute) or self-limited lesion in the liver, characterized by the induction of a focal inflammatory response, enzymatic evidence of liver damage, and hepatic necrosis and apoptosis [6]. Acute or self-limited lesion in the liver provokes ECM deposition and transiently changes the liver architecture for the sake of healing. These lesions are potentially reversible responses to the injury at the early stage of liver fibrosis. However, these situations could aggravate and develop into irreversible liver

ABBREVIATIONS

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMPK	AMP-activated protein kinase
APAP	acetaminophen
ARE	antioxidation response element
AST	aspartate aminotransferase
BDL	bile duct-ligated
DEN	diethylnitrosamine
DMN	dimethylnitrosamine
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECM	extracellular matrix
EGCG	epigallocatechin gallate
ET-1	endothelin-1
FFA	free fatty acid
γ-GCS	γ-glutamylcysteine synthetase
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
GSK-3β	glycogen synthase kinase 3β
GST	glutathione transferase
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HFD	high-fat diet
HO	heme oxygenase
HSC	hepatic stellate cell
ICR	Institute of Cancer Research
iNOS	inducible NO synthase
KEAP-1	kelch-like ECH-associated protein-1
LPS	lipopolysaccharides
MAO	monoamine oxidase
MAPK	mitogen-activated protein kinase
MDA	malondialdehyde
MMP	matrix metalloproteinase
NADPH	nicotinamide adenine dinucleotide phosphate
NAFLD	nonalcoholic fatty liver disease
NAS	NAFLD activity score
NASH	nonalcoholic steatohepatitis
NDMA	*N'*-nitrosodimethylamine
NF-κB	nuclear factor κ-light-chain-enhancer of activated B
NO	nitric oxide
NQO1	NAD(P)H quinine oxidoreductase 1
NRF2	the nuclear factor erythroid 2-related factor 2
3-NT	3-nitrotyrosine
OPN	osteopontin
OS	oxidative stress
PAI-1	plasminogen activator inhibitor-1
PCNA	proliferating cell nuclear antigen
PDGF	platelet-derived growth factor
ROS	reactive oxygen species
α-SMA	α-smooth muscle actin
SOD	superoxide dismutase
SP-1	the specificity protein-1
TAA	thioacetamide
TBARS	thiobarbituric acid reactive substances
TGF-β1	transforming growth factor-β1
TIMP	tissue inhibitor of metalloproteinase
VLDL-TG	very-low-density lipoprotein-triglycerides

cirrhosis and ultimately into HCC with sustained liver injury and overaccumulation of ECM [7]. In normal liver, ECMs maintain a state of dynamic equilibrium [10]. To maintain the balance of production and degradation of ECM, matrix metalloproteinases (MMPs) are activated to remove the over-deposited ECM and protect the liver against irreversible harm. The TIMPs (tissue inhibitor of metalloproteinases) hinder the clearance of ECM, which worsens the situation (▶ **Fig. 2**) [11, 12]. Hepatic stellate cells (HSCs), contributing to approximately 90% of ECM production in myofibroblasts, play a pivotal role during ECM generation and fibrotic scar formation (▶ **Fig. 2**) [13]. In normal conditions, HSCs store vitamin A, control the production of ECM, and regulate local vascular contractility, which are important functions in liver development, metabolism, immune response, and angiogenesis. Upon stimulation, quiescent HSCs are induced into activated HSCs, which are proliferating, fibrogenic, and contractile [13]. Cells derived from the bone marrow derived cells, including circulating fibrocytes and portal fibroblasts, are also transdifferentiate into fibrogenic myofibroblasts during liver injury (▶ **Fig. 2**) [13].

Regulation of HSCs activation and ECM deposition is a potential strategy for the treatment of liver fibrosis [7, 14]. A number of studies have proved the therapeutic agents suppressing HSC activation and ECM accumulation are promising for the treatment of liver fibrosis [15–17].

Oxidative Stress in Liver Fibrosis

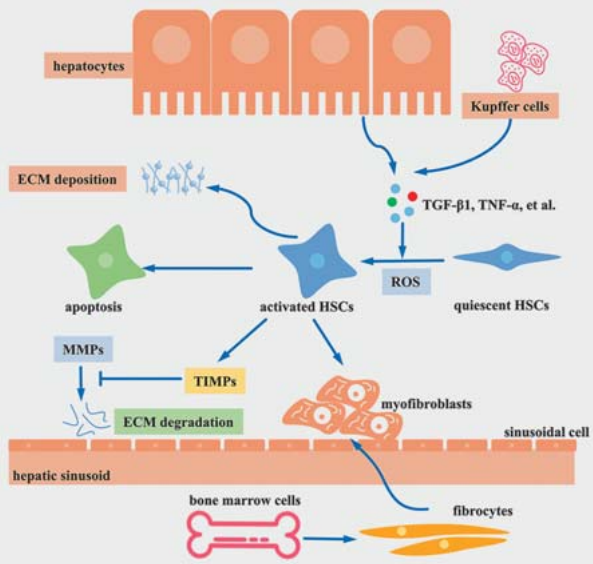
Oxidative stress (OS) is a disturbance in the balance between the production of free radicals and antioxidant defenses and is involved in the pathogenesis of various liver diseases [18]. In liver, many factors, including chronic and excessive alcohol consumption [19], HFD [20], hepatic viral infection [21], and autoimmune response [22], contribute to the onset of OS. OS disrupts the structure and function of biologically relevant macromolecules such as nucleic acids, proteins, lipids, and carbohydrates. In hepatocytes, reactive oxygen species (ROS) are mainly generated by the electron transport chain in the mitochondria, in the endoplasmic reticulum during protein folding and detoxification by cytochrome P450 systems, in the lysosomes during the removal of damaged cellular components, and in the peroxisomes during metabolic or detoxification activities [23, 24]. The prototypic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the plasma membrane and phagosomes also contributes to the production of ROS in Kupffer cells [25]. When the intracellular antioxidant defense system is overwhelmed, excessive ROS induce liver dysfunction and injury to stimulate inflammatory responses and the infiltration of neutrophils [26]. Kupffer cells, endothelial cells, and infiltrating inflammatory cells then secrete transforming growth factor-β1 (TGF-β1) following the liver injury. The cross-talk

between hepatocytes and HSCs is bidirectional. On the one hand, the injured hepatocytes, activated Kupffer cells, and infiltrating neutrophils generate excessive ROS and secrete inflammatory cytokines and TGF-β1 to activate HSCs, which results in overproduction of ECM and the specific inhibitors of MMP, such as TIMP-1 and TIMP-2 [27, 28]. On the other hand, the activated HSCs produce ROS to destroy hepatocytes and activate Kupffer cells. In addition, TGF-β1 induces its own expression in activated HSCs, thereby creating an autocrine loop [29].

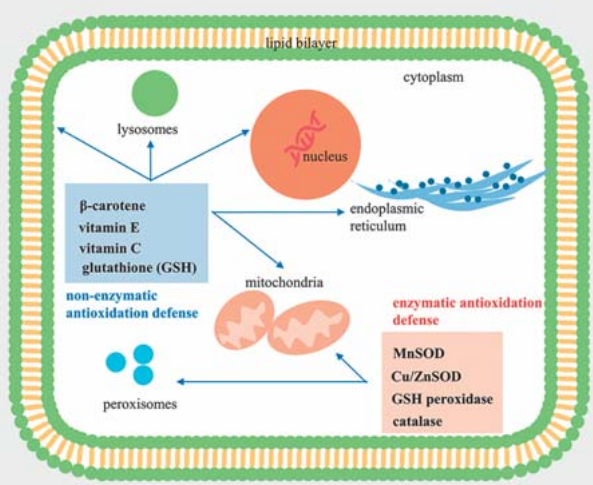
Challenging with free radicals from cellular and xenobiotic metabolism and immune process, liver cells develop their enzymatic and non-enzymatic antioxidative defense systems (▶ **Fig. 3**). Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione transferase (GST), heme oxygenase (HO), and catalase are commonly involved in enzymatic antioxidant defense [30]. Some signaling pathways, including NF-κB (nuclear factor κ-light-chain-enhancer of activated B) and NRF2 (the nuclear factor erythroid 2-related factor 2)/HO-1, are involved in fighting against oxidative damage. The NF-κB subunits are oxidized by ROS, which impair the DNA binding and transcriptional activity of NF-κB. Its inhibitor IκBα is degraded with the help of IKK kinase complexes [31]. Hence, the activation of IKK kinase activity is a potent strategy to enhance the NF-κB activity and strengthen its antioxidant capacity [32]. As a transcription factor, NRF2 dissociates from its inhibitor kelch-like ECH-associated protein-1 and binds to antioxidation response element (ARE) in nucleus to activate the transcription of its target genes, including NAD(P)H quinine oxidoreductase 1, HO-1, and γ-glutamylcysteine synthetase under OS [33].

Apart from the enzymatic antioxidant defense, some endogenous antioxidants, such as ascorbic acid (vitamin C), carotenoids, α-tocopherol (vitamin E), and glutathione (GSH), participate in non-enzymatic antioxidant defense (▶ **Fig. 3**) [30]. Hence, antioxidants strengthening the cellular antioxidative capacity are promising strategies for treatment of liver fibrosis [18, 34].

▶ **Fig. 1** Criteria used to select the articles for the data presented in this review.



▶ **Fig. 2** The general pathogenesis of liver fibrosis.



▶ **Fig. 3** The non-enzymatic and enzymatic antioxidative defense systems.

Antioxidants for the Treatment of Liver Fibrosis

Considering that OS has been implicated in fibrogenic stimulation for decades and overaccumulation of ROS is a crucial part of fibrotic pathway, the application of antioxidants for the treatment of liver fibrosis has been well documented [35, 36]. Recent findings contributed a concept of redox-fibrosis, in which the cellular oxidant and antioxidant systems could serve as potential therapeutic targets [37, 38]. Several anti-fibrotic therapies aimed to modulate OS and the generation of ROS have been put forward, and some of them have already been involved in clinical trials [39]. The safety and efficacy of natural antioxidants have attracted increasing attention. Hence, naturally occurring antioxidants for the treatment of liver fibrosis and the potential mechanisms were summarized (▶ **Table 1**).

Flavonoids

Flavonoids are a group of polyphenolic compounds with a C6-C3-C6 core structure, and most flavonoids possess antioxidant

▶ **Table 1** Naturally occurring antioxidants for the treatment of liver fibrosis.

Compound	Source	Model	Dosage	Mechanisms	Clinical trials	References
SRE (wogonin as a major component)	Scutellariae radix and Rhei rhizome, 1: 2	DMN-induced liver fibrosis in rats	1.25 and 6.25 mg/kg, oral administration daily for 3 wk	↑ SOD, GPx, and catalase; ↓ MDA; ↓ oxidation of protein and DNA; ↓ ROS; ↑ NRF2-ARE signaling; ↓ NF-κB pathway	no report	[44]
Morin	*Maclura pomifera, Maclura tinctoria* and *Psidium guajava*	LX-2 cells; DEN-induced liver fibrosis in rats	50 μM; 50 mg/kg, orally by gavage thrice per week for 6 wk	↓ proliferation; ↓ Wnt signaling; ↓ GSK-3β, β-catenin, and cyclin D1	no report	[45]
		Chronic CCl_4-induced liver fibrosis in rats	30 mg/kg, oral administration daily for 8 wk	↓ MDA and NO; ↑ GSH; ↓ hepatic hydroxyproline content		[46]
Galangin	*Alpinia officinarum* (lesser galangal) and *Helichrysum aureonitens*	CCl_4-induced liver fibrosis in rats	40 and 80 mg/kg, orally by gavage daily for 12 wk	↓ MDA and hydroxyproline concentration; ↑ SOD and GPx; ↓ α-SMA and TGF-β1	no report	[47]
Isorhamnetin	Yellow and red onions	Murine HSCs and LX-2 cells; CCl_4-induced liver fibrosis in rats	50 and 100 μM; 10 and 30 mg/kg, oral administration 5 d per week for 4 wk	↓ α-SMA, PAI-1, and collagen; ↓ ROS; ↓ 4-hydroxynonenal and nitrotyrosine-positive cells and GSH depletion; ↓ TGF-β/Smad signaling	no report	[48]
Myricitrin	*Myrica rubra* (Chinese bayberry)	CCl_4-induced liver fibrosis in mice	30 and 100 mg/kg, orally by gavage daily for 2 d	↓ lipid peroxidation; ↑ GSH level and CYP2E1 expression; ↓ TGF-β1 and α-SMA; ↑ proliferating cell nuclear antigen expression	no report	[49]
Naringin	Grapes, oranges and tomatoes	Nickel- and cadmium-induced toxicity in rats	orally administrated 80 mg/kg for 20 d or 50 mg/kg for 4 wk, respectively	↓ lipid peroxidation; ↑ antioxidant defense	no report	[53, 54]
Naringenin		Ethanol-induced liver fibrosis in rat	50 mg/kg, oral administration daily for 30 d	↓ expression of collagen; ↓ HSCs activation	no report	[55, 56]
		Fructose-treated rat	50 mg/kg, oral administration daily for 45 d	↓ lipid peroxidation and protein oxidation; ↑ SOD, catalase, GPx, GST, and GR; ↑ GSH, vitamins C and E		[57]
		Lead acetate-treated rats	50 mg/kg, orally by gavage daily for 4 wk	↑ GSH, catalase, and GPx		[58]
Pinocembrin	*Penthorum chinense*	HSC-T6 and LX-2 cells	10, 20, and 40 μM	↓ ROS; ↓ TGF-β1; ↓ SMAD nuclear translocation	no report	[62]
Silymarin	The seeds of *Silybum marianum* (milk thistle)	CCl_4-induced liver fibrosis in rats	100 mg/kg, oral administration daily for 10 d, or 200 mg/kg, oral administration three times daily for 8 wk	↓ MDA; ↑ GSH; ↑ Mn-SOD, Cu/Zn-SOD, and GPx activities; ↓ connective tissue growth factor	NCT02006498, NCT00680407, NCT00389376	[64, 70]
		Sodium nitrite-induced liver fibrosis in rats	10 and 25 mg/kg, oral administration daily for 12 wk	↓ MDA; ↑ hepatic GR and GPx; ↑ mitochondrial cytochrome C oxidase function		[71]
EGCG	Green tea	HFD-fed female Sprague-Dawley rats	50 mg/kg, intraperitoneal injection, 3 times per week for 8 wk	↓ expressions of key pathological oxidative and pro-inflammatory markers; ↓ TGF/SMAD, PI3K/Akt/FoxO1, and NF-κB pathways	no report	[75]
Glycyrrhetinic acid	The roots of *Glycyrrhiza glabra* (licorice)	CCl4-induced chronic liver fibrosis in mice	25, 50, and 100 mg/kg, subcutaneous injection daily for 30 d	↓ MAO and MDA; ↑ NRF2, Cu/Zn-SOD, GPx, and catalase	no report	[78]
Glycyrrhizin		CCl_4-induced liver fibrosis in rats	12.5 and 25 mg/kg, intraperitoneal injection daily for 8 wk	↓ SP-1 expression	NCT03349008	[80]
		Fructose-induced metabolic syndrome in rats	50 mg/kg, single intraperitoneal injection	↓ NF-κB signaling; ↓ phosphorylation of MAPKs		[81]

continued

▶ **Table 1** *Continued*

Compound	Source	Model	Dosage	Mechanisms	Clinical trials	References
Astragaloside IV	Radix Astragali	HSCs from male Sprague-Dawley rats	3–100 μM	↓ROS and lipid peroxidation; ↑GSH; ↑NRF2 gene expression	no report	[82]
Ursolic acid	Apples, basil, cranberries, peppermint, rosemary, oregano and prunes	CCl_4-induced liver fibrosis in ICR mice	25 and 50 mg/kg, oral injection daily for 6 wk	↓ROS; ↑SOD, catalase, and GPx; ↑NRF2 expression	no report	[84]
Andrographolide	*Andrographis paniculata*	APAP-induced liver fibrosis in mice	20 and 40 mg/kg, oral administration daily for 4 wk	↓hepatic collagen deposition and HSCs activation; ↓OS; ↑NRF2 nuclear translocation and the expression of antioxidant genes	no report	[86]
Geniposide	Fruits of *Gardenia jasminoides* Ellis	HFD-induced NASH in rats	50 and 100 mg/kg, oral administration daily for 6 wk	↓FFA content; ↑SOD and GPx levels; ↓MDA level	no report	[90]
		Ethanol- tripterygium glycosides- and CCl_4-induced liver injury in mice models	20, 40, and 80 mg/kg, intragastrical administration daily for 7 d; 400 mg/kg, oral administration daily for 6 d	↑SOD, GSH, and catalase		[91–93]
β-carotene	*Dunaliella salina*	CCl_4-induced hepatotoxicity male ICR mice	71, 355, and 710 mg/kg, oral treatment daily for 8 wk	↑SOD, catalase, GPx, and GSH	no report	[94]
Matrine	Dried roots of *Sophora flavescens* Ait	HSC-T6 cells, CCl_4-induced liver fibrosis in rats	1–2 mM; 50 and 100 mg/kg, intragastrical administration daily for 12 wk	↓collagen synthesis; ↓serum hyaluranic acid levels and hepatic hydroxyproline contents	no report	[100]
		High-fructose-diet-induced NAFLD in rats	40, 80, and 160 mg/kg, oral administration daily for 4 wk	↓MDA level; ↑GSH content; ↑NRF2 activity; ↓NF-κB activation		[101]
Berberine	European barberry, goldenseal, goldthread, Oregon grape, phellodendron, and tree turmeric	CFSC-2G HSC and CCl_4-induced liver fibrosis in mice	50 mg/kg, oral administration daily for 6 wk	↓proliferation of HSC; ↓expression of α-SMA; ↑SOD; ↓MDA content; ↑AMPK; ↓TGF-β1	no report	[102]
Armepavine	*Nelumbo nucifera*	TNF-α- or LPS-stimulated HSC-T6 cells and BDL-induced liver fibrosis in rats	1–10 μM; 3 and 10 mg/kg, oral administration twice daily for 3 wk	↓α-SMA AP-1 and collagen; ↓TGF-β1, TIMP-1 and iNOS gene expression; ↑metallothionein genes	no report	[105]
Caffeine	Coffee beans	TAA-induced liver fibrosis in rats and HSC-T6 cells	50 mg/kg, oral administration daily for 8 wk	↓expression of TGF-β and pro-fibrogenic proteins; ↓HSCs activation; ↓expression of α-SMA	no report	[106]
Nicotinic acid	Plants and animals	TAA-induced liver fibrosis in rats	50 mg/kg, oral administration daily for 8 wk	↑GPx levels; ↓TGF-β levels; ↓HSC activation and ECM deposition; ↓activities of MMP-2 and -9	no report	[107]
Betaine	Sugar beet	Ethanol plus CCl_4-treated liver fibrosis in rats	2% w/w in food for 14 wk	↓α-SMA and TGF-β protein expressions; ↓MMP-2, TIMP-1, and TIMP-2 mRNA expressions	no report	[108]
Curcumin	Rhizomes of *Curcuma longa* (turmeric)	LPS-treated rats	30 or 60 mg/kg, oral administration daily for 7 d	↓TBARS level; ↑GSH and SOD levels	NCT02908152	[112]
		CCl_4-induced liver fibrosis in rats and HSCs	200 and 400 mg/kg, oral administration daily for 8 wk	↓OS; ↓HSC activation		[113]

continued

▶ **Table 1** *Continued*

Compound	Source	Model	Dosage	Mechanisms	Clinical trials	References
Resveratrol	*Polygonum cuspidatum* Grapes and berries	DMN-induced liver fibrosis in rats	10 mg/kg, oral gavage daily for 7 d	↓MDA level; ↑SOD and GPx levels	NCT02030977, NCT01446276, NCT01464801, NCT02216552	[119]
		LPS-induced OS in rats	20 mg/kg, intraperitoneal injection daily for 7 d	↓lipoperoxidation; ↓SOD, catalase, and GPx activities		[120]
		NDMA-induced liver fibrosis in rats	10 mg/kg, intraperitoneal injection three times per week for 3 wk	↓α-SMA and MDA; ↑liver glycogen, SOD, and ATPases		[121]
Polydatin	*Polygonum cuspidatum*	Alcohol-induced hepatic injury C57BL/6 mice	50 or 100 mg/kg, oral administration daily for 8 d	↓ALT and AST; ↓OS; ↑mitochondrial content and MMPs levels	no report	[122]
Rosmarinic acid	Plants from the families of Blechnaceae and Lamiaceae	CCl_4-induced liver fibrosis in rats	10, 25, and 50 mg/kg, oral gavage daily for 3 d	↓3-NT and TBARS formation; ↑SOD activity; ↓TGF-β1 and α-SMA expression; ↑NRF2 and HO-1	no report	[123]
Chlorogenic acid	Coffee beans and many plants	PDGF-treated HSC-T6 cells and CCl_4-treated liver fibrosis in rats	30 and 60 mg/kg, intragastrical administration daily for 8 wk	↓ROS; ↓p38 and ERK1/2 phosphorylation; ↓proliferation and pro-fibrotic genes expression; ↓hydroxyproline content and expression of α-SMA, collagen I, collagen III, and TIMP-1; ↑nuclear NRF2; ↓MDA level; ↑GSH, SOD, and catalase levels	no report	[124–126]
Salidroside	*Rhodiola rosea*	High-fat- and high-cholesterol-fed NASH rat	150 and 300 mg/kg, oral administration daily for wk	↓lipid accumulation; ↑antioxidant enzyme levels; ↓CYP2E1 and Nox2 mRNA	no report	[128]
Cinnamaldehyde	In the bark of cinnamon trees and other Species of the genus *Cinnamomum*	CCl_4-injured rats	10 mg/kg, oral administration daily for 6 consecutive d	↓ALT, AST, and lactate dehydrogenase activities; ↓OS and inflammation mediated through TLR2 pathway	no report	[129]
Apocynin	*Apocynum cannabinum*	CCl_4-induced liver fibrosis in female Long Evans rats	100 mg/kg, oral administration daily for 2 wk	↓AST, ALT, and ALP activities; ↓OS markers; ↑catalase and SOD activity	no report	[130]
Cichoric acid	A variety of plant species	Methionine- and choline-deficient diet-fed mice	10 and 30 mg/kg, oral administration daily for 4 wk	↑antioxidant enzymes; ↓inflammation; ↓fibrosis, apoptosis, and lipogenesis-related gene expression; ↑AMPK	no report	[131]
Rhein	*Rheum palmatum*	APAP-induced hepatotoxicity in rats	10, 20, and 40 mg/kg, oral administration daily for 2 d	↓ROS, NO, and MDA; ↑GSH content	no report	[134]
Sauchinone	*Saururus chinensis*	LX-2 cells, CCl_4-induced liver fibrosis in mice	10 and 20 mg/kg, oral administration four times per week for 4 wk	↓phosphorylation of Smad2/3; ↓the transcript levels of PAI-1 and MMP-2; ↓α-SMA; ↓4-hydroxynonenal and nitrotyrosine	no report	[135]
Osthole	Fruits of *Cnidium monnieri* Cusson	TAA-induced liver fibrosis in rats and activated HSCs (HSC-T6 and LX-2)	10 mg/kg, oral administration twice per day for 4 wk; 3 and 10 µg/mL	↓hepatic collagen and α-SMA; ↓expression of fibrosis-related genes; ↓production of fibrosis-related cytokines and chemokines; ↓TGF-β1-induced migration and invasion; ↓TGF-β1- or ET-1-induced HSCs contractility	no report	[137]

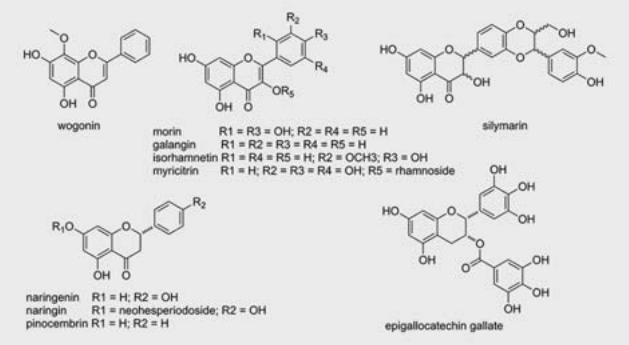
property. Some flavonoids have been reported with protective effect against liver fibrosis (▶ **Fig. 4** and **Table 1**).

Wogonin is a major flavonoid from the dried roots of *Scutellaria baicalensis* Georgi (Lamiaceae) [40]. Wogonin possesses the hepatic protective abilities including anti-virus, anti-inflammation, apoptosis induction of HCC cells, and free radical scavenging [41–43]. The ethanol extract from a herbal combinatorial formula (SRE, 1.25 and 6.25 mg/kg body weight, oral administration daily for 3 wk), containing wogonin as a major ingredient, showed a protective effect against dimethylnitrosamine (DMN)-induced liver fibrosis in rats [44]. Furthermore, the anti-fibrotic effect of SRE is mediated by elevating the levels of SOD, GPx, and catalase in DMN-exposed liver to prevent oxidative damage, decreasing the malondialdehyde (MDA) level to protect liver from DMN-induced lipid peroxidation, and ameliorating the oxidation of protein and DNA to restore liver injury and improve organ functions. SRE attenuates DMN-mediated liver injury by removing the excess accumulated ROS and inducing NRF2-ARE signaling to stimulate the expression of antioxidative enzymes [44].

Morin is a flavonoid isolated from *Maclura pomifera* (Raf.) C. K. Schneid. (Osage orange, Moraceae), *Maclura tinctoria* (L.) Steud. (old fustic, Moraceae), and the leaves of *Psidium guajava* L. (common guava, Myrtaceae). Morin inhibits the proliferation of human HSCs LX-2 cells, suppresses Wnt signaling, and induces G1 cell cycle arrest at the concentration of 50 µM. Morin (50 mg/kg body weight, orally by gavage thrice per week for 6 wk) ameliorates diethylnitrosamine (DEN)-induced liver fibrosis in rats by downregulating the expression levels of glycogen synthase kinase 3β (GSK-3β), β-catenin, and cyclin D1 [45]. The treatment of morin (30 mg/kg body weight, oral administration daily for 8 wk) attenuates the liver index and serum biomarkers of liver function that were enhanced by chronic CCl_4 intoxication, with silymarin (100 mg/kg body weight) as a positive control. Furthermore, morin inhibits the elevated levels of MDA and nitric oxide (NO) and restores GSH to its normal level in hepatocytes. The increased hepatic hydroxyproline content is markedly decreased by the administration of morin [46]. Hence, morin could be employed as a promising preventive natural supplement for liver fibrosis.

Galangin is a flavonol, present in *Alpinia officinarum* Hance (lesser galangal, Zingiberaceae) and *Helichrysum aureonitens* Sch. Bip. (Compositae). In liver fibrotic rats induced by the subcutaneous injection of CCl_4, galangin (40 and 80 mg/kg body weight, orally by gavage daily for 12 wk) reverses the CCl_4-induced increase of hyaluronic acid, laminin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) and decrease of total protein and albumin in serum; colchicin (0.2 mg/kg body weight) was used as a positive control [47]. Galangin markedly reduces hepatic MDA and hydroxyproline concentration and increases the activities of liver SOD and GPx compared with CCl_4-treated rats. In addition, galangin significantly downregulates the expression levels of α-smooth muscle actin (α-SMA) and TGF-β1 [47]. Hence, galangin might inhibit the CCl_4-induced liver fibrosis in rats, probably by removing oxygen free radicals, decreasing lipid peroxidation, and inhibiting HSCs activation and proliferation.

Isorhamnetin commonly exists in pungent yellow and red onions (Amaryllidaceae). Isorhamnetin (50 and 100 µM) inhibits the TGF-β1-induced expression of α-SMA, plasminogen activator



▶ **Fig. 4** Flavonoids for the treatment of liver fibrosis.

inhibitor-1 (PAI-1), and collagen in primary murine HSCs and LX-2 cells [48]. Isorhamnetin increases the nuclear translocation of NRF2 and blocks the TGF-β1-induced ROS production in HSCs. Furthermore, isorhamnetin (10 and 30 mg/kg body weight, oral administration 5 d/wk for 4 wk) prevents the CCl_4-induced increase in serum ALT and AST levels and causes histopathological changes characterized by the decrease in collagen accumulation [48]. Isorhamnetin attenuates the CCl_4-induced increase in the number of 4-hydroxynonenal and nitrotyrosine-positive cells and prevented GSH depletion. Isorhamnetin can inhibit the TGF-β/Smad signaling pathway and relieve OS, thus inhibiting HSC activation and preventing liver fibrosis.

Myricitrin (myricetin-3-*O*-α-rhamnoside) is a flavonoid from *Myrica rubra* Sieb. et Zucc. (Chinese bayberry, Myricaceae), possessing antioxidant and anti-inflammatory activities. Myricitrin (30 and 100 mg/kg body weight, orally by gavage daily for 2 d) ameliorates the CCl_4-induced increase in serum AST and ALT levels and the histopathological changes in the liver with silymarin (100 mg/kg body weight) as a positive control. Liver OS is reduced by myricitrin, as evidenced by the decrease in lipid peroxidation with a concomitant increase in GSH level and CYP2E1 expression. TGF-β1 and α-SMA expression is markedly ameliorated by myricitrin, indicating the inhibition of pro-fibrotic response. Myricitrin also improves the regeneration of hepatic tissue after CCl_4-intoxication, as evidenced by increased proliferating cell nuclear antigen expression [49], indicating its significant anti-fibrotic activity.

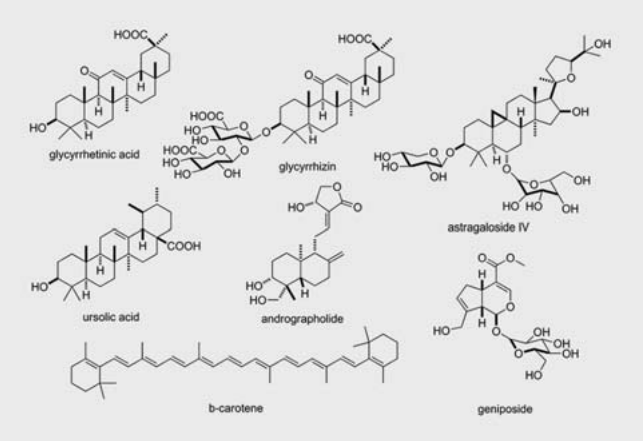
Naringenin and its glycoside naringin are naturally occurring citrus flavanones predominantly found in grapes (Vitaceae), oranges (Rutaceae), and tomatoes (Solanaceae), possessing have a wide range of pharmacological properties, including anti-dyslipidemia, anti-obesity, and anti-fibrosis [50–52]. Naringin attenuates the nickel- or cadmium-induced liver toxicity in rats when orally administrated with 80 mg/kg body weight for 20 d or 50 mg/kg body weight for 4 wk, respectively, by significantly reducing lipid peroxidation and restoring the levels of antioxidant defense [53, 54]. Oral treatment of 50 mg/kg naringenin for 30 d decreases the expression of collagen in ethanol-induced liver fibrotic rats, which is associated with reduced OS and HSC activation [55, 56]. In fructose-administered rats, naringenin (50 mg/kg body weight/day for 45 d) reduces the levels of oxidative markers by inhibiting liver cell leakage, lipid peroxidation, and protein oxidation, and enhances

the antioxidant potential by elevating enzymatic antioxidant activities including SOD, catalase, GPx, GST, and GR in liver. Moreover, naringenin supplementation increases the levels of non-enzymatic antioxidants such as GSH and vitamins C and E in rats [57]. Histopathological evidence indicates that naringenin (50 mg/kg body weight, orally by gavage daily for 4 wk) reduces the liver damage in lead acetate-administered rats by improving GSH, catalase, and GPx levels. This finding demonstrates that naringenin serves as an antioxidant and chelating agent to compete against lead acetate-induced OS and damage in liver [58]. Hence, naringenin and naringin are potential antioxidants for treatment of liver fibrosis.

Pinocembrin is a flavonoid isolated from *Penthorum chinense* Pursh (Penthoraceae), which has been widely used for liver protection for thousands of years [59, 60]. The water extract of *P. chinense* (5.15 and 10.30 g/kg body weight/day for 4 wk) protects the liver against chronic ethanol-induced injury in mice by attenuating ROS generation and MDA level, restoring GSH depletion, and increasing SOD and GPx activities with silymarin (86 mg/kg body weight) as a positive control [61]. In a recent study, pinocembrin from *P. chinense* suppresses the activation of both human HSC LX-2 and rat HSC-T6 cells at the concentrations of 10, 20, and 40 µM, mediating through ROS production alleviation, TGF-β1 inhibition, and prevention of Smad nuclear translocation, which suggest its potential for protection against liver fibrosis [62]. Further *in vivo* studies are needed to verify the anti-fibrotic effect of *P. chinense* and pinocembrin.

Silymarin, a mixture of flavonolignans consisting of silibinin, isosilibinin, silicristin, and silidianin, is isolated from the seeds of *Silybum marianum* (L.) Gaertn. (milk thistle, Compositae) [63, 64]. Silymarin has been used for centuries to treat liver, spleen, and gallbladder disorders [64, 65]. As the most well-studied natural product in the treatment of liver disease, silymarin has been recognized as "liver tonics" [66]. Silymarin is used to combat various liver conditions in both clinical settings and experimental models [63, 64, 67, 68]. As an antioxidant, silymarin mainly reduces free radical production and lipid peroxidation [69]. Silymarin (100 mg/kg body weight, oral administration daily for 10 d) significantly decreases the MDA level and increases the GSH level in CCl_4-induced liver damage, indicating that this substance protects the liver from damage with notable redox functions [64]. Silymarin (200 mg/kg body weight, oral administration 3 times daily for 8 wk) also improves liver fibrosis in CCl_4-treated rats by decreasing the connective tissue growth factor [70]. Silymarin increases the activities of MnSOD, Cu/ZnSOD, and GPx in liver [70]. Silymarin (10 and 25 mg/kg body weight, oral administration daily for 12 wk) also prevents sodium nitrite-induced liver fibrosis in rats by reducing hepatic MDA levels, restoring the activity of hepatic GR and GPx, and inhibiting the deactivation of mitochondrial cytochrome C oxidase function [71].

Several studies have revealed the protective effects of epigallocatechin gallate (EGCG) or green tea polyphenols against liver fibrosis on various animal models [72–74]. Intraperitoneal injection with 50 mg/kg of EGCG thrice per week for 8 wk improves the hepatic histology (decreased number of fatty score, necrosis, and inflammatory foci), reduces liver injury, and attenuates hepatic changes, including fibrosis, by downregulating the expression levels of key pathological oxidative and pro-inflammatory markers in HFD-fed female Sprague-Dawley rats [75]. EGCG treatment also counteracts the activity of TGF/Smad and NF-κB pathways [75]. For the *in vitro* and *in vivo* models of thioacetamide (TAA)-induced hepatic fibrosis, EGCG inhibits the osteopontin (OPN)-dependent injury and fibrosis, primarily by upregulating miR-221 to accelerate OPN degradation [76]. Thus, green tea polyphenols and EGCG are useful supplements in the prevention of nonalcoholic fatty liver disease (NAFLD).



▶ **Fig. 5** Terpenoids for the treatment of liver fibrosis.

Terpenoids

Terpenoids are a large and diverse class of natural products derived from isoprene units. Some terpenoids have been reported with anti-fibrotic effects (▶ **Fig. 5** and **Table 1**).

Glycyrrhetinic acid and glycyrrhizin are the major bioactive constituents isolated from the roots of *Glycyrrhiza glabra* L. (Leguminosae) [77]. In CCl_4-treated mice, glycyrrhetinic acid (25, 50, and 100 mg/kg body weight, subcutaneous injection daily for 30 d) reverses the CCl_4-induced increase in serum monoamine oxidase and MDA and decrease in nuclear NRF2 expression and its target genes, including Cu/Zn-SOD, catalase, and GPx, with silymarin (100 mg/kg body weight) as a positive control. In addition, glycyrrhetinic acid exhibits the antioxidant effects *in vitro* on $FeCl_2$-ascorbate-induced lipid peroxidation in mouse liver homogenates and on 2,2-diphenyl-1-picrylhydrazyl-scavenging activity [78]. These results suggest that glycyrrhetinic acid may be an effective hepatoprotective agent and a viable candidate for treating liver fibrosis. Glycyrrhizin has various pharmacological effects and has been used to treat chronic hepatitis, especially hepatitis C virus (HCV) infection and associated diseases [79]. Glycyrrhizin (12.5 and 25 mg/kg body weight, intraperitoneal injection daily for 8 wk) attenuates CCl_4-induced liver fibrosis in rat by downregulating the expression of the specificity protein-1, the critical factor for the initiation of OS, in both transcriptional and translational levels [80]. In a fructose-induced metabolic syndrome rat model, a single intraperitoneal injection of 50 mg/kg body weight of glycyrrhizin prevents several complications of metabolic syndrome, including lipid peroxidation, protein carboxylation, and mitochondrial ROS generation, which resulted in attenuation of OS in liver by inhibiting the NF-κB inflammatory pathway and pre-

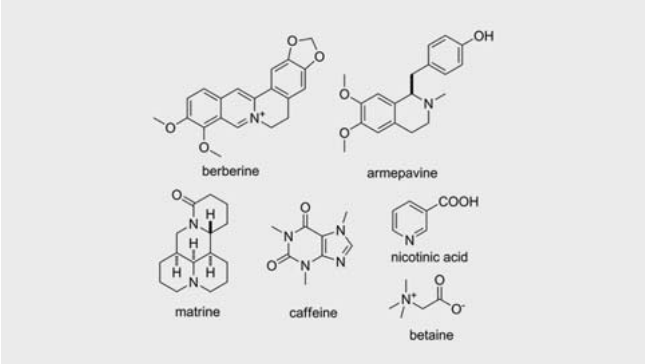
venting the phosphorylation of MAPKs (mitogen-activated protein kinases) signaling pathway [81]. Glycyrrhetinic acid, glycyrrhizin, and the extract of *G. glabra* have shown protective effects against liver fibrosis in various *in vitro* and *in vivo* studies. Clinical trials are needed to further verify the anti-fibrotic effects.

Astragaloside IV, the active component of Radix Astragali (Leguminosae), possesses antioxidant property and anti-fibrotic potential for renal fibrosis, and 3–100 μM astragaloside IV attenuates OS in activated HSCs from male Sprague-Dawley rats by scavenging ROS, reducing lipid peroxidation, elevating the cellular level of GSH, and stimulating NRF2 gene expression. The depletion of cellular GSH by buthionine sulfoximine or abrogation of p38 MAPK with SB-203580 eliminated the inhibitory effects of astragaloside IV on genes relevant to HSC activation [82]. These studies provide novel insights into the mechanisms of astragaloside IV as an anti-fibrogenic candidate in the prevention and treatment of liver fibrosis.

Ursolic acid, a natural pentacyclic triterpenoid, has been found in various plants including apples (Rosaceae), basil (Lamiaceae), cranberries (Ericaceae), peppermint (Lamiaceae), rosemary (Lamiaceae), oregano (Lamiaceae), and prunes (Rosaceae) and possesses many biological activities, including antioxidation and anti-inflammation [83]. Ursolic acid (25 and 50 mg/kg body weight, oral injection daily for 6 wk) prevents CCl_4-induced hepatotoxicity and fibrosis in ICR (Institute of Cancer Research) mice with colchicine (1 mg/kg body weight) as a positive control. The CCl_4-induced profound elevations of OS, as well as inflammation and apoptosis in liver, are suppressed by ursolic acid through modulating the NRF2/ARE signaling pathway [84]. These results suggest that ursolic acid has hepatoprotective actions.

Andrographolide is a labdane-type diterpenoid isolated from *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Acanthaceae), which has a broad range of therapeutic applications including anti-inflammatory and anti-platelet aggregation activities and potential antineoplastic properties [85]. Andrographolide (20 and 40 mg/kg body weight, oral administration daily for 4 wk) decreases hepatic collagen deposition and HSCs activation in APAP (acetaminophen)-induced mice [86]. Andrographolide alleviates liver OS and reduces ROS formation in HSCs. Andrographolide enhances the nuclear translocation of NRF2 and increases the expression of its downstream genes both *in vitro* and *in vivo*. Andrographolide might be clinically applied for the treatment of liver fibrosis.

Geniposide is an iridoid glycoside from the fruits of *Gardenia jasminoides* Ellis (Rubiaceae), which is useful against hyperlipidemia and fatty liver diseases [87, 88]. Geniposide at 5 and 20 μM effectively prevents TGF-β1-induced fibrotic responses in AML12 cells [89]. In HFD-induced nonalcoholic steatohepatitis (NASH) rats, the free fatty acid content is reduced by geniposide (50 and 100 mg/kg body weight, oral administration daily for 6 wk), suggesting its potential to prevent HFD-induced liver injury [90]. Geniposide markedly increases endogenous antioxidants and SOD and GPx levels but decreases the MDA level to protect liver cells from oxidative damage [90]. The antioxidant property of geniposide is related to its ability to reduce free radical formation and enhance free radical scavenging. The hepatoprotective activities of geniposide (20, 40, and 80 mg/kg body weight, intragastrical administration daily for 7 d) are also identified in mice models treated with alcohol, tripterygium glycosides, or CCl_4 with bifendate (150 mg/kg body weight) as a positive control [91, 92]. The ALT, AST, and alkaline phosphatase (ALP) levels are significantly decreased by geniposide in the above models [91, 92]. Moreover, geniposide (400 mg/kg body weight, oral administration daily for 6 d) remarkably elevates the GSH level and increases the SOD and catalase activities in CCl_4-induced liver damage mice with biphenyldicarboxylate pills (100 mg/kg body weight) as positive controls [93]. Thus, geniposide could be a potential candidate for the treatment of liver fibrosis.

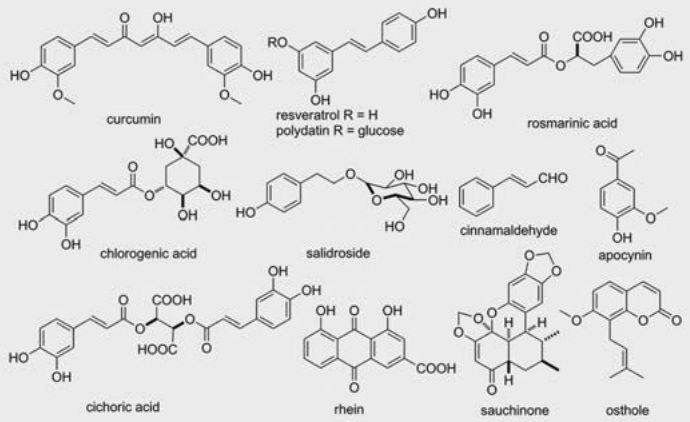


▶ **Fig. 6** Alkaloids for the treatment of liver fibrosis.

Dunaliella salina (Dunal) Teodoresco (Dunaliellaceae) is a unicellular biflagellate green alga from the Chlorophyceae class, which is rich in β-carotene. In CCl_4-induced hepatotoxicity male ICR mice, the oral treatment of *D. salina* extract (71, 355, and 710 mg/kg body weight) daily for 8 wk reverses the decreases in SOD, catalase, GPx, and GSH content and the increase in MDA content in liver. Liver histopathology shows that β-carotene reduces the incidence of liver lesions induced by CCl_4 with silymarin (200 mg/kg) as a positive control [94]. The results suggest that β-carotene exhibits potential protective effects against CCl_4-induced liver damage in mice.

Alkaloids

Alkaloids with protective effects against liver fibrosis were listed in ▶ **Fig. 6** and **Table 1**. Matrine is a primary active alkaloid isolated from the dried roots of *Sophora flavescens* Ait (Leguminosae), a commonly used traditional herb to cure hemafecia, dysentery, jaundice, and anuresis [88, 95]. Matrine has a variety of pharmacological properties, including anti-inflammatory, immunity regulatory, antiviral, and anti-fibrotic effects [96–99]. Matrine (1–2 mM) markedly reduces serum- or TGF-β1-driven collagen synthesis in HSC-T6 cells; matrine (50 and 100 mg/kg body weight, intragastrical administration daily for 12 wk) significantly decreases serum hyaluronic acid levels and hepatic hydroxyproline contents to attenuate CCl_4-induced liver fibrosis [100]. In a high-fructose-diet-induced NAFLD rat model, matrine (40, 80, and 160 mg/kg body weight, oral administration daily for 4 wk) retards the histopathological progression by restoring the increased MDA level, depleting GSH content, facilitating NRF2 translocation to the nuclei, and inhibiting hepatic NF-κB activation [101].



▶ **Fig. 7** Other natural compounds for the treatment of liver fibrosis.

Berberine is an alkaloid found in several plants including European barberry (Berberidaceae), goldenseal (Ranunculaceae), goldthread (Ranunculaceae), Oregon grape (Berberidaceae), phellodendron (Rutaceae), and tree turmeric (Berberidaceae). Oral administration of 50 mg/kg berberine daily for 6 wk ameliorates CCl_4-induced liver fibrosis in mice by decreasing the enzyme release of ALT, AST, and ALP in the serum and elevating SOD and reducing MDA content in the liver tissue. Moreover, berberine treatment activates AMP-activated protein kinase (AMPK), decreases the expression levels of TGF-β1 and α-SMA, and inhibits the proliferation of CFSC-2G HSCs [102]. These results may benefit the development of berberine in the prevention of chronic liver disease.

Armepavine is an active compound from *Nelumbo nucifera* Gaertn. (Nelumbonaceae), exerting anti-inflammatory effects on human peripheral blood mononuclear cells [103] and immunosuppressive effects on T lymphocytes and lupus nephritic mice [104]. In HSC-T6 cells, armepavine (1–10 µM) attenuates TNF-α- and LPS-stimulated α-SMA protein expression and AP-1 activation. Armepavine (3 and 10 mg/kg body weight, oral administration twice daily for 3 wk) suppresses the TNF-α-induced collagen deposition. In bile-duct-ligated-treated rats, armepavine treatment reduces the plasma AST and ALT levels, hepatic α-SMA expression and collagen contents, and fibrosis scores. Moreover, armepavine attenuates the mRNA expression levels of TGF-β1, TIMP-1, and inducible NO synthase but upregulates metallothionein gene expression [105]. Hence, armepavine has anti-fibrotic effects.

The support on the beneficial effects of caffeine on the liver is increasing. Caffeine (50 mg/kg body weight, oral administration daily for 8 wk) protects against TAA-induced liver cirrhosis in rats by restoring the redox equilibrium and inhibiting the expression levels of TGF-β and pro-fibrogenic proteins. Caffeine also inhibits HSCs activation and suppresses the expression of α-SMA [106]. Caffeine may attenuate liver fibrotic processes.

Chronic TAA administration induces liver fibrosis, which is prevented by nicotinic acid. The oral administration of 50 mg/kg body weight nicotinic acid daily for 8 wk prevents the elevation of liver enzymes and restored the GPx levels. Additionally, nicotinic acid decreases the TGF-β levels and attenuates the oxidative processes, reducing the HSC activation and ECM deposition. Nicotinic acid decreases MMP-2 and − 9 activities [107]. Hence, nicotinic acid can be an anti-fibrotic agent against liver injury.

Betaine is an amino acid firstly discovered in sugar beet (*Beta vulgaris* L., Amaranthaceae), together with other beet cultivars. In ethanol with CCl_4-treated liver fibrosis rats, betaine treatment (2 % w/w in food for 14 wk) diminishes the triglyceride level, the α-SMA and TGF-β protein levels, and MMP-2, TIMP-1, and TIMP-2 mRNA levels. Hence, the anti-fibrotic effect of betaine may be related to its suppressive effects on oxidant and inflammatory processes together with the HSC activation in alcoholic liver fibrosis [108].

Other compounds

In addition to the above-mentioned compounds, several other compounds have been reported to protect against liver fibrosis (▶ **Fig. 7** and **Table 1**).

Curcumin is a polyphenolic compound isolated from the rhizomes of *Curcuma longa* L. (turmeric, Zingiberaceae) [109]. Curcumin can treat a wide variety of inflammatory diseases including cancer, diabetes, and fibrosis [109, 110]. Curcumin has excellent antioxidant properties [111]. In LPS-challenged rats, the oral administration of 30 or 60 mg/kg curcumin daily for 7 d decreases the thiobarbituric acid reactive substances (TBARS) level and elevates GSH and SOD levels [112]. Curcumin (200 and 400 mg/kg body weight, oral administration daily for 8 wk) also protects the liver from CCl_4-induced injury by attenuating OS *in vivo* and inhibits HSC activation *in vitro* [113].

Resveratrol is a polyphenolic compound isolated from *Polygonum cuspidatum* Sieb. et Zucc (Polygonaceae). As a natural phytoalexin, resveratrol is also present in various plant species including grapes and berries and acts against environmental stress and fungal infection [114]. Resveratrol possesses anti-aging, anti-carcinogenic, anti-inflammatory, and antioxidant properties [115–118]. In the rat model, the oral gavage of 10 mg/kg resveratrol daily for 7 d protects DMN-induced liver fibrosis by decreasing the MDA and increasing the SOD and GPx levels in liver [119]. Resveratrol (20 mg/kg body weight, intraperitoneal injection daily for 7 d) has a protective effect against LPS-induced OS in rat liver by reversing the LPS-induced lipoperoxidation and offsetting the depletion of SOD, catalase, and GPx activities [120]. In *N'*-nitrosodi-

methylamine-induced liver fibrosis rats, resveratrol supplement (10 mg/kg body weight, intraperitoneal injection 3 times per week for 3 wk) refurbishes the liver architecture by significantly restoring the levels of biomarkers of oxidative damage (MDA, SOD, protein carbonyls, and membrane-bound ATPases) and inhibits the α-SMA expression and HSC activation to obstruct liver fibrosis [121].

Polydatin is a resveratrol glucoside isolated from *P. cuspidatum*. The oral administration of 50 or 100 mg/kg polydatin daily for 8 d alleviates the alcohol-induced hepatic injury by reducing the liver injury markers, ALT and AST, attenuating OS, and restoring the antioxidant balance in the hepatic tissue. Polydatin pre-treatment prevents alcohol-induced mitochondrial damage and refurbishes the MMP levels in the liver [122]. Hence, polydatin may have a potential benefit in preventing alcohol-induced acute hepatic injury.

Rosmarinic acid is a natural phenolic acid found in a variety of plants, especially the families of Blechnaceae and Lamiaceae. Rosmarinic acid (10, 25, and 50 mg/kg body weight, oral gavage daily for 3 d) decreases 3-nitrotyrosine and TBARS formation but increases SOD activity to ameliorate OS in the liver tissue from CCl_4-treated rats. Additionally, rosmarinic acid downregulates the expression levels of TGF-β1 and α-SMA, suggesting the suppression of pro-fibrotic response. The hepatoprotective activity of rosmarinic acid occurs with enhanced NRF2 and HO-1 expression [123]. Thus, rosmarinic acid possesses anti-fibrotic activity against acute liver toxicity.

Chlorogenic acid is a phenolic compound and exerts anti-inflammatory and antioxidant activities. Chlorogenic acid (30 and 60 mg/kg body weight, intragastrical administration daily for 8 wk) prevents CCl_4-induced liver fibrosis by attenuating the inflammation and OS in rats [124–126]. Liver fibrosis is induced in CCl_4-injected rats, characterized by increased hydroxyproline content and the expression of α-SMA, collagen I, collagen III, and TIMP-1, which are alleviated markedly by chlorogenic acid [124]. Furthermore, chlorogenic acid increases the expression of nuclear NRF2 and its related antioxidant genes and suppresses the expression of NLRP3 inflammasome [127]. Chlorogenic acid decreases the MDA level and increases GSH, SOD, and catalase levels in liver tissues. In HSC-T6 cells, platelet-derived growth factor induces ROS production, p38 and ERK1/2 phosphorylation, proliferation and pro-fibrotic genes expression, which were reversed by chlorogenic acid treatment (12.5, 25, and 50 µg/mL) [126]. Hence, chlorogenic acid protects against liver fibrosis by suppressing OS.

Salidroside is a glucoside of tyrosol found from *Rhodiola rosea* L. (Crassulaceae). In high-fat and high-cholesterol-fed NASH rat model, salidroside treatment (150 and 300 mg/kg body weight, oral administration daily for weeks) effectively reduces lipid accumulation and inhibits liver injury in a dose-dependent manner [128]. Salidroside treatment restores the antioxidant enzyme levels and inhibits the expression of CYP2E1 and Nox2 mRNA in the liver, which prevents the initial step of free radical generation from NASH.

Cinnamaldehyde is a polyphenol possessing anti-inflammatory and antioxidant properties. In CCl_4-induced liver injury rats, daily oral administration of 10 mg/kg cinnamaldehyde for 6 consecutive days significantly reverses the CCl_4-induced elevation in serum ALT, AST, and lactate dehydrogenase activities [129]. Furthermore, cinnamaldehyde significantly reduces CCl_4-induced OS and inflammation mediating through toll-like receptor-4 (TLR-4) signaling pathway, as well as the expression of downstream transcription factors such as NF-κB. The protective effect of cinnamaldehyde is comparable to that of the positive control silymarin (100 mg/kg body weight, oral administration). Hence, cinnamaldehyde is a promising candidate for hepatoprotective therapy.

Apocynin, also known as acetovanillone, is a natural organic compound structurally related to vanillin. Female Long Evans rats were administered with CCl_4 orally (1 mL/kg) twice a week for 2 wk and were treated with apocynin (100 mg/kg, orally) daily for 2 wk. Apocynin significantly reduces serum AST, ALT, and ALP activities and inhibits OS markers (MDA and NO levels) in CCl_4 treated rats [130]. Apocynin treatment also restores the catalase and SOD activity in CCl_4 treated rats. Hence, apocynin protects liver damage induced by CCl_4 by inhibiting lipid peroxidation and stimulating the cellular antioxidant system.

Cichoric acid is a hydroxycinnamic acid, an organic compound of the phenylpropanoid class, which occurs in a variety of plant species. The oral administration of chicoric acid (10 and 30 mg/kg) daily for 4 wk reduces the OS by upregulating antioxidant enzymes and decreases the inflammation by inhibiting pro-inflammatory cytokines and NF-κB activation in mice fed with a methionine- and choline-deficient diet [131]. In addition, chicoric acid reduces the fibrosis, apoptosis, and lipogenesis-related gene expression and increases the AMPK activation. Chicoric acid may be effective in the treatment of NAFLD and NASH.

Rhein is a lipophilic anthraquinone derivative found in *Rheum palmatum* L. (Polygonaceae), which has been used as traditional herbal medicine in China for thousands of years [132, 133]. Rhein has various pharmacological effects including anti-inflammatory, antioxidant, and antimicrobial activities [132, 133]. The potential protective effects of rhein were investigated on APAP-induced hepatotoxicity in rats [134]. The results show a reduction in ROS production, NO level, and MDA level and the restoration of GSH content by oral rhein administration (10, 20, and 40 mg/kg body weigh daily for 2 d) in APAP-induced liver injured rat [134]. The anti-fibrotic effect of rhein might be connected to its property in ameliorating OS.

Sauchinone is a lignan found in *Saururus chinensis* (Lour.) Baill. (Saururaceae). Sauchinone (10 and 30 µM) blocks the TGF-β1-induced phosphorylation of Smad2/3 as well as the transcript levels of PAI-1 and MMP-2 in LX-2 cells. Sauchinone (10 and 20 mg/kg body weight, oral administration 4 times per week for 4 wk) significantly inhibits liver fibrosis in CCl4-injured mice, as indicated by the decrease in the regions of hepatic degeneration, inflammatory cell infiltration and the intensity of α-SMA staining in mice. Furthermore, sauchinone inhibits OS, as assessed by the staining of 4-hydroxynonenal and nitrotyrosine [135]. Sauchinone attenuates liver fibrosis and HSCs activation, which might be mediated by suppressing OS.

Osthole is an active component present in many medicinal plants especially in the fruits of *Cnidium monnieri* L. Cusson (Apiaceae), which has been clinically applied due to its various pharmacological properties, such as antioxidation and anti-inflammation [136]. In TAA-treated Sprague-Dawley rats, the oral administration of 10 mg/kg osthole twice per day for 4 wk significantly re-

duced liver injury by diminishing the plasma AST and ALT levels, improving the histological architecture, decreasing the collagen and α-SMA accumulation, and improving hepatic fibrosis scores. In HSCs (HSC-T6 and LX-2), osthole (3 and 10 µg/mL) reduces the expression of fibrosis-related genes, suppresses the production of fibrosis-related cytokines and chemokines, attenuates the TGF-β1-induced migration and invasion in HSCs, and alleviates the TGF-β1- or endothelin-1-induced HSCs contractility [137].

Clinical Trials

With the growing demands for a safe, effective, and economic treatment of liver fibrosis, an increasing number of researchers focused their studies in the research and development of agents against liver fibrosis in recent years. By searching the database of clinical registration in the United States (https://ClinicalTrials.gov/) by using "liver fibrosis" as the keyword, several natural compounds have been involved in clinical trials, including silymarin, glycyrrhizin, curcumin, and resveratrol. The single- and multiple-dose pharmacokinetics of silymarin were examined in patients with NAFLD or HCV to determine whether the disposition of silymarin and therefore its potential efficacy vary among liver disease populations. The efficacy of silymarin may be more readily observed in NAFLD patients because of their higher flavonolignan plasma concentrations and more extensive enterohepatic cycling compared with those in HCV patients [138]. A randomized, double-blind, placebo-controlled trial (NCT02006498) was performed on consecutive adults with biopsy-proven NASH and a NAFLD activity score (NAS) of 4 or more to verify the efficacy of silymarin in treating NASH. Patients were randomly assigned to groups given silymarin (700 mg) or placebo 3 times daily. After 48 wk, a significantly higher proportion of patients in the silymarin group had reductions in fibrosis based on histology and liver stiffness measurements. The silymarin group also had significant reductions in mean AST-to-platelet ratio index, fibrosis-4 score, and NAFLD fibrosis score [139]. Additionally, silymarin has been involved in a phase II clinical trial for noncirrhotic patients with nonalcoholic steatohepatitis (NCT00680407). Taken together, silymarin has been validated as a potential anti-fibrotic agent, but a larger trial is needed to confirm the clinical application of silymarin to reduce liver fibrosis.

A clinical trial was carried out to determine the addition of glycyrrhizin to entecavir in the treatment of chronic HBV (hepatitis B virus) and advanced fibrosis or cirrhosis (NCT03349008), which has not reported its results.

A clinical trial was performed to evaluate the effects of curcumin supplement on the metabolic factors and hepatic fibrosis in NAFLD patients with type 2 diabetes (NCT02908152); however, the results were not reported.

In a randomized, double-blinded, controlled clinical trial (NCT02030977), 50 NAFLD patients were supplemented with either a 500-mg resveratrol capsule or a placebo capsule for 12 wk. Both groups were advised to follow an energy-balanced diet and received physical activity recommendations. In both groups, anthropometric measurements (weight, body mass index, and waist circumference), liver enzymes, and steatosis grade were improved ($p < 005$). Resveratrol supplementation was associated with a significant reduction in liver enzyme ALT, inflammatory cytokines, NF-κB activity, serum cytokeratin-18, and hepatic steatosis grade, as compared with placebo supplementation. For the treatment of NAFLD, 12 wk of supplementation of 500 mg resveratrol, along with lifestyle modification, is superior to lifestyle modification alone [140]. In another clinical trial (NCT01446276), a long-term (6 mo) and high-dose (500 mg 3 times daily) resveratrol treatment did not improve either basal or insulin-mediated VLDL-TG (very-low-density lipoprotein-triglycerides) secretion, oxidation, or clearance rates, nor did it affect palmitate or glucose turnover in nondiabetic, upper-body obese men with NAFLD. Likewise, no changes in body composition or liver fat content occurred following resveratrol compared with placebo treatment [141]. Therefore, more studies are needed to confirm the clinical application of resveratrol in treatment of liver fibrosis.

A Chinese herbal formula Fuzheng Huayu (NCT00854087) has completed the phase II clinical trial. In China, 2 products derived from traditional Chinese herbal medicine Fufang Biejia Ruangan Tablet (NCT01965418) and Fuzheng Huayu Tablet combined with Huangqi Decoction Granule (NCT00540397) have been involved in clinical trials. These agents target the treatment of liver fibrosis, NASH, HCV/HBV infections, NAFLD, or cholestasis. Some natural compounds and herbal medicine formula have been authenticated as anti-fibrotic agents in clinical trials, but further studies should be performed with a large trial.

Conclusion and Perspective

The authors retraced the panorama of liver fibrosis and the role of OS in the development of liver fibrosis. OS, a critical factor that mediates inflammatory response and triggers HSC activation, is a potential therapy target for treating fibrotic liver diseases. Naturally occurring antioxidants have been verified as potential therapeutic agents to balance the intracellular redox state. Plenty of evidence, both basic and clinical, provides a bright perspective on the antioxidative strategy for the treatment and prevention of liver fibrosis.

However, the clinical application of antioxidants in treatment of liver fibrosis is still far away.

1. Redox balance plays a key role in many physiological and pathological processes, and the liver is a central organ for metabolism. Thus, the determination of dosage, period, and route of administration of antioxidants in treating liver fibrosis involves a challenging translational research.
2. Most of the compounds possess a variety of pharmacological activities, indicating that the mechanisms for the treatment of liver fibrosis by antioxidants might partly be attributed to the antioxidative ability. Other mechanisms should also be considered. Natural compounds are supplied in high dosage in cell and animal experiments, and most compounds did not show a dose-effect manner for treating liver fibrosis, resulting in low possibility for clinical application.
3. Most of the reviewed antioxidants have limited oral bioavailability due to their hydrophobicity, quick degradation in the gastrointestinal tract, poor permeation through the intestinal membrane, extensive metabolism in the gut, and transport

mediated by efflux pumps [142]. It might yield failed results on clinical trials. Several strategies could be employed to improve the poor oral bioavailability [142]. Micro and nanonization can improve the aqueous solubility and intestinal absorption. The encapsulation of lipophilic compounds into cyclodextrins can enhance the aqueous solubility and stability. Chitosan-based delivery systems can afford gastric stability, enhanced penetration through the intestinal membrane, and protection against intestinal metabolism. The co-administration of metabolic enzyme and P-glycoprotein inhibitors may enhance oral bioavailability. Despite the significant anti-fibrotic effect of naturally occurring antioxidants in animal models, these limitations render them ineffective in humans. More translational studies are needed to evaluate the effective and safe dose, the duration of treatment, and formulation strategy to realize the clinical application of antioxidants in treating liver fibrosis.

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Conflict of Interest

The authors declare no conflict of interest.

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