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Plant derived antioxidants and antifibrotic drugs: past, present and future

Devaraj Ezhilarasan[1], Etienne Sokal[1], Sivanesan Karthikeyan[2], Mustapha Najimi[1*]

[1]*Institut de Recherche Expérimentale et Clinique, Laboratory of Pediatric Hepatology and Cell Therapy, Université Catholique de Louvain, Brussels, Belgium*

[2]*Department of Pharmacology and Environmental Toxicology, Food and Hepatotoxicology Laboratory, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, India*

PEER REVIEW

Peer reviewer

Dr. Shah Alam Khan, Associate Professor of Medicinal Chemistry, Department of Pharmacy, Oman Medical College, Muscat, Sultanate of Oman.
Tel: 00968-97245361 (165)
E-mail: sakhan@omc.edu.om

Comments

The present review is an eye bird view of past, present and future of some selected plant derived antioxidants and antifibrotic drugs. The article provides valuable and scientific information about the beneficial uses and mechanism of actions of these commonly studied phytochemicals.
Details on Page 743

ABSTRACT

Hepatic fibrosis occurs as a wound-healing process after several forms of chronic hepatic injury. Activation and proliferation of hepatic stellate cells play pivotal role in the pathogenesis of hepatic fibrosis. Many researchers, from the therapeutic perspective, have focused their attention on searching for novel agents with inhibitory effects on hepatic stellate cells proliferation and activation to prevent hepatic fibrogenesis and a number of plant derived antioxidants have been tested as anti-fibrogenic agents, they generally suppress proliferation and collagen synthesis. Plants remain an imperative source of novel drugs, novel drug leads and new chemical entities. The plant based drug discovery resulted primarily in the development of antioxidant, anti-cancer and other anti-infectious agents and continues to contribute to the new leads in clinical trials. This review summarizes some of those most important plant derived anti-fibrotic drugs and their beneficial effects on experimentally induced hepatic fibrosis *in vitro* and *in vivo*. The plant derived antioxidant compounds described herein are curcumin, silymarin, silibinin, baicalein, resveratrol, salvianolic acids, tetrandine, quercetin and berberine. Studies from ours and as demonstrated by pervious workers much information has been accumulated over the past two decades through *in vivo* and *in vitro*. In light of those studies, it has been confirmed that plants derived antioxidants, particularly flavanoids, show a significant influence to block hepatic fibrosis regardless of any etiology. This review outlines recent progress in the use of plant derived drugs against experimentally induced liver fibrosis by *in vitro* and *in vivo* studies and summarizes the possible mechanisms anti-fibrotic effects of these compounds.

KEYWORDS
Curcumin, Hepatic stellate cells, Hepatic fibrosis, Antioxidant, Silibinin

1. Introduction

Liver fibrosis is a dynamic and highly integrated molecular, tissue and cellular process that drives the progression of chronic liver diseases towards liver cirrhosis and hepatic failure. Activation and proliferation of hepatic stellate cells (HSCs) play a central role in the pathogenesis of hepatic fibrosis by producing excessive extracellular matrix (ECM) components[1]. Many researchers, from the therapeutic perspective, have focused their attention on searching for novel agents with inhibitory effects on HSCs proliferation and activation to prevent hepatic fibrogenesis and a number of plant derived antioxidants have been tested as anti-fibrogenic agents, they generally suppress proliferation and collagen synthesis.

Plants remain an important source of new drugs, new drug leads and new chemical entities. Plants have been utilized as medicines for thousands of years[2]. In search

*Corresponding author: Dr. Mustapha Najimi, Research Associate, University of Catholique Louvain, Laboratory of Pediatric Hepatology and Cell Therapy, PEDI-Avenue Mounier 52 bte B1.52.03, 1200 Woluwe-Saint-Lambert, Brussels, Belgium.
E-mail: mustaphanajimi@ uclouvain.be

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of better treatment options, many patients have turned to alternative medicines in the hopes of identifying "natural" substances with less toxicity but equal effectiveness. Many compounds in plants, including those found in vegetables, fruits, wine, and green tea exhibit antioxidant activities and are beneficial to human health. The global market for plant-derived drugs was worth an estimated $18 billion in 2005, this figure grew to nearly $19 billion in 2006 and expected more than $26 billion by 2011, at an average annual growth rate of 6.6% between 2006 and 2011[3]. These reports of plant-derived drug market clearly show the inevitable use of herbal plants against various pathological conditions.

Use of herbal drugs in the treatment of liver diseases has a long tradition, especially, in Eastern medicine. Some herbal extracts have yielded molecules, often related to flavonoids, with proven antioxidant, antifibrotic, or anti-carcinogenic properties, including curcumin, silymarin, silibinin, baicalein, resveratrol, salvianolic acids, tetrandrine, quercetin, berberine (Table 1) *etc*., which can serve as primary compounds for the development of specific anti-fibrotic drugs. It was previously reported that antioxidants derived from medicinal plants, could suppress peroxidation and eliminate reactive oxygen species in HSCs, and consequently, reduce or even reverse hepatic fibrosis in treated animals[4].

Table 1

Plant derived antioxidants or antifibrotic compounds.

Botanical name	Plant part(s)	Active compound(s)	Effects	References
Curcuma longa	Stem or roots	Curcumin	Antioxidant	[11]
			Antifibrotic	[7,8,11]
Silybum marianum	Seeds/fruits	Silymarin	Antioxidant	[13]
			Antifibrotic	[14]
	Seeds	Silibinin or silybin	Antioxidant	[18,19]
			Antifibrotic	[18,19]
Scutellariae radix	Roots	Baicalein	Antifibrotic	[23,24]
Veratrum grandiflorum *Polygonum cuspidatum*	Roots	Resveratrol	Antioxidant	[48]
			Antifibrotic	[29,48]
Salvia miltiorrhiza	Roots	Salvianolic acids A & B	Antioxidant	[32,33,35]
			Antifibrotic	[34,35,36]
Stephania tetrandra	Roots	Tetrandrine	Antifibrotic	[43,44]
Berberis aristata *Berberis aquifolium*	Fruits	Berberine	Antioxidant	[52]
			Antifibrotic	[23,52]
Various fruits & vegetables	Leaves/grains	Quercetin	Antioxidant	[45,49]
			Antifibrotic	[45,49]

Much information from the *in vivo* and *in vitro* studies have been accumulated over the past two decades, therefore, in this work a review of the most studied plant derived drugs against hepatic fibrosis was performed. This review outlines progress in the use of plant derived drugs against experimentally induced liver fibrosis by *in vitro* and *in vivo* studies and summarizes the possible mechanisms anti-fibrotic effects of these compounds (Tables 2 and 3).

2. Antioxidant and antifibrotic properties of plant derived antioxidants

2.1. Curcumin

Curcumin is a natural antioxidant derived from turmeric, a spice used in Chinese and Indian cooking and credited with therapeutic properties derived from the rhizomes of *Curcuma longa*[5]. It possesses several functional groups that exhibit antioxidant activity allowing it to modulate redox signaling pathways in cells. It also activates an intracellular antioxidant defense system through its stimulation of nuclear factor-erythroid-2-related factor 2, a transcription factor, which binds to the antioxidant response element in the regulatory region of several genes coding for intracellular antioxidants, cytoprotective and detoxification proteins[6]. Studies were demonstrated that curcumin inhibited hepatic fibrosis in a rodent model by reducing oxidative stress and inhibiting HSCs activation and collagen gene expression[7]. In HSCs, curcumin exerts wide spectrum of beneficial effects such as anti-oxidative, anti-inflammatory, anti-fibrogenic and anti-proliferative effects. Xu *et al*. reported that curcumin inhibited activation of HSCs *in vitro* by reducing cell proliferation[8], inducing apoptosis and suppressing extra cellular matrix gene expression. Furthermore, it suppressed the activation of HSCs as demonstrated by repressing the activity of Nuclear Factor-κB (NF-κB) and reducing messager RNA (mRNA) levels of collagen and fibronectin and α-smooth muscle actin (α-SMA). Several intracellular signaling pathways are modulated by curcumin treatment in HSCs including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinases, activator protein 1, peroxisome proliferator-activated receptor gamma (PPAR-γ) and NF-κB[8,9] that are said to play pivotal role during the activation and proliferation of HSCs and pathogenesis of hepatic fibrosis.

HSC activation coincides with a dramatic reduction in the abundance of PPAR-γ in both *in vivo* and *in vitro*[10], which might play an important role in hepatic fibrogenesis. Studies demonstrated that curcumin induced gene expression of PPAR-γ in activated HSC *in vitro*, leading to the activation of PPAR-γ signaling. Curcumin dramatically induced the expression of PPAR-γ and revived its trans-activating activity in activated HSC *in vitro*. Activation of PPAR-γ contributed to the inhibitory effect of curcumin on activated HSCs proliferation[8]. This drug noticeably increases the level of intracellular glutathione (GSH), which requires activation of PPAR-γ. *De novo* synthesis of GSH by curcumin brings about the interruption of transforming growth factor-β (TGF-β) signaling by suppressing the gene expression of TGF-β receptors, resulting in inhibition of connective tissue growth factor gene expression. These effects were said ultimately lead to a reduction in the production of ECM, in activated HSCs *in vitro*[11]. Thus, the anti proliferative effect of curcumin may be beneficial to inhibit the proliferation and activation of HSCs, which is perquisite to hepatic fibrosis. The above studies were concretely proved curcumin undoubtedly have antioxidant and anti-fibrogenic properties of curcumin by virtue of its suppression of HSCs activation, proliferation, lipid peroxidation and induction of PPAR-γ receptor activation lead to anti-fibrotic effect. Despite its wide spectrum of beneficial effects and substantial safety, clinical applications of this molecule for hepatic fibrosis have been largely limited to case series or small clinical trials. The poor bioavailability of curcumin is likely the major hurdle for its more widespread use in humans.

Table 2

Summery of antifibrotic studies performed by using plant derived drugs *in vivo*.

Drugs	Drug doses used	Toxicant /method	Animals	Antifibrotic targets/results	Reference
Curcumin ($C_{21}H_{20}O_6$)	200 & 400 mg/kg	CCl_4	SDR	Induction of PPIA-γ Inhibition of PDGF, TGF β, collagen 1A1 Suppression of TNF-α, Interferon-γ, IL-6 Increased GSH, decreased lipid peroxidation	[7]
Silymarin ($C_{25}H_{22}O_{10}$)	50 mg/kg	BDL	WR	Suppression of procollagen type I, TIMP-I, TGF-β mRNA	[13]
	25 & 50 mg/kg			Decreased collagen content and histological fibrosis score	[14]
Silibinin ($C_{25}H_{22}O_{10}$)	100 mg/kg	DMN	WR	Inhibited protein synthesis, attenuated the Hyp and collagen content in the liver	[19]
Baicalein ($C_{15}H_{10}O_5$)	20, 40 & 80 mg/kg	CCl_4	SDR	Decreased hyaluronic acid, laminin, procollagen type III in serum, hydroxyproline and MMPs in liver Inhibited protein synthesis of PDGF-β receptor	[23]
Resveratrol ($C_{14}H_{22}O_3$)	10 mg/kg	CCl_4	WR	Decreased collagen, TGF β1 and NF-κB	[29]
	10 mg/kg	DMN	SDR	Decreased hydroxyproline level, reduction in type I collagen, TGF β1, α-SMA mRNA expression	[4]
Tetrandrine ($C_{38}H_{42}N_2O_6$)	1 & 5 mg/kg	DMN	SDR	α-SMA and NF-κB-positive cells were decreased in the fibrotic liver, reduced hepatic collagen content, attenuated mRNA expression of ICAM-1, α-SMA and TGF-β 1	[42,43]
	5, 10 & 20 mg/kg	BDL		Decreased α-SMA protein expression and reduced Hyp level, suppressed the expression of TIMP-1 and collagen	[42,44]
Salvianolic acids (Sal A) ($C_{26}H_{22}O_{10}$)	Sal A 10 mg/kg Sal B 50 mg/kg	TAA	SDR	Reduction in collagen, TNF-α , IL-6, IL-1β, fibrosis scores, α-SMA expression	[35]
	Sal B 10 & 20 mg/kg	CCl_4	SDR	Decreased hyaluronic acid, procollagen III peptide, laminin and type IV collagen, decreased expression of NF-κB in nucleolus and increased expression of NF-κB and IκBα in cytoplasm	[34]
(Sal B) ($C_{36}H_{30}O_{16}$)	Sal B 10 mg/kg	DMN	WR	Reduced expression of angiotensin II signaling (angiotensin type 1 receptor; AT1R) and inhibited ERK activation	[36]
Quercetin ($C_{15}H_{10}O_7$)	75, 150 & 300 μmol/kg	BDL		Reduced hepatic collagen content and oxidative stress	[45]
Berberine ($C_{20}H_{18}NO_4^+$)	100, 200 & 400 mg/kg	CCl_4	Mice	Decreased α-SMA positive cells in liver	[53]
	50, 100 & 200 mg/kg	CCl_4	WR	Decreased Hyp level, reduced expression of α-SMA and TGF	[52]

CCl_4: carbon tetrachloride; DMN: dimethylnitrosamine; TAA: thioacetamide; BDL: bile duct ligation; SDR: Sprague-Dawley rats; WR: Wistar rats; TIMP-1: tissue inhibitor metalloproteinase 1; Hyp: hydroxyproline.

2.2. Silymarin

Silymarin is a standardized hepatoprotective agent extracted and isolated from the medicinal plant *Silybum marianum*. This extract contains well-defined ingredients including silibinin. Owing to its hepato-tropism and the favorable characteristics under experimental conditions, silymarin has been recommended as a hepatoprotective agent for many types of acute and chronic liver diseases[12]. Silymarin stimulates hepatocyte RNA synthesis, acts as a radical-scavenger, and suppresses HSCs/myofibroblasts proliferation and collagen synthesis *in vitro*. *In vivo* it reduced hepatic collagen accumulation in rat biliary fibrosis secondary to bile duct occlusion even when silymarin treatment was started in an advanced stage of fibrosis[13].

Recently silymarin and verapamil significantly inhibited hepatic fibrosis. The combination of verapamil and silymarin, showed the best protection through their synergistic antifibrotic effect[14]. Moreover, silymarin has shown beneficial effects against several hepatotoxins. It was established that silymarin prevented the hepatic fibrosis and cirrhosis experimentally induced by CCl_4 and serum markers of liver damage and lipid peroxidation were also normalized[15]. When silymarin was given to reverse CCl_4-cirrhosis the results were not positive[16], indicating the fact that silymarin is effective in preventing liver damage but not in reversing a well established cirrhosis. However in a recent clinical trial study, silymarin did not significantly reduce serum transaminases levels more than placebo in participants with chronic hepatitis C virus infection unsuccessfully treated with interferon-based therapy[17].

2.3. Silibinin

Silibinin, a flavonolignane which represents approximately 60% of silymarin, has been identified as the major active agent. A recent study from our laboratory established that silibinin treatment significantly reduced the collagen and hydroxyproline level in the dimethylnitrosamine-induced hepatic fibrosis in rats by virtue of its antioxidant, free radical scavenging and membrane stabilizing properties[18]. Silibinin reduced platelet-derived growth factor (PDGF)-induced DNA synthesis and cell proliferation in human primary HSCs. Silibinin also reduced PDGF-induced cell migration in a dose-dependent fashion. Finally, pre-treatment treatment with (25-50) μmol/L of silibinin significantly reduced the TGF-β-induced *de novo* synthesis of procollagen type I in cell supernatants[19]. Albeit, these results seemed to have some therapeutic effects against experimentally induced hepatic fibrosis, some inconsistent reports were found due to different drug preparation and the vehicle used to dissolve silibinin especially *in vivo*. The bioavailability of silibinin is still a challenging task for the scientists owing to its poor solubility in the water and other biological solvents.

2.4. Baicalein

Scutellariae radix, the root of *Scutellaria baicalensis*

Table 3

Summery of antifibrotic studies performed by using plant derived drugs *in vitro*.

Drugs	Concentration	Duration	Cells/cell line	Results	Reference
Curcumin ($C_{21}H_{20}O_6$)	10, 30, 50 & 100 μmol/L	4, 8, 16 and 24 h	Rat primary HSC	Induction of PPAR-γ receptors expression, decreased cyclin and CDKs protein expression, type I collagen, α-SMA, fibronection mRNA expression	[8]
	20 μmol/L	24 h	Rat primary HSC	Suppressed toll-like receptor-4 leading to inhibition of NF-κB, Suppressed ERK, AKT signaling, Suppressed TGF-β1-induced α-SMA expression and collagen deposition	[9]
Silibinin ($C_{25}H_{22}O_{10}$)	5, 25 & 50 μmol/L	48 h	Human primary HSC	Reduced PDGF-induced DNA synthesis and cell proliferation, also reduced PDGF-induced cell migration in a dose-dependent fashion, inhibition of IκBα phosphorylation and to its capability to inhibit ERK, MEK, and Raf phosphorylation , inhibited HSC proliferation	[18]
Baicalein ($C_{15}H_{10}O_5$)	3, 10, 30 or 100 mmol/L	24 h	Rat primay HSC	Inhibits PDGF induced HSC proliferation and DNA synthesis	[24]
Salvianolic acids (Sal A) $C_{26}H_{22}O_{10}$)	Sal A (5, 10 & 20 μmol/L) or B (100, 200 & 400 μmol/L)	15 min (pre-treatment)	HSC-T6	Sal A (10 μmol/L) and Sal B (200 μmol/L) treatment attenuated PDGF increased accumulation of H_2O_2 and reduced the PDGF-stimulated expressions of α-SMA	[35]
(Sal B) ($C_{36}H_{30}O_{16}$)	Sal B (10^{-5} mol/L)	4 h (pre-treatment)	Rat primary HSC and HSC-T6	Inhibited ang II-stimulated HSC activation including cell proliferation and expression of type I collagen, α-SMA production, reduced the gene expression of TGF-β1 and downregulated AT1R expression and ERK and c-Jun phosphorylation	[36]
Resveratrol ($C_{14}H_{22}O_3$)	100 μmol/L	24 or 48 h	Rat primay HSC	Inhibited HSC proliferation, expression of α-SMA was suppressed by a high dose of resveratrol, suppressed, tyrosine phosphorylation, and mitogen-activated protein (MAP) kinase activation in PDGF-BB-stimulated stellate cells, reduced cyclin D levels	[48]
Tetrandine ($C_{38}H_{42}N_2O_6$)	5 & 10 μg/mL	24 h	Rat primary HSC	Reduced DNA synthesis of HSC without affecting its viability and α-SMA expression	[42]
	0.5-5.0 μmol/L	24 h	HSC-T6	Concentration-dependently inhibited NF-κB transcriptional activity induced by TNF-α, including IκBα phosphorylation and mRNA expressions of ICAM-1, also inhibited TGF-β 1-induced α-SMA secretion and collagen deposition	[43]
Quercetin ($C_{15}H_{10}O_7$)	10 or 50 μmol/L	24 or 48 h	Rat primay HSC	Inhibited HSC proliferation, expression of α-SMA was suppressed by a high dose of resveratrol, suppressed, tyrosine phosphorylation, and mitogen-activated protein (MAP) kinase activation in PDGF-BB-stimulated stellate cells, reduced cyclin D levels	[48]
Berberine ($C_{20}H_{18}NO_4^+$)	0, 1, 2.5, 5, 10, or 20 μg/mL	48 h	Rat HSC (CFSC) cell line	Inhibited CFSC proliferation and induced cell cycle arrest in G1 phase, decreased Akt phosphorylation and FoxO1 phosphorylation	[53]

Georgi, is a conventional herbal medicine, and is widely used for traditional herbal preparations in Japan and China. In Japan, Sho-saiko-to, a traditional herbal preparation containing Scutellariae radix is commonly used to treat patients with chronic hepatitis as an approved prescription drug. The anti-fibrogenic effects of Sho-saiko-to have been demonstrated in experimental hepatic fibrosis and carcinoma. It significantly inhibits hepatic fibrosis by inhibiting the activation HSCs, inhibiting hepatic lipid peroxidation, promoting matrix degradation, and suppressing ECM accumulation[20]. Baicalein is flavonoid extracted from roots of a medicinal plant, *Scutellaria baicalensis* (or Huang Qin in Chinese). It contains a variety of flavones, amino acids and essentials oils. More than 30 flavonoids are found in its dried root, including baicalein (major component), baicalin, wogonin and wogonin-7-O-glucuronide. Among them, baicalein is one of the main active ingredients[21]. The suppressive effects of Sho-saiko-to on proliferation and collagen synthesis were shown in cultured HSCs. Sho-saiko-to arrested HSCs at G0/G1 phase and inhibited cell proliferation[22].

Baicalein has an anti-fibrotic effect in the rat liver. Recently, it has been reported that its administration suppressed protein synthesis of PDGF-β receptor and reduced experimental fibrosis. Baicalein also reduced hydroxyproline levels, which could reduce the availability of hydroxyproline for collagen synthesis in CCl_4 induced-hepatic fibrosis in rats[23]. Inoue and Jackson have reported that baicalein can inhibit HSCs proliferation[24], DNA synthesis and protein synthesis in HSC. Further they have reported that baicalein has strong anti-proliferative effect than retinol and quercetin, which has the same basic chemical structure as baicalein and suggested that this suppressive effect of baicalein on protein synthesis might have been contributed to the anti-fibrogenic effect of Sho-saiko-to in hepatic fibrosis[24]. These studies confirm that baicalein can effectively inhibit the liver fibrosis *in vivo* by down regulating the hydroxyproline, collagen and protein synthesis of PDGF-β receptor and *in vitro* by inhibiting the proliferation, protein synthesis, DNA synthesis of cultured HSCs. However, further studies are warranted for baicalein as a potential treatment in a clinical setting.

2.5. Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) was first isolated from the roots of white hellebore (*Veratrum grandiflorum* O. Loes) in 1940, and later, in 1963, from the roots of *Polygonum cuspidatum*, a plant used in traditional Chinese and Japanese medicine. Initially characterized as a natural phytoalexin present in grapes, peanuts and red wine, has various pharmacological effects including anti-inflammatory and antioxidant properties, modulation of lipid metabolism and prevention of cancer[25,26].

NF-κB plays an important role in HSC activation and fibrogenesis. Critically, NF-κB is constitutively active in

pro-fibrogenic liver myofibroblasts due to an excess of Rel factors via an epigenetic down-regulation of the inhibitor of NF-κB (IκB) protein[27]. Currently, NF-κB is a potential target for anti-fibrotic strategies in liver fibrosis therapy. Importantly, it has been reported that resveratrol inhibits the activation of NF-κB[28]. Resveratrol completely diminished the levels of activation of NF-κB and production of TGF-β on in rat model of CCl_4 induced-hepatic fibrosis and reported this effect was accompanied by prevention of the translocation of NF-κB to the nucleus and attenuation of the pro-fibrotic cytokine TGF-β[29]. Furthermore, Chávez *et al*. recommended from the results of their study that resveratrol is an excellent anti-fibrogenic drug and the protective effects of the compound was more associated with its ability to reduce the pro-fibrogenic cytokine TGF-β might have been by inactivating NF-κB, than to its antioxidant properties[29]. Nonetheless, studies regarding the usefulness of resveratrol are scanty. Hence, further studies are needed to confirm the anti-fibrogenic efficacies of resveratrol[29].

2.6. Salvianolic acids

Salvianolic acids A (Sal A) and B (Sal B), two major phenolic compounds extracted from *Salvia miltiorrhiza*, which is a common herbal medicine that has been clinically used in China for more than one thousand years as a blood-circulation accelerating agent and antioxidant[30] and have been reported to exert free radical scavenging and anti-peroxidative effects[31]. It has been demonstrated that Sal A and Sal B inhibited the proliferation and activation of HSCs, respectively[32,33]. Sal B has been proved capable of inhibiting hepatic fibrosis and reversing chronic hepatitis B by double-blind double-dummy clinical follow-up observations, and was suggested a very promising clinic drug for anti-hepatic fibrosis[30].

The anti-fibrotic effect of Sal B is associated with its ability to up-regulation the expression of NF-κB in the nucleolus and to down-regulation the expression of NF-κB and IκB in the cytoplasm CCl_4 induced-hepatic fibrosis in rats[34]. In other *in vitro* studies, it is interesting to note that Sal A (10 μmol/L) was about 20 times more potent than Sal B (200 μmol/L) in ameliorating PDGF stimulated oxidative stress in HSCs. Moreover, Sal A and B treatment exerted antifibrotic effects through amelioration of thioacetamide and PDGF induced oxidative stress in rat liver and human HSCs respectively, resulting in hepato-protection and reduction of hepatic fibrosis[35].

Recently, Li *et al*. reported different antifibrotic target in which Sal B, attenuated animal liver fibrosis and inhibited Angotensin II (Ang-II) induced HSC activation[36]. The down regulation of Ang-II signaling such as decreasing Ang-II receptor type 1 (AT1R) expression and ERK and c-Jun phosphorylation, and the inhibition of TGF-β expression stimulated by Ang-II is important action mechanism for Sal B against HSC activation and liver fibrosis[36]. This work has been supported that the effect of Sal B relates to Ang-II signal transduction in HSC activation and may provide a new insight to understand the anti-hepatic fibrosis effect of Sal B and the results from these studies suggest that Sal B is a promising drug candidate than Sal A in the treatment of hepatic fibrosis.

2.7. Tetrandrine

Tetrandrine, a bisbenzylisoquinoline alkaloid isolated from the root of *Stephania tetrandra* (*S. tetrandra*), is the major pharmacologically active component of *S. tetrandra*. The root of *S. tetrandra* has been used for the treatment of edema and arthritis in traditional Chinese medicine since antiquity[37]. It has been shown to possess a potently protective effect against the development of liver fibrosis[38].

It has been revealed that tetrandrine at least partially exerted its anti-fibrogenic effects by a direct inhibiting effect on HSCs. The mechanism by which tetrandrine inhibits rat HSCs activation is not clear. Several studies reported that tetrandrine administration has been shown to block the calcium channels in various cell types[39,40]. Other investigators found that these voltage-operated calcium channel plays an important role in the regulation of HSCs contractility which is the marked phenotype of activated HSCs[41]. Hence, in light of these reports, Park *et al*. suggested that inhibitory effect of tetrandrine on HSCs activation, at least in part, through the blocking of calcium channels[42].

The other studies show that (1 and 5 mg/kg) tetrandrine treatment, exerts anti-fibrotic effects in dimethylnitrosamine intoxicated rats and *in vitro* tetrandrine treatment (0.5-5.0 μmol/L) exerted inhibitory effects on HSC-T6 cells. Tetrandrine significantly reduced Iκα expression and TNF-α induced NF-κB activity, Iκα phosphorylation and NF-κB nuclear translocation in HSC-T6 cells, reduced the NF-κB activity stimulated by tumor necrosis factor-α (TNF-α) and hydrogen peroxide in HSC-T6 cells, and also reduced the mRNA expression of the NF-κB responsive gene, intra cellular adhesion molecule-1, induced by TNF-α, tetrandrine concentration dependently attenuated TGF-β1 stimulated α-SMA secretion and collagen deposition in HSC-T6 cells[43]. Along this line Lee *et al*. reported tetrandrine treatment prevented liver fibrosis in bile duct ligated rats by suppressing the expression of TIMP-1 mRNA of HSCs in fibrogenesis, resulting in reduced expression of collagen lead to promotion of matrix degradation[44]. However, further studies are needed to understand the inhibition mechanism of the tetrandrine.

2.8. Quercetin

Quercetin, the most abundant flavonoid in nature, presents in large amounts in vegetable, fruits, tea, and olive oil, and because it contains a number of phenolic hydroxyl groups, it exhibits its therapeutic potential against many diseases, including ischemic heart diseases, atherosclerosis, liver fibrosis, renal injury, and chronically biliary obstruction[45-47]. Quercetin and other natural phenolics inhibit HSCs activation by perturbing signal transduction pathway and cell protein expression[48]. It has been found that both collagen deposition and production of malondialdehyde are reduced in parallel by administration of quercetin to bile duct-ligated rats[45]. Administration of quercetin against CCl_4

induced cirrhosis in mice significantly increases antioxidant defense and prevents oxidative damage[49]. Moreover, quercetin also increased gene expression and functional activity of antioxidant enzymes superoxide dismutase and catalase, and it significantly reduced the pro-inflammatory cytokines expression and NF-κB activation were also inhibited[50].

Several papers were emphasized that the mechanism of action of quercetin antifibrotic effect in experimentally induced liver fibrosis was due its immense antioxidant, scavenging of free radical and membrane stabilizing properties. However further studies are needed to clarify the exact mechanism of action of quercetin on its therapeutic effects.

2.9. Berberine

Berberine is an isoquinoline alkaloid present in a number of important medicinal plant species, such as *Berberis aristata* and *Berberis aquifolium*. Studies have clearly demonstrated that berberine exerted anti-fibrotic effects possibly through activation of AMP-activated protein kinase, blocking NADPH oxidase 4 and Akt expression and reduction in the expression of α-SMA[51]. The effect of berberine on rat liver fibrosis induced by multiple hepatotoxic factors, including CCl_4, ethanol, and high cholesterol, demonstrated that berberine could prevent experimental liver fibrosis by regulating the anti-oxidant system and lipid peroxidation[52].

Sun *et al*. demonstrated that berberine directly inhibits the proliferation of CFSC cells by increasing p21 and p27 expression and inducing G1 arrest, which may be related to regulating Akt/FoxO1 signaling pathway[53]. Moreover, berberine dramatically decreased the number of activated HSCs, the amount of fibrotic septa, and the content of hepatic collagen prevents CCl_4 induced experimental liver fibrosis. Berberine treatment also caused the decrease in the secreation of TGF-β1 and inhibits HSC activation[52]. Further clinical investigations would be necessary to determine whether berberine could be effective for the prevention of liver fibrosis in humans.

In the past two decades, several thousand papers have been published particularly on these substances, used as antioxidants, anti-fibrotic agents and especially as hepatoprotective agents. This publication volume indicates scientific interest in these plant derived drugs. The data reported in this review clearly indicate the increasing interest on these drugs as well as the continuous improvement in knowledge about the molecular actions of these substances. As reported in this review, plant derived drugs such as curcumin, silymarin, silibinin, baicalein, resveratrol, salvianolic acids, tetrandrine, quercetin, berberine *etc*., have been extensively studied against various drug and toxicant induced hepatic fibrosis *in vitro* or *in vivo*. Each of these compounds has their own specific characteristics and mechanism of actions, with an intrinsic beneficial effect. This review revealed that these plant derived drugs were inevitable source for the researchers and they have undoubtedly promising beneficial effects. Owing to their poor bioavailability, these drugs are showing different results in experimental research and clinical trials. Hence, future studies are warranted to improve the bioavailability of these drugs and thereby researchers could come out with better treatment strategy.

3. Conclusion

This review summarizes some of the most important plant derived anti-fibrotic drugs and their beneficial effects on experimentally induced hepatic fibrosis *in vitro* and *in vivo*. Much information regarding the development of anti-fibrotic drugs from plant source have been accumulated over past two decades through *in vivo* and *in vitro* studies. In light of these studies, it has been confirmed that plants derived antioxidants, particularly flavanoids, show a significant influence to block hepatic fibrosis regardless of any etiology. Since the few plant derived compounds were studied so far which have just opened a wide perspective and the need of the hour is worth to screen other medicinal plant extracts and their natural compounds in appropriate model system either by *in vivo* or *in vitro*. There is an urgent need for further scientific assessment of the potential benefits and dangers of the huge range of herbal medications available. Such studies may lead to the development of new drugs particularly suited and specifically tailored to block hepatic fibrosis by (i) inhibition of the HSC proliferation by either cell cycle arrest or via inducing cellular senescence, (ii) clearance of activated and proliferated HSCs by induction of apoptosis or cell death and (iii) promoting the degradation of the extracellular matrix.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Liver plays an important role in the metabolism and detoxification of xenobitotics. However, chronic hepatic injury caused by activation and proliferation of hepatic stellate cells may lead to the development of hepatic fibrosis. Modern medicine available to prevent or treat hepatic fibrosis are themselves associated with liver toxicity, therefore, there is a need to search and explore plant derived antioxidants especially flavonoids as potential candidate for development of new drugs for this disease.

Research frontiers

This review article outlines recent progress made in the use of plant derived phytochemicals such as curcumin, silymarin, silibinin, baicalein, resveratrol, salvianolic acids, tetrandine, quercetin and berberine against experimentally induced liver fibrosis by *in vitro* and *in vivo* studies and their possible mechanism of action. The review article is short, concise and informative.

Related reports

This review is a compilation of *in vivo* and *in vitro* research studies conducted in the past two decades on the plant derived antioxidants to evaluate their role in the prevention of hepatic fibrosis. The reported phytochemicals have been extensively studied and could pave the way for the development of promising antifibrotic drugs.

Innovations and breakthroughs

The data reported in this review article highlights the recent updates on plant derived natural drugs such as curcumin, silymarin, silibinin, baicalein, resveratrol, salvianolic acids, tetrandine, quercetin, berberine *etc.* in the prevention of hepatic fibrosis. This review will help the researcher across the globe in improving their knowledge and understanding about the beneficial uses as well as molecular actions of these natural plant products.

Applications

Natural plant products such as silymarin, quercetin, curcumin *etc.* are well known antioxidant and hepatoprotective agents. These compounds could provide a lead molecule for the development of antifibrotic drugs.

Peer review

The present review is an eye bird view of past, present and future of some selected plant derived antioxidants and antifibrotic drugs. The article provides valuable and scientific information about the beneficial uses and mechanism of actions of these commonly studied phytochemicals.

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