REVIEW

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Protective and therapeutic possibility of medical herbs for liver cirrhosis

VESNA STANKOVIĆ[1], VLADIMIR MIHAILOVIĆ[2], SLOBODANKA MITROVIĆ[1], VLADIMIR JURIŠIĆ[3]

[1]*Institute of Pathology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia*

[2]*Department of Chemistry, Faculty of Science, University of Kragujevac, Kragujevac, Serbia*

[3]*Institute of Pathophysiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia*

Abstract

Liver damage is a serious medical problem worldwide and is caused by primary or secondary metabolic, microbiological, toxicological, immunological and circulatory etiological factors. The objective of this paper is to analyze the hepatoprotective effect of various plants, their biologically active compounds and extracts and the possibility of these compounds to attenuate complex pathophysiology processes during chronic inflammation and the development of liver cirrhosis. This review summarizes several plants whose hepatoprotective effects have been demonstrated and partially describes the mechanisms of inflammation inhibition. It is known that fibrosis includes an oxidative damage, inflammatory and immune response and star-shaped cells and their activation in hepatocytes. Effects of particular phytocompounds and their anti-inflammatory mechanisms have been studied in several cell lines *in vitro*, *in vivo* in different animal models, as well as in some clinical studies. Results suggest that mechanisms include reduction of oxidative stress, suppression of the inflammatory and immune response, as well as the inhibition of the activation of hepatic stellate cells (HSCs), decreasing extracellular matrix (ECM) deposition and induction of apoptosis, protection of hepatocytes from apoptosis and creating apoptotic bodies, which are phagocytic and activate HSCs. Medical herbs are abundant, economical and versatile and thus are potential alternative agents with anti-inflammatory mechanism. They can be a source of bioactive compounds, and with the aim of preventing the formation and progression of fibrosis, they can find wide applications in medical practice. The obtained results should promote further research in order to identify safe and effective protective and therapeutic resources.

Keywords: liver fibrosis, hepatoprotective plants, herbal medicine, hepatic stellate cells, apoptosis, extracellular matrix.

Introduction

Natural products and medical herbs, as conventional or complementary medicines in the prevention or treatment of various liver diseases, have been used in many countries since ancient times. Currently used medicines for liver diseases have been isolated from plants or represent synthetically modified forms of natural products [1]. There are numerous studies that confirm the hepatoprotective effects of some plant species, such as milk thistle [*Silybum marianum* (L.) Gaertn.], turmeric (*Curcuma longa* L.) and liquorice (licorice) (*Glycyrrhiza glabra* L.), which have beneficial effects in human [2, 3]. Also, many of the medicines that are currently recommended by physicians for liver disorders are plant formulations based on plant extracts, compounds isolated from plants, a mixture of compounds of different plants or a mixture of plant extracts. Published data point to a large number of medicinal herbs used in traditional medicine in different countries and different regions of the world for the treatment of liver disease as well as information about their hepatoprotective effect of using well-known experimental models of liver disease. In these experimental models, carbon tetrachloride, paracetamol, D-galactosamine, thioacetamide, sodium fluoride, ethanol and bacterial lipopolysaccharides are commonly used hepatotoxic agents that induce liver damage by initiating the process of inflammation and the generation of reactive oxygen species [4, 5]. Testing of the hepatoprotective effects of herbal preparations by using these experimental models allows monitoring of levels of serum enzymes, oxidative stress parameters and histopathological changes in the liver tissue of experimental animals (*in vivo*) or an increase in vitality and survival of cells of a specific cell line (*in vitro*) [5–10].

The aim of this review is to present the hepatoprotective role of some relevant medicinal herbs and biologically important compounds or extracts together with mechanisms by which complex pathogenic processes leading to the development of cirrhosis over the course of chronic damages are inhibited. Antifibrotic effects could be attained by targeting cell lines and different processes of the pathogenesis, depending on the damaging cause. This review includes the plants whose hepatoprotective effects (Figure 1) have been demonstrated in many studies, as well as the mechanism of their hepatoprotective function, including the mechanism of hepatoprotective activity of chemical compounds present in these plants. The following medicinal plants and their bioactive compounds or extracts are included: *Curcuma longa* L. (curcumin), *S. marianum* (L.) Gaertn. (silymarin), *Ginkgo biloba* L. (*G. biloba* extract), *Salvia miltiorrhiza* Bunge (water extract of *S. miltiorrhiza*, salvianolic acids A and B), *G. glabra* L. (glycyrrhizin, glycyrrhizic acid), *Scutellaria baicalensis* Georg (baicalin), *Bupleurum falcatum* L. (saikosaponins A and D), *Phyllanthus* spp. (ethanol extract of *Phyllanthus rheedii* Wight.), *Berberis aristata* DC.

(berberine), *Panax notoginseng* (Burkill) (ginsenosides Rg1 and Rb2), *Andrographis paniculata* (Burm.f.) (andrographolide), *Picrorhiza kurroa* Royle ex Benth (picroside II) and *Coffea* spp. (caffeine).
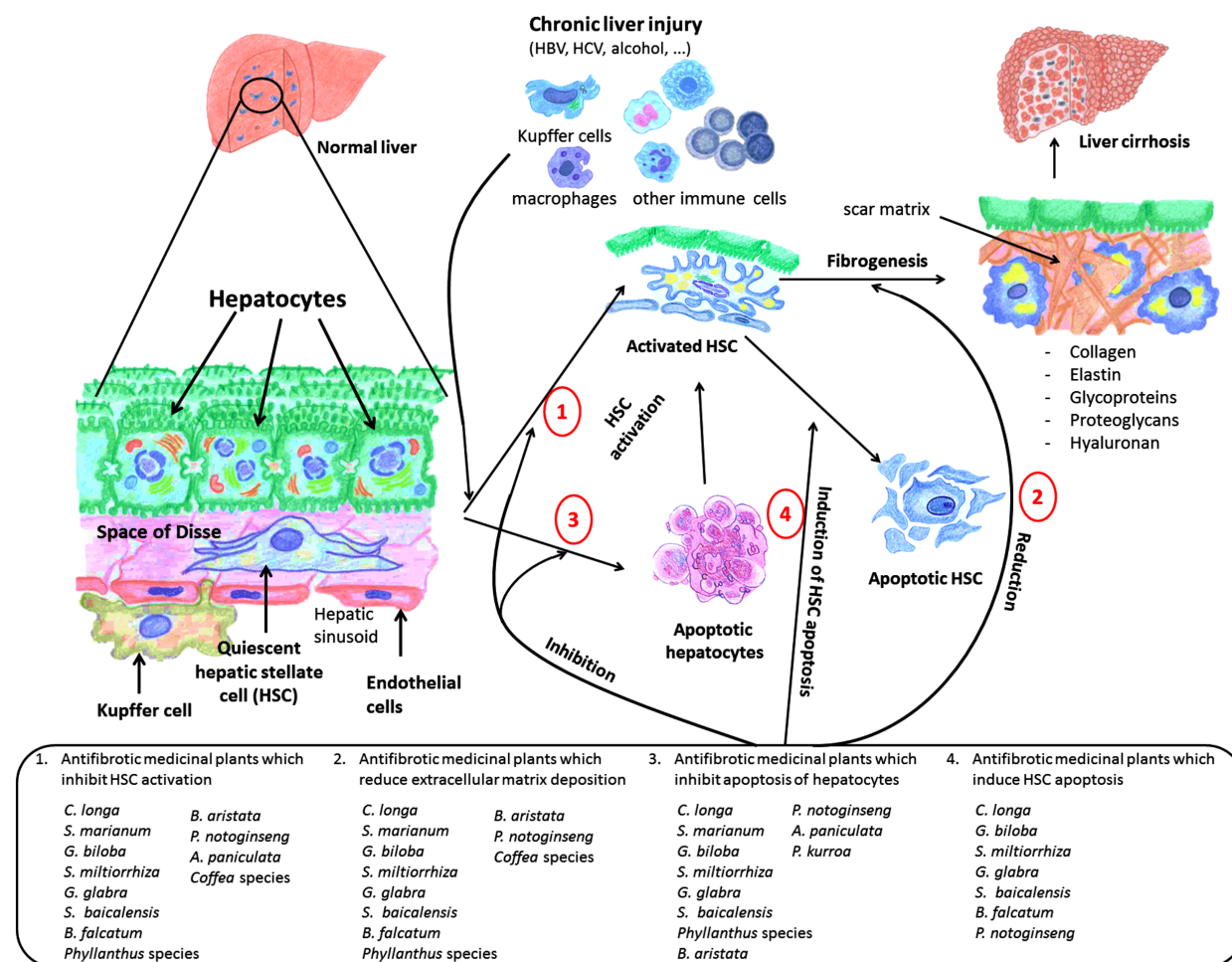

Figure 1 – *Medicinal plants and their mechanism of action in protection and reduction of liver fibrosis.*

Protective mechanisms of medicinal plants in liver fibrosis

Cirrhosis is a progressive, diffuse, irreversible and nodular transformation of the liver parenchyma with the loss of liver function. The main processes that leads to the development of cirrhosis are hepatocyte necrosis, regeneration, progressive, uncontrolled irreversible fibrosis and disorder of intra- and extrahepatic circulation.

Connective tissue proliferation occurs as a response to inflammatory and toxic damage. Fibrosis in the early stages is a dynamic process of synthesis and delay of components of the extracellular matrix (ECM) in Dissse spaces, especially of collagen type I, III, IV, glycoproteins – fibronectin, laminin, elastin and entactin, and undulin and proteoglycans – chondroitin sulphate, heparin sulphate, dermatan sulphate and heparin, with the activation of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [11, 12].

Hepatic stellate cells (HSCs) are localized in perisinusoidal Disse spaces between the basolateral surface of hepatocytes and the anti-luminal side of endothelial cells of the sinusoid [13]. Activated HSCs are the main site of the synthesis of ECM [14], but fibroblasts, hepatocytes and epithelial cells of biliary ductus also contribute [15]. Activation, reactivation, proliferation and prolonged survival of HSCs as well as increased ECM deposition are important steps during the creation of fibrous tissues [16]. Paracrine stimuli from damaged hepatocytes, macrophages, platelets, immune cells and/or activated Kupffer cells trigger this process of activation [17–19]. These activated stellate cells transform into myofibroblasts-like cells, express contractile proteins, synthesize growth factors, chemokines, fibrogenic cytokines, release retinoids and acquire chemotactic features [13–15].

Antifibrotic medicinal plants that inhibit HSCs activation

Transforming growth factor-beta 1 (TGF-β1) is the main cytokine that induces fibrosis generation and is produced by hepatocytes, endothelial, Kupffer cells and the epithelial cells of the bile ducts [12, 20, 21]. Stimuli may lead to an increased production of all matrix components, its remodeling and the induction of new fibrogenic effects [20, 22, 23].

The interaction of TGF-β1 and their membrane receptors induces the phosphorylation of intracellular mediators of Smad proteins that act as transcription factors in the nucleus and trigger cell proliferation [20]. The TGF-β1/ Smad complex is an important profibrogenic pathway. The modulation of expression and interaction of TGF-β1 and their receptors was observed in curcumin (*C. longa*)

[24], and bioactive compounds of *S. miltiorrhiza* [25], *G. glabra* [26, 27] and *Coffea* spp. [28, 29].

During activation, HSC epigenetic regulation of the transcription factor nuclear factor-kappa B (NF-κB) and the change of the expression of peroxisome proliferator-activated receptor gamma (PPARγ) modulate the expression of genes of various types of collagen, alpha-smooth muscle actin (α-SMA) TGF-β, TGF-β receptors, MMP-2 and TIMP-1 and -2 [14, 19]. The inhibition of NF-κB, stimulation by interferon-γ [30], PPARγ ligands [31] and antioxidants inhibit the expression of TGF-β1 and thus the activation of HSC. Suppression of NF-κB and the activation of PPARγ during the application of *G. biloba* extract [32, 33], 18α-glycyrrhizin [34], glycyrrhizic acid [35] and baicalin [36] have an anti-fibrogenic effect.

TGF-β, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), by releasing from damaged endothelial cells, stimulate the activation of HSCs [16, 17] in the same manner as transforming growth factor-alpha (TGF-α), reactive oxygen species (ROS) and lipid peroxides (LPO) released from activated Kupffer cells. Activated Kupffer cells favor cell proliferation, matrix synthesis and MMP-9 by expressing TGF-β1 [16, 37]. Activated Kupffer cells can also influence the proliferation of HSCs and contractility, a decrease in collagen synthesis and an increase in the synthesis of collagenases by the synthesis of anti-inflammatory interleukin-1 (IL-1) cytokine and nitric oxide (NO) [16].

PDGF, EGF, TGF-α, monocyte chemotactic factor (MCF), interleukin-6 (IL-6), connective tissue growth factor (CTGF), endothelin-1, angiotensin-II and vascular endothelial growth factor (VEGF) stimulate the proliferation of collagen synthesized cells, migration of HSCs toward the area of damage and emphasize fibrogenic response [12, 14, 23]. Some herbal extracts achieve their effect by blocking extracellular signal-regulated kinase (ERK) signaling molecules and focal adhesion kinase (FAK), growth factors PDGF, CTGF, VEGF and TGF-α, thus reducing the proliferative response of hepatic stellate cells [19]. Curcumin [38], silymarin [39], *G. biloba* extract [40], *Salvia* extract [25, 41] and caffeine [28, 29] inhibit CTGF through the inhibition of the TGF-β signal. Curcumin [42], salvianolic acids A and B [43, 44] and ginsenoside Rg1 [45] may compromise signaling pathways, which starting interaction of PDGF and platelet-derived growth factor β receptor (PDGF-βR).

Cyclins and cyclin-dependent kinases play a key role in cell cycle regulation, and they present potential therapeutic targets in the inhibition of the proliferation of activated HSCs. A bioactive compound of salvianolic acid (*S. miltiorrhiza*) can inhibit the proliferation of HSC *via* p21 and p27 proteins, which are inhibitors of cyclin-dependent kinases [43].

Endothelin-1, somatostatin, NO and angiotensinogen II potentiate the contractile properties of HSCs by expressing contractile proteins resulting in vascular disorders and the development of portal hypertension [14].

Antifibrotic medicinal plants that reduce ECM deposition

The accumulation of ECM in tissues depends on the balance between their synthesis and degradation [46, 47].

The degradation of collagen and other matrix components is carried out by MMPs, which are produced by fibroblasts, macrophages, Kupffer cells and leukocytes, and inhibited by TIMP [48, 49]. In the initial stage of damage, a transient increase in MMP-3 and MMP-13 is present, and in the advanced stage, the effect of TIMP-1 dominates. Regulation of activation of secreted or inactive precursor collagenase by the system of plasminogen activation and the control of the same *via* urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) is another option of controlling the ECM and the potential effects of bioactive compounds (salvianolic acids A and B, *P. notoginseng* saponins, ginsenoside Rb1) [50, 51].

It has been shown that curcumin, silymarin (a flavonoid mixture consisting of silybin, silydianin and silychristin), *G. biloba* extract, salvianolic acids A and B, glycyrrhizin, glycyrrhetinic acid, baicalin, *P. notoginseng* saponins, ginsenoside Rb1 and caffeine can decrease synthesis of collagen type I and III; *C. longa* can decrease synthesis of fibronectin [52], while *G. biloba* extract and salvianolic acids A and B can decrease synthesis of laminin. The stimulation of MMP-1, -2, -7, -9, and -13 is achieved by the use of curcumin, *G. biloba* extract, salvianolic acids A and B, glycyrrhizin, glycyrrhetinic acid, *P. notoginseng* saponins and ginsenoside Rb1 [53–56], while TIMP is inhibited with the application of curcumin, silymarin, silybinin, silybin, *G. biloba* extract, salvianolic acids A and B, *P. notoginseng* saponins and ginsenoside Rb1. However, *A. paniculata* (andrographolide) showed no effect on the synthesis and degradation of ECM components.

Antifibrotic medicinal plants that induce HSCs apoptosis

Activated HSCs have a prolonged survival. Macrophages in the liver promote the survival of activated HSCs by the activation of NF-κB [57]. NF-κB is a complex protein that controls the transcription of DNA, production of cytokines and survival of cells by the modulation of apoptosis. The inhibition of this pathway leads to selective apoptosis of the HSCs [58] and to the protection of hepatocytes from apoptosis, which may be one of the options for preventing fibrosis [59, 60].

Apoptosis induction correlates with an inhibitory effect on NF-κB [61], gene expression for PPARγ and with the blocking of signaling pathways of TGF-β, PDGF and EGF. It also promotes intracellular adaptive protein FADD (Fas-associated protein with death domain), which, by the engagement of procaspases, activates a signal complex for causing cell death and the control of FLIPs [Fas-associated death domain-like IL-1β-converting enzyme (FLICE)-inhibitory proteins], which are antagonists of caspases [62].

The apoptotic induction of activated HSCs is achieved by increasing the pro-apoptotic Bax and Fas and reducing anti-apoptotic Bcl-2 and Bcl-x1 proteins, freeing cytochrome c, the activation of caspase-8 and caspase-9 and an execution of caspase-3 with the activation of endonucleases for the degradation of DNA.

The most examined option for modulating and possibly to inducing apoptosis of activated HSCs are attributed to the compounds isolated from *C. longa*, *Ginseng* spp. and *S. miltiorrhiza*. However, compounds isolated from

plants *G. biloba* [56], *G. glabra* [34], *B. falcatum* [63], *B. aristata*, *P. kurroa* and *A. paniculata* have almost no effect on apoptosis induction of HSCs.

Curcumin inhibits the effect of Bcl-2 anti-apoptotic proteins, promotes the apoptotic Bax protein [24, 61, 64], induces a release of cytochrome c from mitochondria [62] and activates caspase-3. Compounds tanshinone I and tanshinone IIA from *S. miltiorrhiza* promote apoptosis of HSCs by the expression of Bax and Fas [65] and reducing mitochondrial membrane potential (MMP) [66, 67]. Saponins, as bioactive compounds of *P. notoginseng*, induce apoptosis through tumor necrosis factor-alpha (TNF-α), whose membrane receptor of the intracellular domain binds to the death signaling complex and runs the mitochondrial caspase cascade [68].

Antifibrotic medicinal plants that inhibit apoptosis of hepatocytes

Chronic damage and hepatocyte apoptosis with the phagocytosis of apoptotic bodies by HSCs leads to the activation of HSCs through the Janus kinase (JAK) signal transduction and transcription activation of JAK/STAT (signal transducer and activator of transcription) and protein kinase B (AKT)/NF-κB-dependent pathway, acting thus as a powerful fibrogenic stimulus [69–71]. HSCs cease migration into the zone of apoptotic bodies by binding Toll-like receptor 9 (TLR9) [72].

The most researched cytoprotective effect was observed in experiments that included *C. longa*, *S. miltiorrhiza*, *G. glabra*, *G. biloba* and their chemical constituents that act through the inhibition of the mitochondrial pathway of apoptosis and the reduction of oxidative stress. Curcumin (*C. longa*) and glycyrrhizin (*G. glabra*) achieve their anti-apoptotic effect by reducing the synthesis of ROS, inhibiting LPO [73], reducing NO, increasing the super-oxide dismutase (SOD) enzyme, reducing expression of intercellular adhesion molecule-1 (ICAM-1) [74], inhibiting of cytochrome c and the activation of caspase-3 and regulating the expression of Bcl-2 and tumor necrosis factor receptor 1 (TNFR1) [75]. Protection of hepatocytes can be accomplished by inhibition of the interleukin-18 (IL-18), regardless of the activation of caspase [76] *in vitro* and *in vivo* conditions. The therapeutic strategy is directed toward the protection of hepatocytes from apoptosis and stopping the fibrosis.

The process of fibrosis includes an oxidative stress, inflammatory and immune response, HSCs and their activation, hepatocytes and inflammatory and immune cells [12, 22]. Effects of compounds isolated from medicinal plants and mechanisms for achieving their anti-fibrotic effects were studied by *in vitro* and *in vivo*, with the aim of reducing oxidative stress and suppressing the inflammatory and immune responses. Inhibition of the HSCs activity and induction of apoptosis were also investigated, as well as the effects of excessive ECM deposition, protection of hepatocytes from apoptosis and the creation of apoptotic bodies, which, being phagocytized, activate HSCs. The control of the dynamics of ECM synthesis by inhibiting the activation of HSCs, suppressing and modulating TGF-β1/Smad signaling pathway and its degradation is the therapeutic objective of halting fibrosis and restoring normal structure of the liver.

Clinical studies conducted on herbal preparations for liver disease

Clinical studies involving herbal medicine in the treatment of liver diseases are rare and mainly include silymarin (standardized extract of *S. marianum*), glycyrrhizin (from aqueous extract of *G. glabra*) and herbal medicines as mixtures of different herbal extracts [77, 78].

The mixture of compounds from *S. marianum*, known as silymarin, constitutes of the following: silybin (silybinin, silybinin; approximately 50% to 60%), isosilybin (about 5%), silychristin (about 20%) and silydianin (about 10%), as well as silymonin, isosilychristin, isosilybinin, etc. [79]. One of the first clinical studies with silymarin showed significant differences in survival between patients with liver cirrhosis of different origin who had silymarin treatment and patients with vitamin placebo, where a greater percentage of patients with silymarin therapy for a period of two and four years survived [80]. Similar results were obtained in another study in which patients with liver cirrhosis because of a complication of chronic alcoholism were included [81]. However, both studies had their drawbacks, including high dropout rate, poor adherence to rules and lack of information on infections with hepatitis B and C. Also, these studies did not include histopathological data. A pilot study on the impact of silymarin in patients with hepatitis C showed that there was no reduction in the level hepatitis C virus (HCV)-RNA, but the level of alanine aminotransferase (ALT) in patients who were involved in this study was reduced [77]. Silymarin is the most commonly researched and used herb for liver disease; over forty clinical trials have been performed with *S. marianum* preparations in the last years, including testing *S. marianum* preparations in liver cirrhosis, viral hepatitis, on toxic liver diseases and on the efficacy on patients already being treated with psychotropic drugs. Most of these studies demonstrate positive effects for indications including cirrhosis and alcoholic liver disease, hepatitis and psychotropic drug-induced liver damage [82].

Glycyrrhizin (glycyrrhizic acid or glycyrrhizinic acid), the major constituent of the liquorice (*G. glabra*) root, is a saponin compound with various pharmacological effects and has been used for over 20 years to treat chronic viral hepatitis in Japan [8, 78]. A clinical study conducted in Japan on a medicine containing glycyrrhizin, cysteine and glycine in patients with chronic hepatitis C showed a lower frequency of the development of liver cirrhosis and hepatocellular carcinoma after 15 years in relation to patients who were not treated with this medicine [83]. However, data on the level of HCV-RNA, biochemical parameters, pathomorphological changes and the quality of life of patients in this study were not given.

In addition to studying the extracts of one plant, a number of studies on herbal formulations containing a variety of plant extracts and compounds were included as well. A mixture of 10 different plant extracts, including *S. miltiorrhiza*, *Astragalus membranaceus* and *Spatholobus suberectus* and the main components of these plants, were tested in a clinical study in patients with hepatitis B [78]. Results showed that this preparation improves the clinical symptoms of liver fibrosis of hepatitis B patients

as well as biochemical parameters associated with this type of disease [84]. This herbal mixture reversed fibrosis in the tests in rats and the positive effects on the proliferation of human hepatic stellate cell line (LX-2) and human liver cancer cell line (HepG2 cells) [85].

A mixture of extracts of *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *Cassia occidentalis*, *Terminalia arjuna*, *Achillea millefolium* and *Tamarix gallica* commonly used in Indian traditional herbal recipes has been marketed in the West as LIV.52. In uncontrolled observations in patients with liver disease, this extract was reported to improve serum biochemistry values. Furthermore, it lowered circulating levels of acetaldehyde in healthy adults consuming alcohol [86]. In a controlled trial, LIV.52 was tested in a study in patients with liver disease due to alcoholism; however, it was not shown to have a significant effect [87]. The same mixture in a clinical study showed that patients with liver cirrhosis who had a treatment with this herbal medicine over a period of six months showed better clinical picture than in the group of patients who had placebo treatment [88, 89].

Polyherbal formulation composed of plant extracts of *T. arjuna*, *Withania somnifera*, *Phyllanthus niruri*, *B. aristata*, *Tinospora cordifolia* (Willd.) Miers, *P. kurroa* and *Boerhaavia diffusa* L., in a clinical study, showed that patients with acute viral hepatitis recovered faster compared to the placebo group [90].

All mentioned herbal formulations have been shown to be effective in ameliorating the course of chronic liver disease. Among them, silymarin and LIV.52 were the most researched, but no published clinical studies compared their efficiency in liver disease.

⎙ Conclusions

Medical herbs are abundant, economical, versatile and thus so popular potential antifibrotic agents. They can be a source of bioactive compounds, and with the aim of preventing the formation and progression of fibrosis, they can find wide application in medical practice.

The main activities of bioactive compounds in the pathogenesis of fibrosis, studied in *in vitro* and *in vivo* conditions, are the reduction of oxidative stress, the suppression of the inflammatory and immune response, inhibition of HSCs activation, excessive deposition of ECM, the induction of their apoptosis, the protection of the hepatocytes of the apoptosis and the creation of apoptotic bodies, which, being phagocytic, activate HSCs. The control of the dynamics of ECM synthesis by inhibiting the activation of HSCs, suppressing and modulating TGF-β1/Smad signaling pathway and its degradation are therapeutic targets with the aim of stopping fibrosis and restoring normal structure of the liver.

Based on the presented data, we can conclude that medical herbs may represent a significant source of medical agents that can be used as therapeutics in liver inflammation and fibrosis. From many plants, the chemical compounds responsible for hepatoprotective activity have been identified and the mechanisms of their hepatoprotective activity have been described. In addition, it was shown that mixtures of compounds of one plant species may exhibit a significant hepatoprotective effect as efficiently as or more efficiently than one of the compounds. However, in spite of all evidence of hepato-

protective activity of herbal preparations, it is necessary to work on further investigations to single out or develop a medicine that would contain a single active ingredient or the most effective dose of bioactive compounds and meet the qualities and standards of modern medicine. With this aim, future research in this area should be focused on examining the chemical composition and standardization of herbal medicines and randomized placebo-controlled clinical trials in order to test its clinical efficacy in the treatment and prevention of liver diseases.

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Corresponding author

Vladimir Jurišić, Professor, MD, PhD, Institute of Pathophysiology, Faculty of Medical Sciences, University of Kragujevac, 69 Svetozara Markovica Street, P.O. Box 124, 34000 Kragujevac, Serbia; Phone/Fax +381 34 306 800, e-mail: vdvd@lycos.com