Herbal Products for Liver Diseases: A Therapeutic Challenge for the New Millennium

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Use of herbal drugs in the treatment of liver diseases has a long tradition, especially in Eastern medicine. Standardization has been a problem, and randomized, placebo-controlled clinical trials to support efficacy are lacking. Some herbal extracts promoted for gastrointestinal or biliary disorders contain potent hepatotoxic alkaloids and are harmful. However, some of these extracts have yielded molecules, often related to flavonoids, with proven antioxidative, antifibrotic, antiviral, or anticarcinogenic properties, including glycyrrhizin, phyllanthin, silibinin, picroside, and baicalein, which derive from licorice root, *Phyllanthus amarus*, milk thistle, *Picrorhiza kurroa*, and sho-saiko-to, respectively, that can serve as primary compounds for the development of specific hepatotropic drugs.

BACKGROUND

Natural remedies represent a $1.8 billion market in the United States, and a single herbal preparation, silymarin, which is used almost exclusively for liver diseases, amounts to $180 million in Germany alone.[1] Marketing of herbals tripled between 1992 and 1996,[1] and nearly a third of outpatients attending liver clinics use these products.[2] This is reflected in the internet home pages of hepatitis foundations. Herbal products have been classified as food supplements and thus are exempt from regulations on quality control and proof of efficacy that govern standard pharmaceuticals. This is contentious in view of the biological activity of many herbals and, more worrisome, their occasionally severe toxicity.

Use of herbal medicines can be traced back as far as 2100 B.C. in ancient China (Xia dynasty) and India (Vedic period). The first written reports date back to 600 B.C. with the Caraka Samhita of India and the early notes of the Eastern Zhou dynasty of China that became systematized around 400 B.C. The recipes, once formulated, were usually expanded rather than abandoned during subsequent centuries. Expansion was stimulated by a growing understanding of the natural evolution of frequently encountered diseases and by emerging hypotheses regarding their causes. Hepatitis was and continues to be prominent. Biliary stasis in patients with jaundice, often associated with ascites and encephalopathy, led to the discovery that the liver is responsible for bile production and excretion. However, contrary to the Aristotelian Western world, which preferred the analytical approach to medicine, even when based on unfounded assumptions, the Eastern hemisphere always considered disease a manifestation of a more general imbalance of the dichotomous energies that govern life as a whole and human life in particular. In China these energies are represented by the complementary Yin (representing earth and moon, moistness, darkness and passivity the female aspect) and Yang (representing sun, dryness, light, and activity the male aspect), the balance and timely sequence of which is necessary to maintain health. In the Ayurveda (sanskrit: ayur, life; veda, knowledge) of India, similar forces are agni (strength, health, and innovation) and ama (weakness, disease, and intoxication).

With the revolution of the natural sciences and evidence-based medicine, the divide between Western and Eastern medicines appeared to widen. However, given the limitations of conventional treatment for chronic diseases and tumors, both patients and scientifically trained physicians are giving increased attention to the more holistic approach of Eastern medicine. Although this may represent in part a trend towards mysticism in our modern world, the effectiveness of Eastern medicine is amenable to Western analysis. One explanation is the placebo effect, part of which can be explained by modulation of neurotransmitters or the immune system in the brain, and another is the fact that some herbal drugs contain ingredients that specifically treat disease.

EFFICACY AND SAFETY OF HERBAL PRODUCTS

Any evaluation of herbal products faces major problems. The first is the use of mixed extracts (concoctions) and variations in methods of harvesting, preparing, and extracting the herb, which can result in dramatically different levels of certain alkaloids. The biologically active substances have been structurally defined and standardized for only a few of the herbs. Even then, it may not be known if this molecule is the sole active principle or if efficacy depends on the mixture of compounds.

The second problem is a lack of randomized, placebo-controlled clinical studies. Traditional Eastern medicine relies on empiricism and a holistic philosophy, and controlled studies are considered unnecessary. This is a view shared by many Western supporters of alternative medicine. Also, trials may not use end points, such as death from liver disease, histological fibrosis or inflammation, cancer, and transplantation.

Abbreviation: HBsAg, hepatitis B surface antigen.

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Related to these issues is concern about the safety of herbal remedies. Numerous reports of toxic effects contradict the popular view that herbals are natural and therefore harmless. A survey of the National Poison Information Service for the years 1991-1995 documented 785 cases of possible or confirmed adverse reactions to herbal drugs, among which hepatotoxicity was the most frequent.[3] The real number is probably much higher because of underreporting. Although abnormal liver function tests mostly return to normal once the offending drug is withdrawn, cases of chronic disease and acute liver failure requiring transplantation have been reported.[4] There are groups of plant alkaloids with well established hepatotoxicity (Table 1).[4-6] The pyrrolizidine alkaloids found in herbal teas or enemas containing Crotalaria, Senecio, Heliotropium, or Symphytum damage the hepatic central vein endothelia, causing veno-occlusive disease that may be lethal or require transplantation. Germander (*Teucrium chamaedrys L.*), broadly used in France as an antipyretic for treatment of abdominal discomfort and for weight reduction, contains hepatotoxic alkaloids identified as furano-diterpenoids that, after activation by the hepatic cytochrome P450 3A, deplete glutathione and precipitate hepatocyte necrosis, apoptosis, and cytoskeletal disorganization.[7,8] Greater celandine (*Chelidonium majus*) has resulted in acute hepatitis; extracts of this herb are broadly used in Europe to treat gallstone disease and dyspepsia.[9] Hepatotoxicity can result also from misidentification or mislabeling of a plant, contamination by chemicals such as heavy metals, and incorrect storage that leads to microbial or fungal growth and toxin production. Safety testing is needed. Before this can be implemented, however, preparations must be standardized and must replace in the market the uncontrolled and individualized concoctions currently being offered. Safety concerns notwithstanding, sufficient scientifically useful data have accumulated during the last few years to allow an overview of herbal compounds, some of which appear to be beneficial and may serve as a basis for future drug development.

STUDIES OF DEFINED FORMULATIONS OF HERBAL MEDICINES

Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds, for which pharmacodynamic and pharmacokinetic data are usually available. These resemble the medications of traditional Western medicine. In only a few cases, however, have studies documented their efficacy using accepted parameters of disease progression.

Glycyrrhizin. This group of related, sulfated saponins and lectins from the licorice root has been used for over 20 years to treat chronic viral hepatitis in Japan. It has a well-documented transaminase-lowering effect. The standardized aqueous extract (Stronger Neo-Minophagen C) has to be administered parenterally. A daily dose of 80 mg given for 2 weeks can normalize aspartate transaminase and alanine transaminase in over 60% of patients.[10] The preparation has immunosuppressive and anti-inflammatory effects in cell culture, where glycyrrhizin inhibits $CD4^+$-T cell- and tumor necrosis factor-mediated cytotoxicity.[11] Furthermore, the extract modifies glycosylation and blocks sialylation of hepatitis B surface antigen (HBsAg), which leads to its retention in the trans-Golgi apparatus.[12] In an uncontrolled trial of 17 hepatitis Be antigen–positive patients with chronic hepatitis B, a 4-week course of glycyrrhizin followed by 4 weeks of interferon-alfa produced loss of hepatitis B e antigen in 10 of 17 patients after 6 months.[13] However, only 3 of the 10 patients underwent seroconversion to antibodies to e antigen, and virus titers were not reported. In a small randomized study of 28 patients with chronic hepatitis C who were nonresponders to interferon monotherapy, 13.3% became hepatitis C virus–RNA negative after interferon alone compared with 33.3% after a glycyrrhizin/interferon combination therapy over 3 months.[14] However, this was not statistically significant. In a retrospective analysis of 84 patients with chronic hepatitis C virus infection who were treated with intravenous glycyrrhizin 2 to 7 times weekly for a median of 10.1 years, comparison with a matched group of 109 patients who remained untreated over 9.2 years revealed a 2.5-fold reduction of the relative risk of hepatocellular carcinoma.[15] This could be due to an anti-inflammatory effect of the preparation rather than to its weak antiviral effect. Because of its aldosterone-like activities,[16] use of the drug requires caution and monitoring for hypertension, hyperkalemia, and worsening ascites.

Phyllanthus amarus. This herb and related species are Indian plants that contain phyllantins, hypophyllantins, and polyphenoles with antiviral properties. An aqueous extract inhibited

TABLE 1. Selection of Herbal Preparations With Proven Hepatotoxicity

Causative Plants	Toxic Agents	Symptoms	Mechanism/Pathology
Crotalaria Senecio Heliotropium *Symphytum officinale* (Comfrey)	Pyrrolizidine alkaloids	Veno-occlusive disease	Endothelial cell glutathione depletion, central vein necrosis, thrombosis, and fibrosis
Atractylis gummifera	Atractylate, gummiferin	Hepatitis	Inhibition of oxidative phosphorylation, hepatic necrosis
Callilepsis laureola	Atractylate	Hepatitis	Hepatocyte necrosis
Chelidonum majus (greater celandine)	Chelidonine, sanguinarine, berberine, coptisine?	Hepatitis (cholestatic)	Lymphocyte infiltration
Larrea tridentata (chaparral)	Guaiaretic acid derivatives	Hepatitis	?
Teucrium chamaedrys (germander)	Furano-diterpenoids	Hepatitis	Hepatocyte glutathione depletion and apoptosis
Chinese herbal mixtures (artemisia, hare's ear, chrysanthemum, plantago seed, gardinia, red peony root, etc.)	Largely undefined	Hepatitis	?

NOTE. Data are selected from Larrey and Pageaux,[5] Kaplowitz,[6] Benninger et al.,[9] and Yoshida et al.[4]

woodchuck hepatitis virus DNA polymerase and surface antigen expression[17,18] and several protein kinases such as cAMP-dependent protein kinase, protein kinase C, and myosin light-chain kinase in rat liver.[19] A nonrandomized clinical study showed a remarkable 59% (22 of 37 patients) clearance of HBsAg in chronic carriers who were treated for 30 days compared with only 4% (1 of 23 patients) given placebo.[20] However, these results await confirmation. There was no effect of *P. amarus* on duck hepatitis B virus.[21]

Daphnoretin. This dicoumarin drug extracted from the Chinese herb *Wilkstroemia indica* was shown to suppress HBsAg in Hep3B cells, an effect mediated by activation of protein kinase C.[22] The same investigators reported a powerful suppression of HBsAg by costunlite and dehydrocostus lactone, two alkaloids from *Saussurea lappa* Clarks root.[23] However, no clinical studies with these compounds have been reported.

Silymarin. A standardized extract from the milk thistle *Silybum marianum* contains as its main constituents the flavonoids silybinin, silydianin, and silychristin.[24] Milk thistle extracts were used as early as the 4th century B.C., became a favored medicine for hepatobiliary diseases in the 16th century, and experienced a revival in central Europe in the late 1960s (Table 2). The flavonoid silibinin, which constitutes 60% to 70% of silymarin, has been identified as the major active ingredient.[25,26] Its pharmacological profile is well defined, and studies in cell culture and animal models clearly show its hepatoprotective action with little or no toxicity.[26,27,33-41] Silymarin enhances the activity of hepatocyte RNA-polymerase I,[26] complexes toxic free iron,[33] protects the cell membrane from radical-induced damage,[34] and blocks the uptake of toxins such as *Amanita phalloides* toxin.[32,35] A potent scavenger, it prevents lipid peroxidation and normalizes the lipid profile of hepatocyte membranes.[36] Silymarin provided liver protection in rat models of liver damage induced by carbon tetrachloride and paracetamol.[37,38] Four of 12 dogs fed lyophilized Amanita toxin and given supportive care died from hepatic failure and coma within 35 to 54 hours, whereas all 11 dogs receiving high-dose silymarin survived.[39] In a retrospective analysis of 205 patients with Amanita intoxication, of whom 30 received treatment, the death rate of those given intravenous silymarin was reduced significantly (12.8% vs. 22.4%).[40]

In recent *in vitro* studies, silymarin down-regulated the proinflammatory leukotriene B4 in Kupffer cells.[41] In randomized clinical trials for acute viral hepatitis A or B, oral silymarin either exerted no benefit[29] or accelerated clinical recovery, causing a significantly more rapid normalization of bilirubin and aspartate transaminase than did the control.[30] Similarly, in alcohol-related hepatitis treated with silymarin, transaminase levels dropped more rapidly than in the untreated disease.[42] A 4-month course of silymarin in patients with moderately active alcohol-related liver disease led to a 41% reduction of alanine transaminase, compared with no change in controls.[43] In a randomized trial, 170 biopsy-proven cirrhotic patients, 92 with alcohol-related and 78 with nonalcohol-related liver disease, were treated with silymarin or placebo for a mean of 41 months.[44] Although serum biochemistry values did not differ between the 2 groups, the number of surviving cirrhotic patients with alcohol-related liver disease was significantly higher in the silymarin group, especially in those with Child-Pugh class A cirrhosis. Most of the latter patients continued to drink, which may have influenced the results. Also, the dropout rate was high, although dropouts were counted as therapy failures. A subsequent randomized, placebo-controlled study of 200 patients with alcohol-related cirrhosis, 75 of whom dropped out, could not confirm a survival benefit.[45]

These data point up the difficulty of studying a heterogeneous group of patients and of using death as the endpoint for a condition that progresses over many years. An intermediate endpoint is progression of fibrosis to cirrhosis, which is the primary determinant of morbidity and mortality in patients with chronic liver diseases. *In vitro*, silymarin blocks proliferation of hepatic stellate cells, the main source of excess collagen in fibrosis. This is accompanied by down-regulation of the profibrogenic transforming growth factor β.[46] In liver injury induced by complete occlusion of the biliary system in the rat, oral silymarin reduced collagen accumulation in a dose-dependent fashion.[47] It was similarly antifibrotic when administered from weeks 4 to 6, *i.e.*, starting at a time when liver collagen is already increased 4-fold, a situation encountered in most patients with chronic liver disease. The antifibrotic effect was accompanied by reduced numbers

TABLE 2. History of the Milk Thistle as a Liver Remedy

Century/Year	Use/Indication	Source
4th Century B.C.	General medicinal herb	Theophrastus
1st Century A.D.	Emetic, general medicinal herb	Dioskurides
11th Century A.D.	Ulcers, shingles	Hildegard von Bingen, Causae et curae
1564	Stitch in the side, astringent	A. Lonicerus, Kreuterbuch, Frankfurt
1626	Stitch in the side, pestilence, renal calculi	P.A. Matthiolus, Neues Kreuterbuch, Prague
1755	Liver disease, liver pain	A. von Haller, Medicinisches Lexikon, Frankfurt
1846/1951	Liver disease, icterus, biliary colic	J.G. Rademacher, Erfahrungsheillehre, Berlin
1938	Hepato-cholangiopathies, chronic leg ulcers	G. Madaus, Lehrbuch der biologischen Heilmittel, Leipzig

Year	Characterization/First Clinical Studies	Source (reference)
1968-1974	Characterization of active compounds	Wagner et al.[25] and Sonnenbichler et al.[26]
1971	First animal experimental studies on liver protection	Platt et al.[27]
	Antidote for Amanita phalloides intoxication in the rat	Schriewer et al.[28]
1976-1988	Elucidation of molecular actions of silibinin	Sonnenbichler et al.[26]
1977/1978	First controlled clinical studies in acute viral hepatitis	Bode et al.[29] and Magliulo et al.[30]
1980	First controlled study in alcoholic cirrhosis	Benda et al.[31]
1980-1981	Amanita phalloides antidote in clinical studies	Hruby et al.[32]

of activated stellate cells[48] and a greater than 50% reduction of both procollagen I and tissue inhibitor of metalloproteinase messenger RNA, both being major effectors of fibrogenesis.[49] These data have spawned randomized, placebo-controlled studies of silymarin in patients with chronic viral hepatitis that include follow-up biopsies and a panel of serum markers of liver fibrosis.[50]

Picroliv. Picroliv is an alcoholic extract from the root of *Picrorhiza kurroa* that contains the iridoid glycosides kutkoside and picroside. In the rat these glycosides act as antioxidants[51] and ameliorate the hepatotoxic effects of carbon tetrachloride,[52] thioacetamide, galactosamine,[53] and paracetamol.[54] Despite their wide oral usage in India, no reliable data for human liver disease exist.

TJ-9. TJ-9, commonly prescribed in China as xiao-chai-hu-tang and in Japan as sho-saiko-to, is an aqueous extract from the roots of scutellaria, glycyrrhiza, bupleurum, and ginseng; the pinella tuber; the jujube fruit; and the thew ginger rhizome. Two major alkaloids from scutellaria, baicalin and baicalein, are strong inhibitors of lipid peroxidation.[55] The extract prevented hepatocellular membrane damage and restored mitochondrial function in endotoxin-treated rats, increasing hepatic levels of superoxide dismutase and glutathione.[56,57] Other *in vitro* effects that are related to the observed antitumour activity of sho-saiko-to include up-regulation of the inducible nitric oxide synthase in hepatocytes cultured in the presence of interferon γ[58] and inhibition of proliferation and induction of apoptosis in hepatoma cells.[59,60] The extract modulated the *in vitro* cytokine production in peripheral blood mononuclear cells, stimulated release of tumor necrosis factor-α and granulocyte–colony-stimulating factor in patients with hepatocellular carcinoma and down-regulated synthesis of interleukin-4 and -5 in favor of interleukin-10 in patients with chronic hepatitis *C*.[61,62] Other *in vitro* effects include stimulation of inducible nitric oxide synthase and down-regulation of interleukin-4 and -5 in favor of interleukin-10 in patients with chronic hepatitis *C*.[61,62] In the rat model of dimethylnitrosamine-induced liver injury, the extract sho-saiko-to protected liver synthetic function[63] and restored hepatic retinoid levels.[64] Sho-saiko-to reduced hepatic collagen content in the rat models of fibrosis due to choline-deficiency,[65] dimethylnitrosamine, and pig serum.[66] The latter work identified baicalin and baicalein, which are structurally similar to silibinin,[67] as major active compounds, leading to the hypothesis that these agents may have an antifibrotic activity separable from their effect as inhibitors of lipid peroxidation. Whereas information on the antiviral efficacy of sho-saiko-to is at best rudimentary,[68] a prospective randomized 5-year study of 260 patients with cirrhosis showed a near-significant ($P < .053$) survival benefit for the treated patients; this reached significance in those patients without HBs-Ag.[69]

FORMULAS CONTAINING MIXTURES OF HERBS WITH PARTIALLY KNOWN OR LARGELY UNKNOWN INGREDIENTS

The literature is replete with experimental studies using herbs of largely unknown composition. The following are those preparations for which human studies or mechanistic data exist.

Compound 861. Known as cpd 861, this is an aqueous extract of 10 defined herbs based on traditional Chinese medicine. The aim of traditional Chinese medicine is resolution of blood stasis and liver stagnation, two conditions that form the basis of liver pathology and patient discomfort.[70] The chief herbs used in cpd 861 are *Salvia miltiorrhiza*, *Astragalus membranaceous*, and *Spatholobus suberectus*.[71] Rats with experimental liver fibrosis showed a 50% reduction of the 5-fold increased hepatic collagen level when cpd 861 was administered daily by gavage.[72] This was accompanied by a comparable down-regulation of hepatic messenger RNA for transforming growth factor β1 and for procollagens I, III, and IV, as well as by increased hepatic collagenase activity. Because procollagen messenger RNAs, major effectors of liver fibrogenesis, were also down-regulated in cultures of hepatic stellate cells, a direct antifibrotic effect was proposed.[73] From 1993 to 1995, 60 patients with chronic hepatitis B were treated in an open trial with cpd 861.[71] After 2 years, subjective improvement was reported by 50 patients (83%), and this was accompanied by a reduction in spleen size in 41% and a decrease in liver enzyme levels and serum fibrosis markers such as PIIINP and laminin. In a nonrandomized controlled trial, 22 patients with chronic hepatitis B were treated with cpd 861 for 6 months and compared with 12 matched patients receiving a control herbal medicine.[74] Follow-up liver biopsy results showed a statistically significant improvement in both histological inflammation and fibrosis in the cpd 861 group but no change in the control subjects.

LIV.52. An extract of several plants prepared for ayurvedic medicine has been marketed in the West as LIV.52. Standardization, chemical characterization, functional, and pharmacological studies are not well documented. The extract was reported to improve serum biochemistry values in rats with toxic liver damage,[75] and uncontrolled observations in patients with liver disease seemingly gave similar results.[76] Furthermore, it lowered circulating levels of acetaldehyde in healthy adults consuming alcohol.[77] Therefore, Fleig et al.[78] performed a randomized, placebo-controlled, 2-year clinical trial in 188 patients with alcohol-related cirrhosis. LIV.52 did not affect the survival rate of Child class A and B patients but increased mortality among the 59 Child class C patients (81% in the treated group, compared with 40% in the placebo group). Twenty-two of 23 deaths in the LIV.52 group were related to bleeding or liver disease compared with only 3 of 11 deaths in the placebo group. This result led to immediate withdrawal of the drug. It highlights the danger of ill-defined herbal preparations and the necessity for in-depth preclinical testing.

FUTURE DIRECTIONS

There is no doubt that certain herbal products contain chemically defined components that can protect the liver from oxidative injury, promote virus elimination, block fibrogenesis, or inhibit tumor growth. Although additive effects may be lost, the active molecules must be isolated and tested in suitable culture and animal experiments and finally in randomized, placebo-controlled studies to enable rational clinical use of the agents. Biologically active molecules derived from herbal extracts can serve as suitable primary compounds for effective and targeted hepatotropic drugs.

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