

# Hepatitis C: Epidemiology and Review of Complementary/Alternative Medicine Treatments

by Lyn Patrick, ND

## Abstract

Hepatitis C is emerging as a serious worldwide problem. In the United States the current mortality figures may triple in the next ten years, rivaling HIV. The disease has a latency of 10-30 years and symptoms or signs may not appear until cirrhosis is evident. Adequate diagnosis, including liver biopsy, is essential in assessing the current stage of the viral infection and the need for treatment. Hepatitis C may manifest as hepatic fibrosis, cirrhosis, hepatocellular carcinoma, lichen planus, glomerulonephritis, mixed cryoglobulinemia, or porphyria. The hepatic damage is due both to the cytopathic effect of the virus and the inflammatory changes secondary to immune activation. The use of the botanical components glycyrrhizin, catechin, silymarin and phytosterols, and the antioxidants N-acetylcysteine and vitamin E are reviewed for their efficacy in treating chronic hepatitis and affecting liver damage.

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## Introduction

The World Health Organization has estimated 170 million people worldwide are infected with hepatitis C.<sup>1</sup> The prevalence in the United States is estimated at 3.9 million, approximately four times the current number of those infected with the HIV virus. Due to the latent nature of the disease (infection may precede symptoms by an average of 25 years) only one million of these individuals have actually been diagnosed.<sup>2</sup> An estimated 8-10 thousand deaths occur annually in the United States as a result of hepatitis C-related liver disease, compared to 16,685 AIDS deaths in 1997.<sup>3</sup> Hepatitis C mortality figures are expected to triple by the year 2010, giving hepatitis C a resultant mortality that may rival HIV. Ninety percent of those infected internationally cannot afford treatment and due to the specific characteristics of the virus, a vaccine is not expected.<sup>4</sup> Hepatitis C has been estimated to be the most common cause of chronic liver disease, cirrhosis, and liver cancer in the Western Hemisphere.<sup>5</sup>

Attempts to treat the virus have been disappointing. Interferon alfa is an FDA approved treatment for chronic hepatitis C. In six-month treatment regimens, studies have shown an immediate (20-25%) failure rate determined by lack of clearance of the virus.<sup>6</sup>

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Those who exhibit viral clearance experience a 30-70 percent relapse rate within the first few months of discontinuing therapy. A sustained response lasting at least six months is seen in only 10-15 percent of patients.<sup>7</sup> The side-effect profile of interferon alfa2b is high: nausea, headache, fever, myalgias, fatigue, leukopenia, thrombocytopenia, alopecia, irritability, depression, infrequent thyroid abnormalities, pulmonary complications, and retinal damage. Patients with autoimmune disorders, thyroid dysfunction, decompensated cirrhosis and thrombocytopenia, and post-transplant patients are usually not eligible for treatment with interferon due to the risk of serious side-effects.<sup>7</sup> Although evidence from multiple studies shows interferon does decrease risk for progression to hepatocellular carcinoma, the risk/benefit ratio and cost of treatment may render it prohibitive.<sup>8</sup>

The anti-viral drug ribavirin has been used in combination with interferon and has resulted in significantly improved responses: current studies show a 28-66 percent sustained response after 48 weeks of treatment.<sup>9</sup> The side-effects of the combination therapy are, however, "universal, significant, and possibly serious." Ribavirin frequently causes hemolytic anemia leading to necessary dose reductions and is a known teratogen.<sup>10</sup>

As a result of the worldwide need for treatment options, the National Institutes of Health (NIH) will sponsor an international conference on "Complementary and Alternative Medicine in Chronic Liver Disease" August 22-24, 1999 at NIH in Bethesda, Maryland.

### History of Hepatitis C

In 1975, when the hepatitis A and B antibody tests became available, it was determined the majority of transfusion-related hepatitis was neither hepatitis A nor B.<sup>11</sup> Hepatitis C was isolated in 1989 with viral genome sequencing that led to the first screening antibody assay in 1990. At that point,

incidences in multiple-transfusion recipients were high: 80 percent in more than 1,000 transfusion-dependent thalassemics,<sup>12</sup> and 75 percent of multi-transfused patients in remission from leukemia.<sup>13</sup> In 1992, a more sensitive second-generation EIA was introduced, which led to a significant reduction in contamination of the blood supply. Today, with more sensitive screening, it is estimated the risk of receiving blood from a donor who is infectious but has not yet seroconverted is 1/100,000.<sup>14</sup>

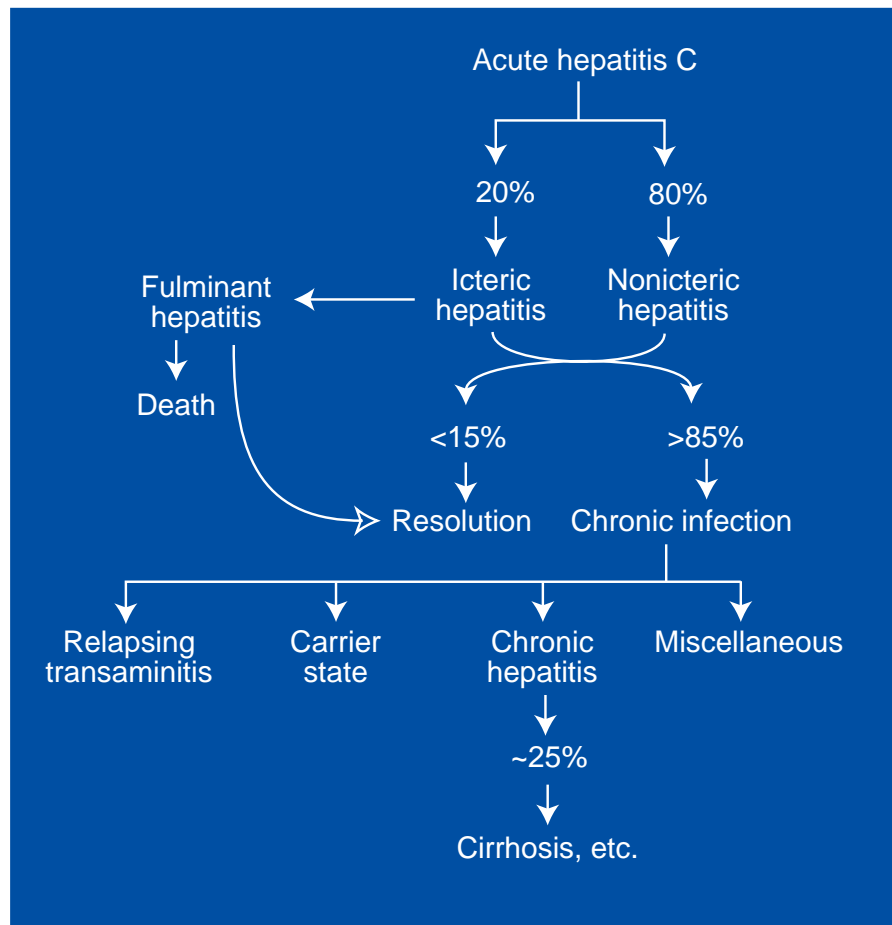
Current diagnostic antibody assays include the recombinant immunoblot assay (RIBA) which is more effective at excluding false-positive results than previous EIA antibody screening assays. Detection of viral load can be accomplished within days of infection via RT-PCR assays. Viral load assessment is necessary in immuno-compromised individuals who may not produce sufficient antibodies or for those symptomatic individuals who may have false-negative antibody results. It is also necessary as a monitoring tool in antiviral therapy.<sup>15</sup>

### The Virus

As a result of host selectivity and hepatitis C viral mutations, the virus now occurs in six different types or genotypes. They appear to vary in virulence and certain genotypes (genotype 1b) may carry a poorer prognosis and be less susceptible to treatment with interferon alfa.<sup>16,17</sup>

Like other RNA viruses, the hepatitis C virus (HCV) genome is "fluid," meaning it changes substantially, even within the same infected individual. Because, like other RNA viruses, HCV has an absence of repair mechanisms that operate during DNA replication, it mutates freely. These mutations lead to production of different viral isolates called "quasi-species" that can occur within any given genotype.<sup>18</sup> A person infected with the 1b genotype (the most difficult to treat) could therefore have many different quasi-

**Figure 1.** Outcome of HCV Infection.



## Transmission

Case-control studies of non-A, non-B hepatitis (hepatitis strains labeled A-G have been identified) have found significant associations between viral infection and a history of blood transfusions at least six months previously, direct patient care or laboratory work, intravenous (IV) drug use, multiple sexual partners, or sexual or household contact with an infected person. The highest prevalence is among hemophiliacs who received factor concentrate transfusions before 1992.<sup>19</sup> Persons with a history of IV drug use account for possibly 50 percent of chronic infections.<sup>19</sup> Approximately 20 percent of hemodialysis

species of that genotype in their body. These mutations have the ability to sidestep the host's immune surveillance mechanisms because the immune system develops antibodies to only a minority of the quasi-species. This is believed to be the reason 85 percent of infected individuals do not develop immunity to HCV and go on to develop chronic infections.

Attempts to develop vaccines have been unsuccessful for the same reason: neutralizing antibodies produced against HCV are specific for certain quasi-species and not for others. It would be very difficult to develop a vaccine that would recognize the genetic variants of this diverse virus.<sup>12</sup>

patients worldwide are reported to show anti-HCV antibodies, independent of receiving blood transfusions and positively associated with increasing years on dialysis.<sup>14</sup> Geographical variations of all sources of HCV exist, however, and hemodialysis is not an exception to this rule. In the United States, 35 percent of hemodialysis patients in one study were HCV infected. Sexual transmission and household exposure transmission is a route of infection, but appears to occur infrequently and accounts for possibly 10-15 percent of all cases.<sup>19</sup> In a Japanese study of 154 infected couples, 18 percent of monogamous HCV spouses were co-infected, the risk increasing for each decade of marriage.<sup>20</sup> Alter<sup>19</sup> estimates the likelihood of sexual transmission is approximately five percent, and neither the U.S. Public Health Service nor the NIH recommend

barrier precautions in monogamous relationships. Perinatal spread is uncommon and, when it occurs, rarely leads to chronic infection of the child unless the mother is co-infected with HIV.<sup>21</sup>

Current risks for acquiring or having acquired hepatitis C include: illegal intravenous drug use (including short-term use in the previous 20 years), being an organ or transfusion recipient prior to 1992, intranasal cocaine use with shared equipment, tattoo or body piercing with nonsterile instruments, using an infected person's razor or toothbrush, and engaging in high-risk sexual behavior (having multiple partners or failing to use a condom).<sup>22</sup>

Prior hospitalization is a risk factor (prevalence in hospitalized patients is 2-20 percent).<sup>23</sup> Patient-to-patient transmission has been implicated in outbreaks of HCV in a hematology ward, and surgeon-to-patient transmission has been identified as a cause in a pediatric oncology unit.<sup>24,25</sup>

## Clinical Course

The current incubation time of HCV is between 2-26 weeks, although 80-90 percent of cases occur within 5-12 weeks post-transfusion.<sup>26</sup> Most patients with acute hepatitis C do not have demonstrable signs or symptoms at the onset of infection. Twelve percent of a cohort of 50 HCV patients had a remembered past history of symptomatic hepatitis; however, due to the high incidence of coinfection hepatitis, the symptomatic episode may have been simultaneous infection with acute hepatitis A.<sup>27</sup> Only 25 percent of acute non-A, non-B hepatitis patients are jaundiced and only 33 percent have significantly elevated alanine aminotransferase (ALT) levels (>800 I.U.).<sup>28</sup>

As mentioned previously, the ability of the virus to mutate appears to prevent effective immune eradication, even in the case of a healthy cellular immune response. This is reflected by the high percentage of cases that become chronically infected: studies range

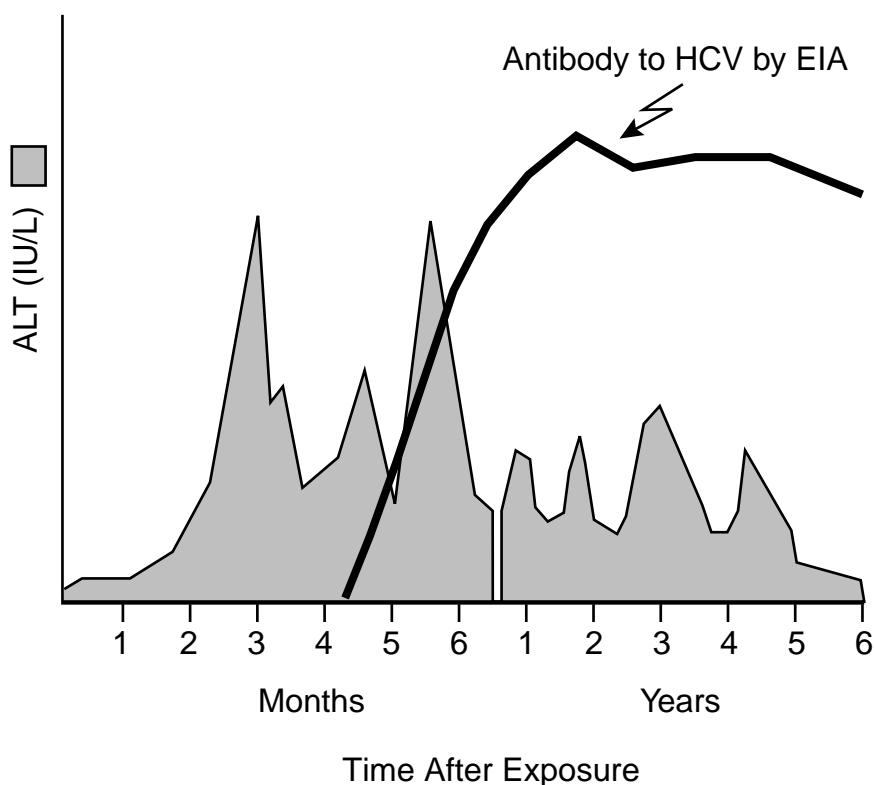
from 90 percent for those with genotype 1b, to 75 percent with genotype 2a or 2b.<sup>29</sup> In the United States, it is generally accepted that at least 85 percent of anti-HCV antibody positive patients will progress to chronic hepatitis (Figure 1).<sup>30</sup>

Chronic hepatitis C is usually characterized by serum ALT levels that have been elevated for 6-12 months after acute onset. ALT levels may normalize within one year, but may again rise and become chronically elevated (Figure 2).<sup>31</sup> Fluctuating transaminases in the absence of alcoholism are accepted to be diagnostic of hepatitis C.<sup>2</sup> However, HCV can and does progress in the absence of signs and symptoms; approximately one-third of those chronically infected with HCV exhibit consistently normal serum ALT levels.<sup>35</sup>

Even in the face of normal liver enzymes and an asymptomatic course, there is a high prevalence of liver disease. In a study of 98 healthy, anti-HCV antibody positive blood donors, 95 percent had histological abnormalities evidenced by liver biopsy and 75 percent were diagnosed with histological evidence of chronic hepatitis.<sup>32</sup> The progression of HCV appears to vary geographically and possibly with genomic type. In Japan, where the predominant genotype is 1b, only two percent of HCV patients appear to recover from acute infections, while the remainder will most commonly progress to chronic hepatitis (30%), cirrhosis (20%), and hepatocellular carcinoma (15%) a mere eight years later.<sup>2</sup> In the United States, progression is slower, with the development of cirrhosis in 20-30 percent of patients in 10-20 years of follow-up.<sup>35</sup>

The most common symptom of HCV is fatigue. In one study of 102 patients referred to a liver unit, fatigue occurred in 35 percent of subjects.<sup>33</sup> The onset of cirrhosis may be relatively asymptomatic with only subtle physical changes: palmar erythema, spider angiomas (blanching with pressure), hypertrophy of the parotids, gynecomastia in men (due to decreased clearance of estrogen in the liver),

**Figure 2.** Clinical Course of Chronic Hepatitis C Infections.



### Co-Infection with HIV

Among HIV-infected persons, HCV appears to progress more rapidly and lead to increased risk for liver disease. In a population of HCV-infected male hemophiliacs in the United Kingdom, liver-related death rates were approximately 20 times higher than the general population, and 94 times higher in men co-infected with HIV and HCV.<sup>36</sup> Other studies have noted increased replication rates of HCV in HIV-positive individuals with a more rapid progression to cirrhosis.<sup>37</sup>

and fibrosis of the palmar tendons of the hand. Once cirrhosis occurs, other symptoms such as muscle weakness, fluid retention, jaundice, bilirubin in the urine, purpura, upper intestinal hemorrhage, and pruritis may follow. HCV can also manifest as arthritis, lichen planus, glomerulonephrosis, and essential mixed cryoglobulinemia (arthritis, purpura, hives, vasculitis, glomerulonephritis, and neuropathy). Although cryoglobulins are evident in approximately 33 percent of patients, the clinical syndrome occurs only in 1-2 percent.<sup>34</sup> Another potential complication of HCV is porphyria cutanea tarda. It is associated with alcohol abuse, iron overload, and estrogen use, and appears as cutaneous vesicles in sun-exposed areas. The condition, if progressive, leads to skin fragility, bruising, and hyperpigmentation.<sup>35</sup>

### Hepatocellular Carcinoma

One of the most concerning aspects of HCV is the risk for hepatocellular carcinoma (HCC). In a cohort of hemophiliacs, an HCV infection of 25 years duration (as compared to those who are HCV-negative) resulted in a 17-fold increase in risk of death from liver disease and a six-fold increased risk of death from liver cancer.<sup>38</sup> In Europe and Japan, 50-75 percent of all patients with HCC have evidence of HCV infection.<sup>39,40</sup> The incidence of HCC varies with different population studies. In general, 20 percent of patients with chronic HCV develop cirrhosis over a ten-year period.<sup>41</sup> In patients with established cirrhosis due to HCV infection, surveillance studies show 3-4 percent may develop HCC in the first 4-5 years.<sup>42</sup> Progression from cirrhosis to HCC usually takes approximately ten years.<sup>43</sup>

## Liver Biopsy and the Pathology of HCV

Progression of HCV is determined by liver biopsy and measurement of serum ALT. When serum ALT is consistently above 200 IU/L it is predictive of chronic active hepatitis.<sup>44</sup> The current histological classification system for liver biopsies in HCV consists of a grading scale based on necrosis and inflammation, and staging based on fibrosis.<sup>45</sup> This system differentiates mild hepatitis (grade 1-stage 1) from more progressive states of hepatitis (grades 2-4). A biopsy without evidence of fibrosis infrequently progresses to cirrhosis.<sup>46</sup>

## Evidence for Oxidative Stress and Cytokine-Induced Inflammation in HCV

Results of 317 liver biopsy samples from patients with HCV showed evidence of HCV-induced liver damage: lymphoid follicles, large droplet fat, bile duct damage, and Mallory body-like material.<sup>47</sup> Scheur and Sherlock sampled 45 HCV patients, 44 percent of whom exhibited developing or established cirrhosis. They concluded the evidence of lymphoid aggregates (lymphocyte clusters) or follicles in the portal tracts and fatty changes, along with lobular activity, are the characteristic changes in hepatitis C.<sup>48</sup> Bach and Thung examined 50 biopsy samples from patients with HCV compared to 21 patients with autoimmune chronic hepatitis, and found similar pathologic traits that distinguished the HCV samples: bile duct damage, bile duct loss, steatosis, lymphoid cell aggregation (follicles), and lobular and piecemeal necrosis.<sup>49</sup>

The mechanisms involved with liver damage in chronic hepatitis C are not completely understood. HCV is thought to be directly cytopathic to hepatic cells, and there is evidence immune mechanisms involved in viricidal activity are responsible for the inflammatory infiltrates (lymphoid follicles) that can

progress to necrosis.<sup>50</sup> Tumor necrosis factor alpha (TNF- $\alpha$ ) is a cytokine secreted by HCV-specific cytotoxic lymphocytes; TNF- $\alpha$  levels are elevated in chronic hepatitis C. Elevated levels of TNF- $\alpha$  have also been correlated with elevated markers of liver damage (serum ALT levels and alpha-glutathione-S-transferase levels) independent of levels of hepatitis C virus in the blood.<sup>51</sup> TNF- $\alpha$  is one of the cytokines secreted by the specific T<sub>H</sub>2 humoral defense arm of the T lymphocyte cell-mediated immune system. T<sub>H</sub>2 cells also secrete the proinflammatory lymphokines interleukin 6 (IL-6), interleukin 4 (IL-4), interleukin 10 (IL-10), and interleukin 1 (IL-1).<sup>52</sup> The other arm of the T lymphocyte system is comprised of T<sub>H</sub>1 cells, which promote cell-mediated defense, and secrete interleukin 2 (IL-2), interleukin 12 (IL-12), and gamma-interferon (IFN- $\gamma$ ).

The T<sub>H</sub>1 and T<sub>H</sub>2 systems are mutually inhibitory, serving as a regulatory system in balancing humoral and cell-mediated responses. In the well-studied immune activation of HIV infection, the T<sub>H</sub>2 system becomes dominant, destroying immune equilibrium and resulting in a progressive reduction of IL-2 and IL-12.<sup>53</sup> In HCV infections, the same dominance of the T<sub>H</sub>2 system appears to exist: IL-4, IL-6, and IL-10 stimulate humoral immunity and lead to the overproduction of TNF- $\alpha$ , resulting in inflammation and suppressing the production of IL-2 and IFN- $\gamma$ .<sup>54</sup> In a healthy immune system, T<sub>H</sub>1 cells also support the transformation of CD+8 suppressor cells into active natural killer/cytotoxic cells that directly inactivate virus. Lirussi<sup>55</sup> evaluated natural killer cytotoxic response of NK cells in 15 chronic HCV patients and compared them with 23 controls. The NK cell activity in the chronic HCV patients was approximately 50 percent that of the healthy group in three different concentrations of NK cells. The authors suggest an impaired immune response appears to favor chronicity of the disease in chronic HCV. Whether impaired activity of the NK cells in

chronic HCV infections is due to a dominance of T<sub>H</sub>2 lymphocytes remains to be seen.

### **Ribavirin: Mechanisms of Action**

Ribavirin is a guanosine analogue with antiviral activity against RNA and DNA viruses. In combination trials with interferon, sustained virological responses have been as high as 47 percent after 24 weeks of treatment.<sup>56</sup> Although repeated studies have shown significant reduction of ALT levels after six months of treatment with ribavirin alone,<sup>57</sup> multiple studies have failed to detect any significant antiviral activity.<sup>58,59</sup> The benefit of ribavirin in post-liver transplant patients with hepatitis C is a result of decreased lobular inflammation and normalization of ALT levels, and not a reduction of viral load.<sup>60</sup>

There is evidence ribavirin specifically inhibits cytokine production by macrophages. Ning<sup>61</sup> assessed the effect of ribavirin in an experimental model of fulminant murine hepatitis (MHV-3). Even though ribavirin had minimal antiviral activity, it significantly reduced macrophage activation and decreased production of IL-4, but did not effect the production of IL-2 or IFN- $\gamma$ . The authors concluded the beneficial effect of ribavirin in this situation was the specific preservation of T<sub>H</sub>1 cytokines and the inhibition of T<sub>H</sub>2 cytokines.

### **Phytosterols**

Phytosterols are a family of plant lipids that have structural similarity to cholesterol but with a fraction of the absorption: a rate 800-1000 times lower than the absorption rate for cholesterol. In humans, less than five percent of phytosterol compounds are absorbed;<sup>62</sup> however, the amount necessary to be physiologically active is small. Beta-sitosterol and its glycoside have been found to exert lymphoproliferative action in picogram to femtogram amounts — at the same concentrations that endogenous hormones are found.<sup>63</sup> Approximately 80 percent of plant phytosterol

content is  $\beta$ -sitosterol with about one percent in the glycoside form  $\beta$ -sitosterol glycoside.<sup>64</sup>

Plant sterols and sterolins have anti-inflammatory,<sup>65</sup> hypocholesterolemic,<sup>63</sup> and insulin regulating activity.<sup>66</sup> They have been used in the treatment of benign prostatic hypertrophy.<sup>67</sup> Sterols and sterolins are thought to be responsible for the activity of *Serenoa repens*, *Silybum marianum*, *Harpagophytum procumbens*, *Ginkgo biloba*, *Eleutherococcus senticosus*, and *Pygeum africanus*.<sup>68</sup>

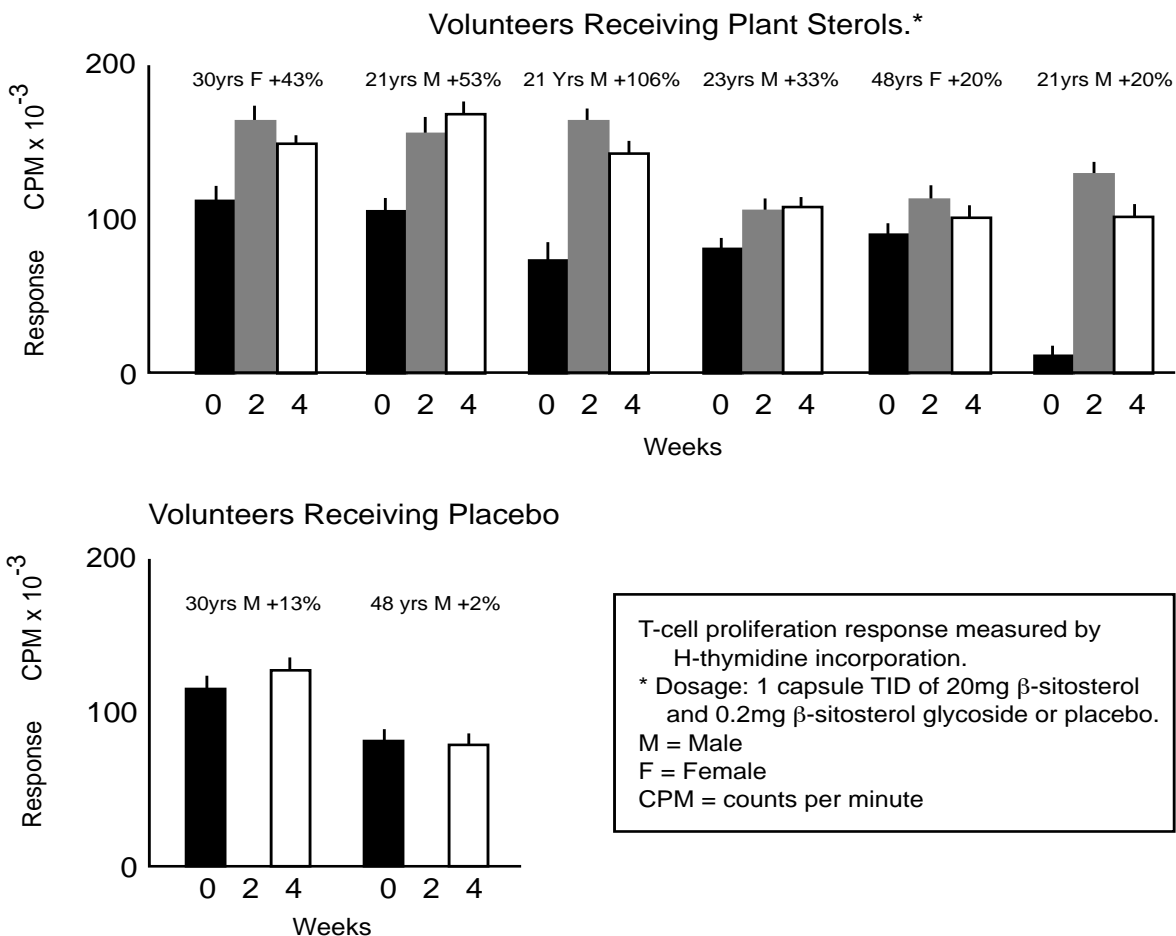
Phytosterols, particularly  $\beta$ -sitosterol and its glycoside  $\beta$ -sitosterolin, have been shown to have immunomodulating properties, preserving T<sub>H</sub>1 cell-mediated immunity while lowering elevated T<sub>H</sub>2 production of inflammatory cytokines.<sup>69</sup>

### **Clinical Trials**

Bouic<sup>64</sup> evaluated the immune-stimulating effect of a combination of  $\beta$ -sitosterol and  $\beta$ -sitosterol glycoside on human lymphocytes, both *in vitro* and *in vivo*. Blood was obtained from eight healthy volunteers on 60 mg  $\beta$ -sitosterol and 0.6 mg  $\beta$ -sitosterol glycoside after two and four weeks. Figure 3 illustrates the T-cell proliferation in six healthy subjects after four weeks of  $\beta$ -sitosterol. Individual increases in T-cell proliferation ranged from 20-920 percent. The sitosterol compound also enhanced *in vitro* production of IFN- $\gamma$ , IL-2, and NK cell activity.

An open trial of  $\beta$ -sitosterol and  $\beta$ -sitosterol glycoside in HIV+ patients for 36 months resulted in a stabilization of CD4+ counts for 24 months, and a significant decrease in IL-6, the cytokine implicated in the induction of HIV replication in infected cells.<sup>70</sup> As a result of preliminary trials, current trials in treating HCV are currently in progress in South Africa.<sup>71</sup>

**Figure 3. Phytosterols: Effect on T-cell Proliferation.**



### Antioxidants in the Treatment of Hepatitis C

Recent evidence has shown oxidative stress and lipid peroxidation play a major role in the fatty liver accumulation (steatosis) that leads to necroinflammation and necrosis of hepatic cells.<sup>72,73</sup> Necrosis, both the piecemeal and bridging types, are associated with a poor prognosis in chronic hepatitis.<sup>74</sup> Fatty tissue accumulation in the liver increases the potential for oxidative stress to trigger lipid peroxidation, leading to cytotoxic intermediates that induce inflammation and fibrosis via immunological pathways.<sup>75</sup> Both in alcoholic and nonalcoholic hepatitis, steatosis (fatty tissue accumulation) and the

lipid peroxidation that follows can lead to activation of stellate cells, the principal cells in the liver responsible for fibrogenesis and, ultimately, cirrhosis.<sup>76</sup>

Understanding the role of lipid peroxidation in liver disease has led to studies using antioxidant therapy in a variety of hepatic disease states. Alcohol-induced hepatitis has a free radical related pathogenesis. Wenzel studied a group of 56 patients with acute alcohol-induced toxic hepatitis. Half of them (n=31) were given 600 mg d-alpha tocopherol, 200 mcg selenium, and 12 mg zinc. This protocol lowered the levels of bilirubin, ammonia, and malondialdehyde (a marker of hepatic free radical production) significantly



when compared to the control group. The hospital stay of the supplemented group was reduced by six days and the mortality was reduced to 6.5 percent (2 of 31 patients) compared to 40 percent (10 of 25 patients) in the control group.<sup>77</sup>

Antioxidants have also been used in combination with interferon alfa2 in children with acute hepatitis B. One study looked at 73 children with acute hepatitis B given tocopherol and interferon alfa2 simultaneously, and found significantly shorter recovery times, higher levels of endogenous alpha-interferon, and a significant increase in the elimination of Hbe antigen with the addition of vitamin E.<sup>78</sup>

Studies using antioxidants in hepatitis C have focused on the effect of a variety of antioxidants, both nutrients and botanicals.<sup>79</sup> Beloqui treated 24 patients with chronic hepatitis C, 14 who had shown no response to interferon after four months. The group was given 600 mg N-acetylcysteine three times daily for 5-6 months in addition to interferon. Serum ALT values steadily declined in all 14 subjects over the 5-6 month period and normalized in 41 percent of the group on combination therapy. The group previously receiving no treatment had no effect from the N-acetylcysteine after one month.

Hoglum<sup>80</sup> treated six patients who had failed interferon therapy and had evidence of fibrosis on liver biopsy. Stellate cell activation in the liver was subsequently measured by the presence of malondialdehyde protein adducts in the biopsy. Treatment with d-alpha tocopherol at the dosage of 1200 I.U. daily for eight weeks was found to stop the fibrogenesis initiated by stellate cell activation. The treatment did not, however, decrease ALT levels, viral titers, or the degree of hepatocellular inflammation.

## Glycyrrhizin

### Phytopharmacology

In Japan, glycyrrhizin has been an accepted treatment of chronic hepatitis for over 20 years.<sup>81</sup> Glycyrrhizin is a conjugate of glycyrrhetic acid and glucuronic acid. Orally administered glycyrrhizin is metabolized in the intestine to glycyrrhetic acid, while intravenous glycyrrhizin cannot be metabolized to glycyrrhetic acid until it is excreted through the bile into the intestines (Figure 4).<sup>82</sup> Both glycyrrhizin and glycyrrhetic acid have been found to possess antiviral activity. In *in vitro* studies Nose<sup>83</sup> found smaller doses of glycyrrhetic acid (5 micrograms/mL) were as effective as larger doses of glycyrrhizin (1000 micrograms/mL) in lowering transaminase levels. On the other hand, in other studies, using murine IFN- $\gamma$  production as a measure of immune modulation, glycyrrhizin was more effective than glycyrrhetic acid.<sup>84</sup>

The first evidence of glycyrrhizin's antiviral effect was found in 1977 in culture studies with herpes simplex virus type 1.<sup>85</sup> In 1990, Crance<sup>86</sup> found complete inhibition of hepatitis A virus antigen expression at concentrations of 1000 and 2000 mcg/mL. The mechanism of glycyrrhizin's antiviral effect was later discovered not to be direct viral inhibition, as previously thought, but inhibition of the virus's ability to penetrate the human hepatocyte. The hepatitis A virus enters cells by the process of endocytosis, a process that glycyrrhizin interrupts by altering cell membrane penetrability.<sup>87</sup> Glycyrrhizin also appears to work as a free radical scavenger: studies with ischemia-reperfusion damage in rat liver (using pre-treatment with subcutaneous glycyrrhizin) significantly decreased lipid peroxides and transaminase levels.<sup>93</sup> As mentioned earlier, glycyrrhizin also acts via immune modulation: intravenous injections in mice induced IFN- $\gamma$  peaks and subcutaneous glycyrrhizin activated murine hepatic T-cells.<sup>88,89</sup>

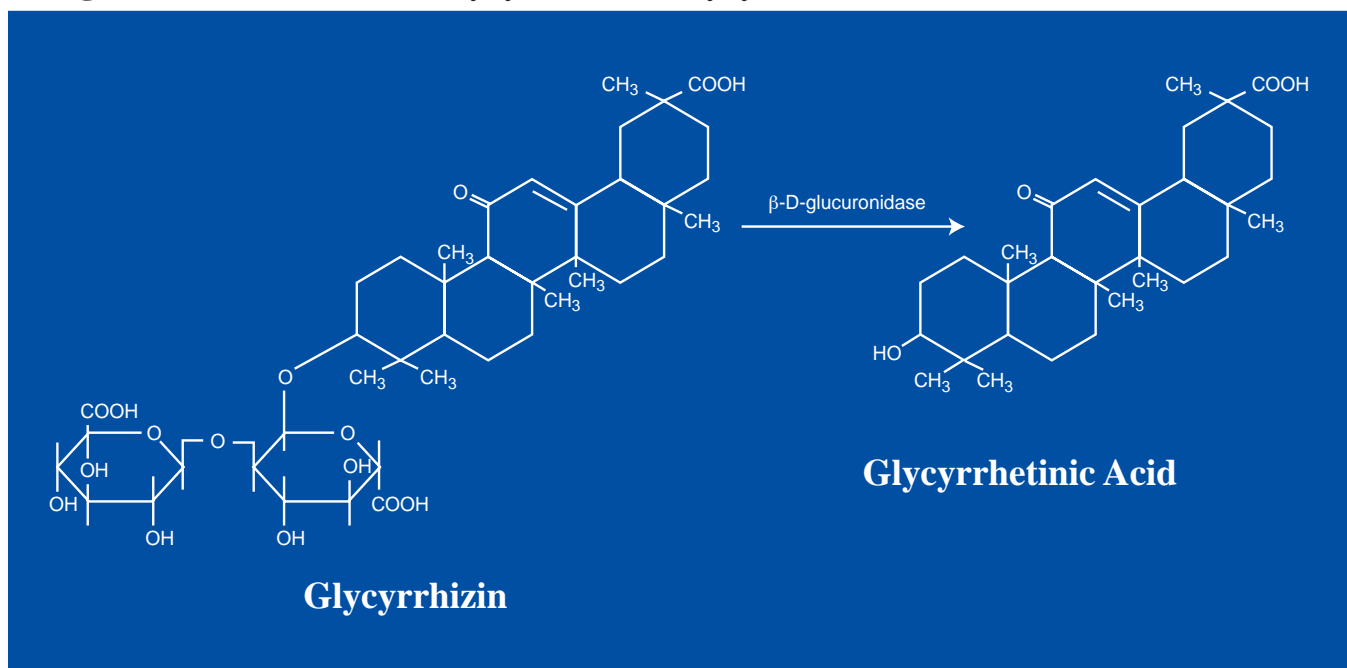
### Clinical Trials

The first randomized trial using intravenous glycyrrhizin was run in 1977 when Suzuki looked at its effect in 133 cases of chronic hepatitis B.<sup>90</sup> The glycyrrhizin was given as Stronger Neo Minophagen C (SNMC) — a solution of 2 mg glycyrrhizin, 1 mg cysteine, and 20 mg glycine per mL. Glycine was added to prevent pseudoaldosteronism, and cysteine was added to assist cysteine-related conjugation reactions in liver detoxification pathways. SNMC(40 mL) was administered

be reduced with a step-wise withdrawal of the daily eight-week dose of 100 mL.<sup>92</sup> A similar phenomenon of transaminase rebound is found after elimination of long-term therapy with ribavirin.<sup>93</sup>

A long-term trial with SNMC in patients with chronic hepatitis C included 84 patients who were given the medication between January 1979 and April 1984.<sup>94</sup> These patients were given 100 mL of SNMC intravenously daily for eight weeks and 2-7 times weekly for 2-16 years (median 10.1 years). They were compared to a control group

**Figure 4.** Metabolism of Glycyrrhizin to Glycyrrhetic Acid



intravenously daily for four weeks. Significant improvements were found in transaminase values and no side-effects were observed.

Later studies found improvements in liver histology: 39 of 45 hospitalized patients had histologically significant improvements in liver biopsy after eight weeks of SNMC at 100 mL daily.<sup>91</sup> Withdrawal of the SNMC caused a rebound of the transaminases which could

of 109 patients who, due to a lack of home health-care services, received only oral botanical and nutritional supplements. On follow-up the serum ALT levels fell to normal in 30 (35.7%) of the group receiving SNMC and in seven (6.4%) of the control group. The 15-year incidence of cirrhosis was 21 percent in the SNMC group and 37 percent in the control group. The incidence of HCC after 15

**Table 1.** Effect of Glycyrrhizin on Acute and Chronic Hepatitis.

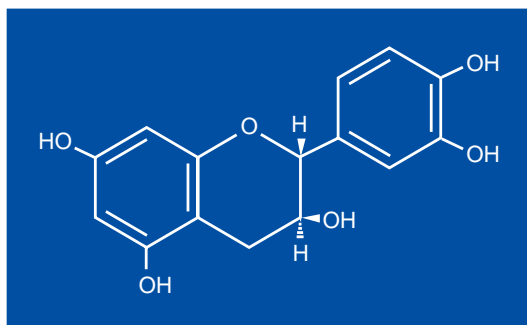
Effect	Acute Hepatitis		Chronic Hepatitis	
	Glycyrrhizin group n=20	Control group n=20	Glycyrrhizin group n=20	Control group n=20
Short-term clinical cure (all liver function tests normal)	17	7	15	2
Marked improvement (ALT < 200; TTT < 10; ZnTT < 14)	3	1	2	0
Improvement (but still ALT > 200; TTT > 10; ZnTTT > 14)	0	7	1	5
No effect	0	5	2	13
*HBsAg +/- before treatment	3/17	5/15	17/3	17/3
HBsAg +/- after treatment	1/19	5/15	9/11	17/3
**HBeAg +/- before treatment	1/19	2/18	10/10	14/6
HBeAg +/- after treatment	0/20	2/18	5/15	14/6

\*HBsAg = Hepatitis B Surface Antigen  
\*\*HBeAg = Hepatitis B Envelope Antigen

years of treatment was 12 percent in the SNMC group and 25 percent in the control group. In this study, patients treated with SNMC for 10 years had incidences of HCC comparable to Japanese interferon-treated patients. The incidence of HCC in lymphoblastoid interferon-treated hepatitis C patients in the

same Japanese hospital was 0.1%, 0.6% and 1.5% per year (for the histologic stages I, II, and III, respectively). The incidence in the SNMC patients was 0.3% for Stage I and II, and 1.3% for stage III.<sup>104</sup> Side-effects related to hypokalemia (10.7%) and hypertension (3.6%) necessitated the use of spironolactone.

**Figure 5.** (+)-Catechin;  
(+)-Cyanidanol; (+)-Cyanidan-3-ol



No one in the study discontinued medication as a result of side-effects. Several questions arise as a result of the data on SNMC; for example, it is not clear to what extent the cysteine and glycine contributed to the positive effects of the glycyrrhizin.

While the metabolism of oral glycyrrhizin is mediated by intestinal bacteria and results in enzymatic conversion to glycyrrhetic acid (Figure 4), both oral and intravenous routes of administration appear to have hepatoprotective properties. Eighty subjects with acute or chronic hepatitis B were given either oral doses of 7.5 g crude glycyrrhiza root, concentrated to contain 750 mg glycyrrhizin, (30 days for the acute group and 90 days for the chronic group; n=20 in each group), and compared to identical groups receiving conventional treatment of inosine plus Poly I:C.<sup>95</sup> Results showed significantly more marked improvement in indices of liver function and negative conversion of HbsAg and HbeAg in the glycyrrhizin group than in the control group (Table 1). In fact, none of the patients in either control group seroconverted. In the glycyrrhizin groups, indicators of liver function returned to normal in 85 percent of subjects with acute hepatitis and 75 percent of those with chronic hepatitis, compared to 35 percent and 10 percent, respectively, in the control groups.

### Catechins

Catechins are a class of flavonoids with hepatoprotective activity. Early research in animals has shown their ability to decrease the hepatotoxicity of ethanol, carbon tetrachloride, phalloidin, and other toxic compounds in rat hepatic tissue.<sup>96</sup> Numerous animal studies have

also demonstrated catechins' antioxidant effects (including the inhibition of lipid peroxidation) and ability to stimulate cell-mediated immunity.<sup>97</sup>

(+)-Cyanidanol-3 is pure catechin (trade names – Catergen, Kanebo, Zyma) derived

from *Uncaria gambir* (Figure 5). In the early 1980s it was the subject of extensive study in the United Kingdom and other parts of Europe as a potential treatment for alcoholic hepatitis.<sup>98</sup> Results were disappointing; evidence for alteration in the course of alcohol-related liver disease was not evident, even at dosages of 2-3 grams daily for six months.<sup>99</sup>

The research on its use in viral hepatitis has been more promising. A double-blind trial compared 3 gm catechin daily (n=58) with placebo (n=66) for 50 days in 124 patients with acute viral hepatitis of various types. Serum AST, ALT, and serum bilirubin were tested every five days. There was a significant difference in effectiveness depending on the type of hepatitis being treated. For patients with hepatitis C the serum AST and ALT levels were significantly lower in the catechin group than the placebo group from the fortieth day on. There were moderate but significant differences in the hepatitis B group in favor of catechin, and no significant difference in the hepatitis A group.<sup>100</sup>

In other clinical trials of acute hepatitis B, catechin was shown to lower serum bilirubin and transaminase levels, and accelerate the disappearance of HbsAg.<sup>101,102</sup> Trials using catechin in chronic hepatitis showed improvements in liver histologies<sup>103</sup> and inhibition of liver lipid peroxidation.<sup>104</sup> In a study with 338 chronic hepatitis B patients, Suzuki<sup>105</sup> showed catechin increased the rates of disappearance of HbeAg in chronic

hepatitis. In this trial, 174 patients received catechin at a dose of 1.5 grams daily for two weeks, followed by 2.25 grams daily for 14 weeks. The HbeAg titer decreased by at least 50 percent in 44/144 patients, and the HbeAg disappeared in 16/144 catechin patients compared to 4/140 placebo patients. The nutrient was well tolerated with the only appreciable side-effect being a transient febrile reaction in 13 patients.

There have been reports of six patients with catechin-related hemolytic anemia, all with demonstrable red blood cell (RBC) antibodies.<sup>106</sup> The hemolysis resolved after discontinuation of the drug and did not return even though autoantibodies to RBCs were still detectable in the blood. Other researchers have reported incidents of hemolysis in patients on catechin but the symptoms remit when the drug is withdrawn and no sequelae or fatalities have been documented.<sup>107</sup> This data, however, was collected from patients who were taking a highly purified pharmaceutical form of catechin (AKA cyanidanol) at a dose of 1-2 grams per day, duplicating the average dose used in treating hepatitis. In the United States, whole botanical sources of catechins, from *Uncaria gambir* for example, are typically used. Catechin content of gambir ranges from 2-10 percent. While these doses are unlikely to result in side-effects, it remains to be seen whether lower concentrations of catechin will afford the same effectiveness as the purified form.

### Silymarin

The pharmacokinetics of the flavonolignans in *Silybum marianum* have been thoroughly reviewed previously in this journal.<sup>108</sup> Due to the antioxidant, antilipid peroxidation, and antifibrotic actions of the silymarin complex, the main component, silybin, has been evaluated for its potential in treating both acute and chronic hepatitis. Multiple trials have shown silymarin, in doses of

70 mg three times daily, can accelerate recovery from acute, symptomatic states in chronic hepatitis, and result in an accelerated return to normal of liver enzyme levels.<sup>109</sup> In a trial assessing the effect of a silybin/phosphatidylcholine complex in chronic hepatitis, eight patients with chronic hepatitis B and/or C were given a silybin/phosphatidylcholine complex equivalent to 120 mg silybin twice daily for 60 days.<sup>110</sup> At the end of the 60-day period, levels of AST, ALT, GGT, total bilirubin, and serum malondialdehyde (a marker of lipid peroxidation in hepatic tissue) were all significantly reduced. The levels of GEC (galactose elimination capacity, a marker for hepatic metabolic activity) were significantly elevated. The therapeutic action in this study was believed to be a result of the botanical complex's ability to stabilize cell membranes by decreasing the phospholipid turnover rate.

The silybin/phosphatidylcholine complex has also been evaluated for a dose-response relationship in a phase-II randomized, open trial in patients with either alcoholic or non-alcoholic chronic hepatitis.<sup>111</sup> Differing doses of either 80 mg twice daily, 120 mg twice daily, or 120 mg three times daily were given to groups of 20 patients for two weeks. Four patients had to discontinue treatment due to gastrointestinal complaints. A statistically significant drop ( $P < .01-.001$ ) in ALT and GGT occurred at doses of 240 mg or 360 mg daily, but not 160 mg.

### Other Potential Botanicals

Several other botanicals hold promise as potential treatments for hepatitis C, although the research to this point has been primarily on hepatitis B. *In vitro* studies have found *Picrorhiza kurroa*, *Phyllanthus niruri*,<sup>112</sup> and *Phyllanthus amarus*<sup>113</sup> have anti-HBsAg activity. *Phyllanthus amarus* appears to exert its antiviral effect, at least in part, by down-regulating HBV mRNA transcription.<sup>114</sup> One of three species of *Phyllanthus* — *niruri*,

(n=42), *amarus* (n=11), and *urinaria* (n=35) — were tested on 123 patients with chronic hepatitis B. Thirty-five control patients received no herbal therapy. Patients receiving *Phyllanthus urinaria* were more likely to become HBeAg negative than those taking the other species.<sup>115</sup> Other studies have found minimal or no effect of *Phyllanthus amarus* on eradication of HBsAg in hepatitis B carriers.<sup>116,117</sup>

## Conclusion

Hepatitis C is a chronic viral infection that is currently treated with pharmaceuticals that have a high side-effect profile. Complementary/alternative therapies include antioxidants, and immunomodulatory and antiviral botanicals and plant extracts. A number of human studies point to their efficacy in treating hepatitis.

Although some of the botanicals discussed in this review have only been evaluated in hepatitis B, there is reason to think that they may have applicability in hepatitis C. Even though the two viruses belong to separate families (hepatitis B is a member of the genus, *Hepadnaviridae*, and hepatitis C, the *Flaviviridae* genus), they are both RNA viruses, and similarities exist in the pathology of chronic hepatitis B and C. Similar to hepatitis C, pathological effects of acute and chronic hepatitis B are not as much the result of the cytotoxicity of the virus, but due more to the host defense mechanisms against the virus.<sup>118</sup> This hypothesis is supported by the evidence in patients with high viral loads who have minimal liver disease and in patients with undetectable viral loads and strong T-cell responses who have severe liver disease.<sup>119</sup> Treatment for both types of chronic hepatitis includes interferon alfa, although it is more effective in chronic hepatitis B, where it appears to be successful in 30-40 percent of chronic infections of adult acquisition.<sup>118</sup> Interferon alfa is both directly antiviral and

immunomodulating, increasing both natural killer cell populations and major histocompatibility complex class I.<sup>120</sup> The similarity here is one of pathogenesis — substances that are both antiviral and immunomodulatory, and which have an anti-inflammatory effect on hepatocytes, may be effective in both types of chronic hepatitis. However, only long-term randomized trials of specific botanicals in chronic HCV using hepatocellular damage and serum markers as end-points will provide conclusive evidence of efficacy.

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