# Hepatotoxicity of Herbals and Dietary Supplements

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INTRODUCTION

Centuries before globalization and the ability to travel widely, indigenous peoples relied for treatment of diseases on what is now referred to as alternative medicine. This included among others, prayer, animal sacrifice, incantations, scarifications, massage, body manipulations, acupressure, acupuncture, and moxibustion (burning mugwort near an acupuncture site). In addition, virtually all peoples of the world utilized products derived from plants and trees for medicinal purposes, a practice dating back centuries, especially in the Far East. Herbals used for treatment were, and continue to be, extracted from the seeds, roots, stems, leaves, berries, barks, and flowers of plants and trees, which vary by region, depending on the local weather, geographic location, geographic elevation, and other conditions [1].

Westernized, pharmaceutically developed medications began to be introduced only a little more than a century ago and, indeed, some drugs, such as digitalis, ephedrine, ipecac, quinine, salicylic acid, and others, including more recently, vincristine and camptothecin, are actually derived from plants. Over the ensuing century, there has been an explosive increase in the numbers and types of commercial drugs developed through chemical and molecular biologic manipulation and synthesis that are evaluated in well-controlled trials to determine their effectiveness and safety, thus clearly revolutionizing medical care. Patients have obviously welcomed and benefitted from these new medicinal discoveries, but some have expressed concern about the often high cost and the fact that a number of drugs are associated with uncomfortable and sometimes serious side effects.

For these reasons, and because many consumers feel that general medical care has become too complex, time restricted, and seemingly less receptive, some have elected to assume greater personal care of their health by turning to the use of complementary and alternative medicine (CAM), which includes the use of herbals and dietary supplements (HDS); their belief is that herbals, having been used for centuries, must be effective and safe. Some will use only herbals for their medical care, referred to as alternative medicine, while others will take them together with commercial drugs, referred to as complementary medicine, hence the term CAM. A recent review of the published data aimed at determining expectations among proponents of CAM use found that the goals of users, in order of prevalence, are to influence the natural history of their disease, promote well-being, reduce side effects, take control of one’s health, relieve symptoms, boost the immune system, provide emotional support, improve their quality of life, cope better with illness, support natural healing, and others [2].

EPIDEMIOLOGY OF AND EXPENDITURE ON HERBALS AND DIETARY SUPPLEMENTS

Prevalence of Complementary and Alternative Medicine Use in the United States

Numerous surveys have been undertaken in the United States and elsewhere to determine the prevalence of use of HDS in the general public and among those with various medical conditions. A telephone survey performed in the United States in 1990 revealed that 34% of the general population was currently utilizing CAM [3], with 2.5% using herbal medicines, and that 67.6% had used at least one CAM therapy during their lifetime [4]. A follow-up survey in 1997 by the same investigators found that the frequency of CAM use had increased to 42%, with 12.1% now admitting to use of herbals [5]. Information has also come from reviews of the National Health and Nutrition Examination Survey (NHANES) databases. In the NHANES I study, covering the period 1971–1974, 23% of respondents reported using vitamin supplements [6].

In the NHANES II study of 1976–1980, the figure for users of supplements was 35% [7]. The figure in the NHANES III survey (1988–1994) rose to 30–42% for men and 42–55% for women [8], while for NHANES IV, covering 1999–2000, the overall figure was 52% [9]. Another source of information in the United States is data from the National Health Interview Survey (NHIS), which covers the noninstitutionalized US civilian population. In this survey of 1999, 28.9% of adults admitted to using at least one CAM therapy in the preceding year, with 9.6% representing herbal medicines [10]. A survey of this database 3 years later revealed that 19% of participants had used herbs or supplements in the previous year, from which it was estimated that 38.2 million US adults had taken herbs or supplements during 2002, with their use being significantly higher in women than men [11].

A far higher frequency of dietary supplement use in the same year (2002) was found in a Health and Dietary Survey sponsored by the US Food and Drug Administration (FDA): 73% of the noninstitutionalized US adult population admitted the use of dietary supplements in the preceding 12 months, with approximately half using herbs or botanicals [12]. Despite variations in the reported rates of HDS use, the data collectively indicate that their use has been increasing in the United States over time. This is noteworthy, since in virtually all surveys, patients are reluctant to inform their health care
providers of their use of CAM therapies unless specifically asked and, even then, somewhat grudgingly. Hence, for reasons to be discussed, it is essential for medical providers to consistently and nondisparagingly inquire whether their patients are presently or have in the past used herbal products, what the products are, and where they were acquired.

Prevalence of Complementary and Alternative Medicine Use in Other Parts of the World

CAM use is, of course, practiced worldwide, and in some countries represents the primary form of medical care. General surveys from Europe attest to the high frequency of herbal use in that continent [13,14], including reports from such countries as Germany [15], Italy [16], Sweden [17], and Spain [18]. High usage is also reported from the Middle East, including Jordan [19], the United Arab Emirates [20], Turkey [21], and Israel [22]. Not surprising is the high frequency of use in the Far East [23–26] and Africa [27–30]; a high frequency has also been reported in Australia [31]. Fact sheets from the World Health Organization (WHO) indicate that in some Asian and African countries, 80% of the population depend on traditional medicine for their primary health care [32].

Complementary and Alternative Medicine Use for Chronic Illnesses

A large body of published data also attests to the frequent use of CAM, including herbas, among persons with a variety of chronic diseases [33]. This includes, but is not limited to, those with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome [27,34], rheumatologic disease [35], cancers [29,36], chronic pain [37], cardiovascular disease [38], diabetes [39], and liver diseases [40–46]. Regarding liver disease, 23% of enrollees in a long-term treatment trial of persons with chronic hepatitis C admitted to their current use (an additional 21% having used them in the past), despite having previously suffered uncomfortable antiviral treatment and now committing themselves to another 3.5 years of treatment with pegylated interferon [42].

Expenditure on Complementary and Alternative Medicine Use

The financial outlay on CAM therapies in the United States, mostly out-of-pocket expenses, is vast. An analysis conducted in 1997 conservatively estimated that expenditure on CAM therapies was US$27 billion, taking into account the cost of both purchasing herbal products and vitamin and CAM professional services [5]. This amount was said to be comparable to the total out-of-pocket expenditures for all physician services. The estimated expenditure 7 years earlier had been US$14.6 billion. In 2007, the estimate had risen to US$33.9 billion in the United States, based on analyses performed at the National Center for Complementary and Alternative Medicine (NCCAM) and the National Institutes of Health (NIH), using data derived from the NHIS [47]. Placing these data in context, the researchers noted that the cost to purchase natural products was “equivalent to about 1/3 of total out-of-pocket spending on prescription drugs,” and that the cost for “CAM practitioner visits was equivalent to approximately 1/4 of total out-of-pocket spending on physician visits.” In their estimate, in 2007 38% of adults and 12% of children in the United States had utilized some form of CAM [47]. More recently, data published by the American Botanical Council showed that during the period 1999–2009 there had been an estimated increase in sales and monetary outlay for herbals in the United States every year in that decade, except for 2002 and 2003 (Table 35-1) [48].

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<th>Year</th>
<th>US$ Total Sales (Billions)</th>
<th>% Change</th>
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<tr>
<td>1999</td>
<td>4.110</td>
<td>+2.7</td>
</tr>
<tr>
<td>2000</td>
<td>4.230</td>
<td>+2.9</td>
</tr>
<tr>
<td>2001</td>
<td>4.356</td>
<td>+3.0</td>
</tr>
<tr>
<td>2002</td>
<td>4.278</td>
<td>-2.7</td>
</tr>
<tr>
<td>2003</td>
<td>4.146</td>
<td>-2.2</td>
</tr>
<tr>
<td>2004</td>
<td>4.290</td>
<td>+3.5</td>
</tr>
<tr>
<td>2005</td>
<td>4.381</td>
<td>+2.1</td>
</tr>
<tr>
<td>2006</td>
<td>4.561</td>
<td>+4.1</td>
</tr>
<tr>
<td>2007</td>
<td>4.759</td>
<td>+4.3</td>
</tr>
<tr>
<td>2008</td>
<td>4.800</td>
<td>+0.9</td>
</tr>
<tr>
<td>2009</td>
<td>5.030</td>
<td>+4.8</td>
</tr>
<tr>
<td>2010</td>
<td>5.200</td>
<td>+3.3</td>
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for healing purposes have also been used in the distant past by Greeks, Egyptians, Indians, and indigenous peoples from Africa and the South Sea Islands and by Native Americans. Unlike Western medicines that are generally designed to treat a specific disease or set of symptoms, the traditional use of herbals is meant to have a broad impact on strengthening the body’s ability to deal with a variety of illnesses. Thus, herbalism is an art rather than a science. Herbals in the traditional Chinese medicine (TCM) culture have consisted of single products believed, through trial and error, to contain pharmacological dosages of diethylstilbestrol [62], indomethacin [63], and warfarin [63,64] and herbal products marketed to treat erectile dysfunction, found to be contaminated with phosphodiesterase type 5 inhibitors [65]. A disturbing finding from the NHANES data obtained between 1999 and 2004 was that blood levels of lead were significantly higher in women who used herbal supplements than among nonusers, the relative difference being 10–24% higher [66].

REGULATION OF DIETARY SUPPLEMENTS

The United States

Preceded by a vigorous and sometimes rancorous debate among stakeholders, the US Congress promulgated the Dietary Supplement and Education Act of 1994 (DSHEA), signed into law on 15 October 1994, during the waning moments of the 103rd Congress. Prior to that time, the FDA permitted manufacturers of drugs but not conventional food and dietary supplements to label their products with claims of disease prevention, mitigation, treatment, or cure. The Act was therefore viewed by the supplement industry as a positive step, since the FDA would now regulate dietary supplements and its ingredients differently from those regulating conventional foods and drugs. The manufacturers of dietary supplements would themselves become responsible for ensuring that supplements and their ingredients were safe before its marketing. Furthermore, manufacturers would not need to register their product with the FDA or get FDA approval before producing or selling their supplements. Manufacturers did have to ensure that their product labeling information was truthful and not misleading and that it complied with current good manufacturing practices (cGMP) for quality control. The role left to the FDA was to take action against any unsafe dietary supplement product, but only once it reached the market. Accordingly, the manufacturer, packager, or distributor of dietary supplements marketed in the United States would be required to notify the FDA of all adverse events associated
with the use of the dietary supplement. Unfortunately, it is estimated that less than 1% of instances of adverse events caused by herbals and dietary supplements are in fact reported to the FDA (Inspector General, Department of Health and Human Services; Government Accountability Office Report to Congressional Requesters, January 2009, Dietary Supplements: FDA Should Take Further Actions to Improve Oversight and Consumer Understanding). In this regard, in a survey published in 2001 [67], while a large proportion of interviewed persons admitted using supplements, most did not inform their regular physicians of this use because they believed that their physicians knew little about these products or were biased against their use, and many were convinced of their value and would continue to use them even if scientifically conducted studies did not prove their effectiveness. However, the majority believed that the FDA should be permitted to review the safety of these products prior to their distribution and, if found unsafe, that they should be removed from use, and furthermore that government regulation should be increased to ensure that claims of safety were in fact true. In 2007, the FDA required a modification to the cGMPs regarding manufacturing, packaging, labeling, and holding operations for dietary supplements and a Final Rule was published on 22 June 2007. More recently, the FDA has developed draft guidance for industry regarding new dietary ingredients notification. Needless to say, there continues to be controversy about the Act, including the question of what actually constitutes a dietary supplement and what represents a new dietary ingredient [68].

Other Regions

Other regions that have had or have begun to develop regulations regarding herbals include the European Union, the United Kingdom, and Canada.

European Union

Current regulations are based on the Traditional Herbals Medicine Products Directive 2004/24/EC announced on 31 March 2004 [69]. The intent was that all traditional medicines in health food shops and pharmacies must be formally registered and the products approved before they could be sold. Acceptable products for licensure were considered to be those whose use was “plausible on the basis of long-standing use and experience” and whose quality and safety could be guaranteed. The duration required to prove their safety was at least 30 years in all, and at least 15 years in the EU. The Directive allowed the passage of 7 years from its announcement for manufacturers to gather the necessary information on their products and on 1 May 2011, the requirement that herbal medicines and their ingredients be registered with evidence of safety went into effect [69]. Based on this Directive, no herbals from China would meet the specifications and they are therefore currently banned in the EU.

United Kingdom

The Herbal Medicines Advisory Committee was established in 2005 to advise on the safety, quality, and efficacy of herbal medicinal products for human use. Although there is no requirement for proof of efficacy, pharmacological effects must be plausible and supported by long-standing use and experience. The herbal medicinal product must be eligible under the Traditional Herbals Medicine Products Directive 2004/24/EC [70]. Prior to this, regulation of the herbal industry was covered by the 1968 Medicines Act. Under the current act, suspected adverse reactions to herbal medicines are reported voluntarily, but are compulsory for manufacturers with registration of a product under the Traditional Herbal Medicines Registration Scheme that has published guidelines for retailers, wholesalers, importers, and manufacturers on its requirements [71].

Canada

Consultations with various stakeholders led to the creation of the Natural Health Products Regulation that addresses the availability and safety of natural health products. All natural health products must have a product license and manufacturers, packagers, labelers, and importers must have site licenses. Licensing requires specific labeling and packaging requirements, cGMP, and evidence of safety and efficacy.

Global Regulatory Environment for Herbal Medicinal Products

The WHO, in response to the proliferation of herbal medicine use in many countries, both developed and underdeveloped, as well as the safety concerns surrounding herbal medicines raised by its member countries, conducted a survey of the 191 member states in 2001 that explored policies, regulations, and areas for more action in the arena of traditional and complementary and alternative medications (TM/CAM). Among 141 respondents, only 45 (32%) reported having a policy on TM/CAM; these policies are loosely defined and include laws, regulations, and consideration of intellectual property issues. Regarding regulations for herbal medicines, 53 (37%) respondents reported having laws and regulations in place as of 2003, although the majority (86 of the 141 respondents;
THE USE OF HERBAL PRODUCTS TO TREAT LIVER DISEASE

Philosophy

There are vast differences in the views of Western and Eastern practitioners regarding the philosophy of health care administration. The Western approach to treatment consists largely of the administration of drugs or the performance of surgery targeting a specific condition or disease. It requires defining the etiology and pathogenesis of the disease, developing and utilizing medications directed specifically at the illness, and conducting rigorous, scientifically defined studies to establish the efficacy and safety of medications; thus, treatment directed at the disease is evidence based. In contrast, the Eastern approaches are integrative and holistic, directed at the body and mind rather than the disease, and based on the view that in order to preserve health, the vital energy that maintains life must flow freely through the entire body [73]. Ideally, with regard to the use of herbals, treatment is administered by an experienced local healer whose knowledge derives from information passed from generation to generation.

In the West, people tend to use herbals or dietary supplements largely to maintain or improve their well-being or health, for bodybuilding purposes, or to treat chronic rather than acute illness; the focus on chronic diseases is because few conventional medications actually cure chronic diseases and many are associated with uncomfortable or even serious adverse effects. Moreover, cost issues can represent a burden for developing countries. The focus on herbals comes from the belief that, having been used for centuries, they are likely to be effective but, more importantly, that being natural they must surely be safe. Western practitioners, however, tend to decry this alternative approach, particularly the use of herbals, because of their seeming unscientific origins, the lack of well-controlled trials to prove their efficacy and safety, the concern of contamination and adulteration, and the not uncommon proclamation of a miracle cure, often delivered as a testimonial. Unsurprisingly, therefore, herbal users tend not to inform their conventional health care practitioners of this fact for fear of being disparaged or reprimanded. However, in view of the prolific use of herbals, because they are sometimes used as a substitute for potentially curative conventional medication by persons with serious disease, and because many have been associated with severe, life-threatening side effects, it is essential that conventional health care practitioners educate themselves about this form of health care and be willing to considerably question their patients about its utilization or advise them on how to use herbals safely.

While HDS are most often employed in Western countries to improve or sustain well-being and general health, for weight reduction, and for muscle-building purposes by bodybuilders, the present discussion will be confined to their recent use for the treatment of liver diseases, predominantly viral hepatitis.

Herbals to Treat Viral Hepatitis

Following the discoveries of the hepatitis A, B, and C viruses (HAV, HBV, and HCV), pharmaceutical companies attempted to identify and evaluate medications in well-designed, controlled trials aimed largely at treating patients with chronic hepatitis B and C. A number of drugs developed clearly represented vitally important advances in the management of these chronic liver diseases but were initially found to be effective in only a limited number of patients, were associated with frequent serious side effects, and their costs were extremely high and, hence, could not be afforded by many affected individuals. Accordingly, viral hepatitis became and continues to be a prime target for self-treatment with herbals.

Various herbal products have been evaluated for treating viral hepatitis, the most prominent being silymarin, glycyrrhizin, Japanese traditional medicine (Kampo medicine; TJ-9, Sho-Saiko-To), TCM, and Phyllanthus amarus [43,74]. Unfortunately, the adherence to generally accepted scientific methods to evaluate drug therapy has been rare; few studies have been conducted as randomized, blinded, controlled trials or have estimated sample size or defined end points ahead of time, have ensured a homogeneous population or disease characteristics, or have measured outcome using appropriate serological tests or other biomarkers, let alone establishing hard end points such as histology or sustained viral clearance. Moreover, there is uncertainty about the quality of the herbals evaluated in studies, whether or not they are appropriately absorbed, and the adequacy of doses given. Finally, reporting of results has lacked uniformity and is often biased by the reporting of only positive results. In this regard, a group of investigators collaborated in the 1990s to develop standards for reporting results of randomized controlled trials, termed the Consolidated...
Standards of Reporting Trials (or CONSORT) Statement [75]. Although this effort focused on reporting conventional drug results, it did include creating a modified version for evaluating herbal medication [76,77].

**Silymarin (Milk Thistle)**

*Silybum marianum* has been used for centuries to treat liver and biliary disorders; in Europe, its use has focused on treating mushroom (*Amanita phalloides*) poisoning [78]. It is also the most common herbal product used by patients with chronic viral hepatitis [40–42]. Its main constituent is silymarin, consisting of four isomers: silybin A and B, isosilybin A and B, silichristin, and sildianin. Numerous studies indicate that it has antioxidant, antifibrotic, and antinflammatory activities, that it neutralizes free radicals and stabilizes cell membranes, and that it is cytoprotective [79–81]. Until recently, there has been no evidence that it has antiviral properties.

Two important early treatment trials brought attention to silymarin as a potential therapy for chronic liver disease, focusing on alcoholic cirrhosis [82,83]. The results of the two studies were, however, contradictory and data were compromised by high dropout rates and the lack of alcohol consumption during treatment. They were followed by a number of other randomized, controlled trials involving patients with alcoholic cirrhosis, acute and chronic viral hepatitis, and primary biliary cirrhosis, almost all administering treatments for only 1–2 months’ duration [43]. A Cochrane review of these trials concluded that silymarin had no significant effect on reducing fibrosis, morbidity, or mortality, but noted that the methodological qualities of the trials were low [84].

In 2007, a standardized silymarin extract (MK-001) was found for the first time to have antiviral activity against HCV [85]. In an in vitro study, standardized silymarin (MK-001) was shown to inhibit infection of human hepatoma Huh7 and Huh7.5.1 cells by the JFH1 (Japanese fulminant hepatitis)-1 virus, expression of tumor necrosis factor (TNF-α) in anti-CD3-stimulated peripheral blood mononuclear cells, and nuclear factor NF-kappa-B (NFkB)-dependent transcription in Huh7 cells. Thus, silymarin appeared to exert anti-inflammatory and antiviral effects. A key study, published 1 year later, reported that silibinin given intravenously dramatically reduced levels of HCV RNA in patients with chronic hepatitis C who had not responded to previous standard treatment with pegylated interferon and ribavirin [86]. This unexpected result was noted in two protocols that involved administering increasing doses of silibinin infused intravenously for 4 h for either 7 or 14 consecutive days, followed by pegylated interferon and ribavirin or by triple therapy for up to 24 or 48 weeks. The inhibitory effect disappeared, however, when the silibinin infusion was completed. Other than mild gastrointestinal symptoms, intravenous treatment with silymarin was well tolerated. A similar antiviral inhibitory effect on HCV and on inhibition of HIV replication was reported in patients coinfected with HIV plus HCV [87], and silymarin was also shown to prevent reinfec tion with HCV of a liver graft following liver transplantation [88]. Additional studies to determine the mechanistic effects of silymarin and silibinin on the HCV and on liver cell necrosis have continued to be performed, yielding improved knowledge of the compound and suggesting that it may have a future role in the treatment of patients with HCV infection [89–92].

On this basis, the NCCAM and the National Institute of Diabetes and Digestive and Kidney Disease, NIH, embarked on scientifically designed studies to evaluate silymarin to treat patients with chronic hepatitis C who had failed to respond to conventional pegylated interferon and ribavirin therapy, as well as for those with nonalcoholic steatohepatitis. Termed SyNCH, the study began as a phase I/II trial using a standardized milk thistle product (Legalon, Madaus AG, Frankfurt, Germany). The phase I study, designed to evaluate absorption characteristics and pharmacokinetics, and to determine effective doses, consisted of administering increasing oral doses of silymarin given at 8-h intervals for 7 days. Among its findings were that steady state exposures suggested nonlinear pharmacokinetics, that the product appeared to be safe, and that it did not produce a meaningful reduction in serum aminotransferase levels or reduce serum levels of HCV RNA at lower doses, but that higher doses may overcome the low bioavailability [93]. Unfortunately, recently reported results of the hepatitis C trial indicate that treatment with oral doses of silymarin, even when pushed to the limit of oral intake, did not lead to reduction of aminotransferase or HCV RNA levels [94]. Clearly, treating by intravenous infusion, although seemingly effective while the infusion continues, is not a viable treatment option. There may, however, be a role for silymarin as an adjunct to the new direct antiviral drugs that have or will become available in the future, or even as an addition to the older standard-of-care drugs, pegylated interferon and ribavirin, in parts of the world where the new drugs are unavailable or cannot be afforded. These possibilities will need to be investigated, however, in future well-controlled trials [95].

**Glycyrrhizin**

Derived from the root of the licorice plant, *Glycyrrhiza glabra*, glycyrrhizin contains glycyrrhetic acid, flavonoids, hydroxycoumarins, and β-sitosterol. It has been commonly used in Japan to treat chronic hepatitis, where it is referred to as Stronger
Neo-Minophagen C (SNMC); the parenteral preparation contains 2% glycyrrhizin, 0.1% cysteine, and 2% glycine [96, 97]. It appears to have antioxidant, anti-inflammatory, and immunosuppressive properties [98, 99]. A number of clinical trials have used this herbal to treat HCV infection, almost all consisting of open-label studies and involving short-term treatment, therefore most being of questionable quality [43: some involved the treatment of hepatitis C, some the treatment of hepatitis B, and at least one was for the treatment of hepatocellular carcinoma (HCC) in chronic hepatitis C. The main effect seen in these short-term studies is an improvement in serum aminotransferase levels without an effect on the HCV. Glycyrrhizin has to be given intravenously (although liquid, powder, and tablet formulations have recently become available but have not yet been fully evaluated in persons with liver disease) and, because of its mineralocorticoid properties, it can cause hypertension, fluid retention, and hypokalemia. Since only a few randomized controlled trials have been performed with this product, additional high quality trials are needed.

**Sho-Saiko-To (TJ-9)**

Used in Japan and China to treat chronic liver diseases, this is an herbal product containing seven herbs (Bupleurum root, ginger rhizome, Ginseng root, Glycyrrhiza root, jujube fruit, Pinellia tuber, and Scutellaria root) [100, 101]. Its name, Sho-Saiko-To, is Japanese and TJ-9 is its label within the Kampo system of medicine, which comprises certain Chinese medicinal herbal combinations adapted to Japanese preference and categorized for composition, indications for use, and presumed health effects. Within this system, another preparation, TJ-108, has an additional apparent antiviral constituent, Gomisin A [102]. Numerous experimental studies indicate that TJ-9 has antifibrotic properties through downregulating RNA expression of genes encoding procollagen alpha I and III, inhibiting TIMP (metalloproteinase inhibitor), reducing expression of α-smooth muscle actin, lipid peroxidation in hepatocytes, and proliferation and activation of hepatic stellate cells [103–106]. One randomized controlled trial and one pilot study with TJ-9 have been performed to treat patients with HBV infection [107, 108] and one randomized, controlled trial was conducted to prevent HCC in patients with chronic HBV infection [109]. Each study suggested some positive effect without being convincingly well performed. A number of serious adverse effects have been reported with this product [110–113]. Clearly, additional well-designed trials are still necessary to fully determine whether this product has any meaningful effect on viral hepatitis.

**Phyllanthus amarus**

Plants of the Phyllanthus genus, found in tropical and subtropical countries, contain alkaloids, flavonoids, lignans, and terpenes. They have been used in India and China to treat diabetes, diarrhea, hepatitis B, and urinary tract conditions [119]. In vitro studies using human cell lines infected with the hepatitis B and woodchuck hepatitis viruses have found that the herbal has an inhibitory effect on HBV polymerase activity and that it decreases mRNA transcription in hepatitis B transgenic mice [120–122]. Several studies have reported contradictory results using this herbal to treat patients with HBV infection: some reported loss of HBsAg and hepatitis B surface antigen (HBsAg), while others have failed to replicate these results. The positive effect, however, appeared more likely when Phyllanthus was used together with interferon [123–126]. Thus, it appears that Phyllanthus may have antiviral activity but, as is true for other herbal products, there is a need for reliable controlled trials to prove that it is indeed an effective product.

Numerous other herbal products have been studied as treatments for liver disease, including Ayurvedic medicines used in India (picrorrhiza and Liv-52), ellagic acid and curcumin, oxymatrin, and a wide array of Chinese herbal concoctions (Bing Gan Ling, Bing Gan Tang, Gansu, Qinggang and Bushen granules, Yi Er Gan Tang, and Yi Zhu decoction) [43, 127, 128]. Valid evidence of efficacy for all these products will remain uncertain until they are studied using appropriate scientific methods.
LIVER INJURY CAUSED BY HERBALS AND DIETARY SUPPLEMENTS

Although there is modestly encouraging but clearly unproven or uncertain evidence for the efficacy of HDS for the treatment of liver diseases—as has been hoped for by the public who use them—there is unquestioned evidence that they are sometimes responsible for causing liver injury, contrary to the belief of users. The real frequency of hepatotoxicity from HDS is unknown. Furthermore, the types of products that cause hepatotoxicity in the United States and their relative frequencies have been described only recently by the NIH-supported Drug-Induced Liver Injury Network (DILIN) study. In a preliminary review of data collected from 93 patients with hepatotoxicity attributable to HDSs prospectively enrolled into the DILIN over an 8-year period, the most commonly implicated products were supplements used for bodybuilding and for weight loss, representing 31% and 18%, respectively, of the 93 collected cases (unpublished). Regarding the bodybuilding supplements, all were men who experienced characteristically protracted periods of jaundice, with modest elevations in aminotransferase levels. In contrast, weight loss supplement injury occurred predominantly among females and was characterized by more prominent elevations in aminotransferase levels.

It seems prudent to distinguish between true herbal drugs and dietary supplements, of which the latter may or may not contain plant extracts, since the reasons for using each are different. Herbal drugs are used mostly to specifically treat or prevent certain diseases, while dietary supplements are usually consumed to compensate for existing or presumed nutrient deficiencies or to promote health in general. Thus, the discussion on hepatotoxicity will be presented in two sections, one focusing on herbal drugs for which adverse hepatic drug reactions have been described and the other describing nutritional supplements that have been found causative for or associated with instances of liver injury.

CAUSALITY ASSESSMENT FOR HEPATOTOXICITY DUE TO HERBALS AND DIETARY SUPPLEMENTS

As is the case for apparent liver injury from conventional medications and xenobiotics, there is no definitive means of diagnosing hepatotoxicity when the injury is thought to be attributable to HDS. Rather, an accurate diagnosis is predicated upon the clinician’s diagnostic acumen and on the willingness of the patient to be forthcoming about the use of products. Causality assessment, the process of determining whether there is a reasonable likelihood that a drug or herbal or dietary supplement is the cause of liver injury, is generally performed using one of several methods [129–134]. More importantly, the assessment must be conducted in a carefully defined methodical fashion (Fig. 35-1); the steps are similar whether the suspect is a conventional medication or an herbal or dietary supplement, with only minor modification. First, when liver injury is identified, the initial step is to obtain a thorough medical history surrounding the event that must include information on the contemporaneous receipt of all medications as well as of HDSs. An active inquiry into the use of HDS is critical since, as has already been stated, patients frequently fail to divulge the use of such products. Second, the use of a product must obviously have preceded the onset of injury but, in general, must have been started no more than a year earlier. Third, all other causes of liver injury must be excluded, including viral, autoimmune, hemodynamic/vascular, metabolic, and inherited diseases (Fig. 35-1). Fourth, attribution to a specific medicinal product is supported if there is improvement of the liver injury following cessation of the suspected product, referred to as dechallenge, with the caveat that, in rare instances, liver injury can be self-perpetuating. Finally, perhaps the most convincing indicator for attribution of injury to a specific product is if there is recrudescence of the injury following serendipitous or surreptitious resumption of that implicated product (rechallenge), often with a more vigorous presentation and more severe outcome. However, because of the possibly greater severity of the reaction, routine intentional rechallenge cannot be advocated.

A characteristic of drug-induced liver injury (DILI) that might be more helpful in implicating herbs than conventional medications is the pattern (hepatocellular, cholestatic, or mixed), duration, or severity of the observed liver injury. For example, bodybuilding supplements have been reported on numerous occasions to typically produce a cholestatic form of liver injury, characterized by protracted jaundice and liver biopsy findings revealing little inflammation but prominent bile pooling [135–137]. Given this extensive clinical experience—the identification of cholestatic liver disease in the setting of bodybuilding supplements—such a presentation permits firmly establishing causation with a high degree of confidence.

LIMITATIONS OF CAUSALITY ASSESSMENT FOR HEPATOTOXICITY DUE TO HERBALS AND DIETARY SUPPLEMENTS

Although several methods have been employed for determining causality for liver injury of conventional
medications, there has not been a distinctive or formal method developed to assess causality for liver injury attributable to HDS, although this is an issue of current investigation [138]. Developing a more specific causality assessment tool is important because there are several aspects of HDS use that can confound the process of causality assessment.

One challenging issue faced in performing causality assessment of possible HDS-induced liver injury is the potential for batch-to-batch, seasonal, and geographic variability in the composition of the product under consideration. This can cause the problem of misattribution, even though the causality assessment process suggests impugning a specific product, since the injurious ingredient or combination of ingredients may no longer be present.

Establishing the timing and duration of use of an agent is an important aspect of determining causality. Current methods of causality assessment assign a lower likelihood of attribution if the latency period between starting the product and the first evidence of liver injury is unusually prolonged. Because of the potential for variability of ingredients from batch to batch or for the introduction of new, more potent ingredients at variable times after initiating treatment, a reliance on latency may not always be relevant.

Finally, many HDSs are complex mixtures, containing a multiplicity of ingredients that make it nearly impossible to identify which specific ingredient is the cause of injury. There are also instances in which an ingredient thought to be harmful is excluded from a product, yet injury from this product continues. This occurred when liver injury was attributed to the commercial product Hydroxycut; the toxic ingredient was believed to be ephedra, which was therefore ordered by the FDA to be excluded from its formulations in 2003 [139–144]. However, cases of liver injury continued to occur, finally leading to its complete market recall in 2009. Clearly, improved detection methods are needed to unequivocally identify the toxic ingredient(s) in an herbal mixture responsible for causing the liver injury.

**HERBAL PRODUCTS ASSOCIATED WITH LIVER INJURY**

Herbals may damage the liver in a manner similar to that attributed to synthetic drugs and the liver injury can be variable, even with the same herbal. As with conventional drugs, there is no uniform mechanism by which injury develops, and risk factors that render an individual susceptible to herbs are known for only a minority of preparations. Hence, clinical signs, symptoms, and findings are identical to those encountered when liver injury is due to synthetic drugs. Clinicians are often misled by not inquiring about the use of herbals and may therefore pursue false lines of suspicion, particularly, if patients do not admit to taking herbals. Liver biopsy may be justified in some instances to assess the type and extent of liver injury, especially when the presentation is that of chronic liver disease, but liver histology lesions specific to herbals are only described for a very few remedies. A careful medication history with specific interrogation for the intake of natural, herbal, or unconventional medication is usually the key investigation, and many
published reports describe delayed recognition of her- 

bals as the true culprit in cases of DILI. A review of her-
bals that have been associated with liver damage is pro-
vided (Table 35-2).

**Herbals Containing Pyrrolizidine Alkaloids**

Among the first herbals found to occasionally cause severe hepatic injury were those containing pyrrolizi-
dine alkaloids such as echimidine, monocrotaline, retronecine, seneciphylline, and symphytum. Extracts from Crotalaria, Heliotropium, Senecio, and Symphytum (comfrey) species are particularly hepatotoxic, with a clear dose dependency. Comfrey (Symphytum officinale) is traditionally used to externally treat bruises and joint injuries with tinctures and ointments, but oral preparations are banned in Europe and North America. Exposure of humans to pyrrolizidine alkaloids occurs predominantly via contaminated food-stuffs such as salads, cereals, and honey [145]. As early as 1920, a syndrome of ascites, hepatomegaly, and eventually cirrhosis was described as Senecio disease in South Africa [146], and was followed by reports from Jamaica of children developing a similar disease after the ingestion of bush tea, which contains Crotalaria species [147]. Contamination of crops with Heliotropium was the reason for a series of pyrrolizidine alkaloid poisonings in India [148,149] and Afghanistan [150], and was followed by instances of liver damage from pyrrolizidine alkaloid intoxication in Arizona, such as in two infants after drinking herbal tea made of Senecio longilobus [151,152]. Similar cases have also been seen in Europe and elsewhere [153–155].

The type of liver injury provoked by pyrrolizidine alkaloids is sinusoidal obstruction syndrome (SOS; also known as hepatic venoocclusive disease), a non-thrombotic obliteration of the sinusoids and lumen of the terminal centrilobular hepatic veins. The resulting venous outflow obstruction causes hepatic congestion and parenchymal necrosis resulting in acute liver failure (ALF) or liver fibrosis and cirrhosis [156]. The mechanism of injury involves a direct toxic effect related to biotransformation of pyrrolizidine alkaloids by microsomal cytochrome P450 (CYP) enzymes into pyrrole derivatives, which then form genotoxic protein adducts [157]. Notably, pyrrolizidine alkaloid toxicity can be enhanced by phenobarbital, a potent inducer of CYP3A4, CYP2B6, and several isoenzymes of the 2C family. Acute toxicity is not species specific and an animal model of SOS using monocrotaline has been characterized [158]. Abundant experimental data also point to an oncogenic potential of comfrey and other pyrrolizidine alkaloid-containing herbs [159]. Treatment of affected patients is symptomatic, and spontaneous recovery upon dechallenge to pyrro-

**Chinese Herbs**

In TCM, the use of herbals dates back as far as 2100 BC and, along with the rising popularity of oriental medicine, their use is increasing worldwide. Most Chinese medicines are mixtures of several different herbs of which one or two are considered the pharmaco-
logically active King herbs, while the remaining con-
stituents enhance the effect of the King herb, alleviate toxicity, or support circumstances considered impor-
tant for regaining health. More than 13,000 different herbal preparations are used in TCM, which makes it extremely difficult to identify the component responsible for the liver injury. Active or toxic ingredi-
ents may vary when plants are harvested during different seasons or extracted through variable proce-
dures. Also, contamination of herbals with microor-
organisms, pesticides, heavy metals, fungal toxins such as aflatoxin, and synthetic drugs has been described [160].

In the United States, large-scale consumption of Jin Bu Huan (Lycopodium serratum) for mild sedative effects has precipitated a total of 11 cases of both acute and chronic hepatitis [161,162]. Unlike many TCM herbal mixtures, Jin Bu Huan is an extract from Lycopodium serratum and (−)-tetrahydropalmiditine is the active ingredient, with opiate-like properties. The precise mechanism by which liver injury is precipitated is currently unclear, but (−)-tetrahydropalmiditine is struc-
turally similar to pyrrolizidine alkaloids (see above). However, liver histology in patients who took Jin Bu Huan showed focal necrosis and portal fibrosis, but no vascular lesions. In all published cases, increased ami-
notransferase levels have normalized after treatment discon-
continuation.

A number of dietary products marketed in the United States contain ma huang (Ephedra spp.) to sup-
port weight reduction. Apart from cardiotoxicity, sev-
eral published reports provide evidence for the risk of hepatotoxicity following the ingestion of ma huang [163–166]. Typically, acute hepatitis occurs after a short period of intake, sometimes with elevated auto-
antibodies, suggesting drug-induced autoimmunity. Alternatively, Bajaj et al. have suggested that ma huang hepatotoxicity may be associated with com-
pound heterozygosity for mutation in the HFE gene (encoding the hereditary hemochromatosis protein), possibly via enhancing oxidative stress [164].
<table>
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<th>Toxic Mechanism</th>
<th>Clinical Presentation</th>
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<td>Toxification of pyrrolizidine alkaloids by CYP3A4</td>
<td>Sinusoidal obstruction syndrome (venoocclusive disease)</td>
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<td><em>Symphytum officinale</em></td>
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<td><em>Crotalaria</em></td>
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<td><em>Senecio longilobus</em></td>
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<tr>
<td><em>Heliotropium</em></td>
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<td>Chinese herbal combinations:</td>
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<tr>
<td><em>Paeonia</em> spp.</td>
<td>Immunostimulation, Atopic dermatitis</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Acute and chronic Hepatitis, hepatic failure</td>
</tr>
<tr>
<td><em>Dictamnus dasycarpus</em></td>
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<tr>
<td>Jin Bu Huan</td>
<td>Sedative</td>
<td><em>Lycopodium serratum</em></td>
<td>Contains (−)-tetrahydropalmistine with structural similarities to PA</td>
<td>Acute and chronic cholestatic hepatitis, fibrosis</td>
</tr>
<tr>
<td>Ma huang</td>
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<td><em>Ephedra sinica</em> (ephrine)</td>
<td>Immunoallergic?</td>
<td>Acute hepatitis, autoimmune hepatitis</td>
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<tr>
<td>Shou Wu Pian</td>
<td>Liver tonic, dizziness, hair loss, constipation</td>
<td><em>Polygonum multiflorum</em></td>
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</tr>
<tr>
<td>Onshidou-Genbi-Kounou</td>
<td>Weight reduction</td>
<td>Unknown; contains <em>Amacharazu</em> tea leaf, barbaloin, saponins, polyphenols</td>
<td>Unknown</td>
<td>Acute hepatitis, liver failure</td>
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<tr>
<td>Chi R Yun</td>
<td>Venereal diseases, contusion, heart failure, growth retardation</td>
<td><em>Breynia officinalis</em></td>
<td>Unknown</td>
<td>–</td>
</tr>
<tr>
<td>Germander:</td>
<td>Weight reduction</td>
<td>Neoclerodane diterpenoids</td>
<td>Hepatocyte apoptosis</td>
<td>Acute and chronic hepatitis (including liver failure), fibrosis (when chronic)</td>
</tr>
<tr>
<td><em>Teucrium chamaedrys</em></td>
<td>Antiinflammatory</td>
<td>Unknown</td>
<td>Unknown</td>
<td>–</td>
</tr>
<tr>
<td><em>Teucrium polium</em></td>
<td>Antioxidant, liver and health tonic, snake bites</td>
<td>Nordihydroguaiaretic acid</td>
<td>Inhibition of COX-1/2 and several cytochrome P450s</td>
<td>Cholestasis, cholangitis, chronic hepatitis, cirrhosis</td>
</tr>
<tr>
<td>Chaparral (Greasewood)</td>
<td>Antioxidant</td>
<td>Nordihydroguaiaretic acid</td>
<td>Inhibition of COX-1/2 and several cytochrome P450s</td>
<td>Cholestasis, cholangitis, chronic hepatitis, cirrhosis</td>
</tr>
<tr>
<td>Larrea tridentata</td>
<td>Antiinflammatory</td>
<td>Unknown</td>
<td>Unknown</td>
<td>–</td>
</tr>
<tr>
<td><em>Atractylis gummifera</em></td>
<td>Antiinflammatory, diuretic, chewing gum</td>
<td>Atractyllosides</td>
<td>Inhibition of gluconeogenesis through interference with oxidative phosphorylation</td>
<td>Acute hepatitis, liver failure</td>
</tr>
<tr>
<td><em>Callilepis laureola</em> (Impila)</td>
<td>Miscellaneous, Zulu remedy (South Africa)</td>
<td>Atractyllosides</td>
<td>Like <em>Atractylis gummifera</em></td>
<td>Like <em>Atractylis gummifera</em></td>
</tr>
<tr>
<td>Pennyroyal oil</td>
<td>Abortifacient, pesticide</td>
<td>Menthofuran</td>
<td>Glutathione depletion through electrophilic metabolites</td>
<td>Fulminant hepatic failure</td>
</tr>
<tr>
<td>Greater celandine</td>
<td>Irritable bowel syndrome</td>
<td>Unknown</td>
<td>Drug-induced autoimmunity?</td>
<td>Chronic hepatitis, fibrosis</td>
</tr>
</tbody>
</table>

(Continued)
Several cases of acute hepatitis and even fulminant hepatic failure have been reported in studies that have investigated the efficacy of Chinese herbal preparations for treating atopic dermatitis [167,168]. The investigators failed to identify the causative agent, but many herbal combinations used to treat eczema have contained *Paeonia* and/or *Dictamnus dasycarpus*. It is noteworthy that of the six cases reported with *D. dasycarpus*, two were fatal [169].

The Shou Wu Pian remedy formulated from the roots and vines of *Polygonum multiflorum* is used as a treatment for dizziness, hair loss, constipation, and, interestingly, as a *liver tonic*. It was first identified in 1996 to have caused acute hepatitis in a 31-year-old pregnant woman, the liver disease subsiding completely after discontinuation of the herb [170]. Following this, there have been additional reports of its potential to cause liver injury [171–175]. Toxicity could be the result of anthraquinones, which are known to be constituents of...
Another herbal marketed for weight loss, Onshidou-Genbi-Kounou, combines several natural compounds (amachazuru, barbaloin, polyphenols, tealeaf, and total saponin), and was the suspected cause of severe acute hepatitis followed by hepatic failure in a woman who took this preparation for several months [177]. Serum levels of liver enzymes were massively elevated to >9,000 IU/L and coagulopathy developed; however, the patient recovered upon cessation of drug intake. Onshidou-Genbi-Kounou contains N-nitrosoflurane-mine, which has only been associated with valvular heart disease. Possibly, Onshidou-Genbi-Kounou contains further unknown hepatotoxins.

Breynia officinalis is marketed under the Chinese proprietary name, Chi R Yun, and is used to treat conjunctivitis, contusions, growth retardation, heart failure, and venereal diseases, in combination with other TCMs. Following the report to the Poison Control Center of Taiwan of the first two cases of liver injury from this herbal, one involving a 43-year-old woman who attempted to commit suicide and developed acute cytolytic hepatitis and the other a 51-year-old woman who used the product to treat her contact dermatitis [178], a case series was described of 19 poisonings that occurred when soup was cooked with B. officinalis, accidentally mistaken for a similar plant, Securinega suffruticosa. The affected persons developed diarrhea, nausea, vomiting, and hepatocellular liver injury without jaundice within 6 months of consuming the soup [179]. The exact mechanism of liver injury remains undetermined, but the close temporal relationship and a positive dechallenge response identifies B. officinalis as the likely cause of liver injury in these cases.

Germander

Germander (Teucrium chamaedrys)-containing capsules and tea bag preparations were approved as weight loss remedies in France, although its active ingredients and precise mechanism of action remain unknown. Subsequent widespread use led to several reports to the French Pharmacovigilance Authorities in 1992 about germander-associated acute, chronic, and even fulminant hepatitis [180]. Liver injury usually developed 2 months after intake and, in those with an acute pattern of liver enzyme alterations, acute cytolytic hepatitis was found histologically. Some patients with a chronic course of liver disease showed histological features of chronic hepatitis with fibrosis and even cirrhosis. Causality assessment linking germander and liver damage was confirmed after accidental reexposure led to an immediate relapse of liver injury. However, all patients recovered after discontinuation of the herbal, except for those with cirrhosis. Systematic analysis of germander preparations demonstrated that they contained flavonoids, glycosides, saponins, and several furane-containing neoclerodane diterpenoids [166]. Regarding the latter, animal experiments have demonstrated the formation of toxic and highly reactive epoxides from these diterpenoids, which are potent inducers of hepatocyte apoptosis [181–184]. Formation of epoxides is enhanced by the induction of CYP3A and by glutathione (GSH) depletion, which may occur during weight loss or regular alcohol consumption.

Subsequently, there have been reports of fulminant hepatic failure following the ingestion of Teucrium polium used as an antiinflammatory and antimicrobial drug and for the treatment of scars [185,186]. There is also a single report of acute hepatitis with jaundice that occurred in a patient who took a traditional Chinese remedy containing Teucrium viscidum for back pain and that resolved spontaneously after the remedy had been stopped [187].

Chaparral

Chaparral (Larrea tridentata; commonly referred to as creosote bush or greasewood) grows in deserts and is a traditional herbal remedy among Native Americans to treat the common cold, bone and muscle pain, and snake bites. Commercial products containing chaparral have been sold for weight reduction and for alleged antiinflammatory, antioxidant, and blood-purifying activities. Furthermore, chaparral has been part of the self-medication taken by patients with HIV infection [188]. In the 1990s, the FDA recorded a series of cases of chaparral-related hepatotoxicity ranging from mild elevations of serum liver enzyme concentrations to fulminant hepatitis, with subsequent liver transplantation for hepatic failure in two cases [189]. Although cholestatic hepatitis accounted for the majority of cases, there were also instances in which cirrhosis developed. L. tridentata was found in all preparations, and biochemical and microbial contamination was excluded. A causal relationship was postulated based on the temporal correlation between intake of chaparral and the onset of liver disease, a consistent pattern of hepatic damage, and the observation that reexposure to chaparral or an increased dose led to relapse or aggravation of clinical signs of liver disease. Chaparral toxicity is believed to be due to nordihydroguaiaretic acid, which inhibits prostaglandin G/H synthases (COX enzymes) and CYP [190].
**Atractylis gummifera** and **Callilepis laureola**

*Atractylis gummifera* has been traditionally used as a natural antipyretic, diuretic, and emetic in North Africa and the Mediterranean, and a secretion from the plant is occasionally consumed by children as chewing gum [191,192]. Toxic hepatitis with an acute onset is well known and commences a few hours after ingestion, accompanied by abdominal pain, headache, and nausea. It is associated with a syndrome of neurovegetative symptoms, hepatoportal failure, and pronounced hypoglycemia [193]. Death due to fulminant hepatic failure is a possibility. Consumption of *A. gummifera* is particularly dangerous during springtime, when toxins are highly concentrated in roots, or when the plant is confused with wild artichoke.

*Callilepis laureola* is the major constituent of *Impila*—a traditional herbal remedy used among the Zulu people from South Africa as a multipurpose treatment for stomach problems, impotence, infertility, and to deter evil spirits. Paradoxically, *Impila* is the Zulu word for health. However, numerous reports provide substantial evidence for potentially fatal nephro- and hepatotoxicity of *C. laureola* [194]. For example, a large series of children were identified retrospectively as having died from *C. laureola* intoxication, presenting with a clinical pattern similar to Reye syndrome with acute hepatorenal failure, hypoglycemia, and multiorgan hemorrhage [195]. Toxicity begins with a sudden onset and mortality is high. Autopsy examinations show centrilobular zonal necrosis of the liver, tubular renal necrosis, and hemorrhages in lungs, skin, and intestine. Despite its well-known toxicity, the underlying mechanism is only partly understood. Studies in rats indicate that atracyloside and carboxy-atriactyloside are potent inhibitors of oxidative phosphorylation and other mitochondrial functions, leading to apoptosis [196]. Popat et al. have shown that *Impila* extracts cause a concentration- and time-dependent loss in viability and mitochondrial GSH content in HepG2 cells that is preventable with *N*-acetylcysteine and *S*-adenosyl-l-methionine, which are precursors of GSH [197].

**Pennyroyal**

Pennyroyal, also referred to as *squawmint oil*, is an herb containing leaves from either *Mentha pulegium* or *Hedeoma pulegioides* that has long been identified as a cause of severe acute liver injury [198]. Its traditional use is as a natural abortifacient and deterrent against fleas. Several reports of fulminant hepatic necrosis due to pennyroyal with lethal outcome have been described [199,200]. Its primary constituents are pulegone and various other monoterpenes characteristically contained in mint species, and particularly in pennyroyal. Hepatotoxicity appears to be due to depletion of GSH by pulegone, subsequently enhanced oxidative stress, and via the primary metabolite of pulegone, menthofuran, which is transformed through CYP into a hepatotoxin [200–203].

**Greater Celandine**

Drugs containing greater celandine (*Chelidonium majus*) are used in Europe to improve bile flow and irritable bowel syndrome. Greater celandine contains at least 20 different alkaloids, including berberine, chelerythrine, coptisine, and chelidonine, of which the latter serves to standardize the extract [204]. However, the therapeutic efficacy of greater celandine for these indications has never been tested in controlled trials. Several reports from European countries, where commercial drug preparations containing greater celandine alkaloids are widely available, have described potential hepatotoxicity of this herbal [204–207]. The largest series of 10 patients from a single German center revealed cholestatic hepatitis together with low titers of autoantibodies, suggesting drug-induced autoimmunity after variable periods of ingestion of different greater celandine preparations [208]. However, the exact mechanism responsible for injury remains unclear and efforts to replicate hepatotoxicity in experimental animals have thus far failed.

**Kava**

In industrialized countries, kava-containing preparations are marketed for the treatment of anxiety disorders and depression, while kava root (*Piper methysticum rhizoma*) has long been used as a traditional psychotropic remedy in Hawaii, Polynesia, and the Fiji Islands. Its sedative activity is caused by kavapyrones, including kavain, dihydrokavain, methysticin, and dihydromethysticin, which act as gamma-aminobutyric acid receptor agonists, thus inhibiting activating neurons in the reticular formation and the limbic system [209–210]. A recent systematic review and meta-analysis of randomized, controlled trials with kava for the treatment of anxiety showed a significant anxiolytic effect, as assessed by the Hamilton Rating Scale for Anxiety, as well as good tolerability [211]. More than 100 cases of liver damage have now been associated with the intake of kava worldwide, comparable to numbers that have led to the banning of synthetic drugs [212]. Consequently, the license to distribute kava products was revoked in the United States, Europe, and Australia [213]. A detailed analysis of 29 cases of adverse hepatic reactions due to kava in Germany using a clinical causality
score reported liver injury with both alcoholic and acetonic kava extracts [214]. The large majority of patients were females who developed cytolytic or cholestatic hepatitis, and nine patients who developed fulminant liver failure with subsequent liver transplantation in eight of the patients: three patients died, two following unsuccessful liver transplantation, and the remaining patients recovered completely after the withdrawal of kava. The mechanism by which liver injury is precipitated remains unclear, since no dose-response pattern can be recognized. However, some patients took doses exceeding several times the recommended daily dose of 120 mg. For most other patients, both the cumulative dose and the latency until the hepatotoxic reaction emerged were highly variable, suggesting drug idiosyncrasy. Along this line, a poor-metabolizer phenotype of CYP2D6 was suggested to be a risk factor for developing kava-related liver damage [215]. Another possible basis for its toxicity relates to the mode of kava extraction, with recent in vitro and animal studies confirming that aqueous kava extracts are less cytotoxic than are organic solvent fractions [216,217]. Notably, modern commercial products rely on alcohol or acetone extraction, a process that may extract toxic compounds (e.g., alkaloids) from the plant.

Black Cohosh (Cimicifuga racemosa)

Black cohosh, a popular herbal from North America for the treatment of menopausal symptoms, joint pain, and myalgia, has been implicated in a number of case reports on liver injury in Australia and North America [218–220]. Clinical presentation ranges from mild serum aminotransferase elevations to fulminant hepatic failure. In several cases, urgent liver transplantation was necessary. Some patients present with features resembling autoimmune hepatitis, such as elevated autoantibodies or skin rashes. In response to these reports, the Dietary Supplement Information Expert Committee of the US Pharmacopeia’s Council of Experts analyzed information from human clinical case reports, adverse event reports, animal pharmacological and toxicological data, historical use, regulatory status, and contemporaneous extent of use associated with black cohosh [221]. All 30 individual reports of liver damage were assigned a causality score of possible, but none was labeled as probable or certain. Based on these results, the Dietary Supplement Information Expert Committee determined that black cohosh products should be labeled with a cautionary statement indicating that hepatotoxicity is a possible adverse outcome. Although meta-analysis of five randomized, controlled clinical trials, including >1,000 patients, revealed no evidence of liver toxicity [222], and reanalysis of individual case report information with liver-specific causality scores raised doubts over black cohosh as being truly hepatotoxic [223], a low incidence of black cohosh hepatotoxicity cannot be excluded by these data. How toxicity may occur is not fully elucidated, but experimental data indicate mitochondrial damage and subsequent apoptosis as a possible molecular mechanism [224].

Miscellaneous

Various other botanicals have been associated with toxic liver damage, such as senna (Cassia angustifolia), which is used as a laxative. Senna was identified as the cause of relatively benign hepatitis in a woman taking approximately 10 times the recommended dose [225]. Causality was confirmed by positive rechallenge. Nadir et al. described a man in whom short-term use of a commercial Cascara sagrada product caused cholestatic hepatitis with subsequent portal hypertension, prolonged prothrombin time, and ascites [226]. C. sagrada contains anthraquinone glycosides and is recognized as an effective laxative. Other frequent etiologies were excluded, but the patient also took amitriptyline, baclofen, and cimetidine at recommended doses, which can occasionally cause liver injury. Further laboratory tests revealed elevated antinuclear and anti-smooth muscle antibody titers of 1:640 and 1:40, respectively, and liver biopsy showed an eosinophilic infiltrate, suggesting drug-induced autoimmunity.

Also, a combination of herbal ingredients, known as Prostata, was suspected to have caused cholestatic hepatitis in a man using this medication for the treatment of benign prostatic hyperplasia [227]. The presumed active ingredient is Serenoa serrulata, which exerts estrogenic and antiandrogenic effects. Either hormone may cause liver injury under certain circumstances.

Centella asiatica has been used in Ayurvedic medicine as a psychophysical regenerator and blood purifier, and for the treatment of dementia, diabetic microangiopathy, skin defects, and obesity [228]. Recently, three female patients who took this herbal for periods of between 1 and 6 months to lose weight developed severe hepatic injury, including granulomatous hepatitis and cirrhosis [229]. Unintentional reexposure resulted in accelerated recurrence of hepatic lesions in two of the patients. Extracts of this herb contain penta cyclic triterpenic saponosides, including asiaticoside, madecassoside, and centellasaponins. The mechanism by which liver damage is precipitated is uncertain. Treatment with ursodeoxycholic acid at 10 mg/kg/day led to normalization of altered liver biochemistries in
all three patients. Another Ayurvedic herbal preparation, termed Liv.52, is freely available through Internet sources and health food stores across North America and Europe, and is taken to strengthen the liver and to influence the natural course of chronic liver diseases. Liv.52 contains Achillea millefolium (yarrow), Capparis spinosa (capsers), Cichorium intybus (wild chicory), Solanum nigrum (black nightshade), Terminalia arjuna (arjuna), and others. Liv.52 has been suggested as useful for the treatment of human alcohol-related liver cirrhosis after experimental data suggested that it may reduce acetaldehyde production [230]. A European randomized controlled clinical trial in 188 patients with alcoholic liver cirrhosis showed no benefit on survival and on surrogate markers of liver injury in cirrhotics with Child-Pugh class A and B, and the study was prematurely terminated because of increased liver-related mortality among those with Child-Pugh class C [231].

A recent case series of seven patients suggests possible liver toxicity from Noni juice (Morinda citrifolia), leading to liver failure requiring liver transplantation in one case [232,233]. Other possible causes for the acute hepatitis were ruled out and liver tests rapidly returned to normal after the cessation of Noni intake. Active components within Noni extracts include flavonoids, glycosides, polyunsaturated fatty acids, vitamins, and anthraquinones. The latter can be transformed into rhein by intestinal bacteria and cause mitochondrial damage [234]. However, experimental studies with Noni have failed to reproduce hepatotoxicity in vitro and in vivo [235].

**DIETARY SUPPLEMENTS ASSOCIATED WITH LIVER INJURY**

**Green Tea (Camellia sinensis)**

Green tea has been consumed for centuries and is currently among the most popular drinks in the world. The first report on liver injury following the ingestion of green tea extracts and preparations was published in 1999 [236] and, since then, there have been numerous cases reported to regulatory agencies worldwide. In response, the US Pharmacopeia performed a systematic review of all reported and published cases from North America, Great Britain, and Australia of liver injury following the ingestion of various different green tea preparations [237]. Thirty-four case reports were retrospectively evaluated, and seven reports pertaining to liver damage were labeled as probable, and the remaining seven cases as possibly linked to green tea. Subsequently, additional cases were made public and, to date, there have been 58 case reports recorded of hepatotoxicity associated with the consumption of green tea extracts, powdered leaves, green tea infusions, and hydroalcoholic and aqueous extracts [238]. Of particular concern is that these included one lethal case. On histological examination, the livers of patients revealed necroinflammatory changes and cholestasis. However, caution should be exercised about assigning unequivocal blame for the liver injury on green tea in some of the reported cases, since in many instances the patients also took other products that have been implicated in causing hepatotoxicity such as C. angustifolia (see above), Ephedra sinica, and Hydroxycut (for both, see below). The basis for the toxicity caused by green tea is uncertain but could be due to (−)-epigallocatechin gallate or its metabolite (−)-epicatechin gallate which, under certain conditions such as fasting, can induce liver damage via increasing oxidative stress [239]. In contrast, however, in vitro and in vivo experimental studies have also demonstrated hepatoprotective properties for green teas [240–242], and a recent systematic review of clinical studies aimed at defining the therapeutic effects of C. sinensis in humans found overall favorable effects from the tea as reflected by reduced mortality, attenuated steatosis, and a reduced incidence of primary liver cancer [243]. Current evidence suggests a causal relationship between intake of green tea-containing products and hepatotoxicity, and whether the risks from green tea consumption outweigh their benefits remains open to speculation. Consequently, in their systematic review, the US Pharmacopeia included a cautionary statement about green tea that reflects this uncertainty [237].

**Herbalife**

There are six published reports describing 34 cases from Argentina, Iceland, Israel, Spain, and Switzerland of severe liver injury following consumption of Herbalife nutritional and herbal supplements for weight control and improvement of nutrition [244–249]. The patterns of injury were mostly hepatocellular, but mixed and cholestatic enzyme patterns were also noted, with severity ranging from mild to severe hepatic damage, including evidence of cirrhosis and ALF requiring liver transplantation. Generally accepted liver disease assessment scoring systems were used in evaluating most of the cases, five of which scored as certain because of a positive rechallenge, while most of the remainder were judged as probable. The cause of liver damage remains speculative, since the patients took up to 17 different Herbalife products at the same time. In addition to the possibility that autoimmune mechanisms played a role in inducing the liver injury among those who had
elevated titers of autoantibodies and plasma cell infiltrates in their liver biopsies, adulteration of individual batches with bacterial pathogens may explain some other cases, in view of a report that two patients were found to have bacterial contamination of several Herbalife products with *Bacillus subtilis* and *Bacillus cereus* [248]. Herbalife runs numerous production sites worldwide, suggesting that spoiled products contaminated with certain germs, chemicals such as softeners, preservatives, and flavor enhancers, pesticides, or heavy metals either added intentionally during the manufacturing process or accidentally contained in the unrefined raw products, i.e., the herb extracts, could have been responsible for local series of cases of hepatotoxicity.

**Usnic Acid**

Usnic acid extracted from lichens and fungi have been marketed as dietary supplements in the United States to aid in weight loss. Efficacy for this indication was postulated based on its function as an uncoupler of the respiratory chain, which in principle can augment weight loss but also cause mitochondrial damage and subsequent hepatocyte death [250]. Several cases of ALF have been reported requiring liver transplantation following the intake of Lipokinetix, a product containing usnic acid and sold as dietary supplement capsules [165,251–254]. In reacting to these reports, the FDA issued a warning about Lipokinetix in 2001 [255]. Onset of liver injury was usually acute with a maximum latency of 3 months and the injury pattern was hepatocellular with massive elevations of alanine aminotransferase and aspartate aminotransferase. Apart from usnic acid, Lipokinetix contained caffeine, diiodothyronine, norephedrine hydrochloride, and yohimbine hydrochloride, which were confirmed by analyzing the used Lipokinetix lots. None of the ingredients was previously associated with liver damage and inadvertent contamination was excluded. These serious events caused the withdrawal of Lipokinetix from the market.

**Hydroxycut**

Owing to numerous reports of liver injury, including cases with acute hepatic failure and subsequent liver transplantation, several Hydroxycut products containing caffeine, chromium polynicotinate, *Garcinia cambogia* (*Garcinia gummi-gutta*), *Gynnema sylvestre*, and green tea were withdrawn by the manufacturer following a warning posted by the FDA in May 2009 [140–145,256]. Hydroxycut preparations were sold to support weight loss and muscle building by conventional retailers, through Internet sources, and via direct television marketing. The clinical presentation consisted of an acute onset after several weeks of intake, presenting with high levels of serum aminotransferases in the majority of cases, while others presented with a more insidious, usually cholestatic course.

**NATURAL TOXINS**

While not ingested intentionally or deliberately added to HDS, certain toxins derived from plants or natural products might contaminate supplements or food during preparation, processing, or storage that may occasionally cause liver injury if ingested accidentally.

**Aflatoxins**

Humans, particularly those in developing countries with humid climate conditions, such as sub-Saharan Africa and East Asia, may be highly exposed to aflatoxins through consumption of maize (corn), peanuts, rice, and other crops contaminated with *Aspergillus flavus* or *Aspergillus parasiticus*. Acute aflatoxicosis presents with abdominal pain, diarrhea, and vomiting, thus rendering aflatoxin poisoning indistinguishable from acute gastroenteritis. Aflatoxins in doses of 2–50 mg daily have been reported to cause acute toxic hepatitis characterized by jaundice, ascites, portal hypertension, and encephalopathy associated with a high mortality [257–259]. The liver biopsy findings in this situation include fatty infiltration and hepatic necrosis, sometimes accompanied by bile duct injury. In addition to causing acute hepatotoxicity, aflatoxins are potent carcinogens, well known to be associated with the development of primary HCC, but also with cancer of the kidneys and large bowel. Because the incidence of HCC is observed to be high in the same regions in which aflatoxin exposure is common, efforts began in the 1960s to determine whether there was a possible link between the HCC risk and aflatoxin exposure. Not only did epidemiological data confirm this association [260] but early experimental studies of *A. flavus*-contaminated groundnut extracts also demonstrated that aflatoxins are in fact capable of inducing acute liver disease in ducks and liver cancer in rodents [261,262]. Indeed, aflatoxin-related hepatocarcinogenicity occurs not only in humans but also in many other species including dogs, nonhuman primates, rodents, and even fish [263]. Chemically, there are four different naturally occurring aflatoxins: AFB1, AFB2, AFG1, and AFG2. AFB1 and AFG1 possess an unsaturated...
bond at the 8,9 position on the terminal furan ring, and epoxidation at this position appears to be critical for their hepatocarcinogenic potency [264].

The metabolism of aflatoxin and the mechanisms of aflatoxin-induced hepatocarcinogenesis are well documented [265]. AFB1 requires metabolic activation to its ultimate carcinogenic form, primarily by the CYP monooxygenase system with the isoenzymes CYP1A2 and CYP3A4 that transform AFB1 to a reactive epoxide (aflatoxin-8,9-epoxide). The epoxide interacts with DNA to generate a promutagenic aflatoxin-N7-guanine adduct and, thus, a mutation in codon 249 of the p53 tumor suppressor gene that is considered crucial for the initiation of human hepatocarcinogenesis [266]. Although the epidemiologic pattern of HCC is changing, i.e., beginning to decline in areas of the world of high HBV endemcity with the advent of the hepatitis B vaccine and possibly increasing in areas plagued by the epidemic of obesity and diabetes, the primary causal risks worldwide continue to be both HBV and HCV infections, as well as exposure to aflatoxin [267]. Indeed, aflatoxin exposure is still considered a major cause of the differences in lifetime HCC risks between Western countries and South East Asia and Africa [267].

Ackee Fruit

Ackee fruit (Blighia sapida) poisoning has been occasionally reported in different developing countries, including Africa, Latin America, and the West Indies [268–270].

Ackee fruits derive from large green leafy trees of West African origin and are consumed either raw or after boiling in milk or water and served on their own or in meat or fish dishes, such as ackee and salt fish. Ackee fruit is a substantial part of the diet in poor, agricultural areas and its taste resembles that of hazelnut or avocado. Toxicity is related to hypoglycin A and hypoglycin B, the former molecule being more toxic than the latter. The ripe fruit flesh of ackee contains only low quantities of hypoglycins, but concentrations in unripe fruits are 10–100 times greater, depending on the season and exposure to sunlight, which significantly reduces hypoglycin concentrations. In view of several larger series of intoxications in Jamaica in the past, the disease related to ackee fruit poisoning is also termed Jamaica vomiting sickness, with a clinical pattern similar to Reye syndrome that includes gastrointestinal symptoms, marked hypoglycemia, and central nervous system abnormalities that typically develop within 6–48 h of ingestion [271]. Lethality is high, particularly in infants and children. Toxicity is believed to be related to methyleneacyclopropylacetic acid, a toxic hypoglycin metabolite that interferes with several cofactors [e.g., coenzyme A (CoA) and carnitine] essential to the β-oxidation of long-chain fatty acids and thus inhibits the transport of long-chain fatty acids into the mitochondria. The reduction in fatty acid metabolism causes an increased use of glucose, and the blockade of the substrate for hepatic gluconeogenesis causes hypoglycemia after the depletion of NADH and hepatic glycogen stores [271]. Findings at postmortem examination of fatal ackee fruit intoxications have included massive steatosis of the liver and kidney, depletion of liver glycogen, diffuse hemorrhages, and generalized hyperemia of the internal organs. Diagnosis is based on the patient’s history of the use of the product and the clinical presentation, since there are no specific biomarkers. There is no definitive therapy for this poisoning other than the use of symptomatic measures such as fluid and glucose replacement. In the light of these risks, ackee fruit is banned in the United States and other Western countries.

**Bacillus cereus**

There are several case reports of fulminant liver failure attributed to Bacillus cereus food poisoning [272–274]. *B. cereus* is a ubiquitous, endospore-forming, aerobic, gram-positive bacterium known for precipitating a type of toxin-mediated food poisoning in the Far East, typically after the ingestion of cooked rice. Although instances of fulminant hepatic failure are extremely rare, those reported have been well characterized, describing acute-onset hepatic failure complicated by lactic acidosis, rhabdomyolysis, cerebral edema, and death in some cases, despite maximal medical care. Certain strains of this bacterium secrete a cyclic peptide toxin, cereulide, which causes vomiting in humans and animals but may also act as a mitochondrial toxin that interferes with fatty acid metabolism, leading to the breakdown of mitochondria derived energy supply [274,275]. Thus, the pathophysiology of *B. cereus*-related liver failure is similar to that observed with ackee fruit, aflatoxin (see above), Reye syndrome, and fatty liver of pregnancy [276]. Diagnosis is based on the coexistence of fulminant hepatic failure, hypoglycemia, lactic acidosis, hyperammonemia, and central nervous system symptoms and should prompt immediate referral to a liver transplant center, since liver transplantation may be the only therapeutic option for some patients.

**Microcystins**

Water contaminated with cyanobacterial hepatotoxic heptapeptides, i.e., microcystins, has been recognized as a possible cause of an outbreak of ALF at a dialysis
center in Caruaru, Brazil [277]. At the clinic, 116 out of 131 patients (89%) experienced visual disturbances, nausea, and vomiting after routine hemodialysis treatment. Subsequently, 100 patients developed ALF and, of these, 76 died. The observed syndrome was thus termed the Caruaru syndrome. Examination of phytoplankton from the dialysis clinic’s water source and in-depth analyses of the clinic’s water treatment system, together with studies on serum and liver tissue of clinic patients, led to the identification of two groups of cyanobacterial toxins: the hepatotoxic cyclic peptide microcystins and the hepatotoxic alkaloid cylindrospermopsin. Authors of this and subsequent analyses concluded that the likely cause of liver injury were microcystins, specifically microcystin-YR, -LR, and -AR [278]. Cyanotoxins—to which class microcystins belong—are also implicated in animal poisoning, human gastroenteritis, dermal contact irritations, and primary liver cancer in humans [279,280]. The (geno) toxic mechanism of microcystins is to act as potent inhibitors of protein phosphatase 1 and 2A, thus leading to increased protein phosphorylation causing cytotoxicity and tumor promotion [281].

CONCLUSIONS

HDS use is extensive, rivaling and often exceeding that of conventional medications. There is general belief that because they have been used for centuries and are pure, they must be effective and safe. Unfortunately, neither of these generalizations is accurate. Efficacy for most herbal products has not been scientifically proven and they are certainly no safer than conventional medications; some of them are less safe. Safety would be enhanced if there were greater oversight of their production and distribution to ensure that they do not contain potentially toxic or otherwise dangerous contents. Asking about their use is an essential component of causality assessment when liver dysfunction is identified with the suspicion that the cause might be DILI.

 DISCLAIMER

The views expressed herein are those of the author and not the US Food and Drug Administration.

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