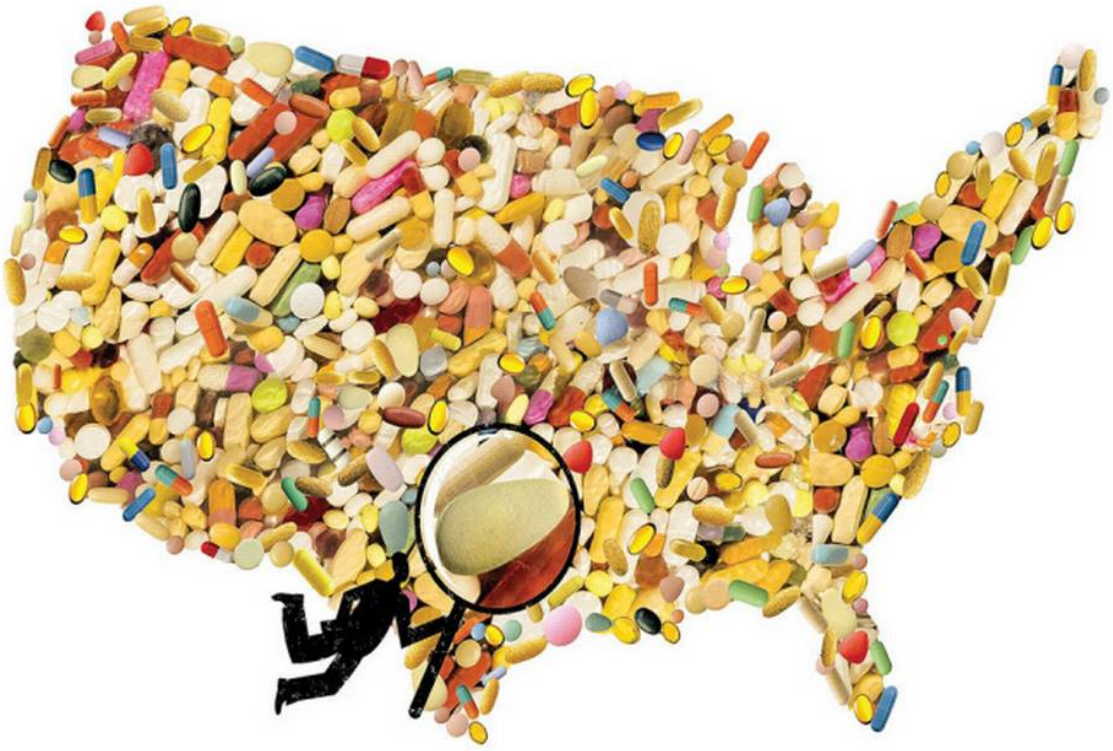
HIDDEN DANGERS: HERBAL AND DIETARY SUPPLEMENT INDUCED HEPATOTOXICITY



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This is to acknowledge that Shannan Tujios, M.D. has disclosed that she does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Tujios will not be discussing off-label uses in her presentation.

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Dr. Tujios is a native Kentuckian who completed her undergraduate degree at Washington University in St. Louis and medical degree from the University of Louisville School of Medicine. She completed internal medicine residency at the University of Michigan and gastroenterology fellowship at UT Southwestern. She returned to the University of Michigan for a fellowship in transplant hepatology and joined UT Southwestern faculty in 2011. Her clinical focus is in liver transplant and general hepatology. She is currently a co-investigator in the Acute Liver Failure Study Group and involved in several clinical trials in the treatment of viral hepatitis.

Purpose and Overview:

The purpose of this presentation is to review how widespread herbal and dietary supplement use under current regulatory system has led to increasing reports of liver injury and be able to recognize, classify and identify mechanisms of hepatoxicity.

Educational Objectives:

1. Recognize the prevalence of herbal and dietary supplement (HDS) use in today's clinical practice.
2. Understand the regulatory oversight of HDS in the United States.
3. Recognize the presentation of possible HDS induced liver injury.
4. Be aware of the unique challenges in diagnosing and studying HDS hepatoxicity.
5. Be familiar with the most common HDS associated with liver injury.

History of Herbal Medicine

While pharmacologic agents were not available until the 19th century, people have been using herbal products to treat illness for centuries. The oldest written evidence was found on a Sumerian clay slab over 5000 years old. In China, the discovery of herbal medicine has been ascribed to emperor Shen Nung (2696 BC). Descriptions of over 365 herbal remedies, including some still used today, were transcribed 2000 years later in the great herbal book, *Shen Nung Pen Tsao Ching*. Numerous herbs including turmeric were mentioned in Aryuvedic books in India nearly 3000 years ago. In Egypt, the Ebers Papyrus (ca. 1550 B.C.) details over 800 formulas of 700 different plants. The ancient Greeks and Romans were herbalists with Dioscorides compiling his *De Materia Medica* ca. 70 A.D. In this five-volume work, over 900 drugs are described from over 600 plant species. It was translated in many languages and served as the primary pharmacopeia for over 1500 years. The native people of Africa, South Pacific Islands and the Americas also had plant centered healing traditions. Traditionally herbal products made of stems, roots, bark, leaves, berries, seeds, and flowers were administered as poultices, compresses, infusions, or decoctions.

In the early 1800s, the discovery and isolation of alkaloids from poppy for opiates (1806) and quinine from bark of *Cinchona* tree to treat malaria (1820) marked the beginning of modern pharmacology.[1] The value of botanicals to modern medicine is undeniable. Aspirin derived from willow bark, digitalis from purple foxglove, vincristine from Madagascar periwinkle and many others. It is estimated that 25 to 40% of modern prescription drugs contain at least one compound now or once derived from or patterned after those found in plants. Nearly 70% of antimicrobials and 50% of anticancer drugs developed in last 30 years are derived from natural products.[2]

Herbal and Dietary Supplements Today

More recently, there has been a growing interest in herbal and dietary supplements used as complementary and alternative medicine (**CAM**) that has spawned a multi-billion dollar industry. In a 1990 telephone survey, 34% of adult Americans reported using CAM with 2.5% using herbals. This increased to 42% using CAM and 12.1% using herbals when the survey was repeated in 1997.[3] The National Health Interview Survey reports 33% of adults using CAM 2002-2012, with non-vitamin dietary supplements most commonly used at 18%. Based on the most recent 2012 survey, an estimated 59 million Americans spend $30.2 billion a year on CAM with $12.8 billion on natural products.[4] If vitamin preparations are included, over half of U.S. adults report using supplements fueling the $37.6 billion a year dietary supplement market. Users are more likely to be non-Hispanic white females over the age of 40 with higher levels of income and education. Most report using supplements for wellness but 40% do so to treat a condition. Because these products are readily available and “natural,” they

are perceived to be effective and safe. However, herbal and dietary supplements (**HDS**) are not classified as drugs and by definition, are not intended to prevent, diagnose, treat, mitigate, or cure diseases.[5] Citing lack of questioning to fear of disapproval from their primary care physicians, over 40% do not disclose use of CAM, including a quarter of those taking supplements.[6]

Regulation of Herbal and Dietary Supplements

In order to ensure the American public had access to safe foods and drugs, the Pure Food and Drug Act was passed in 1906 heralding the beginning of the modern day Food and Drug Administration. In subsequent years further regulations were passed in response to deaths due to poorly manufactured drugs. In 1938, the Food, Drug and Cosmetic Act required premarketing safety data and in 1962, amendments mandated that a drug's efficacy and safety be proved in clinical trials before approval. During this time, vitamins and minerals were considered over-the-counter drugs and regulated as such while herbals were considered foodstuffs.

In 1994, Congress passed the Dietary Supplement Health Education Act (DSHEA). It defines a supplement as a vitamin, mineral, herb or other botanical, amino acid or dietary substance available as a concentrate, metabolite, constituent, extract or any combination meant to supplement the diet. This law now allows dietary supplements to be marketed without FDA approval. Unlike pharmaceutical companies, manufacturers of HDS need not prove safety and efficacy before marketing. Under the Good Manufacturing Practices of 2007, manufacturers are expected to provide truthful labeling, maintain quality standards and ensure safety of their products.[7]

Regulatory environment for HDSs in the United States

Regulation	Responsibilities	
	Manufacturer	FDA
DSHEA (1994)	Identify product ingredients and manufacturer on the label Provide disclaimer noting that product was not evaluated by the FDA for safety and efficacy, and is not intended to diagnose, treat, cure, or prevent disease	Defines supplements as vitamins, minerals, herbs, amino acids (and any concentrate, metabolite, extract thereof) Investigate allegations of attributable toxicity after marketing Conducts premarket review of safety data for new ingredients
cGMP (2007)	Must adhere to standards in identification, purity, strength, composition, and purity of the final dietary supplement Must evaluate the identity, purity, strength, and composition of dietary supplements	Supplements containing contaminants or not containing labeled ingredients are considered adulterated or misbranded

Abbreviation: cGMP, current good manufacturing practice.

Despite these regulations, there is a wide range of variability among products and numerous reports of contaminants and adulterants. In an analysis of 78 bottles of herbal supplements, 80% contained no DNA from the plant advertised on the label. Another study found 59% contained additional botanicals not listed on the label, including potential allergens and toxins. More than 500 "all-natural" products have been found to contain pharmaceuticals, especially in the case of those marketed for weight loss (sibutramine), sexual enhancement (phosphodiesterase-5 inhibitors) and athletic performance (amphetamines and anabolic steroids). The FDA estimates 50,000 adverse events annually from HDS with most being related to renal and liver injury.[9]

Incidence of Herbal and Dietary Supplement-Induced Liver Injury

The true incidence of herbal hepatotoxicity is unknown and thought to be under-reported. It is estimated that less than 1% of adverse reactions to HDS are reported.[10] As with drug-induced liver injury (**DILI**) from conventional pharmaceuticals, most cases of HDS-induced liver injury are idiosyncratic rather than predictable, dose-dependent reactions as seen with acetaminophen. Information is gleaned from case reports, retrospective reviews and recently

Table 1 | Herbal and dietary supplement hepatotoxicity: Prevalence and clinical features among drug-induced liver injury in different reports

Reference	Countries and patient characteristics	Prevalence of HDS hepatotoxicity	Clinical features and prognosis of cases identified as HDS hepatotoxicity
Ibunez *et al.*[9]	Spain (1993–1998) *N* = 103; DILI Population-based, prospective	11%	64% hepatocellular injury 18% mixed injury 18% cholestasis injury
Andrade *et al.*[7]	Spain (1994–2004) *N* = 446; DILI Multi-centre, prospective	2%	89% hepatocellular 11% cholestasis 56% needed hospitalisation 11% death
Chalasani *et al.*[8]	USA (2003–2008) *N* = 300; DILI Multi-centre, prospective	9%	63% hepatocellular 17% cholestasis 21% mixed injury 41% needed hospitalisation 6% ALF 9% chronic DILI
Suk *et al.*[13]	Korea (2005–2007) *N* = 371; DILI Multi-centre, prospective	73% (40% herbs, 14% dietary supplement, 19% folk remedies)	~78% hepatocellular ~10% cholestasis ~12% mixed injury 1.5% death or LT
Wai *et al.*[14]	Singapore (2004–2006) *N* = 31; DILI Multi-centre, prospective	71%	74% hepatocellular 19% cholestasis 7% mixed injury
Devarbhavi *et al.*[15]	India (1997–2008) *N* = 313; DILI Single-centre, retrospective	1.3%	50% mortality
Estes *et al.*[12]	USA (2001–2002) *N* = 20, ALF Single-centre, retrospective	50%	60% underwent LT
Russo *et al.*[11]	USA (1990–2002) *N* = 270; ALF from drug Retrospective, UNOS data	5.1%	All underwent LT
Reuben *et al.*[10]	USA (1998–2007) *N* = 133; ALF from drug Multi-centre, prospective	10%	>90% hepatocellular injury 21% spontaneous recovery 50% underwent LT 29% death

ALF, acute liver failure; DILI, drug-induced liver injury; HDS, herbal and dietary supplement; LT, liver transplantation; UNOS, United Network for Organ Sharing.

prospective databases. The Drug Induced Liver Injury Network (**DILIN**), a multicenter United States research collaboration, found the proportion of cases attributed to HDS increased from 7% in 2004 to 2005 to 20% in 2010 to 2012.[11] Other Western registries report similar percentages while hepatotoxicity due to herbals contributed to over 70% of cases in Korea and Singapore and 40% in China.[12]

Acute liver failure, defined as elevated liver enzymes with INR > 1.5 and encephalopathy, from prospective DILI studies occurred in 1.5 to 11% of HDS cases. The US Acute Liver Failure Study Group reported 253 (9.6%) patients with idiosyncratic DILI with 16.3% of those related to HDS between 1998 and 2015. Mirroring the DILIN's findings, the fraction of HDS among drug-induced acute liver failure increased over time from 12.4% from 1998-2007 up to 21.1% 2007-2015. Alarmingly, HDS-induced acute liver failure resulted in transplant or death more often than cases due to prescription medication (83% vs. 66%).[13] In a recent population study, over 18% of acute liver failure cases were attributed to HDS with 50% resulting in death or liver transplant.[14]

Diagnosing Herbal and Dietary Supplement-Induced Liver Injury

Most adverse drug and herbal reactions are idiosyncratic—due to an individual's own mix of unique characteristics—rather than a predictable occurrence. Proposed mechanisms of drug induced hepatocyte injury include formation of chemically reactive metabolites causing direct cell lysis, damage to bile salt export pumps, mitochondrial inhibition, stimulating apoptotic pathways and formation of drug haptens that activate the immune response.

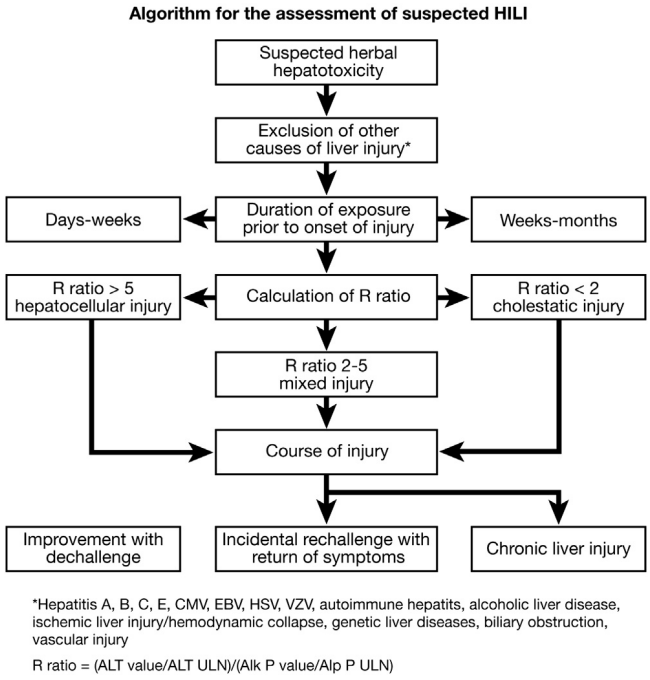
The diagnosis of HDS-induced liver injury follows the same principles as drug-induced liver injury but with some added challenges. Keys to diagnosing drug and HDS liver injury include:

- Exposure must precede the onset of liver injury (although the time from start to injury is highly variable).
- More common causes of liver disease should be excluded.
- Injury typically improves when the causative agent is stopped ("dechallenge").
- Liver injury may recur more rapidly and severely after repeated exposure ("rechallenge").

The clinical presentation of hepatotoxicity can vary, ranging from asymptomatic elevated liver tests to fatigue, nausea and abdominal pain to jaundice and encephalopathy. Once liver injury is recognized and other causes of liver injury are excluded, there must be a high index of suspicion that it may be due to supplement use. Mitigating factors include the patient not divulging HDS, irregularities in HDS and unfamiliarity by both the patient and clinician of potential

culprits. While timing of symptoms with recent beginning of HDS is suggestive, products consumed previously must also be considered as contents may vary over time.

The pattern of liver injury provides a clue as many drugs and some HDS have characteristic signatures. The R ratio (ALT value/ALT ULN)/Alk P value/Alk P ULN) can be calculated to determine if hepatocellular (R ratio > 5), cholestatic (R ratio < 2) or mixed (R ratio 2-5). Clinical improvement after HDS is stopped supports diagnosis but some go on to have progressive liver injury. Incidental re-exposure with return of symptoms and liver test abnormalities strengthens diagnosis as well.[15]



Determining Severity of Liver Injury

Hy's Law

In 1978, Dr. Hyman Zimmerman observed that "drug-induced hepatocellular jaundice is a serious lesion" resulting in 10-50% mortality. This observation has been validated throughout the years and is known as "Hy's Law." When evaluating new drugs, the FDA uses Hy's law (aminotransferases > 3 x ULN with bilirubin > 2 x ULN with normal alkaline phosphatase *probably due to drug* = 1/10 death) to identify signals for severe hepatotoxicity that may occur post marketing. If 1 Hy's law case is identified in 1000, this suggests severe liver injury at a rate of 1/10,000. It is worrisome to find one Hy's Law case in a clinical trial database but finding two is considered highly predictive that the drug has the potential to cause severe liver injury when given to a larger population.[16] It is important to realize that the severity of liver injury is not related to the degree of transaminase elevation but to the presence of hyperbilirubinemia and/or prolonged prothrombin time indicating hepatic dysfunction.

Assigning Causality

Due to the lack of an objective test, attributing liver injury to a drug or HDS can be difficult. DILI can mimic almost any other known liver disease, have differing histologic findings and overall is a rare occurrence. DILI is a diagnosis of exclusion based on clinical assessment and ruling out other potential etiologies with laboratory testing. Several scoring systems have been developed for DILI

and extrapolated for use in HDS-induced liver injury. The Council for International Organizations of Medical Sciences (**CIOMS**) created the Roussel Uclaf Causality Assessment Method (**RUCAM**) in 1989 as the first liver specific causality tool. This score assigns a numerical value to 7 different factors (chronology, risk factors, concomitant drug use, exclusion of other causes, reported history of drug's toxicity, and response to dechallenge) with scores -8 to 14 to grade probability as definite, very likely, probable, possible, unlikely or excluded. In validation studies, RUCAM had 93% positive predictive value and 78% negative predictive value. However, criticism of this method includes weight placed on age, pregnancy, history of hepatotoxicity and unknown alcohol amount as a cofactor.

RUCAM Causality Assessment

Drug: _____ Initial ALT: _____ Initial Alk P: _____ R ratio = [ALT/ULN] ÷ [Alk P/ULN] = _____ ÷ _____ = _____

The R ratio determines whether the injury is hepatocellular (R > 5.0), cholestatic (R < 2.0), or mixed (R = 2.0 – 5.0)

	Hepatocellular Type		Cholestatic or Mixed Type		Assessment
1. Time to onset					
	Initial Treatment	**Subsequent Treatment**	**Initial Treatment**	**Subsequent Treatment**	**Score** (check one only)
○ From the beginning of the drug:					
• Suggestive	5 – 90 days	1 – 15 days	5 – 90 days	1 – 90 days	☐ +2
• Compatible	< 5 or > 90 days	> 15 days	< 5 or > 90 days	> 90 days	☐ +1
○ From cessation of the drug:					
• Compatible	≤ 15 days	≤ 15 days	≤ 30 days	≤ 30 days	☐ +1
Note: If reaction begins before starting the medication or >15 days after stopping (hepatocellular), or >30 days after stopping (cholestatic), the injury should be considered unrelated and the RUCAM cannot be calculated.					
2. Course	**Change in ALT between peak value and ULN**		**Change in Alk P (or total bilirubin) between peak value and ULN**		**Score** (check one only)
After stopping the drug:					
• Highly suggestive	Decrease ≥ 50% within 8 days		Not applicable		☐ +3
• Suggestive	Decrease ≥ 50% within 30 days		Decrease ≥ 50% within 180 days		☐ +2
• Compatible	Not applicable		Decrease < 50% within 180 days		☐ +1
• Inconclusive	No information or decrease ≥ 50% after 30 days		Persistence or increase or no information		☐ 0
• Against the role of the drug	Decrease < 50% after 30 days OR Recurrent increase		Not applicable		☐ -2
○ If the drug is continued:					
• Inconclusive	All situations		All situations		☐ 0
3. Risk Factors:	**Ethanol**		**Ethanol or Pregnancy (either)**		**Score** (check one for each)
○ Alcohol or Pregnancy	Presence		Presence		☐ +1
	Absence		Absence		☐ 0
○ Age	Age of the patient ≥ 55 years		Age of the patient ≥ 55 years		☐ +1
	Age of the patient < 55 years		Age of the patient < 55 years		☐ 0

4. Concomitant drug(s):	**Score** (check one only)
○ None or no information or concomitant drug with incompatible time to onset	☐ 0
○ Concomitant drug with suggestive or compatible time to onset	☐ -1
○ Concomitant drug known to be hepatoxic with a suggestive time to onset	☐ -2
○ Concomitant drug with clear evidence for its role (positive rechallenge or clear link to injury and typical signature)	☐ -3

5. Exclusion of other causes of liver injury:		**Score** (check one only)
Group I (6 causes):	○ All causes in Group I and II ruled out	☐ +2
○ **Acute viral hepatitis** due to **HAV** (IgM anti-HAV), or	○ The 6 causes of Group I ruled out	☐ +1
○ **HBV** (HBsAg and/or IgM anti-HBc), or	○ Five or 4 causes of Group I ruled out	☐ 0
○ **HCV** (anti HCV and/or HCV RNA with appropriate clinical history)	○ Less than 4 causes of Group 1 ruled out	☐ -2
○ **Biliary obstruction** (By imaging)	○ Non drug cause highly probable	☐ -3
○ **Alcoholism** (History of excessive intake and AST/ALT ≥ 2)		
○ **Recent history of hypotension, shock or ischemia** (within 2 weeks of onset)		
Group II (2 categories of causes):		
○ Complications of underlying disease(s) such as autoimmune hepatitis, sepsis, chronic hepatitis B or C, primary biliary cirrhosis or sclerosing cholangitis; or		
○ Clinical features or serologic and virologic tests indicating acute CMV, EBV, or HSV.		

6. Previous information on hepatotoxicity of the drug:	**Score** (check one only)
○ Reaction labeled in the product characteristics	☐ +2
○ Reaction published but unlabeled	☐ +1
○ Reaction unknown	☐ 0

7. Response to readministration:			**Score** (check one only)
○ Positive	Doubling of ALT with drug alone	Doubling of Alk P (or bilirubin) with drug alone	☐ +3
○ Compatible	Doubling of the ALT with the suspect drug combined with another drug which had been given at the time of onset of the initial injury	Doubling of the Alk P (or bilirubin) with the suspect drug combined with another drug which had been given at the time of onset of the initial injury	☐ +1
○ Negative	Increase of ALT but less than ULN with drug alone	Increase of Alk P (or bilirubin) but less than ULN with drug alone	☐ -2
○ Not done or not interpretable	Other situations	Other situations	☐ 0
		TOTAL (add the checked figures)	

Abbreviations used: ALT, alanine aminotransferase; Alk P, alkaline phosphatase; ULN, upper limit of the normal range of values
Modified from: Danan G and Benichou C. J Clin Epidemiol 1993; 46: 1323-30.

Online calculator is available at http://www.pmidcalc.org/8229110.

The DILIN group uses a structured 3 expert opinion process to assign causality based on likelihood percentage. Cases are assigned to one of 5 categories ranging from unlikely to definite with severity ranging from mild to fatal.

DILIN method achieves high inter-observer agreement and is more likely to diagnose DILI when compared to RUCAM. However, DILIN consensus method is cumbersome, not available to the general clinician and subjective. Despite best efforts to objectively diagnose liver injury due to drug and HDS, adjudication remains more of an art rather than science.[17]

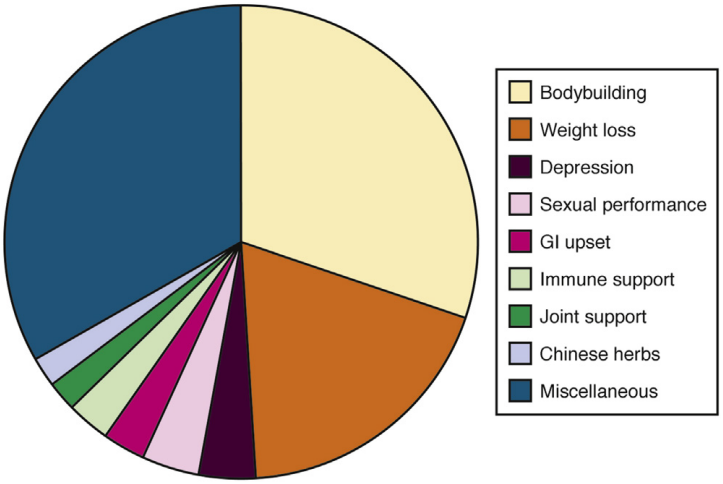
TABLE 6 Comparison of RUCAM, Clinical Practice Expert Opinion, and DILIN Expert Consensus

Criteria	RUCAM	Clinical Practice	DILIN
Adjudication process	Semi-objective quantitative scoring method	Individual expert opinion	Expert consensus
Clinical setting	In real time? In a clinical trial? Retrospective analysis?	Immediate: at the bedside or in the clinic	Case submitted within 6 months of acute DILI onset
Clinical implications	Regulatory? For publication? Mechanistic studies?	Determine if the drug should be stopped vs continued with close monitoring	Epidemiologic data.Identification of pathophysiology and risk factors
Reviewers	1	1	3
Adjudication categories	Highly probable, Probable, Possible, Unlikely, Excluded	Likelihood of 50% or higher usually needed to support clinical decision	Definite, Highly likely, Probable, Possible, Unlikely
Duration of follow-up	1–3 months	Days to months	6 months (or longer)
Nondrug etiologies excluded	Many, but not all (6 group 1 causes: acute HAV, HBV, HCV, biliary obstruction, alcoholism (AST:ALT > 2), ischemic hepatitis; and two group 2 causes: complications of underlying disease, additional viruses as clinically suspected; does not incorporate histology	Usually all (depending on the extent of the evaluation); can include histology	All (including CMV, EBV, HCV, HEV, etc), utilizes hepatic histology read centrally (if available) and long-term follow-up with potential results from biosample testing
Allows for diagnosis of drug-tolerance or adaptation	No	Yes	Yes
Reliance on a positive response to rechallenge	Strong	Rarely	Rarely
Ease of use and generalizability	Not formally tested outside of expert hands	Dependent on clinical experience	Limited to DILIN experts
Reproducibility	$\kappa = 0.34$ May improve with increasing use and familiarity	Unknown for a single expert; improves with discussions to arrive at consensus when more than 1 expert involved, especially when dealing with the same drug (eg, a hepatic adjudication committee for a clinical trial)	$\kappa = 0.6$ Involves discussion to develop a consensus opinion when individual adjudications are divergent

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.
This table has been adapted based on "RUCAM Vs. Expert Opinion," presented by RJ Fontana at Hepatotoxicity DILI Conference XIV: Predicting Serious Drug-induced Liver Injury in Patients, Hyattsville, Maryland, US, March 19, 2014.

Herbal and Dietary Supplements Linked to Liver Injury

Determining the agent(s) responsible in HDS-induced liver injury is uniquely challenging. The permissive regulatory conditions surrounding HDS allow the potential for direct herbal hepatotoxins, contaminants and adulterants to be released on the market without any oversight, determination of safety or efficacy at any stage. To further complicate matters, most cases of HDS-induced liver injury implicate commercial products containing numerous ingredients. Finally, products vary often over time in the list of ingredients contained with no surveillance required. In order to recognize potential culprits, researchers have categorized products by marketed intent. In the DILIN, the most commonly reported products were those used



for bodybuilding but the non-bodybuilding HDS tended to cause a more severe liver injury.[11]

Bodybuilding Supplements

The clinical scenario and pattern of liver injury seen with bodybuilding supplements is virtually diagnostic of anabolic androgenic steroids. Anabolic androgenic steroids are synthetic derivatives of testosterone with restricted use since 1991 due to abuse potential. Despite being labeled as Class III controlled substances, they remain widely available through the Internet as dietary supplements. To circumvent legislation banning these substances, new forms of anabolic steroids are being synthesized making detection of these "designer steroids" more difficult. One study found 23 out of 24 bodybuilding products were adulterated with anabolic steroid compounds, most with no mention on the packaging. [7]

Anabolic androgenic steroids cause several types of liver injury. Focal nodular hyperplasia, peliosis hepatitis, adenomas, hepatocellular carcinoma and even steatosis have all been described though intrahepatic cholestasis is classic and the most frequent form. Typical cases are young men who present with jaundice, pruritus and weight loss, having purchased the product at their gym. Liver enzymes are usually cholestatic though hepatocellular cases have been reported. Most recover but have prolonged jaundice lasting 3-12 months. The exact mechanism of injury is unknown but animal studies showed anabolic steroid induced oxidative stress impaired the bile salt export pump.[18] Given known mutations in ATP8B1/ABCB11, bile homeostasis and export proteins, a genetic susceptibility for steroid induced cholestasis may exist although one has not been identified to date.[19]

Weight Loss Supplements

Hydoxycut®

Hydroxycut® (Iovate Health Sciences, Oakville, Ontario), purported to be "America's #1 selling weight loss supplement brand," is a family of dietary supplements marketed for weight loss. Initial products released in 2002 contained Ma Huang, a botanical containing ephedra alkaloids, and was linked to 2 cases of acute hepatocellular injury with jaundice. After reports of cardiovascular, neurological and hepatic compromise, the FDA banned dietary supplements containing ephedra in 2004. Despite removing ephedra, accounts of liver injury associated with Hydroxycut® continued with 9 additional cases reported. A recent series describes an additional 17 cases of Hydroxycut® associated severe liver injury. Nine men and 8 women were young (mean age 30 years), symptomatic, and presented with hepatocellular injury with transaminases

over 1,000 U/L and jaundice an average 6.4 weeks after starting the supplement. Positive antinuclear antibodies were not uncommon and histology showed cholestatic hepatitis or massive necrosis. In contrast to previous cases with spontaneous recovery, all required hospitalization, 3 underwent liver transplant and one died. The specific ingredient or combination of ingredients in Hydroxycut® responsible for hepatoxicity is unclear. *Cissus quadrangularis*, *Gymnema sylvestre*, *Garcinia cambogia*, *Camellia sinensis* (green tea extract), chromium and caffeine have been suggested as potential hepatotoxins but most speculate that liver injury is due to green tea extract.[20]

In 2009, the FDA issued a public warning regarding risk of severe liver injury associated with Hydroxycut® and the manufacturer recalled its products. Within a month of the recall, new Hydroxycut® formulations containing none of the original botanicals were released, one of which has already been implicated in another case of liver injury.[7] While most patients with Hydroxycut® associated liver injury recover without long-term sequelae, a case of vanishing bile duct syndrome has been published recently.[21]

Herbalife®

Herbalife® (Los Angeles, CA, USA) is one the largest weight management and supplement companies in the world with sales over $5 billion. Products in the form of drinks, energy bars, tablets and capsules are exported to over 60 countries. Since 2005, there have been 11 published reports of 57 cases of liver injury from 7 countries including Switzerland, Israel, Spain, Argentina, Iceland, USA and Venezuela. Most cases are hepatocellular but mixed and cholestatic patterns of liver injury have also been observed. Using various causality scores, most cases were considered probable though definite cases with positive re-challenge were reported. Severity ranges from mild to severe with cases of acute liver failure requiring transplant and chronic injury with eventual cirrhosis reported. Due to the multitude of products containing numerous ingredients, including green tea extract, identifying a potential toxin and mechanism is difficult.[22] Two Herbalife® cases identified *Bacillus subtilis* as a contaminant with dose dependent leakage of LDH from hepatocytes.[23] However, the company maintains they follow strict quality control and challenges the validity of these reports.

Green Tea Extract (Camellia sinensis)

Tea, the most widely consumed beverage next to water, is prepared by pouring hot water over the leaves of the evergreen shrub *Camellia sinensis*. The major bioactive compounds in tea are polyphenol flavonoids called catechins with epigallocatechin gallate (EGCG) making up 30-50% the dry weight of green tea. The average cup of green tea contains 50-150 mg of EGCG. However, concentrated green tea extracts are now a common ingredient in HDS, especially those promoting weight loss.[24]

Since 1999, numerous studies involving over 58 cases have implicated products allegedly containing green tea extract in liver injury. In 2003 Exolise®, an alcoholic extract of *Camellia sinensis*, was withdrawn in France and Spain after 13 cases of acute liver damage. Most reported cases present with hepatocellular injury pattern within 3 months of start and recover after cessation. At least seven patients exhibited accelerated recurrence of liver injury after rechallenge.[25] The US Pharmacopeia reviewed the 34 published cases through 2008 and concluded while there was cause for concern, no advisory label was required.[26] An additional 19 cases of liver injury associated with green tea extract have been identified from 2008 to 2015 with two-thirds involving products with multiple ingredients.[27] Additionally, among the 97 HDS taken by 47 subjects in the DILIN, 51% (49/97) reported containing green tea extracts on the label. On analysis, 73 products contained green tea extract with 40% (29/73) not indicating its presence on the product label. However, there was no statistically significant association between the catechin presence and liver injury causality score, severity or pattern of liver injury. Catechin levels tended to be highest in products used for weight loss but low overall. [28]

The mechanism of green tea extract associated liver injury is unknown but thought due to catechins. EGCG has been shown to be a dose dependent hepatotoxin in mice causing hepatic necrosis with oxidative stress. It is purported that EGCG may induce reactive oxygen species and affect the mitochondrial membrane in certain conditions, such as fasting or genetic predisposition.[29]

In contrast to hepatotoxicity concerns, experimental and clinical data support potential benefits of green tea extract. Furthermore the overall occurrence of liver injury related to green tea appears low. In a meta-analysis of 4 randomized controlled trials of green tea extract that reported adverse liver events, 8 occurred among 1405 subjects (0.5%) versus 1 in 1200 controls (0.1%). Mildly elevated liver enzymes were seen at daily doses 800-1600 mg EGCG with one severely elevated ALT resulting in discontinuation of 1600 mg dose. No serious events occurred.[30]

The safety of green tea extract may vary due to methods of extraction and preparation as well as interaction between ingredients.

Usnic Acid

Usnic acid, derived from lichens, is an uncoupler of oxidative phosphorylation in mitochondria. It is believed decreased efficacy of energy use may lead to thermogenesis and increased fat metabolism for weight loss. However, this mechanism leads to increased oxidative stress and hepatocyte death. Several cases of acute hepatocellular liver injury have been attributed to HDS containing usnic acid, including ones resulting in liver transplant and death. *LipoKinetix®*, a supplement advertised for weight loss containing 100 mg sodium usniate, norephedrine, diiothyronine, yohimbe and caffeine, was linked to at least

11 published cases of acute liver injury prompting an FDA warning in 2001. Ultimately *LipoKinetix®* was removed from the market but other supplements containing usnic acid are still available with severe hepatotoxicity reported with pure usnic acid, *UCP-1®* and *Lipolyz®* supplements.[22]

OxyELITE Pro™

On September 9, 2013, Hawaii Department of Health was notified of seven previously healthy adults presenting May-September 2013 with severe acute hepatitis and liver failure of unknown etiology. All were reported using OxyELITE Pro™ (USPLabs, Dallas, TX, USA), a multi-ingredient supplement containing "proprietary blends of plant-derived extracts" marketed for weight loss.[31] The Hawaii Department of Health with the Centers for Disease Control and the FDA initiated a public health investigation requesting any cases of liver injury associated with weight loss or muscle-building supplements. Clinicians submitted 76 reports, 44 that fulfilled criteria of HDS liver injury, with a total of 36 individuals identified in Hawaii with suspected OxyELITE Pro™ associated liver injury. Nearly two-thirds reported using supplements in addition to OxyELITE Pro™ and time from initial use to onset of symptoms ranged from 1 week to 2 years. Subjects were young (median age 33 years), 57% female, with hepatocellular injury pattern (medians of ALT, AP and TB 1740 IU/L, 141 IU/L, and 9.4 mg/dL). Fourteen patients required hospitalization, 2 received liver transplants and 1 died of cerebral edema.

In early 2013, USPLabs reformulated OxyELITE Pro™ due to the FDA's ban of 1,3-dimethylamylamine (DMAA), an ingredient associated with acute myocardial infarction. Review of the liver injury cases revealed that most reported taking the new DMAA-free formulation of "Super Thermo" OxyELITE Pro™ in the weeks prior to symptoms. Of note, the new formulation contained aegeline, a compound from the native Ayurvedic herbal *Aele marmelos.* The aegeline ingredient, synthesized in China, was a new dietary ingredient and contrary to DSHEA regulations, had not been disclosed to the FDA prior to marketing. No additional known hepatoxins, contaminants or adulterants were found in the products analyzed by the FDA.[32] While all other etiologies of acute liver injury were reportedly excluded, a recent review criticized the causality assessments of the Hawaii cases and concluded that there was insufficient evidence to incriminate OxyELITE Pro™ in all reports.[33] However, an additional seven cases with similar characteristics presenting in 2013 were identified in the continental US through the DILIN, 3 with acute liver failure and 2 requiring liver transplant.[34] The FDA issued a warning letter and OxyELITE Pro™ formulations containing aegeline were removed in November 2013.

Additional HDS marketed for weight loss that have been linked to liver injury include linoleic acid, Ma huang *(Ephedra sinica), Garcinia cambogia,* Germander *(Teucrium chamaedrys)*, Chaparral (*Larrea tridentata*), herbals "Onshidou-Genbi-Kounou" and "Chaso."[35] At least 10 cases of hepatocellular

liver injury including acute liver failure has been linked to *Ephedra.* Due to association with cardiovascular events and stroke, the FDA banned products containing *Ephedra* in 2004.[25] The popular *Garcinia cambogia* has been implicated in 3 cases of liver injury including acute liver failure.[22]

Health Supplements

Black Cohosh (Actaea racemosa, syn *Cimicufuga racemosa)*

Black cohosh is a popular herbal product used primarily for menopausal symptoms. Since 2012, it has been in the top 5 selling botanicals in US with over $40 million in annual sales. It is derived from the roots of *Actaea racemosa*, a plant native to North America. Over 50 reports of hepatotoxicity ranging from elevated transaminases, autoimmune-like hepatitis to acute liver failure prompted a review by the US Pharmacopeia and a cautionary statement added to the label.[36] However, black cohosh has not been associated with liver injury in clinical trials involving over 1200 patients. Several cases of black cohosh hepatotoxicity have been attributed to adulterated products.[37] Driven by high demand, over harvesting of native habitats and increasing costs of authentic black cohosh, Asian *Actaea* are being intentionally substituted for economic gain. Analyses have shown that as many as 25% of preparations labeled as black cohosh contain Asian *Actaea* species instead.[38] US sellers through the Internet have the highest rate of adulterated products (38%) while no adulterated black cohosh preparations were discovered in those obtained from European pharmacies. To further complicate matters, there are now reports of adulterants of the Asian *Actaea* adulterants (www.botanicaladulterants.org, June 2016). While black cohosh appears to be safe, patients may be at risk of liver injury from toxic products erroneously labeled as black cohosh.

Kava (Piper methysticum)

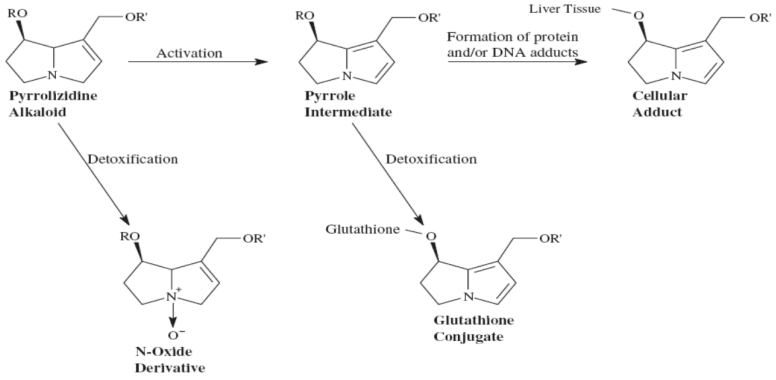
Traditionally a ceremonial beverage in the South Pacific Islands, kava is now sold in a variety of forms as an anxiolytic supplement. Nearly 100 reports worldwide of kava associated liver injury including death lead to its removal in some European markets and an FDA advisory in 2002. Mechanism of hepatoxicity is unknown. It has been postulated that toxicity may be due to alcoholic extraction leading to concentrated kava lactones, increased susceptibility in poor metabolizers with polymorphisms of CYP2D6 or contamination by mold, bacteria or leaf alkaloids. Quality control and daily dose of kava lactones not exceeding 250 mg have been proposed to decrease risk of rare idiosyncratic liver injury.[39]

Pyrrolizidine alkaloids

One of the best-explained plant associated liver injury comes from botanicals containing 1,2 dehydropyrrolizidine alkaloids. More than 350

pyrrolizidine alkaloids have been identified in over 6000 plant species with half of them thought to be hepatotoxic. The concentration of pyrrolizidine alkaloids may fluctuate according to the environment, climate, and season as well the plant age and part. The potential for these agents to cause liver damage has been recognized for over 70 years. First described in Jamaican children drinking bush tea, hepatotoxicity has been reported with *Crotalaria*, *Senecio*, *Heliotropium*, and *Symphytium* (Comfrey) species.

Pyrrolizidine alkaloids are metabolized by cytochrome P450 into active pyrrole derivatives that then form cellular adducts. Inducers of P450 such as phenobarbital enhance toxicity while glutathione repleting N-acetyl-cysteine can be protective. Pyrrolizidine alkaloid adducts act as direct dose-dependent liver toxin damaging the sinusoidal epithelium with liver biopsy shows veno-occlusive disease. Presentation with hepatomegaly and ascites is pathognomonic. Mortality rates reach 20-40% with some developing cirrhosis. In 2001, the FDA banned oral Comfrey.[12]



Vitamin A

It has been known for many years that high doses of vitamin A can result in liver damage. Toxicity is dose-dependent with excess vitamin A stored in the stellate cells, the major mediators of liver fibrosis. Liver biopsy showing lipid droplet laden stellate cells is diagnostic. Hepatotoxicity can present as elevated transaminases, cholestasis, non-cirrhotic portal hypertension or cirrhosis several months to years after taking more than 10 times the recommended daily allowance. Over 50 hepatotoxicity cases due to vitamin A containing supplements have been published. While liver injury rarely occurs with intake less than 50,000 IU per day, there have been reports of injury with 25,000 IU per day and alcoholics may be more susceptible.[22]

Ayurvedic Herbal Products

The majority of India's population use Ayurvedic herbal products with hepatotoxicity rarely reported. However, contents and purity vary with 20-22% of US and Indian-manufactured Ayurvedic products purchased over the Internet found to be contaminated with lead, mercury or arsenic. It is unclear if the few cases of reported liver injury were due to heavy metals or botanical ingredients.[12]

Traditional Chinese Medicine

More than 13,000 herbal preparations have been used for thousands of years as part of Traditional Chinese Medicine yet only a few (less than 60) have been implicated in liver injury.[40] Most products blend 4-5 herbs with 1-2 "King herbs" as the principle ingredient. Labels may only list the "King herb" and additional herbs may alter the constituents making the determination of causative compounds difficult.[12] Plant misidentification, species variation, differing harvest conditions, and extraction process may all contribute to inconsistent herbal products whereas clinical misuse and idiosyncratic reactions may result in hepatoxicity. Herb induced hepatotoxicity accounts for 20-40% of drug induced liver injury in China. The most commonly reported are Lu Cha (*Camillia sinensis*) and mixtures containing *Camillia sinensis* including Chaso and Onshido. Other commonly implicated mixtures include the skullcap (*Scutellaria baicalensis*) containing Xiao Chai Hu Tang used for liver disease and the anti-aging and general tonic Shou Wu Pian (*Polygonum multiflorum*).[40]

U.S. Food and Drug Administration warnings and recalls for liver injury associated with herbal and dietary supplements

Product	Year	Comments
Comfrey	2001	Contained pyrrolizidine alkaloids
Lipokinetix	2001	Contained phenylpropanolamine, caffeine, yohimbine, diiodothyronine, and sodium usniate
Kava Kava	2002	Caused acute liver failure, hepatitis, cirrhosis
Hydroxycut	2009	Caused acute liver failure
Uprizing 2.0	2011	Found to contain Superdrol (anabolic steroid)
OxyElite Pro	2013	Caused acute liver failure

Resources for the Clinician

Liver injury associated with HDS appears to be increasing as use becomes more prevalent. It is imperative to recognize the signs and symptoms of liver injury related to medications and HDS with potential causative agents held. The products reviewed above represent only a fraction of HDS linked to liver injury with additional offenders continuing to be identified. Suspected adverse events related to medications and HDS can be reported to FDA's MedWatch system (http://www.fda.gov/Safety/MedWatch/default.htm). The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and National Institutes of Health have created LIVERTOX®, a current, searchable database of medications, herbs and dietary supplements associated with hepatotoxicity at http://livertox.nih.gov/index.html.

Future Directions

Contrary to popular belief, HDS are no safer than conventional pharmaceuticals and many have been associated with severe liver injury. Supplements marketed for bodybuilding and weight loss containing a multitude of ingredients are especially problematic. Product variability, contamination, and adulteration of HDS remain a concern under current regulation. Coupled with the idiosyncratic nature of HDS-induced liver injury, identifying the causative constituents in this environment is challenging. In order to guide future research, the National Institutes of Health and American Association for the Study of Liver Diseases sponsored a Workshop on Liver Injury from Herbal and Dietary Supplements in May 2015. The most singular finding was that many botanical supplements are generally safe with appropriate selection and judicious use and that the cases of HDS induced liver injury appear to contain adulterants or highly concentrated constituents, far beyond levels found in the plant or standard decoction. Appropriate adjudication and causality assignment are critical to identifying and classifying HDS hepatoxicity cases. Advancements in analyzing the chemical profiles of these products are paramount for future studies to elucidate mechanisms of toxicity and possible susceptibility genes. Meanwhile physicians and scientists should continue to educate the public on the hepatotoxic potential of HDS.

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