Ensuring the safety of botanical dietary supplements

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ABSTRACT
Botanical dietary supplements with a history of safe human use may not require the same level of toxicity testing as synthetic pharmaceutical drugs. Most of the documented examples of acute toxicity caused by botanical dietary supplements have been caused by the substitution of toxic plants for the desired species, probably through misidentification or production errors, or by contamination with pharmaceutical agents, either as a result of poor manufacturing practices or adulteration. Although more difficult to document, chronic toxicities attributed to botanical dietary supplements may be caused by contamination by heavy metals, pesticides, or microbes or by inherent properties of constituents of the botanicals themselves. Like drug-drug interactions, botanical-drug interactions can also be a source of toxicity. Most of these toxicity problems may be prevented by implementing good agricultural practices and good manufacturing practices and applying existing toxicity testing similar to those used in drug development or new toxicity assays under development based on proteomics, genomics, or metabolomics.

KEY WORDS Safety, toxicity, botanical dietary supplements, complementary and alternative medicine, hepatotoxicity, metabolic activation

INTRODUCTION
Consumer interest in complementary and alternative therapies, including the use of botanical dietary supplements, continues to increase throughout the world. In the United States, 42% of the population reported using complementary and alternative medicines, especially botanical dietary supplements (1). Many countries regulate the medicinal and food uses of botanical dietary supplements, and some have specific requirements for herbal products not classified as drugs. For example, the European Union regulates medicinal uses of botanical dietary supplements through the Traditional Herbal Medicinal Product Directive and food supplementation through the Food Supplements Directive 2002/46/EC (2–4). The US Dietary Supplement and Health Education Act of 1994 exempted dietary supplements from regulation as drugs if they are not marketed for the diagnosis, treatment, cure, or prevention of disease (5); thus, they do not require proof of efficacy and do not require premarking approval by the US Food and Drug Administration. However, botanical dietary supplements should still be prepared by the use of good manufacturing practices and should still be tested for safety and efficacy as exemplified by the National Institutes of Health Centers for Botanical Dietary Supplements Research. Toxicity associated with botanical dietary supplements may result from production problems such as the misidentification or mislabeling of the plant species used in a product; use of incorrect parts of plants; contamination or adulteration with pharmaceutical agents; or contamination with pesticides, herbicides, heavy metals, or microbes. Toxicity may also be caused by botanical-drug interactions, the formation of electrophilic metabolites, allergic reactions, botanical-induced autoimmune reactions, or specific systemic or organ-specific reactions. This brief review addresses these potential safety problems and proposes solutions concerning the safe use of botanical dietary supplements.

SAFETY ISSUES RELATED TO THE ACQUISITION OF PLANT MATERIAL
Safety issues regarding the plant material used in botanical dietary supplements include misidentification of a botanical, substitution of a different species, and use of incorrect parts of a plant. Schilter et al (6) reported a risk assessment for botanical dietary supplements including these and other factors such as standardization. To ensure that correct species are obtained, botanicals intended for use in dietary supplements should be cultivated and harvested with the use of good agricultural practices, and field-collected material should be acquired with the use of good collection practices. Each batch of plants used should be identified by using taxonomic examination or biochemical or chemical tests. DNA may be isolated and analyzed by using PCR techniques and compared with authentic material (7), or immunoassays may be used for identification based on species-specific proteins (8). For example, Fong et al (9) used good field collection practice for acquiring rhizomes of Cimicifuga racemosa (L.) Nutt. in the mountains of eastern North America and identified...
specimens by using macroscopic, microscopic, and DNA analyses (10, 11).

When taxonomic or genetic analysis is not possible, such as with plant extracts, processed material should be examined chemically and compared with reference standards for quality assurance and identification. Evaluation may consist of HPLC with ultraviolet absorbance detection, HPLC with evaporative light scattering, or HPLC-mass spectrometry (LC-MS). This approach can detect compounds indicating contaminating plants and thus address safety concerns regarding the composition of botanical dietary supplements.

Toxicity resulting from the misidentification of plant material used in the production of botanical dietary supplements has been reported. End-stage renal disease and in some cases urothelial carcinoma was documented in >40 patients in Belgium who consumed weight-loss formulations based on the Chinese herbs *Stephania tetrandra* and *Magnolia officinalis* (12). Because of a manufacturing error, *Aristolochia fangchi* had been substituted for *Stephania tetrandra*. Aristolochic acid from this plant is known to be nephrotoxic and carcinogenic (13). As a result of this and other occurrences of aristolochic acid toxicity worldwide, the use of *Aristolochia* species in dietary supplements was banned in many countries (14).

**CONTAMINATION AS A TOXICITY RISK**

Even if the botanical constituents of a dietary supplement are nontoxic, nonbotanical contaminants introduced during cultivation, storage, or processing may produce toxic effects. These contaminants may include heavy metals (eg, lead or mercury), pesticides, herbicides, or microbial contaminants (eg, levels of bacteria harmful to human health or toxins such as mycotoxins) that entered the plant during cultivation or pharmaceuticals or industrial chemicals introduced during processing. Chemicals may be introduced as carryover from the processing of other products using the same equipment, added accidentally, or added deliberately as adulterants or processing aids.

Analysis of botanical dietary supplements for heavy metals is routine and uses techniques such as atomic absorption spectros-copy or inductively coupled plasma MS. Assays for microbial content are also well established and should be carried out also as part of routine quality assurance. One outcome of botanical contamination is the formation of mycotoxins, which are toxic fungal secondary metabolites and can be carcinogenic, teratogenic, immunogenic, and neurotoxic. Analyses for pesticide and herbicide residues should be part of routine quality assurance; numerous chromatography-based assays are available. These assays are usually based on gas chromatography with either flame ionization detection, electron capture detection, or MS detection.

A documented example of contamination of botanical dietary supplements by pharmaceutical agents was PC-SPES, which was a popular botanical combination used by men for treating prostate cancer from 1996 until its withdrawal from the market in a popular botanical combination used by men for treating prostate cancer from 1996 until its withdrawal from the market in 1997. Different PC-SPES lots were found to contain warfarin and indomethacin (15, 16) and the synthetic estrogens diethylstilbestrol (15, 17) and ethinyl estradiol (16).

**DETERMINATION OF THE TOXICITY OF BOTANICAL DIETARY SUPPLEMENTS**

**In vitro assays of toxicity**

During drug development, in vitro assays of cytotoxicity, mutagenicity, metabolism, the potential for metabolic activation, and interactions with drug-metabolizing enzymes are carried out. These assays are often difficult to interpret when applied to complex botanical extracts of uncertain composition because they are usually designed for studying individual compounds of known structure and concentration. For example, cytotoxicity assays of botanical extracts are difficult to interpret in terms of predicting toxicity in vivo, because these mixtures and their concentrations are different from the compounds that reach the liver or other organs after oral administration.

A rare example of toxicity screening designed for predicting the formation of electrophilic and potentially toxic metabolites in complex mixtures (18, 19) is ultrafiltration LC tandem MS (LC-MS/MS). This assay is based on the observations that 1) short-lived electrophilic metabolites can be cytotoxic by alkylating cellular biomolecules or participating in redox cycling reactions and 2) one mechanism used by human cells to defend against such reactive intermediates is trapping with glutathione (GSH) to form stable hydrophilic conjugates. Ultrafiltration LC-MS/MS uses incubations of botanical extracts with hepatic microsomes, NADPH, and GSH. If electrophilic and potentially toxic metabolites are formed, they become trapped as GSH conjugates, which are then detected selectively. If GSH adducts are detected with the use of this assay, evidence is obtained for a mechanism of hepatic toxicity. According to Willett et al (20), the most serious safety concern for botanical dietary supplements is the potential for liver injury, and new approaches are needed to identify the determinants of such hepatotoxicity. Combined with in vivo studies to confirm hepatotoxicity (see below), the LC-MS/MS-based toxicity screening assay of Johnson et al (18) is one of the best approaches to address these needs. As an example, Johnson et al (19) evaluated the root of *Piper methysticum* Forst. (kava), which was implicated in liver injury in humans. They found that 2 of the anxiolytic kava lactones, methysticin and 7,8-dihydromethysticin, can be metabolically *O*-demethylated by human cytochrome P450 enzymes to form catechols, which can then be oxidized to quinones (Figure 1). These electrophilic quinones are probably responsible, at least in part, for the hepatotoxicity of kava-containing dietary supplements.

Complementary to ultrafiltration LC-MS/MS screening for cytotoxic metabolites is the Ames test, which is an in vitro assay used to assess the possible mutagenicity of chemicals (21). In this assay, a defective strain of *Salmonella typhimurium* will proliferate only if exposed to mutagenic material that helps reactivate a gene necessary for histidine synthesis. This assay can be modified to screen for mutagenic metabolites by including liver enzymes in the nutrient broth, allowing positive test results to be obtained for metabolically activated natural products, such as safrole from *Sassafras albidum* (Nutall) Nees (Lauraceae). However, the Ames test is an imperfect model for human toxicity because it uses prokaryotes instead of mammalian cells. Unlike ultrafiltration LC-MS/MS toxicity screening, the Ames test provides no information about the identity of mutagenic compounds.
when it is used to screen mixtures such as metabolite preparations or botanical extracts.

### In vivo testing

Classic animal models of chemical and drug toxicity, which can include evaluation of organ toxicity such as hepatotoxicity, systemic toxicity, and teratogenicity, can be used for evaluating the safety of botanical dietary supplements. These in vivo studies have distinct advantages over in vitro models by taking into account issues of pharmacodynamics and pharmacokinetics. As in drug development studies, several animal species should be used to account for interspecies differences. Although animal models can provide evidence of systemic or organ toxicity, they usually do not provide information about mechanisms of toxicity. Therefore, in vitro studies such as metabolic activation experiments are usually needed to obtain this information.

Although in vitro approaches to identifying drug-drug interactions are well established, drug-botanical interactions are more complex because of the multiplicity of compounds in botanical extracts (22). In vivo testing (animal or human) remains the best approach for assessing the potential for drug-botanical interactions.

Unlike most laboratory strains of animals, humans are genetically diverse, consume a varied diet, and are exposed to many environmental factors including pharmaceutical therapy that may be coincident with the consumption of botanical dietary supplements. Although most commercially available botanical dietary supplements do not reflect traditional human use, many have a history of human consumption that may serve as a starting point for clinical evaluation. Therefore, phase I and II clinical trials are ideal settings for the definitive evaluation of the safety as well as the efficacy of botanical dietary supplements. As an example of a phase I clinical trial of a botanical dietary supplement, Piersen et al (23) investigated the maximum tolerated dosage of a red clover extract to determine a safe dosage level for a phase II clinical trial of safety and efficacy with respect to the relief of menopausal hot flashes. The red clover extract was prepared according to good agricultural and manufacturing practices (GAP).
practices and was standardized chemically by isoflavone content and biologically by estrogenicity. Plasma concentrations of liver enzymes indicated no hepatoxicity; plasma concentrations of estrogenic biomarkers, such as estrone, estradiol, follicle-stimulating hormone, and luteinizing hormone, did not change; and there was no clinical evidence of acute toxicity.

Frontiers in toxicity evaluation and prediction

New assays based on the detection or recognition of patterns of diagnostic biomarkers produced by cells or tissues in response to particular toxins are under development. Assays based on proteomics, known as toxicoproteomics (24), measure the effects of xenobiotic exposure on tissues through the identification and quantitative analysis of proteins that are altered in expression and are expected to indicate toxic responses at the cellular level. Assays based on genomics, known as toxicogenomics, interpret how genes are up- or down-regulated in response to cellular exposure to toxins. Assays based on metabolomics, known as toxicometabolomics, may facilitate the identification of changes in the formation of secondary cellular molecules that result from toxicity-induced changes in enzyme expression.

The validity of these assays depends on finding sets of up- or down-regulated proteins, genes, or cellular metabolites that are consistent with specific forms of toxicity. Two approaches are being pursued: patterns of expression are being used as indicators of toxicity, and specific biomarkers are being identified for use in screening assays. For example, specific changes in the expression of genes resulting from in vivo exposure to hepatotoxins such as acetaminophen are being studied with gene microarray analysis (25), and protein changes caused by hepatotoxins such as peroxisome proliferators are being studied with MS-based toxicoproteomics (26). In vivo studies of specific organ toxicities may identify new serum protein markers that can be used to monitor for toxic responses to botanical dietary supplements. In addition, biomarkers of reproductive toxicity may be identified that might help identify and lead to the elimination of products with such risk. Similar toxicometabolomics studies are aimed at identifying new and specific serum or urine markers of toxicity.

These assays have the potential to be used to predict toxic responses to botanical dietary supplements at low doses or after short-term exposure before cytotoxicity, necrosis, or other histologic changes can be detected.

A limitation of these types of assay will be that they will not identify the toxic components of botanical dietary supplements. Some early results suggest that many of the toxic profiles of proteins, genes, etc., are not specific to a unique mechanism or indicative of a specific molecular target (24). However, these patterns are still diagnostic of toxic responses and have the potential to detect many different forms of toxicity instead of being limited to those caused by the electrophilic metabolites. Furthermore, these assays have the potential to be applied to cells growing in vitro or in cells or tissues obtained after in vivo treatment.

CONCLUSIONS

Many toxic responses attributed to botanical dietary supplements may result from contamination by toxic plants or incorrect plant parts, pesticides, herbicides, heavy metals, industrial chemicals, or pharmaceutical agents. Beginning with authenticated plants to prevent misidentification or accidental substitution, then applying good agricultural practices during the acquisition of the plant material and using good manufacturing practices should eliminate most of these problems. Alternatively, toxicity may result from systemic or organ-specific damage by botanical constituents, which would require in vitro and in vivo toxicity studies similar to pharmaceutical development studies. Potential sources of toxicity and approaches to detect and minimize these risks are summarized in Table 1.

Except for the Ames test and the ultrafiltration LC-MS/MS assay of Johnson et al (18), few in vitro toxicity assays are entirely appropriate for botanical dietary supplements. Because animals are also imperfect models for botanical dietary supplements, conventional clinical trials have been the primary sources of toxicity data for these products. To help provide alternative sources of toxicity data, new assays based on toxicoproteomics, toxicogenomics, and toxicometabolomics are being developed to facilitate both in vitro and in vivo screening of the potential of botanical dietary supplements for toxicity.

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### TABLE 1
Sources of toxicity in botanical dietary supplements and approaches to reduce or eliminate these risks

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<td>Use of incorrect plant parts</td>
<td>Good agricultural practices and analytical quality assurance</td>
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<td>Pesticides</td>
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1 LC-MS/MS, liquid chromatography tandem mass spectrometry.
REFERENCES


