Pharmacokinetic Interactions between Drugs and Botanical Dietary Supplements

Alyssa A. Sprouse and Richard B. van Breemen

UIC/NIH Center for Botanical Dietary Supplements Research (AAS, RBvB)
and Department of Medicinal Chemistry and Pharmacognosy (AAS, RBvB),

University of Illinois at Chicago, Chicago, IL
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Corresponding Author:

Richard B. van Breemen

UIC/NIH Center for Botanical Dietary Supplements Research

University of Illinois College of Pharmacy

833 S. Wood Street, Chicago, IL 60612-7231

Telephone: 312-996-9353

E-mail: breemen@uic.edu

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Non-standard Abbreviations:

Rx Drug

BDS Botanical Dietary Supplement

DSHEA Dietary Supplement Health Education Act
Abstract

The use of botanical dietary supplements has grown steadily over the last 20 years despite incomplete information regarding active constituents, mechanisms of action, efficacy, and safety. An important but under-investigated safety concern is the potential for popular botanical dietary supplements to interfere with the absorption, transport and/or metabolism of pharmaceutical agents. Clinical trials of drug-botanical interactions are the gold standard and are usually carried out only when indicated by unexpected consumer side effects or, preferably, by predictive preclinical studies. For example, Phase I clinical trials have confirmed preclinical studies and clinical case reports that St. John’s wort (*Hypericum perforatum*) induces cytochrome P450 3A4/5. However, clinical studies of most botanicals that were predicted to interact with drugs have shown no clinically significant effects. For example, clinical trials did not substantiate preclinical predictions that milk thistle (*Silybum marianum*) would inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and/or CYP3A4. Here, we highlight discrepancies between preclinical and clinical data concerning drug-botanical interactions and critically evaluate why some preclinical models perform better than others in predicting the potential for drug-botanical interactions. Gaps in our knowledge are also highlighted for the potential of some popular botanical dietary supplements to interact with therapeutic agents with respect to absorption, transport and metabolism.
Introduction

In a survey by the U.S. Centers for Disease Control and Prevention, 52 million Americans (4/10 adults) reported using complementary and alternative medicine, especially botanical dietary supplements (Barnes and Bloom 2008), and the Natural Marketing Institute reported that 36 million U.S. adults (approximately 16% of the adult population) used botanical supplements during 2013 (Schultz 2014). A 2011 survey by the Harvard Opinion Research Program found that American consumers used dietary supplements to feel better, improve energy levels, and boost the immune system (Blendon et al. 2013). According to a 2009 Nielsen study, 40% of North Americans and Asians and 30% of Europeans and Latin Americans use dietary supplements (Nielsen Study 2009). Importantly, this does not take into account the various definitions of ‘dietary supplement’ in different parts of the world, some of which include some botanical products as part of the pharmacopeia instead of dietary supplements. The natural products industry generated $5.6 billion in direct sales during 2012 (Schultz 2014), and by a more recent estimate, this industry exceeded $9 billion in sales during 2013 (Lindstrom et al. 2014). From 2012 to 2013, U.S. botanical dietary supplement sales enjoyed an annual increase of 7.9% (Lindstrom et al. 2013).

In the U.S, for example, the botanical dietary supplement market grew rapidly following passage in 1994 of the Dietary Supplement Health Education Act (DSHEA) (Cohen 2012, Cohen 2014). DSHEA defines dietary supplements as neither food nor drugs and therefore liberates them from the regulations of either designation. These products do not require Food and Drug Administration (FDA) approval prior to marketing but must not be adulterated or mislabeled. Although DSHEA has not been amended in over 20 years, the FDA has since imposed regulation 21 CFR part 111 requiring that dietary supplements be produced under dietary supplement current good manufacturing practice (cGMP) conditions. However, cGMP does not
require the botanical dietary supplements industry to investigate possible side effects of the use of these products.

The potential for side effects and other problems resulting from the use of botanical dietary supplements is exacerbated by lack of standardization of these products, patients under-reporting supplement use to their health care providers, and consumers delaying conventional medical care due to reliance on botanical dietary supplements. It is important to note that botanical dietary supplements are used in many different forms such as teas, tinctures, pills, or salves. A wide variety of botanical species are used to produce botanical dietary supplements including different plant parts originating from multiple sources worldwide, all of which contribute to consumer exposure to a wide range of natural products spanning a range of levels. Even scientific studies on the effects of a specific botanical dietary supplement can differ in the species of plants used in the product, the sources of the botanicals, how the botanicals are prepared, how the product is formulated, and how the product is standardized. Each of these variables can affect the biological effects of a botanical dietary supplement and the outcomes of a scientific study.

Among the possible side effects of botanical dietary supplements, as with conventional pharmaceuticals, is interaction with other drugs. This possibility is significant, as 16% of prescription drug users report concurrently taking dietary supplements (Kaufman et al. 2002). This review addresses the potential for botanical dietary supplements to alter the pharmacokinetics of conventional therapeutic agents and, therefore, cause a form of drug-botanical dietary supplement (Rx-BDS) interaction. A review by Tsai et al. (2012) provided a broad overview of drug interactions, toxicities and contraindications for a variety of dietary supplements including botanicals. Both pharmacokinetic and pharmacodynamics interactions were covered, but the depth of Rx-BDS interactions was understandably limited. More recently, Korobkova (2015) reviewed the interactions of natural polyphenols, which can be found in many
botanical dietary supplements, on the activities of cytochrome P450 enzymes (CYP). In particular, Korobkova found that many flavonoids could modulate the activities of CYP3A4, CYP2C9 and CYP1A2 and thereby interfere with drug metabolism. Here, we review the current understanding of these and other Rx-BDS pharmacokinetic interactions, and evaluate the accuracy of preclinical predictive models based on the reality of the clinical evidence.

**Pharmacokinetic Rx-BDS Interactions**

Although the potential for drug-drug interactions must be investigated for all new drugs, and many such interactions have been documented, Rx-BDS interactions remain underexplored. The popularity of botanical dietary supplements worldwide makes this issue particularly urgent. Rx-BDS interactions can include inhibition or induction of 1) cytochrome P450 enzymes involved in drug metabolism; 2) UDP-glucuronosyl transferases; 3) other phase I and phase II enzymes; and 4) drug transporters and drug-efflux proteins (Figure 1). Natural product dietary supplements might inhibit or induce the enzymes responsible for the metabolism of therapeutic agents or their transporters and cause Rx-BDS interactions. When Rx-BDS interactions occur, the pharmacokinetics of therapeutic agents can be altered.

By inhibiting the action of specific drug metabolizing enzymes, natural products in BDS can prolong the half-lives of drugs that depend upon the same enzymes for their degradation, deactivation or conjugation prior to excretion. Longer half-lives will result in prolonged action and even toxicity, especially if drug levels rise unexpectedly after multiple doses. In contrast, inhibition of enzymes responsible for activating pro-drugs would prevent these compounds from exerting their pharmacological effects and would result in loss of pharmacological effects.

On the other hand, enzyme induction would shorten drug half-lives and possibly result in sub-therapeutic levels in the body. Inhibition of drug transporters responsible for uptake would
reduce the absorption of therapeutic agents possibly lowering their efficacy, while induction of drug transporters might cause toxicity due to enhanced blood levels. The opposite is true for efflux drug transporters. An example of a well-documented Rx-BDS interaction is that between St. John’s wort (*Hypericum perforatum*) and drugs metabolized by CYP3A4 (Tirona and Bailey 2006). St. John’s wort induces CYP3A4 through interactions of the natural product constituent hyperforin with the steroid xenobiotic receptor (Wentworth *et al.* 2000). Because 70% of drugs are substrates for CYP3A4, induction of this enzyme can lead to lower efficacy of many therapeutic agents, including oral contraceptives (Hall *et al.* 2003) and the anti-coagulant warfarin (Jiang *et al.* 2004).

**Phase I metabolism.** Cytochrome P450 enzymes are responsible for most phase I metabolism of xenobiotics (Ortiz 1995, Ioannides 1996, Parkinson 1996). These enzymes are expressed primarily in the liver endoplasmic reticulum, although some are abundant in other tissues such as the intestine. The most important cytochrome P450 enzymes in human drug metabolism belong to the 1A, 1B, 2C, 2D, 2E, and 3A subfamilies. The expression and function of these enzymes can be altered by physiological, pathological, genetic, and environmental factors (including exposure to natural products). The following cytochrome P450 enzymes are particularly important in metabolism and Rx-BDS interactions:

**CYP1A1/2 and CYP1B.** Human liver cytochrome P450 is composed of 15-20% CYP1A2, but CYP1A1 is usually not detectable except in smokers. CYP1B (Sutter *et al.* 1994) can metabolize estrogens and some xenobiotic compounds to carcinogens. Substrates for CYP1A2 include acetaminophen, warfarin and caffeine (Wentworth *et al.* 2000, Hall *et al.* 2003). The botanical dietary supplement *Echinacea purpurea* has been reported to inhibit CYP1A2 activity in humans by ~36% (Gorski *et al.* 2004).
CYP3A. Including CYP3A4, CYP3A5 and CYP3A7, the 3A subfamily is the most abundant group of cytochrome P450 enzymes in human liver (30% of the total). Responsible for the metabolism of ~70% of all drugs (e.g., alprazolam, benzphetamine and diazepam) (Shimada et al. 1994), the CYP3A enzymes show broad substrate specificity. CYP3A4 is inducible and can be inhibited by structurally diverse drugs and botanical compounds.

CYP2C8/9/19. Comprising ~25% of CYP450 in human liver (Hall et al. 2003), the 2C subfamily metabolizes many drugs including warfarin, diclofenac, and tolbutamide. Defects in CYP2C19 are rare in Caucasians (2-5%) but affect 12-23% of Oriental subjects.

CYP2D6. Many nitrogen-containing compounds and drugs are metabolized by CYP2D6 including tricyclic antidepressants, morphine and β-blockers (Strobel et al. 1993). Up to 10% of the population have defects in CYP2D6, which can result in exaggerated responses to certain drugs such as tamoxifen and dextromethorphan (Brauch et al. 2009).

CYP2E1. Cytochrome P450 2E1 metabolizes many low mass compounds including acetaminophen, inhalation analgesics, ethanol, and some environmental carcinogens (Guengerich et al. 1991). CYP2E1 is inducible by ethanol and can potentiate acetaminophen toxicity by forming a hepatotoxic quinone imine (Patten et al. 1993).

Phase II metabolism. During phase II metabolism, a substrate is conjugated with a nucleophilic group (thiol, amino, hydroxyl, etc.) donated by a cofactor through a reaction catalyzed by a transferase. Phase II reactions include glucuronidation, phosphorylation, methylation, sulfonation, acetylation, and reaction with glutathione (Testa and Krämer 2008). Most phase II conjugation reactions are catalyzed by the UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) families.

Drug transporters. In addition to first-pass hepatic metabolism, absorption after oral administration is a factor determining the bioavailability of a compound. Lack of absorption might
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explain why many clinical trials of natural products (e.g., milk thistle) have shown no drug interactions although interactions were predicted during preclinical studies.

Serum Binding Competition. The extent to which a drug is bound to serum proteins affects the ability of the drug to be distributed and have therapeutic or toxic effects. If botanical compounds are highly bound to serum proteins, they may compete with other drugs for this protein-binding. Displacement of therapeutic agents from binding sites on serum proteins will increase their rates of elimination, and sudden displacement of drugs from serum proteins by natural products absorbed from a botanical dietary supplement could increase the free drug concentration to toxic levels. For these reasons, botanical compounds which are found to be absorbed should also be tested for serum protein binding.

Preclinical studies of the safety of isolated natural products and those in dietary supplements are essential for determining mechanisms of action, assessing routes of metabolism, and predicting Rx-BDS interactions, but clinical studies must be used to determine the relevance of these results to human health. Due to the popularity of dietary supplements, it is important to determine the safety of these products, and an understudied safety aspect is the potential for Rx-BDS interactions. To determine what Rx-BDS interactions have been investigated and the outcomes of these reports, we reviewed the literature for the most popular botanical dietary supplements (Tables 1 and 2).

State of the Literature

The American Botanical Council reports each year the 40 most popular natural products in the United States based on retail records. We combined the 2012 list created by using SymphonyRI (Blumenthal et al. 2013) with the 2013 list created based on SPINS/IRI (Lindstrom et al. 2014) to provide a comprehensive list of popular botanical dietary supplements. In addition,
we included in our review the additional botanicals goldenseal, noted in a review by Tsai et al. (2012) to cause drug interactions, and hops, due to recent preclinical reports of drug interactions (Yuan et al. 2014). We then examined the data available in the literature for all products on the combined list (Tables 1 and 2). Data for interactions of botanicals with specific drugs were not considered, as these reports often lack confirmation of target enzymes and mechanisms of action. Instead, pharmacokinetic drug interactions of botanicals with specific drug metabolizing enzymes or transporters were included. For simplicity, only positive reports of Rx-BDS interactions were included in the preclinical data columns of Tables 1 and 2, while both negative as well as positive results of Rx-BDS clinical trials were included, as these are the most important evidence of Rx-BDS interactions or the lack thereof.

The 15 botanical dietary supplements listed in Table 1 have been evaluated using both preclinical assays and in clinical trials or only in clinical trials for Rx-BDS interactions. Table 2A summarizes the preclinical data for 13 botanical dietary supplements that have been reported to potentially interact with drugs, while no clinical interaction studies have yet been documented. Examples of botanical dietary supplements with only preclinical evidence of Rx-BDS interactions include bilberry, dandelion, Dong quai, feverfew, grape seed, hops, licorice, red clover, and yohimbe (Table 2A). Most popular botanical dietary supplements, such as kelp, maca, ginger, cinnamon, elderberry, etc., have not been reported to pose risks of Rx-BDS interactions (Table 2B). Indeed, among the 63 most popular natural products in 2012 and 2013 in the United States, 35 have no reports of drug interactions in the literature (Table 2B).

Ten of the dietary supplements listed in Table 1, which include black cohosh, Echinacea, St. John’s wort, milk thistle, and goldenseal, showed potential for Rx-BDS interactions during preclinical studies and were then evaluated in clinical trials. Although preclinical CYP450 inhibition studies are common, CYP450 induction studies are not often conducted. Furthermore,
there are considerable discrepancies between the preclinical inhibition data and the corresponding clinical responses for these botanical dietary supplements. The majority of those dietary supplements (black cohosh, gingko, ginseng, milk thistle, saw palmetto, and valerian) that had been predicted to cause drug interactions using preclinical assays did not produce clinically relevant interactions when tested in humans (Table 1). For example, green tea and kava had been reported to inhibit several drug metabolizing enzymes, but clinical testing of some of these predicted interactions showed no effects. In the case of black cohosh, which had been predicted in preclinical studies to inhibit CYP3A4 and CYP2D6 (Pattern et al. 2003), no clinically observable interactions were observed with CYP3A4, while the predicted inhibition of CYP2D6 was observed in humans but was considered clinically insignificant (Gurley et al. 2004; Gurley et al. 2005).

Only four botanical dietary supplements that were predicted to have drug interactions, St. John’s wort, goldenseal, Echinacea, and garlic oil, have been documented to cause interactions in human trials (Table 1). Even then, only some of the predicted interactions were clinically confirmed. For example, preclinical studies predicted that Echinacea would inhibit CYP2C9, CYP2C19, CYP2D6, and CYP3A4, but a clinical trial carried out by Gorski et al. (2004) found no effects on CYP2C9 or CYP2D6, while inhibition of CYP1A2 and intestinal CYP3A4 were confirmed. Although not predicted by preclinical studies of Echinacea, Gorski et al. observed induction of hepatic CYP3A4 in human subjects. In contrast, a clinical trial by Gurley et al. (2004) found that Echinacea did not inhibit or induce CYP1A2, CYP2D6, CYP2E1, or CYP3A4. These apparently contradictory clinical results of CYP3A4 inhibition/induction by Echinacea can be reconciled in that the intestinal inhibition and hepatic induction of CYP3A4 observed by Gorski et al. (2004) might have offset each other in the study by Gurley et al. (2004), which did not separate these effects. Among the interactions predicted preclinically for garlic dietary
supplements, none have been substantiated in clinical studies except for inhibition of CYP2E1 by garlic oil (Gurley et al. 2002).

In the case of goldenseal, preclinical studies (Table 1) have predicted interactions with CYP2D6, CYP2C9, CYP2C19, and CYP3A4 (Foster et al. 2003; Chatterjee and Franklin 2003; and Budzinski et al. 2000). Clinical trials (Table 1) subsequently confirmed that goldenseal inhibits CYP2D6 (Gurley et al. 2005; Gurley et al. 2008) and CYP3A4/5 (Gurley et al. 2005) but clinical interactions of goldenseal with CYP2C9 and CYP2C19 have not yet been tested. Although preclinical models had not reported any effects of goldenseal on CYP1A2 or CYP2E1, Gurley et al. 2005 investigated this possibility in a clinical trial and found no interactions.

Discussion

The literature on milk thistle (Silybum marianum) and its constituents, silybinin and silymarin, has been extensively reviewed by Brantley et al. 2014. This review indicated that inhibition data had been obtained using recombinant enzymes or human liver microsomes but that no data had been collected regarding induction studies. Although transporter activity and expression were tested, no preclinical absorption data seems to have been produced. From the incomplete pre-clinical studies, it was predicted by some that milk thistle would cause drug interactions, although other researchers disputed this prediction due to low in vivo plasma concentrations and low inhibitory potency. Subsequently, multiple clinical trials of Rx-BDS interactions were carried out using different extracts of milk thistle, and all revealed no drug interaction effects. It was pointed out by Brantley et al. that, to their knowledge, no mathematical modeling had been used to unite the various pre-clinical data as to provide more accurate clinical predictions. In addition to these issues, a variety of milk thistle extracts had been used in the various clinical trials, which further complicated the interpretation of data. The experience with
milk thistle demonstrates how the piecemeal application of some, but not other, preclinical drug interaction studies as well as the failure to unite them with modeling can lead to clinical trials that do not corroborate pre-clinical predictions of drug interactions.

For several other popular botanical dietary supplements, the preclinical testing data for Rx-BDS interactions is incomplete or simply not predictive of clinical effects. In the case of valerian (Table 1), preclinical data predicting Rx-BDS interactions were reported in the same year as the first negative clinical data, so each set of data might have been produced without knowledge of the others. The preclinical data for valerian were obtained using recombinant enzymes (Lefebvre et al. 2004; Strandell et al. 2004) and predicted mild interactions. The first clinical trial of Rx-valerian interactions showed no effects (Donovan et al. 2004), and the lack of clinical effect was confirmed in another clinical trial reported a year later (Gurley et al. 2005).

Although the preclinical data for saw palmetto (Table 1) suggested no inhibition of CYP2D6 or CYP3A4 using recombinant protein (Budzinski et al. 2000), clinical trials were conducted and showed no evidence of Rx-saw palmetto interactions (Markowitz et al. 2003). Interestingly, a later study did show preclinical inhibition of CYP2D6, CYP3A4 and CYP2C9 using recombinant enzymes (Yale and Glurich 2005), which further highlights the problem of incomplete preclinical data used to inform clinical trial decisions. In the case of ginseng (Table 1), preclinical studies with human liver cells predicting drug interactions were not corroborated in a clinical trial (Anderson et al. 2013). A similar outcome was observed for ginkgo (Table 1), when preclinical work with both recombinant protein (Yale and Glurich 2005) and liver microsomes (Ohnishi et al. 2003) predicted inhibition of several CYP450 enzymes, but no Rx-ginkgo interactions was observed in a clinical trial (Gurley et al. 2002).

These many examples indicate that clinical trials often fail to confirm Rx-BDS interactions that were predicted by common preclinical experiments. We assert that this is a failure of current
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pre-clinical models used to predict clinical drug interactions. To correct this problem, we suggest that more rigorous pre-clinical testing of botanical dietary supplements can better inform which botanicals to investigate in clinical trials and can inform the design of these trials.

Recommendations for Future Interaction Studies

In order to avoid expensive human trials that show no effects, we suggest alternative pre-clinical testing methods to predict Rx-BDS interactions more accurately and to provide data for prioritizing botanical dietary supplements for clinical evaluation (Figure 2). This workflow for Rx-BDS studies is based on the FDA Guidance for Industry – Drug Interaction Studies (FDA Guidance 2012) and may also be used to inform experimental design. Our workflow highlights the importance of each preclinical assay before moving to clinical trials. We also suggest that the scheme in Figure 2 should be amended as new and updated preclinical models become available.

To minimize discrepancies between preclinical and clinical trials, the same botanical material or extract should be used at all stages of study. More uniform inter-laboratory results can be obtained by standardizing botanical dietary supplements both chemically, based on active compounds, and biologically through bioassays. The US Pharmacopeial Convention provides guidance on standardization of botanical dietary supplements, and USP Dietary Supplement Reference Standards are available to facilitate standardization (USP 2015). The AOAC International also provides guidance on chemical standardization of botanical dietary supplements (AOAC 2015). The goal of chemical and biological standardization is to ensure that the botanical dietary supplement will have reproducible effects for research purposes as well as for consumers. For additional information regarding standardization of botanical dietary supplements, see our recent perspective (van Breemen 2015).
Another reason for the inconsistencies between preclinical data and clinical results is that the preclinical assays do not take into account bioavailability of the relevant natural products. For example, if the botanical natural products responsible for preclinical inhibition of cytochrome P450 enzymes are not absorbed following oral administration (Shen *et al.* 1997), then they would be unlikely to have any effects on phase I metabolism in humans. Inactivation of these compounds by phase II enzymes via first pass metabolism would also lower their bioavailability and minimize the possibility of Rx-BDS interactions. This reinforces the need for the study of the intestinal absorption and phase I and II metabolism of botanical natural products. Therefore, it is important to start with predictors of bioavailability such as the Caco-2 permeability assay to predict uptake and tissue accumulation. Such studies also allow for the exploration of the effects on drug transporters that can be very important in Rx-BDS interactions. Next, serum-binding assays of bioavailable natural products should be carried out to predict alterations of drug distribution.

The frequency of botanical natural products showing cytochrome P450 inhibition in preclinical studies without similar effects in humans suggests that most preclinical methods are over-estimating inhibition. One possible solution might be the emerging use of human hepatocytes in place of liver microsomes to investigate inhibition as well as induction of drug metabolizing enzymes (Chen *et al.* 2011, Xu *et al.* 2009, Zhao 2008, Li and Doshi 2011). We agree with Mao *et al.*, and others who have also suspected that the use of microsomes tends to overestimate CYP enzyme inhibition, and that incorporating cell membrane permeability and phase II enzyme transformation with intact hepatocytes will provide a more reliable prediction of natural product interactions with CYP enzymes (Mao *et al.* 2011, Li *et al.* 2011). It might be ideal to combine inhibition and induction studies in a single assay by determining both CYP450 activities and expression changes simultaneously. We believe it is important to study both enzyme expression and activity as these complementary data provide different
pieces of information. These data should corroborate each other while providing strong evidence, or lack thereof, of Rx-BDS interactions.

To improve the predictive accuracy of preclinical assays of Rx-BDS interactions, it is ideal to use a model-based form of evaluation of interactions to determine whether clinical studies are necessary (Espié et al. 2009). The most inclusive models are dynamic models such as pharmacologically based pharmacokinetics (PBPK). PBPK uses mathematical models to predict absorption, distribution, metabolism, and excretion. These models integrate preclinical protein/tissue binding, metabolism, transport, and Rx-BDS interaction data with physiochemical data and any pharmacokinetic data available to create a system model of the body. These modeling data could then be used to determine the need for clinical studies, guide the design of Rx-drug interaction experiments, predict the magnitude of interactions, and even predict at-risk populations. When designing these models, it will be important to consider any model assumptions, physiological and biological plausibility, parameters origins, as well as uncertainty and variability.

Importance of Further Investigation

Some botanical dietary supplements have been shown in clinical trials to cause Rx-BDS interactions, but these effects are generally mild to moderate. We suspect this trend will continue with future investigations of drug interactions with the most popular botanical dietary supplements. Occasionally, as in the case of St. John’s Wort, these drug interactions may prove to be significant. For botanical dietary supplements with a long history of use and/or food without incident, the risk for Rx-BDS interactions is likely to be low. However, without preclinical experimentation, these interactions will not be recognized until consumers have already been negatively affected.
Currently, the primary methods for evaluating the potential for Rx-BDS interactions include the use of human liver microsomes and primary human hepatocytes to determine inhibition and induction, respectively, of cytochrome P450 enzymes and the use of Caco-2 human epithelial colorectal adenocarcinoma cell monolayer model to predict absorption and efflux. However, these assays are used sporadically rather than systematically. By using these preclinical assays in tandem along with PBPK modeling, probable Rx-BDS interactions that should be tested in clinical trials can be more accurately predicted. The resulting clinical trials measuring the effects of botanical dietary supplements on cytochrome P450 enzymes using probe drugs will be more likely to produce relevant safety data.

Studies of possible Rx-BDS interactions are especially important considering that manufacturers of botanical dietary supplements are not required to generate these data before production and sale, and because consumers frequently use botanical dietary supplements simultaneously with prescription medications. With the lack of knowledge regarding possible Rx-BDS interactions, we put health at risk, especially for vulnerable populations, who often turn to botanical dietary supplements when conventional medicine fails them. There is an unmet need to carry out studies of potential Rx-BDS interactions that will provide crucial safety information for consumers as well as guide suppliers toward product improvements.
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Authorship Contribution

Participated in research design: Sprouse and van Breemen.

Conducted experiments: Sprouse.

Contributed new reagents or analytic tools: N/A

Performed data analysis: Sprouse and van Breemen.

Wrote or contributed to the writing of the manuscript: Sprouse and van Breemen.
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References


AOAC Stakeholder Panel on Dietary Supplements (SPDS).


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Liang W, Li W, Nikolic D, and van Breemen RB (2003) LC-MS/MS determination of the inhibition of human cytochrome P450 isozymes by a standardized *Trifolium pratense* (red clover) extract. 51st ASMS Conference on Mass Spectrometry and Allied Topics; Montreal, Canada.


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Footnotes

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Figure Legends

Figure 1. Pharmacokinetic Drug-Botanical Interactions. Botanicals can cause pharmacokinetic drug interactions by interfering with drug metabolizing enzymes in the liver, stomach and intestines; drug transporters in kidneys, stomach and intestines that will alter absorption, bioavailability and drug elimination; and proteins in the blood that can alter drug distribution.

Figure 2. Suggested Drug-Botanical Interaction Investigation Work Flow. A) For a botanical dietary supplement, the potential for CYP interactions must first be determined followed by the identification of active compounds. B) For an active compound either alone or in an extract, the absorption, efflux and importance of transporters will first be predicted using the Caco-2 permeability assay. If there is significant absorption, the amount of free-compound in serum will be predicted using rapid equilibrium dialysis. If the properties of the extract or compound are sufficient, drug interaction experiments will then be conducted using the previous experiments to inform concentration decisions. Induction of CYP450 enzyme activity and mRNA expression will be examined using hepatocytes and/or HepaRG cells.
Table 1. Popular natural product supplements with clinical drug interaction data.

(-) No data found. *Clinical interactions have been noted as reported by (Tsai et al. 2014)
†Clinical interactions have been reported as noted by (Hu et al. 2005)
‡Clinical interactions have been noted as reported by (Norwack 2008)

<table>
<thead>
<tr>
<th>Common name</th>
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<th>Preclinical interactions</th>
<th>Clinical interactions</th>
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<td>Induction</td>
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<td>CYP2C19, CYP2D6, CYP3A4, MDR1 (Strandell et al. 2004, Lefebvre et al. 2004)</td>
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</tr>
</tbody>
</table>
Table 2. Popular natural product supplements with no clinical drug interactions data.
*Clinical interactions have been noted as reported by (Tsai et al. 2014)
†Clinical interactions have been reported as noted by (Hu et al. 2005)

<table>
<thead>
<tr>
<th>Common name</th>
<th>Latin binomial</th>
<th>Preclinical interactions</th>
<th>Clinical interactions</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Inhibition</td>
<td>Induction</td>
</tr>
<tr>
<td><strong>A. Popular natural products supplements with preclinical but no clinical drug interaction data</strong></td>
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</tr>
</tbody>
</table>
| Bilberry *
Vaccinium myrtillus | OATP2B1 (Mao et al. 2013) | - | - | - |
| Cannabinoids            |                | - | CYP1A2 (Stout and Cimino 2014) | - |
| Dandelion *
Taraxacum spp. | CYP1A2 (Maliakal and Wanwimolruk 2001) | UDPGT (Zhou et al. 2004) | - |
| Dong quai*†
Angelica sinensis | CYP1A (Lin et al. 1998)
CYP3A4 (Guo et al. 2001) | CYP2D6 (Tang et al. 2006)
CYP3A4 (Gurley et al. 2006) | - |
| Evening Primrose Oil*
Oenothera biennis | Cis-linoleic acid: CYP1A2 (Zou et al. 2002)
CYP2C9, CYP2C19, CYP2D6, CYP3A4 (Netsch et al. 2006) | - | - | - |
| Feverfew leaf
Tanacetum parthenium | CYP2C9, CYP2C19, CYP2D6, CYP3A4 (Li and Doshi 2011) | - | - |
| Grape seed
Vitis vinifera | OATP2B1 (Mao et al. 2013) | - | - |
| Hops
Humulus lupulus | CYP1A2 (Yuan et al. 2014)
CYP2C8, CYP2C9, CYP2C19 (Whitten et al. 2006) | - | - |
| Licorice root*
Glycyrrhiza glabra, G. uralensis and G. inflata | CYP2A1 (Paolini et al. 1999)
CYP2B6, CYP2C8, CYP2C9, CYP2C19 (John et al. 1999, Lefebvre et al. 2004)
UGT1A1 (Guo et al. 2013) | CYP1A2 (Kent et al. 2002)
CYP2B6 (Lefebvre et al. 2004) | - |
| Plant Sterols (e.g.
sitosterol) | MDR1 (Nabekura et al. 2008)
MRP1 (Chow et al. 2006) | - | - |
| Red clover
Trifolium pratense | CYP1B1 (Roberts et al. 2004)
CYP2C8 (Piersen et al. 2004, Liang et al. 2003)
CYP2C9 (Maliakal and Wanwimolruk 2001, Lin et al. 1998) | - | - |

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<tr>
<td></td>
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<td>Inhibition</td>
<td>Induction</td>
</tr>
<tr>
<td>Turmeric</td>
<td>Curcuma longa</td>
<td>CYP1A2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP2B6</td>
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<td></td>
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<td>CYP2C9</td>
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<td>CYP2D6</td>
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<td></td>
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<td>CYP3A4</td>
<td>-</td>
</tr>
<tr>
<td>Yohimbe*</td>
<td>Pausinystalia yohimbe</td>
<td>CYP2D6</td>
<td>-</td>
</tr>
</tbody>
</table>

**B. Popular natural product supplements with no reported preclinical or clinical drug interactions data**

Acai* (Euterpe oleracea); Alfalfa* (Medicago sativa); Aloe vera* (Aloe vera); Artichoke (Cynara spp.); Barley (Hordeum vulgare); Bromelain (Ananas comosus); Cascara sagrada (Frangula purshiana); Cayenne (Capsicum annuum); Chia seed / oil (Salvia hispanica); Cinnamon (Cinnamomum spp.); Coconut oil (Cocos nucifera); Damiana leaf (Turnera diffusa); Elderberry (Sambucus nigra); Eyebright herb (Euphrasia spp.); Fennel (Foeniculum vulgare); Fenugreek (Trigonella foenum-gracecum); Flaxseed (Linum usitatissimum); Ginger (Zingiber officinale); Gotu Kola (Centella asiatica); Gymnema (Gymnema sylvestre); Hawthorn* (Crataegus spp.); Horehound (Marrubium vulgare); Horny goat weed (Epimedium spp.); Horsetail (Equisetum spp.); Horse chestnut seed (Aesculus hippocastanum); Kelp (Laminaria digitata); Maca (Lepidium meyenii); Olive leaf (Olea europaea); Pycnogenol (Pinus pinaster); Red yeast rice* (Monascus purpureus); Senna (Senna alexandrina); Slippery elm bark (Ulmus rubra); Spirulina (Arthrospira spp.); Tribulus (Tribulus terrestris); White kidney bean (Phaseolus vulgaris)
Oral administration

Liver
Interaction with metabolizing enzymes can affect drug metabolism.

Kidney
Interaction with transporters and proteins can affect drug elimination.

Stomach and Intestines
Interaction with transporters and metabolizing enzymes can affect drug absorption and bioavailability.

Transportation in blood
Interaction with bloodstream proteins can affect distribution.