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In vivo effects of goldenseal, kava kava, black cohosh, and valerian on human cytochrome P450 1A2, 2D6, 2E1, and 3A4 phenotypes

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Abstract

Objectives— Phytochemical-mediated modulation of cytochrome P-450 activity may underlie many herb-drug interactions. Single time-point, phenotypic metabolic ratios were used to determine whether long-term supplementation of goldenseal (*Hydrastis canadensis*), black cohosh (*Cimicifuga racemosa*), kava kava (*Piper methysticum*), or valerian (*Valeriana officinalis*) extracts affected CYP1A2, CYP2D6, CYP2E1, or CYP3A4/5 activity.

Methods— Twelve healthy volunteers (6 females) were randomly assigned to receive goldenseal, black cohosh, kava kava, or valerian for 28 days. For each subject, a 30-day washout period was interposed between each supplementation phase. Probe drug cocktails of midazolam and caffeine, followed 24 hours later by chlorzoxazone and debrisoquine were administered before (baseline) and at the end of supplementation. Pre- and post-supplementation phenotypic trait measurements were determined for CYP3A4/5, CYP1A2, CYP2E1, and CYP2D6 using 1-hydroxymidazolam/midazolam serum ratios (1-hour sample), paraxanthine/caffeine serum ratios (6-hour sample), 6-hydroxychlorzoxazone/chlorzoxazone serum ratios (2-hour sample), and debrisoquine urinary recovery ratios (8-hour collection), respectively. The content of purported “active” phytochemicals was determined for each supplement.

Results— Comparisons of pre- and post-supplementation phenotypic ratio means revealed significant inhibition (~40%) of CYP2D6 (difference = −0.228; 95% CI = −0.268 to −0.188) and CYP3A4/5 (difference = −1.501; 95% CI = −1.840 to −1.163) activity for goldenseal. Kava produced significant reductions (~40%) in CYP2E1 only (difference = −0.192; 95% CI = −0.325 to −0.060). Black cohosh also exhibited statistically significant inhibition of CYP2D6 (difference = −0.046; 95%

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CI = -0.085 to -0.007), but the magnitude of the effect (~7%) did not appear clinically relevant. No significant changes in phenotypic ratios were observed for valerian.

Conclusions— Botanical supplements containing goldenseal strongly inhibited CYP2D6 and CYP3A4/5 activity *in vivo*, while kava inhibited CYP2E1 and black cohosh weakly inhibited CYP2D6. Accordingly, serious adverse interactions may result from the concomitant ingestion of goldenseal supplements and drugs that are CYP2D6 and CYP3A4/5 substrates. Kava kava and black cohosh may interact with CYP2E1 and CYP2D6 substrates, respectively. Valerian appears less likely to produce CYP-mediated herb-drug interactions.

Introduction

Since passage of the Dietary Supplement Health and Education Act in 1994, interactions between conventional medications and botanical supplements have become a growing medical concern. Botanical dietary supplements often contain pharmacologically active phytochemicals that, when consumed concomitantly with conventional medications, may result in pharmacokinetic and/or pharmacodynamic herb-drug interactions.¹⁻⁴ A number of significant herb-drug interactions have been linked to phytochemically-mediated alteration of cytochrome P-450 (CYP) activity.⁴ Some of the most noteworthy involve St. John's wort (*Hypericum perforatum*) and various CYP3A4 substrates.⁵⁻¹⁰ St. John's wort contains hyperforin, a phloroglucinol with antidepressant properties and a strong affinity for the orphan nuclear receptor, SXR (steroid-xenobiotic-receptor). As an SXR ligand, hyperforin promotes CYP3A4 gene expression.¹¹ By inducing the activity of intestinal and hepatic CYP3A4, hyperforin can produce marked reductions in the oral bioavailability of many medications rendering them less effective or ineffective.

In vitro evidence from various human CYP inhibition and induction assays suggest that other botanical supplements have the potential to modulate CYP activity.¹²⁻²¹ Corroboration of *in vitro* findings with human *in vivo* studies, however, has been somewhat equivocal. With regard to CYP inhibition, *in vivo* studies of *Echinacea purpurea*^{23,24} saw palmetto^{24,25} and various ginseng supplements^{26,27} seem to complement most *in vitro* findings,^{12,16,22} while those for St. John's wort,^{13,16} *Ginkgo biloba*¹³ and milk thistle^{28,29} are not borne out by the *in vivo* evidence.^{7,24-26,30} Adding to the confusion are garlic supplementation studies in humans that both agree³¹ and disagree^{26,32} with the *in vitro* evidence for CYP3A4 induction.¹⁵ As a whole, such discrepancies can often be traced to higher phytochemical concentrations utilized *in vitro* relative to those achieved *in vivo* following oral administration of botanical supplements. Factors that contribute to these differences include interindividual variations in intestinal absorption and presystemic metabolism, interproduct variability in phytochemical content, and poor product bioavailability and/or dissolution characteristics.

Recently, a practical alternative *in vivo* screening method utilizing single time-point phenotypic metabolic ratios was described for identifying botanical supplements capable of modulating CYP activity.^{24,26} While not intended to supplant concentration-time profiles and area-under-the-curve determinations for calculating drug clearance, phenotypic metabolic ratios of specific "probe drugs" can provide reasonable clearance estimates, thereby allowing multiple CYP enzymes and multiple botanical supplements to be evaluated *in vivo* using a limited blood-sampling scheme.²⁶ This methodology was used to verify the inductive effect of St. John's wort on CYP3A4/5,⁷ and further demonstrated that echinacea, saw palmetto, *Panax ginseng*, and *Ginkgo biloba*, have nominal effects on human CYP activity *in vivo*—findings confirmed by other investigators using more traditional assessments of probe drug clearance (i.e. area-under-the-curve).^{23,25,27,30} Using this approach we describe here, for the first time in humans, the effect of prolonged administration of goldenseal, kava kava, and black cohosh, on CYP1A2, CYP2D6, CYP2E1, and CYP3A4/5 phenotypes. This study also supports a recent

investigation into the effect of valerian on CYP2D6 and CYP3A4³³ and provides further insight into its effect on CYP1A2 and CYP2E1.

Goldenseal (*Hydrastis canadensis*), a botanical supplement taken to prevent the common cold and upper respiratory tract infections, has been shown to inhibit various CYP isoforms *in vitro*.^{12,14,20} Kava kava (*Piper methysticum*), touted for its sedative and antianxiety properties, also appears to inhibit a variety of CYP isoforms *in vitro*, especially CYP3A4/5.^{13,14,18,19,21,34} Like kava kava, valerian (*Valeriana officinalis*) is a popular alternative sedative-hypnotic and anxiolytic that also exhibits modest *in vitro* inhibitory activity of human CYP2D6 and CYP3A4/5.^{16,35} The purported ability of black cohosh (*Cimicifuga racemosa*) to help alleviate menopausal symptoms and premenstrual syndrome has secured its ranking among the top-selling supplements in the United States; however, no studies, *in vitro* or *in vivo*, have evaluated its effect on human CYP activity.

MATERIALS AND METHODS

Study subjects

This study protocol was approved by the University of Arkansas for Medical Sciences Human Research Advisory Committee (Little Rock, AR) and all participants provided written informed consent before commencing the study. Twelve young adults (6 females) (age, mean $\pm$ SD = 24 $\pm$ 3.0 years; weight, 69.3 $\pm$ 14.2 kg) participated in the study and all subjects were in good health as indicated by medical history, routine physical examination, and clinical laboratory testing. All subjects were extensive metabolizers of CYP2D6 as confirmed by debrisoquine urinary recovery screenings.²¹ All subjects were nonsmokers, ate a normal diet, and were not users of botanical dietary supplements. Excluding oral contraceptive use (4 females), subjects were not taking prescription or nonprescription medications. All female subjects had a negative pregnancy test at baseline. Female subjects continued previously prescribed oral contraceptive therapy. All subjects were instructed to use a barrier method of contraception during the study, in addition to any prescribed oral contraceptive. All subjects were asked to abstain from alcohol, caffeine, fruit juices, cruciferous vegetables, and charbroiled meat throughout the study. Adherence to these restrictions was further emphasized five days before each probe drug administration. Subjects were also asked to refrain from taking prescription and nonprescription medications during supplementation periods, and any medication use during this time was recorded. Documentation of compliance to these restrictions was achieved through the use of a food/medication diary.

Due to reports of possible hepatotoxicity associated with prolonged kava kava use,³⁶⁻³⁸ blood chemistry profiles, including assessment of various liver function enzymes (AST, ALT, and GGT), were evaluated in each subject before and at the end of each kava supplementation period.

Supplementation and phenotyping procedure

The ability of goldenseal, kava kava, black cohosh and valerian extracts to modulate human CYP activity was evaluated individually on four separate occasions in each subject. This was an open-label study randomized for supplementation sequence. Each supplementation period lasted 28 days and was followed by a 30-day washout period. This randomly assigned sequence of supplementation followed by washout was repeated until each subject had received all four botanical supplements. Single lots of goldenseal (lot # 303415) and kava kava (lot #V4694K06) were purchased from the same vendor (Wild Oats Markets, Inc. Boulder, CO.). The black cohosh supplement (lot #060706) was a product of Solaray Inc. (Park City, UT), and the valerian supplement (lot #303990) was manufactured by Vitamer (Lake Forest, CA). Product labels were followed regarding the administration of goldenseal root extract (900 mg, three

times daily, no standardization claim), kava kava root extract (1000 mg, twice daily, no standardization claim); black cohosh root extract (1090 mg, twice daily, each capsule standardized to 0.2% triterpene glycosides); and valerian root extract (125 mg, three times daily, no standardization claim). Telephone and electronic mail reminders were used to facilitate compliance, while pill counts and supplementation usage records, were used to verify compliance.

CYP1A2, CYP2D6, CYP2E1 and CYP3A4/5 phenotypes were assessed before (Days -1, 0) and at the end of each supplementation phase (Days 27, 28). Forty-eight hours before supplementation (Day -1) each subject received an oral dose of caffeine (100 mg, oral solution) (Mallinckrodt Baker, Inc. Paris, KY), and midazolam (8 mg) (Bedford Laboratories, Bedford, OH). Blood samples (10 mL) were collected at 1 and 6 hours after probe drug administration and separated by centrifugation (1133 x g) to obtain serum for determining CYP3A4/5 and CYP1A2 activity. To avoid potential interference from midazolam and caffeine, CYP2E1 and CYP2D6 phenotypes were assessed twenty-four hours later.³⁹ The day before supplementation (Day 0), each subject emptied their bladder prior to receiving oral doses of chlorzoxazone (250 mg) (Watson Laboratories, Corona, CA) and debrisoquine (5 mg, oral solution) (Sigma-Aldrich Co., St. Louis, MO). Blood samples were then obtained at 2 hours and urine was collected for 8 hours, at which time the volume was recorded and a 10-milliliter aliquot stored for analysis. All samples were stored frozen at -70°C until analyzed. Phenotypes were again assessed on supplementation Days 27 (CYP1A2, CYP3A4/5) and 28 (CYP2D6, CYP2E1). The CYP modulatory capability of each botanical supplement was evaluated by comparing individual differences in phenotype before and at the end of 28 days of supplementation.

Phenotype Assessment

The justification of specified time points for obtaining metabolite/parent serum ratios to estimate probe drug clearance has been previously addressed.²⁶ Serum ratios of 1-hydroxymidazolam/midazolam determined one hour after dosing were used to estimate CYP3A4/5 activity. CYP1A2 phenotypes were determined from paraxanthine/caffeine serum ratios obtained at six hours. CYP2E1 activity was estimated from 6-hydroxychlorzoxazone/chlorzoxazone serum ratios obtained 2 hours after dosing, while CYP2D6 activity was assessed using 8-hour debrisoquine urinary recovery ratios: [4-hydroxydebrisoquine/(debrisoquine + 4-hydroxydebrisoquine)].

Analytical methods

Serum concentrations of caffeine and paraxanthine were quantified by high performance liquid chromatography (HPLC) with ultraviolet absorbance detection per the method of Holland et al.⁴⁰ Chlorzoxazone and 6-hydroxychlorzoxazone serum concentrations were measured by HPLC using ultraviolet absorbance detection as previously described by Frye and Stiff.⁴¹ The HPLC method described by Frye and Branch employing fluorescence detection was utilized for the quantitation of debrisoquine and 4-hydroxydebrisoquine in urine.⁴² A previously described modification of the HPLC method of Sautou et al.⁴³ was used to determine serum concentrations of midazolam and 1-hydroxymidazolam.²⁶ To optimize the recovery of 6-hydroxychlorzoxazone and 1-hydroxymidazolam, serum samples (250 sL) containing these probe drugs were adjusted to a pH of 5.0 with 0.2M sodium acetate and incubated with β -glucuronidase (250 sL, 2000 units per mL) for 2.5 hours at 37°C

The phytochemical content of each supplement was independently analyzed for specific "marker compounds" by various HPLC and capillary electrophoretic methods at the National Center for Natural Product Research (University of Mississippi, College of Pharmacy). The isoquinoline alkaloid content (hydrastine and berberine) of goldenseal was performed via the method of Abourashed and Khan.⁴⁴ Quantitation of kava lactones (kavain, dihydrokavain,

methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin) was performed per the method of Ganzera and Khan.⁴⁵ Black cohosh was analyzed for triterpene glycosides (cimicifloside, 27-deoxyactein, actein) using reversed phase HPLC with evaporative light scattering detection as described by Ganzera et al.⁴⁶ The valerian supplement was analyzed for valerenic acid content using the capillary electrophoretic method of Mikell et al.⁴⁷

Disintegration tests

An absence of botanical-mediated changes in CYP phenotype could stem from products exhibiting poor disintegration and/or dissolution characteristics. To address this concern, each product was subjected to disintegration testing as outlined in the United States Pharmacopeia 27.⁴⁸ The disintegration apparatus consisted of a basket-rack assembly operated at 29–32 cycles per minute with 0.1 N HCl (37°C) as the immersion solution. One dosage unit of each supplement was placed into each of the six basket assembly tubes. The time required for the complete disintegration of six dosage forms was determined. This process was repeated with an additional six dosage units to assure accuracy. Since there are no specifications for the disintegration time of the botanical supplements used in this study, the mean of six individual dosage forms was taken as the disintegration time for that particular product. A product (e.g. hard gelatin capsule, soft gelatin capsule, uncoated tablet) was considered completely disintegrated if the entire residue passed through the mesh screen of the test apparatus, except for capsule shell fragments, or if the remaining soft mass exhibited no palpably firm core.

Statistics

A repeated measures ANOVA model was fit for each phenotype response using SAS Proc Mixed software (SAS Institute, Inc. Cary, N.C.). Since pre- and post-supplementation phenotypic ratios were determined in each subject for all four supplements, a covariance structure existed for measurements within subjects. Sex, supplement, and supplement-by-sex terms were estimated for each phenotype using a Huynh-Feldt covariance structure fit. If supplement-by-sex interaction terms for a specific phenotypic measure were significant at the 5% level, the focus of the post-supplementation minus pre-supplementation response was assessed according to sex. If the supplement-by-sex interaction was not statistically significant, responses for both sexes were combined. Additionally, a power analysis was performed to estimate the ability to detect significant post- minus pre-supplementation effects. All four phenotype models obtained at least 80% power at the 5% level of significance to detect a Cohen effect size of 1.32 to 1.71 standard deviation units.⁴⁹

RESULTS

General Experimental Observations

No serious adverse events occurred during the course of the study. Headache (4 of 12 subjects) and nausea (3 of 12 subjects) were common complaints during the early phases of black cohosh and goldenseal supplementation. Minor complaints reported for kava kava included drowsiness (3 of 12 subjects) and lower back pain (one subject), while drowsiness (3 of 12 subjects) and nightmares (2 of 12 subjects) were noted for valerian. During the first two weeks of supplementation, three subjects asked and were granted permission to treat their headaches with ibuprofen (200 to 400 mg). These conditions typically subsided two to three weeks before probe drug administration and no other medications were ingested after that time. No significant changes were noted in serum concentrations of AST (21.8 ± 5.2 vs. 21.5 ± 3.7 IU/L), ALT (22.4 ± 12.9 vs. 20.2 ± 7.1 IU/L), or GGT (19.1 ± 11.4 vs. 19.8 ± 10.3 IU/L) following kava kava supplementation.

Effect of Supplementation on CYP Phenotype

The effects of prolonged goldenseal, black cohosh, kava kava, or valerian extract supplementation on CYP phenotypic ratios are shown in Figures 1-4 and Table I. For each phase of the study, no significant differences were observed among mean baseline phenotypic ratios. Of the four botanicals tested, goldenseal produced significant reductions in CYP3A4/5 ($p < 0.0001$, Figure 1A) and CYP2D6 phenotypes ($p < 0.0001$, Figure 2A); kava kava significantly reduced phenotypic ratios for CYP2E1 ($p = 0.009$, Figure 4B); and black cohosh exhibited a statistically significant decrease in CYP2D6 phenotype ($p = 0.02$, Figure 2C). Goldenseal's approximate 40% reduction in CYP3A4/5 and CYP2D6 activity may represent a clinically significant effect. A similar argument could be made for kava kava and its effect (~40% reduction) on CYP2E1. The effect of black cohosh on CYP2D6 (~7% reduction), while statistically significant, may not be clinically relevant. Valerian had no significant effect on any CYP phenotypes. For those supplements that exhibited significant effects on CYP phenotype, no sex-related differences were observed.

Phytochemical Content and Disintegration Testing

HPLC determinations of various "marker" phytochemicals and the daily amount ingested by each subject are represented in Table II. Intra- and interday relative standard deviations for each assay were less than 10%. Except for valerian, all supplements had measurable amounts of "marker" phytochemicals. For the valerian supplement, quantities of valerenic acid were at the limit of quantitation (10 ng/mL), and thus this particular brand may not be representative of the effects of other valerian-containing products on CYP activity.³³ Table II also depicts the mean disintegration time for each supplement dosage form. Disintegration times for hard gelatin capsules containing goldenseal, kava kava, and black cohosh extracts were less than 21 minutes, while those for valerian tablets were almost twice as long.

DISCUSSION

The present study is highlighted by two principal findings. The first is that single time-point phenotypic ratios can provide a practical method for identifying herb-drug interactions that involve CYP inhibition. Although moderate inhibition of CYP2E1 by garlic oil had been demonstrated earlier,²⁶ the significant reductions in CYP2D6 and CYP3A4/5 phenotype following goldenseal extend the method's utility to include these two important CYP isoforms. Previously, this approach had been used to document CYP3A4/5 induction as evidenced by significant elevations in 1-hydroxymidazolam/midazolam ratios following St. John's wort supplementation. The method also demonstrated that an absence of change in mean phenotypic ratios following botanical supplementation could be interpreted as a lack of effect on CYP activity. Such was the case with milk thistle, *Citrus aurantium*, *Ginkgo biloba*, *Panax ginseng*, and saw palmetto extracts—the latter three examples being confirmed by other investigators using more conventional area-under-the-curve assessments.²⁴ Thus, a range of herb-mediated effects on CYP activity (e.g. induction, inhibition, or no effect) can be differentiated with single time-point phenotypic ratios. It must be emphasized, however, that single-time point phenotypic ratios simply provide estimates of probe drug clearance. Yet, even with this limitation, the method's distinct advantage lies in an ability to evaluate multiple CYP enzymes and multiple botanical supplements *in vivo* while using a limited blood-sampling scheme.

The second important finding emanating from the study is that goldenseal appears to inhibit CYP2D6 and CYP3A4/5 *in vivo*, which implies a significant pharmacokinetic herb-drug interaction potential for this supplement. These findings bolster recent *in vitro* investigations demonstrating inhibition of CYP2D6- and CYP3A4-mediated biotransformations by goldenseal extracts. Using human hepatic microsomes, Chatterjee and Franklin⁵⁰ found that

goldenseal extract as well as its two principal isoquinoline alkaloids, berberine and hydrastine, inhibited CYP2D6-mediated bufuralol 1'-hydroxylation. Of the two alkaloids, berberine was more inhibitory toward bufuralol 1'-hydroxylation ($IC_{50} = 45 \mu M$) than hydrastine ($IC_{50} = 350 \mu M$), implying a greater contribution of this phytochemical to CYP2D6 inhibition. When evaluating a series of single-entity herbal tea extracts, Foster et al noted that *Hydrastis canadensis* produced the greatest percent inhibition of cDNA expressed human CYP2D6.¹⁴

Budzinski et al first noted that commercial extracts of *Hydrastis canadensis* were potent *in vitro* inhibitors of CYP3A4.¹² Chatterjee and Franklin later observed that goldenseal extracts as well as individual isoquinoline alkaloids inhibited CYP3A4-mediated testosterone 6 β -hydroxylation.⁵⁰ In the case of CYP3A4, however, hydrastine was more inhibitory ($IC_{50} = 25 \mu M$) than berberine ($IC_{50} = 400 \mu M$). Inactivation of the enzyme appears to stem from the methylenedioxyphenyl moiety of hydrastine interacting with the heme iron of CYP3A4 to produce a stable heme-adduct. These adducts, termed CYP metabolic-intermediate (MI) complexes provide a mechanistic basis for the inhibition of CYP3A4 by goldenseal, and possibly CYP2D6.⁵⁰

Little is known about the pharmacokinetics of goldenseal alkaloids in humans, but animal studies indicate that berberine bioavailability is relatively low.^{51,52} Although the daily dose of isoquinoline alkaloids ingested in the present study was 142 mg (Table II), plasma concentrations of berberine and hydrastine were not determined; nevertheless, the significant effect observed on CYP2D6 phenotype indicates that phytochemicals present in goldenseal can cross the intestinal mucosa. Whether the effect on CYP3A4/5 are limited to intestinal enterocytes or extends to hepatocytes remains to be determined. Interestingly, the only prospective study to date in which the influence of goldenseal supplementation on the pharmacokinetics of a CYP3A4 substrate (indinavir) has been evaluated failed to register any significant effects.⁵³ This may stem from the relatively high oral bioavailability of indinavir, which renders the drug less effective as a probe for assessing herb-mediated changes in intestinal CYP3A4/5 activity.

The statistically significant reduction in debrisoquine urinary recovery ratios following black cohosh supplementation implies a much weaker inhibitory effect on CYP2D6 for this botanical extract than for goldenseal; however, the magnitude of this result (~7%) may not be clinically relevant. A trend toward CYP3A4/5 inhibition (~14%, Table I) was also noted for black cohosh, but the magnitude was not statistically significant ($p = 0.09$). Black cohosh's complex phytochemical makeup^{54,55} coupled with an absence of *in vitro* studies into the extract's effect on CYP isoforms, render a connection between CYP2D6 (and possibly CYP3A4/5) inhibition and a specific marker compound(s) especially difficult. As with many botanical extracts, the pharmacokinetic profile of black cohosh's constituent phytochemicals has yet to be investigated. One group has reported their attempt at quantitating mercapturate conjugates of black cohosh constituents (fukinolic acid, fukiic acid, caffeic acid, and cimicifugate B) in the urine of women who consumed up to 256 mg of a standardized black cohosh extract;⁵⁶ however, none of the target conjugates were detected, which brings into question the bioavailability of these specific phytochemicals. Nonetheless, black cohosh supplements appear to have a good safety profile⁵⁷ and exhibit modest efficacy when used to alleviate perimenopausal and postmenopausal symptoms;⁵⁸ however, their pharmacokinetic herb-drug interaction profile remains uncertain. Therefore, until further studies are conducted, caution should be exercised when taking these supplements in conjunction with conventional medications.

South Pacific islanders have long consumed "traditional" kava beverages prepared from coldwater extracts of powdered kava roots.⁵⁹ These "traditional" preparations are imbibed in social, recreational, and ceremonial settings to imbue psychotropic, hypnotic, and anxiolytic

effects. Since the 1990s, commercial kava extracts, prepared with nonaqueous solvents (acetone, ethanol or methanol) and formulated as tablets and/or capsules, have been marketed as dietary supplements for the alleviation of stress, anxiety, or insomnia.^{34,62} Recently, a spate of reports linking kava use to liver toxicity has prompted the removal of these products from Europe, Australia, and Canada.^{36-38,59,60} In the United States the FDA has issued a warning to consumers alerting them to possible hepatotoxic side effects associated with kava supplementation.⁶¹ For the majority of case reports documenting possible kava-related hepatotoxicity, comedication with prescription drugs and/or other botanical supplements has been a confounding, although common, variable.^{58,59} This raises the question as to whether an underlying kava/drug interaction may be responsible.

Kava lactones, which include methysticin, dihydromethysticin, kavain, dihydrokavain, yangonin, and desmethoxyyangonin, are the active principles of kava extracts. Using c-DNA-expressed CYPs, human liver microsomes, and/or cryopreserved hepatocytes, both kava extracts and specific kava lactones have been shown to inhibit a variety of human CYP isoforms in vitro, with individual IC₅₀ values ranging from 1 to 100 μ M.^{18,19,21,34} Of the kava lactones examined, methysticin, dihydromethysticin, and desmethoxyyangonin appear to be the most potent CYP inhibitors, with CYP3A4 being affected by all three.^{19,21} In contrast, Raucy noted that 100 μ g/mL of kava extract (a concentration not likely achievable in vivo) induced CYP3A4 in human hepatocytes and this effect was SXR-mediated.¹⁵ Collectively, the in vitro studies suggest that kava supplementation may give rise to significant CYP-mediated herb/drug interactions; however, the in vivo data presented herein suggests otherwise.

Phenotypic metabolic ratio comparisons indicated that 28 days of kava supplementation did not significantly affect CYP1A2, CYP2D6 or CYP3A4/5 activity in healthy human volunteers, although CYP2E1 phenotype was decreased appreciably (~40%). In light of the preponderance of in vitro data, it is unclear why we observed an inhibitory effect of kava only on CYP2E1, and not for the other isoforms. This disconnect might stem from lower concentrations of kava lactones realized in vivo relative to those achieved in vitro. Perhaps the daily administered dose of kava lactones (138 mg, Table II) was simply too low to elicit any clinically observable effects on CYPs other than 2E1, a factor that could also account for a lack of any observable kava-related liver toxicity in our subjects. Currently, there are no published studies describing the pharmacokinetics of kava lactones in human subjects, therefore, the relationship between in vitro IC₅₀ values and steady state plasma concentrations of kava lactones remains unknown. Interestingly, methysticin and dihydromethysticin, like hydrastine and berberine, contain methylenedioxyphenyl moieties that can form metabolic-intermediate (MI) complexes with CYP isoforms.¹⁹ Assuming formation of MI complexes lies at the heart of CYP inhibition by kava and goldenseal, other factors (e.g. differences in membrane permeability,⁶² carrier-mediated transport, or other physicochemical properties) may contribute to the in vitro/in vivo discrepancies observed for kava lactones and the isoquinoline alkaloids. A methodological explanation for the observed absence of CYP3A4/5 or CYP2D6 inhibition following kava administration seems unlikely since single-time point phenotypic ratios clearly identified an inhibitory effect for goldenseal.

Regardless of the disparity between previous in vitro studies and the clinical results presented here, a considerable body of evidence suggests that kava can modulate human CYP activity. While we observed no appreciable changes in CYP3A4/5, CYP2D6, or CYP1A2 phenotype at a daily kava lactone dose of 138 mg, these results may not extend to doses exceeding this value. Therefore, to guard against possible CYP-mediated interactions, coadministration of kava with conventional medications should be avoided.

An absence of effect on CYP phenotype for the valerian product used in this study could stem from a paucity of valerenic acid and a long disintegration time (42 minutes, Table II). Wide

variability in valerenic acid (Table II) content among commercial brands is not uncommon; ^{35,63} however, the presence of only trace amounts of the principal marker compound (valerenic acid) render this product less suitable for evaluating valerian's influence on CYP activity in vivo. In vitro examinations of valerian extracts on CYP3A4 activity have provided mixed results even though the same general type of assay (fluorometric microtitre plate assay, GENTEST™) was used.^{12,16,35} Strandell et al¹⁶ and Lefebvre et al³⁵ both found valerian extracts to have moderate to potent CYP3A4/5 inhibitory capabilities, while Budzinski et al ranked them as poor inhibitors.¹² Recently, Donovan et al assessed the effects of a valerian supplement on alprazolam pharmacokinetics and dextromethorphan metabolic ratios in healthy volunteers.³³ The valerian product used by Donovan contained a total valerenic acid content (valerenic acid, acteoxyvalerenic acid, and hydroxyvalerenic acid) of 5.5 mg/tablet. No significant changes in dextromethorphan metabolic ratios and only a slight increase in alprazolam Cmax values led the authors to conclude that "typical doses of valerian are unlikely to produce clinically significant effects on the disposition of medications dependent on the CYP2D6 or CYP3A4/5 pathways of metabolism."³³

In conclusion, single-time point CYP phenotypic ratios indicated that goldenseal supplementation significantly inhibited human CYP3A4/5 and CYP2D6 activity in vivo. Therefore, in order to avoid potentially serious pharmacokinetic interactions, goldenseal supplements should not be taken concomitantly with conventional medications. Black cohosh exhibited mild inhibition of CYP2D6, however, the clinical relevancy of this effect remains uncertain. Of the four CYP isoforms investigated, kava supplementation significantly inhibited CYP2E1 but caused no elevations in serum liver enzymes. Although valerian produced no marked changes in CYP phenotypes, this result may not be representative of products containing greater quantities of valerenic acid. Taken together, these results provide further evidence that botanical supplementation can modulate human CYP activity in vivo, which may lead to significant herb-drug interactions.

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Figure 1. Comparison of pre- and post-supplementation phenotypic ratios (1-hydroxymidazolam/midazolam) for CYP3A4. (A) Goldenseal, (B) Kava kava, (C) Black cohosh, (D) Valerian. Gray circles, Individual values; Black circles, Group means. Asterisks = statistically significant difference from baseline.

Figure 2.

Comparison of pre- and post-supplementation phenotypic ratios (8-hour debrisoquine urinary recovery ratios) for CYP2D6. (A) Goldenseal, (B) Kava kava, (C) Black cohosh, (D) Valerian. Gray circles, Individual values; Black circles, Group means. Asterisks = statistically significant difference from baseline.

Figure 3. Comparison of pre- and post-supplementation phenotypic ratios (Paraxanthine/caffeine) for CYP1A2. (A) Goldenseal, (B) Kava kava, (C) Black cohosh, (D) Valerian. Gray circles, Individual values; Black circles, Group means.

Figure 4. Comparison of pre- and post-supplementation phenotypic ratios (6-hydroxychlorzoxazone/chlorzoxazone) for CYP2E1. (A) Goldenseal, (B) Kava kava, (C) Black cohosh, (D) Valerian. Gray circles, Individual values; Black circles, Group means. Asterisks = statistically significant difference from baseline.

Table 1

Pre- and Postsupplementation Phenotypic Ratios

Phenotypic Ratio (CYP)	Supplement	Presupplementation (mean and 95% CI)	Postsupplementation (mean and 95% CI)	Difference (mean and 95% CI)	Post/Pre Ratios [†] (geometric mean and 90% CI)
OH-MDZ/MDZ (CYP3A4)	Goldenseal	3.93 (3.29 to 4.57)	2.43 (1.76 to 3.09)	-1.50 (-1.84 to -1.16)*	0.60 (0.53 to 0.67)*
	Kava kava	4.03 (3.17 to 4.90)	3.99 (3.33 to 4.64)	-0.04 (-0.07 to 0.04)	1.00 (0.92 to 1.10)
	Black cohosh	3.89 (3.05 to 4.73)	3.35 (2.87 to 3.84)	-0.54 (-1.17 to 0.10)	0.88 (0.78 to 0.98)
	Valerian	3.68 (3.30 to 4.06)	3.68 (2.95 to 4.40)	0.00 (-0.50 to 0.50)	0.96 (0.85 to 1.10)
DMX/CFE (CYP1A2)	Goldenseal	0.47 (0.37 to 0.56)	0.44 (0.32 to 0.55)	-0.03 (-0.12 to 0.06)	0.93 (0.75 to 1.15)
	Kava kava	0.48 (0.36 to 0.61)	0.48 (0.36 to 0.61)	0.00 (-0.11 to 0.11)	1.03 (0.88 to 1.21)
	Black cohosh	0.47 (0.39 to 0.55)	0.43 (0.31 to 0.55)	-0.04 (-0.14 to 0.05)	0.88 (0.73 to 1.06)
	Valerian	0.50 (0.38 to 0.62)	0.47 (0.34 to 0.59)	-0.03 (-0.12 to 0.06)	0.88 (0.74 to 1.05)
OH-CZX/CZX (CYP2E1)	Goldenseal	0.47 (0.39 to 0.56)	0.45 (0.34 to 0.57)	-0.02 (-0.14 to 0.10)	0.93 (0.72 to 1.19)
	Kava kava	0.48 (0.36 to 0.59)	0.28 (0.21 to 0.36)	-0.20 (-0.32 to -0.06)*	0.59 (0.45 to 0.77)*
	Black cohosh	0.45 (0.35 to 0.54)	0.43 (0.34 to 0.53)	-0.02 (-0.09 to 0.06)	0.96 (0.83 to 1.12)
	Valerian	0.46 (0.37 to 0.55)	0.46 (0.36 to 0.57)	0.00 (-0.09 to 0.09)	1.00 (0.84 to 1.20)
HDEB/(HDEB +DEB) (CYP2D6)	Goldenseal	0.64 (0.56 to 0.72)	0.41 (0.30 to 0.52)	-0.23 (-0.27 to -0.19)*	0.60 (0.51 to 0.70)*
	Kava kava	0.62 (0.55 to 0.70)	0.63 (0.55 to 0.71)	0.01 (-0.03 to 0.04)	1.01 (0.96 to 1.06)*
	Black cohosh	0.64 (0.57 to 0.71)	0.59 (0.52 to 0.67)	-0.05 (-0.08 to -0.01)*	0.92 (0.87 to 0.98)*
	Valerian	0.64 (0.58 to 0.71)	0.63 (0.56 to 0.71)	-0.01 (-0.05 to 0.02)	0.98 (0.93 to 1.03)

CI = confidence interval, OH-MDZ = 1-hydroxymidazolam, MDZ = midazolam, DMX = paraxanthine, CFE = caffeine, OH-CZX = 4-hydroxychlorzoxazone, CZX = chlorzoxazone, HDEB = 6-hydroxydebrisoquine, DEB = debrisoquine

[†] = geometric mean of postsupplementation/presupplementation ratios and 90% CI

* = p < 0.05

Table II

Content of phytochemical marker compounds, dosage form, and disintegration times for botanical supplements.

<u>Supplement</u>	<u>Compound</u>	<u>Content (mg/ capsule) (mean ± s.d.)</u>	<u>Daily Dose (mg)</u>	<u>Dosage Form</u>	<u>Disintegration Time (min.) (mean ± s.d.)</u>
Goldenseal	<u>Isoquinoline alkaloids</u>			Hard gelatin capsule	20.4 ± 1.7
	Hydrastine	10.8 ± 0.15	64.8		
	Berberine	12.9 ± 0.15	77.4		
	Total	23.7	142.2		
Black cohosh	<u>Triterpene glycosides</u>			Hard gelatin capsule	4.9 ± 0.1
	Cimiracemoside	0.2 ± 0.02	0.8		
	27-deoxyactein	0.6 ± 0.01	2.4		
	Actein	1.9 ± 0.06	7.6		
	Total	2.7	10.8		
Kava kava	Kava pyrone lactones			Hard gelatin capsule	14.2 ± 2.6
	Kavain	8.4 ± 0.86	33.6		
	Dihydrokavain	7.9 ± 0.81	31.6		
	Methysticin	6.3 ± 0.66	25.2		
	Dihydromethysticin	4.5 ± 0.45	18.0		
	Yangonin	3.6 ± 0.36	14.4		
	Desmethoxyyangonin	3.8 ± 0.40	15.2		
	Total	34.5	138		
Valerian	Valerenic acid	Trace	trace	Uncoated tablet	42.1 ± 10.8