In vitro activity of commercial valerian root extracts against human cytochrome P450 3A4.

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Abstract PURPOSE. Valerian root (*Valeriana officinalis* L.) has been used since antiquity as a medicinal herb. Recent studies have found that certain herbal products used concomitantly with conventional therapeutic products can markedly affect drug disposition. **METHODS.** The *in vitro* effect of aliquots from 14 commercially available single-entity and blended products containing valerian root on cytochrome P450 CYP3A4-mediated metabolism and P-glycoprotein transport has been determined with aqueous, ethanol and acetonitrile extracts. **RESULTS.** Hydroxyvalerenic acid, acetoxyvalerenic acid and valerenic acid content was analyzed and wide variation was found between samples and compared to the concentrations noted on the product labels. Valerian extracts from the products tested also exhibited a marked capacity to inhibit cytochrome P450 3A4-mediated metabolism and P-glycoprotein transport based upon the ATPase assay. **CONCLUSIONS.** There is wide variation between commercially available samples of valerian root. The findings from this study suggest that valerian root may have an initial inhibitory effect when taken with therapeutic products. Further work is warranted to determine whether valerian root can affect other CYP450 isozymes and how the results of this *in vitro* investigation can be extrapolated to *in vivo* situations.

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INTRODUCTION

Valerian is a widely used plant-based medicine (1-3). Valerian (*Valeriana officinalis* L.) native to Europe and Asia is indicated mostly as a mild sedative and as a sleep aid (4). Valerian improves subjective experiences of sleep when taken nightly over one- to two-week periods but long-term safety studies are lacking (5). Valerian is a member of the *Valerianaceae* family that includes up to 250 species (6). Common species include, but are not limited to, *Valeriana wallichii*, *Valeriana edulis* and *Valeriana officinalis*. The latter is composed of five subspecies and is the species most likely to be commonly used in North America. The parts of the plant used for therapeutic purposes are the roots and the rhizomes. The constituents of Valerian include valepotriates (iridoids), the components of the volatile oil, including monoterpenes and sesquiterpenes (valerenic acids), as well as a number of other constituents (7). Valerian products are usually standardized to valerenic acid or sometimes to valeric acid. The identity of the active component(s) in valerian is still uncertain (8).

Reports of drug interactions with many botanicals, including valerian have been noted in the published literature (9-12). Valepotriates have been shown to prolong the action of barbiturates (13) and intra peritoneal administration of valerenic acid produced a dose-related prolongation of pentobarbital sleeping time in mice (14). Other studies showing interactions between valerian constituents and other drugs include a mixture of valepotriates that partially relieved diazepam withdrawal in mice (15). Further evidence of the effect of altered drug metabolism by valerian comes from rodent studies showing an inhibition of methacetin

metabolism with oral administration of some, but not all, valerian constituents (16-17). One clinical case report involved hospitalization due to an intoxication with a mixture of barbital and valerian (18). The potential interaction of valerian and alcohol has often been noted (19); however, this interaction has not always been apparent in human studies (20). Gold et al. (3) identified a series of potential interactions between herbal natural health products (NHPs) including valerian and conventional drug therapy that place older people at risk for an adverse drug event. Valerian may also pose a concern during the perioperative period (1). Dergal et al. (21) determined the range of NHPs and conventional drug therapies used by older adults (aged 65 years and over) attending a memory clinic. Of the 195 patients in the sample, 33 (17%) were 'current users' with 11 potential herb-drug interactions in nine patients including one with valerian and lorazepam.

The therapeutic effects of many herbal medicines have been established; however, definitive pharmacological mechanisms of action on the central nervous system remain to be elucidated for many herbal medications. Although several mechanisms have been identified using purified biomarkers, some studies have provided insufficient information to account for the observed effects of the plant or its extracts. This emphasizes the need to consider the additive and synergistic effects of the multiple constituents (22-23). In addition, components of the plant may act to reduce or antagonize the potential toxicity of other constituents. Interactions with drugs may occur through pharmacokinetic and or pharmacodynamic interactions. All cytochrome P450 (CYP) enzymes exhibit similarity in their structure and general mechanism of action; however, there are significant differences in the detailed activity of individual enzymes as well as in the structures and properties of their active sites. CYP3A4 is the most important isoform in critical tissues, such as hepatic and gastrointestinal tract, responsible for xenobiotic metabolism in humans (21). This enzyme is involved in the biotransformation of over 70% of the drugs in use today (24-25); hence, the possible risk of adverse events due to alterations in this pathway is high.

This study examined whether randomly selected commercial valerian products (capsules, soft gel capsules, caplets, tablets, a tincture and teas) have an affect on the activity of CYP3A4-mediated metabolism. The products were analyzed to determine if they contained authentic valerian marker phytochemicals and the levels of these materials.

MATERIALS AND METHODS

Substrates and Reference Compounds

The capsules, tablets, caplets, soft gels, tincture and the teas were obtained from local commercial outlets but are representative of the North American market. These were all identified with a NHP number and vouchers stored at the University of Ottawa. The moisture content for the capsules, tablets and caplets was determined by drying a known weight of material overnight at 37°C. Dibenzylfluorescein (DBF) and cytochrome P450 CYP3A4 supersomes were obtained from BD GENTEST (Woburn, USA). Hydroxyvalerenic acid (HVA) and valerenic acid (VA) were obtained from Dalton Chemical Laboratories Inc (Toronto, ON). Acetoxyvalerenic acid and hydroxvalerenic acid was obtained from Phytochem, Ichenhausen, Germany. Valerenic acid was also obtained from Extrasynthese, Genay, France. All other chemicals and solvents were of analytical grade.

Stock solutions were prepared at room temperature unless stated otherwise under reduced lighting conditions from the plant material within capsules, tea bags, and whole tablets. Capsules, tablets and caplets were extracted at a concentration of 100 mg/ml, vortexed for 1 min and centrifuged for 15 min at 13,000 rpm. Teas were extracted at a concentration of 25 mg/ml, were polytroned for 1 min, and then centrifuged as above. Soft gels were emptied into a 1.5 ml microfuge tube and 1 ml of solvent was added. The solution was vortexed for 1 min and sonicated for 10 min before being centrifuged as above. The extractions were done in three different solvents: deionized water, 70% ethanol and acetonitrile. All extracts were protected from light and were not kept for more than a day.

Cytochrome P450 Assay Procedure

Aliquots (2µl of organic or 10 µl aqueous) of the valerian root stock solution extracts were tested for their ability to inhibit the metabolism of a CYP3A4 marker substrate using an 200 µl *in vitro* fluorometric microtiter plate assay (26) based upon the method of Crespi et al. (27). The assays were performed in clear-bottom,

opaque-welled microtiter plates (96 well, Corning Costar, model # CS00-3632, Corning, NY). In brief, all control and control-blank wells were balanced to contain an equal volume of 70% ethanol, acetonitrile or deionized water present in the extracts in the test and test-blank wells. All wells had the same amount of NADPH (ß-nicotinamide adenine dinucleotide phosphate, reduced form (Sigma Chemical Co., St-Louis, MO) solution; phosphate buffer (KH_2PO_4, pH 7.4 (0.5 M)); DBF and CYP3A4 enzyme. To compensate for intrinsic fluorescence or quenching of the extracts, two additional controls were required: Control-blank and test-blank wells with denatured CYP3A4. Test samples without DBF were used to determine if CYP3A4-mediated metabolism of the extracts affected either fluorescence or quenching. The enzyme stock solution was thawed in a 37°C sand bath. The enzyme was denatured by boiling for 15 min. All solutions were vortexed prior to addition to the wells except the solutions that required preservation of enzymatic activity. These were gently mixed and were sonicated for 2 sec before being added to the wells. The plates were agitated and incubated at 37°C in the plate reader and read after 20 min.

All assays were conducted in triplicate and repeated at least once with a fresh sample. Nelfinavir mesylate (1.8 μg/well) was used as a positive control to ensure the consistency of the results. The mean and coefficient of variation of each triplicate was calculated. Then, the difference between the test and the test-blank was divided by the difference between the control and the control-blank to compensate for background noise related to the crude product extracts.

P-glycoprotein Assay Procedure

The P-gp ATPase (BD GENTEST) analysis followed the product insert (28) measuring the orthovanadate-sensitive release of phosphate and ATPase activity using a THERMOmax Microplate Reader. Verapamil (Sigma, lot # 56H0925) was examined as a positive control. All assays were conducted in triplicate and repeated once with a freshly prepared sample.

Valerian Content Analysis

Each sample was weighed (approximately 0.5 g) and extracted three times in 8 ml of 70 % ethanol using sonication for 5 min and then centrifuged at 1000 x g for 5 min. The supernatant was collected after each centrifugation and the final extract volume was adjusted to 25 ml. The extracts were filtered through a 0.22 µ PTFE membrane prior to injection. Samples were analyzed for AVA, HVA and VA by HPLC adapted from the method of Bos et al. (29) using a 4 µm LiChrospher 100 RP-18, 75 x 4.6 mm analytical column and 5 µm LiChrospher 100 RP-18 guard column (E. Merck, Toronto, Canada) with a 45°C column oven temperature in a Varian Star HPLC system at 225 nm. The mobile phase consisted of A: 25 mM NaH_2PO_4 pH 2.95 with H_3PO_4 B: acetonitrile. Flow rate 1.2 ml/min in a linear gradient: 40%-70% B in 7 min; 70-85% B in 2 min. Standard curves were generated by HPLC from serial dilution injections of pure standards. HPLC-APCI/MS were performed using an Agilent 1100 series LC/MSD VL with an APCI/MS detector equipped with a Waters YMC ODS-AM column (100 x 2 mm i.d., 3-μM particle size). Elution conditions with a mobile phase system of water (solvent A), acetonitrile (solvent B) and methanol (solvent C) as follows: initial conditions 90:5:5 (A: B: C) changing to 65:30:5 in 10 min, then to 5:90:5 at 11 min with a flow rate of 0.5 ml/min. For MS detection, the conditions were: APCI conducted at 350°C, nebulizer pressure 60 psig, nitrogen-drying gas 5 l/min, capillary voltage 3000 V, corona current 15 μA. The MS data was collected in the scan mode. Peak identities in samples were confirmed by relative retention time; spectral analysis collected over the wavelength range 200-350 nm against published spectra and mass spectral analysis.

All assays were performed under gold fluorescent lighting and samples were kept under reduced lighting conditions.

RESULTS

Fourteen products containing valerian root in capsules, soft gel capsules, caplets, tablets, single entity teas and a tincture were tested (Table 1).

There were marked differences in the information provided by the manufacturers on the product label, making it difficult to compare relative amounts in each product. Daily dose of the tablets, capsules and caplets ranged from 1 to 9 units. Less than half of the product labels stated that the products were standardized to 0.8%. Of these 3 were listed to contain valerenic acid

(VA), 2 samples (NHP 4 and 6) reported standardization to valeric acid which is not the industry standard, and 1 mentioned valerenic acids (VAs). The moisture content determined for the capsules, tablets and caplets ranged from 0.1 to 5.5%. The actual NHP 6 product weight content of 324 mg or 394 mg for capsule and content (Table 1) was considerably lower than the 500 mg stated on the product label; three products (NHP 1, 2, and 4) had higher weights but only NHP 1 indicated the presence of excipients. The excipients in these 3 products ranged from 39 to 610 mg accounting for about 10 to 75% of the content weight.

Table 1: Product information of the valerian root samples examined in this study. Most products had sufficient label information for use and contraindications, form, unit weight, suggested dose, and other ingredients.

NHP	Product and Ingredient list (Dry weight - %)	Suggested Dose
1	Valerian root powdered extract, 200 mg standardized extract 0.8%, 1.6 mg valerenic acid; rice flour, ethylcellulose, maltodextrin; 800 mg cap., cap. and contents 610 mg (95.3%)	one cap. daily, one hr before bedtime
2	Valerian root powder, 400 mg cap., cap. and contents 534 mg, contents 439 mg (95.4%)	1-2 cap. daily at bedtime, or as directed by a physician
3	Valerian root, 530 mg cap., cap. and contents 630 mg, contents 535 mg (94.5%)	3 cap., at bedtime, as desired; do not use if pregnant or nursing
4	Valerian root, 300 mg cap., 0.8 % valeric acid, cap. and contents 480 mg, contents 405 mg (94.8%)	1-2 cap. daily, or as directed by a physician
5	Valerian root, 470 mg cap., MCT, vitamin E and rosemary oil; cap. and contents 574 mg, contents 473 mg (95.0%)	3 cap. 3 x daily or 3 cap. 1/2 hr before bedtime
6	Valerian root, 500 mg cap., powder extract 4:1, 0.8 % valeric acid; excipient - vegetable grade magnesium stearate; cap and contents 394 mg, contents 324 mg (94.6%)	1-2 cap. daily after a meal
7	500 mg soft gel cap., 0.8 % valerenic acid	1-2 cap. daily at bedtime
8	500 mg soft gel cap., 5:1 extract, 0.8 % valerenic acid; soyabean oil, bees wax, lecithin	1-2 cap. up to two times daily, or as directed by a physician or natural health practitioner; not recommended for pregnant or lactating women
9	Valerian root, 400 mg caplets, extract 1:4, 100 mg extract, 0.8 % valerenic acids; (97.7%)	1-2 caplets at bedtime
10	Valerian root, 560 mg tablets, powdered extract 1:4, 140 mg extract; (99.9%)	1-2 tablets at bedtime (for occasional use only)
11	Rhizome and roots of *V. officinalis*, liquid extract 1:8, 200 mg/ml; 60 % alcohol (1.1% residue)	4-8 ml 3 x daily (800-1600 mg/dose), or as recommended by a physician
12	Valerian root, 56 g tea bags	
13	Valerian root, *V. wallichii*, 67.2 g tea bags	
14	Valerian root, 45 g tea bags	

Each product was analyzed to determine content of hydroxyvalerenic acid (HVA), acetoxyvalerenic acid (AVA) and VA (Table 2).

Table 2: Valerenic acids measured in commercial valerian root extracts. The values are given in ppm (μg/g dry weight) and the relative proportions of each (%). Hydroxyvalerenic acid (HVA); acetoxyvalerenic acid (AVA), and valerenic acid (VA).

NHP	HVA	AVA	VA	Total VAs
1*	127.4 (3.6%)	1528.3 (43.8%)	1834.9 (52.6%)	3490.6
2	52.3 (5.4%)	534.9 (54.8%)	389.5 (39.9%)	976.7
3	125.0 (6.7%)	963.0 (51.7%)	773.1 (41.5%)	1861.1
4**	150.0 (3.0%)	2227.8 (44.2%)	2661.1 (52.8%)	5038.9
5	76.5 (3.9%)	908.2 (46.7%)	959.2 (49.3%)	1943.9
6**	95.6 (3.9%)	1014.7 (41.3%)	1345.6 (54.8%)	2455.9
7*	83.3 (3.9%)	989.6 (46.6%)	1052.1 (49.5%)	2125
8*	86.5 (3.0%)	1206.7 (41.5%)	1615.4 (55.5%)	2908.6
9***	149.0 (5.4%)	1182.7 (42.9%)	1423.1 (51.7%)	2754.8
10 H_2O	62.5 (38.2%)	76.9 (47.1%)	24.0 (14.7%)	163.4
10 EtOH	31.9 (30.0%)	53.2 (50.0%)	21.3 (20.0%)	106.4
11 (μg/ml)	2	2	31	35
12	-	-	72.1 (100%)	72.1
12 Ground	15.0 (9.7%)	-	140.0 (90.3%)	155
13	50.0 (29.7%)	45.5 (27.1%)	72.7 (43.2%)	168.2
13 Ground	-	175.0 (81.4%)	40.0 (18.6%)	215
14	-	-	1218.2 (100%)	1218.2

Label claims standardized to * 0.8% valerenic acid ** 0.8% valeric acid *** 0.8% valerenic acids

VA was found in all the samples with a wide quantity range from 21 to 2661 ppm. VA account for 14.7-100% of the recovered related acids. The lowest levels were found in the tablet, tincture and two of the teas. The third tea (NHP 14) had relatively higher VA levels. HVA and AVA were not detected beyond traces in 2 of the tea samples (NHP 12 and 14). NHP 4 had the highest total content of 5038.9 ppm valerenic acids. The lowest levels were found in the tablet (NHP 10), the tincture (NHP 11) and in two teas (NHP 109, 142). In most samples, with the exception of NHP 10 and 13, HVA was the minor constituent accounting for less than 10% of the total related acids.

As natural product samples can contain constituents with different extraction coefficients, the effect on CYP3A4-mediated metabolism was determined for three solvent extracts: water, 70% ethanol and acetonitrile (Tables 3, 4 and 5).

Table 3: Percent inhibition of human cytochrome P450 CYP3A4-mediated metabolism by aliquots of valerian root products in capsules extracted in water, 70% ethanol and acetonitrile. Inhibition (at 20 min.) ± Standard deviation (n = 2 in triplicate unless stated otherwise).

NHP	Inhibition ± SD		
	H_2O	70% EtOH	Acetonitrile
1	NA[1]	NA	NA
2	57.8 ± 1.3	51.2 ± 0.3	87.7 ± 10.5
3	66.2 ± 1.4	47.3[2]	66.0 ± 3.2[3]
4	77.7 ± 0.3	54.1 ± 0.1	83.2 ± 4.3[3]
5	66.9 ± 2.6	70.5 ± 2.4	74.4 ± 9.6[4]
6	66.1 ± 4.7	35.6 ± 3.0	59.5 ± 5.9[3]

[1] Not Available [2] n=1 [3]n=4 [4]n=7

Values obtained with the various capsules (Table 3) were similar with moderate (35-75%) to high (76-88%) inhibition with all samples and extracts. Inhibition of CYP3A4-mediated metabolism tended to be slightly lower in the aqueous extracts and slightly higher in those extracted with acetonitrile. Background fluorescence or quenching was compensated for by the use of appropriate controls and did not change when extracts were incubated with CYP3A4 in the absence of the test substrate DBF.

Results were more variable with the caplet, tablet, tincture and soft gel products examined (Table 4).

Multiple layers were obtained after centrifugation of the soft gel product extracts. Two distinct liquid layers were obtained with water and acetonitrile. The second layer was very small and insufficiently separated when samples were extracted with ethanol. In the soft gel products, the inhibitory effect varied extensively between products and extraction solvent. Layer 1 of the acetonitrile extracts of NHP 8 gave the highest inhibition, whereas the highest inhibition in the aqueous extract of NHP 8 was found in Layer 2. In each instance, these levels were nearly 2-fold greater than those obtained with NHP 7.

The highest levels of inhibition were obtained with the NHP 7 ethanolic extract. The caplet (NHP 9) had moderate activity. Low activity (<35% inhibition) was detected in the tablet (NHP 10) and tincture (NHP 11) products with the highest levels of inhibition in the ethanolic extracts.

Table 4: Percent inhibition of human cytochrome P450 CYP3A4-mediated metabolism by Valerian soft gels, caplets, tablets and tincture extracted in water, ethanol 70 % and acetonitrile. Inhibition (at 20 min.) ± Standard deviation (n = 2 replicates).

NHP		Extraction Solvent		
		H_2O	70% EtOH	Acetonitrile
7	layer 1[1]	10.9 ± 1.0	ND[2]	30.9 ± 8.8
	layer 2	41.9 ± 2.8	68.5 ± 2.9	22.6 ± 9.9
8	layer 1	16.6	ND	80.6 ± 2.4
	layer 2	87.8 ± 1.8	46.2 ± 2.2	8.0 ± 4.7
9		74.7 ± 2.7	50.7 ± 0.6	76.9 ± 10.7
10		ND	34.6 ± 2.3	15.0 ± 0.7
11		ND	27.9 ± 10.0	ND

[1] During centrifugation of the extracts the samples separated into two distinct layers.
[2] Not determined. Insufficient material separated.

The single-entity teas were initially tested at 100 mg/ml but high levels of inhibition required retesting at 25 mg/ml extracts. The one exception (NHP 14) generally had only moderate inhibitory activity (Table 5).

Table 5: Percent inhibition of human cytochrome P450 CYP3A4-mediated metabolism by extracts of valerian teas. Inhibition (at 20 min.) ± Standard deviation (n = 2-7 replicates).

NHP	Extraction Solvent		
	H_2O	70% EtOH	Acetonitrile
12	67.8 ± 7.9 n = 4	61.0 ± 0.9 n = 2	64.8 ± 5.1 n = 7
13	62.5 ± 1.3 n = 5	61.6 ± 0.7 n = 2	74.7 ± 3.7 n = 5
14	59.6 ± 6.0 n = 4	54.9 ± 3.9 n = 2	30.1 ± 7.7 n = 6

The vanadate-sensitive P-glycoprotein ATPase activity was determined for aqueous and ethanolic extracts of capsule products only (Fig. 1).

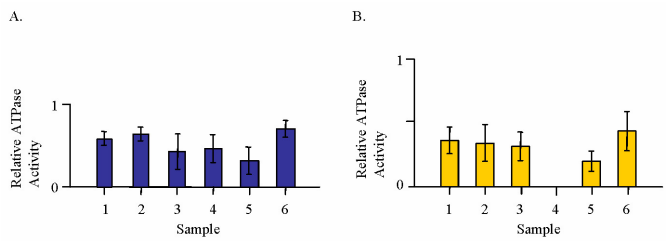

Figure 1: ATPase activity for aliquots of 100 mg/ml extracts from capsules containing valerian root expressed as a ratio to 20 μM verapamil from each individual assay. A. Water extracts. B. 70% ethanolic extracts.

The results expressed relative to 20 μM verapamil show that the water extracts were more active than the 70% ethanolic extracts. The extracts from NHP 6 were the most active effectors in this system. An equivalent amount of authentic reference standards of HVA and VA were examined and found to have similar activity at about 20-25% of that of 20 μM verapamil (Fig. 2).

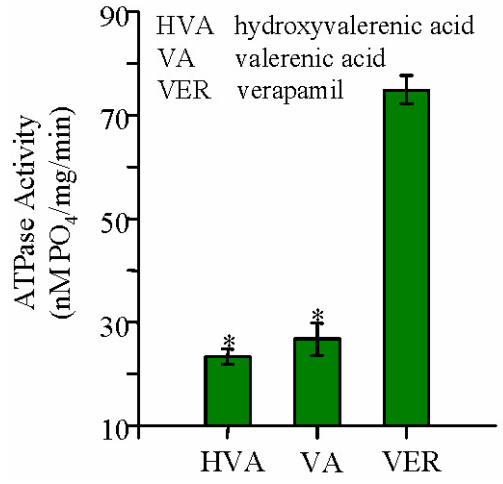

Figure 2: The vanadate-sensitive P-glycoprotein ATPase activity for 20 μM valerenic acid, hydroxyvalerenic acid and verapamil (n=2). * indicates statistical significance.

DISCUSSION

With the increased demand for herbal medicinal preparations, it is increasingly important to elucidate/identify potential adverse effects, including mechanistic interactions between herbal and conventional drugs so that these products can be used safely. The randomly selected samples chosen for this study were found to represent 6 major commercially available categories of valerian products (capsules, tablets, caplets, soft gels, tincture and the teas); and were examined for biomarker content as a surrogate for quality control, and for their ability to affect the major human drug metabolism enzyme and transport protein.

Information printed on some product labels examined in this study stated that the products were standardized to VA, VAs and valeric acid. This is indicative of the confusion created by the industry, as valeric acid is a 5 carbon molecule, which is not related to the much larger VA acids. HPLC analysis of the related VAs revealed all products contained this valerian marker but there was wide variation in content compared to the concentrations stated on the label of some product. With the exception of one tea (NHP 14), the lowest VAs recovery was with tablet, tincture and tea products examined. The highest recovery was observed in the powder capsules. In most samples (NHP 1-9), similar amounts of AVA and VA were present with lesser amounts of HVA. Interestingly two products, the tablet NHP 10 and tea NHP 13 had markedly higher amounts of HVA. The total content of VAs varied from 977 to 5039 ppm and supports the reports of substantial product heterogeneity (29, 30) who found that the concentration of VA and its derivatives ranged from < 0.01 to 6.32 mg/g of product.

Despite the wide variation in actual and reported label weight, and amounts of measured phytochemical constituents, all product extracts were found to have similar moderate to high inhibition of CYP3A4-mediated metabolism and P-glycoprotein ATPase activity. In most cases, the ethanolic extracts were slightly less active than the corresponding water or acetonitrile extracts. There was no apparent relationship or correlation between the amounts of the key HVA, AVA, VA or VAs biomarkers for valerian and this specific biological activity. This finding with CYP3A4 seems to be consistent with Zou et al. (31), who did not find any inhibitory activity with VA. These results are also consistent with our earlier reports with other NHPs (26, 32, 33) and from Williamson (23) that synergistic interactions are important in phytomedicines. The premise being that a whole or partially purified extract of a plant has more pharmacological activity or advantages over a single isolated ingredient. Studies with purified components such as that of Zou et al. (31) can

provide information on whether these compounds have an inhibitory effect but negative findings cannot necessarily be extrapolated to the complex phytomedicine. In this instance, standardized valerian root products contain up to 0.8% VA leaving about 99% of the complex mixture to account for the inhibitory effects observed here. The results here show that the VAs are not the only biologically active constituents contained in these natural products which may elicit a systemic or pre-systemic effect on drug disposition. The results here show that the VAs are not the only biologically active constituents contained in these natural products which may elicit a systemic or pre-systemic effect on drug disposition.

There are several confounding factors that make it difficult to infer clinical assessments based on *in vitro* studies. These can include product heterogeneity resulting from the inherent variability due to harvest, manufacturing processes and the interindividual variability affected by how the product is prepared and consumed. The use of crude extracts presents a second confounding factor in that *in situ* metabolism of a plant constituent could possibly alter the fluorescence or quenching capacity of the sample resulting in a false result. This can be mitigated somewhat by the use of additional controls but it cannot be eliminated under these assay conditions. With these confounding factors in mind, the *in vitro* assay can provide some guidance on whether the product may have a qualitative inhibitory effect (34). Ghosal et al. (35) report that the chemical inhibition and correlation data for DBF with CYP3A4 ($r = 0.92$) indicates that the substrate can be used as specific functional probe for cytochrome P450 3A4. There is no *a priori* basis to extrapolate either positive or negative *in vitro* inhibitory results to acute or chronic clinical exposure. However, similar *in vitro* evidence with extracts from Echinacea (36), garlic (26) and St John's wort (32, 36, 37) predicted a potential pharmacological effect. Clinical studies with *Echinacea* (38), garlic (39, 40), and St. John's wort (41, 42) have demonstrated that prolonged exposure of these NHPs can have a significant pharmacological effect on drug pharmacokinetics affecting both the safety and efficacy of these drugs. This observation is consistent with that of Liu (43) who documented a number of examples where NHPs were shown to have a biphasic effect on drug metabolism, where administration 1 hr before testing was inhibitory but if administered 24 hr prior to the test they caused induction of drug metabolism. Such biphasic effect has also been noted with many conventional drugs. Little information is reported about the normal use of valerian root or what plasma levels can be obtained for the key biomarkers.

The findings from this study suggest that valerian root may have an initial inhibitory effect when taken with therapeutic products. The variability in the HVA, AVA, VA, total VAs content; and inhibitory effects on CYP3A4-mediated metabolism and ATPase activity suggests that these observations may be similar for other products but caution is warranted if attempting to make such an extrapolation to other lots or formulations of valerian root products. Many consumers of herbal products assume that because the products are natural, there will be no side effects from their use; the evidence presented here adds to the increasing body of evidence suggesting that they are not necessarily biologically benign. Further work is warranted to determine whether valerian root affects other CYP450 isozymes and how the results of this *in vitro* investigation can be extrapolated to *in vivo* situations.

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