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Research Article

# Gauging the clinical significance of P-glycoprotein-mediated herb-drug interactions: Comparative effects of St. John's wort, Echinacea, clarithromycin, and rifampin on digoxin pharmacokinetics

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Concomitant administration of botanical supplements with drugs that are P-glycoprotein (P-gp) substrates may produce clinically significant herb-drug interactions. This study evaluated the effects of St. John's wort and *Echinacea* on the pharmacokinetics of digoxin, a recognized P-gp substrate. Eighteen healthy volunteers were randomly assigned to receive a standardized St. John's wort (300 mg three times daily) or *Echinacea* (267 mg three times daily) supplement for 14 days, followed by a 30-day washout period. Subjects were also randomized to receive rifampin (300 mg twice daily, 7 days) and clarithromycin (500 mg twice daily, 7 days) as positive controls for P-gp induction and inhibition, respectively. Digoxin (Lanoxin® 0.25 mg) was administered orally before and after each supplementation and control period. Serial digoxin plasma concentrations were obtained over 24 h and analyzed by chemiluminescent immunoassay. Comparisons of area under the curve $(AUC)_{(0-3)}$, $AUC_{(0-24)}$, elimination half-life, and maximum serum concentration were used to assess the effects of St. John's wort, *Echinacea*, rifampin, and clarithromycin on digoxin disposition. St. John's wort and rifampin both produced significant reductions ($p < 0.05$) in $AUC_{(0-3)}$, $AUC_{(0-24)}$, and $C_{max}$, while clarithromycin increased these parameters significantly ($p < 0.05$). *Echinacea* supplementation did not affect digoxin pharmacokinetics. Clinically significant P-gp-mediated herb-drug interactions are more likely to occur with St. John's wort than with *Echinacea*.

**Keywords:** Digoxin / Echinacea / Herb-drug interactions / Pharmacokinetics / St. John's wort

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## 1 Introduction

Over the last 8 years, a considerable body of work has been published about herb-drug interactions, including several comprehensive reviews [1–10]. Interest in this topic is easily understood given the growing popularity of alternative medicine, the myriad botanicals available worldwide as dietary supplements, the multitude of unique phytochemicals present in these products and the paucity of knowledge regarding their pharmacology. More importantly, almost 25% of all prescription drug users take herbal medicines concomitantly with conventional medications [11–13].

From a clinical perspective, the take home message from herb-drug interaction studies can oftentimes be confusing,

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**Abbreviations: AUC**, area under the curve; $\mathbf{C_{max}}$, maximum serum concentration; $\mathbf{k_e}$, elimination rate constant; **P-gp**, P-glycoprotein; **SXR**, steroid xenobiotic receptor; $\mathbf{T_{max}}$, time of maximum serum concentration; $\mathbf{T_{1/2}}$, elimination half-life

especially when the results of *in vitro* studies utilizing human tissue or cell lines are not supported by human *in vivo* studies [14]. While *in vitro* studies provide insight as to which botanicals may affect drug pharmacokinetics, only *in vivo* studies can provide a definitive means for determining the clinical importance of pharmacokinetic herb-drug interactions. However, statistically significant effects observed among human *in vivo* herb-drug interaction studies might not always translate into clinical significance. For instance, Gurley *et al.* [15] noted that goldenseal supplementation produced a statistically significant increase (14%) in digoxin $C_{max}$ values; however, when compared to the 95% increase produced by clarithromycin, the goldenseal-digoxin interaction appears clinically insignificant. Other recent studies utilizing well-recognized modulators of cytochrome P-450 3A (CYP3A) and P-glycoprotein (P-gp) activity *in vivo*, demonstrated that milk thistle and black cohosh had no clinically significant effects on human CYP3A-mediated drug metabolism [16], or P-gp-mediated drug efflux [17]. Taken together, these examples illustrate that, when possible, *in vivo* effects of botanicals should be compared to appropriate controls in order to gauge the clinical relevance of herb-drug interactions.

P-gp (ABCB1), an ATP-binding cassette protein coded by the *ABCB1* gene, plays a prominent role in the disposition of many xenobiotics through its action as a drug efflux pump. Phytochemical-mediated alterations in P-gp activity may give rise to herb-drug interactions by altering drug absorption, distribution, and elimination [5]. In this report, we describe the effects of St. John's wort and, for the first time in humans, *Echinacea* supplementation on the pharmacokinetics of digoxin, a putative P-gp substrate that exhibits a narrow therapeutic index. As a means of gauging the clinical relevancy of botanical-mediated P-gp interactions, we also compare supplement effects to those of clarithromycin, an inhibitor of P-gp activity [18] and rifampin, an inducer of P-gp expression [19].

## 2 Materials and methods

### 2.1 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. Eighteen young adults (9 females) (age, mean ± SD = 30 ± 5.4 years; weight, 77.5 ± 16.5 kg) participated in the study and all subjects were in good health as indicated by medical history, routine physical examination, electrocardiography, and clinical laboratory testing. All subjects were non-smokers, ate a normal diet, were not users of botanical dietary supplements, and were not taking prescription (including oral contraceptives) or non-prescription medications. All female subjects had a negative pregnancy test at baseline. All subjects were instructed to abstain from alcohol, caffeine, fruit juices, cruciferous vegetables, and char-broiled meat throughout each 2-week phase of the study. Adherence to these restrictions was further emphasized 5 days before digoxin administration. Subjects were also instructed to refrain from taking prescription and nonprescription medications during supplementation periods, and any medication use during this time was documented. Documentation of compliance to these restrictions was achieved using a food/medication diary.

### 2.2 Supplements and supplementation/ medication regimens

The effect of St. John's wort, *Echinacea*, rifampin and clarithromycin on digoxin oral absorption was evaluated individually on four separate occasions in each subject. This was an open-label study randomized for supplementation/ medication sequence ("Supplementation/medication" refers to either St. John's wort, *Echinacea*, rifampin, or clarithromycin). Each supplementation phase (St. John's wort or *Echinacea*) lasted 14 days while each medication phase (rifampin or clarithromycin) was of 7 days duration. Each supplementation/medication phase was followed by a 30-day washout period. This randomly assigned sequence of supplementation/medication followed by washout was repeated until each subject had received all four products. Single lots of St. John's wort (lot # 530812, standardized extract WS 5572) and *Echinacea* (lot #A05551200) were purchased from Nature's Way Products, (Springville, UT) and Gaia Herbs (Brevard, NC), respectively. Rifampin (Rifadin®, Aventis Pharmaceuticals, Kansas City, MO.) and clarithromycin (Biaxin®, Abbott Laboratories, North Chicago, IL) were utilized as positive controls for P-gp induction and inhibition, respectively. Product labels were followed regarding the recommended dosing of St. John's wort extract (300 mg, three times daily, standardized to contain 3% hyperforin); *Echinacea* extract [*E. purpurea* aerial, root, and seed parts 195 mg, and *E. augustifolia* root parts 72 mg (equivalent to 2600 mg crude herb), three times daily, standardized to contain 2.2 mg isobutylamides per capsule]; clarithromycin (500 mg, twice daily) and rifampin (300 mg, twice daily). Rifampin, however, was not administered on the day of digoxin administration [20]. Telephone and electronic mail reminders were used to facilitate compliance, while pill counts and supplementation usage records were used to verify compliance.

### 2.3 Digoxin administration

Following an overnight fast, subjects reported to the University of Arkansas for Medical Sciences General Clinical Research Center for digoxin administration and blood sampling. Prior to digoxin administration, subjects were weighed and questioned about their adherence to the dietary

 

and medication restrictions. Female subjects were administered pregnancy tests and only those with negative test results were allowed to participate. Following the placement of a 20 gauge-indwelling catheter into a peripheral vein of the forearm, an oral dose of digoxin (0.25 mg, Lanoxin®, GlaxoSmithKline, Research Triangle Park, NC) was administered with 240 mL of water. Throughout the study, digoxin doses were administered 24 h before the start of each supplementation/medication phase (baseline) and again on the last day of each phase. Serial blood samples were obtained before and at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after digoxin administration. Each subject's blood pressure, heart rate, and respiration rate was monitored at 1, 2, and 6 h post digoxin administration. Four hours after digoxin administration, subjects received identical meals consisting of a turkey sandwich, potato chips, carrot sticks, and water.

### 2.4 Determination of digoxin serum concentrations

Digoxin serum concentrations were determined by an automated chemiluminescent immunoassay system (ACS:180 Digoxin, Chiron Diagnostics, West Walpole, MA). Calibrations were performed in the range of 0.1–5.0 ng/mL. Serum concentrations greater than 5 ng/mL were diluted and reassayed. The lower LOQ was 0.1 ng/mL. The interday accuracy for digoxin at 0.58, 1.77, and 3.48 ng/mL was 5.4, 3.7, and 2.9%, respectively. The interday precision for digoxin at 0.49, 0.98, and 1.97 ng/mL was 7, 6, and 2%, respectively.

### 2.5 Supplement analysis

The phytochemical content of each supplement was independently analyzed for specific "marker compounds" by ChromaDex (Clearwater, FL). Quantitative determination of hyperforin, hypericin, and various flavonoids in St. John's wort was achieved using a proprietary isocratic HPLC method similar to that described by Liu *et al.* [21]. *E. purpurea* was analyzed for various phenolic acids (*e.g.* chicoric acid, echinacoside, and caftaric acid) and isobutylamide content using a proprietary gradient HPLC method similar to that described by Molgaard *et al*. [22].

### 2.6 Pharmacokinetic analysis

Digoxin pharmacokinetics were determined using standard noncompartmental methods with the computer program WinNonlin (version 2.1; Pharsight, Mountain View, CA). Area under the plasma concentration time curves from 0 to 24 h ($AUC_{(0-24)}$) and 0 to 3 h ($AUC_{(0-3)}$) were determined by use of the trapezoidal rule. Rifampin, clarithromycin, and other P-gp modulators have significant effects on digoxin pharmacokinetics during the absorption phase [18, 19], which was the reason for evaluating $AUC_{(0-3)}$. The terminal elimination rate constant ($k_e$) was calculated using the slope of the log-linear regression of the terminal elimination phase and the elimination half-life ($T_{1/2}$) was calculated as $0.693/k_e$. Peak digoxin concentrations ($C_{max}$) and the times when they occurred ($T_{max}$) were derived directly from the data.

### 2.7 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 [23]. 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 (soft gelatin capsule) 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.

### 2.8 Statistical analysis

A repeated measures ANOVA model was fit for each pharmacokinetic parameter using SAS Proc Mixed software (SAS Institute, Cary, NC, USA). Since pre- and post-supplementation/medication pharmacokinetic parameters were determined in each subject for all four study phases, a covariance structure existed for measurements within subjects. Sex, supplement/medication, and supplement/medication-by-sex terms were estimated for each parameter using a Huynh-Feldt covariance structure fit. If supplement/medication-by-sex interaction terms for a specific parameter measure were significant at the 5% level, the focus of the post-supplementation/medication minus pre-supplementation/medication response was assessed according to sex. If the supplement/medication-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/medication effects. All four models obtained at least 80% power at the 5% level of significance to detect a Cohen effect size of 1.32 to 1.71 SD units [24].

 

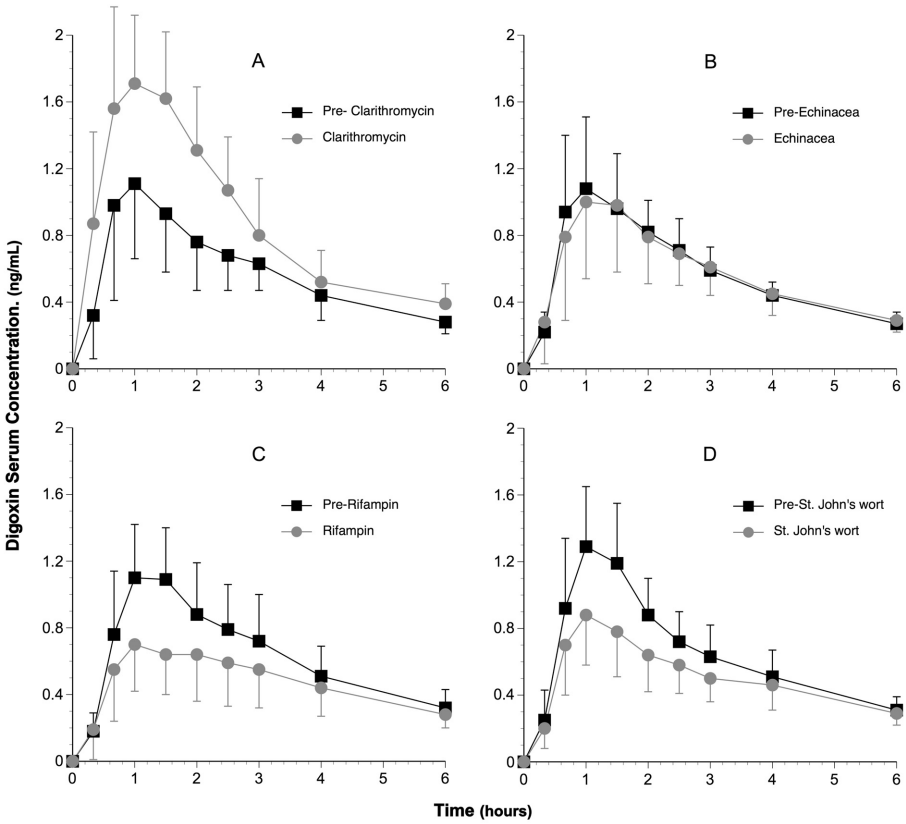


**Figure 1.** Digoxin concentration-time profiles (0-6 h) before and after each supplementation/drug phase. (A) Pre- and post-clarithromycin; (B) pre- and post-*Echinacea*; (C) pre- and post-rifampin; (D) pre- and post-St. John's wort. Black squares = pre-experimental mean serum digoxin concentrations. Gray circles = post-experimental mean serum digoxin concentrations. Error bars = SD.

## 3 Results

### 3.1 General experimental observations

All eighteen subjects completed each phase of the study. Neither spontaneous reports from study participants nor their responses to questions asked by study nurses regarding supplement/medication usage revealed any serious adverse events. While 2 subjects taking St. John's wort noted drowsiness, no significant side effects were reported for *Echinacea*. Nausea, indigestion, and complaints of a metallic taste were frequently noted during clarithromycin phases. Mild indigestion and reddish discoloration of the urine were common conditions reported with rifampin use. No clinically significant changes in blood pressure, heart rate, or respiratory rate were observed after digoxin administration. Examination of pill counts and food/medication diaries revealed no significant deviations from the study protocol.

### 3.2 Effect of supplementation on digoxin pharmacokinetics

The effects of clarithromycin, rifampin, St John's wort, and *Echinacea* on serum digoxin concentration versus time profiles are depicted in Fig. 1. Statistically significant increases ($p < 0.05$) in digoxin $AUC_{(0-3)}$ (68%), $AUC_{(0-24)}$ (46%), $T_{1/2}$ (73%) and $C_{max}$ (75%) were observed after 7 days of clarithromycin administration (Fig. 1A, Table 1). Statistically significant reductions ($p < 0.05$) in digoxin $AUC_{(0-3)}$ (−30%), $AUC_{(0-24)}$ (−25%), and $C_{max}$, (−38%) were noted following rifampin administration (Fig. 1C, Table 1). St. John's wort also produced statistically significant reductions in digoxin $AUC_{(0-3)}$ (−28%), $AUC_{(0-24)}$ (–23%), and $C_{max}$, (−36%) (Fig. 1D, Table 1). No significant effects on digoxin disposition were observed as a result of *Echinacea* supplementation. Digoxin $T_{max}$ (1 h) was not significantly affected by any of the treatments. In addition, no sex-related changes in digoxin pharmacokinetics were noted for any of the supplement/medication interventions.

### 3.3 Phytochemical content and disintegration testing

Results of phytochemical analyses and disintegration testing for St. John's wort and *Echinacea* are presented in Table 2.

## 4 Discussion

Considerable evidence supports the premise that prolonged supplementation with St. John's wort can induce P-gp-mediated drug efflux, resulting in clinically significant

**Table 1**. Digoxin pharmacokinetic parameters before and after supplementation/drug phases (mean ± SD)

| *Supplement/Drug phase* | $AUC_{(0-3)}$ [ng · h/mL] | $AUC_{(0-24)}$ [ng · h/mL] | $T_{1/2}$ [hours] | $C_{max}$ [ng/mL] |
|---|---|---|---|---|
| Pre-Clarithromycin | 2.2 ± 0.8 | 7.3 ± 2.0 | 32.5 ± 9.5 | 1.2 ± 0.3 |
| Post-Clarithromycin | 3.7 ± 1.3* | 10.7 ± 3.4* | 56.3 ± 18.6* | 2.1 ± 0.9* |
| Pre-Rifampin | 2.3 ± 0.7 | 8.1 ± 1.9 | 34.6 ± 11.5 | 1.3 ± 0.4 |
| Post-Rifampin | 1.6 ± 0.6* | 6.1 ± 1.9* | 31.3 ± 10.9 | 0.8 ± 0.3* |
| Pre-St. John's wort | 2.5 ± 0.6 | 7.8 ± 1.6 | 30.1 ± 9.3 | 1.4 ± 0.3 |
| Post-St. John's wort | 1.8 ± 0.5* | 6.0 ± 1.3* | 27.7 ± 12.0 | 0.9 ± 0.2* |
| Pre-Echinacea | 2.2 ± 0.5 | 7.3 ± 1.6 | 32.5 ± 8.9 | 1.3 ± 0.3 |
| Post-Echinacea | 2.1 ± 0.6 | 7.5 ± 1.8 | 31.5 ± 9.6 | 1.2± 0.3 |

*$p < 0.05$.

**Table 2**. Phytochemical analysis and disintegration times for botanical dosage forms ($n = 6$)

| *Supplement* [dosage form] | Compound | Content [mg/dosage unit] | Daily dose [mg] | Disintegration time [min] |
|---|---|---|---|---|
| St. John's wort | Hyperforin | 8.0 ± 0.06 | 24.0 | 44.8 ± 1.8 |
| (coated tablet) | Hypericin | 0.1 ± 0.001 | 0.3 | |
| | Pseudohypericin | 0.3 ± 0.001 | 0.9 | |
| | **Flavonoids** | | | |
| | Rutin | 5.6 ± 0.06 | 16.8 | |
| | Hyperoside | 3.6 ± 0.04 | 10.8 | |
| | Isoquercetin | 2.1 ± 0.03 | 6.3 | |
| | Quercetrin | 0.8 ± 0.01 | 2.4 | |
| | Quercetin | 0.8 ± 0.01 | 2.4 | |
| | Total | 21.3 | 63.9 | |
| *Echinacea* | **Phenolic acids** | | | 6.1 ± 0.7 |
| (soft gel capsule) | Chicoric acid | 3.3 ± 0.03 | 29.7 | |
| | Echinacoside | 1.3 ± 0.01 | 11.7 | |
| | Caftaric acid | 1.4 ± 0.01 | 12.6 | |
| | Cynarin | 0.3 ± 0.001 | 2.7 | |
| | Chlorogenic acid | 0.2 ± 0.001 | 1.8 | |
| | **Isobutylamides** | 1.3 ± 0.01 | 11.7 | |
| | Total | 7.8 | 70.2 | |

reductions in the bioavailability of P-gp substrates like digoxin [25–27], fexofenadine [28–30], and cyclosporine [31–33]. St. John's wort appears to induce P-gp expression through the actions of hyperforin, a phytochemical unique to *Hypericum* species and potent ligand for the orphan nuclear receptor, steroid-xenobiotic-receptor (SXR), or human pregnane xenobiotic receptor (hPXR) [34, 35]. When ingested, hyperforin is taken up by intestinal enterocytes and hepatocytes binding SXR in the nucleus of these cells. In turn, SXR-hyperforin complexes form heterodimers with the retinoid-X-receptor that bind to the drug-response element of the *ABCB1* gene up-regulating its expression [4]. Recent evidence, however, suggests that the magnitude of St. John's wort-mediated drug interactions is a function of hyperforin dose [27, 33, 36]. Administration of St. John's wort for at least 14 days at hyperforin doses lower than 5 mg/day produced no significant changes in digoxin pharmacokinetics, whereas significant reductions in AUC and $C_{max}$ were noted for daily doses in excess of 10 mg [27, 36]. In the present study, subjects exposed to daily hyperforin doses of 24 mg for 14 days exhibited significant reductions in digoxin AUC (–25%) and $C_{max}$ (–35%). The magnitude of these effects was strikingly similar to that reported by Johne *et al.* [25] and Mueller *et al.* [27] whose subjects also ingested hyperforin doses in excess of 10 mg/day for 11–14 days.

Our findings also demonstrated that 14 days of St. John's wort (24 mg/day hyperforin) reduced digoxin exposure comparable to that observed after 7 days of rifampin (600 mg/day). Rifampin, like hyperforin, is a ligand for SXR and a well-recognized inducer of P-gp [37]. Interestingly, rifampin is also a substrate for P-gp [38], as well as an inhibitor of the solute carrier organic anion transporter family, member 1B1 (SLCO1B1, also known as organic anion transporting polypeptide 1B1, OATP1B1), and its simultaneous administration with digoxin may actually increase digoxin exposure [38]. For this reason, we refrained from administering rifampin concomitantly with digoxin on the days of pharmacokinetic sampling. This practice allowed us to better gauge the clinical magnitude of P-gp induction on

 

digoxin disposition. To our knowledge, this is the first direct comparison of St. John's wort to rifampin from an herb-drug interaction perspective.

Unlike rifampin and St. John's wort, clarithromycin markedly increased digoxin AUC, $C_{max}$, and $T_{1/2}$. The extent of these increases was similar to that previously reported by Rengelshausen *et al.* [18] and Gurley *et al.* [15, 17]. Accordingly, these results confirm clarithromycin's clinical utility as a positive control for P-gp inhibition. Although none of the botanical supplements tested here inhibited P-gp, future studies with other botanicals may produce statistically significant differences. By juxtaposing the effects of botanical supplementation to that of clarithromycin, or other clinically-recognized P-gp inhibitors (*e. g.* quinidine), a more meaningful interpretation can be obtained.

*Echinacea*, marketed as an "immune system booster" and alternative treatment for the common cold, ranks among the top-selling botanical supplements worldwide [39]. Due to its popularity, knowledge of its herb-drug interaction profile is much needed. *In vitro* studies suggest that extracts of *Echinacea* species are capable of modulating CYP activity [40 – 45], but no assessments of *Echinacea* on P-gp function have been reported to date. Such studies are warranted given the variety of phytochemicals present in *Echinacea*, particularly the alkamides, which are bioavailable when administered orally [46 – 48]. While a few clinical studies have investigated *Echinacea's* effect on human CYP isoforms [49, 50], to our knowledge this is the first report of *Echinacea's* effect on human P-gp activity *in vivo*. Unlike St. John's wort, the *Echinacea* supplementation regimen used in this study produced no significant effects on digoxin disposition. This finding implies that *Echinacea*, is not a potent modulator of human P-gp *in vivo*, and thus poses no clinically significant interaction risk with digoxin or other P-gp substrates. Previous *in vivo* assessments of human CYP isoforms suggest that *Echinacea* has little impact on CYP2C9, CYP2D6, CYP2E1, and CYP1A2, but may selectively modulate the activity of CYP3A4 at hepatic and intestinal sites [49, 50]. When taken together, the existing clinical evidence suggests that *Echinacea's* drug interaction potential appears to be relatively minor, however, given the inter-product variability in phytochemical content and potency among *Echinacea* supplements [51 – 53], these results may not extend to regimens utilizing higher dosages, longer supplementation periods, or products with improved dissolution and/or bioavailability characteristics.

In summary, botanical supplements may interact with conventional medications, but the magnitude of such interactions is not readily predictable from *in vitro* studies. Clinical studies offer the best means of assessing the drug interaction potential of botanical supplements. However, such studies often neglect to include appropriate benchmarks for gauging the clinical significance of any observed herb-mediated effects. Using rifampin and clarithromycin as positive controls for P-gp induction and inhibition, respectively, the effects of St. John's wort and *Echinacea* on the disposition of digoxin were evaluated. Our findings revealed that St. John's wort products containing sufficient hyperforin are equivalent to rifampin in terms of P-gp induction, whereas, *Echinacea* had no bearing on P-gp activity. These observations illustrate too the spectrum of effects that may occur during human herb-drug interaction studies and provide a means for assessing their clinical relevance. Accordingly, *in vivo* herb-drug interaction studies may be better served when known modulators of drug metabolism and transport are included in their design.

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