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PHARMACOKINETIC INTERACTIONS OF A LICORICE DIETARY SUPPLEMENT WITH CYTOCHROME P450 ENZYMES IN FEMALE PARTICIPANTS

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Abbreviations: ALK, alkaline phosphate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the serum drug concentration-time curve; BMI, body mass index; BUN, blood urea nitrogen; CL, clearance; C_{max} maximum serum concentration; CYP, cytochrome P450; GRAS, generally recognized as safe; SRM, selected reaction monitoring; T_{max}, time to reach maximum concentration; $T_{1/2}$, elimination half-life; UHPLC-MS/MS, ultrahigh-pressure liquid chromatography-tandem mass spectrometry; U.S. FDA, United States Food and Drug Administration;

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ABSTRACT

Licorice, the roots and rhizomes of *Glycyrrhiza glabra* L., has been used as a medicinal herb, herbal adjuvant, and flavoring agent since ancient times. Recently, licorice extracts have become popular as dietary supplements used by females to alleviate menopausal symptoms. Exposure to licorice products containing high levels of glycyrrhizic acid can cause hypokalemia, but independent from this effect, preclinical data indicate that licorice can inhibit certain cytochrome P450 (CYP) enzymes. To evaluate whether or not clinically relevant pharmacokinetic interactions of licorice with CYP enzymes exist, a Phase 1 clinical investigation was carried out using a licorice extract depleted in glycyrrhizic acid (content <1%) and a cocktail containing caffeine, tolbutamide, alprazolam, and dextromethorphan, which are probe substates for the enzymes CYP1A2, CYP2C9, CYP3A4/5, and CYP2D6, respectively. The botanically authenticated and chemically standardized extract of roots from *G. glabra* was consumed by 14 healthy menopausal and post-menopausal female participants twice daily for two weeks. The pharmacokinetics of each probe drug were evaluated immediately before and after supplementation with the licorice extract. Comparison of the average areas under the time-concentration curves (AUCs) for each probe substrate in serum showed no significant changes from licorice consumption, while time to reach peak concentration for caffeine and elimination half-life for tolbutamide showed small changes. According to the U.S. Food and Drug Administration guidance, which is based on changes in the AUC of each probe substrate drug, the investigated licorice extract should not cause any clinically relevant pharmacokinetic interactions with respect to CYP3A4/5, CYP2C9, CYP2D6, or CYP1A2.

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SIGNIFICANCE STATEMENT

Despite GRAS status, the licorice species, *Glycyrrhiza glabra*, has been associated with some toxicity. Preclinical studies suggest that *G. glabra* might cause pharmacokinetic drug interactions by inhibiting several cytochrome P450 enzymes. This Phase 1 clinical study addressed these concerns by evaluating clinically relevant effects with respect to CYP3A4/5, CYP2C9, CYP2D6, and CYP1A2. These results showed that a standardized *G. glabra* extract did not cause any clinically relevant pharmacokinetic drug interactions with four major cytochrome P450 enzymes.

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INTRODUCTION

Licorice root/rhizomes and their extracts are widely used in herbal medicines and dietary supplements, as well as to flavor and sweeten many foods, confections, and pharmaceuticals (Murray et al., 2020). The most commonly used licorice species in confections, medicines, and dietary supplements in western countries is *Glycyrrhiza glabra* L., while the Asian species, *G. uralensis* Fish and *G. inflata* Batalin, are used most often in Traditional Chinese Medicine (Pastorino et al., 2018). As a dietary supplement, *G. glabra* is used as a digestive aid and for its anti-inflammatory and antimicrobial effects. Due to the estrogenicity of licorice constituents such as liquiritigenin (Li et al., 2016a), licorice root extracts are used by menopausal females as an alternative to conventional hormone replacement therapy (Boonmuen et al., 2016).

Despite a long history of safe use as a natural medicine and flavoring agent, as reflects by its GRAS status, licorice has some potential to cause harmful and potentially fatal side effects (Wahab et al., 2021). As an example, a 74-year old woman consuming a large quantity of black licorice had to be admitted to the hospital and treated for hypokalemia (Benge et al., 2020). Glycyrrhizic acid is the primary source of sweetness in licorice, but it is metabolized to glycyrrhetinic acid, which inhibits type-2 11β-hydroxysteroid dehydrogenase (Molhuysen et al., 1950). 11β-Hydroxysteroid dehydrogenase decomposes cortisol in the distal nephron, and inhibition of this enzyme can cause hypokalemia and have other effects related to mineralocorticoid receptor activity (Yoshino et al., 2021).

Another purported safety concern regarding licorice root extracts is the potential for pharmacokinetic drug interactions such as inhibition or induction of drug metabolizing enzymes. For example, the inhibition of cytochrome P450 (CYP) Phase

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I drug metabolizing enzymes can reduce the rate of drug clearance, thereby increasing drug plasma concentrations and causing toxicity due to overdose (Tannenbaum et al., 2014). Induction of CYP enzymes can have the opposite effect of increasing drug clearance, reducing half-life and drug exposure, and lowering efficacy. The CYP superfamily includes the most important Phase I drug metabolizing enzymes, which are responsible for transforming more than 80% of drugs, primarily through oxidative metabolism (Iyer and Sinz, 1999; Tannenbaum et al., 2014).

Consequently, the United States Food and Drug Administration (FDA) has established guidance for clinical evaluation of drug-drug pharmacokinetic interactions involving CYP enzymes (U.S. Food and Drug Administration, 2020), and similar clinical studies have been described for the evaluation of the drug-botanical pharmacokinetic interactions (Yoshino et al., 2021). The approach involves measuring the effect of the test drug or botanical dietary supplement on the pharmacokinetics of a low dose of a probe drug or a cocktail of probe drugs, each of which is metabolized by a specific CYP enzyme. This cocktail of probe substrates is administered immediately before and after intervention with the testing drug or botanical, serial blood samples are obtained over time, and the concentration of each probe substrate is measured for pharmacokinetic modeling. Comparison of the pharmacokinetic profiles of each probe substrate pre- and post-dosing allows conclusions about possible induction or inhibition of the relevant CYP enzymes.

Several preclinical studies have indicated that licorice root extracts can inhibit specific CYP enzymes (Pastorino et al., 2018). However, our laboratory found that an extract of *G. glabra* roots inhibited CYP2B6, CYP2C8, CYP2C9, and CYP2C19 only moderately and CYP3A4 only weakly (Li et al., 2016a). In another study, an extract of *G. glabra* roots inhibited CYP1B1 (Sharma et al., 2017). Glycyrrhetinic acid was

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reported to be a potent inhibitor in vitro of CYP3A4, a weak inhibitor of CYP2C9 and CYP1A2, while altering the pharmacokinetics of the natural product bakuchiol in rats (Li et al., 2016b). Glycyrol from licorice was found to be a strong competitive inhibitor of CYP1A1 and CYP2C9 (Kim et al., 2016). In a clinical study, glycyrrhizic acid (300 mg/day) induced CYP3A4 (Tu et al., 2010).

To assess if such CYP interactions with licorice could cause clinically relevant drug interactions, we carried out a Phase I clinical trial of a standardized extract of *G. glabra* using four drugs recommended by the FDA as probe substrates of CYP3A4, CYP2C9, CYP2D6 and CYP1A2. Cocktails of these four probe substrates have been used previously to study pharmacokinetic interactions between botanical dietary supplements and cytochromes P450 (Chen et al. 2020; van Breemen et al. 2020). Although CYP2D6 is not known from in vitro studies to be inhibited by licorice, it was included because the FDA recommends that potential CYP2D6 pharmacokinetic interactions be investigated for all new therapeutic agents (U.S. Food and Drug Administration, 2020).

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METHODS

Chemicals and reagents

LC-MS grade methanol and acetonitrile were purchased from VWR (Radnor, PA), and LC-MS grade formic acid was purchased from Thermo Fisher (Rockford, IL). Water was prepared using an ElgaPurelab Ultra (Siemens Water Technologies, Woodridge, IL) water purification system. Caffeine, [trimethyl-$^{13}C_3$]-caffeine, tolbutamide, dextromethorphan, [methyl-d_3]-dextromethorphan, alprazolam, and [phenyl-d_5]-alprazolam were purchased from MilliporeSigma (St. Louis, MO). [Butyl-d_9]-4-hydroxy-tolbutamide was purchased from Toronto Research Chemicals

(Toronto, Canada). Blank serum, obtained from individual donors, was purchased from BioIVT (Westbury, NY). The standardized extract of *G. glabra* root used during this clinical investigation was provided by our Botanical Center after being sourced from Natural Remedies (Bangalore, India), botanical authenticated, and tested for microbial and chemical contaminants. The licorice extract was standardized using liquid chromatography-tandem mass spectrometry as described previously (Li et al., 2016a; Simmler et al., 2014), and its use in this pharmacokinetic drug-botanical interaction study was granted an exemption from U.S. Food and Drug Administration Investigational New Drug requirements.

Study design

This phase I clinical study was registered on Clinicaltrials.gov (NCT03948243). The human participant protocol was approved by the University of Illinois at Chicago Institutional Review Board (#2015-0651). Nineteen (19) healthy peri-menopausal and post-menopausal female participants aged 47-66 were enrolled. To ensure that the participants were healthy, each received a physical examination, comprehensive blood chemistry panel, electrocardiogram, and urinalysis. Participants who had chronic diseases or significant medical conditions were excluded. Other exclusions included smoking, alcohol or drug abuse, obesity (BMI>40), allergy or hypersensitivity to caffeine, dextromethorphan, tolbutamide, alprazolam, or licorice, pregnancy, or use of hormone replacement therapy within 8 weeks of the study. Participants were screened for *CYP2D6* phenotype using polymerase chain reaction with primer extension (Alverno Central Laboratory; Hammond, IN) and excluded if their CYP2D6 phenotype indicated low activity.

Beginning one week prior until the end of the study, participants were advised to avoid any food or beverages containing caffeine, citrus, or licorice. Some citrus

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constituents are CYP enzyme inhibitors (Saito et al., 2005), while caffeine was one of the probe substrates. Participants also consumed no non-study dietary supplements or pharmaceuticals beginning two weeks before and until the end of the study (Figure 1).

After fasting overnight, a baseline blood sample (7 mL) was obtained from each participant’s arm vein using an in-dwelling line. Then, an oral cocktail was administered containing 100 mg caffeine (CYP1A2 substrate), 250 mg tolbutamide (CYP2C9 substrate), 30 mg dextromethorphan (CYP2D6 substrate), and 2 mg alprazolam (CYP3A4/5 substrate). Blood samples (7 mL) were drawn at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 48, 72, and 96 h postdosing of the cocktail probe substrates (Figure 1). The blood samples were centrifuged, and the isolated serum samples were stored at -80 °C until analysis. Breakfast was provided to the participants 0.25 h after administration of the cocktail. Blood glucose levels were measured at baseline and 0.5 h, and a comprehensive blood panel was done at 2 h for each participant to ensure they did not have any adverse reactions to the substrates. Vital signs were taken at baseline, 1, 4, 8, 12, 24, 48, 72, and 96 h, and adverse events were assessed daily until day 4 (Figure 1).

On day 8 post dosing with the substrates cocktail (Figure 1), participants started taking 2 capsules of the licorice extract per day (one capsule in the morning and one in the evening) for 14 days, which is the treatment period recommended by the U.S. Food and Drug Administration (2020) for clinical trials evaluating induction of cytochromes P450. Each capsule contained 75 mg *G. glabra* extract standardized to 3.1 mg glabridin, 0.55 mg glycyrrhizic acid, 0.50 mg liquiritin, and 0.47 mg isoliquiritin. After consuming the licorice extract for 14 days, participants returned to clinic on day 22 after fasting overnight (Figure 1). Blood draws, probe cocktail administration, and

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medical examinations were then repeated as described above for days 0 through day 4. Compliance with consumption of the licorice dietary supplement was evaluated by participant self-reporting and a count of returned licorice capsules.

UHPLC-MS/MS

To evaluate changes in the pharmacokinetics of each CYP probe substrate due to licorice, the concentrations of caffeine, tolbutamide, dextromethorphan, and alprazolam in serum obtained at different time points before and after intervention with the licorice dietary supplement were measured using ultrahigh-pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) as described previously (Chen and van Breemen, 2020). For the measurement of caffeine and tolbutamide, serum (50 µL) was mixed with water (50 µL) containing 0.1% formic acid, and serum proteins were precipitated by adding acetonitrile/methanol (9:1; v/v) (300 µL) containing [$^{13}C_3$]-caffeine (1000 ng/mL) and [d_9]-4-hydroxy-tolbutamide (2400 ng/mL). After vortex mixing (0.5 min) and centrifugation at 4°C for 15 min, the supernatant (80 µL) was removed and diluted with 30% aqueous methanol (20 µL) containing 0.1% formic acid prior to UHPLC-MS/MS analysis.

For the measurement of dextromethorphan and alprazolam, serum (100 µL) was mixed with water (100 µL) containing 0.1% formic acid. Serum proteins were precipitated by adding 600 µL of acetonitrile/methanol (9:1; v/v), containing [d_3]-dextromethorphan (28 ng/mL) and [d_5]-alprazolam (15 ng/mL) followed by vortex mixing (0.5 min). After centrifugation for 15 min, the supernatant (800 µL) was removed, evaporated to dryness, and reconstituted in 50 µL of 30% aqueous methanol containing 0.1% formic acid.

UHPLC-MS/MS quantitative analyses were carried out using a Shimadzu (Kyoto, Japan) LCMS-8060 triple quadruple mass spectrometer and Nexera UHPLC

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system. Separations were carried out using a Waters (Milford, MA) Acquity UHPLC BEH C_{18} column (2.1 x 50 mm, 1.7 µm) with a gradient from water containing 0.1% formic acid to acetonitrile as follows: 5-15% acetonitrile 0-0.7 min, 15-55% acetonitrile 0.7-1.5 min, 55-75% acetonitrile 1.5-2 min, and 1 min at 95% acetonitrile, followed by equilibration at 5% acetonitrile for 1 min. The column oven temperature was 40 °C, the flow rate was 0.5 mL/min, and the temperature of the autosampler was 4 °C. The injection volume was 6 µL for the measurement of caffeine and tolbutamide and 15 µL for the measurement of dextromethorphan and alprazolam. Positive ion electrospray tandem mass spectrometry with collision induced dissociation and selected reaction monitoring (SRM) was used for quantitative analysis. The quantifier and qualifier SRM transitions for each analyte and stable isotope labeled internal standard were as follows: caffeine *m/z* 195 to *m/z* 138 and *m/z* 195 to *m/z* 110; [$^{13}C_3$]-caffeine *m/z* 198 to 140 and *m/z* 198 to *m/z* 112; tolbutamide *m/z* 271 to *m/z* 172 and *m/z* 271 to *m/z* 91; [d_9]-4-hydroxy-tolbutamide *m/z* 296 to *m/z* 188 and *m/z* 296 to *m/z* 107; dextromethorphan *m/z* 272 to *m/z* 215 and *m/z* 272 to *m/z* 171; alprazolam *m/z* 309 to *m/z* 281 and *m/z* 309 to *m/z* 205; and [d_5]-alprazolam *m/z* 314 to *m/z* 286 and *m/z* 314 to *m/z* 210. The desolvation line temperature was 250 °C, nebulizing gas flow was 3 L/min, drying gas flow was 10 L/min and dwell time was 25 ms/ion.

Pharmacokinetics modeling and statistics

Pharmacokinetic parameters were calculated using noncompartmental analysis and the log-linear trapezoidal method with Phoenix WinNonlin software (Certara, Princeton, NJ; Version 8.0). The parameters included area under the concentration-time curve (AUC), peak serum concentration (C_{max}), time to reach peak concentration (T_{max}), elimination half-life ($T_{1/2}$), and oral clearance rate (CL/F), and

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were summarized as natural logarithm (mean) ± natural logarithm (standard error, SE) for 14 participants. Following the FDA guidance for clinical drug interaction studies (U.S. Food and Drug Administration, 2020), the geometric mean ratios before and after the dietary supplement intervention at the 90% confidence interval were obtained. Two-way paired t-test was used to determine if each mean pharmacokinetic parameter changed due to consumption of licorice extract. Statistical analyses were carried out using R and GraphPad Prism 7 (GraphPad Software; San Diego, CA).

RESULTS

Out of 19 enrolled participants, only 14 participants completed the clinical trial, because COVID-19 shelter-in-place mandates occurred during the dosing of the final cohort. The study sponsor, NCCIH/NIH, approved early termination and considered the study complete. The number of participants is consistent with recent clinical studies of pharmacokinetic interactions between botanical dietary supplements and drugs metabolized by cytochromes P450. For example, a total of 15 peri-menopausal and post-menopausal female participants was used in a study of red clover pharmacokinetic interactions with cytochromes P450 (Chen et al, 2020), and 16 peri-menopausal and post-menopausal female participants were used in pharmacokinetic interaction study of hops with four cytochromes P450 (van Breemen et al., 2020). In a 2021 clinical study of CYP3A and drug transporter interactions with the botanical goldenseal, 8 male and 8 female participants were studied (Nguyen et al. 2021).

During the present study no serious adverse events was reported. One participant reported an unsteady gait at 12 hours following administration of the

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probe substrate cocktail which resolved at 24 h. Another participant reported redness and itching at the phlebotomy site on day 23 that resolved on day 24.

The serum metabolic panels before and after licorice intervention were compared using paired two-way t-test (Table 1). The metabolic parameters showed no significant changes following 14 days of licorice dosing. Based on counts of returned licorice capsules and self-reporting, 12 of the 14 participants had 100% compliance while the other 2 had 96% compliance.

The age range of participants completing the study was 47-66 years (Table 1). The mean age was 56.28±1.83 (mean ± SE) years (N = 14) while 28.6% of the participants were post-menopausal. The mean BMI of the 14 participants was 28.42±1.67 (mean ± SE) kg/m^2. Six participants were African American, two were Asian, six were Caucasian of whom three were ethnically Hispanic.

The average concentration-time curves of the 14 participants (Figure 2), the semi-log average concentration-time curves (Supplemental Figure), the individual participant area under the concentration-time curve (AUC) values (Figure 3), and other average pharmacokinetics calculations (Table 2) were compared for each probe drug before and after consumption of the licorice extract for 14 days. Dextromethorphan (CYP2D6 substrate) and alprazolam (CYP3A4/5 substrate) showed no significant changes in pharmacokinetics. The T_{max} of caffeine, which is a probe of CYP1A2, showed a 32% decrease from 0.63 ± 1.16 h to 0.43 ± 1.15 h after licorice supplement intervention (Table 2). The $T_{1/2}$ of tolbutamide (CYP2C9 substrate) decreased by 6.8% from 8.58 ± 1.06 h to 8.00 ± 1.06 h (Table 2). However, the AUC values of caffeine and tolbutamide did not show any significant differences between pre-dosing and post-dosing of the licorice extract. Overall, the

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pharmacokinetic profiles of all four probes of cytochrome P450 activity showed either weak or no changes in response to the licorice extract administration.

DISCUSSION

According to the NIH Office of Dietary Supplements Database (DSLD, 2022), there are 946 dietary supplements currently marketed in the United States disclosing *G. glabra* on the labels. Among these, 451 products contain extracts but only 225 labels disclose the amount of *G. glabra* extract in the product. The dosage range for these 225 products is 1 mg/d to 1060 mg/d with a median extract dosage of 100 mg/d and a mean dosage of 175 mg/d. Therefore, the 150 mg/d dosage administered in this investigation was between the median and mean dosages recommended for all available products.

Administration of the standardized *G. glabra* extract at 150 mg/d did not cause any changes of the pharmacokinetic parameters for probe substrates of CYP3A4/5, CYP2D6, CYP1A2, and CYP2C9, except for the T_{max} of caffeine (CYP1A2) and $T_{1/2}$ of tolbutamide (CYP2C9). The change of T_{max} observed in the caffeine pharmacokinetic profile was probably caused by poor dietary compliance, as caffeine is present in many foods and beverages and was detected in some participants' baseline serum. The change in tolbutamide half-life is less clear, but this might have been caused by CYP2C9 polymorphisms.

According to the FDA Guidance for Clinical Drug Interaction Studies (U.S. Food and Drug Administration, 2020), a decrease of 20% in AUC (induction) or an increase of 1.25-fold in AUC (inhibition) is defined as a clinically relevant pharmacokinetic interaction. Therefore, the changes in T_{max} for CYP1A2 and $T_{1/2}$ CYP2C9 do not meet the criteria for clinically relevant drug interactions. In our previous in vitro study of *G. glabra* drug interactions (Li et al., 2017), a similar licorice

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extract caused moderate inhibition for CYP2B6, CYP2C8, CYP2C9, and CYP2C19, and weak inhibition of CYP3A4. In this Phase I clinical trial, these predicted cytochrome P450 inhibition interactions were not observed.

This discrepancy between in vitro predictions of pharmacokinetic licorice-drug interactions and clinically observed interactions might be due, at least in part, to low bioavailability of the active licorice constituents. Low bioavailability can be the result of poor absorption following oral administration or rapid first-pass metabolism. For example, the licorice chalcone isoliquiritigenin, which inhibits CYP2C8, CYP2C9, and to a lesser extent CYP3A4 (Li et al., 2017), is a substrate of an intestinal efflux transporter that can slow its absorption (Dai et al., 2008). Isoliquiritigenin also undergoes rapid glucuronidation in the intestine before reaching the liver, which would lower its bioavailability (Guo et al., 2008). As another example, the licorice constituent, glabridin, which inhibits CYP3A4, is also metabolized rapidly by intestinal UDP-glucuronyltransferases prior to reaching the liver (Guo et al., 2015). For licorice constituents that reversibly inhibit drug metabolism but are metabolized rapidly, simultaneous administration of the licorice dietary supplement and probe drugs might enable detection of inhibition. However, with twice daily administration of the licorice dietary supplement followed by administration of the probe drugs after an overnight fast, the present study was designed to test for longer lasting inhibitory effects. Probably for similar reasons, several previous clinical investigations of botanical dietary supplements, including hops (van Breemen et al., 2020) and red clover (Chen et al., 2020), also found no clinically relevant pharmacokinetic interactions with cytochromes P450 despite in vitro predictions of drug interactions during preclinical studies.

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Despite preclinical data predicting pharmacokinetic interactions between *G. glabra* and cytochromes P450, this investigation indicates that there is no clinically relevant risk of drug interactions for this extract with respect to CYP1A2, CYP2C9, CYP2D6, and CYP3A4/5. In addition, the lack of adverse events or changes in metabolic parameters indicate a high margin of safety. Although the dosage of the *G. glabra* extract in this study (150 mg/d) is widely used in a variety of commercially available dietary supplements, there might be risk of drug interactions at higher doses. Some licorice products contain higher doses of *G. glabra* extract, contain root powder instead of extract, or might contain higher percentages of glycyrrhizic acid. It should be noted that some licorice dietary supplements contain different licorice species such as *G. uralensis* or *G. inflata*, which have not yet been evaluated in clinical trials for drug interactions.

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AUTHOR CONTRIBUTIONS

Wrote the manuscript: J.L. and R.v.B.

Designed the research: R.v.B., S.B., and M.V.

Performed the research: J.L., E.B., S.B., and M.V.

Analyzed the data: J.L. and M.V.

Contributed new reagents/analytical tools and edited the manuscript: S.-N.C. and G.F.P.

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Footnotes

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FIGURE LEGENDS

Figure 1. Clinical study design and schedule of clinical procedures.

Figure 2. Average concentration-time curves for four CYP probe substrates pre- and post-consumption of an extract of licorice from *G. glabra* by 14 female participants for 14 days. The average concentration-time curves for all 14 participants indicate no significant changes in pharmacokinetics for selective probe substrates of CYP1A2, CYP2C9, CYP2D6, or CYP3A4/5. Error bars denote ±SE.

Figure 3. Comparison of individual AUC values for each CYP probe substrate before and after consumption of a *G. glabra* licorice extract for 14 days. The AUC values of caffeine (CYP1A2 substrate) and tolbutamide (CYP2C9 substrate) showed some pharmacokinetics changes for individual female participants following licorice consumption, but the average value does not suggest significant changes. Error bars denote ±SE.

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TABLES

Table 1. Demographics and metabolic panel characteristics of female participants (N=14) before (day 0) and after (day 22) supplementation twice per day with an extract of *G. glabra* licorice. BMI: body mass index; BUN: blood urea nitrogen; ALK: alkaline phosphates; ALT; alanine aminotransferase; AST: aspartate aminotransferase.

Parameter	Day 0	Day 22	% Change in mean
Age (years)	56.28±1.83		
Race (N)			
African American	6 (42.9%)		
Asian	2 (14.2%)		
Caucasian	6 (42.9%)		
Ethnicity (N)			
Hispanic	3 (21.4%)		
Non-Hispanic	11 (78.6%)		
Menopausal status (N)			
postmenopausal	4 (28.6%)		
perimenopausal	10 (71.4%)		
BMI (kg/m^2)	28.42±1.67		
Sodium (mmol/L)	140.71	140.21	-0.50
Calcium (mmol/L)	8.95	8.75	-0.20
Potassium (mmol/L)	3.89	4.04	0.16
Chloride (mmol/L)	106.00	106.79	0.16
Anion gap (mmol/L)	6.58	6.00	-0.58
Glucose (fasting; mg/dL)	96.71	98.93	2.21
BUN (mg/dL)	14.64	14.21	-0.43
Creatinine (mg/dL)	0.77	0.80	0.03
BUNcreat	19.33	17.56	-1.77
Bilirubin (mg/dL)	0.48	0.44	-0.04
ALK (U/L)	71.43	71.64	0.21
ALT (U/L)	11.57	11.64	0.07
AST (U/L)	13.93	16.14	2.21
Albumin (g/dL)	3.81	3.71	-0.11
Protein (g/dL)	6.24	6.30	0.06
CO_2 (mmol/L)	28.07	27.29	-1.43

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Table 2. Pharmacokinetic parameters of cytochrome P450 probe substrate before (day 0) and after (day 22) consumption of a *G. glabra* licorice extract for 14 days. (N = 14 female participants).[a]

Probe substrate (enzyme)	**PK parameter**	**Before licorice (Day 0)**	**After licorice (Day 22)**	**% Change in mean**	**GR (22:0)**	**90% CI**	**p-value**
Alprazolam (CYP3A4/5)	AUC (h*µg/L)	6.60 ± 0.07	6.55 ± 0.08	-0.75	0.95	(0.90, 1.01)	0.65
		ln(735.1 ± 1.1)	ln(699.2 ± 1.1)				
	T1/2 (h)	2.77 ± 0.06	2.75 ± 0.07	-0.72	0.98	(0.93, 1.04)	0.83
		ln(15.96 ± 1.06)	ln(15.64 ± 1.07)				
	Tmax (h)	-0.01 ± 0.24	-0.28 ± 0.23	78.26	0.76	(0.39, 1.49)	0.43
		ln(0.99 ± 1.27)	ln(0.76 ± 1.26)				
	Cmax (µg/L)	3.71 ± 0.09	3.67 ± 0.10	-1.08	0.95	(0.77, 1.17)	0.77
		ln(40.85 ± 1.09)	ln(39.25 ± 1.11)				
	CL/F (L/h)	1.05 ± 0.08	1.00 ±0.07	-5.31	1.05	(0.99, 1.11)	0.65
		ln(2.86 ± 1.08)	ln(2.72 ± 1.07)				
Dextromethorphan (CYP2D6)	AUC (h*µg/L)	3.36 ± 0.26	3.36 ± 0.25	0	1.00	(0.82, 1.22)	1.00
		ln(28.79 ± 1.30)	ln(28.79 ± 1.28)				
	T1/2 (h)	2.09 ± 0.11	1.97 ± 0.12	-5.74	0.88	(0.72, 1.09)	0.47
		ln(8.08 ± 1.12)	ln(7.17 ±1.13)				
	Tmax (h)	0.85 ± 0.07	0.65 ± 0.18	-23.53	0.82	(0.55, 1.22)	0.32
		ln(2.34 ± 1.07)	ln(1.92 ± 1.20)				
	Cmax (µg/L)	1.00 ± 0.24	1.16 ± 0.19	8.87	1.17	(0.89, 1.55)	0.61
		ln(2.71 ± 1.27)	ln(3.19 ± 1.21)				
	CL/F (L/h)	6.94 ± 0.26	6.95 ± 0.25	0.14	1.00	(0.82, 1.23)	0.98
		ln(1032.8 ±1.3)	ln(1043.2 ± 1.3)				
Caffeine	AUC (h*µg/L)	9.78 ± 0.08	9.81 ± 0.09	0.31	1.03	(0.92, 1.16)	0.81

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(CYP1A2)		ln(17676.6 ±1.1)	ln(18215.0 ±1.1)				
	T1/2 (h)	1.47 ± 0.07	1.55 ± 0.06	5.44	1.08	(0.98, 1.18)	0.40
		ln(4.35 ± 1.07)	ln(4.71 ± 1.06)				
	Tmax (h)	-0.46 ± 0.15	-0.83 ± 0.14	80.43	0.69	(0.48, 0.99)	0.10
		ln(0.63 ± 1.16)	ln(0.43 ± 1.15)				
	Cmax (μg/L)	7.90 ± 0.09	7.92 ± 0.08	0.25	1.02	(0.86, 1.21)	0.87
		ln(2697.3 ±2.69)	ln(2751.8 ± 1.08)				
	CL/F (L/h)	1.70 ± 0.09	1.73 ±0.08	1.76	0.97	(0.86, 1.09)	0.81
		ln(5.47 ± 1.09)	ln(5.64 ±1.08)				
Tolbutamide (CYP2C9)	AUC (h*μg/L)	12.67 ± 0.09	12.64 ±0.10	-0.24	0.97	(0.87, 1.08)	0.83
		ln(318061.5 ±1.1)	ln(308661.3 ±1.1)				
	T1/2 (h)	2.15 ± 0.06	2.08 ± 0.06	-3.26	0.93	(0.88, 0.99)	0.42
		ln(8.58 ± 1.06)	ln(8.00 ± 1.06)				
	Tmax (h)	1.32 ± 0.17	1.28 ± 0.14	-3.03	0.97	(0.60, 1.55)	0.86
		ln(3.74 ± 1.18)	ln(3.60 ± 1.15)				
	Cmax (μg/L)	10.04 ± 0.06	9.97 ± 0.07	-0.7	0.93	(0.86, 1.01)	0.46
		ln(22925.4 ± 1.1)	ln(21375.5 ± 1.1)				
	CL/F (L/h)	-0.24 ± 0.09	-0.21 ± 0.10	-12.5	1.03	(0.92, 1.15)	0.83
		ln(0.79 ±1.09)	ln(0.81 ±1.11)				

[a] Except as noted, data are expressed as [ln(mean) ± ln(standard error)]. GR (22:0) = geometric mean ratio of day 22–day 0; 90% CI = 90% confidence interval for the GR (22:0).

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Figure 1

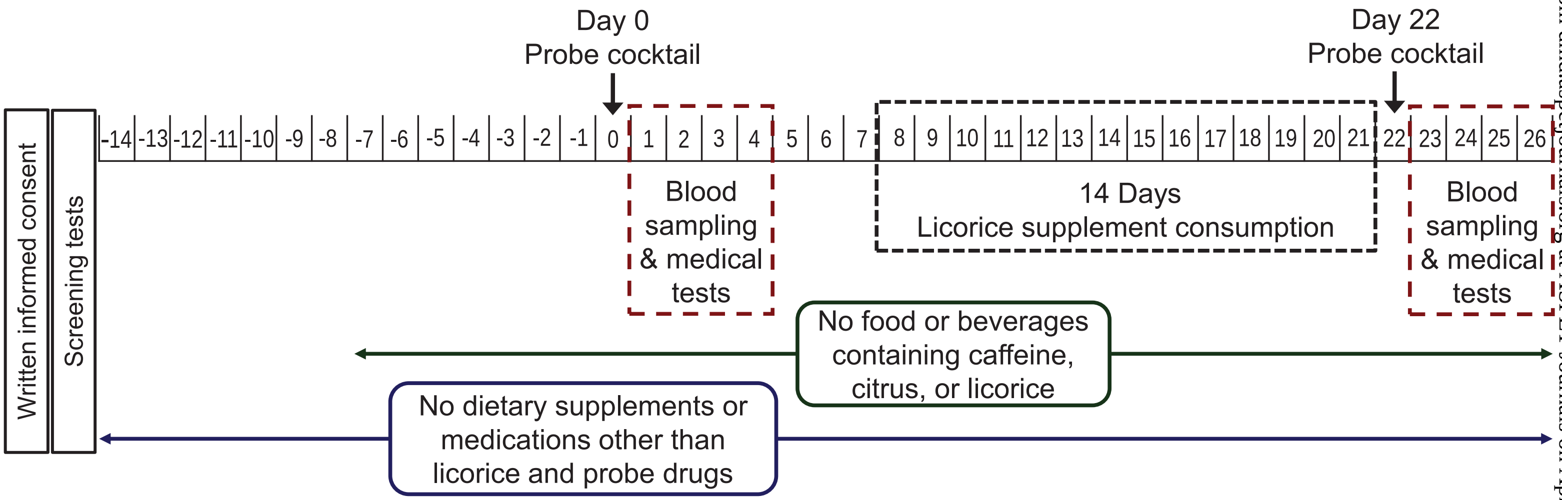

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Figure 2

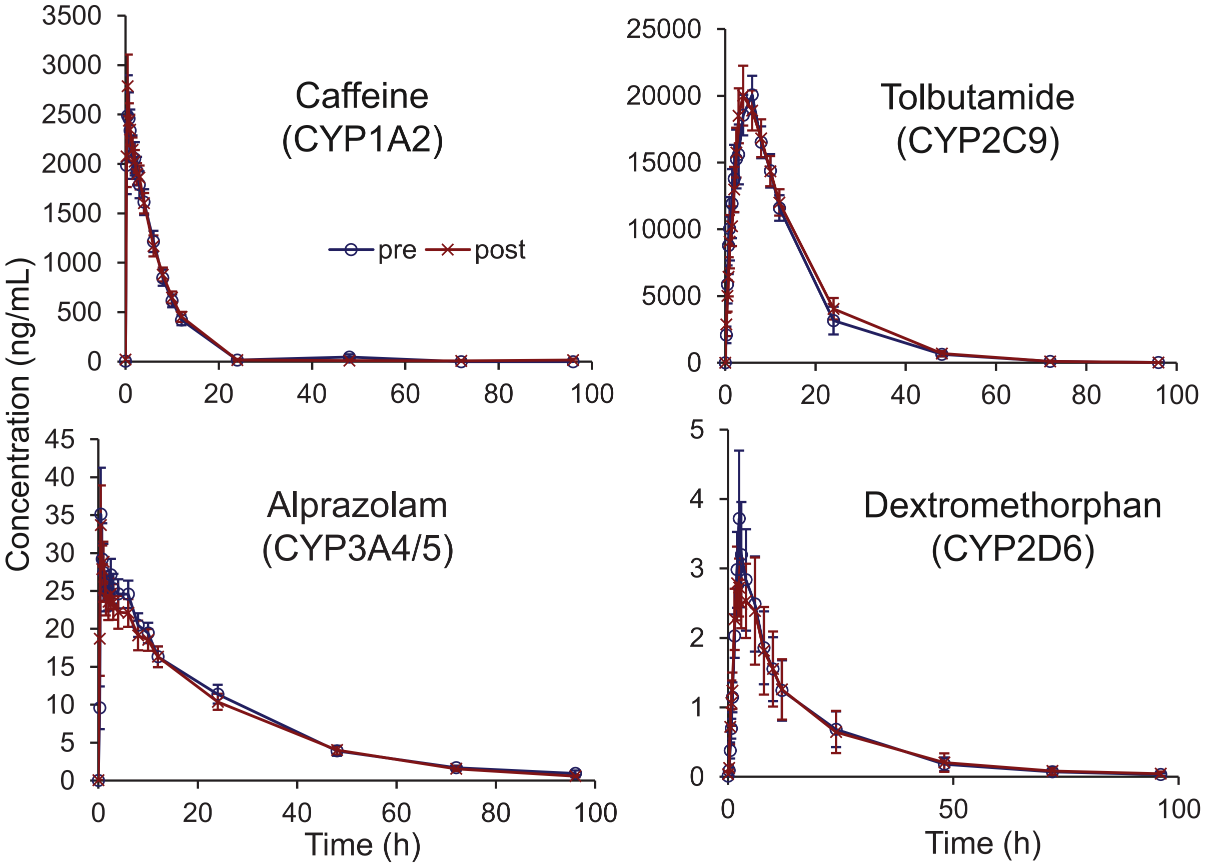

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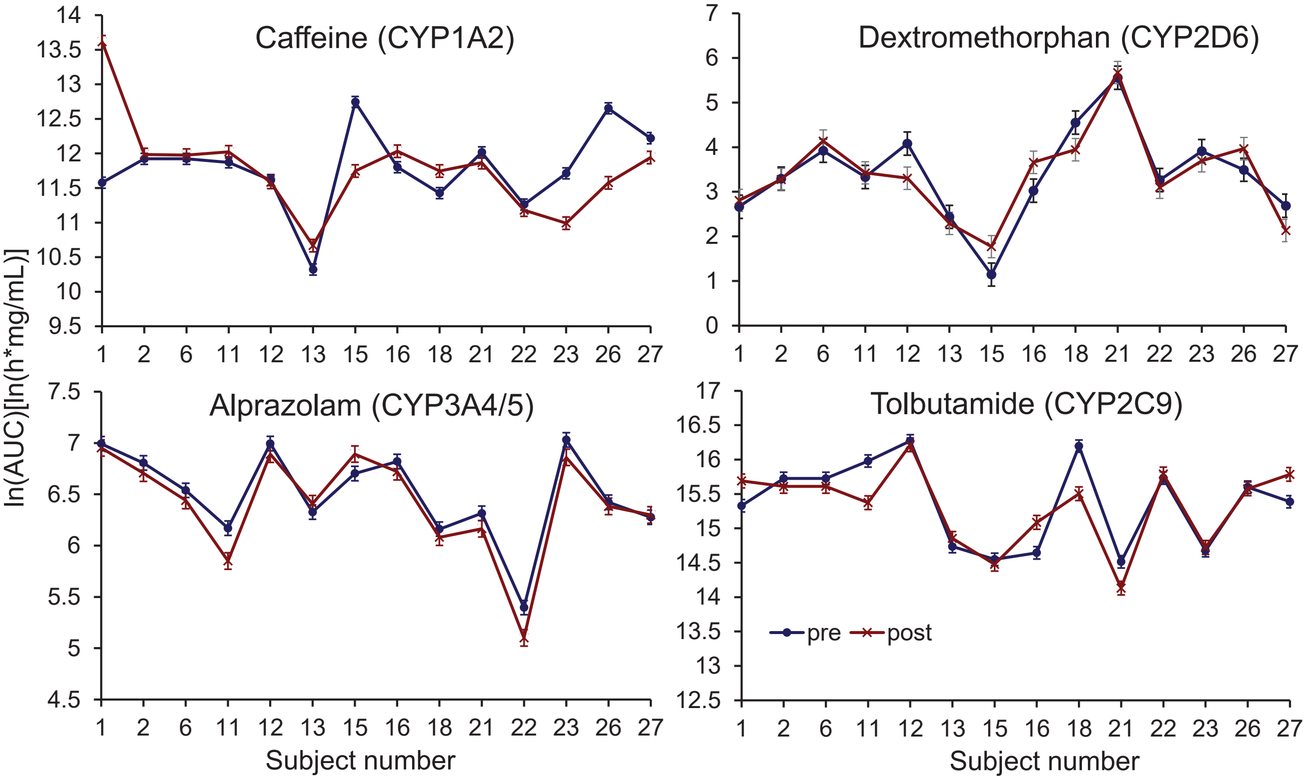
Figure 3



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