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DRUG METABOLISM & DISPOSITION

Special Section on Natural Products: Experimental Approaches to Elucidate Disposition Mechanisms and Predict Pharmacokinetic Drug Interactions

Application of Cryopreserved Human Intestinal Mucosa and Cryopreserved Human Enterocytes in the Evaluation of Herb-Drug Interactions: Evaluation of CYP3A Inhibitory Potential of Grapefruit Juice and Commercial Formulations of Twenty-Nine Herbal Supplements

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ABSTRACT

Commercial formulations of 29 commonly used herbal supplements (HSs) and grapefruit juice were evaluated for drug interaction potential via quantification of their CYP3A inhibitory potential in two in vitro experimental models of human small intestine, cryopreserved human intestinal mucosa (CHIM), and cryopreserved human enterocytes (CHEs). Two CYP3A substrates were used—in the studies with CHIM, CYP3A activity was quantified via liquid chromatography tandem mass spectrometry quantification of midazolam 1'-hydroxylation, whereas in CHE, luciferin-IPA metabolism to luciferin was quantified by luminescence. Upon treatment of CHIM with the estimated lumen concentration of the HS upon each oral administration (manufacturers' recommended dosage dissolved in 200 ml of culture medium), >80% CYP3A inhibition was observed for green tea extract, St. John's wort, valerian root, horehound, and grapefruit juice. Less than 50% inhibition was observed for fenugreek, aloe vera, guarana, soy isoflavone, maca, echinacea, spirulina, evening primrose, milk thistle, cranberry, red yeast rice, rhodiola, ginkgo biloba, turmeric, curcumin, white kidney bean, garlic, cinnamon, saw palmetto berries, panax ginseng, black elderberry, wheat grass juice, flaxseed oil, black cohosh, and ginger root. The results were confirmed in a a dose-response study with HSs obtained from three suppliers for the four inhibitory HSs (green tea extract, horehound, St. John's wort, valerian root) and three representative noninhibitory HSs (black cohosh, black elderberry, echinacea). Similar results were obtained with the inhibitory HSs in CHE. The results illustrate that CHIM and CHE represent physiologically relevant in vitro experimental models for the evaluation of drug interaction potential of herbal supplements. Based on the results, green tea extract, horehound, St. John's wort, and valerian root may cause drug interactions with orally administered drugs that are CYP3A substrates, as was observed for grapefruit juice.

SIGNIFICANCE STATEMENT

In vitro evaluation of 29 popular herbal supplements in cryopreserved human intestinal mucosa identified green tea extract, horehound, St. John's wort, and valerian root to have CYP3A inhibitory potential similar to that for grapefruit juice, suggesting their potential to have clinically significant pharmacokinetic interaction with orally administered drugs that are CYP3A substrates. The results suggest that cryopreserved human intestinal mucosa can be used for in vitro evaluation of drug interactions involving enteric drug metabolism.

Introduction

Herbal medicines and supplements have been used for their putative health benefits and in the treatment of illnesses throughout human history. For the past decade, the use of herbal supplements (HSs) has gained wide acceptance by the general population for the purpose of health enhancement. The retail sales of herbal supplements in the United States have been reported to have increased from $4.8 billion in 2008 to $8.8 billion in 2018 (http://cms.herbalgram.org/herbalgram/issue123/files/HG123-HMR.pdf), with approximately 18–20% of the population acknowledging using herbal products (Bent, 2008; Peregoy et al., 2014). Potential HS-drug interactions are therefore a legitimate concern (Archer et al., 2014; Levy et al., 2017; Ziemann et al., 2019), especially with orally administered drugs. Because oral drug bioavailability is determined by a combination of the fraction absorbed and the fraction escaping metabolism by the intestinal mucosal epithelium, coadministration of drugs with HSs that are inhibitors and inducers of enteric drug metabolism may lead to significant changes in the drug concentration in the systemic circulation.

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ABBREVIATIONS: AUC, area under the curve; CHE, cryopreserved human enterocyte; CHIM, cryopreserved human intestinal mucosa; GFJ, grapefruit juice; HQM, hepatocyte/enterocyte incubation medium; HS, herbal supplement; IVAL, In Vitro ADMET Laboratories.

Because liver is the major organ for drug metabolism, evaluation of drug-drug interactions is traditionally focused on hepatic events, including metabolism- and transporter-mediated drug uptake and efflux. However, there is evidence that enteric drug metabolism can play a significant role in drug interactions involving orally administered drugs. This is illustrated clearly with clinically observed cases of grapefruit juice (GFJ)-drug interactions. Coadministration with GFJ is known to increase the C_{max} and plasma AUC of drugs that are CYP3A substrates, including terfenadine, clopidogrel, saquinavir, cyclosporin, midazolam, nimodipine, triazolam, and verapamil (Schipperijn, 1997; van den Anker and de Wildt, 1997; Bailey et al., 1998b; Fuhr et al., 1998; Holmberg et al., 2014). In a clinical study, pretreatment of patients with GFJ was found to significantly increase the plasma C_{max} and AUC of orally administered but not intravenously administered midazolam, demonstrating that GFJ inhibited enteric but not hepatic CYP3A, therefore establishing that pharmacokinetic drug interactions can occur via the inhibition of enteric drug metabolism (Kupferschmidt et al., 1995). Both clinical and in vitro studies show that the mechanism of GFJ-drug interactions is a result of inhibition of CYP3A-mediated metabolism, with furanocoumarins as the major inhibitors (Bailey et al., 1998a,b, 2003; Paine et al., 2006).

The findings with GFJ illustrate that inhibition of enteric drug metabolism by a perpetrator may lead to clinically significant drug interactions. In our laboratory, we have embarked upon a research program to identify HSs with potent inhibitory potential for enteric drug-metabolizing enzymes. We report here the applications of two in vitro enteric experimental models developed in our laboratory, cryopreserved human intestinal mucosa (CHIM) (Li et al., 2018) and cryopreserved human enterocytes (CHEs) (Ho et al., 2017), in the evaluation of enteric drug interaction potential of commercial formulations of HSs toward CYP3A, one of the most important enteric drug-metabolizing enzymes. Twenty-nine HSs (Table 1) that have been reported to be among the top 40–selling HSs in the United States (Table 2) were evaluated for their inhibitory potential toward enteric CYP3A activity in CHIM and CHEs. GFJ was also evaluated to demonstrate the ability of our in vitro enteric models to identify a complex natural product with proven clinically significant CYP3A inhibitory properties.

Materials and Methods

HSs. The herbal supplements GFJ, orange juice, and cranberry juice used in the study were purchased commercially. The brands, commercial names, and active ingredients indicated in the product labels and the recommended dosages indicated in the product labels are shown in Table 1.

Isolation and Cryopreservation of CHEs and CHIM. CHEs and CHIM were isolated and cryopreserved from human small intestines obtained from the International Institute for the Advancement of Medicine (Exton, PA) and stored in liquid nitrogen as previous described (Ho et al., 2017; Li et al., 2018). CHEs pooled from 10 individual donors (PCHE3107) and CHIM pooled from from five individual donors (PCHIM6031) were used in this study. The demographics for the donors of the human small intestines are shown in Table 3 (CHIM) and Table 4 (CHEs).

Recovery from Cryopreservation. Vials of PCHE3107 and PCHIM6031 (In Vitro ADMET Laboratories, Columbia, MD) were retrieved from liquid nitrogen storage, thawed in a 37°C water bath, and recovered in cryopreserved enterocyte recovery medium [In Vitro ADMET Laboratories (IVAL)]. The recovered CHE and CHIM pellets *were* resuspended in a protein-free cell culture medium, hepatocyte/enterocyte incubation medium (HQM) (IVAL). The CHE cell suspension was evaluated for cell concentration and viability via trypan blue exclusion, and medium was added to adjust to a cell concentration of 2 million viable enterocytes per milliliter (2× the final cell concentration). The CHIM pellet was resuspended with medium to a protein concentration of 1 mg/ml (2× the final protein concentration). Protein concentration was previously determined in CHIM using the Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL).

Herbal Supplement–Drug Interaction Studies. The HSs used were commercially formulated capsules or extracts. The daily recommended oral dose according to the product label of each HS was dissolved in 50 ml

TABLE 1

The common names, scientific names, brand names, and amounts per serving of the herbal supplements evaluated in this study

Each supplement was procured commercially and used directly by dissolution in culture medium without further extraction. The amount per serving represents the weight of herb extract in each recommended administration unit (e.g., per capsule) as indicated on the product label.

Herbal Supplement	Scientific Name	Brand Name	Amount per Serving	Herbal Supplement	Scientific Name	Brand Name	Amount per Serving
Aloe vera	*Aloe vera*	Spring Valley	25 mg	Guarana	*Paullinia cupana*	GNC	250 mg
Black cohosh	*Cimicifuga racemosa*	GNC	40 mg	Horehound	*Marrubium vulgare*	Botanic Choice	250 mg
		Nature's Way	40 mg			Eleclectic Institute	250 mg
		Sundance	540 mg			Nature's Answer	2000 mg
Black elderberry	*Sambucus nigra*	Horbacch	2000 mg	Maca	*Lepidium meyenil*	Sanvall Enterprises	500 mg
		Nature's Way	575 mg	Milk thistle	*Silybum marianum*	GNC	200 mg
		NOW	500 mg	Oregano	*Origanum vulgare*	Nature's Answer	150 mg
Cinnamon	*Cinnamomum cassia*	Spring Valley	500 mg	Red yeast rice	*Monascus purpureus*	Spring Valley	600 mg
Cranberry	*Vaccinium macrocarpon*	James Lake Farm Inc.	500 mg	Rhodiola	*Rhodiola rosea*	Nature's Answer	100 mg
Echinacea	*Echinacea purpurea*	GNC	500 mg	Saw palmetto	*Serenoa repens*	GNC	540 mg
		Nature's Way	1200 mg	Soy isoflavone concentrate	*Glycine max*	GNC	50 mg
		Puritan's Pride	400 mg	Spirulina	*Anthrospira platensis*	Spring Valley	400 mg
Evening primrose oil	*Oenothera biennis*	Spring Valley	610 mg	St. John's wort	*Hypericum perforatum*	GNC	300 mg
Fenugreek	*Trigonella foenum-graecum*	Spring Valley	200 mg			Nature's Bounty	
Flaxseed oil	*Linum Usitatissimum*	Spring Valley	1000 mg			Sundown Naturals	
Garlic	*Allium Sativum*	GNC	500 mg	Turmeric curcumin	*Curcuma longa*	General wellness	500 mg
Ginger root	*Zingiber officinale*	Spring Valley	550 mg	Valerian Root	*Valeriana officinalis*	GNC	5000 mg
Ginkgo	*Ginkgo biloba*	GNC	60 mg			Nature's Bounty	450 mg
Ginseng	*Panex ginseng*	GNC	600 mg			Nature's Way	530 mg
Grapefruit juice	*Citrus paradisi*	Simple Orange Juice	50 ml	Wheat grass juice	*Triticum aestivum*	Sunny Green	100 mg
Green tea extract	*Camellia siensis*	Nature's Bounty	315 mg	White kidney bean	*Phaseolus vulgaris*	Nature's Way	1000 mg
		NOW	400 mg				
		Spring Valley	315 mg				

TABLE 2

Ranking of the herbal supplements evaluated in this study based on US sales in years 2016, 2017, and 2018 fo

Herbal Supplement	Sales Ranking		
	2016[a]	2017[b]	2018[c]
Aloe vera	14	12	16
Black cohosh	5	6	10
Black elderberry	19	15	4
Cinnamon	20	22	18
Cranberry	2	3	NA
Echinacea	3	2	2
Evening primrose oil	NA	40	35
Fenugreek	12	10	9
Flaxseed oil	7	11	12
Garlic	17	18	8
Ginger root	8	9	6
Ginkgo	23	21	22
Ginseng	31	25	23
Green tea extract	4	8	5
Guarana	27	29	29
Horehound	1	1	1
Maca	36	36	33
Milk thistle	16	17	20
Oregano	NA	NA	NA
Red yeast rice	30	27	NA
Rhodiola	28	23	27
Saw palmetto	15	14	11
Soy isoflavone concentrate	NA	NA	NA
Spirulina	NA	NA	NA
St. John's wort	37	37	36
Turmeric curcumin	10	5	3
Valerian root	11	16	19
Wheat grass juice	38	33	17
White kidney bean	NA	NA	NA

*, internet source of the information; NA, not applicable (not ranked in the reports).
[a]http://cms.herbalgram.org/herbalgram/issue115/images/HG15-Mktrpt.pdf.
[b]http://cms.herbalgram.org/herbalgram/issue123/files/HG123-HMR.pdf.
[c]http://cms.herbalgram.org/herbalgram/issue123/files/HG123-HMR.pdf.

(4× the expected intestinal concentration based on the generally accepted intestinal fluid volume of approximately 200 ml. We inadvertently used a 200-ml volume in lieu of the generally accepted volume of 250 ml) of a protein-free medium, HQM (IVAL). The HS solutions were filtered to remove debris with a Nalgene 50-ml 0.2-μM aPES membrane (Thermo Scientific), and pH was adjusted to 7.0–7.2. The resulting filtrates were further diluted by 1:2 serial dilutions with HQM six times to yield dosing solutions representing 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56% after a 1:4 dilution when added to the reaction mixture. GFJ (and other fruit juices evaluated with CHE) was diluted similarly, with the undiluted juice serving as the 4× concentration based on the consumption volume of 50 ml. For the drug interaction studies, 25 μl of the 4× herbal supplements were added in triplicate to 96-well plates, and this was followed by 50 μl of CHEs or CHIM. These were preincubated at 37°C for 15 minutes, and this was followed by the addition of 25 μl of midazolam (Sigma-Aldrich Inc., St. Louis, MI) at 40 μM (4× the final concentration of 10 μM) for studies with CHIM and 25 μl of luciferin-IPA (Promega Inc., Madison, WI) at 12 μM (4× the final concentration of 3 μM) for studies with CHE to each well. The final concentrations of midazolam and luciferin-IPA are within 2× published K_m values for CYP3A. The plates were then incubated at 37°C for 30 minutes. For studies with CHIM, at the end of the incubation period, 200 μl of ice-cold acetonitrile containing 250 nM of the internal standard, tolbutamide (Sigma-Aldrich), was added to each well followed by liquid chromatography tandem mass spectrometry quantification of 1′-hydroxy-midazolam as previously described (Li et al., 2018). For studies with CHE, 40 μl per well of the reaction mixture after incubation was transferred to two opaque white plates for the quantification of luciferin-IPA metabolism (plate 1) and cellular ATP contents (plate 2). Luciferin Detection Reagent (Promega) and ATP Detection Reagent (Perkin Elmer, Shelton, CT) at volumes of 40 μl per well were added to plate 1 and plate 2, respectively, for the quantification of luciferin and ATP, respectively. Luminescence was measured using a plate reader (PerkinElmer Victor3V Multichannel Plate Reader) as previously described (Li, 2009).

Data Analysis

Relative Activity. CYP3A activity is expressed as relative CYP3A activity using the following equations:

$$\textit{Relative CYP3A activity}\ (\%) = \textit{Activity}(\textit{treatment}) \times /\textit{Activity}(\textit{medium control})\ x\ 100,$$

in which CYP3A activity in CHIM was measured as picomoles per minute per milligram protein of 1′-OH midazolam formation from midazolam, and that in CHE was picomoles per minute per million cells of luciferin formation from luciferin-IPA.

TABLE 3

Demographics of the donors of the CHIM used in the preparation of the pooled donor CHIM (PCHIM6031) used in this study

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
Sex	Male	Male	Male	Male	Female
Age	58 y	20 y	20 y	59 y	36 y
Race	Hispanic	Caucasian	Caucasian	Caucasian	Asian
Cause of death	CVA, ICH	CVA, ICH	Suicide	Head trauma	CVA, ICH
BMI	30.27	30.62	26.33	37.88	35.00
Smoking	Yes	No	Yes	No	No
Alcohol	No	No	Yes	No	No
Substance abuse	No	Yes	No	No	No
Medical history	None reported	None reported	Allergies	HTN	HTN
Infectious diseases	HBV-, HCV-, HIV-, CMV+	HBV-, HCV-, HIV-, CMV-	HBV-, HCV-, HIV-, CMV-	HBV-, HCV-, HIV-, CMV+	HBV-, HCV-, HIV-, CMV+
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
Sex	Female	Female	Male	Male	Male
Age	16 y	17 y	34 y	55 y	32 y
Race	Caucasian	Caucasian	Caucasian	Caucasian	Hispanic
Cause of death	Anoxia	Anoxia	Anoxia	CVA, ICH	CVA, ICH
BMI	22.72	23.72	23.8	24.38	20.95
Smoking	No	No	No	No	Yes
Alcohol	No	No	Yes	No	Yes
Substance abuse	Yes	Yes	No	No	Yes
Medical history			Asthma	HTN	Diabetes, HTN
Infectious diseases	HBV-, HCV-, HIV-, CMV+	HBV-, HCV-, HIV-, CMV+	HBV-, HCV-, HIV-, CMV-	HBV-, HCV-, HIV-, CMV+	HBV-, HCV-, HIV-, CMV+

BMI, body mass index; CMV, cytomegalovirus; CVA, cardiovascular arrest; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTN, hypertension; ICH, intracranial hemorrhage.

TABLE 4

Demographics of the donors of the enterocytes used in the preparation of the pooled donor CHEs (PHE3107) used in this study

	Donor 6	Donor 7	Donor 8	Donor 9	Donor 10
Sex	Male	Male	Male	Male	Female
Age	27 y	43 y	41 y	38 y	59 y
Race	African American	Caucasian	Caucasian	African American	Caucasian
Cause of death	CVA 2nd to ICH	GSW/suicide	Anoxia	CVA, ICH	Anoxia
BMI	30.65	26.32	34.59	21.08	30.40
Smoking	Yes	No	Yes	Yes	Yes
Alcohol	Yes	No	Yes	No	No
Substance abuse	Yes	Yes	Yes	Yes	Yes
Medical history	Diabetes, HTN	HTN	GERD, viral meningitis	Asthma	
Infectious diseases	HBV-, HCV-, HIV-, CMV+	HBV-, HCV-, HIV-, CMV-	HBV-, HCV-, HIV-, CMV+	HBV-, HCV-, HIV-, CMV+	HBV-, HCV-, HIV-, CMV+

BMI, body mass index; CMV, cytomegalovirus; CVA, cardiovascular arrest; GERD, gastroesophageal reflux disease; GSW, gunshot wound; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTN, hypertension; ICH, intracranial hemorrhage.

Results with CHE were further normalized by the number of viable cells after HS incubation using the following equations:

$$\text{Relative viability (\%)} = \text{ATP luminenscence (treatment)}/\text{ATP luminescence (medium control)} \times 100$$

$$\text{Normalized CYP3A4 Activity} = \text{CYP3A Activity}/\text{Relative viability}$$

$$\text{Relative CYP3A activity} = \text{Normalized CYP3A activity (treatment)}/\text{Normalized CYP3A activity (medium control)}$$

IC_{50} Determination. IC_{50} for CYP3A inhibition was determined using the Graphpad Prism 8.0 software (www.graphpad.com) via linear regression (log inhibitor concentration vs. response, variable slope, four parameters).

Results

CYP3A Inhibition Studies in CHIM

GFJ Inhibition of Enteric CYP3A Activity. GFJ was used as a "positive control" in the investigation to evaluate whether its known CYP3A inhibitory effect could be observed in CHIM. GFJ at 100% oral concentration yielded a relative CYP3A activity of 4.69% (Fig. 1). To demonstrate dose-response relationship and to evaluate possible intra-experimental variation, GFJ was evaluated at concentrations of 1.56%, 3.13%, 6.25%, 12.5%, 25%, 50%, and 100% in four different plates in the same experiment. Dose-dependent inhibition of CYP3A activity was observed (Fig. 2) with IC_{50} values for GFJ in independent incubations ranging from 4.12% to 8.53% (Table 6).

CYP3A Inhibition by Commercial HS Formulations.

1. Treatment of CHIM with recommended oral dosages.

Relative CYP3A activity in CHIM treated with HSs at the recommended dosages are shown in Fig. 1. The HSs were classified based on their CYP3A inhibitory potential as follows:

a. Relative CYP3A activity <50% (>50% inhibition): green tea, St. John's wort, valerian root, and horehound.
b. Relative CYP3A activity >50% (<50% inhibition): fenugreek, aloe vera, guarana, soy isoflavone, maca, echinacea, spirulina, evening primrose, milk thistle, cranberry, red yeast rice, rhodiola, ginkgo biloba, turmeric curcumin, white kidney bean, garlic, cinnamon, saw palmetto berries, panax ginseng, black elderberry, wheat grass juice, flaxseed oil, black cohosh, and ginger root.

2. Dose-response studies with HSs from three different manufacturers.

To evaluate whether the inhibitory effects were associated with the designated herb for the supplements and to confirm the results with the single concentration study, a dose-response study was performed with HSs obtained from three different brands of the four inhibitory HSs (green tea extract, horehound, St. John's wort, valerian root) and three representative noninhibitory HSs (black cohosh, elderberry, echinacea). Dose-dependent inhibition was observed for the four inhibitory HSs (Fig. 3), with IC_{50} values for the HSs from the three suppliers expressed as percent of the daily recommended dosage, ranging from 27.5% to 49.5% for green tea, 16.1%–70.7% for horehound, 54.8%–86.3% for St. John's wort, and 4.8%–71.3% for valerian root. The three non-inhibitory HSs, except black cohosh from one of the brands, did not exhibit dose-dependent inhibition of CYP3A activity (Fig. 4; Table 5).

Evaluation of Inhibitory Potential of Selected HSs on CYP3A Activity in CHEs.

To confirm the findings in CHIM, selected HSs found in CHIM to be CYP3A inhibitors (horehound, St. John's wort, and valerian root),

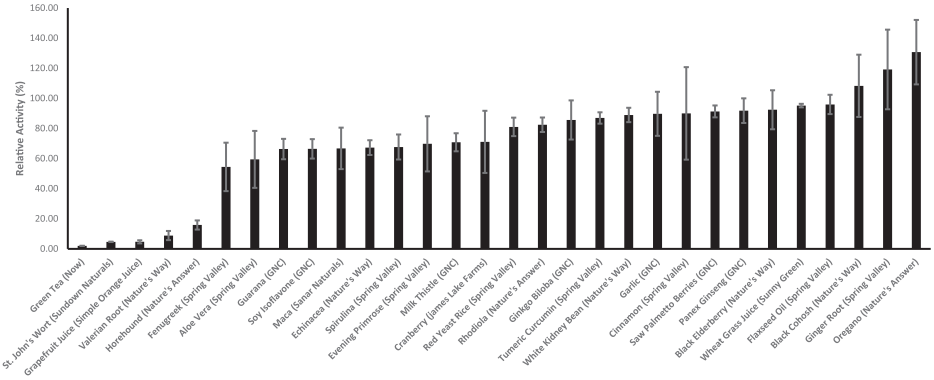

Fig. 1. Relative CYP3A4 activity (activity as percent of that for medium control) in CHIM in the presence of the recommended dosages of commercial formulations of 29 herbal supplements and grapefruit juice was plotted vs. log GFJ concentrations. The brands of the HSs are shown in brackets.

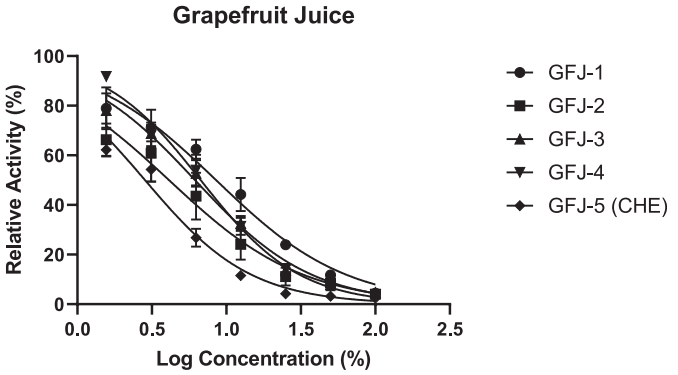

Fig. 2. Effects of GFJ on CYP3A activity (midazolam 1′-hydroxylation) in CHIM. Relative CYP3A4 activity (activity as percent of that for medium control) was plotted vs. log GFJ concentrations. Results of four replicate incubations were shown.

noninhibitors (echinacea, fenugreek, ginkgo biloba, guarana, red yeast rice), and three fruit juices (GFJ, cranberry juice, orange juice) were evaluated in CHE. Dose-dependent CYP3A inhibition was observed for horehound, St. John's wort, valerian root, and grapefruit juice but not for orange juice, echinacea, fenugreek, ginkgo biloba, guarana, and red yeast rice (Fig. 5). The IC_{50} values expressed as percent of daily recommended dosage are 3.97% (GFJ), 52.24% (cranberry juice), 45.08% (horehound), 38.67% (St. John's wort), and 14.26% (valerian root) (Table 6).

TABLE 5

IC_{50} and the 95% likelihood values for CYP3A inhibition in CHIM

Three different brands of noninhibitory (black cohosh, elderberry, echinacea) and inhibitory [green tea, horehound, St. John's wort (SJW), valerian root] HSs and GFJ were evaluated for their inhibitory potential for CYP3A activity in CHIM. The GFJ results were from four replicate plates. The results were derived from nonlinear regression analysis of plots of CYP3A activity (midazolam 1′-hydroxylation) vs. log conc. of each substance using the Graphpad Prism 8.0 software. The different brands are shown in brackets.

Herbal Supplement (Supplier)	IC_{50} (%)	IC_{50} (%) 95% Likelihood
Black cohosh (GNC)	>100	NA
Black cohosh (Sundance)	94.37	65.50–183.2
Black cohosh (Nature's Way)	>100	NA
Elderberry (Nature's Way)	>100	NA
Elderberry (Horbaach)	>100	NA
Elderberry (NOW)	>100	NA
Echinacea (GNC)	>100	NA
Echinacea (Puritan's Pride)	>100	NA
Echinacea (Nature's Bounty))	>100	NA
Green tea (Nature's Bounty)	32.8	27.53–39.45
Green tea (Now)	27.48	22.89–33.11
Green tea (Spring Valley)	49.47	39.42–64.47
Horehound (Nature's Answer)	16.14	13.30–19.59
Horehound (Botanic Choice)	70.65	50.76–114.6
Horehound (Eclectic Institute)	45.37	30.09–78.86
SJW (GNC)	66.39	48.27–104.5
SJW (Sundown Naturals)	54.79	38.56–85.23
SJW (Nature's Bounty)	86.34	69.40–118.7
Valerian (Nature's Way)	4.8	4.23–5.43
Valerian (Nature's Bounty)	71.28	57.71–93.63
Valerian (GNC)	36.96	30.30–46.13
GFJ-1 (plate 1)	8.53	7.32–9.92
GFJ-2 (plate 2)	4.12	3.36–4.96
GFJ-3 (plate 3)	6.16	5.56–6.82
GFJ-4 (plate 4)	6.72	6.14–7.36

NA, not applicable.

Discussion

Drug interaction potential of HSs needs to be accurately defined because of the high probability of their coadministration with prescribed or over-the-counter drugs. HS-drug interaction can occur as a result of the inhibitory and inducing activities for drug-metabolizing enzymes and transporters, as observed with small-molecule drugs, with these events occurring both in the small intestine and in the liver. For inhibitory drug interactions, in which the perpetrator inhibits the metabolic clearance of a victim drug, enteric events may be particularly important. A major reason is that the concentrations of orally administered drugs in the intestinal lumen can be substantially higher than those in the plasma. For instance, oral administration of 500 mg of a drug with a molecular weight of 500 grams per mole will have a concentration of 4.16 mM after dissolution in the intestinal fluid of 240 ml. Also, as described earlier, enteric, not hepatic, events are responsible for clinically significant drug interactions with GFJ.

We report here the CYP3A inhibitory potential of 29 commercial formulations of HSs and GFJ in two in vitro enteric systems, CHIM and CHE. GFJ yielded the expected potent and dose-dependent CYP3A inhibition in both CHIM and CHEs, thereby substantiating that they represent appropriate in vitro experimental models for the evaluation of enteric CYP3A inhibition. Our results led to the identification of green tea extract, St. John's wort, valerian root, and horehound as potent CYP3A inhibitors. It is notable that each HS was evaluated with the actual commercial product, thereby modeling a consumer's enteric exposure upon its ingestion. However, because only the soluble portion of each commercial formulation was evaluated, the effects of components that are soluble in vivo but not in vitro may not be reflected. Our intention was to evaluate whether the commercial products that are readily available in the marketplace would inhibit enteric drug metabolism via exposure of the enterocytes to all the soluble ingredients in the commercial formulations. The correlation of our results with those reported by others (see below) for the herbal supplements evaluated and the finding of similar properties for supplements of different brands provide support for the herbal identity of the supplements evaluated.

Our findings with green tea are consistent with those previously reported. Green tea extracts and commercial formulations have been shown to inhibit CYP3A activity of human liver and intestinal microsomes (Wanwimolruk et al., 2009; Misaka et al., 2013a; Satoh et al., 2016). Pharmacokinetic drug interactions related to CYP3A inhibitory effects of green tea extract include increases in plasma AUC and/or C_{max} of drugs that are CYP3A substrates, such as midazolam (Nishikawa et al., 2004; Misaka et al., 2013b), simvastatin (Misaka et al., 2013b), sildenafil (Werba, 2018), and tacrolimus (Vischini, 2011). Besides CYP3A inhibition, green tea extracts and the associated catechins have been found to be associated with inhibitory activities for UDP-glucuronosyltransferase (Mohamed and Frye, 2011; Tian et al., 2018) and efflux transporters (Zhou et al., 2004).

The pharmacokinetic drug interaction potential with St. John's wort is mainly attributed to the CYP3A induction activity upon prolonged usage, leading to reduced plasma concentration and thereby compromised efficacy of the coadministered CYP3A substrate drugs (Markowitz et al., 2003; Adiwidjaja et al., 2019). Our findings on CYP3A inhibition by St. John's wort are consistent with the reports by others that its major active constituent, hyperforin, has been reported to have both CYP3A-inducing (Hellum et al., 2007) and inhibitory (Obach, 2000; Komoroski et al., 2004) effects. Our results provide additional experimental evidence substantiating a previous report that St. John's wort may have short-term inhibitory and long-term inductive pharmacokinetic drug interactions (Xie and Kim, 2005).

TABLE 6

IC_{50} values for the CYP3A inhibitory potential of selected inhibitory herbal supplements [horehound, St. John's wort (SJW), and fruit juices], GFJ, cranberry juice (CBJ), and orange juice (OJ) in cryopreserved human enterocytes

The IC_{50} values are expressed as percent of the recommended oral dosage. The manufacturer of each of the herbal supplements is shown in parentheses. The results were derived from nonlinear regression analysis of plots of CYP3A activity (luciferin-IPA metabolism) vs. log conc. of each substance using the Graphpad Prism 8.0 software. The different brands are shown in parentheses.

CYP3A Inhibitory Potency	Fruit Juice			Herbal Supplement		
	GFJ	CBJ	OJ	Horehound (Nature's Answer)	SJW (GNC)	Valerian Root (Nature's Way)
IC_{50} (%)	3.97	52.24	>100	45.08	38.67	14.26
IC_{50} (%) (95% likelihood)	3.042–5.028	31.29–121.8	NA	29.53–73.90	23.25–78.70	12.49–16.29

NA, not applicable.

Our finding that valerian is a CYP3A inhibitor is consistent with a clinical study showing that the plasma C_{max} for orally administered alprazolam, a CYP3A substrate, was significantly increased in healthy volunteers taking 1000 mg/day of valerian extract for 14 days (Donovan et al., 2004). Valerian extracts have been found to inhibit CYP3A (Lefebvre et al., 2004), CYP2C8 (Albassam et al., 2015), and UDP-glucuronosyltransferase (Alkharfy and Frye, 2007; Mohamed et al., 2010) activities in human liver microsomes.

We are surprised that there are no previous reports of the CYP3A inhibitory potential of horehound, which caused dose-dependent CYP3A inhibition in both CHIM and CHEs. Most reported studies on herb-drug interactions, both in vitro and in vivo, have not included this herbal supplement, which is a widely used HS (Bent, 2008; Peregoy et al., 2014) that ranked first in total sales in 2016, 2017, and 2018 (Table 2). Our finding that horehound was an inhibitor of CYP3A may explain the finding that aqueous extract of horehound attenuates the hepatotoxicity of cyclophosphamide (Ettaya et al., 2016), a protoxicant that is activated by P450 isoforms, especially CYP2B6 and CYP3A4, to hepatotoxic metabolites (Chang et al., 1997). In our laboratory, we plan to further evaluate horehound as well as its bioactive compounds to elucidate the physiologic relevance of the observed CYP3A inhibitory effects.

The observed CYP3A inhibitory potential of GFJ, green tea, St. John's wort, and valerian root in CHIM and CHEs is therefore consistent with previous in vitro and in vivo findings, suggesting that the two intact cell models of the human small intestine represent physiologically relevant intact cell system for the identification of herbal supplements with drug interaction potential and for mechanistic evaluation of enteric herb-drug and drug-drug interactions. An accurate assessment of the drug interaction potential of herbal supplements requires multidisciplinary research including identification of the perpetrators of drug interactions, evaluation of their effects on enteric metabolism, determination of the amount of the perpetrators absorbed into the portal and systemic circulation, evaluation of interaction of the perpetrators in the plasma with

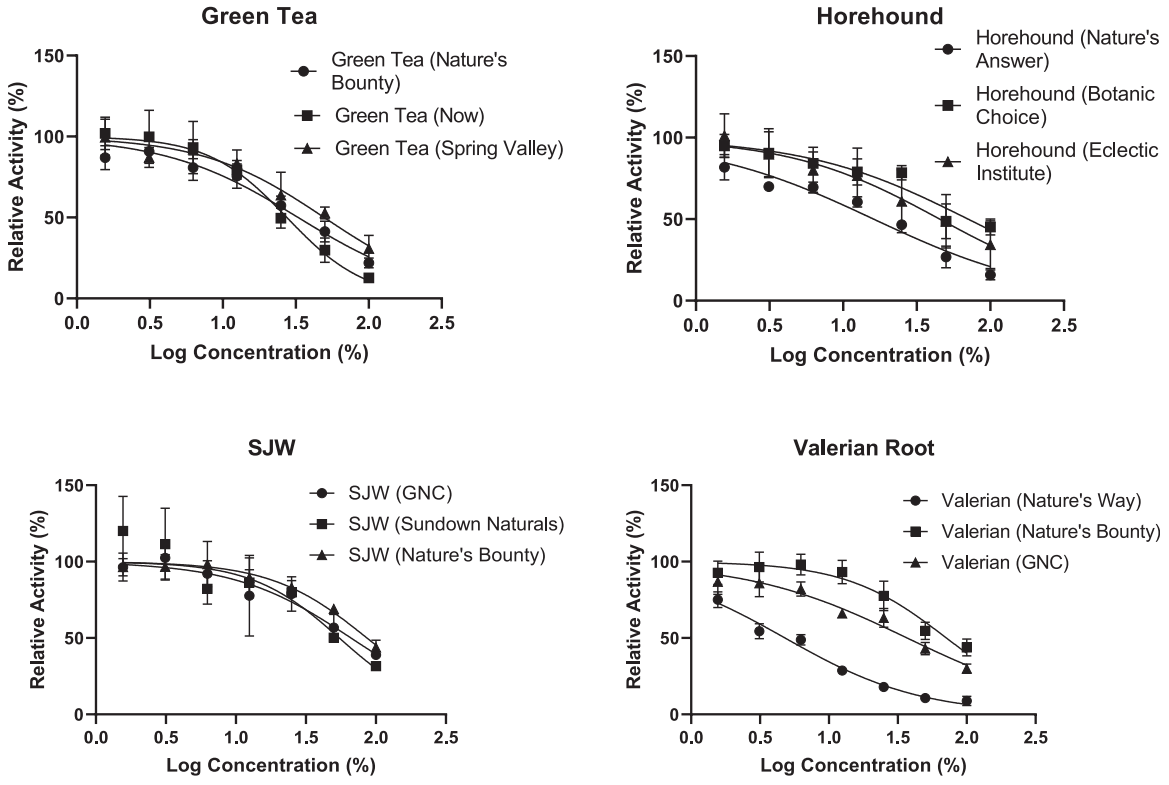

Fig. 3. Dose-dependent inhibition of CYP3A activity (midazolam 1′-hydroxylation) by three brands each of the inhibitory HSs [green tea, horehound, St. John's wort (SJW), and valerian root] in CHIM. Relative CYP3A4 activity (activity as percent of that for medium control) was plotted vs. log HS concentrations. The brands of the HSs are shown in brackets.

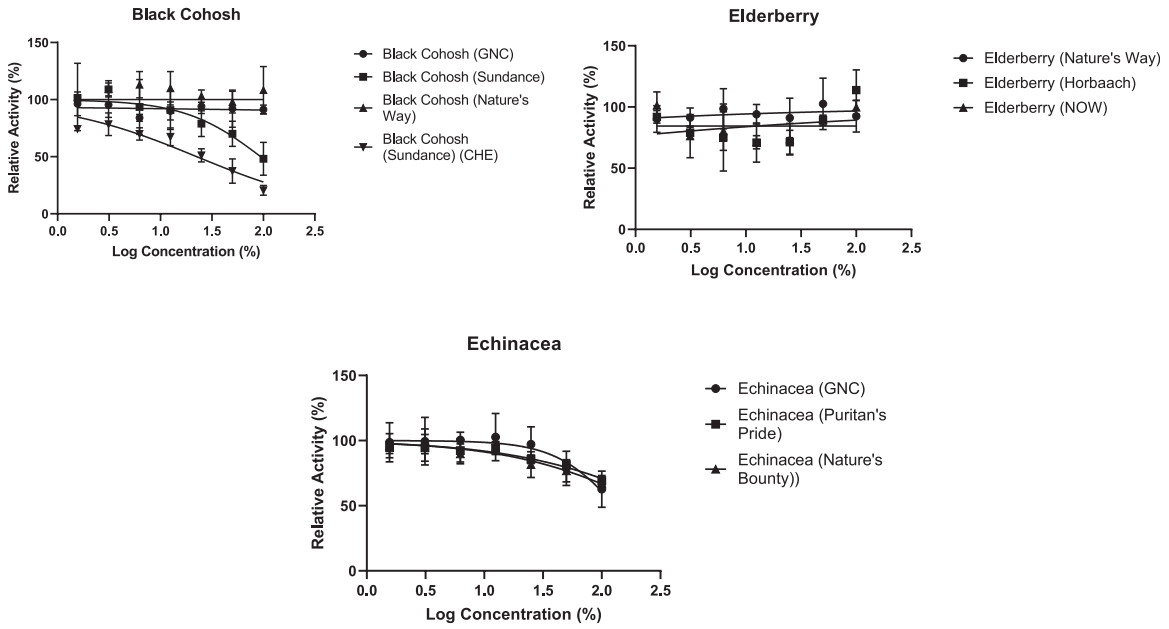

Fig. 4. Relative CYP3A activity (midazolam 1′-hydroxylation) in CHIM upon treatment with three brands each of the noninhibitory HSs (black cohosh, elderberry, and echinacea). Relative CYP3A4 activity (activity as percent of that for medium control) was plotted vs. log HS concentrations. The brands of the HSs are shown in brackets.

hepatic and extrahepatic metabolism, and determination of the effects on victim drugs based on their fractions metabolized (fm) for the affected enteric, hepatic, and extrahepatic pathways. CHIM and CHEs thereby represent in vitro tools to provide information on the enteric events contributing to the drug interaction potential of herbal supplements. Although this report focuses on CYP3A, which is one of the most important enteric drug-metabolizing enzymes, a comprehensive evaluation of enteric drug interaction potential of HSs needs to include all drug-metabolizing enzymes as well as uptake and efflux transporters that are active in the small intestine.

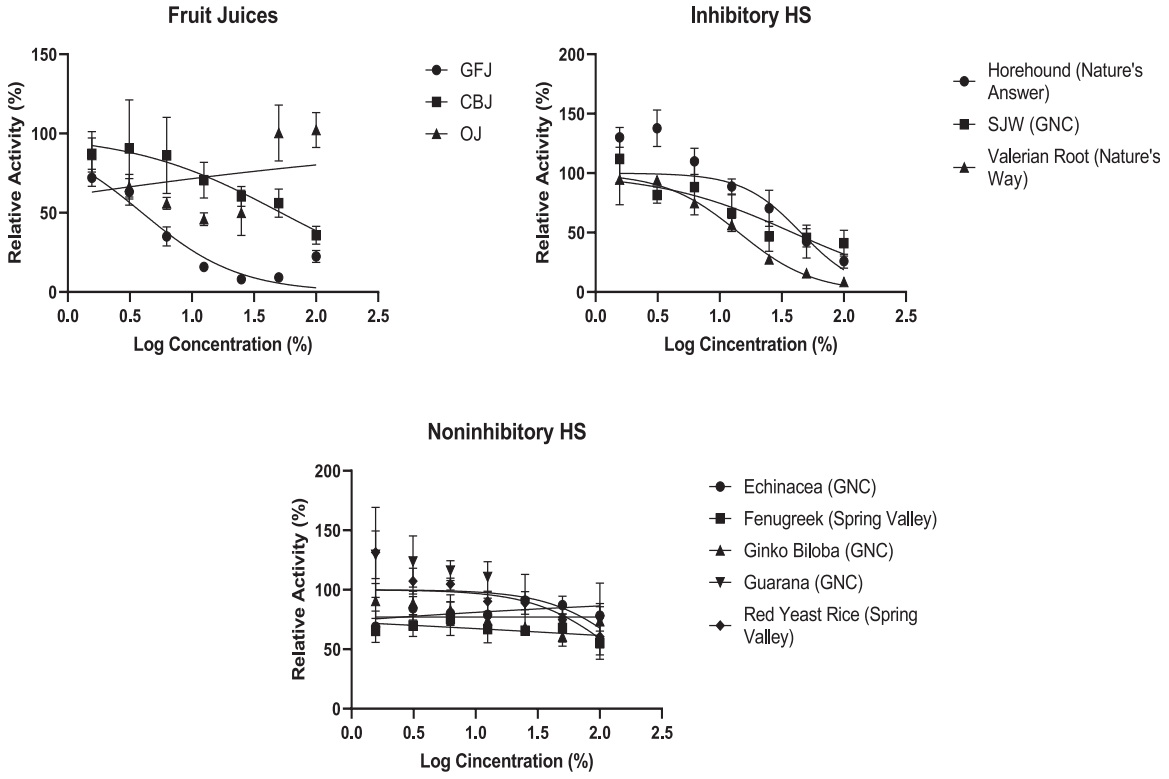

Fig. 5. Relative CYP3A activity (luciferin-IPA metabolism to luciferin) in CHEs upon treatment with GFJ, cranberry juice (CBJ), and orange juice (OJ) (top left); the inhibitory HSs horehound, St. John's wort (SJW), and valerian root (top right); and the noninhibitory HSs echinacea, fenugreek, ginko biloba, guarana, and red yeast rice (bottom). Relative CYP3A4 activity (activity as percent of that for medium control) was plotted vs. log HS concentrations. The brands of the HSs are shown in brackets.

Authorship Contributions

Participated in research design: Loretz, Li.

Conducted experiments: Loretz, Ho, Alam, Mitchell.

Performed data analysis: Loretz, Ho, Alam, Mitchell, Li.

Wrote or contributed to the writing of the manuscript: Loretz, Ho, Alam, Li.

References

Adiwidjaja J, Boddy AV, and McLachlan AJ (2019) Physiologically based pharmacokinetic modelling of hyperforin to predict drug interactions with St john's wort. *Clin Pharmacokinet* **58**: 911–926.

Albassam AA, Mohamed ME, and Frye RF (2015) Inhibitory effect of six herbal extracts on CYP2C8 enzyme activity in human liver microsomes. *Xenobiotica* **45**:406–412.

Alkharfy KM and Frye RF (2007) Effect of valerian, valerian/hops extracts, and valerenic acid on glucuronidation in vitro. *Xenobiotica* **37**:113–123.

Archer M, Proulx J, Shane-McWhorter L, Bray BE, and Zeng-Treitler Q (2014) Development of an alert system to detect drug interactions with herbal supplements using medical record data. *AMIA Annu Symp Proc* **2014**:249–255.

Bailey DG, Dresser GK, and Bend JR (2003) Bergamottin, lime juice, and red wine as inhibitors of cytochrome P450 3A4 activity: comparison with grapefruit juice. *Clin Pharmacol Ther* **73**: 529–537.

Bailey DG, Kreeft JH, Munoz C, Freeman DJ, and Bend JR (1998a) Grapefruit juice-felodipine interaction: effect of naringin and 6′,7′-dihydroxybergamottin in humans. *Clin Pharmacol Ther* **64**:248–256.

Bailey DG, Malcolm J, Arnold O, and Spence JD (1998b) Grapefruit juice-drug interactions. *Br J Clin Pharmacol* **46**:101–110.

Bent S (2008) Herbal medicine in the United States: review of efficacy, safety, and regulation: grand rounds at University of California, San Francisco Medical Center. *J Gen Intern Med* **23**: 854–859.

Chang TK, Yu L, Maurel P, and Waxman DJ (1997) Enhanced cyclophosphamide and ifosfamide activation in primary human hepatocyte cultures: response to cytochrome P-450 inducers and autoinduction by oxazaphosphorines. *Cancer Res* **57**:1946–1954.

Donovan JL, DeVane CL, Chavin KD, Wang JS, Gibson BB, Gefroh HA, and Markowitz JS (2004) Multiple night-time doses of valerian (Valeriana officinalis) had minimal effects on CYP3A4 activity and no effect on CYP2D6 activity in healthy volunteers. *Drug Metab Dispos* **32**:1333–1336.

Ettaya A, Dhibi S, Samout N, Elfeki A, and Hfaiedh N (2016) Hepatoprotective activity of white horehound (Marrubium vulgare) extract against cyclophosphamide toxicity in male rats. *Can J Physiol Pharmacol* **94**:441–447.

Fuhr U, Maier-Brüggemann A, Blume H, Mück W, Unger S, Kuhlmann J, Huschka C, Zaigler M, Rietbrock S, and Staib AH (1998) Grapefruit juice increases oral nimodipine bioavailability. *Int J Clin Pharmacol Ther* **36**:126–132.

Hellum BH, Hu Z, and Nilsen OG (2007) The induction of CYP1A2, CYP2D6 and CYP3A4 by six trade herbal products in cultured primary human hepatocytes. *Basic Clin Pharmacol Toxicol* **100**:23–30.

Ho MD, Ring N, Amaral K, Doshi U, and Li AP (2017) Human enterocytes as an in vitro model for the evaluation of intestinal drug metabolism: characterization of drug-metabolizing enzyme activities of cryopreserved human enterocytes from twenty-four donors. *Drug Metab Dispos* **45**: 686–691.

Holmberg MT, Tornio A, Neuvonen M, Neuvonen PJ, Backman JT, and Niemi M (2014) Grapefruit juice inhibits the metabolic activation of clopidogrel. *Clin Pharmacol Ther* **95**: 307–313.

Komoroski BJ, Zhang S, Cai H, Hutzler JM, Frye R, Tracy TS, Strom SC, Lehmann T, Ang CY, Cui YY, et al. (2004) Induction and inhibition of cytochromes P450 by the St. John's wort constituent hyperforin in human hepatocyte cultures. *Drug Metab Dispos* **32**:512–518.

Kupferschmidt HH, Ha HR, Ziegler WH, Meier PJ, and Krähenbühl S (1995) Interaction between grapefruit juice and midazolam in humans. *Clin Pharmacol Ther* **58**:20–28.

Lefebvre T, Foster BC, Drouin CE, Krantis A, Livesey JF, and Jordan SA (2004) In vitro activity of commercial valerian root extracts against human cytochrome P450 3A4. *J Pharm Pharm Sci* **7**: 265–273.

Levy I, Attias S, Ben-Arye E, Goldstein L, and Schiff E (2017) Potential drug interactions with dietary and herbal supplements during hospitalization. *Intern Emerg Med* **12**:301–310.

Li A. P. (2009) Evaluation of luciferin-isopropyl acetal as a CYP3A4 substrate for human hepatocytes: effects of organic solvents, cytochrome P450 (P450) inhibitors, and P450 inducers. *Drug Metab Dispos* **37:**1598-1603. *Drug Metab Dispos* **37**:1598–1603.

Li AP, Alam N, Amaral K, Ho MD, Loretz C, Mitchell W, and Yang Q (2018) Cryopreserved human intestinal mucosal epithelium: a novel in vitro experimental system for the evaluation of enteric drug metabolism, cytochrome P450 induction, and enterotoxicity. *Drug Metab Dispos* **46**:1562–1571.

Markowitz JS, Donovan JL, DeVane CL, Taylor RM, Ruan Y, Wang JS, and Chavin KD (2003) Effect of St John's wort on drug metabolism by induction of cytochrome P450 3A4 enzyme. *JAMA* **290**:1500–1504.

Misaka S, Kawabe K, Onoue S, Werba JP, Giroli M, Tamaki S, Kan T, Kimura J, Watanabe H, and Yamada S (2013a) Effects of green tea catechins on cytochrome P450 2B6, 2C8, 2C19, 2D6 and 3A activities in human liver and intestinal microsomes. *Drug Metab Pharmacokinet* **28**:244–249.

Misaka S, Kawabe K, Onoue S, Werba JP, Giroli M, Watanabe H, and Yamada S (2013b) Green tea extract affects the cytochrome P450 3A activity and pharmacokinetics of simvastatin in rats. *Drug Metab Pharmacokinet* **28**:514–518.

Mohamed ME and Frye RF (2011) Effects of herbal supplements on drug glucuronidation. Review of clinical, animal, and in vitro studies. *Planta Med* **77**:311–321.

Mohamed MF, Tseng T, and Frye RF (2010) Inhibitory effects of commonly used herbal extracts on UGT1A1 enzyme activity. *Xenobiotica* **40**:663–669.

Nishikawa M, Ariyoshi N, Kotani A, Ishii I, Nakamura H, Nakasa H, Ida M, Nakamura H, Kimura N, Kimura M, et al. (2004) Effects of continuous ingestion of green tea or grape seed extracts on the pharmacokinetics of midazolam. *Drug Metab Pharmacokinet* **19**:280–289.

Obach RS (2000) Inhibition of human cytochrome P450 enzymes by constituents of St. John's Wort, an herbal preparation used in the treatment of depression. *J Pharmacol Exp Ther* **294**: 88–95.

Paine MF, Widmer WW, Hart HL, Pusek SN, Beavers KL, Criss AB, Brown SS, Thomas BF, and Watkins PB (2006) A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice-felodipine interaction. *Am J Clin Nutr* **83**:1097–1105.

Peregoy JA, Clarke TC, Jones LI, Stussman BJ, and Nahin RL (2014) Regional variation in use of complementary health approaches by U.S. adults. *NCHS Data Brief* (146):1–8.

Satoh T, Fujisawa H, Nakamura A, Takahashi N, and Watanabe K (2016) Inhibitory effects of eight green tea catechins on cytochrome P450 1A2, 2C9, 2D6, and 3A4 activities. *J Pharm Pharm Sci* **19**:188–197.

Schipperijn AJ (1997) [Excessive terfenadine level caused by drinking grapefruit juice]. *Ned Tijdschr Geneeskd* **141**:2531–2532.

Tian DD, Kellogg JJ, Okut N, Oberlies NH, Cech NB, Shen DD, McCune JS, and Paine MF (2018) Identification of intestinal UDP-glucuronosyltransferase inhibitors in green tea (*Camellia sinensis)* using a biochemometric approach: application to raloxifene as a test drug via in vitro to in vivo extrapolation. *Drug Metab Dispos* **46**:552–560.

van den Anker JN and de Wildt SN (1997) [Excessive terfenadine level due to drinking grapefruit juice]. *Ned Tijdschr Geneeskd* **141**:1976–1978.

Vischini G (2011) Increased plasma levels of tacrolimus after ingestion of green tea. *Am J Kidney Dis* **58**:329.

Wanwimolruk S, Wong K, and Wanwimolruk P (2009) Variable inhibitory effect of different brands of commercial herbal supplements on human cytochrome P-450 CYP3A4. *Drug Metabol Drug Interact* **24**:17–35.

Werba JP (2018) Update of green tea interactions with cardiovascular drugs and putative mechanisms. *J Food Drug Anal* **26**:S72–S77.

Xie HG and Kim RB (2005) St John's wort-associated drug interactions: short-term inhibition and long-term induction? *Clin Pharmacol Ther* **78**:19–24.

Zhou S, Lim LY, and Chowbay B (2004) Herbal modulation of P-glycoprotein. *Drug Metab Rev* **36**:57–104.

Ziemann J, Lendeckel A, Müller S, Horneber M, and Ritter CA (2019) Herb-drug interactions: a novel algorithm-assisted information system for pharmacokinetic drug interactions with herbal supplements in cancer treatment. *Eur J Clin Pharmacol* **75**:1237–1248.

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