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ORIGINAL ARTICLE

Traditional Herbal Formulas to as Treatments for Musculoskeletal Disorders: Their Inhibitory Effects on the Activities of Human Microsomal Cytochrome P450s and UDP-glucuronosyltransferases

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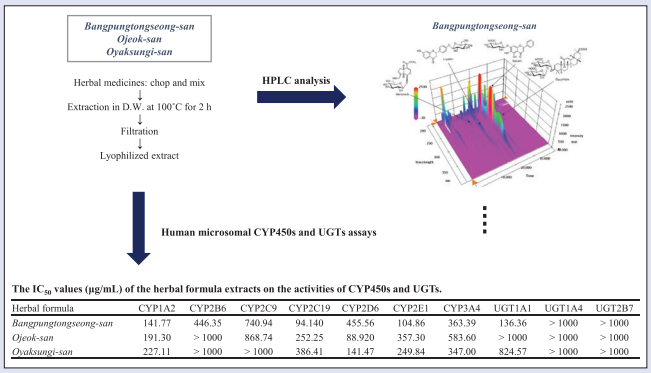
ABSTRACT

Objective: The aim of this study was to assess the influence of traditional herbal formulas, including *Bangpungtongseong-san* (BPTSS; *Fangfengtongsheng-san, Bofu-tsusho-san*), *Ojeok-san* (*OJS; Wuji-san, Goshaku-san*), *and Oyaksungi-san* (OYSGS; *Wuyaoshungi-san, Uyakujyunki-san*), on the activities of the human cytochrome P450s (CYP450s) and UDP-glucuronosyltransferases (UGTs), which are drug-metabolizing enzymes. **Materials and Methods:** The activities of the major human CYP450 isozymes (CYP1A2, CYP3A4, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1) and UGTs (UGT1A1, UGT1A4, and UGT2B7) were investigated using *in vitro* fluorescence-based and luminescence-based enzyme assays, respectively. The inhibitory effects of the herbal formulas were characterized, and their IC_{50} values were determined. **Results:** BPTSS inhibited the activities of CYP1A2, CYP2C19, CYP2E1, and UGT1A1 while it exerted relatively weak inhibition on CYP2B6, CYP2C9, CYP2D6, and CYP3A4. BPTSS also negligibly inhibited the activities of UGT1A4 and UGT2B7, with IC_{50} values in the excess of 1000 μg/mL. OJS and OYSGS inhibited the activity of CYP2D6, whereas they exhibited no inhibition of the UGT1A4 activity at doses <1000 μg/mL. In addition, OJS inhibited the CYP1A2 activity but exerted a relatively weak inhibition on the activities of CYP2C9, CYP2C19, CYP2E1, and CYP3A4. Conversely, OJS negligibly inhibited the activities of CYP2B6, UGT1A1, and UGT2B7 with IC_{50} values in excess of 1000 μg/mL. OYSGS weakly inhibited the activities of CYP1A2, CYP2C19, CYP2E1, CYP3A4, and UGT1A1, with a negligible inhibition on the activities of CYP2B6, CYP2C9, and UGT2B7, with IC_{50} values in excess of 1000 μg/mL. **Conclusions:** These results provide information regarding the safety and effectiveness of BPTSS, OJS, and OYSGS when combined with conventional drugs.

Key words: Cytochrome P450s, herb-drug interactions, traditional herbal formulas, UDP-glucuronosyltransferases

SUMMARY

- *Bangpungtongseong-san* inhibited the activities of human microsomal CYP1A2, CYP2C19, CYP2E1, and UGT1A1, with a negligibly inhibition on the activities of CYP2B6, CYP2C9, CYP2D6, CYP3A4, UGT1A4, and UGT2B7
- *Ojeok-san* (OJS) inhibited the CYP1A2 and CYP2D6 mediated metabolism while showing a comparatively weak inhibition against CYP2B6, CYP2C9, CYP2C19, CYP2E1, CYP3A4, and UGT1A1 in human microsomes
- *Oyaksungi-san* (OYSGS) inhibited the activities of human microsomal CYP2D6, with a relatively weak inhibition on the activities of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1, CYP3A4, UGT1A1, and UGT2B7
- OJS showed no inhibition on the activities of human microsomal UGT1A4 and UGT2B7, and OYSGS did not affect the human microsomal UGT1A4 activity.



The IC_{50} values (μg/mL) of the herbal formula extracts on the activities of CYP450s and UGTs.

Herbal formula	CYP1A2	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4	UGT1A1	UGT1A4	UGT2B7
Bangpungtongseong-san	141.77	446.35	740.94	94.140	455.56	104.86	363.39	136.36	> 1000	> 1000
Ojeok-san	191.30	> 1000	868.74	252.25	88.920	357.30	583.60	> 1000	> 1000	> 1000
Oyaksungi-san	227.11	> 1000	> 1000	386.41	141.47	249.84	347.00	824.57	> 1000	> 1000

Abbreviation used: BPTSS: *Bangpungtongseong-san*, OJS: *Ojeok-san*, OYSGS: *Oyaksungi-san*, CYP450s: cytochrome P450s, UGTs: UDP-glucuronosyltransferases, MSDs: Musculoskeletal disorders, NSAIDs: nonsteroidal anti-inflammatory drugs, EOMCC: 7-ethoxy-methyloxy-3-cyanocoumarin, DBOMF: di(benzyloxymethoxy)fluorescein, BOMCC: 7-benzyloxy-4-trifluoromethylcoumarin, HPLC: High-performance liquid chromatography, PDA: photo diode array, SEM: standard error of the mean, UDPGA: uridine 5′-diphosphoglucuronic acid.

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INTRODUCTION

Drug metabolism is responsible for the biotransformation of xenobiotics, including therapeutic drugs and endogenous/exogenous substances, yielding products that are more soluble in water than are their parent substances. Drug-metabolizing enzymes are classified into two groups, phase I and II enzymes, and cytochrome P450 (CYP450) and UDP-glucuronosyltransferase (UGT) are responsible for the phase I and phase II transformation reactions, respectively.[1-4]

CYP450s participate in the oxidative metabolism of a variety of xenobiotics. CYP450s consist of numerous families and subfamilies

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with similar amino acid sequences. Among them, CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 are considered to play an important role in drug metabolism.[5-8] According to the FDA, drug interactions with CYP2B6 are emerging as important.[9]

UGTs are located in the membrane of the endoplasmic reticulum and can conjugate various endogenous and exogenous compounds. UGTs consist of four superfamilies (UGT1, UGT2, UGT3, and UGT8) which share fifty conserved amino acids among UGT sequence.[10] Among them, UGT1As and UGT2Bs are abundantly expressed in the liver and significantly contribute to the biotransformation and metabolism of xenobiotics.[11] UGTs-catalyzed glucuronidation reactions have been considered to be one of the most important elimination pathways for drugs[4] and this pathway is responsible for more than 35% of phase II drug metabolism reactions.[12] Therefore, many severe herb-drug interactions can be explained by the inhibition of CYP450s and UGTs.

In East Asia, including Korea, China, and Japan, unique traditional medicines have been developed over several thousands of years to diagnose and treat diseases, independent of conventional medicine. Although Western medicine accounts for a major part of medical care in East Asia, the application of Eastern traditional medicines, including herbal medicines, to prevent and treat chronic and intractable diseases in Western countries and Asia has been increasing. Recently, the combination of these two types of medicine, which is termed combined medicine, has been provided to patients and consumers. However, the risks of herb-drug interactions to humans remain unclear.

Musculoskeletal disorders (MSDs), such as arthritis, neuralgia, muscle cramps, and myalgias, are defined injuries and disorders of the muscles, nerves, tendons, ligaments, joints, cartilages, and spinal discs. Medications, including nonsteroidal anti-inflammatory drugs, acetaminophen, opioids and injections of anesthetic, or anti-inflammatory medications in or around the painful sites, are used to treat inflammation or pain in patients with MSDs.[13,14] Among the traditional herbal formulas, *Bangpungtongseong-san* (BPTSS; *Fangfengtongsheng-san, Bofu-tsusho-san*), *Ojeok-san* (OJS; *Wuji-san, Goshaku-san*), and *Oyaksungi-san* (OYSGS; *Wuyaoshungi-san, Uyakujyunki-san*) are commonly used to treat MSDs in Korea. These herbal formulas contain different crude herbs [Table 1] and commonly exhibit anti-inflammatory and anti-analgesic effects.[15-17] In addition, BPTSS has been widely used for the prevention and treatment of obesity, stroke, and hyperlipidemia.[18-21] Furthermore, OJS has been used for the treatment of hyperlipidemia, and OYSGS has been reported to have antiobesity and neuroprotective effects.[22-24] Despite the benefits of these herbal formulas, they can serve as substrates, inhibitors or inducers of CYP450s, or UGTs. Hence, the coadministration of these herbal formulas and conventional drugs, such as aceclofenac, naproxen, aspirin or acetaminophen, for the treatment of MSDs may lead to herb-drug interactions. Therefore, it is necessary to evaluate the potential influence of herbal formulas that contain various herbs and components on drug metabolism in humans.

In this study, the effects of the traditional herbal formulas BPTSS, OJS, and OYSGS on the activities of the major human CYP450s (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4), and UGTs (UGT1A1, UGT1A4, and UGT2B7) were investigated by *in vitro* CYP450 isozyme and UGT isozyme assays.

Table 1: The compositions of the three herbal formulas

Crude drug	Scientific name	Amount (g)		
		Bangpungtongseong-san	***Ojeok-san***	***Oyaksungi-san***
Talcum	*Talcum*	6.38		
Glycyrrhizae Radix et Rhizome	*Glycyrrhiza uralensis*	4.50	2.20	1.13
Gypsum Fibrosum	*Gypsum*	2.63		
Scutellariae Radix	*Scutellaria baicalensis*	2.63		
Platycodonis Radix	*Platycodon grandiflorum*	2.63	3.00	3.75
Saposhnikoviae Radix	*Ledebouriella seseloides*	1.69		
Paeoniae Radix	*Paeonia lactiflora*	1.69	3.00	
Cnidii Rhizoma	*Cnidium officinale*	1.69	2.60	3.75
Angelicae Gigantis Radix	*Angelica gigas*	1.69	3.00	
Rhei Radix et Rhizoma	*Rheum undulatum*	1.69		
Ephedrae Herba	*Ephedra sinica*	1.69	3.70	5.63
Menthae Herba	*Mentha pulegium*	1.69		
Forsythiae Fructus	*Forsythia koreana*	1.69		
Natrii Sulfas	*Natrii sulfas*	1.69		
Schizonepetae Spica	*Schizonepeta tenuifolia*	1.31		
Atractylodis Rhizoma Alba	*Atractylodes japonica*	1.31		
Gardeniae Fructus	*Gardenia jasminoides*	1.31		
Zingiberis Rhizoma Crudus	*Zingiber officinale*	6.25	3.70	3.75
Atractylodis Rhizoma	*Atractylodes lancea*		7.50	
Citri Unshius Pericarpium	*Citrus unshiu*		3.70	5.63
Magnoliae Cortex	*Magnolia officinalis*		3.00	
Angelicae Dahuricae Radix	*Angelica dahurica*		2.60	3.75
Aurantii Fructus Immaturus	*Citrus unshiu*		3.00	3.75
Zingiberis Rhizoma	*Zingiber officinale*		3.00	1.88
Hoelen	*Poria cocos*		3.00	
Pinelliae Tuber	*Pinellia ternata*		2.60	
Cinnamomi Bark	*Cinnamomum cassia*		2.60	
Allii Radix	*Allium fistulosum*		3.70	
Linderae Radix	*Lindera strychnifolia*			5.63
Bombycis Corpus	*Bombyx mori*			3.75
Zizyphi Fructus	*Zizyphus jujuba*			3.75
Total amount (g)		44.16	55.90	46.15
Yield (%)		17.70	21.00	24.40

MATERIALS AND METHODS

Chemicals and materials

Reference standards, albiflorin, paeoniflorin, geniposide, liquiritin, baicalin, glycyrrhizin, ferulic acid, cinnamaldehyde, naringin, and 6-gingerol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Hesperidin and neohesperidin were purchased from Biopurify Phytochemicals (Chengdu, China). Nodakenin was purchased from NPC BioTechnology, Inc., (Daejeon, Korea). The purity of all reference standards was ≥ 98.0%. High-performance liquid chromatography (HPLC)-grade methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ, USA). Glacial acetic acid of analytical reagent grade was purchased from Junsei (Tokyo, Japan).

Vivid® CYP450 Screening Kits (Vivid® CYP1A2 Blue, Vivid® CYP2B6 Blue, Vivid® CYP2C9 Blue, Vivid® CYP2C19 Blue, Vivid® CYP2D6 Blue, Vivid® CYP2E1 Blue, and Vivid® CYP3A4 Green) were purchased from Invitrogen Co., (Camarillo, CA, USA). These kits use 7-ethoxy-methyloxy-3-cyanocoumarin as a substrate for CYP1A2, CYP2D6, CYP2C19, and CYP2E1. In addition, di (benzyloxymethoxy) fluorescein was used as a substrate for CYP3A4, and 7-benzyloxy-4-trifluoromethylcoumarin was used as a substrate for CYP2B6 and CYP2C9. UGT-Glo™ UGT1A1 and UGT2B7 Screening Systems were purchased from Promega (Madison, WI, USA). The recombinant human UGT1A4 enzyme was purchased from Corning Inc. Life Science (Tewksbury, MA, USA). α-Naphthoflavone, ketoconazole, miconazole, sulfaphenazole, quinidine, sodium diethyldithiocarbamate trihydrate, diclofenac, and lopinavir were obtained from Sigma Chemical Co., (St. Louis, MO, USA). All other chemicals were of analytical grade.

Preparation of herbal formula extracts

The crude herbs forming the herbal formulations of BPTSS, OJS, and OYSGS were purchased from a traditional herb market, Omniherb (Yeongcheon, Korea) and HMAX (Jecheon, Korea). All herbs were taxonomically confirmed by Professor Je-Hyeun Lee, Dongguk University, Korea. To obtain the water decoction of the three herbal formulas, each herbal medicine was chopped and mixed as shown in Table 1. The extraction of each herbal formula was performed in distilled water at 100°C for 120 min using an electric extractor (COSMOS-660; Kyungseo Machine Co., Incheon, Korea). The solution was filtered using a standard sieve (No. 270, 53 μm; Chung Gye Sang Gong Sa, Seoul, Korea), evaporated to dryness at 40°C under vacuum (Eyela N-11, Tokyo, Japan), and freeze-dried (PVTFD10RS, IlShinBioBase, Yangju, Korea). The powdered extracts were stored at 4°C, and the yields of the three herbal formula extracts are shown in Table 1. Voucher specimens (2008-KE04, 2008-KE23, and 2008-KE27) have been deposited at the K-herb Research Center, Korea Institute of Oriental Medicine.

High-performance liquid chromatography analysis

For quality assessment of the three formulas of BPTSS, OJS, and OYSGS, a chromatographic analysis was performed using a Shimadzu Prominence LC-20A series (Kyoto, Japan), equipped with a solvent delivery unit, an on-line degasser, a column oven, an autosampler, and a photo diode array (PDA) detector. The data were acquired and processed using LCsolution software (Version 1.24, Shimadzu Co., Kyoto, Japan). The constituents in each formula were separated on a Phenomenex Gemini C18 column (250 mm × 4.6 mm, 5 μm, Torrance, CA, USA) for OJS and OYSGS and a Phenomenex Luna C18 column (250 mm × 4.6 mm, 5 μm, Torrance, CA, USA) for BPTSS, with the column temperature set to 40°C. The mobile phases consisted of 0.1% (v/v) acetic acid in distilled water (A) and 0.1% (v/v) acetic acid in acetonitrile (B). The gradient elutions of the mobile phases are shown in Table 2. The flow-rate and injection volume were 1.0 mL/min and 10 μL, respectively. For HPLC analysis of each formula, 200, 200 and 400 mg of lyophilized BPTSS, OJS, and OYSGS extract were dissolved in 20 mL of distilled water, respectively, and then, the solution was filtered through a SmartPor GHP 0.2 μm syringe filter (PALL Life Sciences, Ann Arbor, MI, USA) before HPLC analysis.

Cytochrome P450 isozyme assay

The assays were performed using the Vivid® CYP450 Screening Kits according to the protocol provided by the manufacturer. The Vivid® CYP450 Screening Kits are designed to assess the metabolic activity of the predominant human CYP450 isozymes involved in hepatic drug metabolism: CYP1A2, CYP3A4, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1. The Vivid® Substrate and Fluorescent Standards were reconstituted, and a standard curve was prepared. A test sample of 40 μL diluted in solvent, a positive inhibition control (a compound that inhibits the respective CYP450 enzyme), or a solvent control was added to each well. The solutions were mixed after adding 50 μL of the Master Pre-Mix containing P450 BACULOSOMES® in the Vivid® CYP450 Reaction Buffer and Regeneration System (consisting of glucose-6-phosphate and glucose-6-phosphate dehydrogenase), and the plate was incubated for 20 min to allow the samples to interact with the CYP enzymes. After preincubation, the reaction was started by adding 10 μL of the Vivid® Substrate and $NADP^+$. The regeneration system converts $NADP^+$ into NADPH, which is required to start the CYP450 reaction. The enzymatic reaction is initiated by the addition of a mixture of $NADP^+$ and the appropriate Vivid Substrate. The fluorescence intensity was measured using an EnVision2103 Multilabel Reader (PerkinElmer Inc., MA, USA) for 15 min at the excitation and emission wavelengths of 485 and 535 nm, respectively, for CYP3A4. For CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1, the fluorescence intensity was measured for 60 min at the excitation and emission wavelengths of 415 and 460 nm, respectively, using a SpectraMax® i3 (Molecular Devices Co., Sunnyvale, CA, USA).

The inhibition percentage (%) was obtained via the following equation: $\%\ \text{Inhibition} = (1 - [S_1 - S_0]/[C_1 - C_0]) \times 100$, where C_1 is the fluorescence of the control after incubation, C_0 is the initial fluorescence of the control, S_1 is the fluorescence of the test sample after incubation, and S_0 is the initial fluorescence of the test sample in the linear section.

Table 2: Solvent gradient for analysis of high-performance liquid chromatography-photo diode array

Time (min)	*Bangpungtongseong-san*		Time (min)	*Ojeok-san*		Time (min)	*Oyaksungi-san*	
	A (%)[a]	B (%)[b]		A (%)	B (%)		A (%)	B (%)
0	95	5	0	85	15	0	85	15
40	25	70	20	75	25	40	35	65
45	0	100	40	45	55	45	0	100
50	0	100	45	0	100	50	0	100
55	95	5	50	0	100	55	85	15
70	95	5	55	85	15	70	85	15
			70	85	15			

[a]1.0% (v/v) acetic acid in water; [b]1.0% (v/v) acetic acid in acetonitrile

The background fluorescence of the herbal formulas was corrected by subtracting the values obtained from the incubation without substrates. The CYP450 inhibition of each sample was expressed regarding IC_{50} as calculated from the log-dose inhibition curve (SigmaPlot, version 12.5, Systat Software, Inc., CA, USA). The data were expressed as the mean ± standard error of the mean (SEM) (n = 3). α-Naphthoflavone, ketoconazole, sulfaphenazole, quinidine, and sodium diethyldithiocarbamate trihydrate were used as positive controls for CYP1A2, CYP3A4, CYP2C9, CYP2D6, and CYP2E1, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19.

UDP-glucuronosyltransferase isozyme assay

The assays were performed using the UGT-Glo™ Screening Systems according to the manufacturer's protocol. The assay systems provide a luminescent method for measuring the activity of UGTs. Two glucuronidation reactions were set up in parallel to measure UGT activity. Both reactions contained a source of UGT (UGT1A1, UGT1A4, or UGT2B7) and the proluciferin substrates (UGT multienzyme substrate or UGT1A4 substrate), but only one of them contained the uridine 5'-diphosphoglucuronic acid (UDPGA) cofactor. Ten microliters of 4× concentrated test sample; diclofenac, which is a known inhibitor of the UGT1A1 and UGT2B7 isozymes; lopinavir, as a UGT1A4 inhibitor; or vehicle was added each well (white opaque 96 well-plate, Corning Inc., NY, USA). Then, 10 µL of UDPGA (plus-UDPGA reaction set) or distilled water (minus-UDPGA reaction set) was added to the relevant wells. Twenty microliters of the prepared 2× control reaction mixture (minus-UGT enzyme) and 2× UGT reaction mixture (UGT1A1, UGT1A4, or UGT2B7) were added to the appropriate wells. The reaction solution was mixed and incubated at 37°C for 90, 180, or 60 min, respectively, for UGT1A1, UGT1A4, and UGT2B7. The final contents of the reactant were 0.1 mg/mL UGT enzyme and 20 µM enzyme substrate in the presence or absence of 4 mm UDPGA. After incubation, 40 µL of the reconstituted luciferin detection reagent plus D-cysteine was added to all wells. After 20 min of incubation at room temperature, the luminescence signal was detected using a SpectraMax® i3.

The detected data were converted to the calculated the difference using the following percent of substrate consumed (%SC) equation: % Substrate consumed = (background corrected difference)/(average minus-UDPGA values) × 100. The inhibition percentage (%) was obtained via the following equation: % Inhibition = (1– [S/C_{AVR}]) × 100, where S is the %SC of each sample or the control wells, and C_{AVR} is the average %SC of the control wells. The UGT inhibition of each sample was expressed regarding IC_{50} as calculated using computer software (SigmaPlot) capable of generating a four parameter logistic curve fit. The data were expressed as the mean ± SEM (n = 2).

RESULTS

High-performance liquid chromatography analysis of herbal formulas

The developed HPLC-PDA method was subsequently applied for the quality control of the three formulas of BPTSS, OJS, and OYSGS. Consequently, the marker compounds in BPTSS, OJS, and OYSGS eluted within 40, 45, and 35 min, respectively, and the typical three-dimensional chromatograms are shown in Figure 1. The correlation coefficient (r^2) of all analytes showed good linearity (≥0.9997). Using the optimized chromatography conditions, the amounts of the various marker compounds in BPTSS, OJS, and OYSGS are summarized in Table 3.

Table 3: The contents of marker compounds in herbal formula extracts

Compound	Contents in extract (mg/g)		
	Bangpungtongseong-san	*Ojeok-san*	*Oyaksungi-san*
Geniposide	5.59±0.012		
Baicalin	13.53±0.120		
Liquiritin	6.06±0.010	1.53±0.040	0.86±0.010
Glycyrrhizin	6.92±0.070	1.85±0.000	0.92±0.012
Naringin		6.29±0.050	9.25±0.056
Hesperidin		4.52±0.100	5.98±0.020
Neohesperidin		4.10±0.060	5.68±0.025
Ferulic acid		0.40±0.000	0.33±0.000
Albiflorin		0.30±0.000	
Paeoniflorin		2.14±0.010	
Nodakenin		1.00±0.000	
Cinnamaldehyde		0.25±0.000	
6-Gingerol		0.15±0.000	

The data are presented as the mean±SD from three independent experiments in triplicate

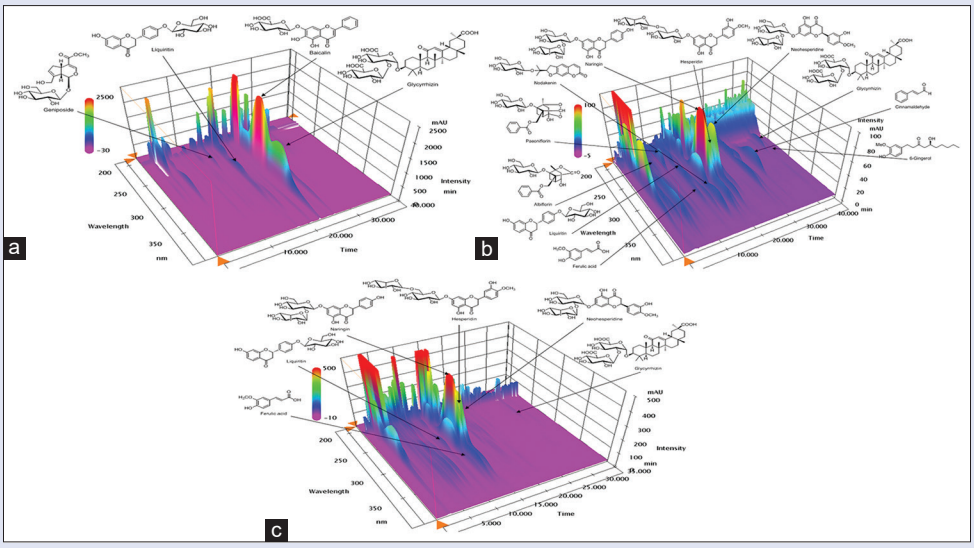

Figure 1: The three-dimensional chromatograms of *Bangpungtongseong-san* (a), *Ojeok-san* (b) and oyaksungi-san (c) from high-performance liquid chromatography-photo diode array

Effects of herbal formulas on the activities of cytochrome P450s

In vitro CYP450 isozyme assays were performed to evaluate whether the three traditional herbal formulas influence the activities of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. As shown in Figures 2-4 and Table 4, α-naphthoflavone, sulfaphenazole, quinidine, diethyldithiocarbamate, and ketoconazole

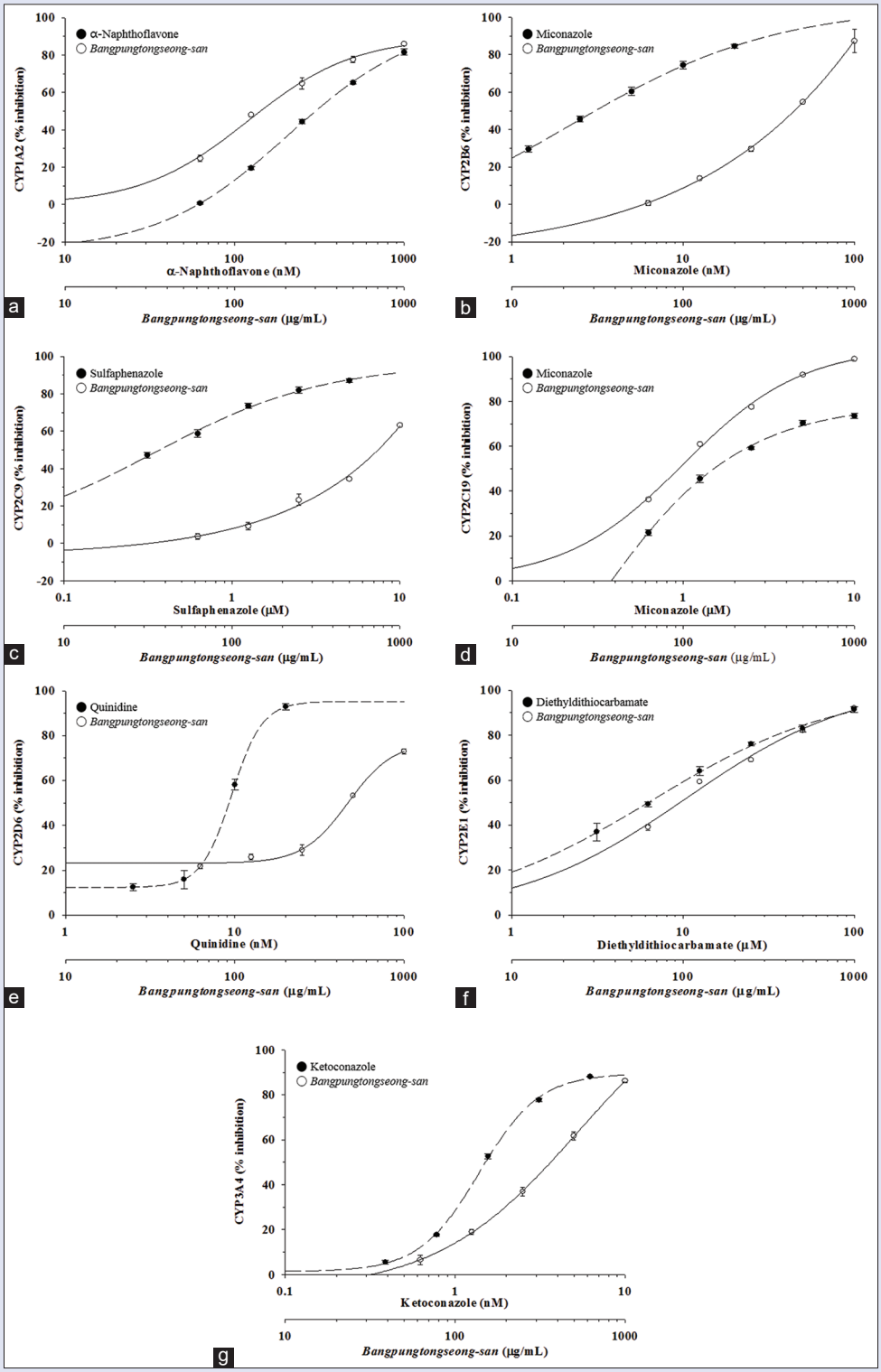

Figure 2: The effects of *Bangpungtongseong-san* on the activities of CYP1A2 (a), CYP2B6 (b), CYP2C9 (c), CYP2C19 (d), CYP2D6 (e), CYP2E1 (f), and CYP3A4 (g). The fluorescence-based enzyme assays of the CYP450 isozymes were established *in vitro*. α-Naphthoflavone, sulfaphenazole, quinidine, sodium diethyldithiocarbamate trihydrate, and ketoconazole were used as positive controls for CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19. The data are presented as the mean ± standard error of the mean (n = 3)

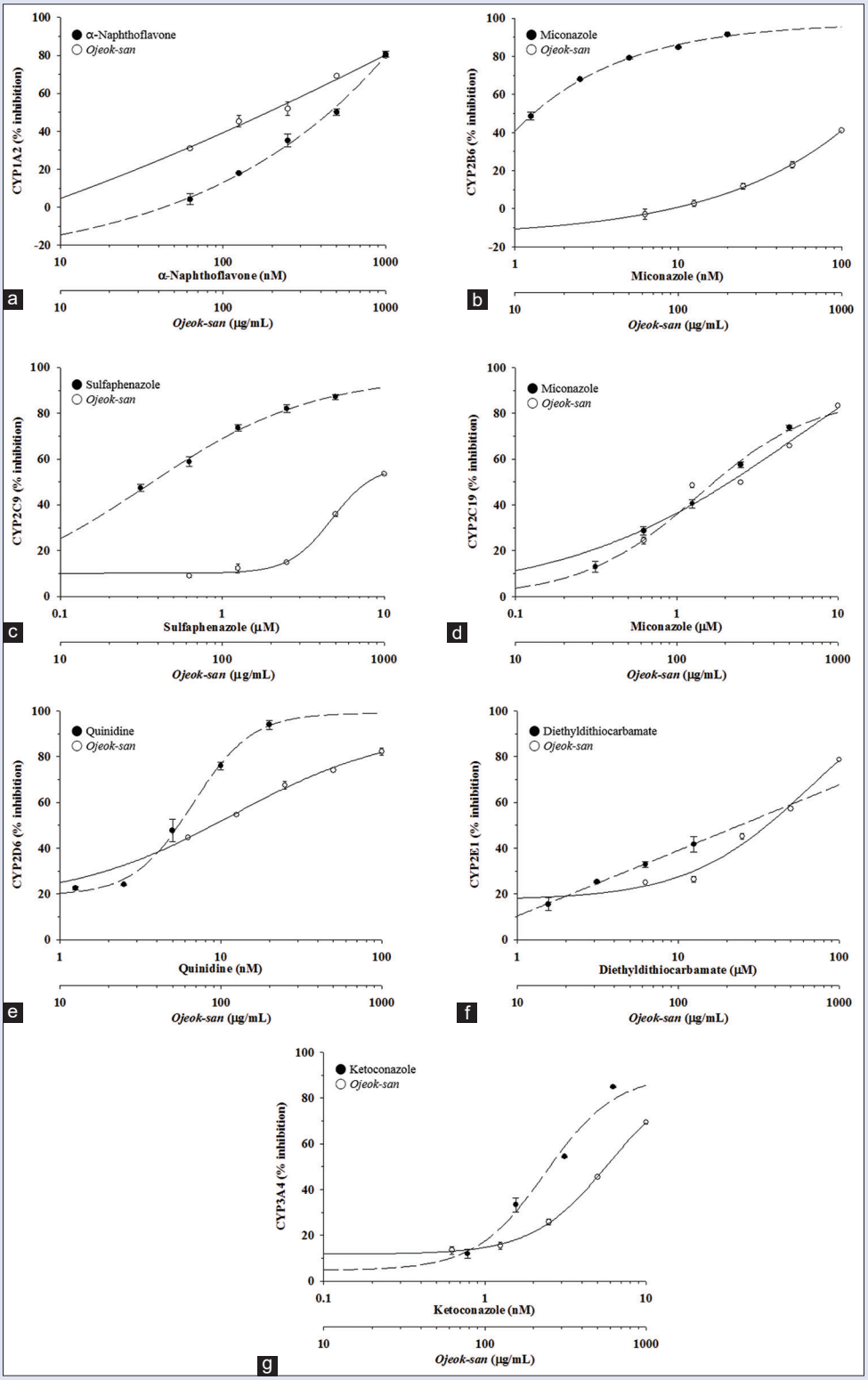

Figure 3: The effects of *Ojeok-san* on the activities of CYP1A2 (a), CYP2B6 (b), CYP2C9 (c), CYP2C19 (d), CYP2D6 (e), CYP2E1 (f), and CYP3A4 (g). The fluorescence-based enzyme assays of the CYP450 isozymes were established *in vitro*. α-Naphthoflavone, sulfaphenazole, quinidine, sodium diethyldithiocarbamate trihydrate, and ketoconazole were used as positive controls for CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19. The data are presented as the mean ± standard error of the mean (n = 3)

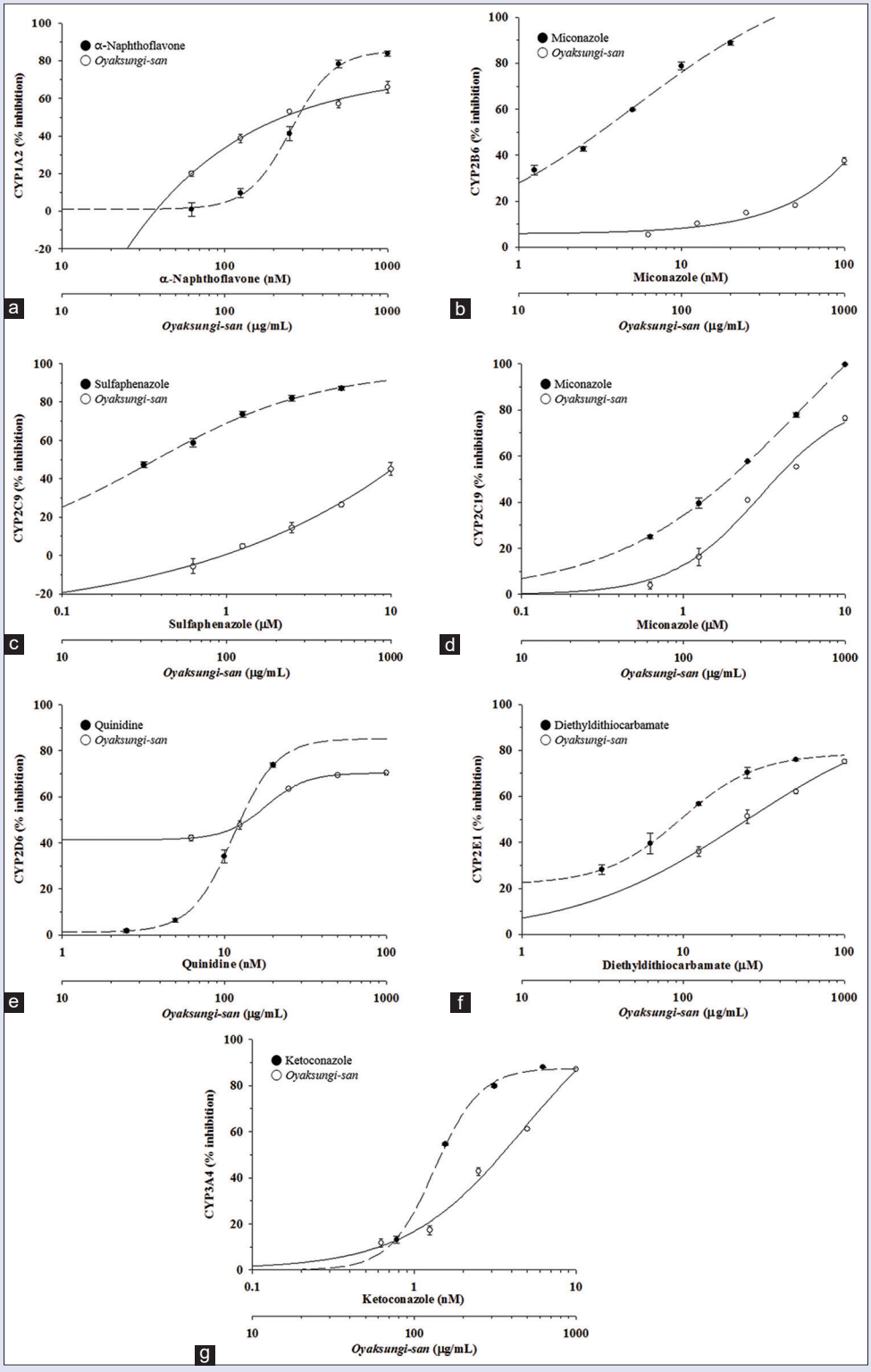

Figure 4: The effects of oyaksungi-san on the activities of CYP1A2 (a), CYP2B6 (b), CYP2C9 (c), CYP2C19 (d), CYP2D6 (e), CYP2E1 (f), and CYP3A4 (g). The fluorescence-based enzyme assays of the CYP450 isozymes were established *in vitro*. α-Naphthoflavone, sulfaphenazole, quinidine, sodium diethyldithiocarbamate trihydrate, and ketoconazole were used as positive controls for CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19. The data are presented as the mean ± standard error of the mean (n = 3)

Table 4: The IC_{50} values (μg/mL) of the herbal formula extracts on the activities of CYP450 isozymes

Herbal formula	CYP1A2	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
Bangpungtongseong-san	141.77	446.35	740.94	94.140	455.56	104.86	363.39
Ojeok-san	191.30	> 1000	868.74	252.25	88.920	357.30	583.60
Oyaksungi-san	227.11	> 1000	> 1000	386.41	141.47	249.84	347.00
Positive control	0.28-0.45 μM	1.29-3.49 μM	0.38 μM	1.56-1.93 μM	5.23-11.72 nM	6.47-12.41 μM	1.33-2.70 nM

α-Naphthoflavone, sulfaphenazole, quinidine, sodium diethyldithiocarbamate trihydrate and ketoconazole were used as positive controls for CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19. The values are the means of triplicate experiments

were used as positive controls that inhibited the activities of CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 in a dose-dependent manner, with IC_{50} values ranging from 0.28 to 0.45 μM, 0.38 μM, 5.23 to 11.72 nM, 6.47 to 12.41 μM, and 1.33 to 2.70 nM, respectively. In addition, miconazole, which was used as a positive control, inhibited the activities of CYP2B6 and CYP2C19 in a dose-dependent manner, with IC_{50} values ranging from 1.29 to 3.49 μM and 1.56 to 1.93 μM, respectively [Figures 2-4 and Table 4].

Effects of *Bangpungtongseong-san* on the activities of cytochrome P450s

As presented in Figure 2 and Table 4, BPTSS inhibited of the activities of CYP1A2, CYP2C19, and CYP2E1, with respective IC_{50} values of 141.77, 94.14, and 104.86 μg/mL. In contrast, BPTSS exerted a relatively weak inhibition on the activities of CYP2B6, CYP2C9, CYP2D6, and CYP3A4, with IC_{50} values of 446.35, 740.94, 455.56, and 363.39 μg/mL, respectively [Figure 2 and Table 4].

Effects of *Ojeok-san* on the activities of cytochrome P450s

As shown in Figure 3 and Table 4, OJS exerted the most potent inhibition of the activity of CYP2D6, with an IC_{50} value of 88.92 μg/mL. In addition, OJS inhibited the activity of CYP1A2, with an IC_{50} value of 191.30 μg/mL, whereas it showed competitively weak inhibition on the activities of CYP2C9, CYP2C19, CYP2E1, and CYP3A4, with respective IC_{50} values of 868.74, 252.25, 357.30, and 583.60 μg/mL [Figure 3 and Table 4]. Conversely, OJS inhibited the activity of CYP2B6 in a dose-dependent manner, but the inhibition at 1000 μg/mL OJS did not reach 50%.

Effects of oyaksungi-san on the activities of cytochrome P450s

As demonstrated by the data in Figure 4 and Table 4, OYSGS inhibited the CYP2D6 activity, with an IC_{50} value of 141.47 μg/mL, followed by the activities of CYP1A2 (IC_{50} = 227.11 μg/mL), and CYP2E1 (IC_{50} = 249.84 μg/mL). In addition, OYSGS inhibited the activities of CYP3A4 and CYP2C19, with similar IC_{50} values of 347.00 and 386.41 μg/mL, respectively [Figure 4 and Table 4]. In contrast, OYSGS exhibited the negligible inhibition of both the activities of CYP2B6 and CYP2C9, with IC_{50} values in excess of 1000 μg/mL.

Effects of herbal formulas on the activities of UDP-glucuronosyltransferases

In vitro UGT isozyme assays were performed to investigate the effects of the three traditional herbal formulas on the activities of UGT1A1, UGT1A4, and UGT2B7. As shown in Figures 5-7 and Table 5, diclofenac, which was used as a positive control for the inhibition of the activities of UGT1A1 and UGT2B7 in a dose-dependent manner, had IC_{50} values ranging from 295.87 to 823.95 μM and 41.15 to 81.97 μM, respectively. Lopinavir was used as a positive control for UGT1A4, and it was inhibited the CYP1A4 activity in a dose-dependent manner, with an IC_{50} value ranging from 51.88 to 96.41 μM.

Table 5: The IC_{50} values (μg/mL) of the herbal formula extracts on the activities of UGT isozymes

Herbal formula	UGT1A1	UGT1A4	UGT2B7
Bangpungtongseong-san	136.36	> 1000	> 1000
Ojeok-san	>1000	> 1000	> 1000
Oyaksungi-san	824.57	> 1000	> 1000
Positive control	295.87-823.95 μM	51.88-96.41 μM	41.15-81.97 μM

Dicofenac was used as a positive control for UGT1A1 and UGT2B7. Lopinavir was used as a positive control for UGT1A4. The values are the means of duplicate experiments

Effect of *Bangpungtongseong-san* on the activities of UDP-glucuronosyltransferases

As shown in Figure 5 and Table 5, BPTSS inhibited the UGT1A1 activity, with an IC_{50} value of 136.36 μg/mL. In contrast, BPTSS inhibited the activities of UGT1A4 and UGT2B7 in a dose-dependent manner, but inhibition at 1000 μg/mL did not reach 50% [Figure 5 and Table 5].

Effect of *Ojeok-san* on the activities of UDP-glucuronosyltransferases

OJS showed a dose-dependent inhibition on the UGT1A1 activity, but the IC_{50} value was higher than 1000 μg/mL [Figure 6 and Table 5]. In contrast, OJS did not affect the activities of UGT1A4 and UGT2B7 at doses <1000 μg/mL [Figure 6].

Effect of oyaksungi-san on the activities of UDP-glucuronosyltransferases

As presented in Figure 7 and Table 5, OYSGS exhibited a competitively weak inhibition on the UGT1A1 activity, with an IC_{50} value of 824.57 μg/mL. Furthermore, OYSGS inhibited the activity of UGT2B7 in a dose-dependent manner, but inhibition at 1000 μg/mL did not reach 50% [Figure 7]. Conversely, OYSGS showed no inhibition of the UGT1A4 activity at doses of <1000 μg/mL [Figure 7].

DISCUSSION

According to the increasing interest in the importance of herb-drug interactions in clinical settings,[25,26] in the study, the effects of traditional herbal formulas (BPTSS, OJS, and OYSGS) that are used to treat MSDs on the activities of CYP450 isozymes (CYP1A2, CYP3A4, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1) and UGT isozymes (UGT1A1, UGT1A4, and UGT2B7) were examined. Several reports have demonstrated the influence of herbal extracts or the components present in these herbal formulas on the activities of CYP450s and UGTs. Among them, Glycyrrhizae Radix and Cnidii Rhizoma of BPTSS, OJS, and OYSGS inhibit the activity of human

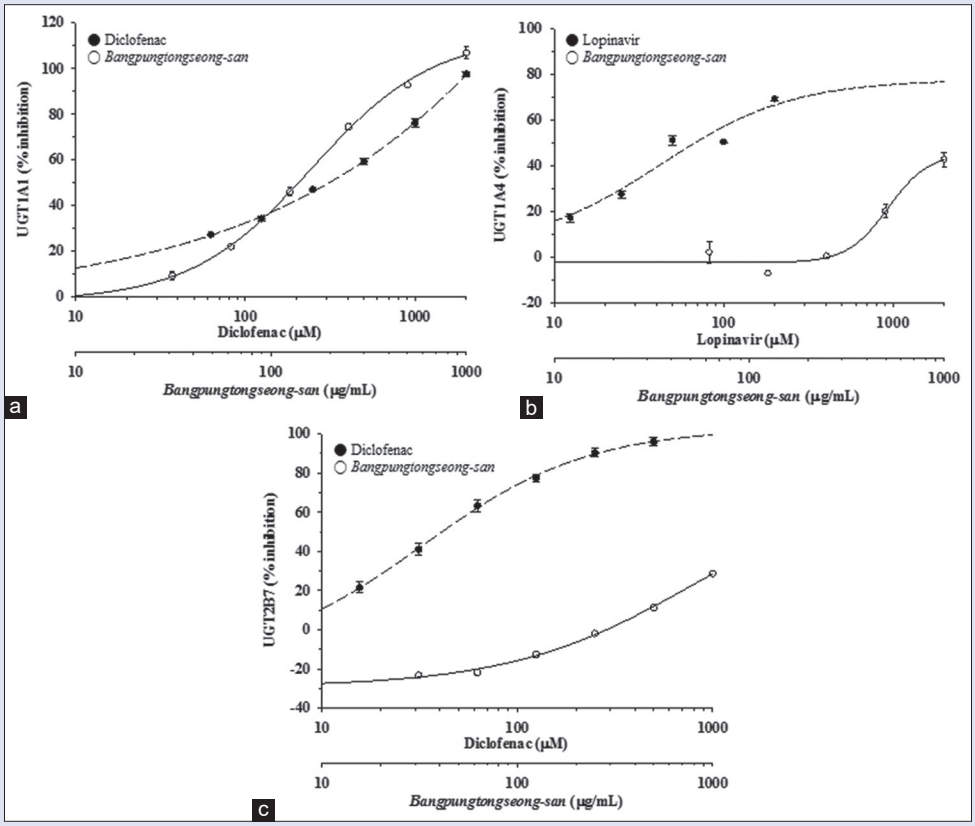

Figure 5: The effects of *Bangpungtongseong-san* on the activities of UGT1A1 (a), UGT1A4 (b), and UGT2B7 (c). The luminescence-based enzyme assays of the UGT isozymes were established *in vitro*. Diclofenac was used as a positive control for UGT1A1 and UGT2B7. Lopinavir was used as a positive control for UGT1A4. The data are presented as the mean ± standard error of the mean (n = 2)

UGT1A1 and rat CYP1A1, respectively.[27,28] In addition, it has been reported that Scutellariae Radix of BPTSS induces and suppresses the levels of CYP1A and CYP2B in rats, respectively.[29] Scutellariae Radix also inhibits the activity of UGT1A1 in humans.[27] Cinnamomi Bark of OJS inhibits the activities of CYP1A2, CYP2C8, CYP2C9, CYP2D6, and UGT1A1 in humans and CYP1A2 and CYP2C11 in rats.[27,30] Pseudoephedrine, one of the components in Ephedrae Herba of BPTSS, OJS, and OYSGS, inhibits the activities of CYP1A1/2 and CYP2E1 in rats.[31] Moreover, decursin in Angelicae Gigantis Radix of BPTSS and OJS has inhibitory effects on the activities of CYP1A1/2, CYP3A12, and CYP2D15 in canines.[32] The chromatographic analysis of the various marker components in these herbal formulas was performed using an HPLC-PDA. Among them, geniposide of BPTSS has been reported to decrease the activities of liver microsomal CYP2E1 in mice.[33] Furthermore, baicalin of BPTSS has an inhibitory effect on CYP1A1, CYP2B1, and CYP2C11 in mice.[34] Glycyrrhizin, which is one of the marker compounds of BPTSS and OYSGS, has been reported to inhibit the activities of CYP1A2 in human.[35] In addition, naringin of OJS and OYSGS reduces the CYP1A2 protein level in mice.[36] Cinnamaldehyde of OJS showed inhibits UGT1A1 in humans.[27] However, the major and/or active compounds of herbal formulas are not known. It is difficult to specifically comprehend the metabolizing mechanisms of medicinal herbs, including herbal formulas because they contain a complex group of hundreds of constituent molecules. To date, the effects of herbal formulas, such as BPTSS, OJS, and OYSGS on the activities of CYP450s and UGTs have not been elucidated. In this study, we investigated the inhibitory effects of BPTSS, OJS, and OYSGS on the activities of human CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, UGT1A1, UGT1A4, and UGT2B7 to assess their clinical significance in herb-drug interactions.

The CYP3A family contributes to approximately 50% of the total CYP450 activity in the human liver and is involved in the metabolism of approximately 60% of therapeutic substances.[37] CYP3A4 is the dominant CYP3A enzyme and is expressed in the human liver and gastrointestinal tract.[38] CYP3A4 also has genetic polymorphisms. Some of the most serious CYP-mediated drug interactions are caused by the accumulation of substrates that are metabolized by CYP3A4, such as astemizole, terfenadine, and cisapride. CYP2D6 exhibits genetic polymorphisms and represents <5% of the total CYP proteins. More than 80 drugs in current clinical use are metabolized by CYP2D6, which has aroused great interest because of its large number of substrates (30–50 drugs) and its genetic polymorphism. CYP2C19 also exhibits genetic polymorphisms and has a number of commonly used substrates, including the benzodiazepine diazepam, the proton-pump inhibitor omeprazole, propranolol, and the antidepressive amitriptyline.[39] Few drugs, other than chlorzoxazone and several inhalation anesthetics, are metabolized by CYP2E1. Moreover, CYP2E1 is responsible for the metabolism of ethanol, and chronic ethanol consumption can induce CYP2E1.[40,41] In this study, BPTSS inhibited the activities of CYP1A2, CYP2C19, CYP2E1, and UGT1A1, which may have clinical implications. Therefore, caution should be exercised when coadministering BPTSS with substrates/inhibitors of CYP1A2, CYP2C19, CYP2E1, or UGT1A1. In addition,

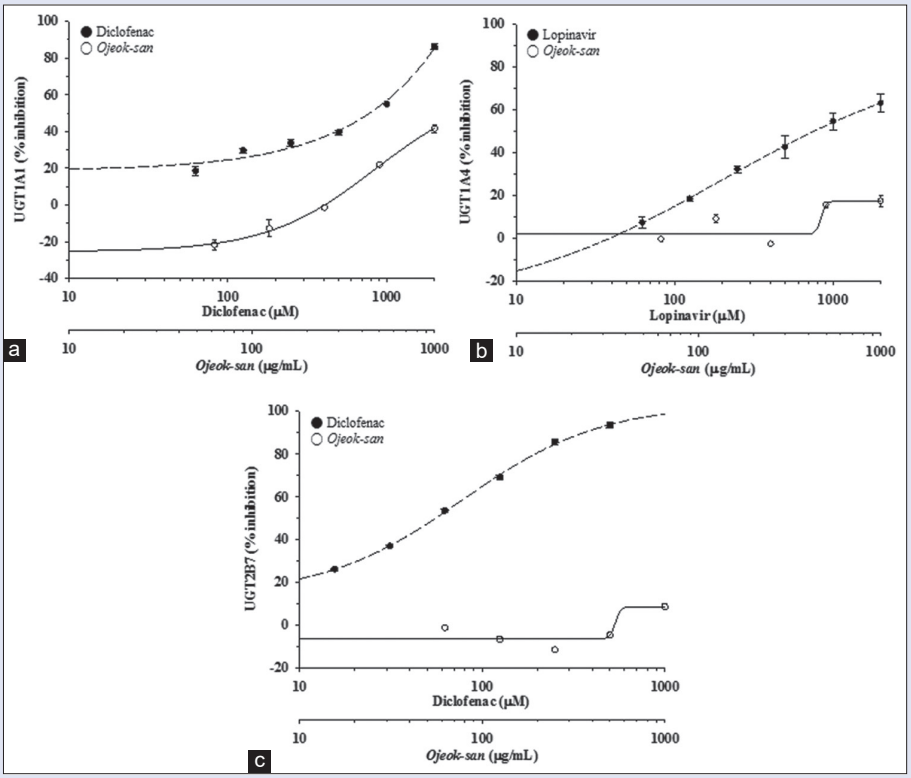

Figure 6: The effects of *Ojeok-san* on the activities of UGT1A1 (a), UGT1A4 (b), and UGT2B7 (c). The luminescence-based enzyme assays of the UGT isozymes were established *in vitro*. Diclofenac was used as a positive control for UGT1A1 and UGT2B7. Lopinavir was used as a positive control for UGT1A4. The data are presented as the mean ± standard error of the mean (n = 2)

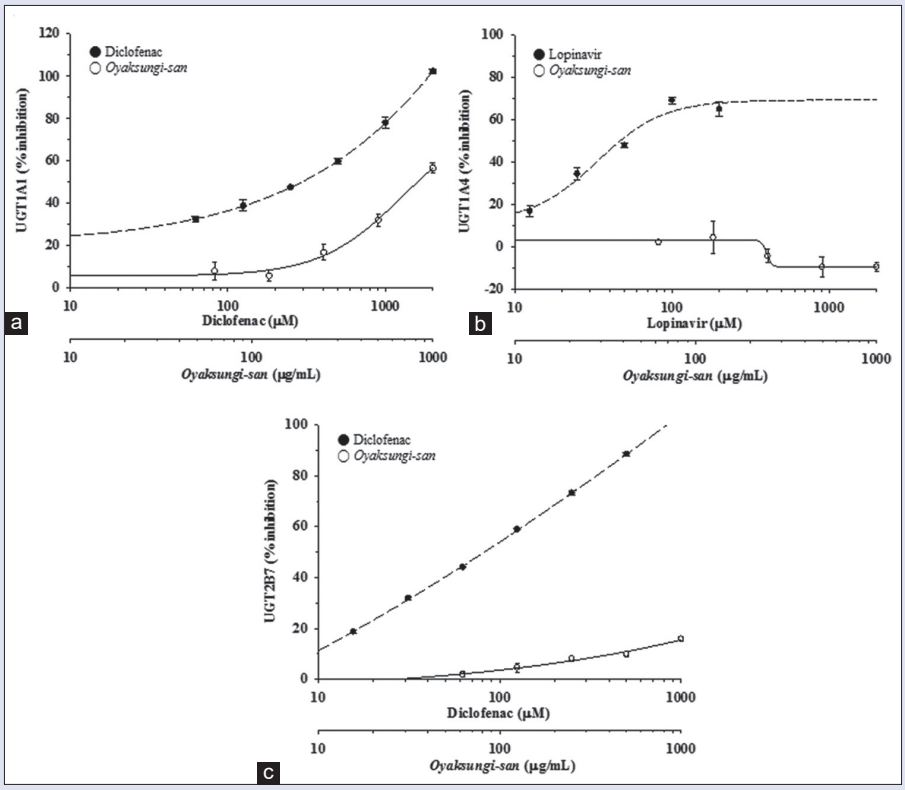

Figure 7: The effects of oyaksungi-san on the activities of UGT1A1 (a), UGT1A4 (b), and UGT2B7 (c). The luminescence-based enzyme assays of the UGT isozymes were established *in vitro*. Diclofenac was used as a positive control for UGT1A1 and UGT2B7. Lopinavir was used as a positive control for UGT1A4. The data are presented as the mean ± standard error of the mean (n = 2)

these results suggest that glycyrrhizin and geniposide of BPTSS would contribute to the inhibition of CYP1A2 and CYP2E1, respectively, by BPTSS.[33,35] In contrast, BPTSS are unlikely to inhibit the metabolism of drugs metabolized by CYP2B6, CYP2C9, CYP2D6, CYP3A4, UGT1A4, and UGT2B7. OJS inhibited the CYP2D6 activity most potently, followed by CYP1A2, CYP2C19, CYP2E1, CYP3A4, and CYP2C9. Thus, attention should be paid when OJS is administered simultaneously with drugs that are metabolized by CYP2D6, and OJS may influence the metabolic reactions mediated by CYP1A2, CYP2C9, CYP2C19, CYP2E1, or CYP3A4 at a high concentration. In contrast, OJS negligibly inhibited the activities of CYP2B6 and UGT1A1, with IC_{50} values in excess of 1000 μg/mL, and OJS not influence the activities of UGT1A4 and UGT2B7 at doses <1000 μg/mL. Thus, OJS would not affect CYP2B6, UGT1A1, UGT1A4, or UGT2B7-mediated metabolism in the clinic. OYSGS showed potent inhibition of the activity of CYP2D6 followed by CYP1A2, CYP2E1, CYP3A4, CYP2C19, and UGT1A1, but it had no significant inhibition on CYP2B6, CYP2C9, UGT1A4, and UGT2B7 at concentrations of over 1000 μg/mL. These findings indicate that OYSGS is an inhibitor of CYP2D6 and that caution is necessary to reduce its adverse effects when it is coadministered with a substrate/inhibitor of CYP2D6. Furthermore, OYSGS has a relatively low potential to be involved in herb-drug interactions when administered simultaneously with substrates or inhibitors of CYP2B6, CYP2C9, UGT1A4, or UGT2B7.

In general, aceclofenac and aspirin, which are used for the treatment of rheumatoid arthritis 2 and osteoarthritis, are metabolized by CYP2C9.[42,43] In addition, naproxen, which is used to treat pain or inflammation caused by arthritis, ankylosing spondylitis, tendinitis, and gout, is a substrate of CYP1A2 and CYP2C9,[44] and the drug also inhibits the UGT2B7 activity.[45] Acetaminophen, which is widely used to treat muscle aches, arthritis, backache, and toothache, is metabolized by CYP3A4, CYP2D6, and CYP2E1.[46-48] Therefore, BPTSS, OJS, and OYSGS are unlikely to cause clinically relevant herb-drug interactions when coadministered with aceclofenac or aspirin for the treatment of MSDs, whereas caution should be exercised to decrease the side effects when any of the three herbal formulas are co-administered with naproxen or acetaminophen.

CONCLUSIONS

This study provided information regarding the risks and benefits potentially associated with the use of BPTSS, OJS, and OYSGS. Caution is necessary when BPTSS is administered together with a substrate/inhibitor of CYP1A2, CYP2C19, CYP2E1, or UGT1A1. Furthermore, herb-drug interactions can occur when OJS or OYSGS are used in combination with other drugs that are metabolized by CYP2D6, to a greater extent than those that are metabolized by other isozymes.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Guengerich FP. Characterization of human microsomal cytochrome P-450 enzymes. Annu Rev Pharmacol Toxicol 1989;29:241-64.
2. Miners JO, Smith PA, Sorich MJ, McKinnon RA, Mackenzie PI. Predicting human drug glucuronidation parameters: Application of *in vitro* and in silico modeling approaches. Annu Rev Pharmacol Toxicol 2004;44:1-25.
3. Oguri K, Yamada H, Yoshimura H. Regiochemistry of cytochrome P450 isozymes. Annu Rev Pharmacol Toxicol 1994;34:251-79.
4. Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: Metabolism, expression, and disease. Annu Rev Pharmacol Toxicol 2000;40:581-616.
5. Bertz RJ, Granneman GR. Use of *in vitro* and *in vivo* data to estimate the likelihood of metabolic pharmacokinetic interactions. Clin Pharmacokinet 1997;32:210-58.
6. Degtyarenko KN. Structural domains of P450-containing monooxygenase systems. Protein Eng 1995;8:737-47.
7. Meunier B, de Visser SP, Shaik S. Mechanism of oxidation reactions catalyzed by cytochrome p450 enzymes. Chem Rev 2004;104:3947-80.
8. Werck-Reichhart D, Feyereisen R. Cytochromes P450: A success story. Genome Biol 2000;1:REVIEWS3003.
9. Food and Drug Administration. Drug interaction studies-Study design, data analysis, implications for dosing, and labeling recommendations; 2012.
10. Guillemette C, Lévesque É, Rouleau M. Pharmacogenomics of human uridine diphospho-glucuronosyltransferases and clinical implications. Clin Pharmacol Ther 2014;96:324-39.
11. Stingl JC, Bartels H, Viviani R, Lehmann ML, Brockmöller J. Relevance of UDP-glucuronosyltransferase polymorphisms for drug dosing: A quantitative systematic review. Pharmacol Ther 2014;141:92-116.
12. Evans WE, Relling MV. Pharmacogenomics: Translating functional genomics into rational therapeutics. Science 1999;286:487-91.
13. Colebatch AN, Marks JL, Edwards CJ. Safety of non-steroidal anti-inflammatory drugs, including aspirin and paracetamol (acetaminophen) in people receiving methotrexate for inflammatory arthritis (rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, other spondyloarthritis). Cochrane Database Syst Rev 2011;11:CD008872.
14. de Leon-Casasola OA. Opioids for chronic pain: New evidence, new strategies, safe prescribing. Am J Med 2013;126 3 Suppl 1:S3-11.
15. Lee CK, Song JM. Immunoregulative action of *Bangpoongtongsungsan*. J Sasang Const Med 1998;10:589-613.
16. Moon YH, Lee DI, Lee SY. Studies on the anti-inflammatory and analgesic activities of *Ohyaksungisan*. Korean J Pharmacogn 1996;27:184–9.
17. Moon YH, Park YJ. Studies on the anti-inflammatory and analgesic activities of *Ohjuksan*. Korean J Pharmacogn 1994;25:258-63.
18. Akagiri S, Naito Y, Ichikawa H, Mizushima K, Takagi T, Handa O, *et al. Bofutsushosan*, an oriental herbal medicine, attenuates the weight gain of white adipose tissue and the increased size of adipocytes associated with the increase in their expression of uncoupling protein 1 in high-fat diet-fed male KK/Ta mice. J Clin Biochem Nutr 2008;42:158-66.
19. Kim KS, Jeon HY, Jeon SY, Hong S, Kang HJ, Kim JS. Mechanism study of *Bangpungtongseongsan* (BTS) on the cerebral hemodynamics in mice. Korean J Orient Intern Med 2002;23:91-8.
20. Lee NH, Lee KS. Effect of *Bangpoomgtonsungsan* on the hypertention and hyperlipidemia. J Korean Orient Med 1975;12:44-55.
21. Shimada T, Kudo T, Akase T, Aburada M. Preventive effects of *Bofutsushosan* on obesity and various metabolic disorders. Biol Pharm Bull 2008;31:1362-7.
22. Kim JH, Soh KS, Jeong CG, Kim KH. Effects of *Ojuck-san* on hyperlipidemia in rats. Korean J Orient Prev Med Soc 2004;8:185-202.
23. Jung MJ, Lee JH, Yeom SR, Lee SK, Song YS, Kim KB, *et al.* Effects of *Ohyaksungi-san (Wuyaoshungi-san)* and *Jungsongouhyul* pharmacopuncture on pain reduction and nerve regeneration after crush injury in rat sciatic nerve. J Orient Rehabil Med 2009;19:51-72.
24. Jung SY, Lee MJ. The effects of *Ohyaksunki-san(Wuyaoshunqisan)* on increasing body fat of rat induced by high fat diet. J Orient Rehabil Med 2007;17:23-39.
25. Eric Y, Kathy A. Interaction of herbal constituents with cytochrome P450 enzymes. Altern Complement Ther 2007;13:239-47.

26. Sparreboom A, Cox MC, Acharya MR, Figg WD. Herbal remedies in the United States: Potential adverse interactions with anticancer agents. J Clin Oncol 2004;22:2489-503.
27. Katoh M, Yoshioka Y, Nakagawa N, Yokoi T. Effects of Japanese herbal medicine, Kampo, on human UGT1A1 activity. Drug Metab Pharmacokinet 2009;24:226-34.
28. Son YH, Kim HG, Nam GS. Effect of *Cnidii Rhizoma* water extract on chemopreventive enzymes for hepatocarcinoma. Korean J Pharmacogn 2003;34:297-302.
29. Kang JJ, Chen YC, Kuo WC, Chen T, Cheng YW, Kuo ML, *et al.* Modulation of microsomal cytochrome P450 by *Scutellariae* Radix and *Gentianae scabrae* Radix in rat liver. Am J Chin Med 1996;24:19-29.
30. Park HJ. Effects of Herbal Medicines Including *Cinnamomi Cortex* on Activity of Several Cytochrome P450 Isoforms. PhD Thesis. Wonkwang University, Department Oriental Pharmacy; 2009.
31. Wu W, Liu L, Han F, Chen Y. Effect of pseudoephedrine and ephedrine on the activities of cytochrome P450 enzymes in rat liver microsomes. China J Tradit Med Pharm 2011;26:1804-7.
32. Abd El-Aty AM, Shah SS, Kim BM, Choi JH, Cho HJ, Hee-Yi, *et al. In vitro* inhibitory potential of decursin and decursinol angelate on the catalytic activity of cytochrome P-450 1A1/2, 2D15, and 3A12 isoforms in canine hepatic microsomes. Arch Pharm Res 2008;31:1425-35.
33. Ma T, Huang C, Zong G, Zha D, Meng X, Li J, *et al.* Hepatoprotective effects of geniposide in a rat model of nonalcoholic steatohepatitis. J Pharm Pharmacol 2011;63:587-93.
34. Hou YN, Zhu XY, Cheng GF. Effects of baicalin on liver microsomal cytochrome P450 system. Acta Pharm Sin 2000;35:890-2.
35. Park JH, Park JY, Ju YS. Inhibitory effects of licorice ethanol extracts and glycyrrhizin on cytochrome P450 drug-metabolizing enzymes in human liver microsomes. Korean J Orient Prev Med Soc 2003;7:65-74.
36. Ueng YF, Chang YL, Oda Y, Park SS, Liao JF, Lin MF, *et al. In vitro* and *in vivo* effects of naringin on cytochrome P450-dependent monooxygenase in mouse liver. Life Sci 1999;65:2591-602.
37. Cascorbi I. Genetic basis of toxic reactions to drugs and chemicals. Toxicol Lett 2006;162:16-28.
38. Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. Adv Drug Deliv Rev 2002;54:1271-94.
39. Bibi Z. Role of cytochrome P450 in drug interactions. Nutr Metab (Lond) 2008;5:27.
40. Badyal DK, Dadhich AP. Cytochrome P450 and drug interactions. Indian J Pharmacol 2001;33:248-59.
41. Goshman L, Roller K. Clinically significant cytochrome P450 drug interactions. J Pharm Soc Wis 1999;3:23-38.
42. Bigler J, Whitton J, Lampe JW, Fosdick L, Bostick RM, Potter JD. CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk. Cancer Res 2001;61:3566-9.
43. Ihm CH, Hwang IT, Kim EY, Kang WK. Pharmacokinetic study of aceclofenac and its metabolites, and application to bioequivalence study. Korean J Clin Pharm 2006;16:52-6.
44. Miners JO, Coulter S, Tukey RH, Veronese ME, Birkett DJ. Cytochromes P450, 1A2, and 2C9 are responsible for the human hepatic O-demethylation of R- and S-naproxen. Biochem Pharmacol 1996;51:1003-8.
45. Joo J, Kim YW, Wu Z, Shin JH, Lee B, Shon JC, *et al.* Screening of non-steroidal anti-inflammatory drugs for inhibitory effects on the activities of six UDP-glucuronosyltransferases (UGT1A1, 1A3, 1A4, 1A6, 1A9 and 2B7) using LC-MS/MS. Biopharm Drug Dispos 2015;36:258-64.
46. Dong H, Haining RL, Thummel KE, Rettie AE, Nelson SD. Involvement of human cytochrome P450 2D6 in the bioactivation of acetaminophen. Drug Metab Dispos 2000;28:1397-400.
47. Snawder JE, Roe AL, Benson RW, Roberts DW. Loss of CYP2E1 and CYP1A2 activity as a function of acetaminophen dose: Relation to toxicity. Biochem Biophys Res Commun 1994;203:532-9.
48. Thummel KE, Lee CA, Kunze KL, Nelson SD, Slattery JT. Oxidation of acetaminophen to N-acetyl-p-aminobenzoquinone imine by human CYP3A4. Biochem Pharmacol 1993;45:1563-9.

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