Expression of Hepatic Cytochrome P450s in Rats Administered with *Guibi-tang,* a Traditional Herbal Formula

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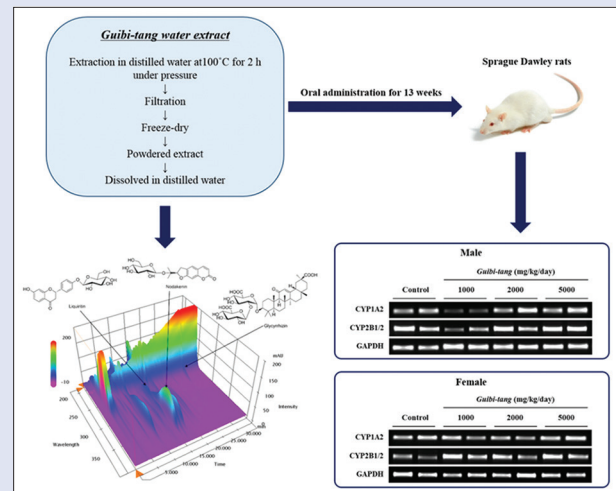
ABSTRACT

Objective: The aim of this study was to investigate the possible herb-drug interactions between the traditional herbal formula *Guibi-tang* (GBT; *Guipi-tang*, *Kihi-to*) and conventional drugs. **Materials and Methods:** GBT was orally administered to either male or female Sprague Dawley (SD) rats once daily at doses of 1000, 2000, or 5000 mg/kg/day for 13 weeks. The messenger ribonucleic acid (mRNA) expression of drug-metabolizing enzyme cytochrome P450 isozymes (cytochrome P450s; CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1) was analyzed in hepatic tissues by reverse transcription-polymerase chain reaction. **Results:** Repeated oral administration of GBT did not significantly influence the mRNA expression of hepatic CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1 in male rats. By contrast, in female rats, the mRNA expression of hepatic CYP1A2 and 2B1/2 was significantly increased by repeated GBT treatment. **Conclusion:** Our findings indicate that caution is required in females when GBT is taken concomitantly with conventional drugs metabolized by CYP1A2 or 2B1/2. Our results provide information regarding the safety and effectiveness of GBT for clinical use.

Key words: Cytochrome P450, *Guibi-tang,* herb–drug interactions, rat liver

SUMMARY

- Repeated oral administration of *Guibi-tang* (GBT) for 13 weeks did not affect the messenger ribonucleic acid (mRNA) expression of hepatic CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1 in male rats
- Repeated oral administration of GBT for 13 weeks induced mRNA expression of hepatic CYP1A2 and 2B1/2 but not for CYP1A1, 2C11, 2E1, 3A1, 3A2, and 4A1 in female rats.

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INTRODUCTION

The utilization of herbal medicines as complementary and alternative medicines is becoming increasingly popular for preventing and treating various diseases.[1] Many patients take herbal medicines together with conventional drugs because they believe that the combination is safe and has synergistic efficacy. However, precise information regarding herb–drug interactions is limited.

Cytochrome P450s (CYP450s), comprised a superfamily of hemoproteins, are involved in various oxidative reactions and play an essential role in the metabolism of xenobiotics. Therefore, it is important to study the induction or inhibition of CYP450s by herbal formulas to predict herb–drug interactions and reduce safety problems.

A traditional herbal formula *Guibi-tang* (GBT; *Guipi-tang*, *Kihi-to*) consists of 12 medicinal herbs [Table 1], and it has been used to treat psychoneurosis including memory impairment, insomnia, and neurosis-related peptic ulcers in Korea, China, and Japan.[2] Biologically, GBT has been reported to have anti-stress effects and beneficial effects on gastrointestinal and immune-mediated diseases.[3,4] However, there are no studies on the effect of GBT on hepatic CYP450 expression.

Table 1: Composition of *Guibi-tang*

Scientific name	Family	Parts of using	Amount (g)	Origin
A. gigas	Umbelliferae	Radix	3.750	Bonghwa, Republic of Korea
D. longan	Sapindaceae	Arillus	3.750	Vietnam
Z. jujuba	Rhamnaceae	Semen	3.750	China
P. tenuifolia	Polygalaceae	Radix	3.750	China
P. ginseng	Araliaceae	Radix	3.750	Yeongju, Republic of Korea
A. membranaceus	Leguminosae	Radix	3.750	Jecheon, Republic of Korea
A. macrocephala	Compositae	Rhizoma	3.750	China
P. cocos	Polyporaceae	Sclerotium	3.750	Pyeongchang, Republic of Korea
A. lappa	Compositae	Radix	1.875	China
G. uralensis	Leguminosae	Rhizoma	1.125	China
Z. officinale	Zingiberaceae	Rhizoma	3.750	Ulsan, Republic of Korea
Z. jujuba	Rhamnaceae	Fructus	3.750	Yeongcheon, Republic of Korea
Total			43.000	

A. gigas: *Angelica gigas*; *D. longan*: *Dimocarpus longan*; *Z. jujuba*: *Ziziphus jujuba*; *P. tenuifolia*: *Polygala tenuifolia*; *P. ginseng*: *Panax ginseng*; *A. membranaceus*: *Astragalus membranaceus*; *A. macrocephala*: *Atractylodes macrocephala*; *P. cocos*: *Poria cocos*; *A. lappa*: *Aucklandia lappa*; *G. uralensis*: *Glycyrrhiza uralensis*; *Z. officinale*: *Zingiber officinale*

In the present study, we investigated the influence of GBT on hepatic messenger ribonucleic acid (mRNA) expression of CYP450s (CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1) in Sprague Dawley (SD) rats administered for 13 weeks.

MATERIALS AND METHODS

Plant materials

Twelve raw materials of GBT were purchased from Kwangmyungdang Medicinal Herbs (Ulsan, Republic of Korea) as dried herbs. Each herbal medicine was authenticated taxonomically by Professor Je-Hyun Lee, College of Oriental Medicine, Dongguk University, Gyeongju, Republic of Korea. Voucher specimens (2012–KE22-1 ~ KE22-12) have been deposited at the K-herb Research Center, Korea Institute of Oriental Medicine.

Chemicals and reagents

Liquiritin and nodakenin (purities ≥98.0%) were purchased from NPC BioTechnology Inc. (Yeongi, Republic of Korea). Glycyrrhizin (purity ≥98.0%) was purchased from Wako Fine Chemicals (Osaka, Japan). High-performance liquid chromatography (HPLC)-grade methanol, acetonitrile, and water were purchased from J. T. Baker (Phillipsburg, NJ, USA), and glacial acetic acid was obtained from Merck AG (Darmstadt, Germany).

Preparation of *Guibi-tang* decoction

A decoction of GBT, which is composed of 12 medicinal herbs [Table 1], was prepared at K-herb Research Center, Korea Institute of Oriental Medicine (Daejeon, Republic of Korea). Briefly, mixtures (61.92 kg, i.e., about 1440 times the composition of a single dose) of 12 herbal medicines were extracted in distilled water (620 L) at 100°C for 120 min under pressure (98 kPa) by an electric extractor (COSMOS-660, Kyung Seo Machine Co., Incheon, Republic of Korea). The extracted solution was filtered using a standard sieve (no. 270; 53 μm; Chung Gye Sang Gong Sa, Seoul, Republic of Korea) and freeze dried (IlShinBioBase, Dongducheon, Republic of Korea). The amount of lyophilized extract was about 18.23% of the original weight (11.29 kg). These powdered extracts were used in the experiment and were stored at 4°C.

High-performance liquid chromatography analysis of *Guibi-tang*

The GBT extract was analyzed for quality control using the Shimadzu Prominence LC-20A series HPLC system (Kyoto, Japan) at 0, 1, and 13 weeks. This HPLC system consists of solvent delivery unit (LC-20AT), online degasser (DGU-20A$_3$), column oven (CTO-20A), auto sample injector (SIL-20AC), and photodiode array (PDA) detector (SPD-M20A). Data were collected and processed by LCsolution software (Version 1.24, Shimadzu, Kyoto, Japan). The compounds were analyzed using a Phenomenex Gemini C$_{18}$ column (250 mm × 4.6 mm; 5 μm, Torrance, CA, USA). Column oven temperature was maintained at 40°C and the mobile phases consisted of 1.0% (v/v) acetic acid in distilled water (A) and 1.0% (v/v) acetic acid in acetonitrile (B). The gradient flow was as follows: 10%–70% B for 0–30 min, 70%–100% B for 30–35 min, 100% B for 35–40 min, and 100%–10% B for 40–45 min. The re-equilibration time was 15 min. The flow rate and injection volume were 1.0 mL/min and 10 μL, respectively. For HPLC quantitative determination, 400 mg of the lyophilized GBT sample was dissolved in 20 mL of distilled water. The solution was filtered through a 0.2 μm syringe filter (Woongki Science, Seoul, Republic of Korea) before HPLC analysis.

Animals

Forty male- and 40 female-specific pathogen-free SD rats (5 weeks old upon receipt, Orient Bio, Gyeonggi-do, Seongnam, Republic of Korea) were used after acclimatization for 14 days before study initiation with an evaluation of health status. The animals were maintained in environmentally controlled rooms at 22°C ± 3°C under a relative humidity of 50% ± 20% with a 12-h light-dark cycle and 12-15 air changes per hour.

This study was performed at the Korea Testing and Research Institute, and the study protocol was approved by the Institutional Animal Care and Use Committee according to the Guidelines for Toxicity Tests of Drugs and Related Materials (Document No. 2012-86) as prepared by the Korea Food and Drug Administration. These experiments were performed according to the Organization for Economic Cooperation and Development Principles of Good Laboratory Practice (1997).

Experimental groups and treatment

Healthy male and female rats were randomly assigned to eight experimental groups. Each group consisted of 10 rats of each gender. Since oral administration is the clinically intended route for GBT, it was administered in the present study by oral gavage after being dissolved in distilled water. Distilled water was given to the animals in the vehicle control group. GBT (1000, 2000, or 5000 mg/kg/day) was administered orally to rats for 13 weeks. At the end of the administration period, the rats were sacrificed after overnight fasting and liver tissues were removed. Livers were immediately frozen and stored at −81°C.

Isolation of total ribonucleic acid and cDNA synthesis

The total RNA was isolated from livers using TriZol reagent (Invitrogen, Camarillo, CA, USA) according to the manufacturer's instructions. The RNA pellet was dissolved in RNase-free water. Total RNA concentration and purity were determined by measuring the absorbance at 260 and 280 nm using NanoDrop 2000 (Thermo Scientific, Rockford, IL, USA). One microgram of total RNA was converted to cDNA using an iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). The obtained cDNA was stored at −20°C or used directly for the reverse transcription-polymerase chain reaction (RT-PCR).

Reverse transcription-polymerase chain reaction

To confirm the level of mRNA expression in rat liver, eight rats were randomly selected from each group. The level of mRNA expression in rat hepatic tissues was analyzed by RT-PCR. The primer sequences and PCR conditions used for CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, 4A1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplification are described in Table 2.[5] All primers were synthesized by Bioneer Co. (Daejeon, Republic of Korea). GAPDH was used as the reference gene for normalization of target gene expression. Reaction mixtures comprised 1 μL of cDNA, 0.1 μL of rTag polymerase, 0.4 μL of 2.5 mM dNTPs, and 0.3 μL (10 pmole) of each forward and reverse primer. Amplicon size and reaction specificity were confirmed by electrophoresis in 1%–2% agarose gels stained with LoadingSTAR (Dynebio, Gyeonggi-do, Seongnam, Republic of Korea). The gels were photographed and the band intensities were quantified using commercially available ChemiDoc™ XRS + imaging system (Bio-Rad).

Statistical analysis

The data are expressed as the mean ± standard error of the mean (n = 8). The experimental data were analyzed using GraphPad InStat (Version 3.05, GraphPad Software, Inc., CA, USA). One-way analysis of variance was used to detect significant differences between the control and treatment groups. Dunnett's test was used for multiple comparisons. The differences were considered significant at $P < 0.05$.

RESULTS

Quantification of the three compounds in *Guibi-tang*

The optimized HPLC-PDA method was applied for the quantitative analysis of the three compounds at 0, 1, and 13 weeks in GBT decoction. The retention times of liquiritin, nodakenin, and glycyrrhizin were about 14.41, 15.33, and 29.42 min, respectively [Figure 1]. The quantitative determination of each component was conducted at 254 nm for glycyrrhizin, 280 nm for liquiritin, and 330 nm for nodakenin. The concentrations of liquiritin, nodakenin, and glycyrrhizin at 0, 1, and 13 weeks in GBT were 0.60–1.71 mg/g, 0.63–1.66 mg/g, and 0.59–1.58 mg/g, respectively [Table 3].

Effect of *Guibi-tang* on hepatic expression of cytochrome P450s

To investigate the influence of GBT on the hepatic CYP450s expression, both male and female rats were treated orally with GBT (1000, 2000,
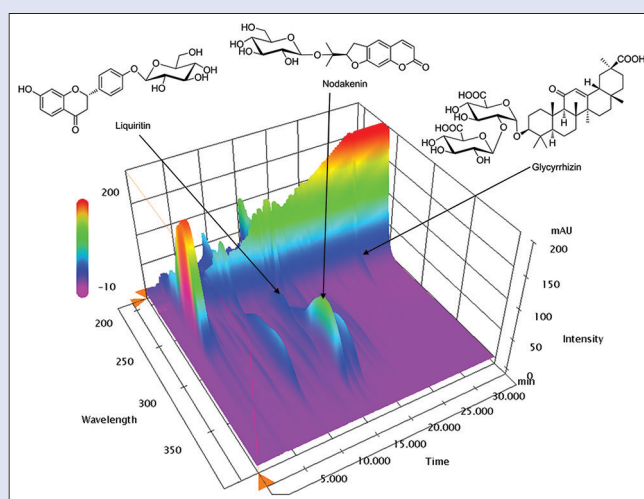

Figure 1: Three-dimensional chromatogram of *Guibi-tang* analyzed by high-performance liquid chromatography-photodiode array

Table 2: Oligonucleotide sequences of primers used for the reverse transcription-polymerase chain reaction analysis and polymerase chain reaction conditions

	Primer sequences (5'→3')	Product size (bp)	PCR conditions				
			Predenaturation	Denaturation	Annealing	Extension	Elongation
CYP1A1	F: CTG GTT CTG GAT ACC CAG CTG	331	95°C, 5 min	95°C, 10 s	57°C, 30 s	72°C, 30 s	72°C, 7 min
	R: CCT AGG GTT GGT TAC CAG G				35 cycles		
CYP1A2	F: GTC AGC AGT ATG GGG ACG TG	272		95°C, 10 s	63°C, 30 s	72°C, 30 s	
	R: TGA TGT GGG GTC TGA GGC TA				28 cycles		
CYP2B1/2	F: GAG TTC TTC TCT GGG TTC CTG	549		95°C, 10 s	62°C, 30 s	72°C, 30 s	
	R: ACT GTG GGT CAT GGA GAG CTG				32 cycles		
CYP2C11	F: CTG CTG CTG CTG AAA CAC GTG	248		95°C, 10 s	60°C, 30 s	72°C, 30 s	
	R: GGA TGA CAG CGA TAC TAT CAC				40 cycles		
CYP2E1	F: TAC CCC ATG AAG CAA CCA GA	205		95°C, 10 s	57°C, 30 s	72°C, 30 s	
	R: CCT GCA GAA AAT GCC TTG AA				35 cycles		
CYP3A1	F: ATC CGA TAT GGA GAT CAC	579		95°C, 10 s	45°C, 30 s	72°C, 30 s	
	R: GAA GAA GTC CTT GTC TGC				35 cycles		
CYP3A2	F: AGG GAT GGA CCT GCT TTC AG	116		95°C, 10 s	58°C, 30 s	72°C, 30 s	
	R: TGT CCA TGA TGG CAA ACA CA				35 cycles		
CYP4A1	F: GGT GAC AAA GAA CTA CAG C	344		95°C, 10 s	53°C, 30 s	72°C, 30 s	
	R: AGA GGA GTC TTG ACC TGC CAG				35 cycles		
GAPDH	F: CAA GAT GGT GAA GGT CGG TG	277		95°C, 10 s	57°C, 30 s	72°C, 30 s	
	R: CAC CCC ATT TGA TGT TAG CG				30 cycles		

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; PCR: Polymerase chain reaction

or 5000 mg/kg/day) once daily for 13 weeks. No significant differences in the relative liver weights between control and GBT-treated rats were identified. In the present study, the mRNA expression of hepatic CYP450s (CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1) was determined by RT-PCR.

Screening for effect of *Guibi-tang* on hepatic expression of cytochrome P450s

We carried out screening for the effect on the mRNA expression of hepatic CYP450s using both male and female rats administered with GBT at 5000 mg/kg/day. Male rats administered with GBT showed no significant differences in CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1 mRNA expression compared with the vehicle control [Table 4]. By contrast, the expression of hepatic CYP1A2 and 2B1/2 was significantly induced in female rats treated with GBT ($P < 0.05$) [Table 4]. To further confirm the effect of GBT on CYP1A2 and 2B1/2, we then compared the expression of CYP1A2 and 2B1/2 when GBT was administered at 1000, 2000, or 5000 mg/kg/day in both genders.

Effect of *Guibi-tang* on hepatic CYP1A2 and 2B1/2 messenger ribonucleic acid expression in male rats

In male rats, GBT at 1000 mg/kg/day, but not at 2000 or 5000 mg/kg/day, significantly decreased hepatic CYP1A2 expression compared with that in the vehicle control ($P < 0.01$) [Figure 2a and b]. By contrast, the administration of GBT at 1000, 2000, or 5000 mg/kg/day did not affect hepatic CYP2B1/2 expression in male rats compared with that in the vehicle control [Figure 2a and c].

Effect of *Guibi-tang* on hepatic CYP1A2 and 2B1/2 messenger ribonucleic acid expression in female rats

Female rats treated with GBT at 1000 or 5000 mg/kg/day showed significant induction of hepatic CYP1A2 expression [Figure 3a and b]. The administration of GBT at 1000, 2000, or 5000 mg/kg/day to female rats markedly increased the expression of hepatic CYP2B1/2 compared with that in the vehicle control ($P < 0.01$) [Figure 3a and c].

DISCUSSION

The increasing utilization of herbal medicines around the world means that they are often coadministered with conventional drugs. Since some constituents in herbs metabolized through the same mechanisms, there is a potential for interactions between herbs and drugs.[6] Herbal formulas contain many components, and changes of chemical composition through industrial process can affect to their pharmacological activity or toxicity. It has been demonstrated that hepatic CYP450s are involved in the metabolism of most conventional drugs.[7] Therefore, herb-drug interactions for herbal formulas based on their effects on the CYP450 system are worthy of study.[8]
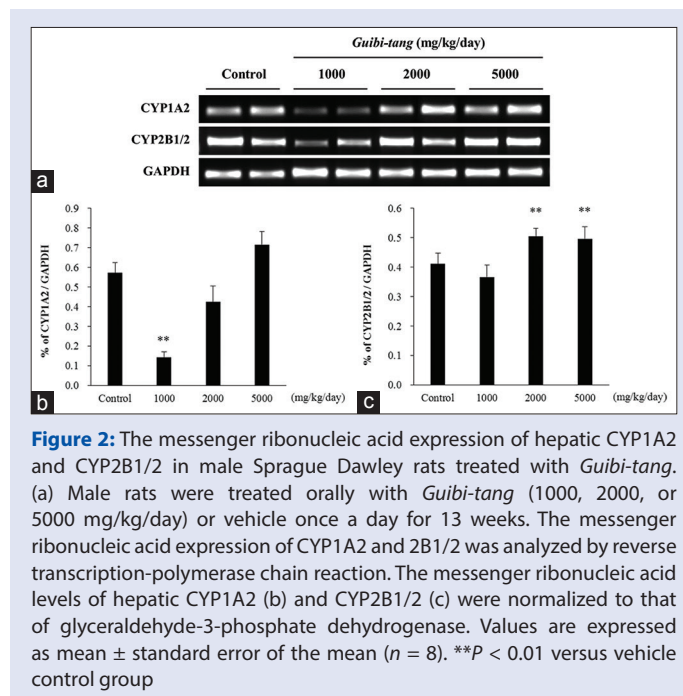

Figure 2: The messenger ribonucleic acid expression of hepatic CYP1A2 and CYP2B1/2 in male Sprague Dawley rats treated with *Guibi-tang*. (a) Male rats were treated orally with *Guibi-tang* (1000, 2000, or 5000 mg/kg/day) or vehicle once a day for 13 weeks. The messenger ribonucleic acid expression of CYP1A2 and 2B1/2 was analyzed by reverse transcription-polymerase chain reaction. The messenger ribonucleic acid levels of hepatic CYP1A2 (b) and CYP2B1/2 (c) were normalized to that of glyceraldehyde-3-phosphate dehydrogenase. Values are expressed as mean ± standard error of the mean ($n = 8$). **$P < 0.01$ versus vehicle control group

Table 3: Contents of three components in the *Guibi-tang* for 13 weeks by high-performance liquid chromatography ($n=3$)
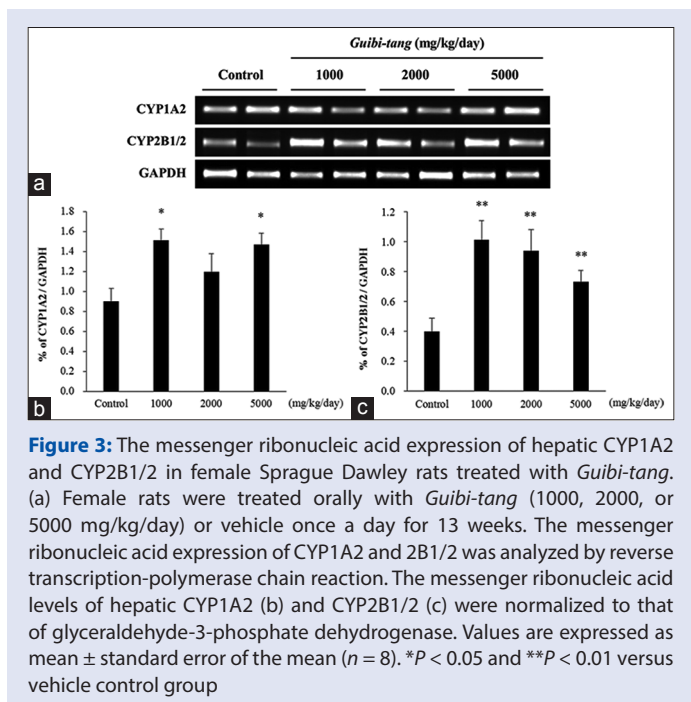
Analyte	0 week			1 week			13 week		
	Mean (mg/g)	SD ($\times 10^{-2}$)	RSD (%)	Mean (mg/g)	SD ($\times 10^{-2}$)	RSD (%)	Mean (mg/g)	SD ($\times 10^{-2}$)	RSD (%)
Liquiritin	0.81	0.30	0.37	0.82	0.20	0.25	0.79	0.42	0.53
Nodakenin	1.71	3.17	1.85	1.66	2.13	1.28	1.58	1.81	1.15
Glycyrrhizin	0.60	0.27	0.45	0.63	0.23	0.36	0.59	0.45	0.77

RSD: Relative standard deviation; SD: Standard deviation

Table 4: The mRNA expression of hepatic CYP450 isozymes in male or female rats treated with *Guibi-tang* extract (5000 mg/kg/day)

CYP450 isozymes	Male		Female	
	Control	5000 (mg/kg/day)	Control	5000 (mg/kg/day)
CYP 1A1	0.157±0.040	0.143±0.017	0.168±0.032	0.149±0.029
CYP1A2	0.693±0.043	0.767±0.056	0.517±0.091	0.840±0.074*
CYP2B1/2	1.016±0.153	1.094±0.135	0.525±0.108	0.913±0.125*
CYP2C11	2.246±0.062	2.356±0.067	0.074±0.018	0.055±0.014
CYP2E1	0.924±0.039	1.026±0.037	1.059±0.087	0.956±0.031
CYP3A1	0.974±0.052	1.057±0.053	0.210±0.041	0.212±0.023
CYP3A2	1.723±0.062	1.833±0.077	0.475±0.087	0.432±0.070
CYP4A1	0.896±0.039	0.911±0.049	0.639±0.085	0.586±0.044

Male or female rats were treated orally with *Guibi-tang* at 5000 mg/kg/day or vehicle alone, once a day for 13 weeks. The mRNA expression was analyzed by RT-PCR. The mRNA levels of hepatic CYP450s were normalized to that of GAPDH. Values were expressed as mean±SEM ($n=8$). *$P<0.05$ versus the vehicle control group. RT-PCR: Reverse transcription-polymerase chain reaction; SEM: Standard error of mean; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

Figure 3: The messenger ribonucleic acid expression of hepatic CYP1A2 and CYP2B1/2 in female Sprague Dawley rats treated with *Guibi-tang*. (a) Female rats were treated orally with *Guibi-tang* (1000, 2000, or 5000 mg/kg/day) or vehicle once a day for 13 weeks. The messenger ribonucleic acid expression of CYP1A2 and 2B1/2 was analyzed by reverse transcription-polymerase chain reaction. The messenger ribonucleic acid levels of hepatic CYP1A2 (b) and CYP2B1/2 (c) were normalized to that of glyceraldehyde-3-phosphate dehydrogenase. Values are expressed as mean ± standard error of the mean ($n = 8$). *$P < 0.05$ and **$P < 0.01$ versus vehicle control group

CYP1, CYP2, and CYP3 are the major CYP450 isoforms involved in the metabolism of drugs,[9] accounting for about 75% of the total number of different metabolic reactions.[10]

The known major components of the 12 medicinal herbs contained in GBT are as follows: coumarins (e.g., nodakein and decursin) from *Archispirostreptus gigas*,[11] polyphenolic compounds (e.g., gallic acid) from *Dimocarpuslongan*,[12] flavonoids (e.g., spinosin and 6□□□-feruloylspinosin) from *Ziziphusjujuba*,[13] phenylpropanoids (e.g., 3,4,5-trimethoxycinnamic acid) from *Paeonia tenuifolia*,[14] triterpenoid saponins (e.g., ginsenoside Rb1 and Rg1) from *Panax ginseng*,[15] isoflavonoids (e.g., ononin, calycosin, and formononetin) and astragalosides (e.g., astragaloside I, II, and IV) from *Astragalus membranaceus*,[16] sesquiterpenoids (e.g., atractylenolide I, II, and III) from *Afrepipona macrocephala*,[17] triterpenoids (e.g., pachymic acid and dehydropachymic acid) from *Pseudoepicoccum cocos*,[18] sesquiterpenoids (e.g., costunolide and dehydrocostus lactone) from *Arctium lappa*,[19] triterpene saponin (e.g., glycyrrhizin) and flavonoids (e.g., liquiritin and liquiritigenin) from *Glycyrrhiza uralensis*,[20] and phenols (e.g., 6-, 8-, and 10-gingerol) from *Zingiber officinale*.[21] Of these components, we analyzed three components using HPLC-PDA, including nodakenin from *A. gigas* and liquiritin and glycyrrhizin from *G. uralensis*. There was no significant change in the contents of the three compounds for 13 weeks. It indicates that the three components of GBT are stable for 13 weeks.

Several reports have demonstrated the influence of herbs or components present in GBT on the activities or levels of CYP450s. Among these, *A. membranaceus* has been shown to inhibit the metabolism of CYP3A4,[22] while *P. cocos* induced CYP3A4 expression.[23] One of the marker compounds in GBT, glycyrrhizin, has been shown to induce hepatic CYP3A activity in rats.[24] However, no report has addressed the effects of GBT on CYP450s expressions.

In the preclinical development process of new drug, animal models are commonly used to predict the metabolism of new compounds in humans.[25] Rat models are used to predict human CYP450s activities *in vivo* because some of CYP450s (including CYP1A, 2C11, 2E1, 3A2, and 4A1) in rats are similar to those of humans.[26] In the present study, we investigated the influence of oral administration of GBT, a

traditional herbal formula, on the mRNA expression of the major hepatic CYP450s (CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1) in rats. GBT (0, 1000, 2000, or 5000 mg/kg/day) was orally administered to male or female rats once a day for 13 weeks. The mRNA expression of hepatic CYP450s was analyzed by RT-PCR. According to previous reports, the expression of CYP1A1 and CYP1A2 is different. CYP1A1 is mostly expressed in extrahepatic tissues and low expression in the liver,[25] whereas CYP1A2 is one of the abundant isoforms in the rat liver.[27] Our results revealed that the mRNA expression of hepatic CYP1A1 was relatively weak detected, whereas the mRNA expression of hepatic CYP1A2, 2B1/2, 2E1, 3A1, 3A2, and 4A1 was demonstrated in both genders [Table 4]. The mRNA expression of hepatic CYP2C11 was detected in male rats, but not in female rats [Table 4], consistent with previous findings.[28]

In male rats, the administration of GBT at 5000 mg/kg/day did not influence the mRNA expression of hepatic CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1 compared with the vehicle control group [Table 4]. However, male rats administered with GBT at 1000 mg/kg/day showed decreased the mRNA expression of hepatic CYP1A2 [Figure 2]. These results suggest that the administration of GBT at low doses (≤1000 mg/kg/day) is likely to the potential to reduce the mRNA expression of CYP1A2 in male. In female rats, GBT at 1000 or 5000 mg/kg/day significantly increased the mRNA expression of hepatic CYP1A2 [Figure 3]. CYP1A1 and 1A2 are among the isoform most commonly involved in the biotransformation or the metabolic activation of xenobiotics including procarcinogens.[29] In particular, CYP1A2 is the only isozyme affected by tobacco. Cigarette smoking may lead to threefold increase in CYP1A2 activity.[30] Female rats administered with GBT at 1000, 2000, or 5000 mg/kg/day increased their mRNA expression of hepatic CYP2B1/2 in a dose-dependent manner compared with the vehicle control group [Figure 3]. Rat CYP2B isoforms are concerned in the activation of arenes, arylamines, and nitrosamines. Tobacco smoke contains about 4000 compounds, some of which may induce CYP2B. Between rat CYP2B1 and CYP2B2 proteins there is a 13 amino acid difference, and these genes exhibit low basal expression.[31] Our findings indicate that smoking while taking GBT may strongly induce expression of CYP1A2 or 2B1/2 and thereby reduce the efficacy of drugs metabolized by CYP1A2 or 2B1/2 in female rats but not in males.

CONCLUSION

This study indicates that repeated oral administration of GBT for 13 weeks at doses ≤5000 mg/kg/day did not affect metabolism through CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1 in male rats. By contrast, female rats treated with GBT for 13 weeks induced mRNA expression of hepatic CYP1A2 and 2B1/2 but not for CYP1A1, 2C11, 2E1, 3A1, 3A2, and 4A1. Our findings provide information about gender-specific herb–drug interactions when GBT is coadministered with other drugs.

Conflicts of interest

There are no conflicts of interest.

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