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

Seong Eun Jin¹, Hyeyung Ha¹, Chang-Seob Seo¹, Hyeun-Kyoo Shin¹, Soo-Jin Jeong²,³

¹K-herb Research Center, Korea Institute of Oriental Medicine, ²KM Convergence Research Division, Korea Institute of Oriental Medicine, ³Korean Medicine Life Science, University of Science and Technology, Daejeon, Republic of Korea

Submitted: 17-03-2017 Revised: 13-06-2017 Published: 31-01-2018

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 isoforms (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 CYP1A2 and 2B1/2 but not for CYP1A1, 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

INTRODUCTION

The utilization of herbal medicines as complementary and alternative medicines is becoming increasingly popular for preventing and treating various diseases. 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. Biologically, GBT has been reported to have anti-stress effects and beneficial effects on gastrointestinal and immune-mediated diseases. 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      | Bonghw, 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. macrophala   | Compositae      | Rhizoma        | 3.750      | China                         |
| P. cocos        | Polyporaceae    | Sclerotum      | 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. macrophala: Atractylodes macrophala; 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 Kgwangmyungdang 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 (IIShinBioBase, 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 Prominance 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), 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 (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 µL/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, 3A1, 3A2, 4A1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplification are described in Table 2. 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, 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.

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 | 35 cycles | 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 TGG GGC TTC TCA GGC TA | | | 28 cycles | | |
| CYP2B1/2 F: GAG TTC TTC TCT TCG GGC TGC CTG | 549 | 95°C, 10 s | 62°C, 30 s | 72°C, 30 s | | |
| R: ACT GTG GGT CAT CAG 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 CTG TGC 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 TGT 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

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,
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. 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. Therefore, herb–drug interactions for herbal formulas based on their effects on the CYP450 system are worthy of study.

**Table 3:** Contents of three components in the Guibi-tang for 13 weeks by high-performance liquid chromatography (n=3)

| Analyte    | Mean (mg/g) | SD (×10⁻²) | RSD (%) | Mean (mg/g) | SD (×10⁻²) | RSD (%) | Mean (mg/g) | SD (×10⁻²) | RSD (%) |
|------------|-------------|-------------|---------|-------------|-------------|---------|-------------|-------------|---------|
| Liqiritin  | 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±0.0566  | 0.517±0.091   | 0.640±0.074*    |
| CYP2B1/2       | 1.016±0.153   | 1.094±0.135   | 1.025±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 (5000 mg/kg/day) or vehicle 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
CYP1, CYP2, and CYP3 are the major CYP450 isoforms involved in the metabolism of drugs, 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., nodakenin and decursin) from Archispirostreptus gigas,[11] polyphenolic compounds (e.g., gallic acid) from Dimocarpus longan,[12] flavonoids (e.g., spinosin and 6'-, 8'-, and 10'-gingerol) from Zingiber officinale, [13] sesquiterpenoids (e.g., atractylenolide) from Pseudopiptadenium macrocephala,[14] triterpenoid saponins (e.g., ginsenoside Rβ1 and Rg1) from Panax ginseng,[15] isoflavonoids (e.g., ononin, calycosin, and formononetin) and astragalsides (e.g., astragaloside I, II, and IV) from Astragalus membranaceus,[16] sesquiterpenoids (e.g., atracylenolide I, II, and III) from Afropipana macrocephala,[17] triterpenoids (e.g., pachymic acid and dehydropachymic acid) from Pseudopiptadenium 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 licoritn 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, 3A1, 3A2, and 4A1) 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;[27] whereas CYP1A2 is one of the abundant isoforms in the rat liver.[28] 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.[29]

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 isofrom most commonly involved in the biotransformation or the metabolic activation of xenobiotics including procarcinogens.[30] In particular, CYP1A2 is the only isozyme affected by tobacco. Cigarette smoking may lead to threefold increase in CYP1A2 activity.[31] 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 CYP2B1 isoforms are concerned in the activation of ares, alylamine, 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.[32] 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 in male rats. Our findings provide information about gender-specific herb-drug interactions when GBT is coadministered with other drugs.

Acknowledgements
This research was supported by a grant for “Construction of Scientific Evidences for Herbal Medicine Formulas (K17251)” from the Korea Institute of Oriental Medicine.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.
REFERENCES

1. Kelly JP, Kaufman DW, Kelley R, Rosenberg L, Anderson TE, Mitchell AA, et al. Recent trends in use of herbal and other natural products. Arch Intern Med 2005;165:2816.
2. Hur J, Dongubogam. Seoul, Korea: Namsandang; 2004. p. 98.
3. Kim HJ, Choi JH, Lim SW. The defensive effect of Keuib-tang on the gastric mucus membrane of mice injured by stress and ethanol. J Korean Med 2003;24:155-68.
4. Busta I, Xie H, Kim MS. The use of Guipi-tang in small animals with immune-mediated blood disorders. J Vet Clin 2009;26:181-4.
5. Mrozikiewicz PM, Bogacz A, Karasievwicz M, Mikołajczuk PL, Orazowsky M, Seremak M. The effect of standardized Echinacea purpureae extract on rat cytochrome P450 expression level. Pyrtome 2010;17:830-3.
6. Venkataramanan R, Komoroski B, Strom S. In vitro and in vivo assessment of herb drug interactions. Life Sci 2003;78:2105-15.
7. Flockhart DA, Oesterheld JR. Cytochrome P450-mediated drug interactions. Child Adolesc Psychiat Clin N Am 2000;9:43-76.
8. Ito AA, Ernst E. Interactions between herbal medicines and prescribed drugs: A systematic review. Drugs 2001;61:163-75.
9. Kobayashi K, Unadaga K, Shimada N, Chiba K. Selectivies of human cytochrome P450 inhibitors toward rat P450 isoforms: Study with cDNA-expressed systems of the rat. Drug Metab Dispos 2003;31:833-4.
10. Furge LL, Guengerich FP. Cytochrome P450 enzymes in drug metabolism and chemical toxicology. An introduction. Biochem Mol Biol Educ 2006;34:66-74.
11. Ahn NJ, Lee MK, Kim YC, Sung SH. The simultaneous determination of coumarins in Angelica gigas root by high performance liquid chromatography-diode array detector coupled with electrospray ionization/mass spectrometry. J Pharm Biomed Anal 2009;49:258-66.
12. Sudarpoen Y, Hui WE, Erben G, Württele G, Changbumrung S, Ulrich CM, et al. Isolation and characterization of ellagitannins as the major polyphenolic components of longan (Dimorpusorum lour) seeds. Phytochemistry 2012;77:226-37.
13. Niu C, Zhang Y. Quantitative analysis and chromatographic fingerprinting of the Senen Zizyphi Spinoseae by ultra-high-performance liquid chromatography coupled with diode-array detector. J Sep Sci 2011;34:2989-96.
14. Kawashima K, Miyako D, Ishino Y, Makino T, Saito K, Kano Y, et al. Anti-stress effects of 3,4,5-trimethoxyxynamic acid, an active constituent of roots of Angelica tenuifolia (Onji). Biol Pharm Bull 2004;27:1317-9.
15. Shan SM, Luo JG, Huang F, Gong LY. Chemical characteristics combined with bioactivity for comprehensive evaluation of Panax ginseng C.A. Meyer in different ages and seasons based on HPLC-DAD and chemometric methods. J Pharm Biomed Anal 2014;89:76-82.
16. Song JZ, Yiu HH, Qiao CF, Han QB, Xu HX. Chemical comparison and classification of radix astragali by determination of isolavonoids and astragalosides. J Pharm Biomed Anal 2008;47:399-406.
17. Tsai CJ, Liang JW, Lin HR. Sesquiterpenoids from Atractylodes macrocephala act as farnesoid X receptor and progesterone receptor modulators. Biogor Med Chem Lett 2012;22:2328-9.
18. Li G, Xu ML, Lee CS, Woo MH, Chang HW, Son JK, et al. Cytotoxicity and DNA topoisomerase inhibitory activity of constituents from the sclerotium of Poria cocos. Arch Pharm Res 2004;27:829-33.
19. Li A, Sun A, Liu R. Preparative isolation and purification of costunolide and dehydrocostus lactone from Aucklandia Japa Decne by high-speed countercurrent chromatography. J Chromatogr A 2005;1076:193-7.
20. Zhang Q, Ye M. Chemical analysis of the Chinese herbal medicine Gan-Cao (Licorice). J Chromatogr A 2009;1216:1954-69.
21. Zick SM, Ruffin MT, Dunic Z, Normolle D, Brenner DE. Quantitation of 6-, 8- and 10-gingerols and 6-shogaol in human plasma by high-performance liquid chromatography with electrochemical detection. Int J Biomed Sci 2010;6:233-40.
22. Pao LH, Hu OY, Fan HY, Lin CC, Liu LC, Huang PW, et al. Herb-drug interaction of 50 Chinese herbal medicines on CYP3A4 activity in vitro and in vivo. Am J Chin Med 2012;40:57-73.
23. Song HY, Shao JW, Chen JF, Wang T, Lin FP, Guo YH, et al. Transcriptional regulation of cytochrome P450 3A4 by four kinds of traditional Chinese medicines. Zhongguo Zhong Yao Za Zhi 2008;33:1014-7,1089.
24. Tai T, Huang X, Su Y, Ji J, Su Y, Jiang Z, et al. Glycyrhrizin accelerates the metabolism of triptolide through induction of CYP3A in rats. J Ethnopharmacol 2014;152:358-63.
25. Martignoni M, Groothuis GM, de Kanter R, Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol 2006;2:875-94.
26. Bogaards JJ, Bertrand M, Jackson P, Oudshoorn MJ, Weaver RJ, van Bladeren PJ, et al. Determining the best animal model for human cytochrome P450 activities: A comparison of mouse, rat, rabbit, dog, micropig, monkey and man. Xenobiota 2000;30:113-62.
27. Wu R, Cui X, Dong W, Zhou M, Simms HH, Wang P, et al. Suppression of hepatocyte CYP1A2 expression by Kupffer cells via AHR pathway: The central role of proinflammatory cytokines. Int J Mol Sci 2008;10:339-46.
28. Chen J, Murray M, Liddle C, Jiangu XM, Darrell GC. Downregulation of male-specific cytochrome P450s 2C11 and 3A2 in bile duct-ligated male rats: Importance to reduced hepatic content of cytochrome P450 in cholestasis. Hepatology 1995;22:580-7.
29. Cheung FJ, Gonzalez FJ, Humanized mouse lines and their application for prediction of human drug metabolism and toxicological risk assessment. J Pharm Biomed Anal 2008;29:289-99.
30. Badyal DK, Dadhich AP. Cytochrome P450 and drug interactions. Indian J Pharmacol 2001;33:248-59.
31. Gonzalez FJ. The molecular biology of cytochrome P450s. Pharmaco Rev 1988;40:243-88.