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Research Paper

Evaluation of the impact of *Polygonum capitatum*, a traditional Chinese herbal medicine, on rat hepatic cytochrome P450 enzymes by using a cocktail of probe drugs

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ABSTRACT

Ethnopharmacological relevance: *Polygonum capitatum* is a well-known Miao medicinal plant that has been used for many years for its unique therapeutic effects on various urological disorders, including urinary calculus and urinary tract infections. To investigate the effect of *Polygonum capitatum* on cytochrome P450 (CYP) isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2E1, and CYP3A4) *in vivo* using a "cocktail" approach by administering five probe drugs to rats. This study assessed the potential of *Polygonum capitatum* to interact with co-administered drugs.

Materials and methods: An aqueous extract of dried whole *Polygonum capitatum* was prepared and administered orally to rats at a dose of 0.58 g/kg or 1.74 g/kg twice daily for 7 or 14 consecutive days. A cocktail of caffeine (1.0 mg/kg), tolbutamide (1.0 mg/kg), omeprazole (2.0 mg/kg), chlorzoxazone (4.0 mg/kg) and midazolam (4.0 mg/kg) was then administered on the eighth or fifteenth day to evaluate the effects of *Polygonum capitatum* on CYP1A2, 2C9, 2C19, 2E1, and 3A4, respectively. Blood samples were collected at a range of time-points and the plasma concentrations of the probe drugs were simultaneously quantified using ultra high-performance liquid chromatography–tandem mass spectrometry. Pharmacokinetic parameters were calculated to evaluate the effects of *Polygonum capitatum* on the activities of these CYP enzymes in rats.

Results: *Polygonum capitatum* pre-treatment had no significant effect on the pharmacokinetic parameters of caffeine, omeprazole or chlorzoxazone. However, the pharmacokinetics of tolbutamide and midazolam were affected significantly ($P < 0.05$) by *Polygonum capitatum*, which induced more rapid metabolism of these probe compounds.

Conclusions: These results suggested that *Polygonum capitatum* could induce CYP2C9 and CYP3A4, and did not influence CYP1A2, CYP2C19 or CYP2E1. Therefore, the clinical dose of drugs metabolized by human CYP2C9 or CYP3A4 may need to be adjusted in patients taking *Polygonum capitatum*, as this herbal medication may result in reduced effective concentrations of these drugs.

1. Introduction

Herbs are increasingly employed worldwide in alternative and complementary therapies. Herbal remedies have increased in popularity as alternative medicines in the Western world over recent decades, and a study published in 2008 estimated that they were used by approximately 20% of the general population in the US (Bent, 2008). Taking herbs together with prescribed western medication places patients at a potential risk for herb–drug interactions (HDIs) (Han et al., 2012). A 2007 survey demonstrated that 15% of patients receiving conventional pharmacotherapy were also taking herbal medicines and potential adverse HDIs were observed in 40% of these patients (Bush et al., 2007). Some patients take the decision to medicate with several different herbs and herbal preparations themselves, without consulting their doctor (Kelly et al., 2005).

Many studies have produced evidence for the induction or inhibition of drug-metabolizing enzymes by herbs (Zhou et al., 2003; Gorski et al., 2004; Gao et al., 2013). Consequently, many significant HDIs have been reported (Ueng and Chen, 2004). Large numbers of undesired effects can occur, including impaired bioavailability of drugs with narrow therapeutic indexes, altered plasma or tissue levels, or enhanced bioactivation of drugs to reactive or toxic intermediates. Further research is required to provide more comprehensive information regarding these potential HDIs (Ernst, 2000; Izzo, 2004; Magee, 2005).

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Polygonum capitatum [Chinese name: Tou-hua-liao, Latin name: *Polygonum capitatum* Buch Ham. ex D Don] belongs to the Polygonaceae family and is a well-known Miao herb which has been widely used in China for the treatment of various urological disorders, including urinary calculus and urinary tract infections (Editorial Committee of Chinese Materia Medica, 2005). A number of *Polygonum capitatum*-based drugs (*e.g.*, Relinqing® granules and Milin® capsules) have been approved by the Chinese State Food and Drug Administration (Liao et al., 2011). Both Relinqing® granules and Milin® capsules are made using aqueous extracts of the whole *Polygonum capitatum* plant, with some other excipients. Relinqing® is the best-selling Chinese patent drug for treatment of urinary system diseases. It is always co-administered with quinolones, macrolides and other antibiotics. Cytochrome P450 (CYP) enzymes are essential for the metabolism of many medications, including the majority of antibiotics (Theuretzbacher and Zeitlinger, 2011). However, no systematic study of the impact of *Polygonum capitatum* on hepatic CYP enzyme activities has been reported to date.

Numerous HDIs have been investigated by using cocktails of probe drugs, in which the activities of several CYP enzymes can be assessed through determination of the clearance of the probe drugs. This approach gains many advantages over the traditional methods since it can characterize the inhibition or induction potential of herbs widely used toward the CYP enzymes identified *in vitro* or *in vivo*, assess several enzymes in one trial to avoid costly and time-consuming drug interaction trials, and obtain complete *in vivo* information about potential CYP-based drug interactions. Among the CYPs identified, five human CYP enzymes (*i.e.*, CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4) are responsible for approximately 80% of CYP-mediated drug metabolism (Lewis, 2003; Daly, 2004; Ingelman-Sundberg, 2004).

To investigate the effects of *Polygonum capitatum* on rat hepatic CYP enzymes, this study adopted a probe cocktail approach using caffeine (CYP1A2), tolbutamide (CYP2C9), omeprazole (CYP2C19), chlorzoxazone (CYP2E1) and midazolam (CYP3A4). Extremely low doses of the probes were employed: 1 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg and 4 mg/kg, respectively. This minimized the potential for pharmacological interactions within the cocktail, without dropping below the drug assay detection limits (Rodeiro et al., 2009). An ultra-high-performance liquid chromatography–tandem mass spectrometry (UPLC–MS) method was used for the simultaneous detection of these five probe compounds and an internal standard, providing a highly selective and sensitive assay with low limits of quantification.

2. Materials and methods

2.1. Materials and plant material

Omeprazole (product no. 20130408), tolbutamide (product no. 20120910) and chlorzoxazone (product no. 20121115) were obtained from Dalian Meilun Biology Technology Co., Ltd. (Liaoning, China); midazolam (product no. 20081213) was obtained from the Jiangsu Nhwa Pharmaceutical Co., Ltd. (Jiangsu, China); caffeine (product no. 12003) was obtained from the China National Institute of Metrology and the internal standard (IS), puerarin (product no. 0752-9605) was obtained from the China National Institutes for Food and Drug Control. High-performance liquid chromatography (HPLC)-grade acetonitrile and formic acid were supplied by Merck (Darmstadt, Germany). Distilled water was obtained from Watsons Group Co., Ltd. (Hong Kong); all other reagents were of analytical grade and obtained from Kermel Technology Co., Ltd. (Tianjin, China).

Whole *Polygonum capitatum* plants were collected from the Good Agricultural Practice (GAP) Base of Tou-hua-liao in Shibing (Guizhou, China) in August 2011, and a specimen (with accession number of PC 20110819) was deposited at the Herbarium of Guiyang Medical University for future reference.

2.2. Preparation of Polygonum capitatum extract

Whole dried *Polygonum capitatum* herbs (3 kg) were boiled in 30 L water for 2 h and then filtered. The residual herbs were added to 24 L of water and boiled for another 2 h. The two extracts were then combined, dried, ground and the resulting powder was used in subsequent experiments. The extraction yield was 17.6% (g g^{-1}), in which gallic acid, protocatechuic acid, myricitrin, hirsutrin, quercitrin, quercetin-3-O-a-L-rhamnoside, and quercetin were determined by UPLC–MS.

2.3. Animals and treatment

Specific pathogen-free male Sprague–Dawley rats (200 ± 20 g) were provided by Guiyang Medical University Laboratory Animal Center (Certificate no. SCXK 2002-0001). Before the experiments, the rats were allowed one week of acclimatization in the animal quarters under air conditioning (25 ± 1 °C) and an automatically controlled photoperiod of 12 h light daily, fed with standard rodent chow and tap water *ad libitum*. The experimental procedures were in compliance with the guidelines of the Committee on the Care and Use of Laboratory Animals in China. A low dose (0.58 g/kg) or high dose (1.74 g/kg) of *Polygonum capitatum* water extract was administered twice a day in our study. In view of the clinical use of *Polygonum capitatum*, we studied the effect of *Polygonum capitatum* on rat CYP enzymes after rats had received oral *Polygonum capitatum* for 7 consecutive days (short period) or 14 consecutive days (long period).

Thirty rats were randomly divided into 5 groups ($n=6$ per group): blank control group (BCG); short period and low dosage group (7d-L); short period and high dosage group (7d-H); long period and low dosage group (14d-L); long period and high dosage group (14d-H). The BCG received water for 10 days. The *Polygonum capitatum* extract powder was dissolved in water and each group of animals was administered either 0.58 g/kg or 1.74 g/kg of the extract by oral gavage (i.g.) twice a day for either 7 or 14 consecutive days, as appropriate.

2.4. Sample preparation and analytical methods

On either the eighth or fifteenth day, 5 ml/kg of probe cocktail solution, containing caffeine (1.0 mg/kg), tolbutamide (1.0 mg/kg), omeprazole (2.0 mg/kg), chlorzoxazone (4.0 mg/kg) and midazolam (4.0 mg/kg), was injected through the caudal vein of all rats in each group. Heparinised blood samples were then collected 5 min, 10 min, 20 min, 40 min, 1 h, 2 h, 3 h, 5 h, 8 h, 12 h, and 24 h after dosing and immediately centrifuged at 5000 rpm at room temperature for 5 min to obtain 100-μL rat plasma samples. These were stored at −20 °C until UPLC–MS analysis.

To each 100 μL of rat plasma sample, the internal standard (IS) (100 μL of 2 μg/mL puerarin) was added before extraction with 100 μL of methanol, vortexing for 5 min and centrifugation for 10 min at 15,000 rpm. The organic layer was transferred to another tube and then evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 400 μL of the mobile phase, centrifuged at 15,000 rpm for 5 min, and 1 μL of the solution was injected into UPLC–MS.

An Acquity TM UPLC™ system was used for separation and tandem MS was used for detection. Chromatographic separation was achieved on a Waters Acquity BEH C18 column (2.1 × 50 mm^2, i.d. 1.7 μm, Waters, Wexford, Ireland). The mobile phase consisted of acetonitrile containing 0.1% formic acid (A) and water containing

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0.1% formic acid (B). The elution gradient was as follows: 10% A (0 min), 65% A (3 min), 90% A (3.5 min), 10% A (4.0 min), and 10% A (4.5 min). The flow rate was 0.35 mL min^{-1}, and the column temperature was set at 45 °C.

A Waters TQD Quantum triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source was used for mass analysis and detection. The mass spectrometer was operated in either positive mode or in negative mode, with the main working parameters set as follows: desolvation temperature, 350 °C; nebulizer gas (N_2), 650 L/h; source heater, 120 °C. The selected or single ion recording (SIR) mode was chosen for quantification of the probe substrates (Table 1). The scan time for each analyte was set at 0.1 s. Data acquisition and processing were conducted on Micromass Masslynx v4.1. The probes were quantified according to a validated UPLC–MS method.

2.5. Method validation

The linearity was evaluated by analyzing calibration standards at each concentration level on three consecutive days. The accuracy and precision were assessed by analyzing QC samples in six replicates at three concentration levels on three days. The extraction recovery was evaluated at three concentration levels and for the I.S. at one concentration level by comparing the peak areas of the analytes obtained from six samples with the analytes spiked before and after extraction. Matrix effect was assessed by comparing the peak areas of the analytes obtained from six samples with the analytes spiked after extraction, at three concentration levels, to those for the neat standard solutions at the same concentrations. The stability of the analytes in plasma at three concentration levels was evaluated under a different storage and process conditions.

2.6. Statistical analysis

Data were presented as mean ± standard deviation (S.D.). Pharmacokinetic parameter calculations were carried out using the DAS 2.0 pharmacokinetic program (Mathematical Pharmacology Professional Committee of China, Shanghai, China), and generated by a non-compartmental model (statistical moment). Statistically significant differences between the pharmacokinetic parameters of the treatment groups and BCG were assessed by one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of statistical significance set at 0.05.

3. Results

3.1. Method validation

Fig. 1 shows the typical chromatograms of blank plasma, the plasma spiked with five cocktail probe drugs and the I.S., and a plasma sample obtained from a rat 10 min after intravenous administration of five cocktail probe drugs. No interference from endogenous substances was observed at the retention time of the analytes and the I.S.

Calibration curves showed good linearity over the range of 0.03–6.00 μg/mL for omeprazole (r=0.9998), 0.03–5.00 μg/mL for caffeine (r=0.9994), 0.29–50.00 μg/mL for midazolam (r=0.9997), 0.21–35.35 μg/mL for chlorzoxazone (r=0.9993) and 0.06–10.20 μg/mL for tolbutamide (r=0.9995). The LLODs of omeprazole, caffeine, midazolam, chlorzoxazone and tolbutamide were 6.48, 9.35, 2.09, 1.91 and 3.28 ng/mL, respectively.

The intra- and inter-day RSDs of the five analytes were less than 12.1% and 16.4%, and the accuracy was from −8.9% to 9.1% for all the analytes. The mean extraction recoveries of three level QC samples were 93.5 ± 5.5%, 83.4 ± 11.4% and 87.5 ± 11.5% for omeprazole, 94.8 ± 8.3%, 79.7 ± 5.9% and 80.3 ± 5.9% for caffeine, 98.3 ± 9.6%, 102.1 ± 16.6% and 85.8 ± 13.6% for midazolam, 102.0 ± 16.7%, 96.7 ± 12.9% and 80.9 ± 6.1% for chlorzoxazone and 96.5 ± 11.3%, 88.4 ± 2.5% and 79.1 ± 4.5% for tolbutamide. The mean recovery of I.S. was 92.3 ± 3.6%. For the five analytes at three QC levels in rat plasma, the matrix effects calculated were between 82.4% and 92.3%; it was demonstrated that the plasma matrix effect was negligible for the assay.

The stability study showed that the five analytes were all stable under all testing conditions, including short-term storage (6 h at room temperature), long-term storage (2 weeks at −20 °C), freeze-thaw cycling, and post-preparative storage. The REs calculated from the QCs under all testing conditions ranged from −13.6% to 11.2%.

3.2. Determination of main components in Polygonum capitatum

The major active components of *Polygonum capitatum* extracts, gallic acid, protocatechuic acid, myricitrin, hirsutrin, quercitrin, quercetin-3-O-a-L-rhamnoside and quercetin were determined by UPLC–MS and compared with authenticated standards. The results showed that 1 g of aqueous extract contained 14.41 ± 0.95 mg (R.S.D.=6.57%), 0.43 ± 0.03 mg (R.S.D.=5.92%), 0.25 ± 0.02 mg (R.S.D.=8.26%), 0.56 ± 0.03 mg (R.S.D.=4.54%), 2.11 ± 0.15 mg (R.S.D.=7.22%), 0.46 ± 0.03 mg (R.S.D.=5.38%) and 1.10 ± 0.05 mg (R.S.D.=4.56%) of these constituents, respectively (n=6).

3.3. Effects of Polygonum capitatum on rat hepatic CYP1A2

The pharmacokinetic parameters ($AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $MRT_{(0-t)}$, $MRT_{(0-\infty)}$, $t_{1/2}$, CL, and V) of caffeine in rats from the different *Polygonum capitatum* extract treatment groups are presented in Table 2. There were no significant differences between the treatment groups and the BCG, indicating that *Polygonum capitatum* did not influence rat CYP1A2 activity *in vivo*.

3.4. Effects of Polygonum capitatum on rat hepatic CYP2C9

CYP2C9 activity was evaluated by comparing tolbutamide pharmacokinetics in the study groups (Table 3). The mean plasma concentration–time curves of tolbutamide in the indicated study groups are presented in Fig. 2a. In all groups of rats pre-treated with *Polygonum capitatum* extract, the $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $MRT_{(0-t)}$, $MRT_{(0-\infty)}$ and $t_{1/2}$ of tolbutamide were decreased significantly, as compared to those observed in the BCG. In addition, CL was significantly increased in all groups pre-treated with *Polygonum capitatum* extract and V increased significantly in the 7d-H, 14d-L and 14d-H groups. Taken together, these results indicated that tolbutamide metabolism was accelerated to varying degrees in these treatment groups, as compared with the BCG. These suggested that *Polygonum capitatum* extract had the potential to induce CYP2C9 activity *in vivo*.

Table 1
SIR transitions and cone voltages for the detection of the CYP probe drugs and internal standard.

Probe drugs	Polarity	Molecular mass	Parent (m/z)	Cone (V)
Caffeine	ESI+	194	195	35
Tolbutamide	ESI−	270	269	40
Omeprazole	ESI+	345	346	35
Chlorzoxazone	ESI−	169	168	35
Midazolam	ESI+	325	326	30
Puerarin (IS)	ESI+	416	417	40

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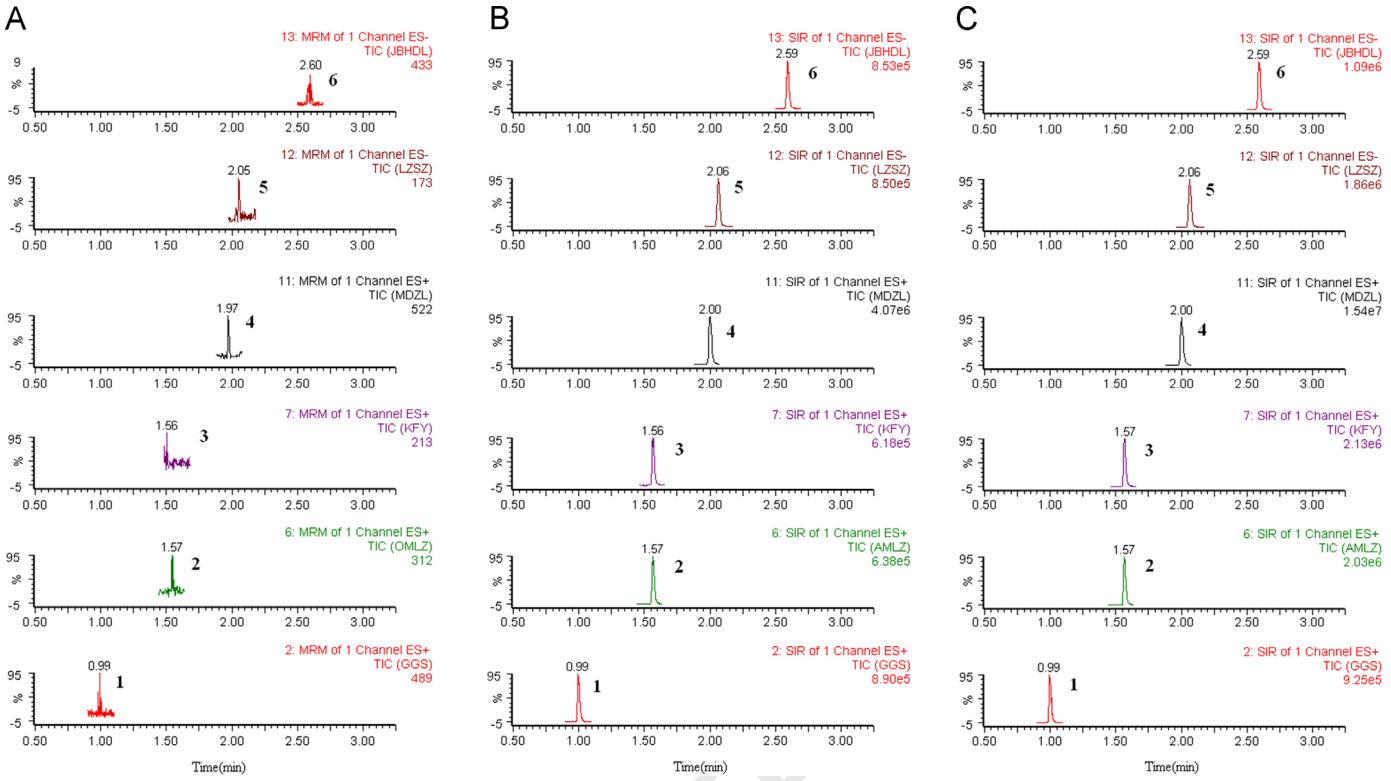

Fig. 1. Chromatograms of five cocktail probe drugs and puerarin (internal standard [I.S.]) in rat plasma. blank plasma sample (A); plasma sample spiked with five cocktail probe drugs and I.S.(B); and plasma sample obtained from a rat 10 min after intravenous administration of five cocktail probe drugs(C). 1. Puerarin (I.S.), 2. Omeprazole, 3. Caffeine, 4. Midazolam, 5. Chlorzoxazone, and 6. Tolbutamide.

Table 2
Effects of *Polygonum capitatum* extract on the pharmacokinetic parameters of caffeine (1 mg/kg).

Parameters	BCG	Treatment groups			
		7d-L	7d-H	14d-L	14d-H
$AUC_{(0-t)}$ (mg/L h)	0.68 ± 0.24	0.74 ± 0.32	0.65 ± 0.17	0.79 ± 0.50	0.43 ± 0.20
$AUC_{(0-\infty)}$ (mg/L h)	0.70 ± 0.24	0.80 ± 0.39	0.70 ± 0.19	0.86 ± 0.56	0.45 ± 0.23
$MRT_{(0-t)}$ (h)	0.15 ± 0.05	0.23 ± 0.08	0.22 ± 0.03	0.24 ± 0.06	0.20 ± 0.06
$MRT_{(0\infty)}$ (h)	0.20 ± 0.07	0.29 ± 0.14	0.28 ± 0.07	0.32 ± 0.16	0.23 ± 0.09
$t_{1/2}$ (h)	0.29 ± 0.21	0.21 ± 0.12	0.21 ± 0.05	0.26 ± 0.13	0.18 ± 0.07
CL (L/h/kg)	1.58 ± 0.56	1.52 ± 0.66	1.88 ± 0.42	1.54 ± 0.75	1.61 ± 0.97
V (L/kg)	0.68 ± 0.55	0.40 ± 0.22	0.50 ± 0.10	0.49 ± 0.16	1.04 ± 0.65

BCG, blank control group (water for 10 days); 7d-L, 0.58 g/kg i.g. *Polygonum capitatum* extract twice daily for 7 consecutive days; 7d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 7 consecutive days; 14d-L, 0.58 g/kg i.g. *Polygonum capitatum* extract twice a day for 14 consecutive days; 14d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 14 consecutive days. Values are presented as mean ± S.D. ($n=6$).

Table 3
Effects of *Polygonum capitatum* extract on the pharmacokinetic parameters of tolbutamide (1 mg/kg).

Parameters	BCG	Treatment groups			
		7d-L	7d-H	14d-L	14d-H
$AUC_{(0-t)}$ (mg/L h)	54.45 ± 0.98	33.63 ± 8.52**	21.18 ± 6.21**	27.68 ± 3.72**	28.09 ± 5.53**
$AUC_{(0-\infty)}$ (mg/L h)	61.48 ± 1.45	34.99 ± 8.93**	22.33 ± 6.82**	30.21 ± 5.48**	29.32 ± 5.34**
$MRT_{(0-t)}$ (h)	8.03 ± 0.20	5.98 ± 0.75**	4.78 ± 1.48**	6.40 ± 0.65**	5.28 ± 2.27**
$MRT_{(0-\infty)}$ (h)	11.20 ± 0.79	6.96 ± 1.46**	5.86 ± 2.09**	8.60 ± 2.69*	6.39 ± 1.92**
$t_{1/2}$ (h)	8.10 ± 0.65	4.99 ± 1.51**	4.47 ± 1.78**	6.58 ± 1.06*	5.46 ± 0.70**
CL (L/h/kg)	0.016 ± 0.004	0.031 ± 0.007**	0.050 ± 0.018**	0.034 ± 0.006**	0.035 ± 0.006**
V (L/kg)	0.19 ± 0.01	0.21 ± 0.06	0.28 ± 0.06**	0.31 ± 0.05**	0.28 ± 0.07*

BCG, blank control group (water for 10 days); 7d-L, 0.58 g/kg i.g. *Polygonum capitatum* extract twice daily for 7 consecutive days; 7d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 7 consecutive days; 14d-L, 0.58 g/kg i.g. *Polygonum capitatum* extract twice a day for 14 consecutive days; 14d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 14 consecutive days. Values are presented as mean ± S.D. ($n=6$).

* $P<0.05$ when compared with related parameters of BCG group.
** $P<0.01$ when compared with related parameters of BCG group.

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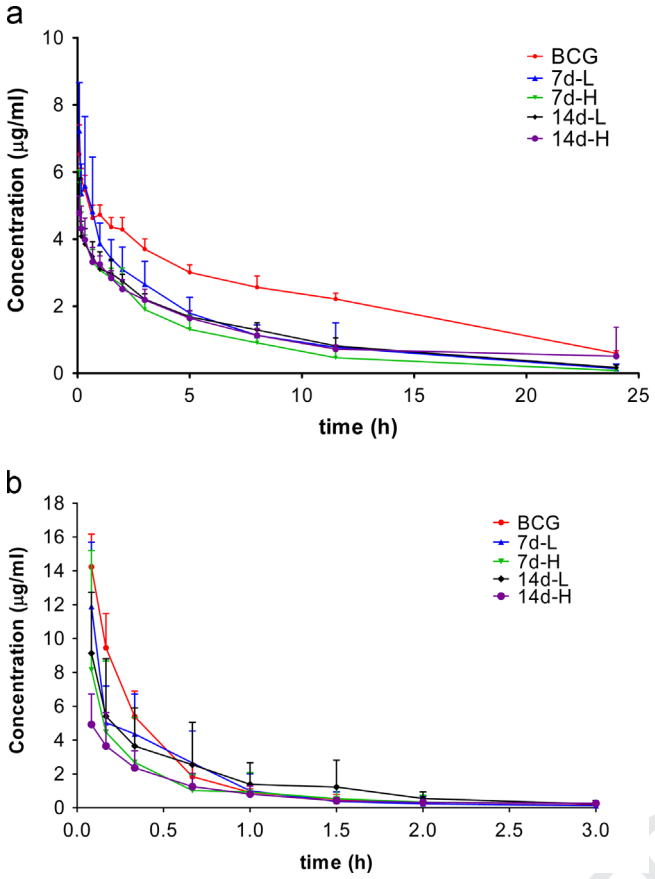

Fig. 2. Mean plasma concentration–time curves of (a) tolbutamide (1 mg/kg, i.v.) and (b) midazolam (4 mg/kg, i.v.) in untreated and *Polygonum capitatum* extract pre-treated rats ($n=6$). BCG (blank control group) received water for 10 days; 7d-L received 0.58 g/kg, i.g. *Polygonum capitatum* extract twice a day for 7 consecutive days; 7d-H received 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 7 consecutive days; 14d-L received 0.58 g/kg i.g. *Polygonum capitatum* twice a day for 14 consecutive days; 14d-H received 1.74 g/kg i.g. *Polygonum capitatum* twice a day for 14 consecutive days. Error bars represent S.D.

3.5. Effects of Polygonum capitatum on rat hepatic CYP2C19

CYP2C19 activity was investigated by analysis of the pharmacokinetics of omeprazole in rats pre-treated with *Polygonum capitatum* extract, as shown in Table 4. No significant differences were observed between the different treatment groups and the BCG. These results indicated that *Polygonum capitatum* did not influence rat hepatic CYP2C19 activity *in vivo*.

3.6. Effects of Polygonum capitatum on rat hepatic CYP2E1

CYP2E1 activity was investigated by analysis of the pharmacokinetics of chlorzoxazone in rats pre-treated with *Polygonum capitatum* extract, as shown in Table 5. Chlorzoxazone pharmacokinetic parameters were not significantly altered in the different treatment groups, as compared with the BCG. These results indicated that *Polygonum capitatum* did not influence rat hepatic CYP2E1 activity *in vivo*.

3.7. Effects of Polygonum capitatum on hepatic CYP3A4

CYP3A4 activity was evaluated by comparing the pharmacokinetics of midazolam in the study groups (Table 6). The mean plasma concentration–time curves of midazolam in the indicated study groups are presented in Fig. 2b. The $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of midazolam were decreased significantly in all groups pre-treated with *Polygonum capitatum* extract, compared with those observed in the BCG group. The $MRT_{(0-t)}$, $MRT_{(0-\infty)}$, $t_{1/2}$, CL and V were significantly increased in some of the pre-treated groups (Table 6). Taken together, these results indicated that midazolam metabolism was accelerated to varying degrees in these treatment groups. These suggested that *Polygonum capitatum* extract had the potential to induce CYP3A4 activity *in vivo*.

4. Discussion

Polygonum capitatum has been used for many years in traditional medicine, where it plays an indispensable role in the treatment of urinary system infections, pyelonephritis and kidney stones. This traditional Miao medicinal plant was researched and developed in the 1980s in Guizhou province. Previous pharmacological studies demonstrated that aqueous extracts of *Polygonum capitatum*, mainly composed of fatty acid esters, triterpenoids, steroids, flavonoids, gallic acid and its analogs, as well as other phenolic compounds (Li and Gong, 2007; Liu et al., 2008; Yu et al., 2008; Yang et al., 2009; Zhang et al., 2010; Zhao et al., 2010; Liao et al., 2011, 2012), possessed antibacterial, anti-inflammatory, hypothermic, analgesic, anti-oxidant and diuretic activities (Ren et al., 1995; Li and Gong, 2007; Liu et al., 2007, 2008; Liao et al., 2011). The commercially available *Polygonum capitatum* products are generally composed of aqueous extracts and for this reason, this type of extract was used in the present study.

Polygonum capitatum-based drugs such as Relinqing® granules and Milin® capsules are frequently prescribed concomitantly with antibiotics such as azithromycin, levofloxacin and clarithromycin in China, to enhance treatment of urinary tract infections (Ou Yang et al., 2006; Hong et al., 2009; Su and Liu, 2013). There is no doubt that this has the potential to increase the risk for HDIs in patients. Therefore, it is very important to elucidate the potential HDIs between *Polygonum capitatum* and other co-administered drugs, so that *Polygonum capitatum* can be used safely and effectively.

The main CYP enzymes closely related to drug metabolism in the human liver include CYP1A2, CYP2A6, CYP2C, CYP2D6, CYP2E1 and CYP3A4; they each account for 15%, 5%, 20%, 2%, 10% and 30% in total CYPs, respectively (Yuan et al., 2002). It is well known that a highly selective probe drug is an efficient tool for the assessment of CYP enzyme activity. Moreover, the probes should be safe for trial subjects, readily available and straightforward (Turpault et al., 2009). As a classic probe drug for CYP1A2 (Kalow and Tang, 1993), the metabolism of caffeine is mainly conducted by the metabolizer of CYP1AC2 by over 80%, and its property of pharmacology (such as the rapid distribution and relative low protein binding rate) makes caffeine the classic probe drug for CYP1A2 (Kalow and Tang, 1993). Chlorzoxazone has been unique to the CYP1A2 presently (Palmer et al., 2001). Both omeprazole and midazolam are listed as sensitive substrates in the Food and Drug Administration draft guidance. Yet tolbutamide is a well-documented CYP2C9 probe drug. In the present study, we investigated the effect of aqueous extract of *Polygonum capitatum* on the activities of these five CYP isoforms in rats through examining the pharmacokinetics of five probe drugs (caffeine, tolbutamide, omeprazole, chlorzoxazone and midazolam). The results showed that *Polygonum capitatum* extract could increase the activities of CYP2C9 and CYP3A4 in varying degrees; however, the increased CYP2C9 and CYP3A4 are not in a dose- or time-dependent manner. On contrary, there are no significant effects on the activities of CYP1A2, CYP2C19 or CYP2E1. These results indicate *Polygonum capitatum*-containing preparations accelerate drug metabolism of CYP3A4 and CYP2C9 substrates.

It is well known that antibiotics can exert their therapeutic effect when their plasma-drug concentration is maintained above the minimal inhibitory concentration (MIC). If the plasma concentration

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Table 4
Effects of *Polygonum capitatum* extract on the pharmacokinetic parameters of omeprazole (2 mg/kg).

Parameters	BCG	Treatment groups			
		7d-L	7d-H	14d-L	14d-H
$AUC_{(0-t)}$ (mg/L h)	0.74 ± 0.22	0.57 ± 0.26	0.58 ± 0.14	0.71 ± 0.45	0.47 ± 0.21
$AUC_{(0-\infty)}$ (mg/L h)	0.76 ± 0.22	0.61 ± 0.32	0.63 ± 0.18	0.78 ± 0.55	0.49 ± 0.23
$MRT_{(0-t)}$ (h)	0.22 ± 0.07	0.23 ± 0.08	0.22 ± 0.04	0.23 ± 0.06	0.19 ± 0.06
$MRT_{(0-\infty)}$ (h)	0.24 ± 0.07	0.29 ± 0.15	0.27 ± 0.07	0.30 ± 0.15	0.23 ± 0.10
$t_{1/2}$ (h)	0.20 ± 0.04	0.22 ± 0.13	0.21 ± 0.06	0.25 ± 0.12	0.17 ± 0.09
CL (L/h/kg)	3.15 ± 1.35	4.01 ± 1.79	4.08 ± 0.89	3.45 ± 1.66	4.77 ± 1.76
V (L/kg)	0.82 ± 0.24	1.10 ± 0.62	1.06 ± 0.23	1.04 ± 0.35	1.04 ± 0.65

BCG, blank control group (water for 10 days); 7d-L, 0.58 g/kg i.g. *Polygonum capitatum* extract twice daily for 7 consecutive days; 7d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 7 consecutive days; 14d-L, 0.58 g/kg i.g. *Pcapitatum capitatum* extract twice a day for 14 consecutive days; 14d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 14 consecutive days. Values are presented as mean ± S.D. ($n=6$).

Table 5
Effects of *Polygonum capitatum* extract on the pharmacokinetic parameters of chlorzoxazone (4 mg/kg).

Parameters	BCG	Treatment groups			
		7d-L	7d-H	14d-L	14d-H
$AUC_{(0-t)}$ (mg/L h)	6.78 ± 1.54	7.21 ± 3.23	6.08 ± 1.64	6.70 ± 1.18	5.82 ± 1.21
$AUC_{(0-\infty)}$ (mg/L h)	7.32 ± 1.49	7.59 ± 3.75	6.69 ± 1.98	7.05 ± 1.41	6.49 ± 1.06
$MRT_{(0-t)}$ (h)	0.88 ± 0.13	0.79 ± 0.15	0.76 ± 0.18	0.80 ± 0.12	0.81 ± 0.13
$MRT_{(0-\infty)}$ (h)	1.05 ± 0.15	0.95 ± 0.28	1.02 ± 0.41	0.95 ± 0.24	1.14 ± 0.27
$t_{1/2}$ (h)	0.88 ± 0.15	0.63 ± 0.30	0.71 ± 0.39	0.77 ± 0.18	0.81 ± 0.31
CL (L/h/kg)	0.57 ± 0.12	0.62 ± 0.24	0.67 ± 0.25	0.59 ± 0.12	0.63 ± 0.12
V (L/kg)	0.71 ± 0.12	0.63 ± 0.18	0.59 ± 0.20	0.65 ± 0.12	0.73 ± 0.25

BCG, blank control group (water for 10 days); 7d-L, 0.58 g/kg i.g. *Polygonum capitatum* extract twice daily for 7 consecutive days; 7d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 7 consecutive days; 14d-L, 0.58 g/kg i.g. *Polygonum capitatum* extract twice a day for 14 consecutive days; 14d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 14 consecutive days. Values are presented as mean ± S.D. ($n=6$).

Table 6
Effects of *Polygonum capitatum* extract on the pharmacokinetic parameters of midazolam (4 mg/kg).

Parameters	BCG	Treatment groups			
		7d-L	7d-H	14d-L	14d-H
$AUC_{(0-t)}$ (mg/L h)	8.23 ± 1.60	4.69 ± 2.20**	3.44 ± 3.28**	4.99 ± 2.49*	2.75 ± 1.30**
$AUC_{(0-\infty)}$ (mg/L h)	8.49 ± 1.69	4.94 ± 2.57*	3.89 ± 3.81*	5.44 ± 2.77*	2.92 ± 1.34**
$MRT_{(0-t)}$ (h)	0.43 ± 0.13	0.37 ± 0.14	0.41 ± 0.08	0.62 ± 0.11*	0.50 ± 0.07
$MRT_{(0-\infty)}$ (h)	0.49 ± 0.15	0.45 ± 0.32	0.62 ± 0.41	0.70 ± 0.10*	0.65 ± 0.17
$t_{1/2}$ (h)	0.39 ± 0.05	0.39 ± 0.30	0.59 ± 0.44	0.53 ± 0.08**	0.54 ± 0.15*
CL (L/h/kg)	0.49 ± 0.08	1.05 ± 0.58*	1.99 ± 1.21**	0.98 ± 0.49*	1.59 ± 0.60**
V (L/kg)	0.28 ± 0.06	0.49 ± 0.27	1.24 ± 0.57**	0.90 ± 0.47**	1.27 ± 0.72**

BCG, blank control group (water for 10 days); 7d-L, 0.58 g/kg i.g. *Polygonum capitatum* extract twice daily for 7 consecutive days; 7d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 7 consecutive days; 14d-L, 0.58 g/kg i.g. *Polygonum capitatum* extract twice a day for 14 consecutive days; 14d-H, 1.74 g/kg i.g. *Polygonum capitatum* extract twice a day for 14 consecutive days. Values are presented as mean ± S.D. ($n=6$).

* $P<0.05$ when compared with related parameters of BCG group.
** $P<0.01$ when compared with related parameters of BCG group.

of antibiotic is lower than the MIC, it will possibly lead to failure of therapy even drug resistance. According to information from Drugbank (⟨http://www.drugbank.ca⟩) and references, some chemicals, such as sulfamethoxazole, ibuprofen, losartan, naproxen, warfarin, tenoxicam, and tolbutamide used in clinics, are substrates of CYP2C9, while some drugs, such as clarithromycin, azithromycin, cortisone, cyclosporine, diltiazem, lovastatin, nifedipine, verapamil, and vincristine, are substrates of CYP3A4 (Guengerich, 1997; Zeng, 2007). Therefore, it is worth to note that when *Polygonum capitatum*-based drugs (*e.g.*, Relinqing® granules) are used in combination with substrates of CYP2C9 and CYP3A4 such as sulfamethoxazole and clarithromycin, their metabolism will be accelerated, plasma-drug concentrations will be reduced, and the MIC may not be obtained. Therefore, clinicians should adjust the dosage of drugs metabolized by these enzymes in patients who take *Polygonum capitatum*-containing preparations.

5. Conclusion

The present study indicates that *Polygonum capitatum* extract increases the activities of CYP2C9 and CYP3A4, and accelerates the metabolism of antibiotics such as sulfamethoxazole and clarithromycin in combination used in clinics. These findings provide a safety reminder for combination used of *Polygonum capitatum*-containing preparations and antibiotics.

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References

Bent, S., 2008. Herbal medicine in the United States: review of efficacy, safety, and regulation: grand rounds at University of California, San Francisco Medical Center. Journal of General Internal Medicine 23, 854–859.

Bush, T.M, Rayburn, K.S., Holloway, S.W., Sanchez-Yamamoto, D.S., Allen, B.L., Lam, T., So, B.K., de Tran, H., Greyber, E.R., Kantor, S., Roth, L.W., 2007. Adverse interactions between herbal and dietary substances and prescription medications: a clinical survey. Alternative Therapies in Health and Medicine 13, 30–35.

Editorial Committee of Chinese Materia Medica, 2005, State Administration of Traditional Chinese Medicine, Miao's Material Medica, Chinese Materia Medica (Zhonghua Bencao). Guizhou Science and Technology, Guiyang, pp. 223–224.

Ernst, E., 2000. Herb-drug interactions: potentially important but woefully underresearched. European Journal of Clinical Pharmacology 56, 523–524.

Daly, A.K., 2004. Pharmacogenetics of the cytochromes P450. Current Topics in Medicinal Chemistry 4, 1733–1744.

Gao, J.W., Shi, Z., Zhu, S.Z., Li, G.Q., Yan, R., Yao, M., 2013. Influences of processed rhubarbs on the activities of four CYP isozymes and the metabolism of saxagliptin in rats based on probe cocktail and pharmacokinetics approaches. Journal of Ethnopharmacology 145, 566–572.

Gorski, J.C., Huang, S.M., Pinto, A., Hamman, M.A., Hilligoss, J.K., Zaheer, N.A., Desai, M., Miller, M., Hall, S.D., 2004. The effect of *echinacea* (*Echinacea purpurea* root) on cytochrome P450 activity *in vivo*. Clinical Pharmacology and Therapeutics 75, 89–100.

Guengerich, F. Peter, 1997. Role of Cytochrome P450 Enzymes in Drug-Drug Interactions, Advances in Pharmacology. Academic Press, New York p. 43.

Han, Y.L., Li, D., Ren, B., Jing, G.P., Meng, X.L., Zhou, Z.Y., Yu, Q., Li, Y., Wan, L.L., Guo, C., 2012. Evaluation of impact of Herba Erigerontis injection, a Chinese herbal prescription, on rat hepatic cytochrome P450 enzymes by cocktail probe drugs. Journal of Ethnopharmacology 139, 104–109.

Hong, K., Yuan, R.P., Jiang, H., Zhao, L.M., Liu, D.F., Ma, L.L., Huang, Y., Chen, Q., 2009. A randomized study comparing combined Relinqing and azithromycin for the treatment of nongonococcal urethritis in men. The Chinese Journal of Human Sexuality 17, 7–9.

Ingelman-Sundberg, M., 2004. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. Trends in Pharmacological Sciences 25, 193–200.

Izzo, A.A., 2004. Herb-drug interactions: an overview of the clinical evidence. Fundamental & Clinical Pharmacology 19, 1–16.

Kalow, W., Tang, B.K., 1993. The use of caffeine for enzyme assays: a critical appraisal. Clinical Pharmacology & Therapeutics 53, 503–514.

Kelly, J.P., Kaufman, D.W., Kelley, K., Rosenberg, L., Anderson, T.E., Mitchell, A.A., 2005. Recent trends in use of herbal and other natural products. Archives of Internal Medicine 165, 281–286.

Lewis, D.F., 2003. P450 structures and oxidative metabolism of xenobiotics. Pharmacogenomics 4, 387–395.

Li, Y.M., Gong, Y., 2007. The research progress on the chemical component and the pharmacology of *Polygonum Capitatum* Ham ex D. Don. Journal of Guizhou University (Natural Sciences) 24, 205–207.

Liao, S.G., Zhang, L.J., Sun, F., Zhang, J.J., Chen, A.Y., Lan, Y.Y., Li, Y.J., Wang, A.M., He, X., Xiong, Y., Dong, L., Chen, X.J., Li, Y.T., Zuo, L., Wang, Y.L., 2011. Antibacterial and anti-inflammatory effects of extracts and fractions from *Polygonum capitatum*. Journal of Ethnopharmacology 134, 1006–1009.

Liao, S.G., Zhang, L.J., Wang, Z., Sun, F., Li, Y.J., Wang, A.M., Huang, Y., Lan, Y.Y., Wang, Y.L., 2012. Electrospray ionization and collision-induced dissociation tandem mass spectrometric discrimination of polyphenolic glycosides: exact acylation site determination of the O-acylated monosaccharide residues. Rapid Communications in Mass Spectrometry 26, 2483–2492.

Liu, M., Luo, C.L., Zhang, Y.P., Qiu, D.W., 2007. Analgesic, anti-inflammatory, and diuretic effects of *Polygonum capitatum* and *Toddalia asiatica*. Guizhou Medical Journal 31, 370–371.

Liu, Z.J., Qi, J., Zhu, D.N., Yu, B.Y., 2008. Chemical constituents from *Polygonum capitatum* and their antioxidation activities *in vitro*. Journal of Chinese Medicinal Materials 31, 995–998.

Magee, K.A., 2005. Herbal therapy: a review of potential health risks and medicinal interactions. Orthodontics & Craniofacial Research 8, 60–74.

Ou Yang, X.R., Min, Y.G., Jiang, W.X., Guo, Z.X., 2006. Clinical observation of relinqing granules and clarithromycin combined treating nongonococcal urethritis. Journal of Dermatology and Venereology 28, 33–34.

Palmer, J.L., Scott, R.J., Gibson, A., Dickins, M., Pleasance, S., 2001. An interaction between the cytochrome P450 probe substrates chlorzoxazone (CYP2E1) and midazolam (CYP3A). Britain Journal of Clinical Pharmacology 52, 555–561.

Ren, G., Chang, F., Lu, S., Zhong, H., Zhang, G., 1995. Pharmacological studies of *Polygonum capitatum* Buch. Ham. ex D. Don. China Journal of Chinese Materia Medica 20, 107–109.

Rodeiro, I., Donato, M.T., Jimenez, N., Garrido, G., Molina-Torres, J., Menendez, R., Castell, J.V., Gómez-Lechón, M.J., 2009. Inhibition of human P450 enzymes by natural extracts used in traditional medicine. Phytotherapy Research 23, 279–282.

Su, Z.R., Liu, L.Q., 2013. Clinical observation of relinqing granules and levofloxacin combined treating urinary system infection. Strait Pharmaceutical Journal 25, 159–160.

Theuretzbacher, U., Zeitlinger, M., 2011. Antibacterial distribution and drug-drug interactions in cancer patients, Principles and Practice of Cancer Infectious Diseases. Springer, pp. 443–454.

Turpault, S., Brian, W., Van Horn, R., Santoni, A., Poitiers, F., Donazzolo, Y., Boulenc, X., 2009. Pharmacokinetic assessment of a five-probe cocktail for CYPs 1A2, 2C9, 2C19, 2D6 and 3A. Britain Journal of Clinical Pharmacology 68, 928–935.

Ueng, Y.F., Chen, R.M., 2004. The role of cytochrome P450 in herb-drug interactions. Current Pharmacogenomics 2, 209–218.

Yang, Y., Cai, F., Yang, Q., Yang, Y.B., Sun, L.N., Chen, W.-S., 2009. Study on chemical constituents of *Polygonum capitatum* Buch Ham. ex D. Don (I). Academic Journal of Second Military Medical University 29, 937–940.

Yuan, R., Madani, S., Wei, X.X., Reynolds, K., Huang, S.M., 2002. Evaluation of cytochrome P450 probe substrates commonly used by the pharmaceutical industry to study *in vitro* drug interactions. Drug Metabolism and Disposition 30, 1311–1319.

Yu, M., Li, Z.L., Li, N., Li, X., 2008. Chemical constituents of the aerial parts of *Polygonum capitatum*. Journal of Shenyang Pharmaceutical University 25, 633–635.

Zeng, S., 2007. Drug Metabolism. Zhejiang University Press, Zhejiang.

Zhang, L.J., Liao, S.G., Zhan, Z.H., Chen, X.J., Lan, Y.Y., 2010. A study on the phenolic constituents of *Polygonum capitatum*. Lishizhen Medicine and Materia Medica Research 21, 1946–1947.

Zhao, H.X., Bai, H., Wang, Y.S., Nie, T.T., 2010. Progress on chemical constituents and analytical methods of *Polygonum capitatum* Buch.-Ham. ex D. Don. Food and Drug 12, 446–448.

Zhou, S., Gao, Y., Jiang, W., Huang, M., Xu, A., Paxton, J.W., 2003. Interactions of herbs with cytochrome P450. Drug Metabolism Reviews 35, 35–98.

Glossary

HDIs: Herb–drug interactions;
CYP: Cytochrome P450;
UPLC-ESI-MS: Ultra-performance liquid chromatography–electrospray ionization mass spectrometry;
$AUC_{(0-t)}$: Area under a curve extrapolated to last measurable concentration at time t;
$AUC_{(0-\infty)}$: Area under a curve extrapolated to infinite time;
$MRT_{(0-t)}$: Mean residence time extrapolated to last measurable concentration at time t;
$MRT_{(0-\infty)}$: Mean residence time extrapolated to infinite time;
$t_{1/2}$: Half-life;
CL: Total body clearance;
V: Volume of distribution.