

Original Article

Effect of *Breynia fruticosa* on CYP450 isoforms activity in rats

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Abstract: *Breynia fruticosa* (L.) Hook. f. is the one distributed abundantly in the south of China and has been used as a Chinese folk medicine for the treatment of chronic bronchitis and wounds. In order to investigate the effects of *Breynia fruticosa* on the metabolic capacity of cytochrome P450 (CYP) enzymes, a cocktail method was employed to evaluate the activities of CYP2B6, CYP2D6, CYP2C19, CYP1A2, CYP3A4, CYP2C9. The rats were randomly divided into *Breynia fruticosa* group (Low, High) and control group. The *Breynia fruticosa* group rats were given 1, 2 g/kg (Low, High) *Breynia fruticosa* decoction by continuous intragastric administration for 7 days. Six probe drugs bupropion, metoprolol, omeprazole, phenacetin, testosterone and tolbutamide were given to rats through intragastric administration, and the plasma concentrations were determined by UPLC-MS/MS. Statistical pharmacokinetics difference for omeprazole, phenacetin and tolbutamide in rats were observed by comparing *Breynia fruticosa* group with control group. Continuous 7 days-intragastric administration of *Breynia fruticosa* induces the activities of CYP2B6, CYP1A2 and CYP2C9 of rats. Induction of drug metabolizing enzyme by *Breynia fruticosa* would reduce the efficacy of other drug. Additionally, there is no significant liver and kidney morphological difference found after *Breynia fruticosa*.

Keywords: CYP450, *Breynia fruticosa*, cocktail, UPLC-MS/MS, rat

Introduction

The genus *Breynia* (Euphorbiaceae) is comprised of 25 species of plants, seven of which are found in China [1]. *Breynia fruticosa* (L.) Hook. f. is the one distributed abundantly in the south of China and has been used as a Chinese folk medicine for the treatment of chronic bronchitis and inflammation by the "Dai" ethnic minority in southern China [2, 3]. Previous phytochemical studies on *Breynia* plants led to the identification of sulfur-containing spiroketal glycosides, alkaloids, and terpenoid and phenolic glycosides, along with several other components [4, 5].

Cytochrome P450 (CYP) enzymes are responsible for most biotransformation steps of xenobiotics and endogenous molecules [6]. Varia-

tions of their activity by inhibition or induction can influence the pharmacokinetics and thereby the effect of drugs (of abuse). Enzyme inhibition by co-administered drugs and/or genetic variations of their expression can increase the risk of adverse reactions [7] or reduce the desired effect [8]. Such drug-drug interactions were described as a major reason for hospitalization or even death [9].

So far, no study on the effects of *Breynia fruticosa* on the metabolic capacity of CYP enzyme was reported. Therefore, in this study, six probe drugs were employed to evaluate effect of *Breynia fruticosa* on the metabolic capacity of CYP2B6, CYP2D6, CYP2C19, CYP1A2, CYP3A4, CYP2C9. The effects of *Breynia fruticosa* on rat CYP enzyme activity will be evaluated according to the pharmacokinetic parameters changes of

six specific probe drugs (bupropion, metoprolol, omeprazole, phenacetin, testosterone and tolbutamide).

Material and methods

Chemicals

Bupropion, metoprolol, omeprazole, phenacetin, testosterone and tolbutamide (all > 98%) and the internal standard diazepam (IS) were obtained from Sigma-Aldrich Company (St. Louis, USA). Ultra-pure water was prepared by Millipore Milli-Q purification system (Bedford, USA). Methanol and acetonitrile (HPLC grade) were obtained from Merck Company (Darmstadt, Germany).

Animals

Sprague-Dawley rats (male, 220 ± 20 g) purchased from Shanghai SLAC Laboratory Animal Co., Ltd. Animals were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All twenty-four rats were housed at Laboratory Animal Research Center of Wenzhou Medical University. All experimental procedures were approved ethically by the Wenzhou Medical University Administration Committee of Experimental Animals.

Breynia fruticosa decoction

These raw materials (*Breynia fruticosa* (L.) Hook. f.) were obtained from the Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University, China, and stored in an environment of normal atmospheric pressure and decoction at 100°C for 30 minutes, and then the residues were discarded, the final decoction concentration was fixed at 1.0 g/mL. The decoction was stored at 4°C.

Pharmacokinetics

Twenty-four rats (220 ± 20 g) were randomly divided into three different dosages of *Breynia fruticosa* groups (Low-group, High-group and control group with 8 rats in each group). Three different *Breynia fruticosa* group (Low-group, High-group) were respectively give *Breynia fruticosa* decoction 1.0, 2.0 g/kg one time by intragastric administration at every morning, and last for 7 days. Control group were give saline by same administration method. At 8 days morning, six probe drugs bupropion, me-

toprolol, omeprazole, phenacetin, testosterone and tolbutamid were mixtured in corn oil and given to the rats of two *Breynia fruticosa* groups and control group by intragastric administration at a single dosage 10 mg/kg for bupropion, metoprolol, omeprazole, phenacetin, testosterone, 1 mg/kg for tolbutamide.

Blood (0.3 mL) samples were collected into heparinized 1.5 mL polythene tubes from the tail vein at 0.0833, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h after intragastric administration of six probe drugs. Plasma (100 μ L) was obtained from blood sample after centrifugation at 4000 g for 10 min. In a 1.5 mL centrifuge tube, 200 μ L of acetonitrile (containing 50 ng/mL IS) was added into 100 μ L of collected plasma sample. After vortex-mixing for 1.0 min, the sample was centrifuged at 13000 g for 15 min. Then supernatant (2 μ L) was injected into the UPLC-MS/MS system for analysis.

Concentration of plasma probe drugs versus time was analyzed by Version 3.0 Data Analysis System (Wenzhou Medical University, China). The main pharmacokinetic parameters of the *Breynia fruticosa* group and control group were analyzed by SPSS 18.0 statistical software; statistical significance was assessed by t-test ($P < 0.05$ was considered as statistically significant).

UPLC-MS/MS determination of probe drugs

The concentration of bupropion, metoprolol, omeprazole, phenacetin, testosterone and tolbutamid in rat plasma were simultaneously determined by a sensitive and simple UPLC-MS/MS method [10]. The compounds were analyzed by a UPLC-MS/MS with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer that equipped with an electrospray ionization (ESI) interface (Waters Corp., Milford, MA, USA). Data acquisition and instrument control were performed on the Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

Bupropion, metoprolol, omeprazole, phenacetin, testosterone, tolbutamid and diazepam (IS) were separated using a Waters BEH C18 column (2.1 mm $\times$ 100 mm, 1.7 μ m) at constant temperature 40°C. The mobile phase consisted of 0.1% formic acid and acetonitrile with gradient elution at a flow rate of 0.4 mL/min and an injection volume of 2 μ L. The mass spectro-

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Table 1. Pharmacokinetic parameters of bupropion and metoprolol in control-group and *Breynia fruticosa*-group rats (mean $\pm$ SD, n = 8)

Parameters		AUC _(0-t) ng/mL*h	AUC _(0-∞) ng/mL*h	t _{1/2z} h	CL _z /F L/h/kg	V _z /F L/kg	C _{max} ng/mL
Bupropion (CYP2B6)	Control	403.9 $\pm$ 150.8	431.2 $\pm$ 166.2	1.3 $\pm$ 0.6	26.7 $\pm$ 10.8	53.1 $\pm$ 34.2	194.0 $\pm$ 102.9
	Low	216.1 $\pm$ 102.3*	228.0 $\pm$ 102.2*	1.2 $\pm$ 0.6	55.6 $\pm$ 32.8*	99.1 $\pm$ 59.8	87.1 $\pm$ 45.5*
	High	229.5 $\pm$ 139.2*	238.9 $\pm$ 148.7*	1.1 $\pm$ 0.3	60.3 $\pm$ 39.1*	86.5 $\pm$ 48.4	95.3 $\pm$ 63.0*
Metoprolol (CYP2D6)	Control	947.3 $\pm$ 227.4	1046.7 $\pm$ 334.5	0.9 $\pm$ 0.5	10.3 $\pm$ 2.8	12.5 $\pm$ 3.9	458.1 $\pm$ 124.1
	Low	449.3 $\pm$ 196.8**	589.9 $\pm$ 227.9**	2.0 $\pm$ 1.4	20.0 $\pm$ 9.3*	56.6 $\pm$ 39.9**	217.1 $\pm$ 124.7**
	High	861.0 $\pm$ 315.3	946.0 $\pm$ 376.4	1.0 $\pm$ 0.2	12.5 $\pm$ 6.1	16.4 $\pm$ 6.3	390.2 $\pm$ 136.2

Compared *Breynia fruticosa*-group with the control group, *P < 0.05.

Table 2. Pharmacokinetic parameters of omeprazole and phenacetin in control-group and *Breynia fruticosa*-group rats (mean $\pm$ SD, n = 8)

Parameters		AUC _(0-t) ng/mL*h	AUC _(0-∞) ng/mL*h	t _{1/2z} h	CL _z /F L/h/kg	V _z /F L/kg	C _{max} ng/mL
Omeprazole (CYP2C19)	Control	425.6 $\pm$ 126.7	432.1 $\pm$ 129.4	0.6 $\pm$ 0.1	25.6 $\pm$ 9.9	23.4 $\pm$ 10.4	465.4 $\pm$ 261.1
	Low	344.1 $\pm$ 140.6	363.6 $\pm$ 134.9	1.0 $\pm$ 0.3*	32.4 $\pm$ 16.4	46.8 $\pm$ 26.0*	297.3 $\pm$ 200.2
	High	408.7 $\pm$ 171.3	415.5 $\pm$ 171.1	0.8 $\pm$ 0.6	29.4 $\pm$ 15.9	34.6 $\pm$ 27.4	494.6 $\pm$ 204.9
Phenacetin (CYP1A2)	Control	7532.1 $\pm$ 2172.5	7533.0 $\pm$ 2172.9	0.4 $\pm$ 0.1	1.4 $\pm$ 0.5	0.8 $\pm$ 0.3	5398.5 $\pm$ 1002.0
	Low	1075.9 $\pm$ 457.8**	1079.1 $\pm$ 457.7**	0.8 $\pm$ 0.2**	11.1 $\pm$ 5.5**	13.0 $\pm$ 8.1**	1257.5 $\pm$ 540.6**
	High	1289.3 $\pm$ 391.8**	1291.6 $\pm$ 393.8**	0.9 $\pm$ 0.6*	8.4 $\pm$ 2.3**	10.2 $\pm$ 6.8**	1515.1 $\pm$ 587.6**

Compared *Breynia fruticosa*-group with the control group, *P < 0.05, **P < 0.01.

Table 3. Pharmacokinetic parameters of testosterone and tolbutamide in control-group and *Breynia fruticosa*-group rats (mean $\pm$ SD, n = 8)

Parameters		AUC _(0-t) ng/mL*h	AUC _(0-∞) ng/mL*h	t _{1/2z} h	CL _z /F L/h/kg	V _z /F L/kg	C _{max} ng/mL
Testosterone (CYP3A4)	Control	166.7 $\pm$ 46.1	245.1 $\pm$ 105.3	5.7 $\pm$ 3.9	48.2 $\pm$ 23.4	341.1 $\pm$ 177.2	94.4 $\pm$ 38.9
	Low	403.2 $\pm$ 104.4**	420.2 $\pm$ 101.5**	2.5 $\pm$ 0.9*	25.1 $\pm$ 6.1*	92.9 $\pm$ 46.7**	105.8 $\pm$ 32.4
	High	140.9 $\pm$ 36.7	161.2 $\pm$ 39.3	5.0 $\pm$ 5.6	65.6 $\pm$ 17.0	438.5 $\pm$ 463.7	47.5 $\pm$ 13.7*
Tolbutamide (CYP2C9)	Control	101949.4 $\pm$ 12782.9	106586.0 $\pm$ 13578.0	5.2 $\pm$ 0.3	0.010 $\pm$ 0.001	0.071 $\pm$ 0.008	9556.1 $\pm$ 1075.7
	Low	46934.3 $\pm$ 5210.1**	50427.8 $\pm$ 6172.2**	5.8 $\pm$ 1.7	0.020 $\pm$ 0.002**	0.165 $\pm$ 0.039**	3716.0 $\pm$ 399.3**
	High	46786.8 $\pm$ 9890.2**	50548.5 $\pm$ 10797.9**	5.9 $\pm$ 1.8	0.021 $\pm$ 0.004**	0.170 $\pm$ 0.043**	4632.9 $\pm$ 1061.4**

Compared *Breynia fruticosa*-group with the control group, *P < 0.05, **P < 0.01.

metric detection was performed in a positive mode. Nitrogen was used as the cone gas (50 L/h) and desolvation gas (1000 L/h). The mass conditions were set as follows: source temperature 150°C; capillary voltage 2.5 kV; desolvation temperature 500°C. The multiple reaction monitoring (MRM) mode was used for quantitative analysis.

The LLOQ for each probe drug in plasma was 2 ng/mL. The RSD of the six probe drugs were less than 15%. The calibration plot of the probe drugs is in the range of 2-2000 ng/mL (r > 0.995). The intra-day and inter-day accuracy ranged from 90% to 115%. The matrix effects

were more than 82% or less than 113%. The extraction recoveries were better than 85%.

Histopathology

After pharmacokinetic properties analysis, rats were deeply anesthetized with 10% chloral hydrate (i.p., 20 mg/kg). The liver and kidney of control group and *Breynia fruticosa* treated groups were rapidly isolated and immersed in freshly prepared 4% w/v formaldehyde (0.1 M phosphate buffer, pH 7.2) for 48 h, and then embedded in paraffin. Then 5 μ m-thick histological sections were prepared and stained with routine HE method (hematoxylin and eo-

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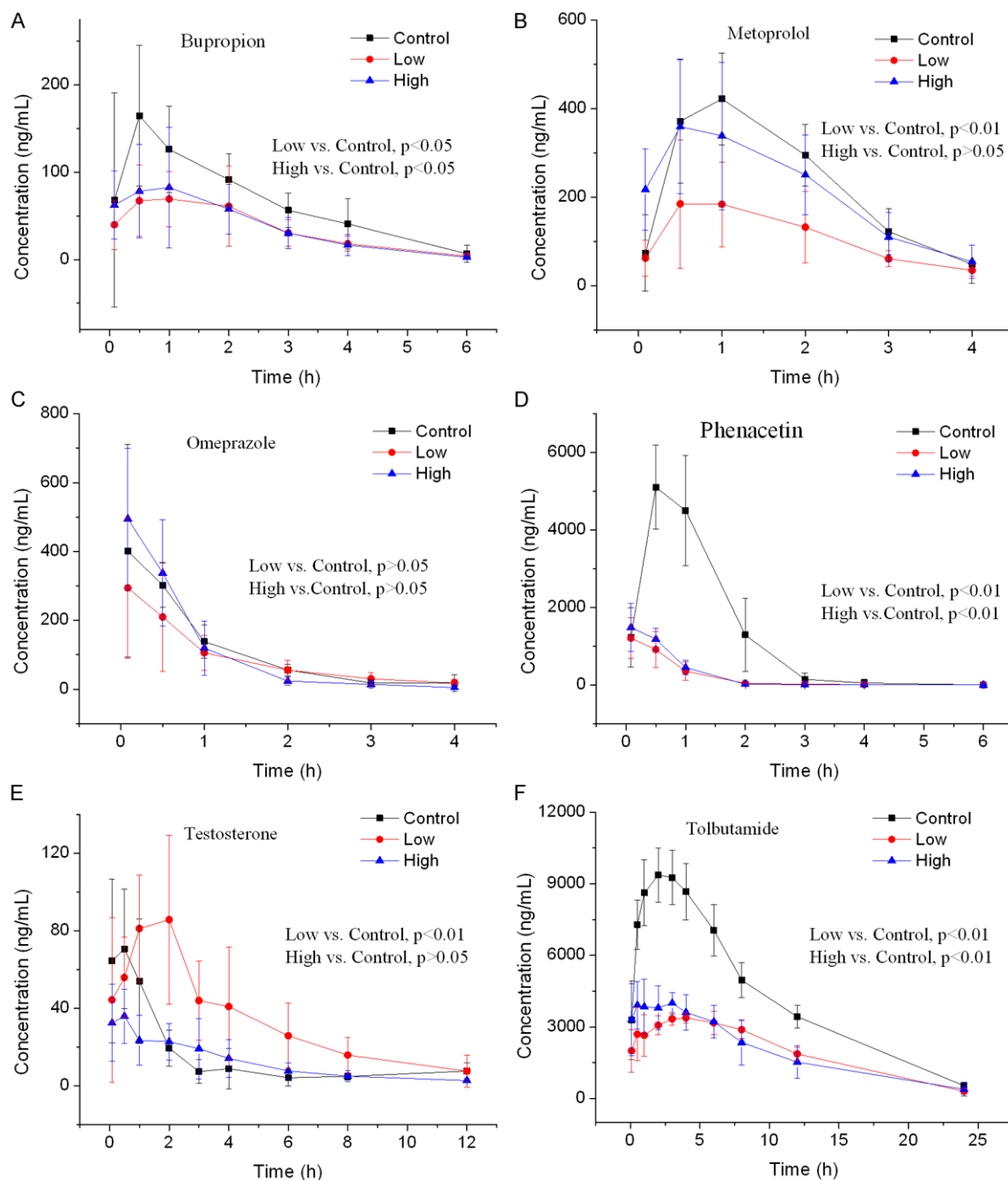


Figure 1. The pharmacokinetic profiles of bupropion (A), metoprolol (B), omeprazole (C), phenacetin (D), testosterone (E), tolbutamide (F) in control group and *Breynia fruticosa* group (Low, High) rats ($n = 8$).

sin). The morphological changes of liver and kidney were observed under light microscope.

Results

Pharmacokinetics

The main pharmacokinetic parameters of bupropion, metoprolol, omeprazole, phenacetin, testosterone and tolbutamid calculated from

non-compartment model analysis were summarized in **Tables 1-3**. The representative profiles of concentration of drugs (bupropion, metoprolol, omeprazole, phenacetin, testosterone and tolbutamid) vs. time were presented in **Figure 1**.

From the **Table 1**, compared with the control group, there difference in pharmacokinetic behaviors can be observed, low and high group

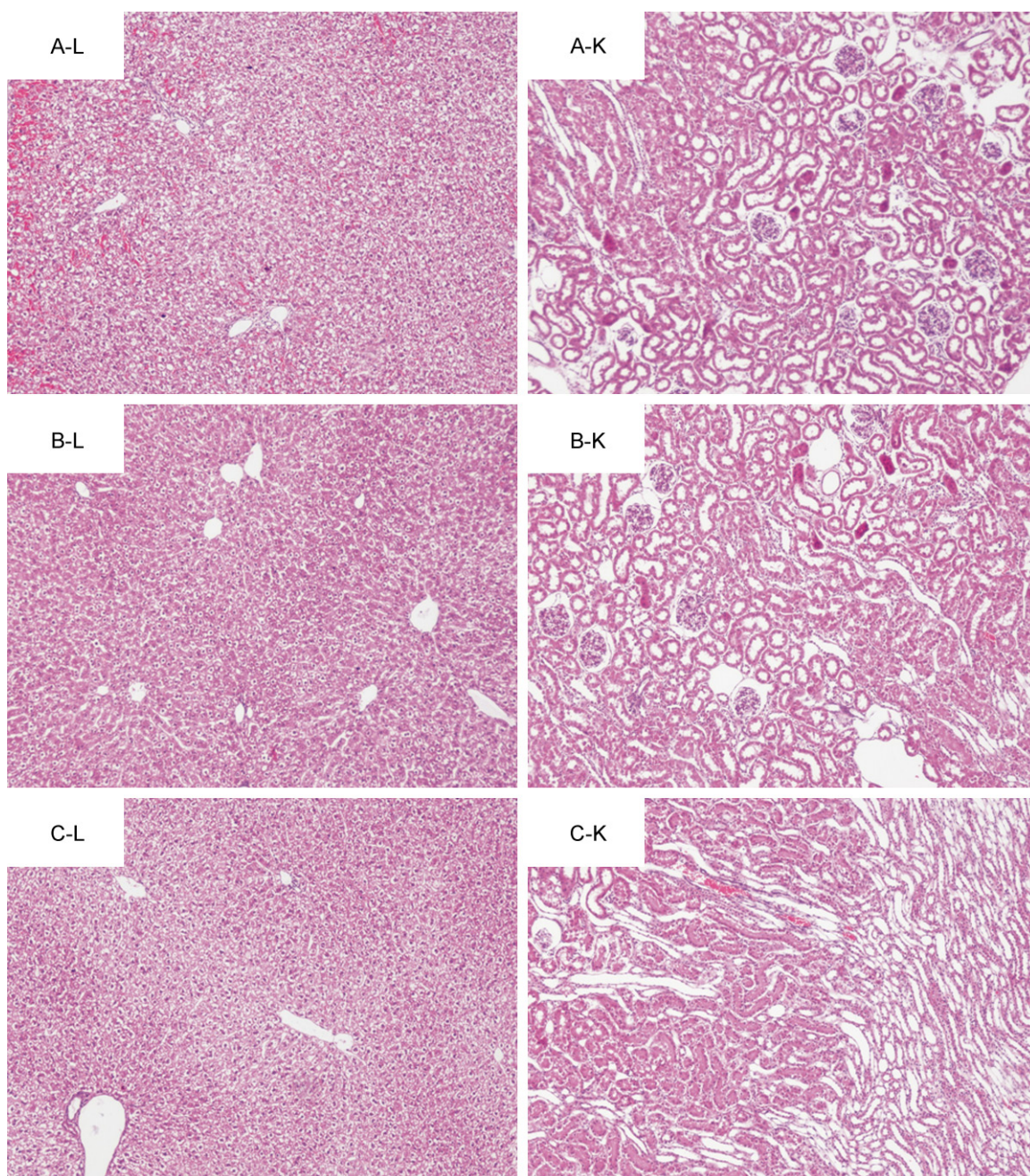


Figure 2. Morphological changes of liver (L) and kidney (K) in control-group (A) and low dosage group (B) and high dosage group (C) (hematoxylin-eosin, $\times 40$).

($P < 0.05$) for bupropion, while compared with the control group, low group ($P < 0.01$) and high group ($P > 0.05$) for metoprolol. While from the **Table 2**, no difference in pharmacokinetic behaviors ($AUC_{(0-t)}$, CL , C_{max}) can be observed between low, high dosage group and control group for omeprazole. The pharmacokinetic behaviors of phenacetin in low and high dosage group compared with the control group ($P <$

0.01). From the **Table 3**, compared with the control group, no difference in pharmacokinetic behaviors can be observed between high dosage group and control group for testosterone ($P > 0.05$), and there difference in pharmacokinetic behaviors for low dosage group ($P < 0.01$). While for tolbutamide, compared with the control group, there difference in pharmacokinetic behaviors ($P < 0.01$).

Morphological changes of liver and kidney

There is no significant morphological difference between control group, low dose group and high dose group in the liver and kidney, **Figure 2**.

The liver lobules are intact and liver cells were arranged tightly along with central veins at low magnification. The hepatocytic plates are separated by sinusoids. There was no hepatocyte swelling, cytoplasm rarefaction and inflammatory cell infiltrating. The nucleus of liver cells is round, clear and fine luster.

The glomerulus and its tubule can be recognized clearly. The glomerular capillary loops are thin and delicate. Endothelial and mesangial cells are normal in number. And the surrounding tubules are normal. There was no glomerulus hypertrophy, glomerulus extracellular matrix accumulating, basement membrane thickening, capillary adheres and glomerulosclerosis in the low and high dose group.

Discussion

In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [11]. In pharmacokinetic interactions, approximately 65% of drug-drug interactions occur in metabolic sites, and drug metabolic enzymes are considered to be the most important interactive sites. A large number of drugs are metabolized by CYP enzymes in the liver, and more than 90% of drug-drug interactions occur at the CYP-catalyzed step [12, 13]. Similarly, supplement-drug interactions involving CYP activity are occasionally found to cause considerable adverse events. For these reasons, we evaluated the effects of *Breynia fruticosa* on the activity of CYP enzymes *in vivo*. We selected CYP isoforms CYP2B6, CYP2D6, CYP2C19, CYP1A2, CYP3A4, CYP2C9 because more than 90% of drugs are known to be metabolized by these 6 CYP enzymes [14, 15].

There no significant difference for AUC, CL and C_{max} of omeprazole ($P > 0.05$) between the *Breynia fruticosa* group (Low, High) and control group was observed. It suggested that the *Breynia fruticosa* was not able to induce or inhibit the activity of CYP2C19 enzyme. The pharmacokinetic parameters of bupropion, phenacetin, tolbutamide experienced obvious

change with decreased $AUC_{(0-t)}$ ($P < 0.05$ or $P < 0.01$), increased CL ($P < 0.05$ or $P < 0.01$) and decreased C_{max} ($P < 0.05$ or $P < 0.01$). This result indicates that the 7 days-intragastric administration of *Breynia fruticosa* induces the metabolism of bupropion (CYP2B6), phenacetin (CYP1A2) and tolbutamide (CYP2C9) in rat, these results was consistent with **Figure 1**. The pharmacokinetic parameters of metoprolol experienced obvious change with decreased $AUC_{(0-t)}$ (Low, $P < 0.01$; High, $P > 0.05$) and increased CL (Low, $P < 0.05$; High, $P > 0.05$). It could not indicate that the 7 days-intragastric administration of *Breynia fruticosa* induces the metabolism of metoprolol (CYP2D6) in rat. The similar results were found in the testosterone, the pharmacokinetic parameters experienced obvious change with increased $AUC_{(0-t)}$ ($P < 0.01$) and decreased CL ($P < 0.05$) for low dosage group, while decreased $AUC_{(0-t)}$ and increased CL ($P > 0.05$) for high dosage group. It could not indicate that the 7 days-intragastric administration of *Breynia fruticosa* inhibit the metabolism of testosterone (CYP3A4) in rat.

As *Breynia fruticosa* is always administrated in combination with other drugs, interactions between *Breynia fruticosa* and other drugs would increase the risk of either diminished efficacy or adverse effects. In our study, we found that 7 days-intragastric administration of *Breynia fruticosa* induce the metabolism of bupropion (CYP2B6), phenacetin (CYP1A2) and tolbutamide (CYP2C9). Therefore, the metabolism and elimination of drugs would change if they are administrated in combination with *Breynia fruticosa*.

Conclusion

The results observed in this study would provide us valuable information regarding the interactions of *Breynia fruticosa* with other drugs. Induction of drug metabolizing enzyme CYP2B6, CYP1A2 and CYP2C9 by *Breynia fruticosa* would reduce the efficacy of other drug. Additionally, there is no significant liver and kidney morphological difference found after *Breynia fruticosa*.

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Disclosure of conflict of interest

None.

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