

## Original Article

# Effect of *Alangium chinense* on CYP450 isoforms activity of rats

Chongliang Lin<sup>1\*</sup>, Jinzhang Cai<sup>2\*</sup>, Yanyan Xu<sup>3\*</sup>, Zixia Lin<sup>4</sup>, Qing Zhang<sup>4</sup>, Ke Su<sup>4</sup>, Yangping Shentu<sup>4</sup>, Congcong Wen<sup>4</sup>, Lufeng Hu<sup>1</sup>, Guanyang Lin<sup>1</sup>

<sup>1</sup>The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China; <sup>2</sup>The Second Affiliated Hospital & Yuying Children's Hospital, Wenzhou Medical University, Wenzhou 325000, Zhejiang, China; <sup>3</sup>Department of Pharmacy, Lishui Central Hospital, Lishui 323000, Zhejiang, China; <sup>4</sup>Laboratory Animal Centre of Wenzhou Medical University, Wenzhou 325035, Zhejiang, China. \*Equal contributors.

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**Abstract:** *Alangium chinense* (Lour.) Harms (Alangiaceae) is a deciduous shrub common in China, and the roots, flowers, and leaves of this plant have historically been used in traditional Chinese medicine. In order to investigate the effects of *Alangium chinense* on the metabolic capacity of cytochrome P450 (CYP) enzymes, a cocktail method was employed to evaluate the activities of CYP2B1, CYP2D1, CYP1A2, CYP3A2, CYP2C11. The rats were randomly divided into *Alangium chinense* group (Low, High) and control group. The *Alangium chinense* group rats were given 0.6, 1.2 g/kg (Low, High) *Alangium chinense* by continuous intragastric administration for 7 days. Five probe drugs bupropion, metoprolol, 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 metoprolol and phenacetin in rats were observed by comparing *Alangium chinense* group with control group. Combined with PCR results, continuous 7 days-intragastric administration of *Alangium chinense* inhibits the activities of CYP2D1 and CYP1A2 of rats. Enzyme inhibition by co-administered drugs and genetic variations of their expression can increase the risk of adverse reactions.

**Keywords:** CYP450, *Alangium chinense*, cocktail, UPLC-MS/MS, rat

### Introduction

Plants of the genus *Alangium* are distributed in the tropics and subtropical area of the Eastern Hemisphere, nine species of which are known to occur in South China. *Alangium chinense* (Lour.) Harms (Alangiaceae) is a deciduous shrub common in China, and the roots, flowers, and leaves of this plant have historically been used in traditional Chinese medicine [1]. Previous phytochemical investigations have demonstrated that this plant contains alkaloids and phenolic glycosides [2, 3]. In Hunan herbal medicine it is used for snake bites, circulation, contraception, hemostasis, numbness, poison, rheumatism, and wounds.

Cytochrome P450 (CYP) enzymes are responsible for most biotransformation steps of xenobiotics and endogenous molecules. Variations 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 (of abuse) and/or genetic variations of their expression can increase the risk of adverse reactions [4] or reduce the desired effect [5]. Such drug-drug interactions were described as a major reason for hospitalization or even death [6].

So far, no study on the effects of *Alangium chinense* on the metabolic capacity of CYP enzyme was reported. Therefore, in this study, five probe drugs were employed to evaluate effect of *Alangium chinense* on the metabolic capacity of CYP2B1, CYP2D1, CYP1A2, CYP3A2, CYP2C11. The effects of *Alangium chinense* on rat CYP enzyme activity will be evaluated according to the pharmacokinetic parameters changes of five specific probe drugs (bupropion, metoprolol, phenacetin, testosterone and tolbutamide).

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**Table 1.** The sequences of the primers used in polymerase chain reaction

Isoenzymes	Reverse	Forward
CYP1A2	GTTGACAATCTTCTCAGG	GTCACCTCAGGAATGCTGTG
CYP2B1	CTCCCTGTCTTTTCATTCTGT	GACAGAAGGATGAGGGAGGAA
CYP2C11	GCATCTGGCTCCTGTCTTTC	AAAAGCACAAATCCGCAGTCT
CYP2D1	GAGGACCACACCTTGAGAGC	TGAGATGTCGCTTTGGGGAC
CYP3A2	ATCAGGGTGAGTGCCAGTTCATAC	CTTCACAAACCGGAGGCCTTTTGGT

## Material and methods

### Chemicals

Bupropion, metoprolol, 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.

### Alangium chinense decoction

These raw materials (*Alangium chinense* (Lour.) Harms (Alangiaceae) 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 0.5 g/mL. The decoction was stored at 4°C.

### Pharmacokinetics

Twenty-four rats (220 ± 20 g) were randomly divided into four different dosages of Alangium chinense groups (Low-group, High-group and

control group with 8 rats in each group). Three different Alangium chinense group (Low-group, High-group) were respectively give Alangium chinense 0.6, and 1.2 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, five probe drugs bupropion, metoprolol, phenacetin, testosterone and tolbutamide were mixed in corn oil and given to the rats of three Alangium chinense groups and control group by intragastric administration at a single dosage 10 mg/kg for bupropion, metoprolol, phenacetin, testosterone, 0.1 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 five probe drugs. Plasma (100 µL) was obtained from blood sample after centrifugation at 4000 g for 10 min. In a 1.5 mL centrifuge tube, 200 µL of acetonitrile (containing 50 ng/mL IS) was added into 100 µL of collected plasma sample. After vortex-mixing for 1.0 min, the sample was centrifuged at 13000 g for 15 min. Then supernatant (2 µL) 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 Alangium chinense 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, phenacetin, testosterone and tolbutamide in rat plasma were simultaneously determined by a sensitive and simple UPLC-MS/MS method [7]. The compounds were analyzed by a UPLC-MS/MS with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer that

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**Table 2.** Pharmacokinetic parameters of probe drugs from control group and Alangium chinense group rats (mean  $\pm$  SD, n=8)

Parameters		AUC <sub>(0-t)</sub> ng/mL*h	AUC <sub>(0-∞)</sub> ng/mL*h	t <sub>1/2z</sub> h	CL <sub>z</sub> /F L/h/kg	V <sub>z</sub> /F L/kg	C <sub>max</sub> ng/mL
Bupropion (CYP2B1)	Control	57.7 $\pm$ 35.5	65.2 $\pm$ 34.9	1.2 $\pm$ 0.7	203.6 $\pm$ 123.4	352.4 $\pm$ 230.8	42.7 $\pm$ 35.3
	Low	58.5 $\pm$ 15.7	60.3 $\pm$ 15.2	0.8 $\pm$ 0.2	173.0 $\pm$ 34.3	205.8 $\pm$ 77.0	38.1 $\pm$ 15.9
	High	79.6 $\pm$ 39.8	82.6 $\pm$ 39.4	0.8 $\pm$ 0.5	146.3 $\pm$ 67.8	196.6 $\pm$ 189.8	56.7 $\pm$ 24.6
Metoprolol (CYP2D1)	Control	94.9 $\pm$ 42.2	132.4 $\pm$ 59.5	1.6 $\pm$ 0.7	99.8 $\pm$ 73.7	201.9 $\pm$ 104.2	41.9 $\pm$ 23.0
	Low	188.0 $\pm$ 66.5**	240.7 $\pm$ 80.4**	1.8 $\pm$ 1.1	45.6 $\pm$ 14.5	113.0 $\pm$ 63.5	85.3 $\pm$ 42.2*
	High	297.3 $\pm$ 151.8**	460.9 $\pm$ 262.4**	2.6 $\pm$ 1.0*	26.4 $\pm$ 10.9*	94.1 $\pm$ 36.6*	121.7 $\pm$ 53.8**
Phenacetin (CYP1A2)	Control	1868.1 $\pm$ 1059.8	1871.1 $\pm$ 1059.0	0.4 $\pm$ 0.1	6.9 $\pm$ 3.4	4.4 $\pm$ 3.2	1885.5 $\pm$ 919.6
	Low	3017.9 $\pm$ 1665.0	3019.6 $\pm$ 1664.7	0.3 $\pm$ 0.1	4.2 $\pm$ 2.1	2.3 $\pm$ 1.7	2547.0 $\pm$ 1214.7
	High	4069.3 $\pm$ 1886.4*	4074.3 $\pm$ 1888.3*	0.4 $\pm$ 0.1	3.3 $\pm$ 2.3*	1.7 $\pm$ 1.2*	3666.0 $\pm$ 1491.8*
Testosterone (CYP3A2)	Control	46.2 $\pm$ 15.9	50.0 $\pm$ 20.2	1.2 $\pm$ 0.7	223.9 $\pm$ 77.2	347.2 $\pm$ 184.4	54.9 $\pm$ 23.0
	Low	47.4 $\pm$ 13.6	54.8 $\pm$ 18.7	1.4 $\pm$ 1.1	208.4 $\pm$ 95.5	366.2 $\pm$ 185.2	66.6 $\pm$ 35.5
	High	67.1 $\pm$ 22.6	73.8 $\pm$ 29.1	1.0 $\pm$ 0.7	155.1 $\pm$ 59.8	197.9 $\pm$ 84.5	65.1 $\pm$ 13.9
Tolbutamide (CYP2C11)	Control	12965.6 $\pm$ 2407.9	13931.2 $\pm$ 2882.2	5.7 $\pm$ 1.0	0.007 $\pm$ 0.002	0.061 $\pm$ 0.012	1231.6 $\pm$ 261.2
	Low	12694.0 $\pm$ 1723.4	13677.6 $\pm$ 2023.9	6.1 $\pm$ 1.0	0.008 $\pm$ 0.001	0.065 $\pm$ 0.010	1383.3 $\pm$ 231.6
	High	13534.6 $\pm$ 2193.1	14347.9 $\pm$ 2578.6	5.4 $\pm$ 1.0	0.007 $\pm$ 0.001	0.055 $\pm$ 0.009	1516.9 $\pm$ 305.9

Alangium chinense group was compared with the control group, \*P<0.05, \*\*P<0.01.

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).

The LLOQ for each probe drug in plasma was 2 ng/mL. The RSD of the five 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 85% to 115%. The matrix effects were more than 85% or less than 115%. The extraction recoveries were better than 80%.

### Histopathology

After pharmacokinetic properties analysis, rats were deeply anesthetized with 10% chloral hydrate (i.p., 20 mg/kg). The some liver of control group and Alangium chinense 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  $\mu$ m-thick histologic sections were prepared and stained with routine HE method (hematoxylin and eosin). The morphological changes of liver were observed under light microscope.

### RT-PCR analysis

After pharmacokinetic properties analysis, rats were deeply anesthetized with 10% chloral

hydrate (i.p., 20 mg/kg). The some liver of control group and Alangium chinense treated groups was removed, frozen and store at 80°C.

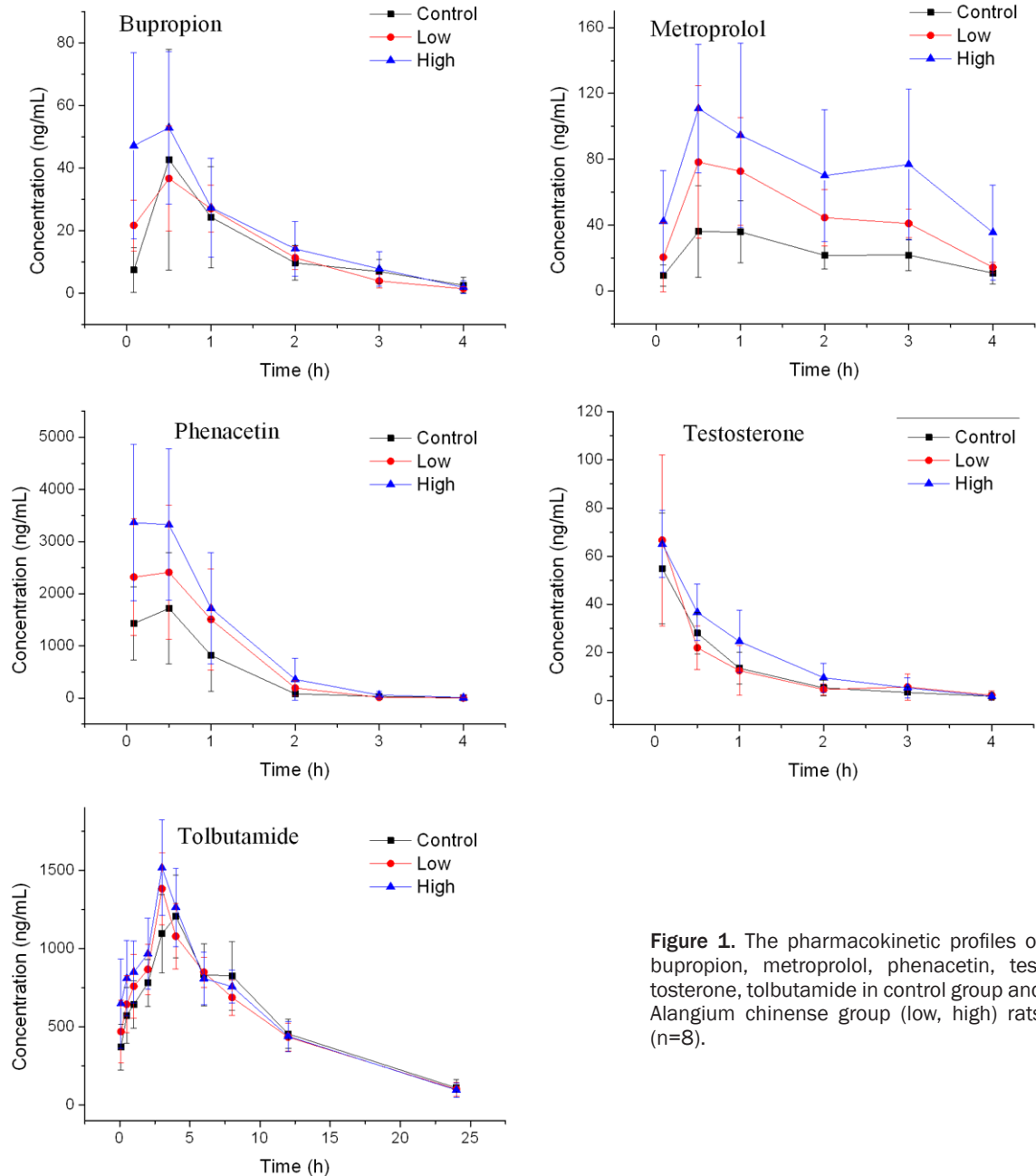
The livers were processed for isolation of total RNA by using TRIzol reagent (Invitrogen, Calsbad, CA, USA) according to the instruction of the manufacturer. The RNA concentration was determined, and the quality of the isolated RNA was assessed using the 260/280 nm absorbance ratio (1.8-2.0 indicates a highly pure sample). RNA integrity was confirmed by running samples on 1% agarose gel. The RNA pellet was stored at -80°C until use [8, 9].

We have used 2  $\mu$ L RNA in a 20  $\mu$ L reaction mixture utilizing RevertAid™ M-MuLVRT (Fermentas, Hanover, MD, USA) according to the supplier's instructions. Resulting reverse transcription products were stored at -80°C until assay.

Reactions were performed in a final volume of 20  $\mu$ L that contained Platinum SYBR Green qPCR SuperMix-UDG 12  $\mu$ L, 2  $\mu$ L cDNA, 0.6  $\mu$ L each of specific oligonucleotide primer (10  $\mu$ M), and 4.8  $\mu$ L DEPC-treated autoclaved distilled water.

PCR was carried out using initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 58°C (CYP1A2), 55°C (CYP2B1), 56°C (CYP2C-11), 63°C (CYP3A2) for 30 s, extension at 72°C

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**Figure 1.** The pharmacokinetic profiles of bupropion, metroprolol, phenacetin, testosterone, tolbutamide in control group and Alangium chinense group (low, high) rats (n=8).

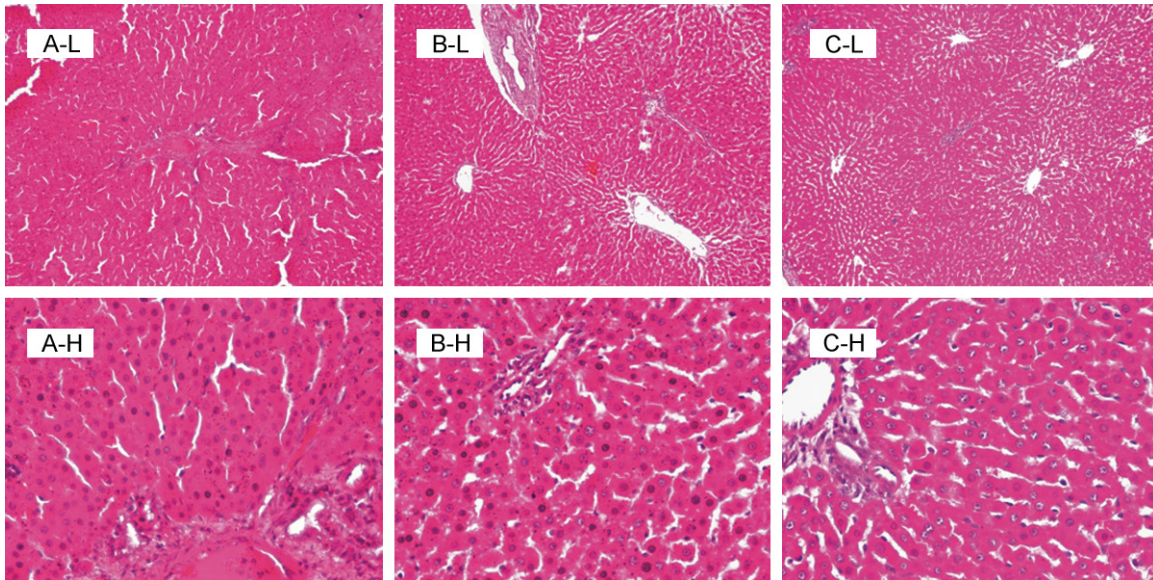
for 30 s and final extension at 72°C for 45 s. The sequences of the forward and reverse primers used in this experiment are summarized in **Table 1**.

### Results

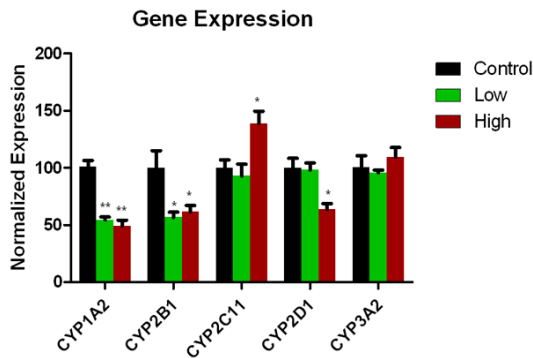
#### Pharmacokinetics

The main pharmacokinetic parameters of bupropion, metroprolol, phenacetin, testosterone and tolbutamide calculated from non-compartment model analysis were summarized in **Table 2**. The representative profiles of concentration of drugs (bupropion, metroprolol, phenacetin, testosterone and tolbutamide) vs. time were presented in **Figure 1**.

From the **Table 2**, no difference in pharmacokinetic behaviors can be observed between Alangium chinense group and control group for bupropion, testosterone and tolbutamide. While for metroprolol, compared with the control group,  $AUC_{(0-t)}$  increased (low,  $P < 0.01$ ; high,



**Figure 2.** Morphological changes of liver in control-group (A) and low dosage group (B) and high dosage group (C) (hematoxylin-eosin, L×100, H×400).



**Figure 3.** Effect of Alangium chinense on mRNA expression of CYP1A2, CYP2B1, CYP2C11, CYP2D1 and CYP3A2 in liver of rat (n=6), \*P<0.05 vs. Control; \*\*P<0.01 vs. Control group.

P<0.01), CL decreased (low, P>0.05; high, P<0.01),  $C_{max}$  increased (low, P<0.05; high, P<0.01). While for phenacetin, compared with the control group,  $AUC_{(0-t)}$  decreased (low, P>0.05; high, P<0.01), CL increased (low, P>0.05; high, P<0.01),  $C_{max}$  decreased (low, P>0.05; high, P<0.05).

#### Morphological changes of liver

According to pathological examination of live, there was no significant morphologic difference between control group and two experiment groups, low dose group and high dose group. Under low magnification, the liver lobules were

intact, liver cells attached to each other tightly and arranged radially along with central veins in experiment groups which were similar to control group. Under high magnification, the nucleuses of liver cells in low dose group and high dose group were all found to be round, clear and fine luster which were no different with control group. There were no swelling liver cells and infiltrating inflammatory cells were observed. The structures of liver sinusoid were recognized clearly in two experiment groups, hepatic cells were not found the fat vacuoles (**Figure 1**).

#### Effects of Alangium chinense on the mRNA expression of CYP450 in rat liver

After 7 days-intragastric administration of Alangium chinense (**Figure 3**), the levels of CYP1A2, CYP2B1 and CYP2D1 in the Alangium chinense group were decreased compared with the control group (P<0.05 or P<0.01), the mRNA expression levels of CYP1A2, CYP2B1 and CYP2D1 in the Alangium chinense groups were obviously lower. After 7 days-intragastric administration of Alangium chinense (**Figure 3**), the mRNA expression levels of CYP2C11 in the Alangium chinense group were increased compared with the control group (P<0.05); and the levels of CYP3A2 in the Alangium chinense group were not decreased or increased compared with the control group.

## Discussion

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. In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [10-15]. 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 [16, 17]. Similarly, supplement-drug interactions involving CYP activity are occasionally found to cause considerable adverse events. For these reasons, we evaluated the effects of acute Alangium chinense poisoning on the activity of CYP enzymes *in vivo*. We selected CYP isoforms CYP1A2, CYP2D1/CYP2D61, CYP3A2/CYP3A4, CYP2C11/CYP2C9 and CYP2B1/CYP2B6 because more than 90% of drugs are known to be metabolized by these 6 CYP enzymes [18, 19].

There no significant difference for AUC, CL and  $C_{max}$  of bupropion, testosterone and tolbutamide ( $P>0.05$ ) between the Alangium chinense group (low, high) and control group was observed. It suggested that the Alangium chinense was not able to induce or inhibit the activity of CYP2B1, CYP3A2 and CYP2C11 enzyme. The pharmacokinetic parameters of metoprolol experienced obvious change with increased  $AUC_{(0-t)}$  ( $P<0.01$ ),  $C_{max}$  ( $P<0.05$ ) and decreased CL ( $P<0.05$ ) after the dosage increase. The mRNA expression levels of CYP2D1 in the Alangium chinense groups were obviously lower. This result indicates that the 7 days-intragastric administration of Alangium chinense could inhibit the metabolism of metoprolol (CYP2D1) in rat. The pharmacokinetic parameters of phenacetin experienced obvious change with the dosage increase, there no difference in pharmacokinetic behaviors can be observed between Alangium chinense (low) group and control group, while the high group compared to control group,  $AUC_{(0-t)}$  ( $P<0.05$ ) and  $C_{max}$  ( $P<0.05$ ) increased, and CL (high,  $P<0.05$ ) decreased. The mRNA expression levels of CYP1A2 in the Alangium chinense groups were obviously lower. This result indicates that the 7 days-intragastric administration of Alangium chinense could slightly inhibit the metabolism of phenacetin (CYP1A2) in rat.

As Alangium chinense is always administrated in combination with other drugs, interactions between Alangium chinense 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 Alangium chinense inhibit the metabolism of metoprolol (CYP2D1) and phenacetin (CYP1A2). Therefore, the metabolism and elimination of drugs would change if they are administrated in combination with Alangium chinense.

After the pharmacokinetic profiles evaluation by cocktail method, we also investigated the hepatotoxicity of Alangium chinense by observing the pathological changes of liver after Alangium chinense administration. The pathological changes of liver were observed at three difference dosages with no changes in high dosage and change in low dosage, **Figure 2**. A more systematic and comprehensive study to investigate the hepatotoxicity of Alangium chinense will be carried out.

## Conclusion

The results observed in this study would provide us valuable information regarding the interactions of Alangium chinense with other drugs. Inhibit of drug metabolizing enzyme CYP2D1 and CYP1A2 by Alangium chinense would increase the plasm concentration of other drug. Enzyme inhibition by co-administered drugs and genetic variations of their expression can increase the risk of adverse reactions.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Drs. Lufeng Hu and Guanyang Lin, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China. Tel: (86) 57755579706; E-mail: hulufeng@163.com (LFH); guanyanglinwzmc@gmail.com (GYL)

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