

Original Article

Effect of *Sceptridium ternatum* on CYP450 isoforms activity of rats

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Abstract: *Sceptridium ternatum* Lyon, a common Chinese herb, has been used in treatment of allergic asthma and whooping cough. In order to investigate the effects of *Sceptridium ternatum* 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 *Sceptridium ternatum* group (Low, High) and control group. The *Sceptridium ternatum* group rats were given 1.2, 12 g/kg (Low, High) *Sceptridium ternatum* 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 bupropion, metoprolol, phenacetin and tolbutamide in rats were observed by comparing *Sceptridium ternatum* group with control group. Continuous 7 days-intragastric administration of *Sceptridium ternatum* may induce the activities of CYP2B1, CYP2D1, CYP1A2 and CYP2C11 of rats. Induction of drug metabolizing enzyme would reduce the efficacy of other drug. Additionally, *Sceptridium ternatum* did not cause hepatotoxicity.

Keywords: CYP450, *Sceptridium ternatum*, cocktail, UPLC-MS/MS, rat

Introduction

Sceptridium ternatum (Thunb.) Lyon, also named Herba *Sceptridium*, is a common herb in Zhejiang Province, China [1]. It has been considered to have anti-toxic effects in the body. *Sceptridium ternatum* (Thunb.) Lyon has been prescribed to treat asthma and whooping cough [2]. Cytochrome P450 (CYP) enzymes are responsible for most biotransformation steps of xenobiotics and endogenous molecules [3-8]. 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 [9] or reduce the desired effect [10]. Such drug-drug interactions were described as a major reason for hospitalization or even death [11].

So far, no study on the effects of *Sceptridium ternatum* on the metabolic capacity of CYP enzyme was reported. Therefore, in this study, five probe drugs were employed to evaluate effect of *Sceptridium ternatum* on the metabolic capacity of CYP2B1, CYP2D1, CYP1A2, CYP3A2, CYP2C11. The effects of *Sceptridium ternatum* 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).

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-

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Table 1. Pharmacokinetic parameters of probe drugs from control and *Sceptridium ternatum* groups of 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 (CYP2B1)	Control	270.6 \pm 74.7	285.4 \pm 74.5	0.8 \pm 0.2	37.1 \pm 9.2	46.0 \pm 20.4	183.1 \pm 66.0
	Low	72.8 \pm 27.5**	86.1 \pm 57.1**	0.9 \pm 0.9	146.0 \pm 57.4**	149.0 \pm 44.0**	47.6 \pm 10.9**
	High	45.4 \pm 17.2**	54.8 \pm 26.3**	0.7 \pm 0.1	219.7 \pm 100.5**	210.5 \pm 91.3**	40.7 \pm 20.7**
Metoprolol (CYP2D1)	Control	1384.1 \pm 194.9	1412.9 \pm 202.0	1.2 \pm 0.1	7.2 \pm 1.2	13.0 \pm 2.6	615.7 \pm 152.6
	Low	599.0 \pm 179.1**	613.5 \pm 183.5**	1.2 \pm 0.7	18.0 \pm 6.9**	30.3 \pm 16.0**	259.2 \pm 81.7**
	High	546.4 \pm 171.8**	563.7 \pm 179.7**	1.3 \pm 0.6	19.6 \pm 7.3**	33.5 \pm 12.2**	212.9 \pm 78.7**
Phenacetin (CYP1A2)	Control	4311.3 \pm 1559.6	4320.9 \pm 1558.1	0.6 \pm 0.3	2.9 \pm 2.0	2.4 \pm 1.8	4038.4 \pm 1495.2
	Low	2576.3 \pm 1789.9	2579.1 \pm 1789.3	0.8 \pm 0.6	5.3 \pm 2.7	6.3 \pm 5.0	2464.3 \pm 1477.8
	High	1117.8 \pm 600.0**	1119.1 \pm 600.0**	0.8 \pm 0.6	12.4 \pm 8.0**	16.1 \pm 14.0*	1315.7 \pm 717.7**
Testosterone (CYP3A2)	Control	112.1 \pm 65.7	145.1 \pm 139.3	2.6 \pm 2.8	100.7 \pm 45.1	260.2 \pm 77.6	51.6 \pm 19.0
	Low	113.0 \pm 46.0	121.5 \pm 51.0	1.9 \pm 0.6	99.8 \pm 52.7	255.7 \pm 123.9	45.7 \pm 21.1
	High	122.5 \pm 51.7	153.4 \pm 53.1	4.0 \pm 3.5	73.8 \pm 30.8	365.9 \pm 259.4	52.4 \pm 26.5
Tolbutamide (CYP2C11)	Control	29875.1 \pm 7816.6	34115.3 \pm 9882.6	7.6 \pm 1.6	0.003 \pm 0.001	0.034 \pm 0.006	2488.8 \pm 247.4
	Low	27226.6 \pm 4059.6	35136.6 \pm 10253.9	10.5 \pm 4.0	0.003 \pm 0.001	0.043 \pm 0.008*	1912.0 \pm 335.1**
	High	22831.9 \pm 4218.4*	26409.6 \pm 5539.7	8.0 \pm 2.4	0.004 \pm 0.001	0.045 \pm 0.012*	1596.3 \pm 297.7**

Sceptridium ternatum group was compared with the control group, *: P<0.05, **: P<0.01.

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 \pm 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.

Sceptridium ternatum decoction

These raw materials (*Sceptridium ternatum* (Thunb.) Lyon) 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 2.0 g/mL. The decoction was stored at 4°C.

Pharmacokinetics

Twenty-four rats (220 \pm 20 g) were randomly divided into three different dosages of *Sceptridium ternatum* groups (Low-group, High-

group and control group with 8 rats in each group). Two different *Sceptridium ternatum* group (Low-group, High-group) were respectively give *Sceptridium ternatum* decoction 1.2 and 12 mg/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 two *Sceptridium ternatum* 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. 100 μ L of plasma 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

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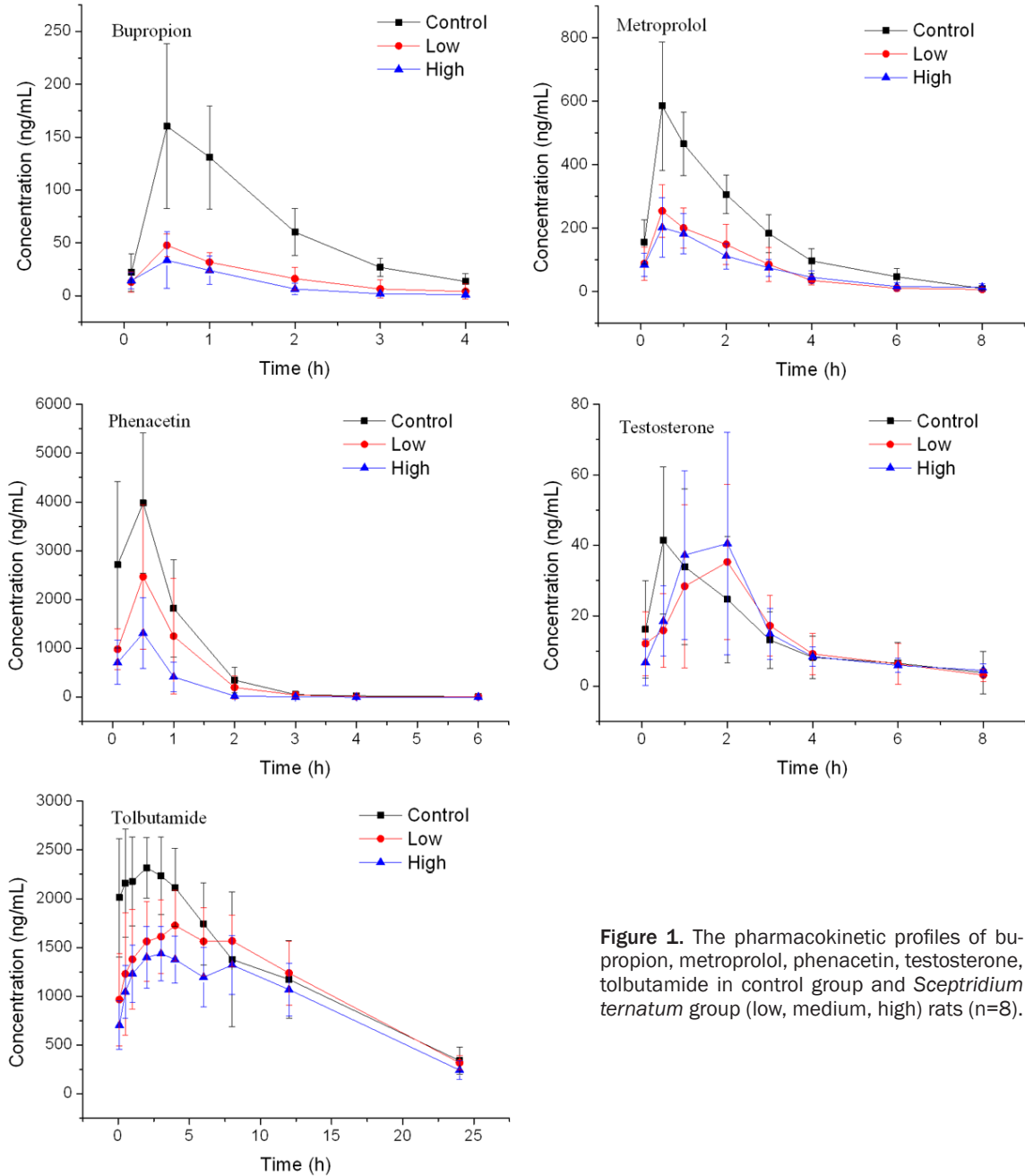


Figure 1. The pharmacokinetic profiles of bupropion, metroprolol, phenacetin, testosterone, tolbutamide in control group and *Sceptridium ternatum* group (low, medium, high) rats (n=8).

Sceptridium ternatum 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, metroprolol, phenacetin, testosterone and tolbutamid in rat plasma were simultaneously determined by a

sensitive and simple UPLC-MS/MS method [12]. 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). 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 ($r > 0.995$).

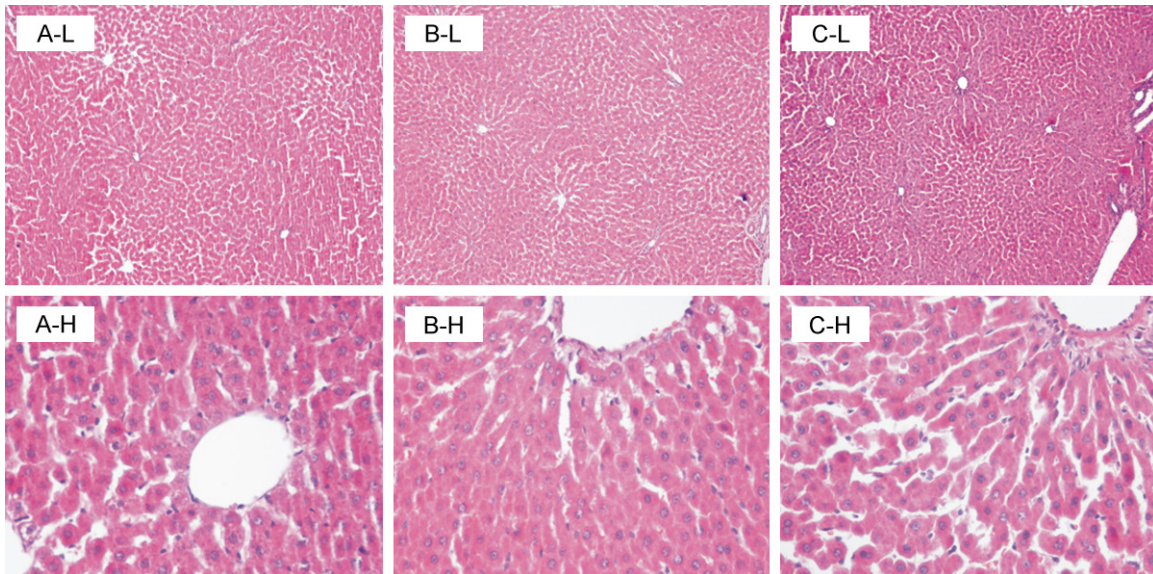


Figure 2. Morphological changes of liver in control-group (A) and low dosage group (B) and high dosage group (C) (hematoxylin-eosin, L \times 100, H \times 400).

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 *Sceptridium ternatum* 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 eosin). The morphological changes of liver were observed under light microscope.

Results

Pharmacokinetics

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

From the **Table 1**, no difference in pharmacokinetic behaviors can be observed between *Sceptridium ternatum* group and control group for testosterone. While from the **Table 1**, the pharmacokinetic behaviors of bupropion in

high dosage group compared with the control group, $AUC_{(0-t)}$ decreased ($P < 0.01$), CL increased ($P < 0.01$), C_{max} decreased ($P < 0.01$), and the similar results were found for metoprolol.

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, $P < 0.01$), and the similar results were found for tolbutamide.

Morphological changes of liver

There is no significant difference between control group, low dose group and high dose group according to pathological examination of liver. There was no liver cells swelling, dissolved, necrosis and inflammatory cell infiltrating were observed. Under low magnification, the hepatocytic plates were separated by sinusoids, liver lobules were intact and liver cells arranged tightly along with central veins which can be recognized clearly. Under high magnification, the nucleuses of liver cells in three groups were all found to be round, clear and fine luster (**Figure 2**).

Discussion

There no significant difference for AUC, CL and C_{max} of testosterone between the *Sceptridium ternatum* group and control group was

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observed. It suggested that the *Sceptridium ternatum* was not able to induce or inhibit the activity of CYP3A2 enzyme.

The pharmacokinetic parameters of bupropion and metoprolol experienced obvious change with decreased $AUC_{(0-t)}$, C_{max} and increased CL after the dosage increase. This result indicates that the 7 days-intragastric administration of *Sceptridium ternatum* could induce the metabolism of bupropion (CYP2B1) and metoprolol (CYP2D1) in rat.

The pharmacokinetic parameters of phenacetin and tolbutamide experienced obvious change, decreased $AUC_{(0-t)}$ ($P < 0.05$), C_{max} and increased CL after the dosage increase. It indicates that the 7 days-intragastric administration of *Sceptridium ternatum* could slightly induce the activity of the metabolism of phenacetin (CYP1A2) and tolbutamide (CYP2C11) in rat.

As *Sceptridium ternatum* is always administrated in combination with other drugs, interactions between *Sceptridium ternatum* 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 *Sceptridium ternatum* induce the CYP2B1, CYP2D1, CYP1A2 and CYP2C11. Therefore, the metabolism and elimination of drugs would change if they are administrated in combination with *Sceptridium ternatum*.

In conclusion, the results observed in this study would provide us valuable information regarding the interactions of *Sceptridium ternatum* with other drugs. Induction of drug metabolizing enzyme would reduce the efficacy of other drug. *Sceptridium ternatum* may not cause hepatotoxicity.

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Disclosure of conflict of interest

None.

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