ORIGINAL ARTICLES

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Effects of *Polygonum multiflorum* on the activity of cytochrome P450 isoforms in rats

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Received June 20, 2014, accepted August 8, 2014

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Pharmazie 70: 47–54 (2015) *doi: 10.1691/ph.2015.4693*

Polygonum multiflorum is a traditional Chinese medicinal herb used in clinical medicine to nourish the liver and kidney. However, in recent years, there have been increased reports of clinical adverse reactions associated with *Polygonum multiflorum* preparations, especially due to liver injury. The cocktail method can be used to assess the influence of *Polygonum multiflorum* on the activity of cytochrome P450 (CYP450) isoforms CYP2B6, CYP2C19, CYP2C9, CYP1A2, CYP3A4, and CYP2D6, which were reflected by changes in pharmacokinetic parameters in six specific probe drugs: bupropion, omeprazole, tolbutamide, phenacetin, midazolam, and metoprolol. Comprised the experimental rats were randomly divided into five groups: control group, alcohol extraction A group, alcohol extraction B group, water extraction A group, and water extraction B group. Each group five male rats and five female rats. Each of the groups received treatments by gavage as follows: control group was given normal saline, alcohol extraction A group was given 15 g/kg alcohol extract of *Polygonum multiflorum* (E15), alcohol extraction B group was given with 30 g/kg alcohol extract (E30), water extraction A group was given 15 g/kg water extract (W15), and water extraction B group was given 30 g/kg water extract (W30). The extract solution was orally administered once a day for 28 consecutive days. The mixture of six probe drugs was given by gavage, and blood samples were collected through the tail vein at different time points. Probe drug concentration in rat plasma was measured by liquid chromatography-mass spectrometry (LC-MS). In the treatment and control groups, *Polygonum multiflorum* alcoholic extract inhibited the activity of CYP2C19 and CYP2C9 and induced the activity of CYP1A2. *Polygonum multiflorum* aquous extract inhibited the activity of CYP2B6, CYP2C19, CYP2C9, CYP1A2, and CYP2D6. Pathological sections showed that in the alcohol extract group the liver was degenerated inconspicuously, and in the water extract group, the cytoplasm had vacuoles and particulate matter. The arrangement of liver cells was irregular.

1. Introduction

The roots of *Polygonum multiflorum* (Chineses name: He-Shou-Wu) are a traditional Chinese medicinal herb used in clinical medicine to nourish the liver and kidney. Ancient herbal books rarely mentioned its safety issues while most of them claimed that it is “non-toxic” but in recent years, clinical adverse reactions have been increasingly reported (Wu et al. 2012), especially liver injury (Dong et al. 2014; Jung et al. 2011; Zhang et al. 2009). The British Medicines and Healthcare Products regulatory agency (MHRA) has issued information regarding *Polygonum multiflorum*’s adverse effects including acute liver injury, significantly increased transaminase, and obvious jaundice. Adverse reactions can take several days to three months to manifest, but most take approximately one month (Dong et al. 2014). Thus, it is important to further clarify the relationship between the different components of the *Polygonum multiflorum* and its toxic effects on the liver, to study its overall oral toxicity, to understand its toxic characteristics and mechanisms, how to reduce clinical adverse reactions, and how to use *Polygonum multiflorum* in a more safe and reasonable manner.

Cytochrome P450 (CYP450) is a supergene family that encodes more than 500 different enzymatic proteins (Zhang et al. 2012). In the human body, CYP450 enzymes are found mainly in the liver and small intestine. Such enzymes have many isozymes, which are divided into multiple genetic/subgenomic families, and enzymes of the same family have similar functions (He et al. 2014). CYP450 enzymes comprise a superfamily of hemoproteins, and three families (CYP1, CYP2, and CYP3) are mainly involved in the metabolism of drugs in both humans and rats (Kobayashi et al. 2003). CYP450 is the most important oxidase of the microsomal mixed-function family of oxidases, which is closely related to the metabolism of endogenous and exogenous substances (Ingelman-Sundberg et al. 2007; Nithipatikom and Gross 2010; Walsh and Miwa 2011). Among the CYP isoforms, families 1 through 3 are the major enzymes involved in drug metabolism, accounting for about 75% of the total number of different metabolic reactions (Furge and Guengerich, 2006). More than 90% of marketed drugs are metabolized by the CYP1A2, 2D6, 2C9, 2C19, 2B6, and 3A_ isoforms (Oh et al. 2012). In order to assess various individual CYP450 activities, probe drugs have been widely used in many clinical

investigations in the field of drug metabolism and pharmacogenetics (Kozakai et al. 2012; Turpault et al. 2009).

Cocktail probing is a method of using several drugs metabolized by different enzymes at the same time–depending on the ratio of its metabolites to prototype drug–to measure changes in metabolism. Now it has become an effective experimental method for observing selective regulating functions of individual CYP enzymes. The use of this cocktail method is increasingly more widespread and can be used to determine the activity of CYP450 enzymes *in vitro* and *in vivo*. It also has important applications for quantitative determination of *in vivo* activity of drug metabolizing enzymes.

In this paper, the cocktail probe drugs approach is used to evaluate the induction or inhibition effects of *Polygonum multiflorum* that resulted in liver toxicity by observing the activities of rat cytochrome P450 isoforms CYP2B6, CYP2C19, CYP2C9, CYP1A2, CYP3A4, and CYP2D6, which are reflected in the changes in pharmacokinetic parameters from six specific probe drugs: bupropion, omeprazole, tolbutamide, phenacetin, midazolam, and metoprolol. Tissue pathological sectioning also revealed the mechanism of *Polygonum multiflorum*-induced liver toxicity in rats.

2. Investigations and results

2.1. Method validation

Figure 1 shows the chromatograms of a blank plasma sample, a blank plasma sample spiked with probe drugs and internal standard substance (IS), and a plasma sample detected by LC-MS. No other endogenous substances were observed during the retention time of the variables and IS, which is confirmed by the clear resolution of the peaks shown. Calibration curves for six probe drugs were generated by linear regression of peak area ratios against concentrations. The calibration plot of the probe drugs in the range of 20-5000 ng/mL were $Y = 0.0006X + 0.0723$ ($r = 0.9936$) for bupropion, $y = 0.0002x + 0.0482$ ($r = 0.9925$) for omeprazole, $y = 0.00005x + 0.017$ ($r = 0.9922$) for tolbutamide, $y = 0.0002x - 0.0037$ ($r = 0.9997$) for phenacetin, $y = 0.0014x + 0.2379$ ($r = 0.9948$) for midazolam, and $y = 0.0009x + 0.0583$ ($r = 0.9983$) for metoprolol. The peak area ratio and concentration of each probe drug had good linear relationships within the concentration range. The lower limit of quantitation (LLOQ) for each probe drug in plasma was 10ng/mL. Table 1 shows the results for intra-day precision, inter-day precision, accuracy, and the recovery rate of extraction. The relative standard deviation (RSD%) of the six probe drugs in 20 ng/mL, 500ng/mL, and 2000 ng/mL were less than 15%. The intra-day relative error (RE%) ranged from −6.55 to 10.54% while the inter-day RE% ranged from −11.88 to 11.47%. The results demonstrated that the values were within the acceptable range, and the method was accurate and precise. The extraction recoveries ranged from 89.36 to 99.68%. Table 1 shows the result of the matrix effect, and the percentage of concentration was between 85% and 115%. Results showed that using this method, the plasma ion inhibition or interference of the matrix is negligible.

2.2. Pharmacokinetic study

The main pharmacokinetic parameters after administration of bupropion, omeprazole, tolbutamide, phenacetin, midazolam, and metoprolol from non-compartment model analysis are summarized in Table 2

Compared with the control group, pharmacokinetics parameters of midazolam in the experimental group showed almost no change.

Compared with the control group, the alcohol extraction group of bupropion showed no statistical differences in pharmacokinetics parameters. In the water extraction group, the plasma concentration-time curve (AUC0-t) of W15 and W30 groups increased from 1857.27 to 5509.63 ng/mL h and 2982.58 ng/mL h, respectively, which had significant differences ($p < 0.05$). Plasma clearance (CL) of W15 and W30 groups were reduced from 5.81 to 2.74 L/h kg and 3.64 L/h kg, respectively, which had significant differences ($p < 0.01$, $p < 0.05$). Maximum plasma concentration (C_{max}) of W15 and W30 groups were increased from 749.30 to 1177.08 ng/mL and 1099.29 ng/mL, respectively, which had significant differences ($p < 0.05$).

Compared with the control group, the AUC_{0-t} of omeprazole was increased in the alcohol extraction group and the water extraction group. For E30, W15, and W30 groups, AUC_{0-t} increased from 970.84 ng/mL h to 1522.06 ng/mL h, 4375.96 ng/mL h and 2964.94 ng/mL h, respectively, with significant differences ($p < 0.05$, $p < 0.01$, $p < 0.01$). CL was reduced in all the groups, of which the E30, W15, and W30 groups were reduced from 8.67 L/h kg to 4.98 L/h kg, 2.54 L/h kg, and 3.51 L/h kg respectively, with significant differences ($p < 0.05$, $p < 0.01$, $p < 0.01$, respectively). C_{max} increased in all the groups, specifically the E30, W15, and W30 groups increased from 610.30 ng/mL to 917.05 ng/mL, 2243.16 ng/mL, and 2232.00 ng/mL, respectively, with significant differences ($p < 0.05$, $p < 0.01$, $p < 0.01$).

Compared with the control group, the AUC_{0-t} of tolbutamide increased in the alcohol extraction and water extraction group. The E15, E30, W15, and W30 groups increased from 67909.22 ng/mL h to 360300.70 ng/mL h, 228784.19 ng/mL h, 463695.63 ng/mL h, and 351217.72 ng/mL h, respectively, all of which had significant differences ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.01$, respectively). All CL reduced from 0.02 to 0.003 L/h kg, 0.005 L/h kg, 0.002 L/h kg, 0.003 L/h kg, respectively; all had significant differences ($p < 0.01$). All C_{max} increased from 9257.71 to 34854.88 ng/mL, 21800.93 ng/mL, 47889.30 ng/mL, and 35724.45 ng/mL, respectively; all had significant differences ($p < 0.01$).

Compared with the control group, AUC_{0-t} of phenacetin was reduced in the alcohol extraction group, E15 and E30 reduced from 7872.74 ng/ml h to 3833.78 ng/mL h, and 3724.57 ng/ml h, respectively, with significant differences ($p < 0.05$). In the water extraction group, AUC_{0-t} increased to 19722.62 ng/mL h and 14281.43 ng/mL h, respectively, with significant differences ($p < 0.01$, $p < 0.05$). CL increased in the alcohol extraction group, in which E15 and E30 increased from 1.54 L/h kg to 4.44 L/h kg and 3.63 L/h kg, respectively, with statistical differences ($p < 0.05$). CL decreased in the water extraction group, where W15 and W30 were reduced to 0.54 L/h kg and 0.87 L/h kg, respectively, with significant differences ($p < 0.05$, $p < 0.01$). C_{max} was reduced in the alcohol extraction group, where E15 and E30 reduced from 5726.51 ng/mL to 3098.85 ng/ml and 3044.28 ng/mL, respectively, with significant differences ($p < 0.05$, $p < 0.01$). C_{max} increased for the water extraction group, where W15 and W30 increased to 9193.26 ng/mL and 7676.91 ng/mL, respectively, with statistically significant differences ($p < 0.01$, $p < 0.05$).

Comparing the alcohol extraction group with the control group, there was no significant difference in the plasma concentration-time curve (AUC_{0-t}), plasma clearance (CL), or maximum plasma concentration (C_{max}) for metoprolol. In the water extraction group, AUC_{0-t} of metoprolol increased, and the W15 and W30 were increased from 3008.02 ng/mL h to 7564.82 ng/mL h and 5770.34 ng/mL h, with significant differences ($p < 0.01$, $p < 0.05$). CL was reduced from 3.16 L/h kg to 1.41 L/h kg and 2.00 L/h kg, with significant differences ($p < 0.05$, $p < 0.01$). C_{max} was increased from 1316.63 ng/mL to 2176.79 ng/mL and 1747.03 ng/mL, with significant differences

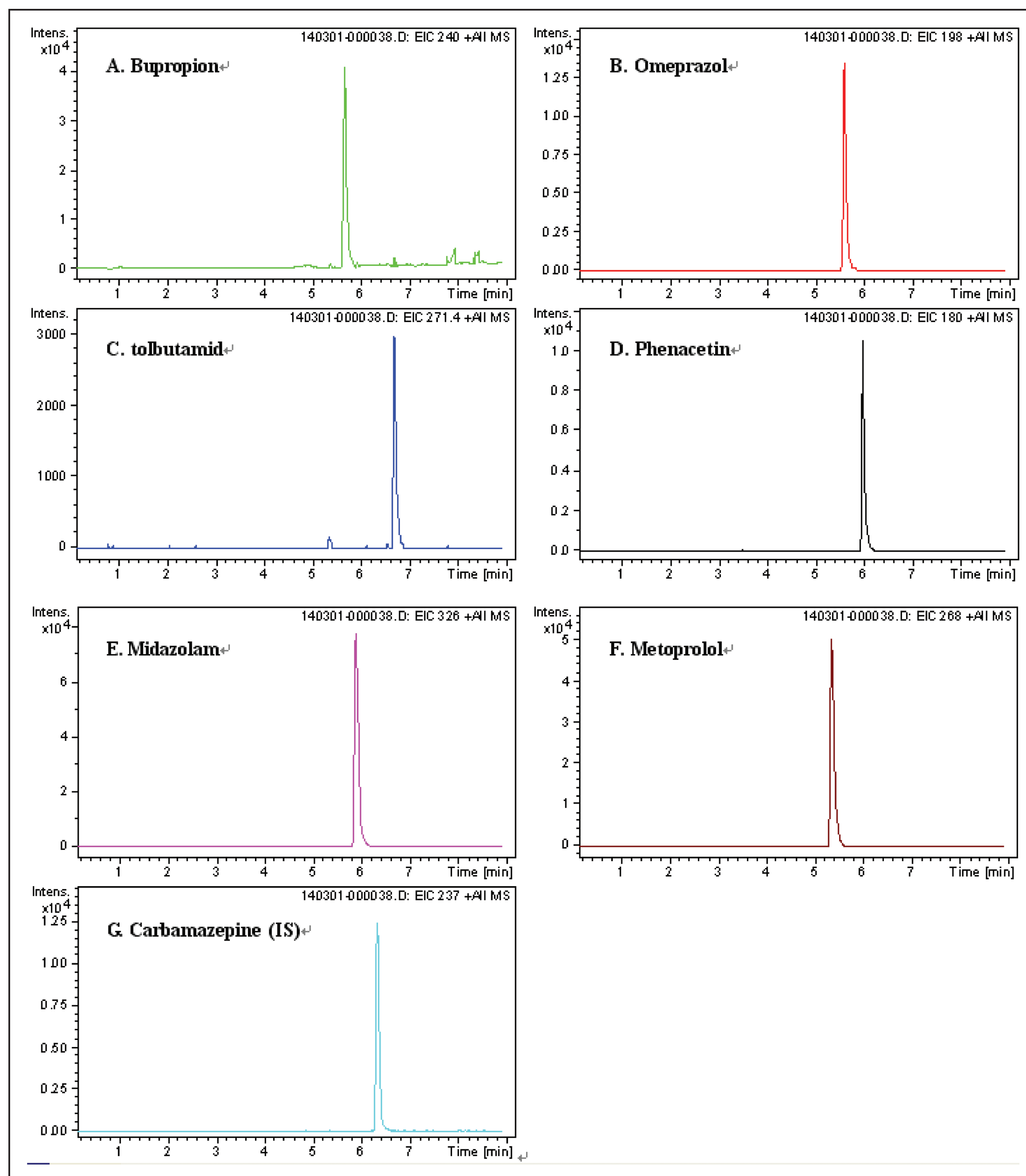


Fig. 1: LC-MS Chromatograms. A. Blank plasma spiked with bupropion; B. Blank plasma spiked with omeprazole; C. Blank plasma spiked with tolbutamide; D. Blank plasma spiked with phenacetin; E. Blank plasma spiked with midazolam; F. Blank plasma spiked with metoprolol; G. Blank plasma spiked with carbamazepine (IS).

($p < 0.01$, $p < 0.05$). Figure 2 shows the concentration-time curve of bupropion, omeprazole, tolbutamide, phenacetin, midazolam, and metoprolol.

2.3. *Liver pathological sections*

As can be seen from Fig. 3, compared with control group, the arrangement of liver cells was relatively regular and had less degenerated cells in the *Polygonum multiflorum* alcohol extraction group. Liver cell cytoplasm of the water extraction group had particulate matter and vacuoles, and the arrangement of liver cells was irregular.

3. Discussion

Compared to the control group, AUC of bupropion increased ($p < 0.05$), CL was reduced ($p < 0.05$), and C_{max} was increased in the *Polygonum multiflorum* water extraction group, indicating that the water extract inhibited the activity of CYP2B6 enzyme. Compared to the control group, in E30, W15, and W30, AUC of omeprazole increased ($p < 0.05$, $p < 0.01$, $p < 0.01$), CL reduced ($p < 0.05$, $p < 0.01$, $p < 0.01$), and C_{max} increased, ($p < 0.05$, $p < 0.01$, $p < 0.01$), indicating that high doses of *Polygonum multiflorum* alcohol extract and water extract can inhibit the activity of CYP2C19 enzyme. For tolbutamide, AUC increased ($p < 0.05$), CL reduced ($p < 0.01$), C_{max} increased ($p < 0.01$) for *Polygonum multiflorum* water extract and alcohol extract groups compared to the control group, indicating that water and alcohol extract can inhibit the activity of enzyme CYP2C9.

Compared to the control group, phenacetin's AUC decreased ($p < 0.05$), CL increased ($p < 0.05$), C_{max} reduced ($p < 0.05$) in the *Polygonum multiflorum* alcohol extract group, indicating that it can induce the activity of CYP1A2 enzyme. While the *Polygonum multiflorum* water extract group can inhibit

Table 1: Precision, accuracy and recovery for probe drugs in rat plasma (mean ± S.D., n = 10)

Probe drugs	Conc. (ng/mL)	RSD (%)		RE (%)		Recovery (%)	Matrix effect (%)
		Intra-day	Inter-day	Intra-day	Inter-day		
Bupropion	20	8.56	10.49	7.24	9.24	91.56 ± 6.35	93.21 ± 7.55
	500	9.32	8.52	−6.55	7.55	98.56 ± 3.52	87.57 ± 4.24
	2000	4.25	5.69	3.49	−5.32	96.25 ± 6.79	93.45 ± 1.06
Omeprazole	20	5.26	6.35	9.45	10.22	92.55 ± 1.35	85.89 ± 5.36
	500	7.41	7.24	−5.36	3.46	89.28 ± 8.44	88.26 ± 4.32
	2000	5.36	6.52	3.25	−2.12	98.13 ± 2.55	93.25 ± 1.78
tolbutamide	20	7.25	8.47	8.69	9.35	93.69 ± 6.37	88.85 ± 6.99
	500	5.24	5.23	−5.66	−6.99	95.47 ± 4.26	94.55 ± 2.33
	2000	4.29	4.22	5.74	4.78	89.64 ± 2.14	98.65 ± 2.33
Phenacetin	20	10.25	10.55	10.25	−11.88	90.44 ± 8.69	87.96 ± 5.89
	500	8.26	9.62	−3.82	5.46	97.89 ± 7.16	95.26 ± 1.23
	2000	6.26	5.29	4.36	5.63	90.11 ± 9.36	90.44 ± 9.26
Midazolam	20	11.58	12.28	10.54	11.47	96.22 ± 1.56	89.74 ± 1.66
	500	8.54	9.22	−4.33	−5.25	90.74 ± 8.58	98.88 ± 4.36
	2000	6.22	8.25	5.87	4.22	99.68 ± 7.69	97.83 ± 8.74
Metoprolol	20	12.35	11.24	9.54	8.74	92.36 ± 10.79	90.67 ± 5.99
	500	9.26	7.36	3.58	5.66	98.12 ± 6.36	88.44 ± 7.24
	2000	6.51	5.34	−1.61	−3.58	89.36 ± 5.22	89.69 ± 4.51

the activity of CYP1A2 enzyme, for phenacetin, the AUC increased ($p < 0.01$, $p < 0.05$), CL reduced ($p < 0.01$, $p < 0.05$), C_{max} increased ($p < 0.01$, $p < 0.05$).

Compared to the control group, the pharmacokinetic parameters of midazolam did almost not almost change, indicating that *Polygonum multiflorum* alcohol and water extracts did not induce or inhibit the activity of CYP3A4. Compared to the control group, in the *Polygonum multiflorum* water extract group, metoprolol's AUC increased ($p < 0.01$, $p < 0.05$), CL reduced ($p < 0.01$, $p < 0.05$), C_{max} increased ($p < 0.01$, $p < 0.05$), indicating that *Polygonum multiflorum* water extract can inhibit the activity of CYP2D6.

In addition, pathological sections revealed that degeneration in the *Polygonum multiflorum* alcohol extract group was not obvious, while the cytoplasm in the water extract group had particulate matter and vacuoles, and the arrangement of liver cells was relatively irregular.

Polygonum multiflorum extract exerted certain effects on rat liver CYP450 enzyme activity. Results demonstrated that *Polygonum multiflorum* alcohol extract can inhibit the activity of CYP2C19 and CYP2C9 and induce the activity of CYP1A2. *Polygonum multiflorum* water extract can inhibit the activity of CYP2B6, CYP2C19, CYP2C9, CYP1A2, and CYP2D6 in rats.

Firstly, it is better to avoid using large doses of *Polygonum multiflorum* for long periods of time, for it may inhibit the activity of some CYP450 enzymes. More than 90% of marketed drugs are metabolized by the CYP1A2, 2D6, 2C9, 2C19, 2B6, and 3A isoforms. When these are combined with large doses of *Polygonum multiflorum*, close attention must be paid in order to avoid high plasma drug concentrations resulting in toxic reactions.

4. Experimental

4.1. Chemicals and reagents

Bupropion, omeprazole, tolbutamide, phenacetin, midazolam, and metoprolol (all > 98%) and internal standard substance (IS) carbamazepine were bought from Sigma-Aldrich Company (St. Louis, U.S.A.). HPLC grade acetonitrile and methanol were from Merck Company (Darmstadt, Germany). All other chemicals were of analytical grade. Ultra-pure water (resistance > 18 mΩ) was prepared by a Millipore Milli-Q purification system (Bedford, U.S.A.).

4.2. Animals

Male and female Sprague-Dawley rats (200 ± 12 g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. The animal license number was SCXK (Shanghai) 2012-0002. All fifty rats were housed at Wenzhou Medical University Laboratory Animal Research Center. Animal were housed under controlled conditions (22 °C) With a natural light-dark cycles. All experimental procedures were conducted according to the Institutional Animal Care guidelines and with ethics approval from the Administration Committee of Experimental Animals, Laboratory Animal Center of Wenzhou Medical University.

4.3. Instuments and Conditions

All analyses were performed with a 1200 Series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, a degasser, an autosampler, a thermostated column compartment, and a Bruker Esquire HCT mass spectrometer (Bruker Technologies, Bremen, Germany) equipped with an electrospray ion source and controlled by ChemStation software (Version B.01.03 [204], Agilent Technologies, Waldbronn, Germany).

Polygonum multiflorum preparations were purchased from the First Affiliated Hospital of Wenzhou Medical University, Guizhou origin. In this study, the method of alcohol extraction was as follows: *Polygonum multiflorum* was crushed into a crude powder and eight times the amount of 80% ethanol was added followed by heating under reflux for extraction three times. Each extraction time was 1 h, combined with alcohol extracting solution, then concentrated and frozen at 4 °C. The method of water extraction was as follows: *Polygonum multiflorum* was crushed into a crude powder, and eight times the amount of water was added and heated at reflux for extraction three times. Each extraction time was 1 h, combined with water extracting solution, then concentrated and frozen at 4 °C. Using a water bath, it was also heated to 37 °C before each administration.

Chromatographic separation was achieved on a 150 mm × 2.1 mm, 5 μm, Agilent Zorbax SB-C_{18} column at 30 °C. A gradient elution program was conducted for chromatographic separation with mobile phase A (0.1 % formic acid in water) and mobile phase B (acetonitrile) as follows: 0−4.0 min (10−80 % B), 4.0−8.0 min (80−80 % B), 8.0−9.0 min (80−10 % B), 9.0−13.0 min (10−10 % B). The flow rate was 0.4 mL/min.

Quantification was performed by peak-area method. The determination of target ions was performed in selective ion monitoring mode (180 m/z for phenacetin, 271 m/z for tolbutamide, 240 m/z for bupropion, 268 m/z for metroprolol, 326 m/z for midazolam, 198 m/z for metoprolol and 237 m/z for IS). Drying gas flow was set to 7 L/min and temperature to 350 °C. Nebulizer pressure and capillary voltage of the system was adjusted to 25 psi and 3500 V, respectively.

4.4. Drugs and groups

The 50 rats were randomly divided into five groups: Control group, E15 group, E30 group, W15 group, W30 group. Each group had five male rats

Table 2: Pharmacokinetic parameters of bupropion, omeprazole, tolbutamide, phenacetin, midazolam and metoprolol (mean ± S.D.), Control group n = 10, experimental group n = 10

Pharmacokinetic	parameters	bupropion	omeprazole	tolbutamide	phenacetin	midazolam	metoprolol
Half-life($t_{1/2}$)(h)	control	3.98 ± 1.36	14.32 ± 5.21	6.96 ± 3.64	0.70 ± 0.20	18.23 ± 13.39	19.73 ± 46.68
	E15	8.89 ± 6.73	9.16 ± 4.98	7.99 ± 7.79	0.57 ± 0.18	10.27 ± 6.46	5.80 ± 3.23
	E30	11.90 ± 7.39	27.82 ± 29.92	7.21 ± 8.41	0.61 ± 0.21	11.41 ± 8.99	6.73 ± 2.88
	W15	3.06 ± 1.11	8.39 ± 7.73	8.19 ± 4.64	1.53 ± 1.36	10.38 ± 9.34	5.70 ± 6.71
	W30	3.58 ± 4.19	11.52 ± 13.15	6.44 ± 4.22	0.97 ± 0.74	11.07 ± 11.69	5.60 ± 5.80
AUC_{0-t}(ng/ml·h)	control	1858.27 ± 615.12	970.84 ± 224.20	67909.22 ± 34247.49	7872.74 ± 3736.81	3353.95 ± 1051.46	3008.02 ± 806.36
	E15	2044.43 ± 947.66	1287.41 ± 320.04	360300.70 ± 121941.76*	3833.78 ± 2841.93*	2312.69 ± 947.17	3477.47 ± 1429.51
	E30	1532.20 ± 588.84	1522.06 ± 481.59 *	228784.19 ± 83846.51**	3724.57 ± 1959.75 *	3152.44 ± 1674.37	2835.13 ± 1158.42
	W15	5509.63 ± 3946.52*	4375.96 ± 1880.03**	463695.63 ± 70642.20 **	19722.62 ± 5076.08**	5182.88 ± 2239.61	7564.82 ± 2601.68 **
	W30	2982.58 ± 901.27*	2964.94 ± 870.59**	351217.72 ± 142805.14**	14281.43 ± 6143.61*	3161.86 ± 1095.43	5770.34 ± 2351.21 *
$AUC_{0-\infty}$(ng/ml·h)	control	1890.79 ± 647.26	1221.70 ± 309.76	76661.17 ± 39773.87	8320.28 ± 4190.62	3696.41 ± 1163.37	3560.46 ± 1399.57
	E15	2255.69 ± 993.75	1483.80 ± 443.34	417266.37 ± 117737.08	3866.63 ± 2838.95	2562.27 ± 1005.74	3571.58 ± 1496.68
	E30	1705.15 ± 638.62	2568.78 ± 1492.53	280997.74 ± 168891.95	3757.16 ± 1959.38	3405.38 ± 1627.78	2952.89 ± 1217.37
	W15	5570.66 ± 4026.41	4629.02 ± 1956.60	550614.50 ± 123078.72	19818.79 ± 5097.06	5454.60 ± 2059.21	7997.26 ± 3089.72
	W30	3000.30 ± 897.48	3159.86 ± 1105.40	394513.73 ± 170404.87	14647.31 ± 6627.78	3310.44 ± 1175.92	5803.95 ± 2336.12
Average dwell time from zero to time(MRT0-t)(h)	control	4.01 ± 0.56	5.88 ± 0.75	7.03 ± 0.61	1.09 ± 0.32	2.62 ± 0.61	2.03 ± 0.31
	E15	5.12 ± 1.33	5.67 ± 1.51	7.65 ± 1.75	0.98 ± 0.12	4.41 ± 1.0040	3.30 ± 0.90
	E30	4.75 ± 1.17	5.89 ± 1.66	7.56 ± 1.15	0.93 ± 0.17	3.41 ± 0.71	2.83 ± 0.82
	W15	4.50 ± 0.89	3.40 ± 0.97	7.51 ± 1.27	2.08 ± 0.88	3.40 ± 1.00	3.51 ± 0.93
	W30	3.08 ± 0.37	2.93 ± 0.50	7.08 ± 0.95	1.42 ± 0.56	2.83 ± 0.46	2.78 ± 0.29
CL(L/h/kg)	control	5.81 ± 1.85	8.67 ± 2.31	0.02 ± 0.01	1.54 ± 0.90	2.93 ± 0.86	3.16 ± 1.11
	E15	5.48 ± 3.28	7.29 ± 2.30	0.0030 ± 0.0010 **	4.44 ± 1.54*	4.42 ± 1.56	3.41 ± 1.98
	E30	6.51 ± 2.09	4.89 ± 2.44 *	0.0050 ± 0.0020 **	3.63 ± 1.39*	3.65 ± 1.99	3.86 ± 1.44
	W15	2.74 ± 1.83 **	2.54 ± 1.05 **	0.0020 ± 0.0010 **	0.54 ± 0.14 **	2.20 ± 1.22	1.41 ± 0.52 **
	W30	3.64 ± 1.24 *	3.51 ± 1.22 **	0.0030 ± 0.0010 **	0.87 ± 0.49*	3.32 ± 1.01	2.00 ± 0.83 *
Apparent volume of distribution (V)(L/kg)	control	41.96 ± 32.00	171.95 ± 47.72	0.14 ± 0.067	1.50 ± 0.91	72.64 ± 50.03	50.79 ± 106.07
	E15	62.10 ± 41.77	87.55 ± 37.05	0.028 ± 0.030	3.94 ± 3.22	59.60 ± 24.78	24.03 ± 8.88
	E30	114.67 ± 98.27	132.47 ± 66.55	0.035 ± 0.025	2.92 ± 1.57	58.83 ± 50.62	35.81 ± 17.70
	W15	10.98 ± 7.52	28.60 ± 20.57	0.020 ± 0.0080	1.16 ± 1.03	41.98 ± 48.85	9.78 ± 10.14
	W30	18.80 ± 22.83	46.82 ± 31.97	0.025 ± 0.010	1.18 ± 1.18	45.45 ± 44.073	18.86 ± 23.50
Cmax(ng/ml)	control	749.30 ± 298.40	610.30 ± 268.24	9257.71 ± 3448.27	5726.51 ± 1392.40	1779.60 ± 393.56	1316.63 ± 236.44
	E15	543.18 ± 346.57	771.03 ± 271.74	34854.88 ± 10006.04**	3098.85 ± 1863.16 *	898.10 ± 391.69	1200.42 ± 693.37
	E30	490.45 ± 231.85	917.05 ± 283.67*	21800.93 ± 4605.81 **	3044.28 ± 1262.58 *	1481.66 ± 787.00	1224.03 ± 372.40
	W15	1177.08 ± 608.96*	2423.16 ± 950.89 **	47889.30 ± 9367.72 **	9193.26 ± 720.21 **	2083.39 ± 772.59	2176.79 ± 599.17 **
	W30	1099.29 ± 312.05*	2232.00 ± 915.62 **	35724.45 ± 10439.77**	7676.91 ± 1900.99 *	1548.77 ± 522.84	1747.03 ± 450.84 *

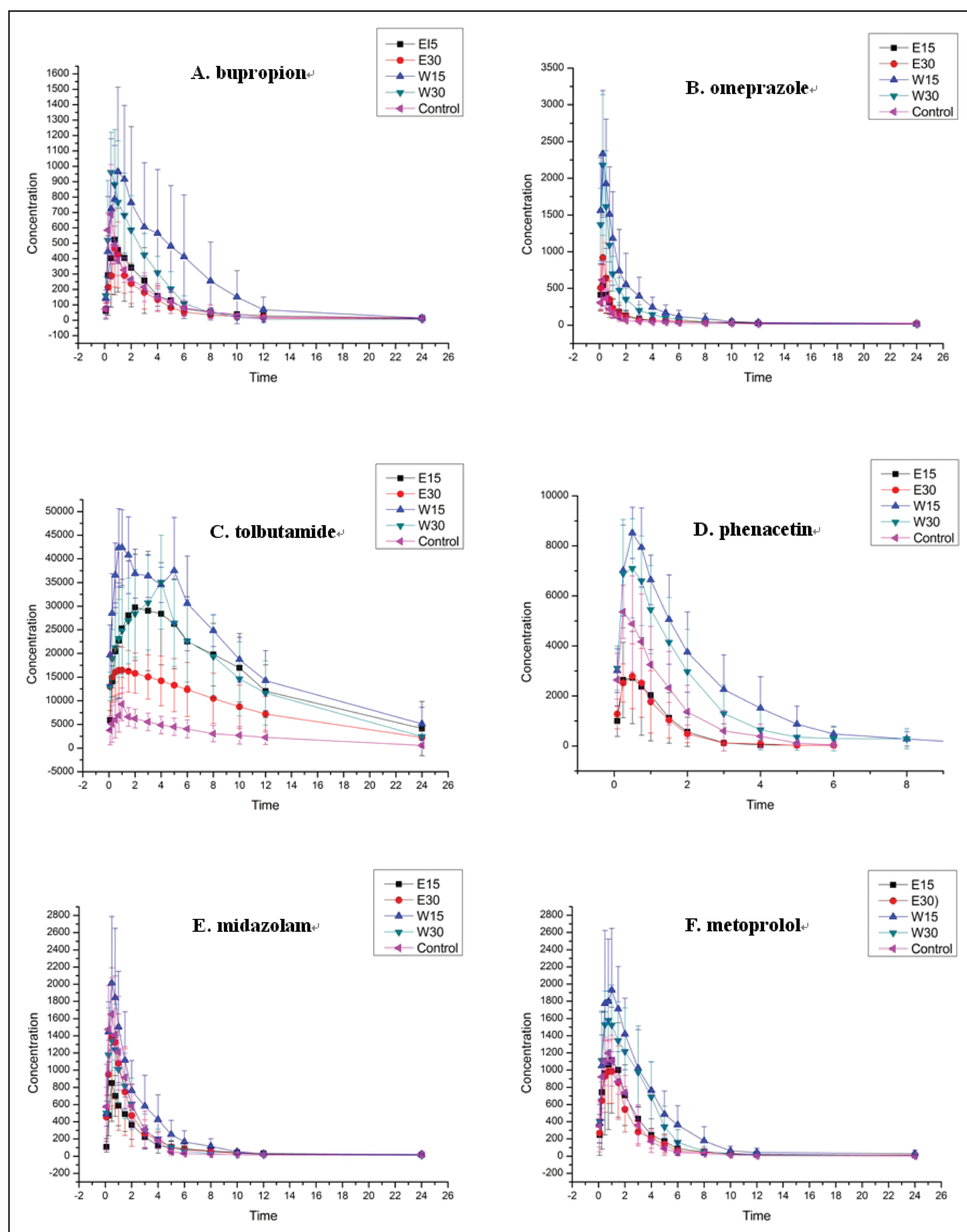


Fig. 2: Pharmacokinetic profiles of bupropion (A), omeprazole (B), tolbutamide (C), phenacetin (D), midazolam (E), metoprolol (F).

and five female rats. Treatment was administered by gavage. The control group was given normal saline, E15 and E30 groups were given 15 g/kg and 30 g/kg of alcohol extract of *Polygonum multiflorum*, respectively. W15 and W30 groups were given 15 g/kg and 30 g/kg of water extract of *Polygonum multiflorum*, respectively. The dosing volume was 15 mL/kg, administered orally once a day for 28 consecutive days.

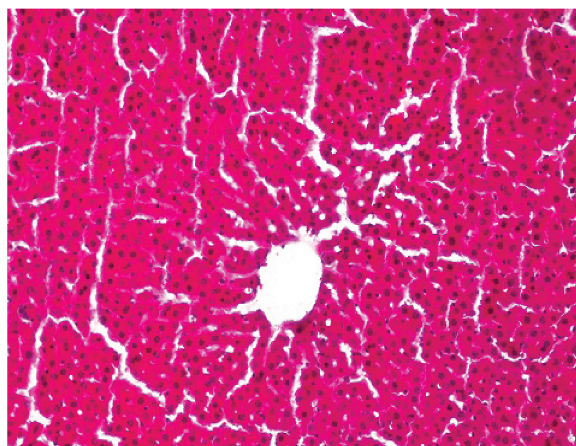
4.5. Pharmacokinetic studies

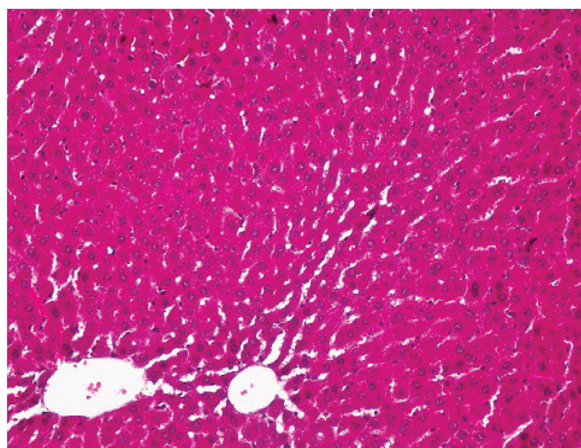
4.5.1. Preparation of standard solutions

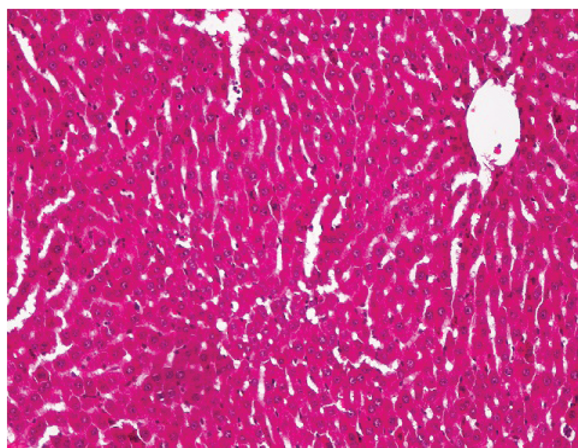
Stock solutions of 1.0 mg/mL each of bupropion, omeprazole, tolbutamide, phenacetin, midazolam, metoprolol, and IS were prepared in methanol. The working standard solutions of each drug were prepared by serial dilution of the stock solution with methanol. All of the solutions were stored at 4 °C and brought to room temperature before use. The calibration standards were prepared by spiking blank rat plasma with appropriate amounts of bupropion, omeprazole, tolbutamide, phenacetin, midazolam, and metoprolol. Calibration plots of each probe drug were constructed in the range of 20-5000 ng/mL for plasma (25, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL). The analytical standards and QC samples were stored at −20 °C.

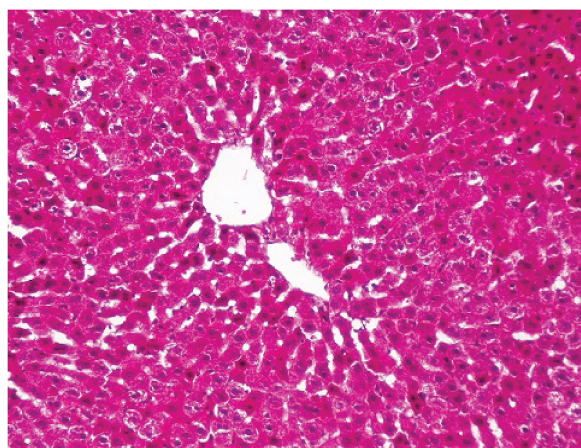
4.5.2. Method validation

Quality control samples required for each batch were freshly prepared and were generated using least-squares linear regression. The lower limit of quantitation (LLOQ) was estimated in the process of calibration curve construction and defined as the lowest concentration for which precision was less than 20% and accuracy within 80-120%. Intra-assay precision was evaluated









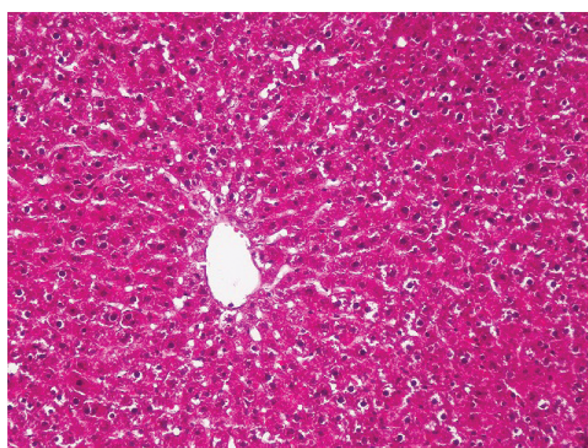


Fig. 3: Typical histopathological section photographs of rat liver changed after administration by ethanol (E15,E30) and water extracts of *Polygonum multiflorum* (W15,W30). (× 200).

by analysis of six replicates of the three QC samples in one run. Inter-assay precision was evaluated by analysis of six replicates of QC samples on three consecutive days.
Recoveries at three QC levels were determined by comparing the peak areas of extracted plasma standards with the peak areas of post-extraction plasma blanks spiked with equivalent concentrations using six replicates. The matrix effect was investigated at three QC levels using three replicates by comparing the peak areas of spike-after-extraction samples with neat standard solutions.

After successfully completing the modeling, the SD rats were administered a mixture of six probe drugs by gavage at 1 mL/kg dose. The concentrations of bupropion, omeprazole, tolbutamide, phenacetin, midazolam, and metoprolol were 10 mg/mL, 10 mg/mL, 1 mg/mL, 10 mg/mL, 10 mg/mL, and 10 mg/mL, respectively. The administered doses of the probe drugs bupropion, omeprazole, tolbutamide, phenacetin, midazolam, and metoprolol were 10 mg/kg, 10 mg/kg, 1 mg/kg, 10 mg/kg, 10 mg/kg, and 10 mg/kg, respectively. After oral administration of the drug probe, blood samples

were collected from the tail vein of rats (0.3 mL) into heparinized 1.5 mL polythene tubes at 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48 h after administration. The samples were immediately centrifuged at 13000 r/min for 10 min, and 100 μL plasma was obtained for each sample.

In a 1.5 mL centrifuge tube, 0.2 mL acetonitrile (with 200 ng/mL IS) were added to 0.1 mL of collected plasma sample. Afterwards, the tube was vortex-mixed for 1.0 min and the sample was centrifuged at 13000 r/min for 10 min. The supernatant (5 μL) was injected into the LC-MS system for analysis. Plasma probe drug concentration *versus* time data for each rat was analyzed by DAS software (Version 3.0, Drug Clinical Research Center of Shanghai University of T.C.M. and Shanghai BioGuider Medicinal Technology Co., Ltd., China). The pharmacokinetic parameters of the test group and control group probe drugs with t-test inspection were analyzed by SPSS 16.0 statistical software. A p value of < 0.05 was considered statistically significant.

4.6. Histopathological detection

Using 10 % neutral formalin, tissue sections were fixed and then dehydrated using graded ethanol, hyalinized with xylene, paraffin embedded, sliced, and stained with Hematoxylin and Eosin (H&E) stain. Pathological changes were then observed under an optical microscope.

Acknowledgments: This study was supported by grants from the Education of Zhejiang province, China, No.Y201327183, the key academic subject (clinical Chinese pharmacy) of the Twelfth-Five Program of state administration of traditional Chinese medicine.

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