Pharmacokinetic and tissue distributions study of adenosine, 4-hydroxybenzyl alcohol and Parishin C from *Gastrodia elata* extract in rats

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Abstract: The plant *Gastrodia elata* is a type of the orchid plant *Gastrodia elata* Bl. which contains glycosides, phenols, polysaccharides, sterols, and organic acids and a variety of active ingredients are proved to have certain pharmacological activities. To understand the process in the body of *Gastridua elata,* we used HPLC to study pharmacokinetics and tissue distributions of adenosine, 4-hydroxybenzyl alcohol and Parishin C in rats. The results showed that the three ingredients could be detected in plasma and different organizations at various time points. There was no significant difference in systemic clearance at three ingredients and it may be show that the three ingredients distributed (0.475±0.025, 0.518±0.033, 0.699±0.051) quickly and eliminated (5.37±0.87, 4.54±0.69, 5.34±0.82) slowly in plasma. There was the highest content of adenosine in spleen, followed by liver and lung. The highest content of 4-hydroxybenzylacohol in liver, and was higher in spleen. Parishin C was highest in heart, followed by liver and spleen. It is obvious that the contents of three ingredients are all higher in liver. The trends of the three ingredients' contents in *G. rhizome* extract were consistent with the contents in the plasma after intravenous administration.

Keyword: *Gastrodia elata*; HPLC, internal standard method, pharmacokinetic, tissue distribution.

INTRODUCTION

The plant *Gastrodia elata* is a type of the orchid plant *Gastrodia elata* Bl. It contains glycosides, phenols, polysaccharides, sterols and organic acids and a variety of active ingredients are proved to have certain pharmacological activities. The property of plant *Gastrodia elata* is gan, ping, and belongs to liver meridian. It is often used to treat men who are dizziness, headache, numbness, rheumatic, etc (Gao, 2002). Modern research has proved that *Gastrodia elata* contains glycosides, phenols, polysaccharides, sterols, and organic acids and a variety of active ingredients are proved to have certain pharmacological activities. Such as gastrodine has been proved to have analgesic action and effect of anti-epilepsy and protection the nerve cells (An *et al*., 2003; Hu *et al*., 2007; Hsieh *et al*., 1997; Kim *et al*., 2001; Li *et al*., 2003; Liu *et al*., 2005; Ojemann *et al*., 2006; Wu *et al*., 1996; Zeng *et al*., 2007). 4-Hydroxybenzylacohol is the hydrolysis product of gastrodine, the study has found that it has the protective effect on the nervous also (Wu *et al*., 1996; Yu *et al*., 2005). And studies had shown that gastrodine could be hydrolyzed to p-hydroxy benzaldehyde in the brain to play a role of neuroprotection (You *et al*., 1994). Parishin C is another kind of typical active ingredient in *Gastrodia elata* which is the ester that condensed by gastrodine and citric acid, and studies had shown that it can significantly reduce the neural separation behavior (Shin *et al*., 2010). For the better understanding of the effect of efficacy material in *Gastrodia elata,* many researchers had studied the pharmacokinetic and tissue distribution of *Gastrodia elata* and its active ingredients (Tao *et al*., 2012; Wang, 2007; Zheng, 2015). In this research, we adopted oral way and used HPLC to study pharmacokinetics and tissue distributions of adenosine, 4-hydroxybenzyl alcohol and Parishin C in rats at the same time. Quercetin was the internal standard. We expect this research can provide reference for the pharmacological activities effects of the active ingredients in *Gastrodia elata.*

MATERIALS AND METHODS

Gastrodia elata was obtained in northeast in the autumn, and Professors Kang Tingguo and Zhang Hui from the Liaoning University of Traditional Chinese Medicine determined it to be authentic herbs. Adenosine and Quercetin were obtained from the China Institute of Food and Drug (test batch number 110879-200202 and 100081-201408, Purity ≥98%, Beijing, China). 4-Hydroxybenzyl alcohol and Parishin C were bought from Bioko Beijing Century Biotechnology Co., Ltd. (Batch Numbers 111970, 150718, purity ≥98%, Beijing, China). Methanol was acquired from Tianjin Branch of the European Chemical Reagent Co., Ltd., chromatography purity, Tianjin, China). The instrumentation used was as follows: Agilent 1100 series High Performance Liquid Chromatograph, ECOSIL chromatographic column (120-5-C18, 250×4.6mm, Germany), Adjustable High-speed dispersing device (Jiangsu Jintan Splendor Equipment Manufacture Co., Ltd., Jiangsu, China), Fast Vortex Mixer (Purchase of Medical Equipment Co., Ltd., Jiangsu, China), HC-2518

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High Speed Refrigerated Centrifuge (HC-2518, Anhui Zhongke Scientific Instrument Co., Ltd., Anhui, China) Male Sprague-Dawley (SD) rats (10–12 weeks old, weighing 200–220 g) were obtained from the Liaoning Changsheng Biotechnology Co., Ltd. (Benxi, China). Animal welfare and experimental procedures were strictly in accordance with the Guidelines of the Committee on the Care and Use of Laboratory Animals of China (Liaoning University of Traditional Chinese Medicine, license: SYXK(辽)2013-0009). Before administration of drugs, the rats were fasted for 24 h with free access of water.

Chromatographic conditions

The chromatographic conditions were as follows: elution with methanol (A) -water (B) (gradient) elution conditions are shown in table 1, column temperature 40□, injection volume 10520μL, volume flow 1.0 mL•min^{-1}, and the detection wavelength 270 nm.

Preparation of the reference solutions

The reference solutions were prepared from standard reference solids, accurately weighed, diluted with methanol containing dissolved adenosine, 4-hydroxybenzyl alcohol, Parishin C and quercetin the concentrations were 0.1890, 0.1845, 0.1660 and 0.1472 mg•mL^{-1} respectively.

Preparation of the test solution

G. rhizome was accurately weighed, 50% methanol was added. First, the mixture was boiled for 1 h after the mixture had been soaked overnight, then be conventional filtered, finally the solvent was evaporated to 1g• mL^{-1} (According to the original herbs) under reduced pressure.

General procedure of sample preparation for HPLC

A liquid-liquid extraction was performed prior to HPLC analysis. First, 20 μL internal standard solution content was added to 200 μL of plasma or tissue samples. The mixture was extracted with 3mL methanol by vortex for 2

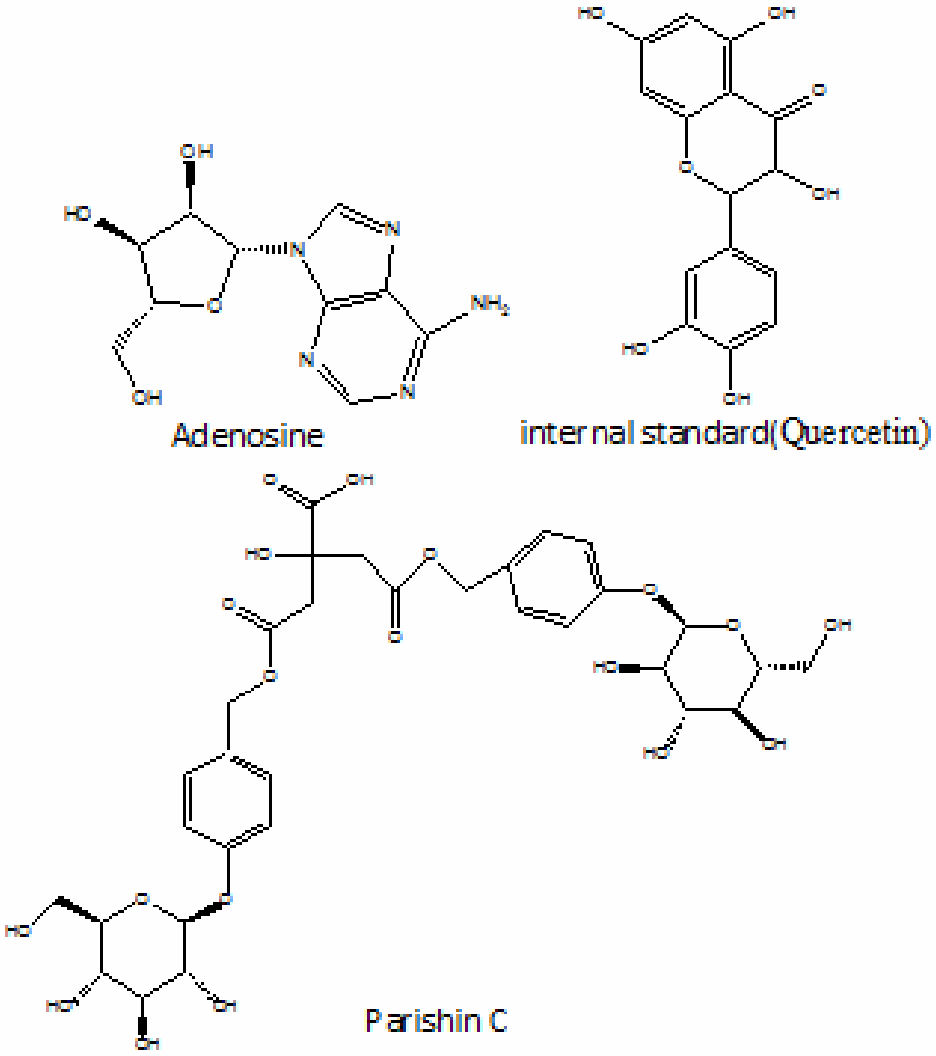


Fig. 1: The structures of Adenosine, Parishin C and internal standard (Quercetin).

Table 1: Mobile phase elution

Time (minute)	A (%)	B (%)
0	4	96
15	8	92
40	20	80
60	60	40

Table 2: The standard curve and its linear range (C is the ratio of peak area, A is the injection volume)

Sample	Reference name	Standard curve line	The linear range (μg)	r
Plasma	Adenosine	C=58.37A-30.98	0.378-1.134	0.9948
	4-Hydroxybenzylalcohol	C=11.19A-4.640	0.676-2.028	0.9925
	Parishin C	C=2.858A-1.096	0.664-1.992	0.9981
Lung	Adenosine	C=3.621A-2.827	0.756-2.268	0.9982
	4-Hydroxybenzylalcohol	C=14.05A+2.34	0.112-0.338	0.9925
	Parishin C	C=0.5337A+0.1662	0.332-1.992	0.9972
Liver	Adenosine	C=1.381A-1.052	0.756-4.536	0.9932
	4-Hydroxybenzylalcohol	C=3.658A-2.672	0.676-4.056	0.9917
	Parishin C	C=2.858A-1.096	1.328-4.986	0.9981
Heart	Adenosine	C=63.70A-0.3109	0.252-0.756	0.9955
	4-Hydroxybenzylalcohol	C=9.226A-0.913	0.135-0.507	0.9919
	Parishin C	C=1.699A-0.106	1.328-5.976	0.9941
Spleen	Adenosine	C=0.4106A-0.1074	2.268-6.804	0.9956
	4-Hydroxybenzylalcohol	C=2.238A-1.192	0.676-2.028	0.9914
	Parishin C	C=0.3223A+0.1294	1.328-5.976	0.9945
Kidney	Adenosine	C=69.55A-8.4	0.126-0.378	0.9908
	4-Hydroxybenzylalcohol	C=32.33A-12.82	0.338-1.014	0.9956
	Parishin C	C=3.602A-2.134	0.664-3.984	0.9951
Brain	Adenosine	C=3.624A+0.012	0.378-1.134	0.9938
	4-Hydroxybenzylalcohol	C=1.374A-0.3156	0.225-0.676	0.9944
	Parishin C	C=0.2325A+0.0082	0.332-0.996	0.9908

Table 3: The main pharmacokinetic parameters of adenosine, 4-hydroxybenzylacohol and parishin C after intragastric administration (n=5) *t*1/2α: Distribution phase half-life, *t*1/2β: elimination phase half-life; AUC0-∞: Area under the concentration-time curve from zero up to infinite time; CLs: systemic clearance.

Parameter	Unit	Ingredients		
		adenosine	4-hydroxybenzylacohol	parishin C
$t_{1/2\alpha}$	h	0.475±0.025	0.518±0.033	0.699±0.051
$t_{1/2\beta}$	h	5.37±0.87	4.54±0.69	5.34±0.82
$AUC_{0-\infty}$	mg•h/L	9.36±1.04	16.3±2.44	12.8±1.57
CLs	L/h/kg	0.107±0.014	0.0613±0.0024	0.0779±0.0033

min. After centrifugation at 5,000 rpm for 20 min, the upper organic layer was transferred to a clean tube and evaporated to dryness at 40°C. The residue was reconstituted in 50 μL of methanol by vortex for 2 min.

Standard curve drawing and specificity study

We had used the blank plasma and tissue to draw the standard curve by using internal standard method. Sample volume (A) was used for the abscissa, and the ratio of sample and internal standard's peak area (C) as the vertical axis. The resulting standard curve (table 2) shown a good linear relationship. The Chromatograph charts were shown in fig. 2.

Precision, accuracy, recovery and stability

The precision and accuracy of the method were preformed. For all the samples spiked with analytes at three concentration levels, the RSD% of both intra-day and inter-day precision was below 9.12%, and the accuracy was within the range of -7.83 to 10.2%. The results demonstrated that the method is accurate and reproducible for determination of three compounds in rat plasma and tissues (data not shown).

The extraction recoveries of three compounds ranged from 89.54 to 92.17% in tissue and plasma samples. The data indicated the biosample preparation procedure was

satisfied and achieve the acceptable extraction recovery. The stability tests were designed to cover the anticipated condition that the samples may experience. The results shown that the three compounds and internal standard remained stable in short-term, freeze-thaw and long-term (data not shown).

Pharmacokinetics of adenosine, 4-hydroxybenzyl alcohol and Parishin C in rats

The test solution was administered by oral route at dose of 4 mL to SD rats (n=5). At the time points of 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 10h post taking oral, blood samples (0.5mL) were collected in heparinized tubes from the orbital vein, and then centrifuged at 11,000 rpm for 20 min to obtain plasma. The plasma was stored at -20^{o}C (Tang *et al*., 2015).

STATISTICAL ANALYSIS

Data were analyzed using SPSS (Statistical Product and Service Solutions, version 17.0, IBM). Unpaired comparisons were tested using one-way ANOVA for three or more groups and unpaired t tests with Welch's correction for two groups. We tested for paired differences among conditions using the Friedman test with Dunn's post test for multiple comparisons as well as Wilcoxon matched-pairs signed rank test where appropriate. The statistical hypothesis test used to compare each dataset is specified below. Statistical significance is defined as $P<0.05$.

Tissue distribution study

Thirty rat were divided into ten groups (n=3 per group) randomly and the test solution was administered by oral route at dose of 4 mL. After taking oral drug, the rats were sacrificed at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 h following administration, and the tissue specimens including lung, liver, heart, spleen, kidney and brain were collected. The tissues were sensed in saline and blotted dry with filter paper, and then weighed for wet weigh and homogenized in ice-cold physiological saline solution. The tissue homogenates were stored at -20^{o}C (Tang *et al*., 2015).

RESULTS

Pharmacokinetics of Adenosine, 4-hydroxybenzyl alcohol and Parishin C in Rats

We used the 3P97 software (Chinese Pharmacology Society: Beijing, China, 1987) to calculate the pharmacokinetic parameters (Chen *et al*., 1999). We also chose the a appropriate pharmacokinetic model based on the lowest Akaike's information criterion (AIC) value, lowest weighted squared residuals, lowest standard errors of the fitting parameters, then dispersion of the residual under equal weight scheme (Chen *et al*., 2001).

The mean plasma concentration-time profiles of three components in rats was shown in fig. 3 and the corresponding pharmacokinetic parameters were summarized in table 3. All the RSD% of three compounds' pharmacokinetic parameters was below 6.12 %, the range of all the parameters was accordance with the requirements of the pharmacokinetics.

Tissue distribution study

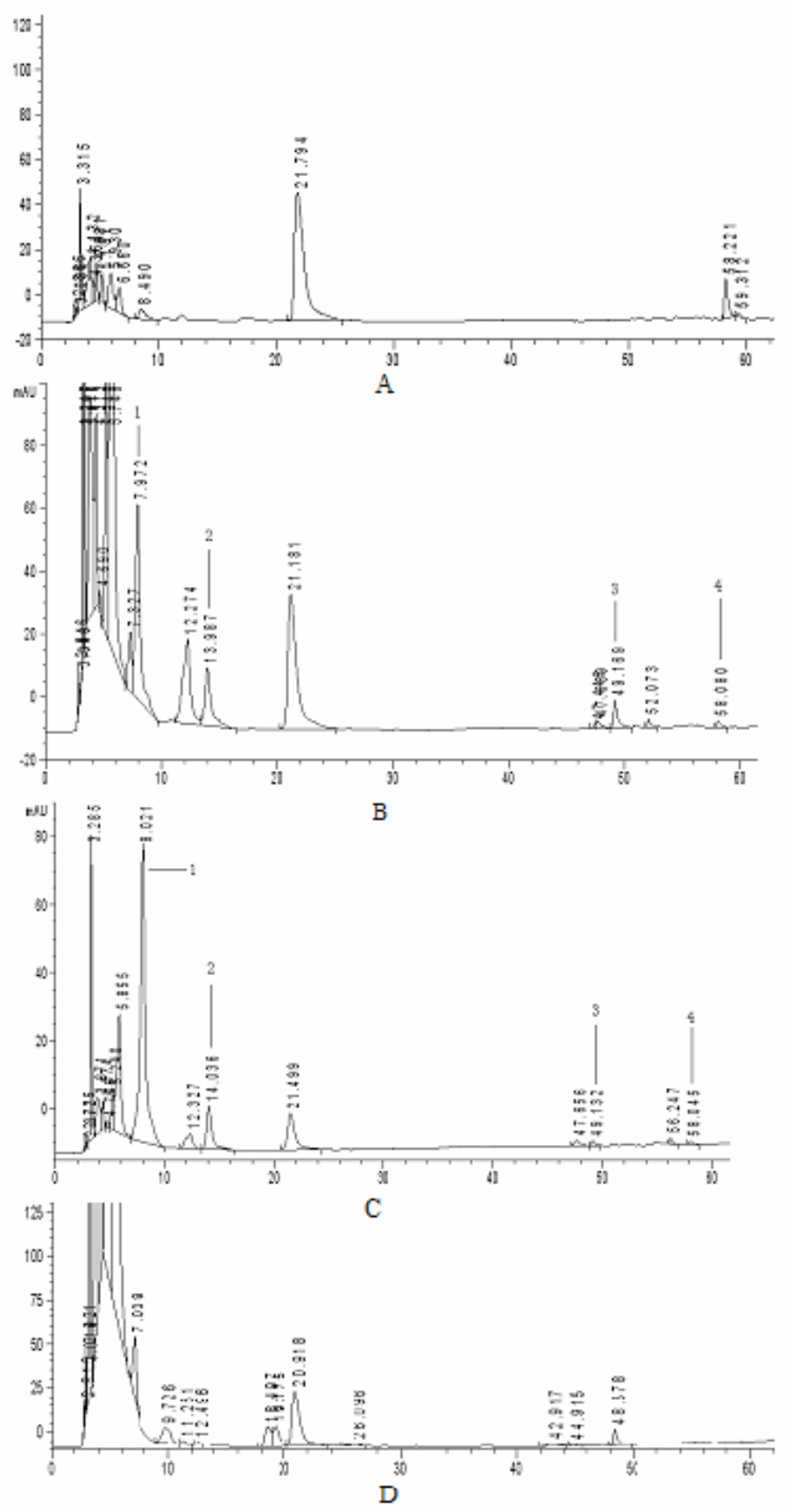
The tissue distribution of adenosine, 4-hydroxy-benzylacohol and parishin C following intragastric administration in rats at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0 h were presented in fig. 4, 5, 6.

DISCUSSION

Three were some pharmacokinetic studies of *Gastrodia elata,* but the target compounds were gastrodin, 4-hydroxybenzylalcohol (Tang *et al*., 2015) and Parishin, gastrodin (Zhang *et al*., 2008) respectively. Both of studies above were only focus on the pharmacokinetic. We had studied the pharmacokinetic and tissue distribution of adenosine, 4-hydroxybenzylacohol and parishin C from *Gastrodia elata* Extract simultaneously.

From the results of pharmacokinetics, we could see that all the ingredients could be detected from 0.5 to 10h after intragastric administration. And the content of adenosine and 4-hydroxybenzylacohol in plasma reached the peak at 2h, Parishin C reached the peak at 3h. The data of adenosine and Parishin C were fitted with one-compartment model and 4-hydroxybenzylacohol was fitted with a two-compartment model. The three ingredients' distribution phases (adenosine $t_{1/2\alpha}$, 0.475 h; 4- hydroxybenzylacohol $t_{1/2\alpha}$, 0.518 h; Parishin C $t_{1/2\alpha}$, 0.699 h) are very fast and the elimination phases(adenosine $t_{1/2\beta}$, 5.37 h; 4- hydroxybenzylacohol $t_{1/2\beta}$, 4.54 h; Parishin C $t_{1/2\beta}$, 5.34 h) are slowly. The AUC value of adenosine was least (9.36), 4-hydroxybenzylacohol was biggest (16.3), Parishin C was larger (12.8). There was no significant difference in systemic clearance at three ingredients, and it may be show that the three ingredients eliminated quickly in plasma.

From the results of tissue distribution, we could see that adenosine can be detected from 0.5 to 10 h after intragastric administration. There was the highest content of adenosine in spleen, followed by liver and lung. The content was lower in the brain and heart, the lowest in kidney. The content was highest at 4 h (0.678 mg. mL^{-1}), and then decreased slightly, risen again after 7 h (0.654 mg. mL^{-1}), began to decrease after 10 h (0.516 mg. mL^{-1}) in spleen. The content was higher at 2 h (0.316 mg. mL^{-1}), and then decreased, reached a maximum at 8 h (0.434 mg. mL^{-1}) in liver. And the content reached the maximum and then decreased gradually in lung. The results above suggest adenosine may have good accumulation in the spleen, poor in the kidney.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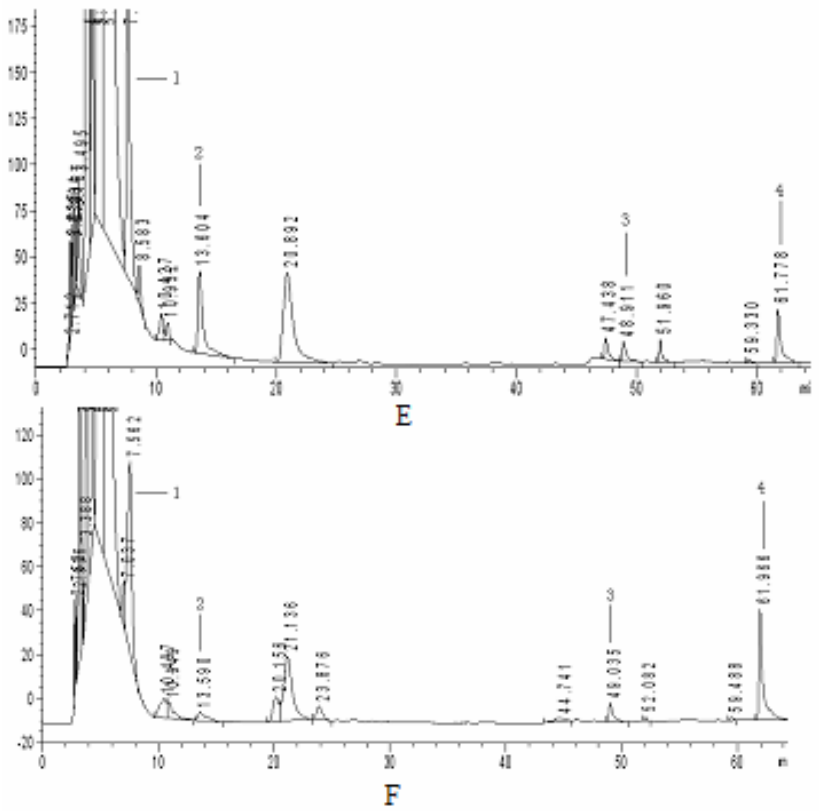

Fig. 2: Representative chromatograms of plasma (A blank, B blank plasma spiked with standard substances 1, 2, 3 and internal standard substance 4, C plasma sample at 3 h following intragastric administration) and brain (D blank, E blank tissue spiked with standard substances 1,2,3 and internal standard substance 4, F tissue sample at 3 h following intragastric administration) (1 is adenosine, 2 is 4-hydroxybenzyl alcohol, 3 is Parishin C, 4 is internal standard substance quercetin).

4-hydroxybenzylacohol could be detected from 0.5 to 10 h. There was the highest content in liver, and the content of different time points changed variations, the content was highest at 6 h (0.401 mg. mL^{-1}). The content was higher and reached the peak at 2h (0.152 mg. mL^{-1}) in spleen. The content was normal in kidney, heart and brain. And the content of 4-hydroxybenzylacohol was lowest in lung. The results above suggest 4-hydroxybenzylacohol might have good accumulation in liver and spleen, poor in lung.

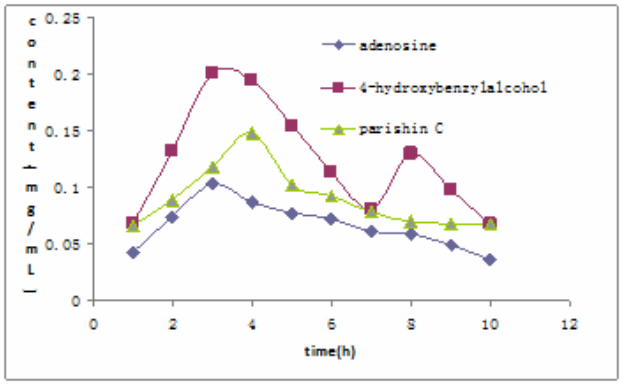

Fig. 3: Plasma content-time profiles of adenosine, 4-hydroxybenzylacohol and parishin C (n=5).

Parishin C can be detected from 0.5 to 10 h also, but the content was higher in all tissues. There was the highest content of Parishin C in heart, followed by liver and spleen. The content of Parishin C reached the peak at 4h in heart, 7h in liver, 4 h in spleen and 10 h in kidney. There were lower content in lung and brain.

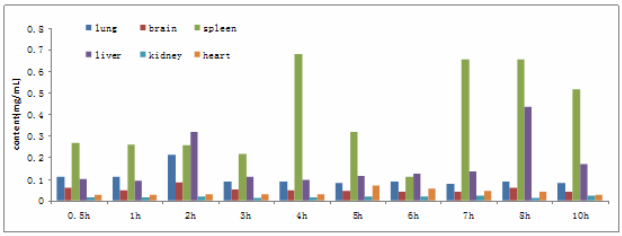

Fig. 4: Tissue content-time profiles of adenosine.

It was obvious that the contents of three ingredients were all higher in liver, this may be associated with *Gastrodia elata* belonging to the liver meridian. Preliminary experimental results had shown that the content of adenosine, 4-hydroxybenzylacohol and Parishin C in the *G. rhizome* are 0.0200, 0.183 and 0.113mg•g^{-1} respectively. The trends of the three ingredients' contents in *G. rhizome* extract were consistent with the contents in the plasma. But there were significant difference in the tissues. The

average content of Parishin C was highest, adenosine was higher and 4-hydroxybenzylacohol was lowest. It may be the different ingredients have different accumulation rate, or different ingredients have different pharmacological effects.

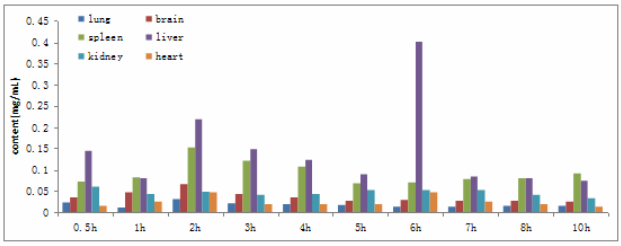

Fig. 5: Tissue content-time profiles of 4-hydroxybenzylacohol.

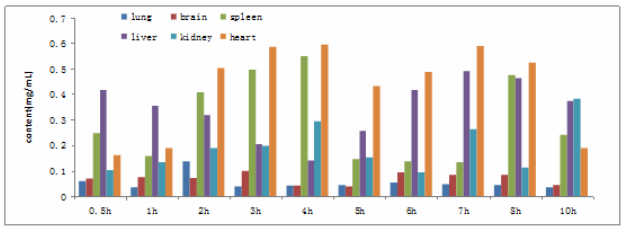

Fig. 6: Tissue content-time profiles of Parishin C.

CONCLUSION

According to the results of pharmacokinetics and tissue distribution, we could see that all the ingredients can be detected from 0.5 to 10h in plasma and tissues after intragastric administration. The trends of the three ingredients' contents in *G. rhizome* extract were consistent with the contents in the plasma after intragastric administration. But there were significant difference in the tissues. The average content of Parishin C was highest, adenosine was higher and 4-hydroxybenzylacohol was lowest.

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