A study of pharmacokinetic interactions among co-existing ingredients in *Viscum coloratum* after intravenous administration of three different preparations to rats

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ABSTRACT

Background: *Viscum coloratum* (Komar) Nakai, known as Hujisheng in china, has been widely used as a herb medicine to treat a variety of diseases, including cardiovascular diseases, cancer, hypertension, hepatitis and hemorrhage. **Objective:** The aim was to investigate pharmacokinetic interactions among co-existing ingredients in *V. coloratum* after intravenous administration of three different preparations (four monomer solutions, the mixture of them and *Viscum coloratum* extracts) to rats. **Materials and Methods:** After protein precipitation pretreatment with plasma samples, high performance liquid chromatographic methods were developed and applied to quantitatively determinate the four components [syringin (Syri), homoeriodictyol-7-*O*-*β*-*D*-glycoside (Hedt-III), homoeriodictyol-7-*O*-*β*-*D*-apiose (1 → 2)-*β*-*D*-glycoside (Hedt-II) and homoeriodictyol-7-*O*-*β*-*D*-apiosiyl-(1 → 5)-*β*-*D*-apiosyl-(1 → 2)-*β*-*D*-glycoside (Hedt-I)].The pharmacokinetic parameters (Area under the curve [$AUC_{(0-t)}$], $AUC_{(0-\infty)}$, $t_{1/2}$) were calculated using DAS 2.1 software (Chinese Pharmacological Society,Shanghai,China) and compared statistically by One-way analysis of variance using SPSS software (18.0, Chicago, IL, USA) with $P < 0.05$ considered statistically significant. **Results:** Good linearities were achieved in the measured concentration range with R^2 it0.9920. Precision, accuracy and extraction recovery were all within the acceptable range. For Syri, there was a significant difference only on $t_{1/2}$ among three treatment groups. For Hedt-I, Hedt II and Hedt-III, three flavonoid glycosides, the change of $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and $t_{1/2}$ were markedly distinctive and even converse. **Conclusion:** Complex, extensive pharmacokinetic interactions were observed among these components in *V. coloratum*. They were mutually influenced by the *in vivo* absorption, distribution, metabolism and elimination. The result suggested traditional Chinese medicine was a complicated system, and we should take a scientific and dialectic view in the research and development processes.

Key words: Co-existing ingredients, pharmacokinetic interactions, *Viscum coloratum*

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INTRODUCTION

In recent years, traditional Chinese medicine (TCM) is developed rapidly and gain more and more interesting due to their beneficial effects and relatively low toxicities. TCM products are usually used as a single herb or combination of herb and drugs to obtain better treatment effect.[1] No matter which way, the powerful clinical therapeutic effects are the result of various interactions, which may be antagonistic, synergic or additive occurring among these multiple ingredients.[2] As a complexity system, these interactions are also generally reflected in the pharmacokinetic profiles of complexed constituents. The *in vivo* pharmacokinetic behavior of herbs or herb-herb is resulted from mutual interactions of these individual components, not equivalent to a simple adduct. Pharmacokinetics of any individual ingredient cannot represent that of the whole herb. Therefore, it is significantly necessary to study differences of pharmacokinetic profiles among Multicomponents for development and clinical applications of TCM. Actually, many researches have investigated interactions in Pharmacokinetics or Pharmacodynamics among components of herb medicine. For example, it was surmised that formula compatibility could significantly influence the pharmacokinetics of the Er-Mu preparation by affecting P-gp and cytochrome P450.[3] Perplexing, significant pharmacokinetic interactions were observed

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among the major water-soluble constituents in the Dan-shen injection-induced by content variation.[4] Other components in Qingfu Guanjieshu could effectively influence the pharmacokinetic behavior and metabolic profile of paeonol in rats.[5] These findings strongly suggest that more attention should be paid to effects of Multicomponent Pharmacokinetic interactions on the integrity of complete prescriptions.

In this paper, pharmacokinetic interactions among co-exiting ingredients in herb extracts are investigated taking *Viscum Coloratum* (Komar.) Nakai (*V. Coloratum*) for an example. *V. Coloratum,* known as Hujisheng in china, has been widely used as a herb medicine to treat a variety of diseases including cardiovascular diseases, cancer, hypertension, hepatitis, and hemorrhage.[6-8] Moreover, *V. Coloratum* is frequently used as a basic composition unit of Chinese herbal formulas for achieving mutual reinforcement, assistance, restraint, and detoxication.[9-11] *V. Coloratum* mainly contains flavonoids, phenylpropanol glycoside, triterpenoids, and alkaloids.[12] Among these ingredients, Syringin (Syri) is chosen as a marker to evaluate the quantity of *V. Coloratum* in Chinese Pharmacopoeia.[13] Homoeriodictyol-7-*O*-*β*-*D*-glycoside (Hedt-III), Homoeriodictyol-7-*O*-*β*-*D*-apiose (1 → 2)-*β*-*D*-glycoside (Hedt-II) and Homoeriodictyol-7-*O* -*β*-*D*-apiosiyl-(1 → 5)-*β*-*D*-apiosyl-(1 → 2)-*β*-*D*-glycoside (Hedt-I), the three flavonoid glycosides are most common and highest content ingredients in *V. Coloratum* according to our research group findings.[14] The four components display many beneficial properties, such as anti-coagulant,[15] anti-inflammatory,[16] anti-oxidant,[17] anti-tumor[18] and anti-fungal activity.[19] In consideration of the above, we choose Syri, Hedt-I, Hedt-II and Hedt-III (structures illustrated in Figure 1) as the bioactive marker components to investigate pharmacokinetic interactions of co-existing ingredients in *V. Coloratum*.

In the present study, for the first time different preparations, the four monomer components (MONO), the mixture solution of them (MIX) and *Viscum Coloratum* extracts (VCE) were intravenous (*i.v.*) administrated to rats. Then difference between the pharmacokinetic parameters was systematically and comprehensively investigated and compared to reveal the potential pharmacokinetic interactions of Syri, Hedt-I, Hedt-II, Hedt-III and other unknown ingredients in VCE. It was expected that the results of this study would provide some references for the apprehension of pharmacodynamics of *V. Coloratum*.

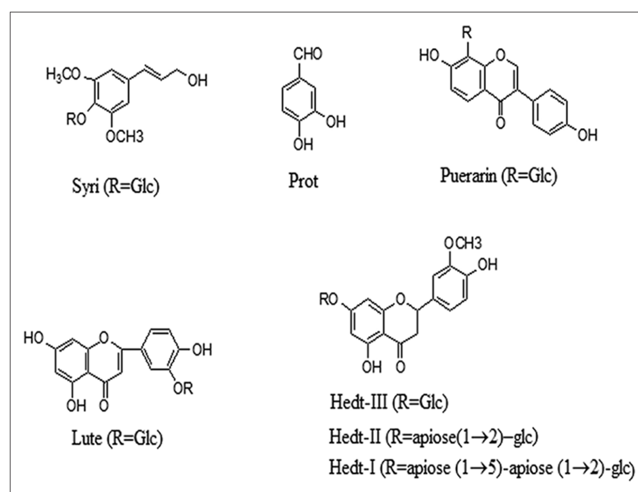

Figure 1: The chemical structures of syringin, homoeriodictyol-7-*O*-*β*-*D*-apiosiyl-(1→5)-*β*-*D*-apiosyl-(1→2)-*β*-*D*-glycoside, homoeriodictyol-7-O-β-D-apiose(1→2)-*β*-*D*-glycoside, homoeriodictyol-7-o-*β*-*d*-glycoside, puerarin, protocatechualdehyde and luteolin-7-O-*β*-*D*-glycoside

MATERIALS AND METHODS

Reagents and chemicals

Syringin, Hedt-I, Hedt-II and Hedt-III were all separated and purified from *V. Coloratum* by us and structures of these compounds were identified by spectroscopy. The purity were all above 98% by high performance liquid chromatographic (HPLC). Puerarin (Puer), Protocatechualdehyde (Prot) and luteolin-7-*O*-*β*-*D*-glycoside (Lute) (internal standard [IS]) were ordered from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and Acetonitrile (HPLC grade) were purchased from the Concord Tech Reagent Company (Tianjin, China). Glacial acetic acid and tween-80 (analytical grade) were purchased from the Tianjin Damao Chemical Reagent Factory (Tianjin, China). Distilled water prepared from demineralized water was used throughout the experiments.

Experimental animals

Male and female pathogen-free Wistar rats (200–250 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University, and the protocol was approved by the Animal Ethics Committee of this institution. All rats were fasted for 12 h and allowed free access to water prior to the experiments.

Apparatus and chromatographic conditions

Reverse phase (RP)-HPLC was carried out on a liquid chromatography (LC) system consisting of an LC-10AT*vp*

liquid chromatograph and an SPD-10A*vp* ultraviolet–visible is detector (Shimadzu, Japan). The chromatographic conditions were shown in Table 1. The column temperatures were all set at 30°C.

Preparation of *Viscum Coloratum* extracts, monomer components and mixture solution of them solutions for injection

The powder of *V. Coloratum* (40 g) was extracted by refluxing with ethanol (800 mL) for 3 h and extracts was filtrated, combined to give brown syrup. Then, the syrup was applied on a polyamide column and eluted by water (100 mL). The water elution was collected and concentrated to 10 mL. Then pH was adjusted to 6–7 by 0.1 mol/L sodium hydroxide solutions. The active carbon (0.1%), tween-80 (0.15%) and water were added to 20 mL, then filtrated intensively and sterilized.

The MONO was prepared by dissolving Syri, Hedt-I, Hedt-II and Hedt-III in normal saline and adding 0.04 mL tween-80 with the concentration of 0.27 mg/mL, 1.54 mg/mL, 1.50 mg/mL and 0.52 mg/mL, respectively, and the MIX was prepared by blending the four compounds to obtain the same concentration with the MONO. The concentrations of four components in MONO and MIX were equal to VCE.

Preparation of standard solutions and quality control samples

Stock solutions of Syri, Hedt-I, Hedt-II and Hedt-III were separately prepared in 25 mL volumetric flask by dissolving the reference substance in methanol and appropriate amounts of stock solutions were mixed to make a pooled standard solution. The working solutions were obtained by serial dilution of the pooled standard solution. quality control (QC) sample was prepared at high, medium and low concentrations in the same manner as the standard solution.

The IS solutions, Puer for groups of VCE and MIX, Prot for group of MONO (Syri) and Lute for group of MONO (Hedt-I, Hedt-II, and Hedt-III), were prepared by dissolving the reference substance in methanol and further diluting to give a final concentration of 20, 6 and 40 μg/mL, respectively.

Pharmacokinetic study

All Wistar rats were divided randomly into six groups for the i.v. administration with samples (MONO-Syri, MONO-Hedt-I, MONO-Hedt-II, MONO-Hedt-III, MIX and VCE) equivalent to 1.35, 7.7, 7.5 and 2.6 mg/Kg of Syri, Hedt-I, Hedt-II and Hedt-III, respectively. Blood samples were collected from ophthalmic venous plexus in heparinized tubes pre-dose and at 0.017, 0.083, 0.25, 0.50, 1.0, 1.5, 2.0 and 3.0 h. Following centrifugation at 4000 rpm for 10 min, plasma samples were collected to tightly seal plastic tubes (Heparin lithium anticoagulation) and kept frozen at −80°C until analysis.

Plasma sample preparation

To each 100 μL plasma sample, 250 μL methanol and 50 μL IS solution were added and vortexed for 1 min. Following centrifuging at 12,000 rpm at 4°C for 5 min, the supernatant was evaporated to dryness under nitrogen gas at 40°C. Then the residue was reconstituted in 200 μL mobile phase. After vortexed dissolving for 30 s and centrifuged at 12,000 rpm at 4°C for 5 min, a portion of the supernatant (20 μL) was injected into the HPLC system for analysis.

Method validation

This method was fully validated according to the currently accepted FDA bioanalytical method validation guidance[20] with respect to specificity, linearity, precision and accuracy, recovery and stability. The selectivity was assessed by comparing the chromatograms of six different sources of blank plasma processed by protein precipitation pretreatment. The linearity was assessed by analyzing the calibration curves using least-squares linear regression of the peak area ratios of the analytes to the IS versus the nominal concentration of the calibration standards with a weighed factor ($1/C^2$). The lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration curve with an acceptable accuracy within ± 20% and the precision below 20%. The precision was expressed as the RSD and accuracy was calculated as the RE. Three levels

Table 1: Chromatographic conditions

	Syri, Hedt-I, Hedt-II, Hedt-III (determination of VCE and MIX)	Syri (determination of MONO)	Hedt-I, Hedt-II, Hedt-III (determination of MONO)
Column	Synergi C_{18} (250×4.6 mm, 4 μm)	Diamonsil C_{18} (200×4.6 mm, 5 μm)	Synergi C_{18} (250×4.6 mm, 4 μm)
Mobile phase	0-30 min B_1/A (10:90-30:70)	B_2/A (30:70)	B_2/A (45:55)
Flow rate	1.0 mL/min	1.0 mL/min	1.0 mL/min
Wavelength	280 nm	265 nm	280 nm

A: 0.5% glacial acetic acid; B_1: Acetonitrile; B_2: Methanol; VCE: *Viscum coloratum* extracts; MONO: Monomer solution; MIX: Mixture solution of them; Hedt-I: *D*-apiosiyl-(1→5)-*β*-*D*-apiosyl-(1→2)-*β*-*D*-glycoside; Hedt-II: Homoeriodictyol-7-*O*-*β*-*D*-apiose (1→2)-*β*-*D*-glycoside; Hedt-III: Homoeriodictyol-7-*O*-*β*-*D*-glycoside; Syri: Syringin

Table 2: Calibration curves for Syri~Hedt-III in plasma samples (*n*=7)

VCE, MIX	Regression equation	R^2	Linear range (μg/ml)	LLOQ (μg/ml)	MONO	Regression equation	R^2	Linear range (μg/ml)	LLOQ (μg/ml)
Syri	$y=3.64\times10^{-2}x+2.19\times10^{-3}$	0.9964	0.20-60.00	0.20	Syri	$y=1.74\times10^{-2}x-2.93\times10^{-2}$	0.9947	0.20-60.00	0.20
Hedt-I	$y=2.67\times10^{-2}x+1.91\times10^{-3}$	0.9978	0.30-60.00	0.30	Hedt-I	$y=4.68\times10^{-2}x+4.43\times10^{-3}$	0.9974	0.30-60.00	0.30
Hedt-II	$y=4.10\times10^{-2}x+5.52\times10^{-3}$	0.9921	0.30-59.60	0.30	Hedt-II	$y=8.74\times10^{-2}x-2.53\times10^{-3}$	0.9938	0.30-59.60	0.30
Hedt-III	$y=3.09\times10^{-2}x+5.20\times10^{-3}$	0.9923	0.28-56.00	0.28	Hedt-III	$y=6.28\times10^{-2}x+1.77\times10^{-3}$	0.9954	0.28-56.00	0.28

VCE: *Viscum coloratum* extracts; MIX: Mixture solution of them; LLOQ: Lower limit of quantification; MONO: Monomer solution; Hedt-I: *D*-apiosiyl-(1→5)-*β*-*D*-apiosyl-(1→2)-*β*-*D*-glycoside; Hedt-II: Homoeriodictyol-7-*O*-*β*-*D*-apiose (1→2)-*β*-*D*-glycoside; Hedt-III: Homoeriodictyol-7-*O*-*β*-*D*-glycoside; Syri: Syringin

Table 3: Precisions, accuracies and recoveries for Syri~Hedt-III in rat plasma (*n*=6) following intravenous admininstration of VCE and MIX

VCE, MIX	Spiked concentration (μg/ml)	Intra-day RSD (%)	Inter-day RSD (%)	Accuracy RE (%)	Recovery (%)	MONO	Spiked concentration (μg/ml)	Intra-day RSD (%)	Inter-day RSD (%)	Accuracy RE (%)	Recovery (%)
Syri	0.60	5.5	6.9	−3.4	84.9±4.1	Syri	0.60	7.6	5.4	−2.6	91.9±2.8
	3.00	2.3	11.6	2.5	91.1±2.2		3.00	6.9	6.0	0.1	77.4±3.9
	48.00	5.2	9.2	−0.1	81.7±4.5		48.00	5.2	5.6	5.5	87.1±3.2
Hedt-I	0.60	5.7	9.9	−7.9	75.1±4.7	Hedt-I	0.60	5.3	11.8	0.1	80.0±7.4
	3.00	7.6	1.5	−3.9	72.3±7.3		3.00	5.1	3.6	4.1	71.5±11.8
	48.00	6.0	2.1	5.6	80.4±5.0		48.00	8.0	6.8	2.0	78.6±2.9
Hedt-II	0.60	6.3	9.2	−0.2	70.2±4.1	Hedt-II	0.60	5.4	9.3	−1.2	88.8±4.4
	2.98	6.2	7.7	−4.9	84.0±5.0		2.98	5.8	3.2	3.2	93.9±5.6
	47.68	6.4	4.7	4.7	80.6±3.9		47.68	5.3	3.4	3.3	82.3±1.5
Hedt-III	0.56	2.9	5.8	−1.9	71.8±4.6	Hedt-III	0.56	6.2	10.8	−7.7	86.5±2.8
	2.80	6.2	8.3	0.6	87.0±9.5		2.80	7.3	9.4	−3.4	93.4±2.9
	44.80	7.1	5.1	0.8	78.2±3.9		44.80	6.8	11.1	0.7	90.0±4.7

VCE: *Viscum coloratum* extracts; MIX: Mixture solution of them; MONO: Monomer solution; Hedt-I: *D*-apiosiyl-(1→5)-*β*-*D*-apiosyl-(1→2)-*β*-*D*-glycoside; Hedt-II: Homoeriodictyol-7-*O*-*β*-*D*-apiose (1→2)-*β*-*D*-glycoside; Hedt-III: Homoeriodictyol-7-*O*-*β*-*D*-glycoside; RSD: Relative standard deviation; RE: Relative error; Syri: Syringin

Table 4: Stability of Syri~Hedt-III in rat plasma

Components	0 h	24 h (ambient temperature)	20d (−20°C)	3 freeze-thaw cycles
Syri	0.59	0.57	0.56	0.58
	3.02	3.12	3.17	3.17
	47.89	49.90	48.26	47.04
Hedt-I	0.58	0.53	0.56	0.55
	2.91	2.96	3.11	2.94
	46.33	48.33	47.18	46.14
Hedt-II	0.60	0.63	0.57	0.59
	3.09	3.19	3.26	2.88
	48.02	48.92	49.02	47.36
Hedt-III	0.57	0.61	0.54	0.59
	2.80	2.77	2.67	2.69
	44.66	46.66	43.89	45.23

Hedt-I: *D*-apiosiyl-(1→5)-*β*-*D*-apiosyl-(1→2)-*β*-*D*-glycoside; Hedt-II: Homoeriodictyol-7-*O*-*β*-*D*-apiose (1→2)-*β*-*D*-glycoside; Hedt-III: Homoeriodictyol-7-*O*-*β*-*D*-glycoside

of QC samples in six replicates were analyzed during the same day using the same calibration curve to determine the intra-day precision. Three batches of QC samples were analyzed on three consecutive days to evaluate the inter-day precision. The recoveries were determined by comparing the responses of the analytes extracted from replicate QC samples (n = 18) with the response of analytes from non-extracted standard solutions at equivalent concentrations. The stability was studied under a variety of storage conditions: Performing three cycles of freezing (−20°C)-thawing (room temperature), 24 h storage at room temperature and in a freezer at −20°C for at least 1 month.

Pharmacokinetic data analysis

All measurements were expressed as the mean ± standard deviation (SD). The mean concentration-time curve and other pharmacokinetic parameters were computed by the DAS 2.1 software package (Chinese Pharmacological Society, Shanghai, China). And statistical analysis was performed by One-way analysis of variance using SPSS software (18.0, Chicago, IL, USA) with $P < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Validation of the assay

Selectivity

The selectivity of the method toward endogenous plasma matrix was evaluated with plasma of six rats. The typical chromatograms obtained from blank plasma, blank plasma spiked with the analytes (at LLOQs) and

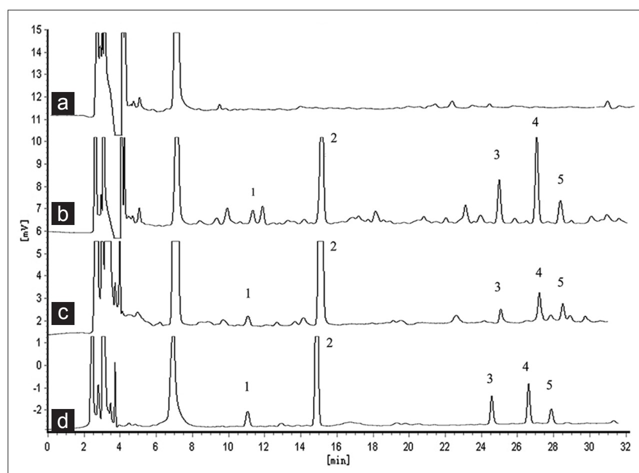

Figure 2.1: Chromatograms of blank plasma (a), plasma spiked with Syri ~ Hedt-III (limit of quantification) and IS (20 μg/ml) (b), the rat plasma sample 0.5 h after i.v. administration of 9.8 g/kg *Viscum coloratum* extracts (c) and the rat plasma sample 0.5 h after i.v. administration of Syri~Hedt-III mixture (d). 1: Syri, 2: IS, 3: Homoeriodictyol-7-*O*-*β*-*D*-apiosiyl-(1→5)-*β*-*D*-apiosyl-(1→2)-*β*-*D*-glycoside, 4: Homoeriodictyol-7-*O*-*β*-*D*-apiose(1→2)-*β*-*D*-glycoside, 5: Hedt-III, Syri: Syringin, Hedt-III: Homoeriodictyol-7-*O*-*β*-*D*-glycoside, IS: Internal standard

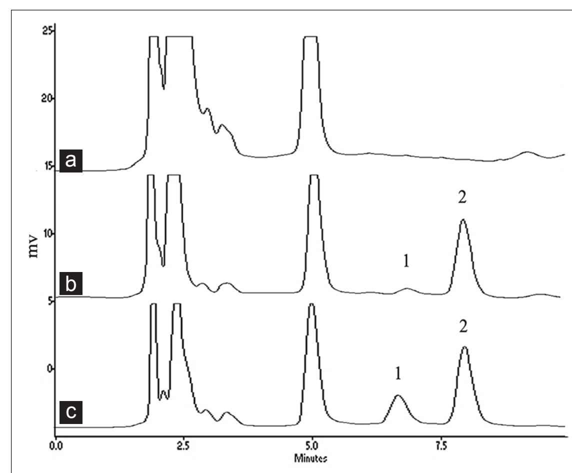

Figure 2.2: Chromatograms of blank plasma (a), A blank rat plasma sample spiked with Syringin (Syri) (limit of quantification) and IS. (6 μg/ml) (b) and a rat plasma sample 0.5 h after i.v. administration of 1.35 mg/kg Syri (c). 1: Syri, 2: IS, IS: Internal standard

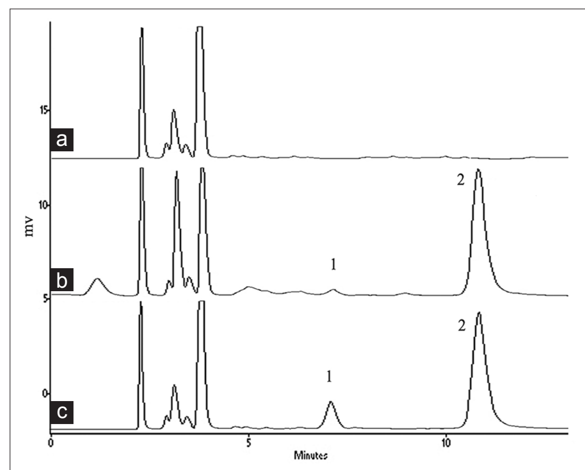

Figure 2.3: Chromatograms of blank plasma (a), a blank rat plasma sample spiked with Hedt-I (limit of quantification) and IS (40 μg/ml) (b) and a rat plasma sample 0.5 h after i.v. administration of 7.7 mg/kg Hedt-I (c). 1: Hedt-I, 2: Internal standard Hedt-I: Homoeriodictyol-7-*O*-*β*-*D*-apiosiyl-(1→5)-*β*-*D*-apiosyl-(1→2)-*β*-*D*-glycoside

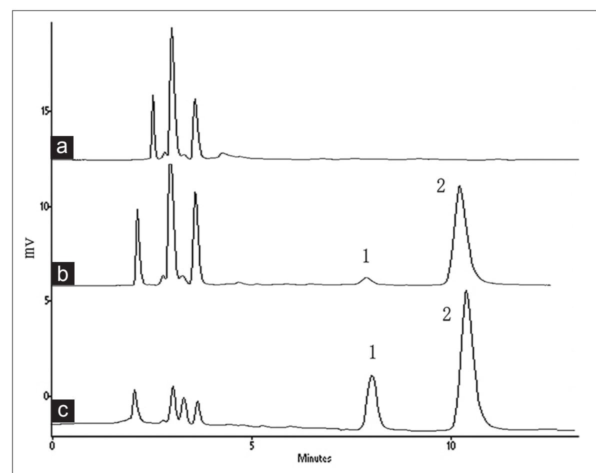

Figure 2.4: Chromatograms of blank plasma (a), a blank rat plasma sample spiked with Hedt-II (limit of quantification) and IS (40 μg/ml) (b) and a rat plasma sample 0.5 h after i.v. administration of 7.5 mg/kg Hedt-II (c). 1: Hedt-II, 2: Internal standard, Hedt-II: Homoeriodictyol-7-*O*-*β*-*D*-apiose(1→2)-*β*-*D*-glycoside

IS, and plasma sample after i.v. administration of the preparations are presented in Figures 2.1-2.5. With the above chromatography conditions, there was no significant interference and no cross-interference observed at the retention times of any analytes or the IS.

Linearity and calibration curve

The results of calibration curves, linearity (R^2) and LLOQ were listed in Table 2. The residuals, difference between the back-calculated concentrations of the calibration standards and their nominal concentration, were no more than ± 15% at all concentrations, which demonstrated that the values were all within the acceptable range.

Precision, accuracy and extraction recovery

The results of intra- and inter-day precision, accuracy and extraction recovery of four analytes in QC samples are summarized in Table 3. The intra- and inter-day precisions (RSD) of these analytes were not more than 11.8% and accuracies (RE) were between -7.9% and 5.5% for the entire QC levels indicating acceptable precision and accuracy of the present method. Mean absolute recoveries of Syri, Hedt, Hedt-I, and Hedt-II from rat plasma were 77.4–91.9%, 71.5–80.4%, 70.2–93.9% and 71.8–93.4%

Table 5: Pharmacokinetic parameters of Syri~Hedt-III following intravenous administration of MONO (Syri~Hedt-III), MIX and VCE to Wistar rats, respectively, (n=6)

Parameter	Syri			Hedt-I			Hedt-II			Hedt-III		
	MONO	MIX	VCE	MONO	MIX	VCE	MONO	MIX	VCE	MONO	MIX	VCE
$AUC_{(0-t)}$ (μg·h/ml)	3.13±0.44	2.73±0.18	2.52±0.49	13.52±0.83	10.45±2.57	12.09±1.79	16.86±2.7[b]	6.57±3.22[a]	10.10±2.17[a]	2.75±0.54	3.43±1.15	5.97±0.80[ab]
$AUC_{(0-\infty)}$ (μg·h/ml)	3.26±0.41	2.94±0.19	2.72±0.55	13.74±0.76	10.57±2.55	12.37±1.93	16.96±2.6[b]	6.71±3.15[a]	10.23±2.17[a]	2.89±0.51	3.69±1.17	6.30±0.93[ab]
$t_{1/2}$ (h)	0.38±0.03[b]	0.52±0.07[a]	0.51±0.09[a]	0.50±0.12	0.41±0.29	0.51±0.12	0.37±0.07	0.45±0.16	0.39±0.04	0.34±0.18	0.74±0.39[a]	0.66±0.25[a]

[a]P<0.05, compared with MONO and [b]P<0.05, compared with MIX using one-way analysis of variance (one-way ANOVA). Hedt-I: *D*-apiosiyl-(1→5)-*β*-*D*-apiosyl-(1→2)-*β*-*D*-glycoside; Hedt-II: Homoeriodictyol-7-*O*-*β*-*D*-apiose (1→2)-*β*-*D*-glycoside; Hedt-III: Homoeriodictyol-7-*O*-*β*-*D*-glycoside; VCE: *Viscum coloratum* extracts; MIX: Mixture solution of them; MONO: Monomer solution; AUC: Area under the curve; Syri: Syringin

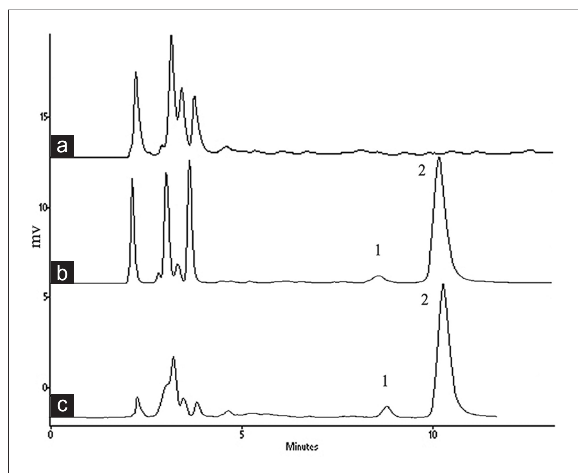

Figure 2.5: Chromatograms of blank plasma (a), a blank rat plasma sample spiked with Hedt-III (limit of quantification) and IS (40 μg/ml) (b) and a rat plasma sample 0.5 h after i.v. administration of 2.6 mg/kg Hedt-III (c). 1: Hedt-III, 2: Internal standard, Hedt-III: Homoeriodictyol-7-*O*-*β*-*D*-glycoside

at three QC levels, respectively. The protein precipitation used in the experiment was proved to be simple, rapid and successful.

Stability

The results of stability showed there was no obvious substance loss under the conditions that plasma samples might experience during this study, and these are summarized in Table 4.

Pharmacokinetic analysis

The RP-HPLC methods were successfully applied to pharmacokinetic study of Syri, Hedt-I, Hedt-II, and Hedt-III in rat plasma after *i.v.* administration of MONO, MIX, and VCE. The pharmacokinetic parameters are presented in Table 5, and the mean plasma concentration-time profiles are illustrated in Figure 3.

For Syri, the result of One-way ANOVA showed there was a significant difference on $t_{1/2}$ between mix and mono, also between VCE and MONO while there was no difference between VCE and MIX. The result might indicate that the three components (Hedt-I, Hedt-II, and Hedt-III) in VCE reduced Syri's elimination much more than the other unknown ingredients in VCE. In addition, the result showed no significant differences on area under the curve ($AUC_{(0-t)}$) and $AUC_{(0-\infty)}$ among MONO, MIX, and VCE. The reason that $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of Syri were not impacted by the other ingredients in VCE might due to the un-interrupted metabolizing enzymes of Syri.

For Hedt-I, Hedt II and Hedt-III, three flavonoid glucosides, although the only difference between these three was the number of linked glucose, the effects on their pharmacokinetic profiles were markedly distinctive and even converse. The result showed there were no difference on $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and $t_{1/2}$ for Hedt-I among MONO, MIX, and VCE. We hypothesized that non-Hedt-I components in VCE did not have any pharmacokinetic effects. For Hedt-II, there was no significant difference on $t_{1/2}$ among MONO, MIX, and VCE. Furthermore, there is no difference on $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ comparing group of MIX and VCE. However, both $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were decreased comparing VCE/MIX group to MONO group. This may suggest that Syri, Hedt-I and Hedt-III could accelerate Hedt-II's metabolism by affecting metabolizing enzymes and thus reduced its bioavailability. Comparing to VCE group, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were reduced for both MIX and MONO groups. This may show that the unknown ingredients in VCE could inhibit Hedt-III's metabolism. Variation of $t_{1/2}$ was same with that of Syri. The result suggested that Syri, Hedt-I, and Hedt-II might reduce Hedt-III's elimination.

In summary, results of the present study indicated that interactions among co-exiting components in VCE were complex and significant, which needed special attention. To a large extent, the powerful therapeutic effect of TCM depended on multiple factors and TCM was also based on overall functional state of patients, which was a different point of view from western medicine.[21] The reason and mechanism leading to these differences in the pharmacokinetic properties

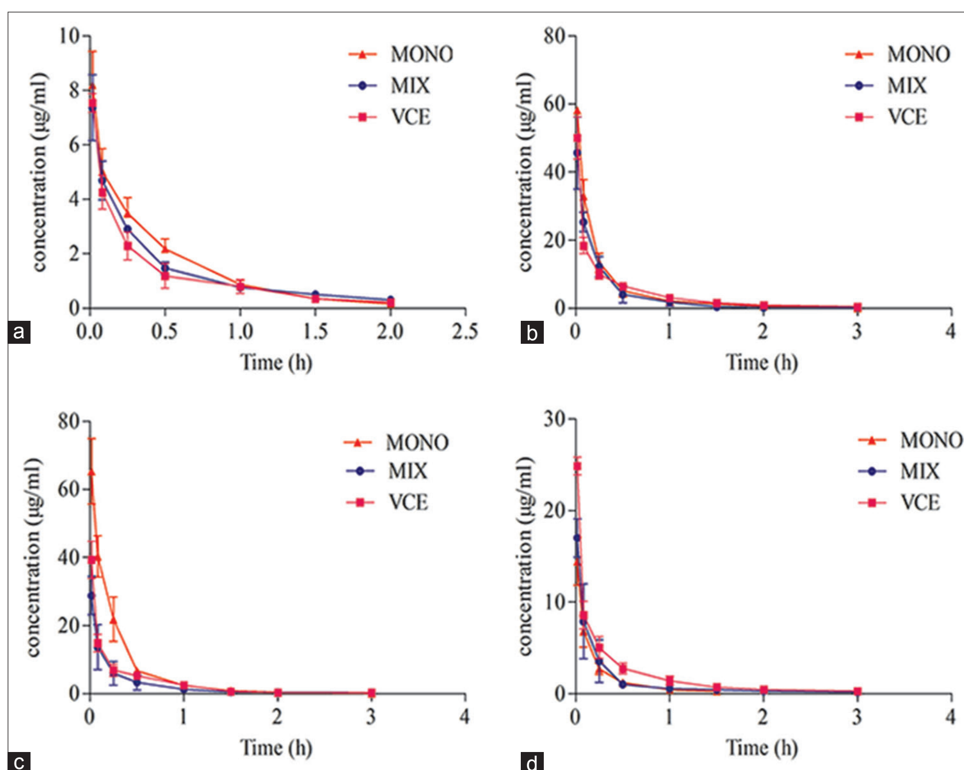

Figure 3: Mean plasma concentration-time profile of syringin (a), homoeriodictyol-7-*O*-*β*-*D*-apiosiyl-(1→5)-*β*-*D*-apiosyl-(1→2)-*β*-*D*-glycoside (b), homoeriodictyol-7-*O*-*β*-*D*-apiose(1→2)-*β*-*D*-glycoside (c) and homoeriodictyol-7-*O*-*β*-*D*-glycoside (d) after i.v. administration of different solutions to rats

among different treatments had not been satisfactorily explained and need to be further investigated. To our knowledge, the pharmacokinetic interactions were mainly based on the mutual influence in absorption, distribution, metabolism and elimination. Especially the metabolism and plasma protein binding were the big players.[22]

CONCLUSION

In this paper, we delivered firstly pharmacokinetics interactions among Syri, Hedt-I, Hedt-II, Hedt-III and unknown ingredients of VCE in rat after *i.v.* administration different preparations. Three reliable RP-HPLC methods were established validated and successfully applied to comparison of these different experiment groups. The study demonstrated that co-administration of components in VCE could cause an alteration in their pharmacokinetic profiles, which would be helpful for understanding the action mechanism and further development of clinical applications of the Chinese traditional herbal medicines.

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