

Effect of *Radix Platycodonis* and *Radix Cyathulae* in Xuefu Zhuyu Tang on tissue distribution of paeoniflorin in blood-stasis mice by HPLC: Experimental evidence on *Shi* ingredients in traditional formula compatibility

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Xuefu Zhuyu Tang (XFZY), a famous formula in traditional Chinese medicine, has been demonstrated to show good therapeutic effects on diseases caused by blood stasis syndrome. Two of its eleven herbs, *Radix Platycodonis* and *Radix Cyathulae*, have been considered as *Shi* ingredients in the hierarchy of traditional formula compatibility and proven possessing synergistic properties that strengthen the formula's potency of activating blood circulation and resolving blood stagnation. However, its mechanism is still not clearly elucidated. In our previous study, we observed their effects on paeoniflorin pharmacokinetics of XFZY in rats. In this study, we continued by detecting and comparing their effect on the tissue distribution of paeoniflorin after oral administration of XFZY and its three variants (XFZY without *Radix Platycodonis* or/and *Radix Cyathulae*) in blood-stasis mice via HPLC assay. The results indicated that combining usage of *Radix Platycodonis* and *Radix Cyathulae* increased the distribution of paeoniflorin in the lung and kidney and introduced the paeoniflorin into the liver, spleen and heart. It might explain their synergistic properties that strengthen the formula's effect of invigorating blood and dissolving stasis and provide experimental evidence to understand the pharmacological effects of *Shi* herbs in the hierarchy of traditional formula compatibility.

Keywords: Blood stasis syndrome, Paeoniflorin, *Radix cyathulae*, *Radix platycodonis*, *Shi* ingredient, Tissue distribution, Traditional formula compatibility, Xuefu Zhuyu Tang

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Blood stasis syndrome (BSS), an important pathological concept in traditional Chinese medicine (TCM)^{1,2}, has been proven to correlate with the occurrence and development of a wide range of diseases such as coronary heart disease, hypertension, different types of cancer, diabetes, arrhythmia, obesity, dyslipidemia, gastric ulcer, hepatitis B, psoriasis, primary glomerular disease and rheumatoid arthritis³⁻⁵. With the ever-increasing prevalence of such diseases and the ever-emerging acceptance of complementary and alternative therapy, the use of TCM has become a worldwide trend⁶. Hence, TCM formulae of promoting blood circulation and removing blood stasis have been exploited as important therapeutic approaches.

Xuefu Zhuyu Tang (XFZY), one of the most effective and commonly-used formulae for BSS^{7,8},

was first recorded in the medical classic *Correction of Errors in Medical Works* by Qing-ren Wang (1768–1831)⁹. Its protective actions of reducing inflammation and oxidation, modulating angiogenesis, inhibiting platelet aggregation and improving hemorheology have been well documented¹⁰⁻¹³. This formula has become a focus of attention for many researches due to its favorable potency of activating blood circulation and dissolving blood stasis. It consists of 11 herbs that embody the compatibility principle of “*Jun, Chen, Zuo, and Shi*” (also known as “sovereign-minister-assistant-courier”). In TCM, most formulae achieve their potency through actions between/among diverse ingredient herbs rather than a single herb. Hence, a critical issue for an optimized application of formulae is to understand their prescription compatibility.

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In XFZY, *Radix Platycodonis* (*Platycodon grandiflorum* (Jacq.) A.DC.) and *Radix Cyathulae* (*Cyathula officinalis* KC Kuan) have been regarded as *Shi* (guide, messenger, conductant or envoy) ingredients that are to guide the action of the formula on the target channel or area of the body. Pharmacologically, they have been proven effective in increasing the formula's potency of activating blood circulation and resolving blood stagnation¹⁴, but its mechanism has not been fully elucidated. In our previous study, we observed their effects on paeoniflorin pharmacokinetics of XFZY in rats and revealed that combining usage of both herbs increased the absorption amount and rate of in rats and accelerated its elimination from the blood¹⁵. In this study, to provide more experimental evidence on the *Shi* herb in the hierarchy of TCM formula compatibility and optimize their clinical applications, we continued by detecting and comparing their effect on the tissue distribution of paeoniflorin after oral administration of XFZY and its three variants (XFZY without *Radix Platycodonis* or/and *Radix Cyathulae*) in blood-stasis mice via HPLC assay.

Methodology

Animals

A total of 48 SPF Kunming mice (4 weeks of age, weighing 25±5 g, half male and half female), were obtained from Experimental Animal Center of Sichuan Biological Products Institute. All the experiments were conducted in accordance with the guidelines and ethics for the use of experimental animals and approved by the local Institutional Review Committee on Animal Care and Use. Four mice were kept in each cage and conditioned in a breeding room at 25°C with a relative humidity of 50%±10% under a dark/light cycle of 12 h. All mice were fed under the above conditions for one week with free access to water and rodent chow at all times.

Medicinal materials and reagents

XFZY consists of *Persicae Semen* (*Prunus persica* (L.) Batsch and *Prunus davidiana* (Carr.) Franch.), *Radix Angelicae Sinensis* (*Angelica sinensis* Oliv. Diels), *Rhizoma Chuanxiong* (*Ligusticum chuanxiong* Hort), *Flos Carthami* (*Carthamus tinctorius* L.), *Radix Paeoniae Rubra* (*Paeonia lactiflora* Pall. and *Paeonia veitchii* Lynch), *Radix Rehmanniae* (*Rehmannia glutinosa* (Gaertn.) Libosch. ex Fisch. et Mey.), *Fructus Aurantii* (*Citrus aurantium* L), *Radix Bupleuri* (*Bupleurum chinense* DC. and *Bupleurum scorzonifolium* Willd.), *Radix Platycodonis*, *Radix*

cyathulae, and *Radix et Rhizoma Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat., and *Glycyrrhiza glabra* L.) at differential ratio of 12:9:4.5:9:6:9:6:3:4.5:9:6. All herbs, commercially available as dried matter, were purchased from Sichuan Ben Cao Tang Pharmaceutical Co., Ltd. with identification of two herbology professors (Chengdu, China).

Paeoniflorin among several identified effective constituents of XFZY was considered the appropriate index for detection^{8,15}. Paeoniflorin reference substance (purity 98%, batch No.: 121207) were obtained from Chengdu Pufei De Biotech Co., Ltd.; Riboflavin reference substance (purity 98%, batch No.: 130114) were obtained from Chengdu PureChem-Standard Co., Ltd.; Glucan T500 was obtained from Beijing YaAnDa Biotechnology Co., Ltd.; and chromatographically pure carbinol and acetonitrile were required.

Instruments and analytical conditions

The instruments used were Ultraviolet-visible (UV-Vis) detector (SPD-10Avp); high performance liquid chromatography (HPLC, LC-10ATvp) (Shimadzu Corporation, Japan); FA1004 electronic scales (Shanghai Liangping Instrument Co. Ltd. Shanghai, China); LG15-W high speed micro centrifuge (Beijing medical centrifuge factory, Beijing, China); WH-1 Mini vortex mixer (Shanghai Huxi Analytical Instrument Co. Ltd. Shanghai, China); Milli-Q water purification system (Millipore, Billerica, MI, USA). MVIS-2035 auto-hemorheology analyzer (Chongqing Tianhai Medicare Instrument Co. Ltd. Chongqing, China).

The chromatographic separation was obtained on Diamonsil C₁₈ chromatographic column (250 mm×4.6 mm, 5 μm). The chromatographic conditions were recorded as follows: injection volume was 20 μL; chromatograms were monitored at 230 nm; column temperature was maintained at 30°C; mobile phase consisted of acetonitrile and water (16:84) with a flow rate of 1.0 mL min⁻¹; and the geniposide were set as internal standard substance.

Preparation of medicinal solution

According to the traditional formulae, the amount of 78 g XFZY, 73.5 g XFZY-JG (XFZY without *Radix Platycodonis*), 69 g XFZY-NX (XFZY without *Radix Cyathulae*), and 64.5 g XFZY-JG-NX (XFZY without *Radix Platycodonis* and *Radix Cyathulae*) were multiplied by 45 to 3 510 g, 3 307.5 g, 3 105 g, and 2 902.5 g, respectively, and immersed in 35 100 mL,

33 075 mL, 31 050 mL, and 29 025 mL of water (v/v, 1:10), respectively, for 30 min at room temperature. XFZY, XFZY-JG, XFZY-NX, and XFZY-JG-NX were decocted 30 min for 3 times, respectively. After filtration and concentration in a rotatory evaporator for 8 h, the aqueous extract of each sample was dried at 45°C in a vacuum drying oven to obtain 426 g original extract powder for XFZY, 1 054 g for XFZY-JG, 948 g for XFZY-NX, and 650 g for XFZY-JG-NX. The mass fraction of paeoniflorin in original extract powder was 2.290 mg g⁻¹ for XFZY, 2.620 mg g⁻¹ for XFZY-JG, 3.366 mg g⁻¹ for XFZY-NX, and 3.120 mg g⁻¹ for XFZY-JG-NX. An amount of 4 g extractive powder of each sample was accurately weighed, dissolved in 4.0 mL, 4.6 mL, 5.9 mL, and 5.4 mL of water, and swirled to mix thoroughly and obtain 4 medicinal solutions for oral gavage.

Glucan T500 solution at concentration of 25% was prepared by dissolving 1.0 g Glucan T500 in 4 mL of 0.9% sodium chloride injection. All solutions were stored at 0°C until analysis.

Preparation of stock solutions and quality control samples

Paeoniflorin stock solution was prepared by dissolving 6.63 mg paeoniflorin in 90% methanol aqueous solution at concentration of 130.0 µg mL⁻¹. Internal standard stock solution was prepared by dissolving 1.82 mg geniposide in 90% methanol aqueous solution at concentration of 35.67 µg mL⁻¹. Internal working standard solution was prepared by diluting 12.5 mL stock solution 90% methanol aqueous solution at concentration of 8.92 mg L⁻¹. Quality control (QC) samples were prepared in the same manner at paeoniflorin concentrations of 0.0208, 0.26, and 2.6 mg L⁻¹ for the lung, 0.026, 0.208, and 2.08 mg L⁻¹ for the kidney, and 0.026, 0.208, and 2.08 mg L⁻¹ for the serum. All solutions were stored at 0°C until analysis.

Method validation

Specificity: The specificity of this method was investigated by comparing chromatograms of blank kidney, lung, and serum homogenate samples; blank kidney, lung, and serum homogenate samples spiked with standard solution and IS; and treated kidney, lung, and serum homogenate samples.

Linearity and quantification: Calibration curves were obtained by linear regression of the peak area ratio of paeoniflorin to IS (*Y*-axis) and the standard concentration, which constituted the range of the calibration curve (*X*-axis). The calibration line was

corrected by a weighted factor ($1/X^2$), described as $Y=a+bX$. Each QC sample was recalculated using the calibration curve.

Precision and accuracy: Accuracy and precision of the method were determined by repeat analyses of QC samples. The intraday precision and accuracy were evaluated by determining QC samples five times within a single day, whereas the interday precision and accuracy were assessed by determining QC samples over three consecutive days.

Recovery rate: The recovery of paeoniflorin was performed by comparing the peak area of the added paeoniflorin in kidney, lung, and serum homogenates with the same amount of paeoniflorin added to the mobile phase. QC samples at three concentrations were evaluated in the recovery experiments ($n=5$ for each QC).

Stability: The stability was evaluated by measuring QC samples at three concentrations under different conditions ($n=4$ for each QC). Freezing thawing stability was investigated after QC samples underwent three cycles of freezing at -20°C freezer and thawing at 37°C water bath; the short-term stability was assessed by exposing QC samples at room temperature for 8 h; and the long-term stability was estimated after storing QC samples at -20°C for 7 d.

Tissue distribution assay

Animal groupin: Mice were randomized into two groups: normal group and blood-stasis model group, with 24 in each group. Then, mice in each group were randomly sub-assigned to 4 groups, with 6 in each sub-group.

Animal experimental model: The animals were fasting for 12 h and allowed free access to water before modeling. A 25% glucan T500 solution (20 mL kg⁻¹) was injected intravenously through caudal vein of the mice in the blood-stasis model group, and 0.9% physiological saline for the normal group. The level of whole blood viscosity, plasma viscosity, hematocrit, and fibrinogen were 7.08±0.89 mPa s (200 s⁻¹), 2.23±0.37 mPa s (200s⁻¹), 65.46±8.46 %, and 7.25±0.68 g/L in the blood-stasis group.

Medicinal solution administration: Ten minutes after modeling, mice in the normal and the blood stasis group were treated orally with 6 mL kg⁻¹ of XFZY, XFZY-JG, XFZY-NX, and XFZY-JG-NX solution, respectively. Each group was administered equal volume of paeoniflorin at 13.742 mg kg⁻¹.

Specimen collection and processing: The mice were sacrificed 30 min after administration. Tissues including the heart, liver, spleen, lung, and kidney were obtained from each mouse, cleaned with 0.9% physiological saline, absorbed by filter paper, weighed, and homogenized in normal saline solution (250 mg/mL). The obtained tissue homogenates were stored separately at -20°C.

Before analyzing, 90% methanol aqueous solution was added to the liver, lung, and kidney (4 mL g⁻¹); and heart and spleen (8 mL g⁻¹). For each sample, 20 µL working internal standard solution was added to 500 µL tissue homogenate. The mixtures were vortexed for 20 s and centrifuged at 10 000 r min⁻¹ for 10 min. Supernatant of each sample was transferred into a 1.5 mL centrifuge tube individually and evaporated under nitrogen in a water bath at 40°C, and 100 µL of water were added for redissolution. The obtained samples were centrifuged at 10 000 r min⁻¹ for 10 min. Finally, 20 µL supernatant of each sample was taken for analysis.

Data analysis

Statistical analysis was performed using the SPSS 13.0 software and the data were presented as the standard error of the mean. The concentration-peak area curves of paeoniflorin were obtained through the DAS 2.0 software (Shanghai, China). Comparisons on peak area and concentration ratio of paeoniflorin between two groups were statistically evaluated by the Mann-Whitney U test; the concentration of paeoniflorin was changed into napierian logarithm and evaluated by a one-way analysis of variance (ANOVA). Statistical significance were considered at $p < 0.05$.

Results

Method validation

Specificity: Representative chromatograms were shown in Fig. 1. The paeoniflorin and endogenous impurities were confirmed separate well, the degree of separation was found to be 1.8, and the retention time was 15.9 min for paeoniflorin and 9.5 min for internal standard substance.

Linearity and sensitivity: The equation for the calibration curves and linear range of paeoniflorin were presented in Table 1. The range of calibration curves were shown in Fig. 2. Results showed that calibrations curves were linear and acceptable. The range of concentration and limits were suitable for the tissue distribution study of paeoniflorin.

Precision and accuracy: The precision was presented as the relative standard deviation (RSD), the data of intra-day and inter-day accuracy and precision of paeoniflorin were demonstrated in Table 2. The results showed the intra-accuracy ranged from -2.5% to 1.8%, the inter-accuracy -1.4% to 1.4%, the intra-precision 1.7% to 3.1%, and the inter-precision 1.9% to 3.0%. It indicated that the precision and accuracy of the present method were within the acceptable limits.

Recovery: The extraction recoveries were presented in Table 2. Data revealed that the lung samples was well within 62.1%~89.0%; the kidney samples was well within 67.7%~90.7%; and the serum samples was well within 80.6%~87.4%. The SD of extraction recoveries was 0.8%~6.9% for the lung samples, 2.0%~3.1% for the kidney samples, and 1.7%~5.5% for the serum samples.

Stability: The results of stability under different storage conditions were presented in Table 3. Results revealed that samples were stable under various experimental conditions.

Tissue distribution assay

A validated HPLC method was applied to investigate paeoniflorin distribution in the heart, liver, spleen, kidney, and lung of blood-stasis mice 30 min after oral administration of XFZY and its three variants. The data of tissue distribution and statistical analysis were presented in Table 4.

The results displayed that upon the comparison with XFZY-JG-NX, sole usage of *Radix Platycodonis* (XFZY-NX) increased the distribution concentration of paeoniflorin in the kidney of normal group, and the lung/serum concentration rate and distribution of paeoniflorin in the lung and kidney of blood-stasis group; sole usage of *Radix Cyathulae* (XFZY-JG) introduced the paeoniflorin into the tissue of heart, liver, and spleen in the blood-stasis group; and combing usage of *Radix Platycodonis* and *Radix Cyathulae* (XFZY) increased the lung/serum concentration rate and distributed more paeoniflorin into the tissue of heart, liver and spleen in the blood-stasis group. The comparisons between the normal and the blood-stasis group were also performed for XFZY and its three variants. The findings revealed that in the blood-stasis group, the combing usage of *Radix Platycodonis* and *Radix Cyathulae* (XFZY) significantly increased the lung/serum ratio and the concentration of paeoniflorin in the lung and kidney and distributed

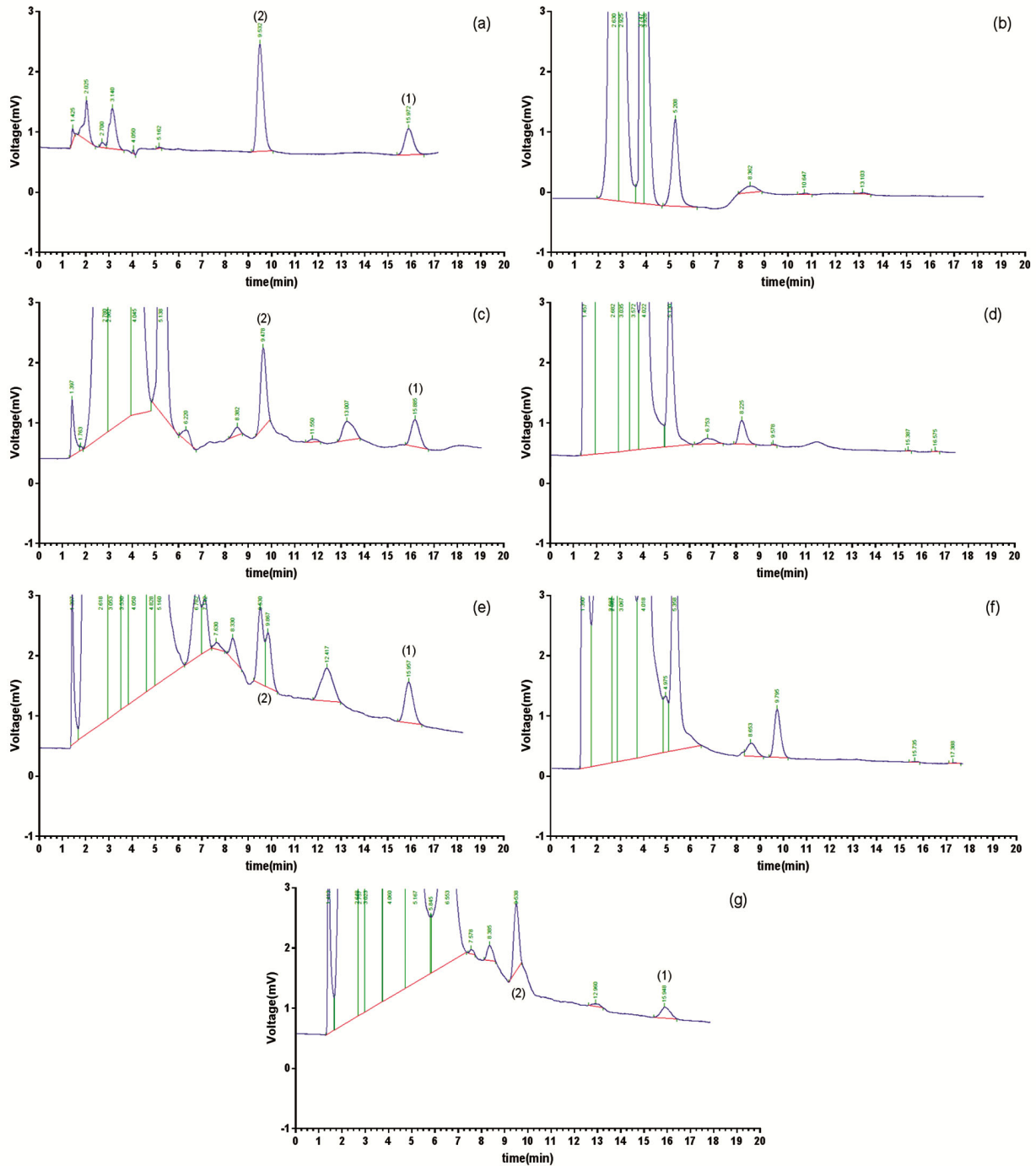


Fig. 1 — HPLC chromatograms of paeoniflorin (t/min) (a), reference substance (b), blank serum (c), serum (d), blank kidney (e), kidney (f), blank lung (g), lung. (1) paeoniflorin, (2) internal standard substance.

the paeoniflorin into more tissues, including liver, spleen and heart; sole usage of *Radix Cyathulae* (XFZY-JG) increased the concentration of paeoniflorin in the lung and kidney, and distributed the paeoniflorin into the tissue of heart, liver, spleen; sole usage of *Radix Platycodonis* (XFZY-NX) increased the lung/serum ratio and the kidney/serum

ratio and uplifted the concentration of paeoniflorin in the lung and kidney; removal of both *Radix Platycodonis* and *Radix Cyathulae* (XFZY-JG-NX) increased the kidney/serum ratio and the concentration of paeoniflorin in the kidney, whereas decreased the lung/serum ratio and the concentration of paeoniflorin in the lung.

Table 1 — Calibration curves and linear range of paeoniflorin

Compound	Tissue	Calibration curves	Correlation coefficient (<i>r</i>)	Linear rang (ng/mL)
Paeoniflorin	Serum	$Y = 17\,748.848X - 200.472$	$r = 0.999\,1$	0.026~2.08
	Lung	$Y = 17\,663.323X - 300.424$	$r = 0.999\,7$	0.0208~2.60
	Kidney	$Y = 17\,721.875X - 291.167$	$r = 0.999\,9$	0.026~2.08

Table 2 — Intra-assay and Inter-assay precision, accuracy, and recovery of paeoniflorin in the serum, lung, and kidney

Compound	Tissue	QC Concentration (mg/L)	Intraday		Interday		Recovery (% mean ± SD)
			Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	
Paeoniflorin	Serum	0.026	2.9	0.1	2.6	0.1	87.4 ± 5.5
		0.208	2.8	1.0	2.0	1.0	80.6 ± 2.0
		2.08	3.1	-0.1	3.0	0.0	87.1 ± 1.7
	Lung	0.0208	2.8	-2.5	2.2	-1.4	62.1 ± 6.9
		0.26	2.9	1.8	2.7	0.7	83.1 ± 0.8
		2.6	2.0	0.7	2.0	0.9	89.0 ± 1.9
	Kidney	0.026	2.6	-0.5	2.4	0.1	67.7 ± 2.0
		0.208	1.7	-2.1	1.9	-1.4	84.7 ± 2.9
		2.08	2.5	1.2	2.1	1.4	90.7 ± 3.1

Table 3 — Stability of paeoniflorin in the serum, lung, and kidney (*n*=4)

Compound	Tissue	QC Concentration (mg/L)	Freeze-thaw stability		Short-term stability		Long-term stability	
			Mean ± SD	RE (%)	Mean ± SD	RE (%)	Mean ± SD	RE (%)
Paeoniflorin	Serum	0.026	0.0248 ± 0.0011	-4.6	0.0261 ± 0.0005	0.4	0.0257 ± 0.0003	-1.2
		0.208	0.2062 ± 0.0072	-0.9	0.2078 ± 0.0018	-0.1	0.2082 ± 0.0032	0.1
		2.08	2.0136 ± 0.0821	-3.2	2.1061 ± 0.0235	1.3	2.0348 ± 0.0508	-2.2
	Lung	0.0208	0.0198 ± 0.0010	-4.8	0.0221 ± 0.0004	6.3	0.0207 ± 0.0001	-0.3
		0.26	0.2538 ± 0.0085	-2.4	0.2611 ± 0.0034	0.4	0.2648 ± 0.0026	1.9
		2.6	2.6986 ± 0.1011	3.8	2.6155 ± 0.0205	0.6	2.5146 ± 0.0461	-3.3
	Kidney	0.026	0.0278 ± 0.0009	6.9	0.0254 ± 0.0004	-2.3	0.0268 ± 0.0003	2.9
		0.208	0.1996 ± 0.0131	-4.0	0.2064 ± 0.0065	-0.8	0.2086 ± 0.0035	0.3
		2.08	1.9748 ± 0.0328	-5.1	2.021 ± 0.0141	-2.8	1.9555 ± 0.0236	-6.0

Discussion

Formula is the major form of TCM's clinical application and its potency is attributable to the synergistic and/or additive action between/among diverse ingredient herbs. Hence, to reveal the scientificity and rationality of its compatible rank is of great importance for uncovering the compatibility myth of the formula and optimizing its clinical application. Among the four ranks of the ingredients in the hierarchy of formula compatibility, *Shi* (guide, messenger, conductant or envoy) is capable of directing the actions of the formula to the target channel or areas of the body and reflects the selective therapeutic effects of Chinese medicinals on a certain area or tissue of the body. It is crucial for the therapeutic effect of a formula and has been the focus of increasing researches. In this study, we validated the effect of *Shi* ingredients in the context of a

formula. The effect *Radix Platycodonis* or/and *Radix Cyathulae* on the tissue distribution of paeoniflorin in blood-stasis mice was investigated through "decomposing formula" (removing *Radix Platycodonis* or/and *Radix Cyathulae* from XFZY) and comparing XFZY with its three variants.

XFZY has been proven effective in treating various diseases caused by BSS¹⁶⁻²⁰. Among its 11 ingredients, *Radix Platycodonis* and *Radix Cyathulae* have been categorized into the *Shi* ranking. They are potent in improving XFZY's effect of invigorating blood circulation and removing blood stasis¹⁴. Traditionally, the former is bitter, acrid and neutral, enters the lung and carries the effect of other herbs upwards; whereas the latter is bitter, sweet, sour and neutral, enters the kidney and liver and conducts blood downwards. In this study, we demonstrated under the blood-stasis state, combining use of *Radix*

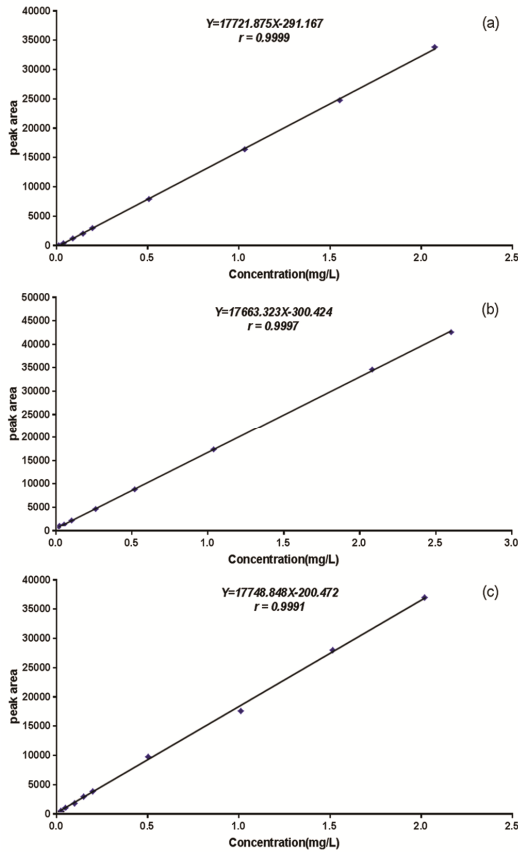


Fig. 2 — A-C curve of (a), kidney sample (b), lung sample (c), serum sample

Platycodonis and *Radix Cyathula* exerted significant effects on the distribution of paeoniflorin in the lung and kidney, which is consistent with the TCM wisdom. Sole usage of *Radix Platycodonis* also presented an effect on the kidney, while using *Radix Cyathula* solely or together with *Radix Platycodonis* introduced the paeoniflorin into more tissues, including the heart, liver and spleen. Herein, we infer the therapeutic effect of the *Shi* herbs may be attributed to their influences on the tissue distribution of the effective constituent.

In TCM, the human body is an integrated and balanced whole; all the component parts are interconnected via channels. The amount of medicinal concentration found in a tissue is also closely correlated with its concentration in the serum. Therefore, we detected both indexes and further calculated the lung-serum concentration ratio (paeoniflorin concentration in the lung/paeoniflorin concentration in the serum) and kidney-serum concentration ratio (paeoniflorin concentration in the kidney/paeoniflorin concentration in the serum). The

Table 4 Paeoniflorin tissue distribution of mice 30 min after oral gavage (n=6)

Group	Treatment	Peak area							Concentration/ $\mu\text{g}\cdot\text{mL}^{-1}$		Tissue concentration / % Serum concentration	
		Heart	Liver	Spleen	Lung	Kidney	Serum	Lung	Kidney	Lung	Kidney	
Normal	XFZY(a)	0**	0**	0**	125** ^{c,ΔΔ}	2464*	1568	0.0127**	0.1527* ^{b,Δ}	0.0996 ^{c,ΔΔ}	8.2* ^{c,Δ}	153.8
	XFZY-JG(b)	0*	0**	0*	27** ^{c,ΔΔ,Δ}	860** ^{c,ΔΔ}	1120** ^{c,Δ,Δ,Δ}	0.0072** ^{c,Δ}	0.065** ^{a,Δ,Δ,Δ}	0.0744** ^{c,ΔΔ,Δ}	12.3	85.9
	XFZY-NX(c)	0	0	0	1709 ^{a,Δ,Δ,Δ}	2898 ^{b,ΔΔ,Δ}	6587 ^{b,Δ}	0.1138 ^{b,Δ}	0.1800 ^{b,ΔΔ,Δ}	0.3824 ^{a,Δ,Δ,Δ}	38.6 ^{b,Δ}	86.3
Blood-Stasis	XFZY-JG-NX(d)	0	0	0	573 ^{b,Δ}	1148 ^{c,ΔΔ}	3168 ^{b,Δ,Δ}	0.0466	0.0812 ^{c,Δ}	0.1898 ^{b,Δ}	27.5	50.9**
	XFZY(a)	150** ^{c,Δ,Δ,Δ}	384** ^{b,Δ,Δ,Δ,Δ}	138** ^{c,ΔΔ,Δ,Δ}	1386** ^{d,Δ}	7131*	4410	0.0955**	0.4188* ^{d,Δ}	0.2598	38.7* ^{b,Δ,Δ}	177.4
	XFZY-JG(b)	112*	81** ^{a,Δ,Δ,Δ,Δ,Δ}	117* ^{c,Δ}	627** ^{c,Δ}	4361**	4001**	0.0525** ^{c,ΔΔ}	0.2625**	0.2367**	19.6 ^{a,Δ,Δ,Δ}	139.9
	XFZY-NX(c)	22 ^{a,Δ}	0 ^{a,Δ,Δ,Δ,Δ}	0 ^{a,Δ,Δ,Δ}	5765 ^{b,Δ,Δ,Δ}	10056	7860	0.3434 ^{b,ΔΔ,Δ}	0.5839 ^{d,Δ}	0.4541 ^{d,Δ}	68.6 ^{b,ΔΔ,Δ,Δ}	144.4
	XFZY-JG-NX(d)	0 ^{a,Δ,Δ}	0 ^{a,Δ,Δ,Δ,Δ}	21 ^{a,Δ}	222 ^{a,Δ,Δ,Δ}	3010	2469	0.0211 ^{c,Δ}	0.1863 ^{a,Δ,Δ}	0.1504 ^{c,Δ}	12.6 ^{a,Δ,Δ,Δ}	125.4**

(1) 0: paeoniflorin undetected. (2)*: $p \leq 0.05$ for the comparison between the normal and the blood-stasis group for the same formula; **: $p \leq 0.01$ for the comparison between the normal and the blood-stasis group for the same formula. (3) a, b, c, and d for XFZY, XFZY-JG, XFZY-NY, and XFZY-JG-NX, respectively; Δ : $p \leq 0.05$ for comparison between two formulae; $\Delta\Delta$: $p \leq 0.01$ for comparison between two formulae. (4) Major parameters including lung/serum concentration ratio, kidney/serum concentration ratio, lung concentration, and kidney concentration

results demonstrated that sole usage of *Radix Platycodonis* or *Radix Cyathula*, and combining *Radix Platycodonis* with *Radix Cyathula* increased the lung-serum and kidney-serum concentration ratio. Herein, we infer that the accelerated ratio may imply an increased distribution of the active constituents from the blood to the tissues.

Syndrome (zheng, pattern) is the abstraction and generalization of the pathological changes at a certain stage of disease²¹, which reflects the changes of multi-system of the human body and provides further stratification of the patient's condition. It is the target of treatment and the underlying principle for the TCM's strategy of "different treatments for same disease, and same treatment for different diseases". The key to utilizing TCM appropriately and maximizing its efficacy is to accurately interpret syndrome. Therefore, the establishment and adoption of animal model that reflects certain syndrome is of great importance in the pharmacological research of TCM. In this study, the mouse model used was a simulation of the BSS, and the results presented that for the same formula, significant differences on peak area of paeoniflorin and concentration of paeoniflorin in different tissues were noted between the normal group and the BSS group ($p < 0.05$). BSS exerted influences on the tissue distribution of paeoniflorin, hence the adoption of syndrome-based experimental animal is necessary for the performing corresponding pharmacokinetic studies.

Conclusion

This study validated that for BSS, combining usage of *Radix Platycodonis* and *Radix cyathulae* in XFZY could fortify the distribution of paeoniflorin in the lung and kidney, which are consistent with TCM wisdom. Moreover, they could distribute the paeoniflorin into more tissues, including the heart, liver and spleen. This may partly explain why combination of these two herbs demonstrated a more potent effect on activating blood flow and relieving blood stasis.

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