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RESEARCH ARTICLE

Pharmacokinetic profile of 2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-*D*-glucoside in mice after oral administration of *Polygonum multiflorum* extract

Guiyuan Lv[1,2], Hui Gu[1], Suhong Chen[2], Zhaohuan Lou[1], and Letian Shan[1]

[1]*Zhejiang Chinese Medical University, Hangzhou, P.R. China and* [2]*Wenzhou Medical College, Wenzhou, P.R. China*

Abstract

Context: Stilbene glycoside (2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-*D*-glucoside) is a main bioactive component of *Polygonum multiflorum*, a traditional Chinese medicine (TCM) commonly used in clinic for anti-aging treatment. Its medicinal activities, such as anti-oxidation, anti-inflammation and endothelial protection, have been extensively studied, but its pharmacokinetic property is still unclear.

Objective: A pharmacokinetic study was undertaken to quantitatively determine *P. multiflorum* stilbene glycoside (*PM-SG*) in mouse plasma after oral administration of 100 mg/kg *P. multiflorum* extract.

Materials and methods: A sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with liquid-liquid phase extraction method was employed for this study. Pharmacokinetic parameters of *PM-SG* were determined in mice applying both compartmental and non-compartmental analyses.

Results and discussion: The calibration curve for *PM-SG* in the plasma was linear ($r^2 > 0.99$) over the range of 0.66 to 56.40 μg/ml, and the concentration-time curve was plotted with the maximum concentration (C_{max}) and time to reach maximum concentration (T_{max}) of 29.62 μg/ml and 60 min, respectively. The intra- and inter-day variations were less than 3% for relative standard deviation (RSD) and relative error (RE), with a good recovery of more than 97% (RSD <3%). All pharmacokinetic parameters estimated by compartmental and non-compartmental models reached a same conclusion that *PM-SG* was rapidly absorbed and widely distributed throughout the body with a great efficiency of utility, followed by quick elimination and clearance.

Conclusions: This was the first report on determination of the pharmacokinetic profile of *PM-SG* in mice after oral administration. The result may provide a meaningful basis for evaluating the clinical applications of such a bioactive compound from herbal medicines.

Keywords: Pharmacokinetics, stilbene glycoside, traditional Chinese medicine, RP-HPLC, compartmental model

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Introduction

Polygonum multiflorum Thunb. (Polygonaceae), well known as 'He-Shou-Wu' in the East and 'Fo-ti' in the West, is one of the most important traditional Chinese medicine (TCM) widely used in clinic for health promotion and disease treatment for over a thousand years. It was recorded for the first time by the ancient medical book '*Rihuazi Materia Medica*' in the Song Dynasty of China (793 AD) and listed in the Chinese Pharmacopoeia[1]. *P. multiflorum* possesses many effects, such as anti-oxidation, detoxification, anti-tumor, lubricating intestine, etc., to treat neurological disorders, cardiovascular disorders, and other diseases commonly associated with aging[1–5]. Traditionally, it was orally used in the form of extract (decoction) in which many constituents were involved[1,6]. Recent chromatographic studies have revealed that a major bioactive constituent, identified as stilbene glycoside, are responsible for the medicinal effects of this herb[7–9].

Stilbene glycoside (2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-*D*-glucoside, $C_{20}H_{22}O_9$) of *P. multiflorum* (*PM-SG*), as the major active phenolic constituent in the herb

Address for Correspondence: Suhong Chen, Academy of Traditional Chinese Medicine, Wenzhou Medical College, Wenzhou 325035, P.R. China. Tel. and Fax: +86–571-86613601. E-mail: letian.shan@hotmail.com

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(structure in Figure 1), has been reported to possess anti-oxidative, anti-tumor, anti-inflammatory, cholesterol reduction, liver protective and endothelial protective activities, and has attracted much attention and generated a great deal of research interest for its therapeutic application[10–14]. As a herbal component, *PM-SG* always exerts its effect via various extracts of *P. multiflorum* in which *PM-SG* is present as the main component. To date, many of such *P. multiflorum* extracts have been developed into new drugs approved by the SFDA (State Food and Drug Administration, P.R. China), e.g. a commercially available herbal preparation named Shou-wu-pian (Certificate No.: Z20025008) has been developed as tonic to nourish the liver and kidney and improve the blood microcirculation. Our preliminary study has demonstrated a *PM-SG*-enriched (>50%) *P. multiflorum* extract possessing satisfactory lipid-lowering and hepatoprotective activities against fatty liver, by which a China Patent of Invention (Patent No.: ZL 2004 1 0029566.3) has been taken out. However, although there are relatively extensive investigations on the pharmacological profile of *PM-SG*, no report has hitherto described its pharmacokinetic property including absorption, distribution, elimination, and clearance in mice. The pharmacokinetic study of such a herbal component can advance our knowledge of its *in vivo* mechanism of action and help us to understand and predict a variety of events related to the efficacy and toxicity of its source herb. Moreover, due to the complexity of the extract where *PM-SG* exists, it is very necessary to explore the pharmacokinetic property of *PM-SG* from the extract. Therefore, the importance of studying the pharmacokinetics of *PM-SG* is evident, and such a pharmacokinetic study is undoubtedly required.

Based on the reported investigations, a hypothesis is put forward that *PM-SG* from the extract can be rapidly absorbed and widely distributed throughout living body for exerting its favorable therapeutic effects and then can be quickly eliminated and cleared from the body for minimizing the side effect. In this study, to test this hypothesis and clarify the pharmacokinetic profile of *PM-SG* after oral administration of *P. multiflorum* extract to mice, a sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) with UV detection coupled with liquid-liquid phase extraction method was developed and applied.

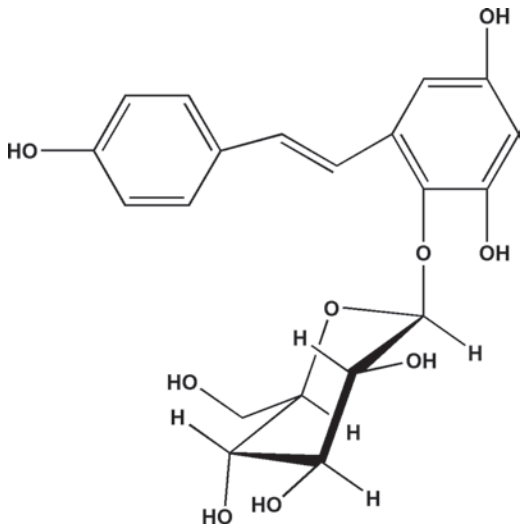

Figure 1. Chemical structure of *Polygonum multiflorum* stilbene glycoside (2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-*D*-glucoside, *P. multiflorum* stilbene glycoside (*PM-SG*)). *PM-SG* ($C_{20}H_{22}O_9$, molecular weight 406) is an active component extracted from *P. multiflorum*.

Materials and methods

Materials and reagents

Roots of *P. multiflorum* were purchased from Zhejiang Traditional Chinese Medical University Electuary Factory (Hangzhou, P.R. China), and were authenticated by authors. The reference standard of *PM-SG* (>99% purity) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Ministry of Health, Beijing, P.R. China). Methanol and acetonitrile (HPLC grade) were purchased from Merck KGaA (Darmstadt, Germany). The distilled water was obtained and purified using Milli-Q system (Millipore, Milford, MA). Other commercial reagents and solvents were of analytical grade.

Preparation of *PM-SG* extract of *P. multiflorum*

A multiple extraction procedures was applied to prepare *PM-SG*, as described by Lv et al.[13] Briefly, the crushed dried roots of *P. multiflorum* were extracted with 60% (v/v) ethanol using the refluxing system. After evaporation, the concentrated ethanolic extract was subjected to open column chromatography packed with macroporous resin. The column was stepwisely eluted with each of five different ethanol concentrations (10%, 20%, 30%, 40%, and 50%). Of these, the 40% aqueous-ethanol fraction was obtained and concentrated by evaporation. More than 50% *PM-SG* are contained in the obtained product.

Animals

Male ICR (Institute for Cancer Research) mice, weighing 30–40 g, were purchased from the Animal Supply Center of Zhejiang Academy of Medical Science (Certificate No.: SCXK2003-0001, Hangzhou, P.R. China). The animals were kept in an environmentally controlled breeding room (temperature: 25 ± 1°C, humidity of 55 ± 5%, and a 12/12 h light/dark cycle) for 1-week acclimatization before experiment. All mice were fed rodent laboratory chow with tap water *ad libitum* and were fasted for 12 h before administration but had free access to water. All procedures were in strict accordance with the P.R. China legislation on the use and care of laboratory animals and with the guidelines established by the Institutional Animal Ethics Committee and Committee for the Purpose of Control and Supervision of Experiments on Animals in P.R. China.

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Instruments and conditions

The RP-HPLC analysis was carried out on an Agilent Technologies 1200 Series liquid chromatograph (Agilent Technologies, Palo Alto, CA), equipped with G1311A quaternary solvent-delivery system, G1329A autosampler, G1316A thermostatted column compartment, G1322A vacuum degasser and G1315B diode array detector (DAD). Chromatographic separation was performed on an Ultimate XB-C_{18} column (150 mm × 4.6 mm i.d., 5 μm; Welch, MD) coupled with Agilent C_{18} pre-column (15 mm × 4.6 mm i.d., 5 μm; Palo Alto, CA) at temperature of 25°C. The ratio of mobile phase consisting of acetonitrile-H_2O was 20:80 (v/v) for plasma samples after oral administration. The flow rate was 1.0 ml/min and the sample injection volume was 10 μl. The detection wavelength was 320 nm. The obtained chromatographic data were acquired and analyzed by using Agilent ChemStation software.

Pharmacokinetic studies

Treatment of animals

Fifty ICR mice were equally partitioned into ten groups ($n = 5$). Nine of these were orally administered with *PM-SG* solution at a dose of 100 mg/kg, while the other one as the control group was treated with physiological saline at an equal volume. All mice were anaesthetized with ether before blood sampling, and each one was sampled only once.

Preparation of plasma sample

The blood sample (0.5 ml) of each mouse was collected from ophthalmic venous plexus as described by Zhang et al.[15] according to a specific schedule at times of 0 (control group), 10, 20, 30, 50, 60, 70, 90, 120 and 180 min after dosing. The blood samples were immediately transferred into heparinized tubes to separate the plasma by centrifugation at 4000*g* for 15 min. Each plasma sample (0.1 ml) obtained was mixed with a triple volume of methanol in a centrifuge tube. The mixture was vortexed for 30 s and centrifuged at 4000*g* for 15 min at 4°C to remove the protein from the organic phase. Each supernatant was collected and transferred into a clean tube, and then was evaporated to dryness under a N_2 stream. The residue was reconstituted with methanol (0.8 ml) and stored at –20°C before analysis.

Preparation of the standard solution, calibration standard and quality control samples

The stock solution of *PM-SG* was prepared through dissolving the reference substance in methanol to a concentration from 10.60 to 97.80 μg/ml. Then each stock solution was subsequently diluted by methanol to obtain working standard solution at target concentration. All standard solutions were stored at 4°C. Calibration standards of *PM-SG* at concentrations of 0.66, 1.33, 2.65, 10.60, 34.00, and 56.40 μg/ml were prepared by spiking the appropriate amount of above standard solutions into 0.2 ml drug-free mice plasma. The calibration curve was constructed by plotting the peak area versus the concentration of the calibration standard. Linear regression analysis was employed to calculate the slope, intercept and correlation coefficient. Quality control (QC) sample was prepared at high, medium, and low concentrations in the same manner as the calibration standard, and was used to assess precision, accuracy, recovery and stability of the method.

Method validation

A sensitive quantification method for determining *PM-SG* concentration in mouse plasma using above-mentioned RP-HPLC system was established and validated. The limit of quantitation (LOQ) was defined as the lowest concentration that could be accurately and precisely quantified corresponding to a signal-to-noise ratio of 10 and the limit of detection (LOD) was determined as the amount that could be detected with a signal-to-noise ratio of 3. Replicates of QC sample at each concentration was analyzed to examine the precision and accuracy via evaluation of relative standard deviation (RSD) and relative error (RE), respectively. The intra-day variance of the precision and accuracy was determined by assaying the QC samples in a single day, while the inter-day variance was obtained over four consecutive days. Using the QC samples, recovery and stability of *PM-SG* were also determined. The recovery was calculated by comparing the mean peak areas of extracted samples with that of unextracted samples in standard solutions. The stability was assessed by measuring sample concentrations under room temperature (about 25°C) at the storage time of 0, 5, 10, 15, 24, and 36 h.

Pharmacokinetic analysis

Compartmental analysis

Compartmental pharmacokinetic analysis of all data was carried out using the DAS 2.0 software (Drug and Statistics 2.0, the Committee of the Mathematic Pharmacology, the Chinese Society of Pharmacology, Hefei, P.R. China). An appropriate pharmacokinetic model was chosen through the lowest Akaike's information criterion (AIC) value, the lowest weighted squared residuals, the lowest standard errors of fitting parameters, and the dispersion of residual under equal weight scheme[16–19]. The compartmental pharmacokinetic parameters, including area under concentration-time curve (AUC_{0-t} and $AUC_{0-\infty}$), elimination half-life ($t_{1/2}$), absorption half-life ($t_{1/2}K_a$), apparent central volume of distribution (V_1/F), oral clearance (CL/F), first-order absorption rate constant (K_a), elimination rate constant (K_e) and lag time (T_{lag}), were obtained using relevant equations[20,21].

Non-compartmental analysis

Non-compartmental pharmacokinetic analysis was also processed by the DAS 2.0 software. Maximum concentration (C_{max}) and the time to reach maximum concentration (T_{max}) of *PM-SG* in plasma were directly obtained from the observed values. Other non-compartmental pharmacokinetic parameters, such

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as AUC_{0-t}, $AUC_{0-\infty}$, area under the first moment curve ($AUMC_{0-t}$ and $AUMC_{0-\infty}$), variance of retention time (VRT_{0-t} and $VRT_{0-\infty}$), mean residence time (MRT_{0-t} and $MRT_{0-\infty}$), terminal elimination half-life ($t_{1/2Z}$), apparent volume of distribution (V_Z/F) and clearance (CL_Z/F), were determined by standard formulas[20–22].

Results

Chromatography

Under the conditions as described above, the RP-HPLC chromatograms of blank plasma, blank plasma spiked with *PM-SG* and the plasma after oral administration of

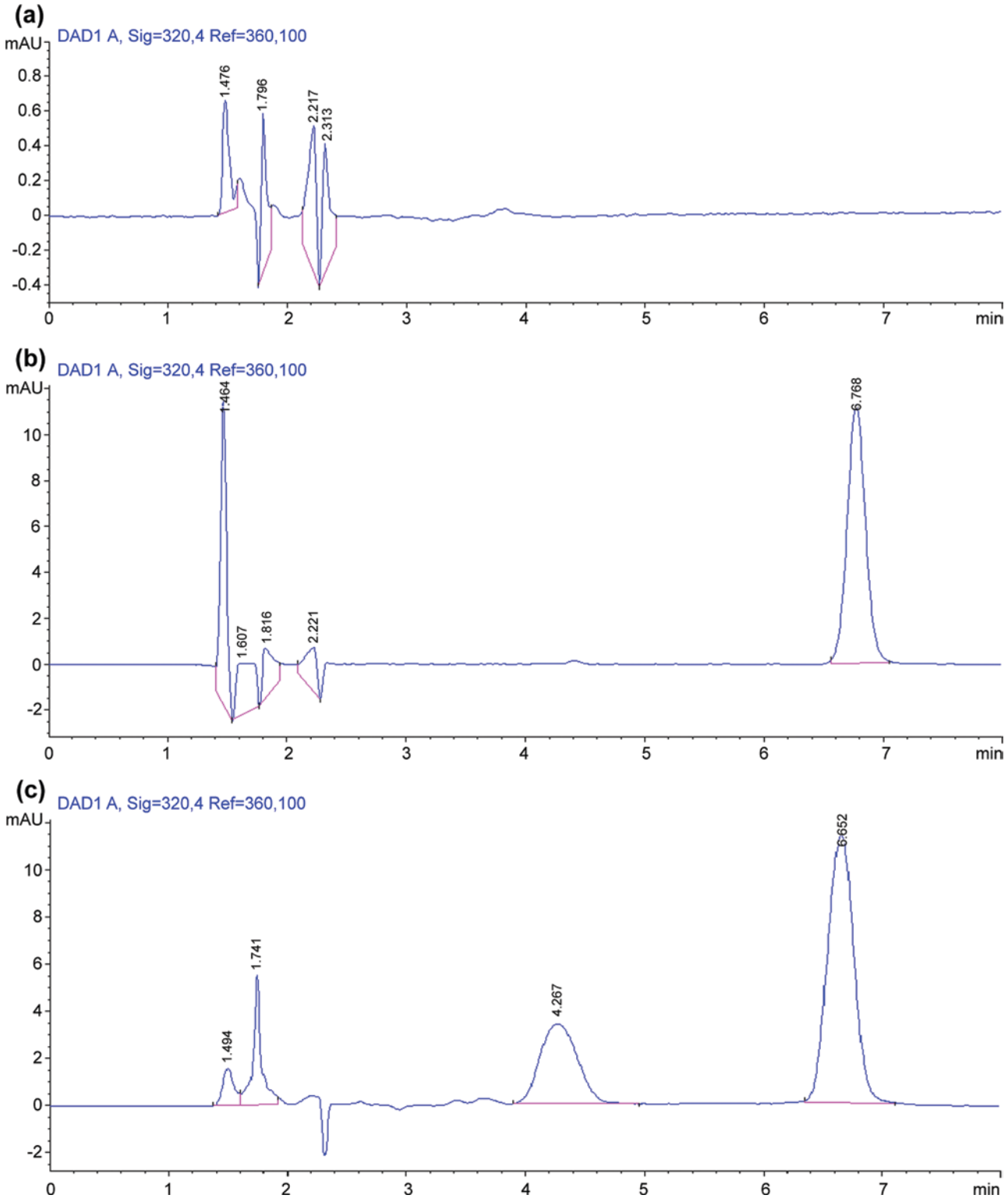

Figure 2. Chromatograms of *Polygonum multiflorum* stilbene glycoside (*PM-SG*) in mouse plasma: (A) blank plasma; (B) blank plasma spiked with *PM-SG*; (C) plasma sample after oral administration of *P. multiflorum* extract solution at a dose of 100 mg/kg. Retention time of *PM-SG* was 6.786 min.

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100 mg/kg *PM-SG* solution, were illustrated in Figure 2. The retention time (T_r) was 6.786, and the peak of the analyte was identified by comparing its T_r with that of the standard. There was no interfering peak within the elution time for either reference standard or the sample.

Method validation

The RP-HPLC method was shown to be suitable for the quantification of *PM-SG* in mouse plasma. The linear regression was obtained between the peak area (X) and concentration (Y) of the standard solution. Its calibration curve displayed good linearity over the concentration range from 0.66 to 56.40 μg/ml, and the regression equation was calculated as follows: $Y = 0.0737X - 1.4168$ (correlation coefficient, $r^2 > 0.998$). The measured LOQ and LOD values were less than 1.5 ng/ml and 0.3 ng/ml, respectively, indicating excellent sensitivity of this method. The overall intra-day and inter-day variations were less than 3% for RSD, with a range from –1.7 to 2.1% for RE, suggesting an acceptable precision and accuracy for this assay. A satisfactory reliability and reproducibility was demonstrated by the recovery which was higher than 97% (RSD < 3%). Further, stability test showed that *PM-SG* at different concentrations in plasma sample were all stable within at least 36 h at room temperature, since no obvious degradation of the analyte occurred during this time course (all RE < 4.0%).

Pharmacokinetic studies

In this study, the validated analytical method was employed to study the pharmacokinetic profile of *PM-SG* after oral administration of *P. multiflorum* extract solution at a dose of 100 mg/kg. The mean plasma concentration-time profile was presented in Figure 3. Pharmacokinetic analysis of the concentration-time data was carried out using DAS 2.0 software.

Compartmental study

According to AIC value and model diagnostics, the plasma concentration-time data were found to fit best

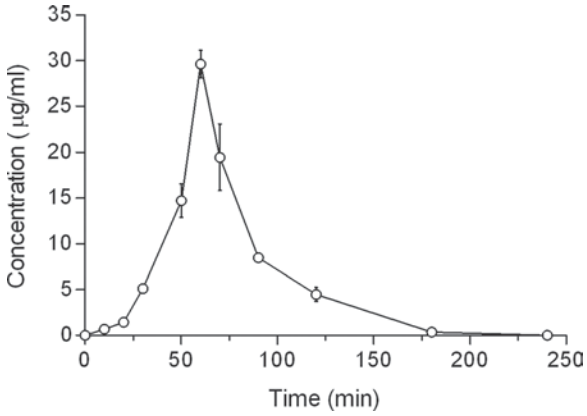

Figure 3. Mean plasma concentration-time profile of *Polygonum multiflorum* stilbene glycoside (*PM-SG*) in mouse plasma after oral administration of *P. multiflorum* extract solution ($n = 5$).

Table 1. Compartmental pharmacokinetic parameters of *Polygonum multiflorum* stilbene glycoside (*PM-SG*) in mouse plasma after oral administration ($n = 5$)

Parameter	Value	Parameter	Value
$t_{1/2}$ (min)	17.21	$t_{1/2}K_a$ (min)	13.66
V_1/F (ml/kg)	2114.00	K_a (min^{-1})	0.05
CL/F (ml/min/kg)	85.00	K_e (min^{-1})	0.04
AUC_{0-t} (μg/ml·min)	1160.18	T_{lag} (min)	27.65
$AUC_{0-\infty}$ (μg/ml·min)	1174.40		

AUC, area under concentration-time curve; CL/F, oral clearance; K_a, first-order absorption rate constant; K_e, elimination rate constant; $t_{1/2}$, elimination half-life; T_{lag}, lag time.

an open one-compartment model. The estimated compartmental parameters were shown in Table 1. A rapid absorption and elimination process were observed from the parameters $t_{1/2}K_a$, $t_{1/2}$, K_a and K_e with low levels. The value of CL/F (85.0 ml/min/kg) was as high as the mouse hepatic blood flow rate (90.0 ml/min/kg)[23], indicating that *PM-SG* can be cleared quickly from the mouse body via hepatic clearance. The V_1/F was 2114.0 ml/kg, which was much larger than the total blood volume of mouse (about 80.0 ml/kg) and even exceeded the total body water in mouse (about 725.0 ml/kg)[23], indicating that *PM-SG* can be widely distributed into extravascular systems. The AUC_{0-t} and $AUC_{0-\infty}$ values were extremely high (both than 1100.0 μg/ml·min) after oral administration of *PM-SG* at a dose of 100 mg/kg, which suggested a great absorption and utility of this analyte in mouse plasma. The T_{lag}, determined as the time span after *PM-SG* administration until the first measurable concentration in plasma exceeded the LOQ, was pronounced and found to be as long as 27.7 min, which can plausibly be explained by a prolonged gastric transit time of *PM-SG*. In sum, it is concluded that *PM-SG* displayed a fast and effective absorption and distribution with rapid elimination and clearance in mice, showing that it can be transported from blood into tissues and removed from them rapidly.

Non-compartmental study

To fully evaluate the pharmacokinetic profile of *PM-SG*, a non-compartmental analysis was performed in this study. Table 2 showed the analysis result of the plasma concentration data. A rapid absorption, extensive distribution, great utility, and fast elimination and clearance of *PM-SG* in mice were represented by the relatively low T_{max} (60 min), high V_Z/F (2043.0 ml/kg), high AUC (both more than 1.3×10^3 μg/ml·min), high AUMC (about 9.7×10^4 μg/ml·min), low $t_{1/2Z}$ (18.9 min), and high CL_Z/F (75.0 ml/min/kg) values, respectively. These findings were in good agreement with that of above one-compartment analysis. Further, the parameter MRT and VRT, which were not used in compartmental analysis, revealed an acceptable time value for 63.2% of the administered dose of *PM-SG* to be eliminated by all processes. Together with the compartmental

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Table 2. Non-compartmental pharmacokinetic parameters of *Polygonum multiflorum* stilbene glycoside (*PM-SG*) in mouse plasma after oral administration ($n=5$)

Parameter	Value	Parameter	Value
C_{max} (μg/ml)	29.62	$MRT_{0\infty}$ (min)	74.36
T_{max} (min)	60.00	VRT_{0t} (min^2)	733.66
AUC_{0-t} (μg/ml·min)	1322.30	$VRT_{0\infty}$ (min^2)	861.51
$AUC_{0-\infty}$ (μg/ml·min)	1331.86	$t_{1/2Z}$ (min)	18.86
$AUMC_{0-t}$ (μg/ml·min)	97047.74	V_Z/F (L/kg)	2043.00
$AUMC_{0-\infty}$ (μg/ml·min)	99032.73	CL_Z/F (L/min/kg)	75.00
MRT_{0t} (min)	73.39		

AUC, area under concentration-time curve; AUMC, area under the first moment curve; CL/*F*, oral clearance; K_a, first-order absorption rate constant; K_e, elimination rate constant; MRT, mean residence time; V_z, apparent volume of distribution; $t_{1/2}$, elimination half-life; T_{lag}, lag time.

analysis, the obtained parameters showed that *PM-SG* had a favorable pharmacokinetic profile in mouse plasma after oral administration.

Discussion

TCM are used to treat diseases clinically in the form of combined herbal mixtures. The therapeutic activities of TCM are possessed by the main active compound as well as a combination and interaction of various components inside. As the main compound of *P. multiflorum*, *PM-SG* has attracted intense interest for its medicinal effects[10–14] and is responsible for the therapeutic activity of its source herb. Due to the routine application of *P. multiflorum* in a manner of extract, *PM-SG* always exerts effects together with other minor components in the extract, indicating more complex of pharmacokinetic profile of *PM-SG* than that of pure stilbene glycoside. It is thereby necessary to determine the pharmacokinetic profile of *PM-SG* in the presence of other minor components. However, up to our knowledge, no pharmacokinetic study of this compound in any herb has been hitherto published. Therefore, here we validated and developed a RP-HPLC with liquid-liquid extraction to perform such a study in mice.

A *P. multiflorum* extract with more than 50% *PM-SG* was preferentially used for this study due to its certain advantage over any other form of *PM-SG*. For instance, it can be deduced from TCM theory that this extract composed of not only *PM-SG* but also other active compounds possesses more or better effects than that of a single pure *PM-SG*, owing to an entire efficacy exerted by various active compounds inside and a positive synergic interaction between *PM-SG* and other compounds[24]. Likewise, this extract is more appropriate for this study than crude extract because the latter contains more compounds with less content of *PM-SG* resulting in more complex combination pattern of those compounds and lesser degree of significance of *PM-SG* effect. Another important reason why *P. multiflorum* extract was used is to better mimic the clinical situation in which a fine extract (decoction) of this herb was commonly used in practice[1,6]. Moreover, our preliminary study has investigated the same extract of *P. multiflorum* and found that it possessed favorable hepatoprotective effect against fatty liver disease, for which a China Patent of Invention (Patent No.: ZL 2004 1 0029566.3) was obtained. Thus, the present pharmacokinetic study using this extract becomes a continuing study of our preliminary one, indicating that adopting such an extract for this study would be reasonable. However, what interaction between *PM-SG* and other compounds in this extract would occur and whether the interaction would be significantly effective, remains unknown. The underlying mechanism of such interaction needs to be explored.

The RP-HPLC method has been successfully applied to previous analytical and pharmacokinetic studies of bioactive component from TCM[25–31]. In this study, with some modifications, we developed this method with liquid-liquid phase extraction to make it more suitable for determining the pharmacokinetic profile of *PM-SG*. For example, to obtain a high quality chromatogram with a baseline separation of *PM-SG* in plasma as quickly as possible, various HPLC columns and analytical conditions were tested by trial and error and the result indicated the Ultimate column under the present condition was most applicable for the analysis due to its ultimate performance. Zhang et al. have adopted a chromatographic method to perform quantitative determination of *PM-SG* for QC, and their result showed the intra-day and inter-day precision (represented by %RSD) of less than 1.01 and 2.88% respectively and the recovery ranging from 100.03 to 102.62% of less than 2% RSD[32]. Comparatively, our present study using the RP-HPLC method also achieved a similar good performance (specificity, reliability, etc.) of *PM-SG* analysis as theirs, due to its similar good intra- and inter-day precisions (both <3%) and recovery (>97% with RSD <3%) to that of Zhang et al, indicating that our method is reliable and satisfactory for such a pharmacokinetic study and even suitable for the QC study. Together with other results of the complete method validation in our study, it can be concluded that the present RP-HPLC coupled with liquid-liquid extraction possesses high specificity, sensitivity, precision, accuracy, recovery and stability. By using this method, *PM-SG* in mice plasma was clearly detected and identified by its retention time (Figure 2). Interestingly, a new peak at retention time 4.267 min can be found in plasma after *PM-SG* administration (Figure 2c), indicating a possible metabolite of *PM-SG in vivo*. After mass spectrometric analysis by another study (data not shown), we identified this peak as an α-*D*-glucuronic acid conjugate of *PM-SG*. Further study is required to determine how such a metabolite would be produced and what effect it has.

Generally, either of compartmental and non-compartmental analysis can be used for the pharmacokinetic study. However, Hamidi has reported that the non-compartmental analysis does have some limitations

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for determining the pharmacokinetic properties of active compound[22]. Therefore, in this study, we adopted both compartment and non-compartment models to perform a full evaluation of pharmacokinetic profile of *PM-SG*. According to the minimum AIC criterion, a one-compartment open kinetic model was proposed to analyze the pharmacokinetics of *PM-SG*. Satisfyingly, after measurement of pharmacokinetic parameters by DAS software, both of the two model analyses reached a same conclusion that *PM-SG* can be rapidly and effectively absorbed from mice gastrointestinal tract into the blood as well as tissues and then quickly eliminated and cleared from the body, demonstrating our hypothesis that *PM-SG* possesses a favorable pharmacokinetic profile in mice.

In summary, since *PM-SG* has been reported to possess various therapeutic effects *in vitro* and *in vivo*[10–14], it would be very useful to obtain its pharmacokinetic profile for improving understanding of when, where and how it can be effective. Therefore, we performed the present study to support the clarification of mechanism of action of *PM-SG*, which can help us to better understand the pharmacology of this active compound. To some extent, our result indicated a substantial evidence of the *in vivo* effects of *PM-SG*, since the favorable pharmacokinetic profile of *PM-SG* in mice has been demonstrated. Taken together with the findings from our preliminary pharmacological study, this study may provide useful guidance (e.g. dosage regimen and application strategy) for developing *PM-SG* as a candidate of new drug for disease treatment such as anti-fatty liver. To clarify *PM-SG*'s underlying mechanism of action will be the next step so that it can proceed to the human trials.

Conclusions

A simple, sensitive, specific and reliable RP-HPLC coupled with liquid-liquid extraction method has been developed and adopted for the determination of *PM-SG* in mouse plasma after oral administration of *P. multiflorum* extract. Our study provided satisfactory precision and accuracy of the assay with adequate recovery and stability of the analyte. The result of this study well described the favorable pharmacokinetic profile of *PM-SG*, contributing to a better understanding of the pharmacological mechanism of action of *P. multiflorum*, and providing a solid basis for investigating the material basis of the clinical efficacy of such a TCM. Up to our knowledge, this is the first report of pharmacokinetic study of *PM-SG* in mouse plasma after oral administration.

Declaration of interest

This study was supported by the Key Project of the Science and Technology Department of Zhejiang Province of China (No. 2002C23030). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

Authors' contributions

Lv and Chen contributed to the conception and design of the study. Gu and Lou carried out the experimental work. Shan contributed to the data analysis of the study, and drafted, revised and finalized this manuscript. All authors read and approved the final version of the manuscript.

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