Comparative pharmacokinetics of three triterpene acids in rat plasma after oral administration of Poria extract and its formulated herbal preparation: GuiZhi-FuLing capsule

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A B S T R A C T

A sensitive liquid chromatographic-mass spectrometric technique coupled with liquid−liquid extraction method was developed and validated for simultaneous determination of dehydro-tumulosic acid, tumulosic acid and polyporic acid C in rat plasma. The analytes were separated on a Kromasil C18 column with a total running time of 12.5 min. Author had compared the pharmacokinetics of dehydro-tumulosic acid, tumulosic acid and polyporic acid C after oral administration of the extract of Poria and its formulated herbal preparation (GuiZhi-FuLing capsule). The improved pharmacokinetic profiles of the three compounds were found in the GuiZhi-FuLing capsule, indicating the more effective absorption and the slower elimination, compared with the Poria extract. Furthermore, this study revealed that as far as the Poria extract was concerned, it is very valuable to be used as a clinical instruction of GF capsule.

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1. Introduction

GuiZhi-FuLing (GF) capsule, a popular traditional Chinese medical formulation, is derived from GuiZhi-FuLing Wan, originated in Jin Kui Yao Lue by an eminent Chinese physician Zhang Zhongjing in Han Dynasty (150–219 AD). This prescription composes of five herbs (1:1:1:1:1, g/g), including Cinnamomi Ramulus (the dried twig of Cinnamomum cassia Presl), Poria (the dried sclerotia of Poria cocos (Schw.) Wolf), Moutan Cortex (the dried root bark of Paeonia suffruticosa Andr.), Persicae Semen (the dried mature seed of Prunus persica (L.) Batsch), and Paeoniae Radix Alba (the dried root of Paeonia lactiflora Pall.). According to the statement of the Chinese Pharmacopoeia (2010 Edition) [1], the usage of this GF capsule is for oral administration after meals with three capsules per time and three times per day. The course of treatment is usually 3 months. In pharmaceutical research, GF capsule was reported to prevent the progression of atheromatous plaque by creating a sounder antioxidant defense system [2–4]. Clinically, GF capsule has been mainly used to treat gynecological diseases, e.g. uterine fibroids, endometriosis, pelvic inflammatory disease, ovarian cyst, dysmenorrhea. A study indicated that GF capsule could improve the syndrome of blood stasis in postmenopausal women [5]. Wang et al. reported the GF capsule is used for treatment of gynecological malignant tumor by promoting softening and absorption of the proliferative lesion, stimulating the immune system, and inhibiting the growth of cancer cells [6]. In addition, the GF capsule was reported to exhibit anti-atherosclerogenic activity in human [7].

Poria, one of the herbs in GF capsule, is a widely used Chinese herbal medicine: this is generally used in combination with other herbs in different prescriptions to treat edema, nephrosis, chronic gastritis, acute gastroenteric catarrh dizziness
and nausea [8–10]. In addition, Poria has been reported to have sedative and diuretic action to patients [11,12]. The major constituents of Poria are triterpenoid compounds containing dehydro-tumulosic acid, tumulosic acid and polyporic acid C. Pharmacological studies have demonstrated that dehydro-tumulosic acid, tumulosic acid and polyporic acid C of Poria exhibited activities in anti-inflammatory, anti-tumor, antivirus, and pro-immunology [13–17]. Therefore, the pharmacokinetics of dehydro-tumulosic acid, tumulosic acid and polyporic acid C were urgently required to study for evaluating clinical applications of Poria and GF capsule.

The determination of dehydro-tumulosic acid in rat plasma after administration was reported by using LC [18]; however, simultaneous determination of dehydro-tumulosic acid, tumulosic acid and polyporic acid C has not been reported. Here, a simple, rapid and sensitive liquid chromatographic-mass spectrometric (LC-MS) method was established, which was applied successfully to the comparative pharmacokinetic studies of the three major compounds in rat plasma after oral administration of Poria extract and GF capsule.

2. Experimental procedures

2.1. Materials and reagents

Cinnamomi Ramulus, Poria, Moutan Cortex, Persicae Semen, and Paoniae Radix Alba were all purchased from Shenyang Tongrentang Drug Co., Ltd. (Shenyang, China). All the crude drugs were of high quality and authenticated by one of the authors, associate professor Ying Jia of Pharmacognosy Department, Shenyang Pharmaceutical University. The reference standards of dehydro-tumulosic acid, tumulosic acid and polyporic acid C (purity >98%) were isolated from Poria by the author’s laboratory (Department of Pharmaceutical Analysis, Shenyang Pharmaceutical University, Shenyang, China), and the structures were characterized by spectral methods, including MS, 1H- and 13C-NMR spectra (data not shown). The data were consistent with those sedative and diuretic action to patients [11,12]. The major constituents of Poria were triterpenoid compounds containing dehydro-tumulosic acid, tumulosic acid and polyporic acid C. Pharmacological studies have demonstrated that dehydro-tumulosic acid, tumulosic acid and polyporic acid C of Poria exhibited activities in anti-inflammatory, anti-tumor, anti-virus, and pro-immunology [13–17]. Therefore, the pharmacokinetics of dehydro-tumulosic acid, tumulosic acid and polyporic acid C were urgently required to study for evaluating clinical applications of Poria and GF capsule.

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2.2. Animals

Female pathogen-free Wistar rats weighted 230–250 g were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University. The rats were bred in an air-conditioned animal quarter at a temperature of 22 ± 2 °C and a relative humidity of 50 ± 10%, with a natural light-dark cycle for 7 days before the experiment. Before the drug administration, they were fasted overnight and free access to water. All the animals used in this work received humane care in compliance with institutional animal care guidelines.

2.3. Instruments and LC–MS conditions

The assay was performed on a Shimadzu (Japan) LC-MS 2010EV system equipped with an electrospray ionization (ESI) interface. The liquid chromatographic separations were performed on a Kromasil C18 column (150 mm × 4.6 mm, 5 μm), which was preceded by a C18 guard column (4.0 mm × 2.0 mm, 5 μm, Phenomenex, Torrance, CA). The mobile phase was acetonitrile–3 mM ammonium acetate buffer solution (75:25, v/v) at a flow rate of 0.8 mL/min (25% of the eluent was split into the inlet of mass spectrometer) within a running time of 12.5 min. The injection volume was 20 μL. The analytes and IS were ionized by ESI source in negative ion mode under the following source conditions: nebulizing gas 1.5 L/min, CDL temperature 200 °C, heat block temperature 200 °C, detector voltage 1.75 kV, and other parameters were fixed as the tuning file. Analysis was carried out by selected ion monitoring (SIM) mode for dehydro-tumulosic acid [M–H]−/m/z 483.30, tumulosic acid [M – H]−/m/z 485.20, polyporic acid C [M–H]−/m/z 481.20, and IS [M–H]−/m/z 469.30.

2.4. Preparation of Poria extract and GF capsule

GF capsule was prepared in accordance with the process as stated in the Chinese Pharmacopeia (2010 Edition) [1]. Meanwhile, the extract of Poria, according to the preparation method of GF capsule, was prepared in the following procedures. The crude drug (in powder form of 5 g) was extracted twice by refluxing with 50 mL of 90% ethanol, 1 h for each time. The extracted solution obtained by filtration was concentrated under reduced pressure as sample I. Then the residue was extracted by water in the same method as ethanol, of which the concentrated solutions was as sample II. Afterward, the samples I, II and the fine power of Poria (20 g) were mixed uniformly with Poria extract, and the mixture was used for animal studies.

To calculate the contents of the analytes, the dried powder of Poria extract and GF capsule were weighed right amount, ultrasonic extracted with methanol, filtered through 0.45 μm membrane, and then quantitatively analyzed by LC external standard method. In 1 g of Poria extract, the amounts of dehydro-tumulosic acid, tumulosic acid and polyporic acid C were having 1.07 mg, 0.93 mg, 0.38 mg, respectively. In 1 g GF capsule, the amounts of dehydro-tumulosic acid, tumulosic acid and polyporic acid C were 0.31 mg, 0.32 mg, and 0.11 mg, respectively. Finally, Poria extract and GF capsule were re-dissolved in water before the administration to rats.

2.5. Standard solution and quality-control samples

The stock solutions (in acetonitrile) of dehydro-tumulosic acid, tumulosic acid, polyporic acid C and IS were prepared at concentrations of 0.20 mg/mL, 0.20 mg/mL, 0.23 mg/mL and 0.10 mg/mL, respectively. A series of mixed working standards having 200–10,000 ng/mL for dehydro-tumulosic acid, 100–5,000 ng/mL for tumulosic acid and 115–5,750 ng/mL for polyporic acid C were obtained by diluting a mixture of the stock solutions with acetonitrile. In addition, the stock solution of the IS was diluted to a concentration of 500 ng/mL with acetonitrile. All the solutions were stored under 4 °C.
The calibration standards of dehydro-tumulosic acid (20, 40, 200, 400, 600, and 1000 ng/mL), tumulosic acid (10, 20, 100, 200, 300, and 500 ng/mL) and polyporic acid C (11.5, 23.0, 115, 230, 345, and 575 ng/mL) were prepared by adding 20 μL of the mixed working standard solution to blank plasma. Three levels of quality-control (QC) samples at concentrations of 40, 400, and 800 ng/mL for dehydro-tumulosic acid, 20, 200, and 400 ng/mL for tumulosic acid and 23, 230 and 460 ng/mL for polyporic acid C in plasma were prepared separately in the same fashion.

2.6. Biosample collection

Twelve female Wistar rats were randomly divided into two groups (6 rats per group). After oral administration of Poria extract at a dosage of 1.60 g/kg of rat weight (containing 1.71 mg/kg for dehydro-tumulosic acid, 1.48 mg/kg for tumulosic acid, and 0.61 mg/kg for polyporic acid C) and GF capsule at a dosage of 4.80 g/kg (containing 1.49 mg/kg for dehydro-tumulosic acid, 1.54 mg/kg for tumulosic acid, and 0.53 mg/kg for polyporic acid C) to rats, the blood samples (approx. 0.5 mL) were collected from the suborbital vein into heparinized tubes before administration and 0.08, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h after dosing, and then immediately centrifuged at 13000 rpm for 5 min. The plasma samples were stored at −20 °C and analyzed within 2 weeks.

2.7. Biosample preparation

Plasma samples (200 μL) were spiked with 20 μL of IS, 20 μL of acetonitrile and 20 μL of 0.5 M hydrochloric acid, then the mixtures were extracted with 2 mL of ether by vortexing for 3 min. After centrifugation at 13000 rpm for 5 min, the organic phase was transferred to a polypropylene tube and evaporated to dryness at room temperature under a gentle stream of nitrogen. The residue was reconstituted with 100 μL of the mobile phase, and 20 μL of which was used for LC−MS analysis.

2.8. Method validation

2.8.1. Specificity

Specificity was assessed by a comparison of the chromatograms from blank plasma with those obtained from the corresponding plasma spiked with dehydro-tumulosic acid, tumulosic acid, polyporic acid C and IS, and a plasma sample after the oral administration of Poria extract and GF capsule.

2.8.2. Linearity and LOQ

Linearity was assessed by analyzing the calibration curves (20–1000 ng/mL for dehydro-tumulosic acid, 10–500 ng/mL for tumulosic acid and 11.5–575 ng/mL for polyporic acid C) in plasma using least-square linear regression of three analytes-to-IS peak area ratios versus the normalized standard concentration with a weighed (one per square of concentration) factor. The limit of quantification (LOQ) was defined as the lowest concentration of the calibration curve with acceptable accuracy and precision.

2.8.3. Precision and accuracy

The precision and accuracy were evaluated by QC samples at low, medium and high concentrations. Precision was expressed as the relative standard deviation (RSD in %), and accuracy was expressed as relative error (RE in %). The intra-day, inter-day precision and accuracy were evaluated over a period of three consecutive days with six replications at each concentration per day (n = 18) and calculated with calibration curves obtained daily.

2.8.4. Extraction recovery and matrix effect

The extraction recovery and matrix effect at three QC concentrations were evaluated in a set of six replicates. The recoveries of dehydro-tumulosic acid, tumulosic acid, polyporic acid C were determined by comparing the peak areas from the extracted samples with those from post-extracted blank plasma spiked with the analytes at the same concentration. The recovery of the IS was determined in the same way at the concentration of 100 ng/mL. The matrix effect was measured by comparing the peak areas of analytes added into post-extracted blank with analytes dissolved in matrix component-free reconstitution solvent.

2.8.5. Stability

Stability studies were investigated at three QC concentrations under different storage conditions: three freeze (−20 °C)–thaw (room temperature) cycles, 24 h storage at room temperature, frozen (−20 °C) for 14 days.

2.9. Data analysis

The concentrations of dehydro-tumulosic acid, tumulosic acid, polyporic acid C in the plasma, after administration of Poria extract and GF capsule at different time, were calculated from the daily calibration curve. Maximum plasma drug concentration (C_{max}), and the time to reach maximum plasma drug concentration (T_{max}) were observed directly from the harvested data. All concentration–time data were analyzed using the DAS 2.1 software package (Chinese Pharmacological Society). The comparison of pharmacokinetic data was performed by SPSS 16.0 (Statistical Package for the Social Science) using independent samples t-tests after their natural logarithmic transformation or the Mann–Whitney test. P<0.05 was considered statistically significant for all the tests.

3. Results

3.1. Biosample preparation

Blood sample preparation was based on liquid−liquid extraction in our study. Several organic solvents were tested, including ethyl acetate, ether and methyl tert-butyl ether. The liquid−liquid extraction with ether could effectively extract all the target analytes, and which could remove proteins and other interfering components in rat plasma. Moreover, the adoption of hydrochloric acid in the extraction procedure increased the recovery of the analytes. Therefore, ether accompanied with hydrochloric acid was proved to be a simple, efficient solvent for extracting dehydro-tumulosic acid, tumulosic acid, polyporic acid C and the IS.
3.2. LC–ESI–MS optimization

According to the carboxyl groups of the analytes, the amounts of dehydro-tumulosic acid, tumulosic acid, polyporic acid C and IS (see Fig. 1) were analyzed in an ESI negative ion mode, and full-scan mass spectrums of them after direct injection in the mobile phase were obtained. The results indicated that the ions of three analytes were all \([M-H]^-\) ions, and the responses were very stable and showed good linearity in SIM mode. Whereas, there were hardly any responses to these analytes under positive ion mode, such as \([M+H]^+, [M+Na]^+, [M+K]^+\) or \([M+H_2O]^+\). The quantitative analysis was carried out in SIM as follow: \([M-H]\ m/z 483.30\) for dehydro-tumulosic acid, \([M-H]\ m/z 485.20\) for tumulosic acid, \([M-H]\ m/z 481.20\) for polyporic acid C, and \([M-H]\ m/z 469.30\) for the IS.

To increase the sensitivity of these analytes, ammonium acetate (as pH regulator) was included in the mobile phase. The response to dehydro-tumulosic acid, tumulosic acid, polyporic acid C, or IS was improved greatly. Thus the concentration of ammonium acetate in the mobile phase was optimized from 1–5 mM. Finally, acetonitrile-3 mM ammonium acetate buffer solution (75:25, v/v) was adopted as the mobile phase for sufficient and stable ionization responses for the analytes.

3.3. Method validation

3.3.1. Specificity

No endogenous interference was observed at the retention times of dehydro-tumulosic acid (6.9 min), tumulosic acid (7.6 min), polyporic acid C (9.7 min), or IS (11.2 min), which showed the specificity of the analyses (see Supplementary figure). Typical chromatograms obtained from blank plasma, blank plasma spiked with a mixture of dehydro-tumulosic acid, tumulosic acid, polyporic acid C and IS, and rat plasma obtained after oral administration of Poria extract and GF capsule were shown in Fig. 2.

3.3.2. Linearity and LOQ

The linearity was evaluated on three separate days with two sets of calibration curves per day. The calibration curves showed good linearity over a concentration range of 20–1000 ng/mL for dehydro-tumulosic acid, 10–500 ng/mL for tumulosic acid and 11.5–575 ng/mL for polyporic acid C, respectively. The typical calibration curves were as follows: \(y = 9.66 \times 10^{-3}x + 5.60 \times 10^{-2}\) \((r = 0.9956)\) for dehydro-tumulosic acid, \(y = 1.89 \times 10^{-2}x - 3.82 \times 10^{-2}\) \((r = 0.9934)\) for tumulosic acid and \(y = 2.57 \times 10^{-2}x + 7.99 \times 10^{-3}\) \((r = 0.9963)\) for polyporic acid C. The LOQ for dehydro-tumulosic acid, tumulosic acid and polyporic acid C were 20 ng/mL, 10 ng/mL and 11.5 ng/mL with the RSD of six replications at 8.7%, 10.4% and 9.5%, respectively (Table 1). In addition, the LOD of the three analytes was calibrated of 4 ng/mL for dehydro-tumulosic acid, 2 ng/mL for tumulosic acid and 3 ng/mL for polyporic acid C.

3.3.3. Precision and accuracy

Intra-day and inter-day precision, and accuracy for the assays of dehydro-tumulosic acid, tumulosic acid and polyporic acid C were summarized in Table 1. The criteria for acceptability of all results including that the precision and accuracy were all within ±15% from the nominal values.

3.3.4. Extraction recovery and matrix effects

The recoveries of dehydro-tumulosic acid, tumulosic acid, and polyporic acid C were 73.1–76.4%, 71.4–77.5%, and 70.9–72.0%, respectively (Table 1), which showed that the recoveries of analytes were stable at different concentrations. The mean extraction recovery of IS was 90.3%. Here, the matrix effect were within the range of 97.0–102.7% (Table 1), indicating that no significant matrix effect was observed for dehydro-tumulosic acid, tumulosic acid or polyporic acid C. The mean matrix effect for the IS was 100.5%.

3.3.5. Stability

The stability of dehydro-tumulosic acid, tumulosic acid and polyporic acid C in rat plasma under different conditions

Fig. 1. Chemical structures of dehydro-tumulosic acid (A), tumulosic acid (B), polyporic acid C (C), the internal standard glycyrrhetinic acid (D).
was summarized in Table 2. The results showed that the three analytes were stable in plasma at three freeze–thaw cycles, at room temperature for 24 h and at −20 °C for at least 14 days.

3.4. Pharmacokinetics studies

The validated method was successfully applied to the comparative pharmacokinetic studies of dehydro-tumulosic acid, tumulosic acid, and polyporic acid C in rat plasma after administration of Poria extract and GF capsule. The concentration–time curves of dehydro-tumulosic acid, tumulosic acid, and polyporic acid C; and Table 3 shows the main pharmacokinetic parameters. \( \text{AUC}_{0-\infty} \), \( \text{AUC}_{0-\infty} \), and \( C_{\text{max}} \) were evaluated by an independent-samples \( t \)-test, meanwhile, \( T_{1/2} \) and \( T_{\text{max}} \) were calculated using a non-parametric statistical test (Mann–Whitney test) [22,23]. A statistical analysis was performed by using an analysis of variance with \( P<0.05 \) as the minimal level of significance.

As shown in Table 3 and Fig. 3, the main differences in pharmacokinetics of dehydro-tumulosic acid, tumulosic acid, and polyporic acid C have been reflected in \( C_{\text{max}} \), \( \text{AUC} \), and \( T_{1/2} \).

Table 1

<table>
<thead>
<tr>
<th>Chemical spiked (ng/ml)</th>
<th>Inter-day RSD(^a) (%)</th>
<th>Intra-day RSD (%)</th>
<th>Accuracy RE(^b) (%)</th>
<th>Recovery(^c) (%)</th>
<th>Matrix effect(^d) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydro-tumulosic acid</td>
<td>40</td>
<td>12.7</td>
<td>5.8</td>
<td>4.6</td>
<td>76.4 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1.6</td>
<td>6.2</td>
<td>4.8</td>
<td>74.3 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>2.0</td>
<td>6.2</td>
<td>−5.4</td>
<td>73.1 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>12.5</td>
<td>6.3</td>
<td>9.0</td>
<td>77.5 ± 5.8</td>
</tr>
<tr>
<td>Tumulosic acid</td>
<td>200</td>
<td>5.8</td>
<td>3.4</td>
<td>7.7</td>
<td>71.4 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>3.9</td>
<td>5.3</td>
<td>−10.3</td>
<td>75.3 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>8.5</td>
<td>6.8</td>
<td>−7.6</td>
<td>72.6 ± 6.2</td>
</tr>
<tr>
<td>Polyporic acid C</td>
<td>230</td>
<td>3.7</td>
<td>5.2</td>
<td>8.2</td>
<td>70.9 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>460</td>
<td>5.8</td>
<td>6.7</td>
<td>3.6</td>
<td>71.8 ± 7.0</td>
</tr>
</tbody>
</table>

A: the extracted plasma samples. B: the post-extracted blank plasma spiked with the three analytes.

\(^a\) Relative standard of derivation, where \( n = 6 \).

\(^b\) Relative Error, where \( n = 6 \).

\(^c\) Recovery (\%) = 100 × (A/B). The data was presented as mean ± SD, where \( n = 6 \).

\(^d\) Matrix effect (\%) = 100 × (A/B). The data was presented as mean ± SD, where \( n = 6 \). A: the post-extracted blank plasma spiked with the three analytes. B: the standard solution containing equivalent amounts of the three analytes.
There are no significant differences in the \(C_{\text{max}}\) values of dehydro-tumulosic acid and tumulosic acid in the GF capsule and the Poria extract group (Table 3). But, a remarkable decrease \((P<0.05)\) in the \(C_{\text{max}}\) value of polyporic acid C at \((199.0\pm25.4)\) vs \((311.5\pm4.6)\) ng/mL was observed after orally administered GF capsule compared with the Poria extract. After oral administration of the GF capsule, \(AUC_{(0-t)}\) and \(AUC_{(0-\infty)}\) of the three analytes increased remarkably \((P<0.05)\), as compared with the values after oral administration of the Poria extract. The elimination of the three analytes from rat plasma was rapid having the \(T_{1/2}\) of \(2.5\pm0.5, 4.2\pm1.4,\) and \(2.3\pm0.7\) (h), and could be detected only in 24 h in the Poria extract group. On the other hand, in the GF capsule group, the three analytes were eliminated more slowly with the \(T_{1/2}\) of \(8.9\pm2.7, 10.6\pm2.3,\) and \(7.9\pm2.9\) (h), which could be detected up to 36 to 48 hours. The results indicated that oral administration of GF capsule will lead to a better absorption \((AUC)\) and slower elimination rate \((T_{1/2})\) of dehydro-tumulosic acid, tumulosic acid and polyporic acid C, as compared with that of the intake of Poria extract. However, it can be seen from the plasma concentration–time curves (Fig. 3), in GF capsule group, \(AUC\) was significantly increased in the elimination phase compared with the Poria extract group. Particularly, the \(T_{1/2}\) was calculated based on the second peak \([24]\) in the GF capsule group. Therefore, it can be speculated that the slower elimination rate was a greater contribution to the improvement of the bioavailability of these compounds in GF capsule group. In addition, although a remarkable decrease in the \(C_{\text{max}}\) values of polyporic acid C was observed the GF capsule compared with the Poria extract group, according to the pharmacokinetic results mentioned above, it can be inferred that the bioavailability of GF capsule group is better than the Poria extract group.

### 4. Discussion and conclusions

The typical bimodal phenomenon was appeared in rats after oral administrated the GF capsule while the phenomenon did not exist after administrated the Poria extract (Fig. 3). The bimodal phenomenon, which happened very often in the pharmacokinetic study of traditional Chinese medicine \([24,25]\), might result from the enterohepatic circulation, the transformation from other similar compound, or the pH of different regions in intestinal tract. Comprehensive analysis of these pharmacokinetic results including \(C_{\text{max}}\).
P-gp in the intestine. Then, paeoniflorin was pumped out by As for the terpenoids, the three analytes might be substrates with the theory of to treat complicated gynecological diseases. It is coincident herbs might affect multiple targets, which enable GF capsule the three analytes about P-gp could lead to an increase in ab-
group, the competitive inhibition between paeoniflorin and
in rat plasma: the LOQ was calibrated of 20 ng/mL for

As hydro-tumulosic acid, 10 ng/mL for tumulosic acid and polyporic acid C

Table 3
The pharmacokinetic data for dehydro-tumulosic acid, tumulosic acid and polyporic acid C after oral administration of Poria extract and GF capsule.

<table>
<thead>
<tr>
<th>Data</th>
<th>Unit</th>
<th>Dehydro-tumulosic acid</th>
<th>Tumulosic acid</th>
<th>Polyporic acid C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Poria&lt;sup&gt;a&lt;/sup&gt;</td>
<td>GF capsule&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Poria</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;(0→t)&lt;/sub&gt;</td>
<td>ng·h/mL</td>
<td>2110 ± 613</td>
<td>2907 ± 431*</td>
<td>2310 ± 643</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;(0→∞)&lt;/sub&gt;</td>
<td>ng·h/mL</td>
<td>2173 ± 584</td>
<td>3425 ± 696*</td>
<td>2467 ± 641</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>ng/mL</td>
<td>486.9 ± 50.5</td>
<td>417.6 ± 22.4*</td>
<td>417.3 ± 41.5</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>h</td>
<td>2.5 ± 0.5</td>
<td>8.9 ± 2.7*</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>h</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Poria extract at 1.60 g/kg of rat weight.
<sup>b</sup> GF capsule at 4.80 g/kg of rat weight.
<sup>* Mean ± SD, where n = 6.

*P<0.05, **P<0.01 compared with the Poria extract.

AUC, T<sub>1/2</sub>, and the bimodal phenomenon showed almost the same trend. The similar pharmacokinetic results in some traditional Chinese medicine study [26,27]. The possible reasons for these phenomena were mainly summed up to two points. On one hand, Moutan Cortex and Paeoniae Radix Alba are the major ingredients in GF capsule; the two herbs contain a large amount of glycosides, e.g. paeoniflorin, oxy-paeoniflorin, paenoesin, and benzoylpaeoniflorin [28–31]. The extremely low bioavailability of paeoniflorin in animal was a result of the intestinal flora metabolism [32,33]. These aforementioned glycosides might be rapidly degraded by intestinal bacterium similar to that of paeoniflorin. In particular, Poria is known to have robust antibacterial activity [34]. In GF capsule, the competitive inhibition between glycosides and the three analytes could reduce the degradation of the analytes by intestinal flora, which therefore could lead to a secondary absorption and an outcome of the double peaks. On the contrary, Poria by itself should not have the possibility of herb–herb interactions.

On the other hand, Walle et al. have reported that some terpenoids, such as paclitaxel can be P-gp substrates [35]. As for the terpenoids, the three analytes might be substrates P-gp in the intestine. Then, paeoniflorin was pumped out by P-glycoprotein, which is an ATP-dependent drug transporter at the apical membrane [36,37]. For this reason, in GF capsule group, the competitive inhibition between paenoesin and the three analytes about P-gp could lead to an increase in absorption compare with the Poria extract group. The compatible rationality of Poria combined with other four herbs might be presented based on traditional Chinese medical science by our ancestors. The huge number of activity ingredients in five herbs might affect multiple targets, which enable GF capsule to treat complicated gynecological diseases. It is coincident with the theory of “synergistic effects”, and the penetrating research is still in progress.

In summary, a simple, specific and sensitive LC–MS method was established and validated for simultaneous determination of dehydro-tumulosic acid, tumulosic acid and polyporic acid C in rat plasma: the LOQ was calibrated of 20 ng/mL for dehydro-tumulosic acid, 10 ng/mL for tumulosic acid and 11.5 ng/mL for polyporic acid C. The developed method was employed for the first time to a comparative pharmacokinetic study of the three analytes between the GF capsule and the Poria extract group. Results of the present study indicated that the pharmacokinetic process of the three analytes in GF capsule group were significantly different compare with the Poria extract group: AUC<sub>0→∞</sub> increased and the elimination step down, which might prolong the potency of the three analytes in vivo. Furthermore, this study revealed that as far as the Poria extract was concerned, it is very valuable to be used as a clinical instruction of GF capsule.

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References


