Feeble Antipyretic, Analgesic, and Anti-inflammatory Activities were Found with Regular Dose 4’-O-β-D-Glucosyl-5-O-Methylvisamminol, One of the Conventional Marker Compounds for Quality Evaluation of Radix Saposhnikoviae

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ABSTRACT

Introduction: 4’-O-β-D-glucosyl-5-O-methylvisamminol (GML) is a conventional marker compound for quality control of Radix Saposhnikoviae. Despite that, neither pharmacodynamic or pharmacokinetic information is available with regard to GML. As such, the aim of this study was to assess the conventional evaluation indices for the quality of Radix Saposhnikoviae. Materials and methods: Pyretic animal model, hot plate test, and ear edema model were established to evaluate and compare the antipyretic, analgesic, and anti-inflammatory effect of the chromone derivatives cimifugin, prime-O-glucosylcimifugin (PGCN), and GML in Radix Saposhnikoviae. High performance liquid chromatography separation and analysis was used to obtain pharmacokinetic parameters. Simulated gastric fluid and simulated intestinal fluid was used to investigate the metabolite profiles of PGCN and GML in gastrointestinal tract. Results: Cimifugin exerted a marked dose-dependent antipyretic, analgesic, and anti-inflammatory effect, whereas the effects of PGCN were relatively lower. GML had feeble pharmacodynamic effects. Pharmacokinetic study showed that only cimifugin was detected in the plasma sample of cimifugin and PGCN-treated animals, with drug concentration in the former much higher than the latter. No components were traced in the plasma samples from GML-treated rats. Stability study showed that PGCN and GML was predominantly biotransformed into cimifugin and 5-O-methylvisamminol, respectively. The latter was proven to be extremely unstable in liver tissue. Conclusions: A feeble antipyretic, analgesic, and anti-inflammatory activities was observed when GML was orally delivered. Given that Radix Saposhnikoviae extract is generally administered orally, we speculate that this compound might be a nonpharmacologically active agent in real usage. Thus, it might be unscientific to evaluate the quality of Radix Saposhnikoviae based on the content of GML. Key words: 4’-O-β-D-glucosyl-5-O-methylvisamminol, chromone, pharmacological activity, pharmacokinetics, Radix Saposhnikoviae

INTRODUCTION

Radix Saposhnikoviae (Fangfeng in Chinese), the dried root of perennial herb Saposhnikovia divaricate (Turcz.) schischk under Umbelliferae, is a common traditional herbal medicine for the treatment of pyrexia, rheumatism, headache, vertigo, generalized aching, convulsion, and arthralgia and inflammatory symptoms in traditional clinical practice in Asian countries such as China, Japan, and Korea for thousands of years.1,2,3 It is noteworthy that these pharmacological activities of Radix Saposhnikoviae have also been confirmed by modern scientific experiments using its extracts.4,5,6 Phytochemical studies on this herb revealed the existence of abundant compounds in Radix Saposhnikoviae, such as chromones, coumarins, polycyclic ethers, and polysaccharides, among which chromones and coumarins are the main components of Radix Saposhnikoviae.4,7,8 Of these chemicals, chromones have been identified as the main bioactive constituents most relevant to its pharmacological activity, pharmacokinetics, Radix Saposhnikoviae

SUMMARY

GML-derived cimifugin, which represents the potential pharmacodynamic component of Radix Saposhnikoviae chromones, in plasma was almost nil in contrast to cimifugin and PGCN. And thus, feeble antipyretic, analgesic, and anti-inflammatory activities were found with GML. Abbreviations used: AUC: area under concentration-time curve, DNP: 2,4-Dinitrophenol, HPLC: high performance liquid chromatography, HPLC-MS: high performance liquid chromatography-mass spectrophotography, GML: 4’-O-β-D-glucosyl-5-O-methylvisamminol, MVL: 5-O-methylvisamminol, PGCN: prime-O-glucosylcimifugin, SIF: alkaline phosphatase. SIF: simulated intestinal fluid.

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Prime-O-glucosylcimifugin (PGCN) and 4'-O-β-D-glucosyl-5-O-methylvisamminol (GML) are the two major types of chromones in Radix Saposhnikoviae with clear chemical structure (Figure 1) and thus both of them were selected as marker compounds to assess Radix Saposhnikoviae quality in Chinese pharmacopoeia, whereas the latter is used as an identification marker in Japanese Pharmacopoeia. It has been documented that PGCN exhibited significant antipyretic, analgesic, and anti-inflammatory bioactivities. Cimifugin, the aglycone of PGCN, is another primary component in Radix Saposhnikoviae. A previous study showed that the cimifugin in rat plasma was much higher than that of PGCN after oral administration of Radix Saposhnikoviae extract, although the latter has much higher content than the former. As thus, it was speculated that the cimifugin might represent the potential pharmacodynamic component of Radix Saposhnikoviae, and other chrome derivatives such as PGCN and GML act as prodrugs and would be absorbed into blood in forms of cimifugin through biotransformation in the intestine or stomach. Indeed, this hypothesis was confirmed by a recent study, in which cimifugin and 5-O-methylvisammiol (MVL) were obtained from the incubation system composed of PGCN and intestine flora. In spite of that, neither pharmacodynamic or pharmacokinetic information is available with regard to GML, the another marker compound for quality control of Radix Saposhnikoviae. Therefore, the aims of this study were to investigate the pharmacodynamic effects of GML, mainly in terms of anti-inflammatory, analgesic, and anti-inflammatory activities, as well as its pharmacokinetics. Cimifugin and PGCN were used in this investigation for comparison.

MATERIALS AND METHODS

Animals
Healthy adult Sprague-Dawley rats (180–220 g) and Kunming mice (20–25 g) were used for the study. Animals were kept at standard conditions of temperature (25 ± 1°C) and 12-h light/12-h dark cycle and relative humidity of (70 ± 10%) throughout the experimental period. The rats and mice were given free access to standard laboratory pellets and water. All experimental procedures involving animals were conducted in accordance with the guidelines of the National Institutes of Health guidelines. The experimental protocols were also reviewed and approved by the Ethical Committee of Heilongjiang University of Chinese Medicine (approval number: HUCM2014-00348). All efforts were made to minimize animal suffering and to reduce the number of animals used. The health status of animals was monitored throughout the experiment protocols, and no obvious adverse health effects were observed following administration of cimifugin, PGCN, or GML.

Reagents and treatment
Cimifugin, PGCN, and GML (all purity ≥98%) were purchased from Shanghai Jinsui Bio-Technology Co., Ltd. (Shanghai, China). Cimifugin, PGCN, and GML were dissolved in saline for administration to animals. 2,4-Dinitrophenol (DNP) was provided by Chengdu Xiya Chemical Co. Ltd. (Chengdu, China). Xylene and pepsin were provided by Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol was obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). All other reagents were analytical purity. Distilled water was prepared from demineralized water and used throughout the experiment.

Evaluation of antipyretic effect of cimifugin, PGCN, and GML
The antipyretic activity of the three chromone derivatives cimifugin, PGCN, and GML was evaluated using Sprague-Dawley male rats (180–220 g). The body temperature of each rat was determined using digital thermometer. Pyrexia was induced by subcutaneous injection of single dose of 30 mg/kg body weight (b.w.) DNP. To determine and compare the antipyretic activities of cimifugin, PGCN, and GML, the treatment groups were given 1, 2, and 4 mg/kg b.w. p.o. doses of these three agents, respectively. The rats in control group were administered with equivalent amounts of saline orally. The rectal temperature of each group was recorded before and 30, 60, 90, 120, and 180 min following drug administration.

Evaluation of analgesic effect of cimifugin, PGCN, and GML
Hot plate test was performed to evaluate the analgesic effect of the drugs. Kunming female mice of 20–25 g were used. Experimental animals were subjected to pretesting on a hot plate set at 55 ± 0.5°C. Animals having latency time (time for which rat remains on the hot plate surface without licking or flicking of paws or jumping off) 5–30 s were included in this study. These mice were randomly divided into different groups, receiving increasing oral doses (1, 2, and 4 mg/kg b.w.) of cimifugin, PGCN, and GML, respectively. The mice in control group were administered with equivalent amounts of saline orally. The latency time of each group was recorded before and 15, 30, 60, 90, and 120 min following drug administration. The maximal latency (cut-off) time was set at 30 s to prevent tissue damage.

Evaluation of anti-inflammatory effect of cimifugin, PGCN, and GML
To evaluate the anti-inflammatory effects of these three agents, mouse ear edema model was established as described previously. Briefly, Kunming male mice of 20–25 g were used. These mice were randomly divided into different groups, receiving oral doses (1, 2, and 4 mg/kg b.w.) of cimifugin, PGCN, and GML. The mice in control group were administered with equivalent amounts of saline orally. After 30 min, the inner and outer sides of the right ear (auricle) were treated topically with xylene (0.02 mL/mouse) for 1 h, with left ear untreated as a control. Subsequently, the mice were euthanized by cervical dislocation. Punch biopsy samples of 8-mm diameter were obtained from the same parts of the left and right ears. The ear edema index was reflected by weight.

Figure 1: Chemical structures of prime-O-glucosylcimifugin (PGCN), cimifugin, and 4'-O-β-D-glucosyl-5-O-methylvisamminol (GML).
difference between right and left ears of each animal.

**Pharmacokinetic measurement of cimifugin, PGCN, and GML**

Forty male Sprague–Dawley rats in each group were used in this experiment. They were randomly divided into four groups, each consisting of 10 rats. The rats in these four groups were orally administered with 6 mg/kg cimifugin, 2 mg/kg cimifugin, 6 mg/kg PGCN, and 6 mg/kg GML, respectively. 0.5, 1, 1.5, 2, 3, 5, 8, and 12 h after the drug delivery, rats were anesthetized by ether inhalation and 0.5 mL blood samples from rats were collected in heparinized tube via the orbit vein. Meanwhile, the blank plasma was obtained from saline orally administered Sprague–Dawley rats. All these plasma was centrifuged immediately at 3000 rpm for 10 min to yield plasma. Seventy percent perchloric acid 20 μL was added to 100 μL of plasma samples. The mixture was vortexed and centrifuged at 3000 rpm for 10 min. The supernatant were subjected to 0.45 μm microporous membrane with the filtrate collected and used for subsequent HPLC analysis. Chromatographic separation and analysis were performed using an L-2000 Elite HPLC system. The analytes were subjected to a Kromasil C18 column (200 mm 4.6 mm i.d., particle size 5 μm), equipped with a Shim-pack security guard column. The mobile phase was a methanol (A)–water mixture with a linear gradient elution at a flow rate of 1.0 mL/min at 25°C. The elution program was as follows: 40–45% A (0–5 min); 45–60% A (5–10 min); 60–80% A (10–15 min); 80–95% A (15–20 min); 95–40% A (20–30 min). The injection volume was 20μL and detection wavelength was set at 254 nm.

**Metabolism studies of PGCN and GML in SGF and SIF**

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to Chinese Pharmacopeia (2010). Briefly, SGF was prepared by dissolving and mixing 16.4 mL diluted HCl (36% HCl 234 mL diluted with water to 1 L) and purified pepsin (10 g) and diluting with water to 1 L. This test solution had a pH of 1.2. For SIF preparation, 6.8 g potassium dihydrogen phosphate was dissolved in 500 mL water. The pH was adjusted to 6.8 with 0.4% NaOH, this solution was mixed with diluted 23.4% HCl 234 mL diluted with water to 1 L. This test solution had a pH of 1.2. For SIF preparation, 6.8 g potassium dihydrogen phosphate was dissolved in 500 mL water. The pH was adjusted to 6.8 with 0.4% NaOH, this solution was mixed with diluted 23.4% HCl 234 mL diluted with water to 1 L. The mixture was incubated at 37°C in a shaking water bath. After 15 min incubation, the mixture was added and the mixture was incubated at 37°C in a shaking water bath. After 15 min, the metabolic stability of this chemical was evaluated.

**RESULTS**

**Antipyretic effects of cimifugin, PGCN, and GML**

The cimifugin, PGCN, and GML in Radix Saposhnikoviae varied a lot in antipyretic activity. Single dose injection of 30 mg/kg b.w. DNP induced a remarkable rise of rectal temperature in a time-dependent fashion. As shown in Table 1, cimifugin p.o. significantly and dose dependently (1, 2, and 4 mg/kg) attenuated hyperthermia in rats. The antipyretic effects at any dose started at 0.5 h after injection of DNP and remained significant at least up to 3 h. When compared with cimifugin, antipyretic activity of PGCN appeared relatively weaker; 4 mg/kg PGCN revealed less pronounced antipyretic activity than 1 mg/kg dose of cimifugin. In addition, its onset of antipyretic response was delayed, with reversal of hyperthermia starting at 2 h after administration of PGCN 1 and 4 mg/kg. As for GML, no significant pyretic effect was found (P>0.05).

**Analgesic effects of cimifugin, PGCN, and GML**

Results showed that cimifugin p.o. exhibited a dose-dependent increase in latency time, starting at 0.5 h and lasting at least 2 h, indicating the significant analgesic activity of cimifugin. However, results from PGCN were much weaker, as the analgesic effect of this agent at 4 mg/kg was much weaker, as the analgesic effect of this agent at 4 mg/kg was

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**Table 1: Effect of Cimifugin, PGCN, and GML on 2,4-dinitrophenol-induced pyrexia**

|       | 0.5 h     | 1 h     | 1.5 h    | 2 h     | 3 h     |
|-------|-----------|---------|----------|---------|---------|
| Saline| 1.26 ± 0.31| 1.98 ± 0.29| 2.46 ± 0.23| 3.24 ± 0.21| 2.84 ± 0.13|
| Cimifugin| 0.85 ± 0.40*| 1.45 ± 0.35**| 2.16 ± 0.27**| 2.43 ± 0.23**| 1.92 ± 0.19**|
|        | 0.81 ± 0.35**| 1.34 ± 0.24**| 1.76 ± 0.27**| 1.94 ± 0.38**| 1.34 ± 0.20**|
|        | 0.68 ± 0.34**| 1.13 ± 0.24**| 0.88 ± 0.38**| 0.85 ± 0.36**| 0.58 ± 0.56**|
| PGCN  | 1.10 ± 0.59| 1.91 ± 0.35| 2.20 ± 0.23*| 2.61 ± 0.26**| 2.10 ± 0.18**|
|        | 1.01 ± 0.19| 1.71 ± 0.34| 2.13 ± 0.22**| 2.78 ± 0.52**| 2.17 ± 0.22**|
|        | 0.86 ± 0.31*| 1.57 ± 0.20**| 1.99 ± 0.31**| 2.54 ± 0.33**| 2.01 ± 0.24**|
| GML   | 1.05 ± 0.42| 1.91 ± 0.52| 2.32 ± 0.53| 2.99 ± 0.53| 2.71 ± 0.49|
|        | 0.98 ± 0.25| 1.74 ± 0.33| 2.41 ± 0.31| 3.07 ± 0.27| 2.76 ± 0.38|
|        | 1.24 ± 0.32| 2.00 ± 0.28| 2.55 ± 0.28| 3.07 ± 0.31| 2.70 ± 0.39|
only equivalent to that of cimifugin at 1 mg/kg. GML hardly had any
analgesic activity [Table 2].

**Anti-inflammatory effects of cimifugin, PGCN, and GML**
In the xylene-induced ear edema, cimifugin induced a dose-dependent
reduction in edema. Although PGCN also exhibited significant anti-
inflammatory effects, its activity was less pronounced than cimifugin;
the anti-inflammatory action of this agent at 4 mg/kg was only equivalent
to that of cimifugin at 1 mg/kg. GML hardly had any analgesic activity,
although an anti-inflammatory tendency was found [Table 3].

**Pharmacokinetic study of cimifugin in rat plasma**
At first, the metabolite profiles of the three agents in plasma were
qualitatively investigated. After oral administration of cimifugin, PGCN,
or GML at a single dose of 6 mg/kg, rat blood sample were collected and
pretreated by protein precipitation. Then these samples were separated
on a Kromasil HPLC C18 column. Only cimifugin was identified,
whereas not any constituent was detected in the plasma of GML-treated
rats in the whole process of the experiment [Figure 2]. Based on the
qualitative results, cimifugin concentrations in rat plasma were further
quantitatively measured before and 0.5, 1, 1.5, 2, 3, 5, 8, and 12 h after
oral administration of cimifugin (2 and 6 mg/kg), PGCN (6 mg/kg), or
GML (6 mg/kg). The concentration–time curve and pharmacokinetic
parameters were presented in Figure 3 and Table 4, respectively. These
results demonstrated that when orally administered single same dose
of the three agents (all at 6 mg/kg), cimifugin was associated with a
significant higher systemic exposure (Cmax and AUC) than PGCN and
GML. In actual fact, both Cmax and AUC were 0 in the plasma of GML
administered rats, which was consistent with the qualitative results as
shown in Figure 2h(1)–h(3).

**Metabolism studies of PGCN and GML in SGF and SIF**
As only cimifugin was detected in the plasma of either cimifugin- or
PGCN-treated rats and no constituents were found in GML-treated
animals, we speculated that these drugs may be metabolized in the gut
or directly excreted in the feces. However, the latter seems not to be the
case, as no traces of chromes were found in the rat stools throughout the
experiment (data not shown). Thus, we investigated the metabolism of
PGCN and GML in simulated gastrointestinal environment. As shown in
Table 5, the vast majority (99.64%) of PGCN was transformed into

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**Table 2:** Analgesic effect of cimifugin, PGCN, and GML in hot plate test

| Treatment | Dose (mg/kg) | 0 h | 0.5 h | 1 h | 1.5 h | 2 h |
|-----------|--------------|-----|-------|-----|-------|-----|
| Saline    | 0            |     |       |     |       |     |
|           | 8.82 ± 1.62  | 10.46 ± 1.78 | 9.71 ± 1.35 | 10.11 ± 2.10 | 10.82 ± 2.30 |
| Cimifugin | 1            | 8.38 ± 0.89  | 11.45 ± 1.48 | 11.52 ± 0.60* | 11.85 ± 1.30* | 12.69 ± 1.44* |
|           | 2            | 8.17 ± 1.18  | 12.64 ± 2.80* | 14.01 ± 1.75** | 15.48 ± 2.24** | 14.57 ± 1.80** |
|           | 4            | 9.00 ± 1.17  | 13.88 ± 3.63** | 13.23 ± 3.28** | 14.77 ± 0.69** | 15.48 ± 2.27** |
| PGCN      | 1            | 8.29 ± 1.72  | 9.51 ± 1.71  | 9.48 ± 1.40  | 9.67 ± 1.16  | 10.61 ± 1.38  |
|           | 2            | 8.43 ± 1.62  | 11.83 ± 1.50  | 11.05 ± 1.87* | 12.17 ± 2.53* | 12.36 ± 1.71*  |
|           | 4            | 9.18 ± 1.34  | 11.65 ± 1.31  | 11.52 ± 1.51* | 12.25 ± 1.28** | 12.38 ± 1.31* |
| GML       | 1            | 9.27 ± 1.46  | 9.25 ± 1.56  | 9.55 ± 1.89  | 9.82 ± 1.08  | 10.43 ± 1.39  |
|           | 2            | 8.88 ± 1.35  | 11.40 ± 2.26  | 10.88 ± 1.47  | 10.96 ± 1.27  | 10.38 ± 1.75  |
|           | 4            | 8.64 ± 1.6   | 10.72 ± 1.51  | 8.63 ± 2.53  | 9.76 ± 3.50  | 10.09 ± 1.44  |
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Table 3: Effect of cimifugin, PGCN, and GML on xylene-induced ear edema in mice

| Treatment | Dose (mg/kg) | Increase in ear edema weight (mg) | % Inhibition of edema weight (%) |
|-----------|--------------|-----------------------------------|---------------------------------|
| Saline    | 0            | 7.01 ± 3.31                       | –                               |
| Cimifugin | 1            | 4.57 ± 0.33**                     | 35                              |
|           | 2            | 3.70 ± 0.29**                     | 48                              |
|           | 4            | 2.16 ± 0.36**                     | 70                              |
| PGCN      | 1            | 5.83 ± 0.23*                      | 16                              |
|           | 2            | 5.45 ± 0.33**                     | 22                              |
|           | 4            | 4.32 ± 0.42**                     | 38                              |
| GML       | 1            | 6.64 ± 0.18                       | 5                               |
|           | 2            | 6.42 ± 0.23                       | 9                               |
|           | 4            | 6.13 ± 0.25                       | 12                              |

cimifugin in SIF and a small proportion (1.99%) of transformation in SGF. As for GML, cimifugin metabolite was found neither in SGF nor in SIF. HPLC analysis showed that ~33.37% of GML degraded into a new compound, except for a small quantity of the drugs transformed into PGCN in SGF (3.09%) and SIF (0.38%). By HPLC-MS analysis, this new compound is confirmed to be the deglucosed derivative of GML, that is, MVL [Figure 4]. Stability studies in Figure 5 found that <20% cimifugin was degraded in the liver tissue homogenate and hardly any transformation occurred in plasma. In contrast, up to 44.9 and 36.0% MVL rapidly disappeared within a short span of 15 min co-incubation with in vitro hepatic system and plasma, respectively, suggestive of bio-inspired instability properties of this metabolite.

DISCUSSION

To the best of our knowledge, this study is the first report on antipyretic, analgesic, and anti-inflammatory activities and pharmacokinetics of GML. The present investigation showed that this compound was not absorbed into blood and does not possess antipyretic, analgesic, and anti-inflammatory effects when orally administered, although it has long been used for quality control for Radix Saposhnikoviae in many editions.
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Figure 4: Mass spectrum analysis of the new metabolite of 4'-O-β-D-glucosyl-5-O-methylvisamminol (GML) in simulated intestinal fluid (SIF). The related substance was identified as 5-O-methyvisammiol (MVL)

Figure 5: In vitro stability studies of 5-O-methyvisammiol (MVL) in rat hepatic system and plasma. One hundred microliters of simulated intestinal fluid (SIF) containing MVL or cimifugin was first coincubated with 30% in vitro liver tissue homogenate or plasma sample of rats at 37ºC in a shaking water bath. Fifteen minutes later, metabolic stability of these two chemicals was evaluated and compared by HPLC analysis of parent drugs left using relative peak area, which was obtained by designating the actual peak area at 0 min as a value of 100%.

of Chinese Pharmacopoeia.

Regarding the pharmacodynamic effects of the three chromone derivatives in Radix Saposhnikoviae, only PGCN, as far as we know, was documented to possess antipyretic, analgesic, anti-inflammatory, anti-platelet aggregation, and other actions. However, both previous and our present study showed that it was the aglycone, cimifugin, rather than the parent drug (PGCN) that represented the potential pharmacodynamic component. This is compatible with a previous study which suggested that PGCN was of a moderately absorbed compound. It is worthwhile to note that in this study only cimifugin, but not any trace of PGCN, was found in the plasma samples of PGCN orally administered rats, which is inconsistent with previous reports, which also found a certain amount of parent drug, although its content in blood is much lower than that of cimifugin. We speculated that the discrepancy might be primarily due to the dose used for study. Indeed, Our used dose of PGCN was 2–6 mg/kg, which was much lower than those used in other studies (2–6 vs. 10 mg/kg). In spite of that, the dose in our study might represent the real in vivo process of PGCN, because the dose of 2–6 mg/kg rat b.w. was designated based on the actual usage dosage and human–rat dose conversion.

As aforementioned, because except for cimifugin, no other components were found in the blood, the pharmacological effects of orally administered PGCN might totally arise from its dyglycosated metabolite cimifugin. Indeed, we herein found that cimifugin monomer solution took comparatively stronger antipyretic, analgesic, and anti-inflammatory effects, and PGCN have comparatively weaker pharmacological activities when it is orally administered. But when it comes to GML, no antipyretic, analgesic, and anti-inflammatory activities were found with this compound when it was orally used. Moreover, we reckon that GML might not produce any pharmacodynamic effects when orally administered, because not any form of this compound was traced in the blood for this delivery route.

It is rather curious that GML was not traced either in the blood or in the feces. As thus, we detected its biotransformation products in gastrointestinal using SGF and SIF. As a result, an extremely unstable metabolite MVL was obtained in the SIF. We thus hypothesized that GML might first be metabolized to unstable MVL, which then was absorbed into the blood and rapidly degraded by the enzymes in the blood and liver; the degradation process was perhaps exceedingly fast, and thereby we failed to detect MVL in the plasma samples. This hypothesis warrants further confirmation.

CONCLUSION

To conclude, no antipyretic, analgesic, and anti-inflammatory action was observed with GML. Meanwhile, this compound is hard to be absorbed into bloodstream. Given that Radix Saposhnikoviae extract is generally administered orally, we speculate that this compound might be a nonpharmacologically active agent in real usage. Thus, it might be
unscientific to evaluate the quality of Radix Saposhnikoviae based on the content of GML.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Kim MK, Yang DH, Jung M, Jung EH, Eom HY, Suh JH. Simultaneous determination of chromones and coumarins in Radix Saposhnikoviae by high performance liquid chromatography with diode array and tandem mass detectors. J Chromatogr A 2011; 1218: 6319-30.
2. Okuyama E, Hasegawa T, Matsushita T, Fujimoto H, Ishibashi M, Yamazaki M. Analgesic components of saposhnikovia root (Saposhnikovia divaricata). Chem Pharmaceut Bull 2001; 49: 154-60.
3. Kang J, Zhou L, Sun JH, Ye M, Han J, Wang BR. Three new compounds from the roots of Saposhnikovia divaricata. Chem Pharmaceut Bull 2001; 49: 154-60.
4. Li Y, Zhao L, Zhang H, Jia J, Lv L, Zhou G. Comparative pharmacokinetics of prim-O-glucosylcimifugin and cimifugin by liquid chromatography-mass spectrometry after oral administration of Radix Saposhnikoviae extract, cimifugin monomer solution and prim-O-glucosylcimifugin monomer solution to rats. Biomed Chromatogr 2012; 26: 1234-40.
5. Dai J, Chen X, Cheng W, Liu X, Fan X, Shen Z. A sensitive liquid chromatography-mass spectrometry method for simultaneous determination of two active chromones from Saposhnikovia root in rat plasma and urine. J Chromatogr B 2008; 868: 13-9.
6. Tai J, Cheung S. Anti-proliferative and antioxidant activities of Saposhnikovia divaricata. Oncol Rep 2007; 18: 227-34.
7. Zhang ZQ, Tian YJ, Zhang J. Studies on the antioxidative activity of polysaccharides from radix Saposhnikoviae. Zhong yao ca i = Zhongyaozhi 2008; 31: 268-72.
8. Kang J, Sun JH, Zhou L, Ye M, Han J, Wang BR. Characterization of compounds from the roots of Saposhnikovia divaricata by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrometry 2008; 22: 1899-911.
9. Committee CP. Pharmacopeia of the people's republic of china 2010 Beijing, China Chemical Industry Press
10. Zhao B, Yang XB, Yang XW, Liu JX. Biotransformation of prim-O-glucosylcimifugin by human intestinal flora and its inhibition on NO production and DPPH free radical. J Asian Natural Products Res 2012; 14: 886-96.
11. Li YY, Wang H, Chen J, Zhao L, Zhang H, Chai YF. RRLC-TDF/MS in identification of constituents and metabolites of Radix Saposhnikoviae in rat plasma and urine. J Chromatogr B 2012; 868: 13-40.
12. Kang JY, Khan MN, Park NH, Cho JY, Lee MC, Fuji H, Antipyretic, analgesic, and anti-inflammatory activities of the seaweed Sargassum fulvellum and Sargassum thunbergii in mice. JEthnopharmacol 2008; 116: 187-90.
13. Wei W, Wu XM, Li YJ. Methodology of pharmacological experiment. 2010; 4th ed Beijing: People's Medical Publishing House
14. Xue BY, Li W, Li L, Xiao YQ. A pharmacodynamic research on chromosome glucosides of fangfeng. Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = 2000; 25: 297-9.
15. Chen N, Wu Q, Chi G, Soromou LW, Hou J, Deng Y. Prime-O-glucosylcimifugin attenuates lipopolysaccharide-induced acute lung injury in mice. International immunopharmacology 2013; 16: 139-47.
16. Zhao B, Yang XB, Yang XW, Wu Q, Wang Y, Zhang LX. Intestinal permeability of the constituents from the roots of Saposhnikovia divaricata in the human Caco-2 cell monolayer model. Planta Medica 2011; 77: 1531-5.