



# The metabolism of gelsevirine in human, pig, goat and rat liver microsomes

Hua-Hai Zhang<sup>1,2</sup> | Wen-Jia Yang<sup>3</sup> | Ya-Jun Huang<sup>4,5</sup> | Wen-Jing Li<sup>6</sup>  |  
Shuo-Xin Zhang<sup>1,2</sup> | Zhao-Ying Liu<sup>4,7</sup> 

<sup>1</sup>College of Forestry, Northwest A&F University, Yangling, China

<sup>2</sup>Qinling National Forest Ecosystem Research Station, Huoditang, China

<sup>3</sup>Yangling Demonstration Zone Hospital, Yangling, China

<sup>4</sup>College of Veterinary Medicine, Hunan Agricultural University, Changsha, China

<sup>5</sup>Hunan Prima Drug Research Center Co., Ltd., Changsha, China

<sup>6</sup>College of Landscape Architecture and Arts, Northwest A&F University, Yangling, China

<sup>7</sup>Hunan Engineering Technology Research Center of Veterinary Drugs, Hunan Agricultural University, Changsha, China

## Correspondence

Shuo-Xin Zhang, College of Forestry, Northwest A&F University, Yangling, Shaanxi 712100, China.

Email: sxzhang@nwsuaf.edu.cn

Zhao-Ying Liu, College of Veterinary Medicine, Hunan Agricultural University, Changsha, Hunan 410128, China.

Email: liu\_zhaoying@hunau.edu.cn

## Funding information

National Natural Science Foundation of China, Grant/Award Number: 31972737; Scientific Research Fund of Hunan Provincial Education Department, Grant/Award Number: 18A088

## Abstract

Gelsemium is a small genus of flowering plants from the family Loganiaceae comprising five species, three of which, *Gelsemium sempervirens* (L.) J. St.-Hil., *G. elegans* Benth and *G. rankinii* Small, are particularly popular. Compared with other alkaloids from *G. elegans*, gelsemine, gelsevirine and koumine exhibit equally potent anxiolytic effects and low toxicity. Although the pharmacological activities and metabolism of koumine and gelsemine have been reported in previous studies, the species differences of gelsevirine metabolism have not been well studied. In this study, the metabolism of gelsevirine was investigated by using liver microsomes of humans, pigs, goats and rats by means of HPLC-QqTOF/MS. The results indicated that the metabolism of gelsevirine in liver microsomes had qualitative and quantitative species differences. Based on the results, the possible metabolic pathways of gelsevirine in liver microsomes were proposed. Investigation of the metabolism of gelsevirine will provide a basis for further studies of the in vivo metabolism of this drug.

## KEYWORDS

*gelsemium*, gelsevirine, goat, HPLC-QqTOF, human, metabolites, MS, pig

## 1 | INTRODUCTION

*Gelsemium* is a small genus of flowering plants from the family Loganiaceae, comprising five species, three of which, *Gelsemium sempervirens* (L.) J. St.-Hil., *G. elegans* Benth and *G. rankinii* Small, are particularly popular. They are species native to the southwestern United States and East Asia (Zhang & Wang, 2015). Among them,

*G. elegans* is also known as Gouwen, Duan chang cao or Dacha yao in China, is highly toxic and has been used traditionally for the treatment of pain, spasticity, and skin ulcers in Chinese folkloric medicine (Xu et al., 2006).

Previous studies have identified more than 200 compounds, including alkaloids, steroids and iridoids, in *Gelsemium*. Among these compounds, alkaloids and iridoids are regarded as the two most

H. H. Zhang and W. J. Yang contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors *Veterinary Medicine and Science* Published by John Wiley & Sons Ltd

active groups that are likely to be responsible for the observed pharmacological effects. Based on their chemical structures, diverse and complex alkaloids have been classified into the following six types: gelsedine-type, gelsemine-type, humantenine-type, koumine-type, sarpagine-type and yohimbane-type (Kitajima, 2006; Liu et al., 2017; Zhang et al., 2009). Recently, many studies have been conducted on the pharmacological, phytochemical, toxicological aspects of *Gelsemium*. Both in vivo and in vitro experiments have demonstrated that *Gelsemium* exhibits a diverse set of antitumor, analgesic, anti-inflammatory, immunomodulatory, anxiolytic and protective neurotropic biological effects (Jin et al., 2014; Kitajima et al., 2003); however, its toxicity restricts its appropriate dosage and clinical use (Wang et al., 2017). Alkaloids such as gelsemine, koumine, gelsemine, gelsevirine, gelsenicine, sempervirine and humantenine and their analogs are the major active components in *Gelsemium* (Chen et al., 2017; Liu et al., 2012). Compared with other alkaloids from *G. elegans*, gelsemine, gelsevirine and koumine exhibit equally potent anxiolytic effects and reduced toxicity (Su et al., 2011). The pharmacological activities and metabolism of koumine and gelsemine have been reported by other researchers, (Xiao et al., 2017; Yang et al., 2017) and the metabolism of gelsevirine was examined in vitro in our previous study (Zhang et al., 2019). The species differences in gelsevirine metabolism have not been well studied. Comparative metabolism is an important way to interpret different toxicities by comparing the varieties, quantities and production rates of metabolites in different species.

The aim of the present work was to investigate the metabolism of gelsevirine in liver microsomes of humans, pigs, goats and rats using HPLC-QqTOF/MS. This work will greatly improve the understanding of the metabolic characteristics of gelsevirine among different species. Moreover, it will further facilitate the toxicity and food safety explanations of *G. elegans* in animals.

## 2 | MATERIALS AND METHODS

### 2.1 | Chemicals

Gelsevirine was purchased from Shanghai Kangbiao Chemicals Ltd. (Shanghai, China), nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Roche Chemical Co. (Beijing, China), and formic acid and acetonitrile (HPLC grade) was purchased from ROE (Newark, DE, USA) and Merck (Darmstadt, Germany), respectively. Deionized water was obtained with a Milli-Q purification system (Bedford, MA, USA). All other chemicals and reagents used were of the highest analytical grade.

### 2.2 | Identification of gelsevirine in human, pig, goat and rat liver microsomes

The metabolism of gelsevirine was investigated by using human, pig, goat and rat liver microsomes. Rat, pig and goat liver microsomes

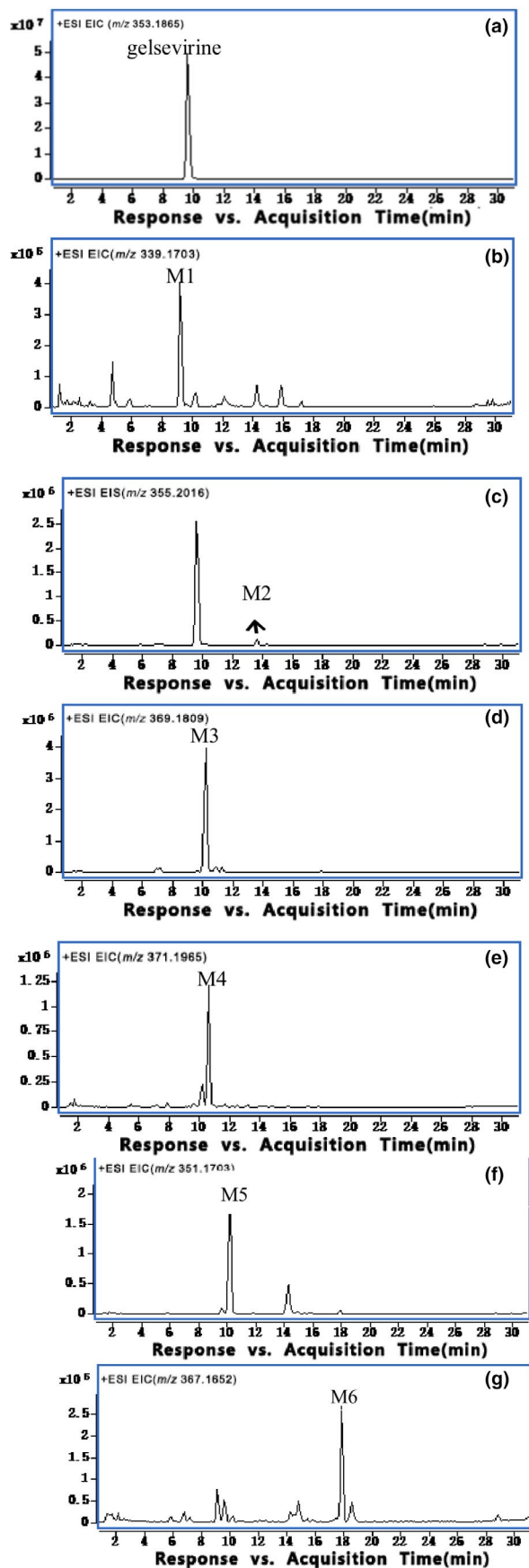
were prepared following the protocol in a previous study (Zhang et al., 2013), and all of them were preserved at  $-70^{\circ}\text{C}$  before use. Human liver microsomes were purchased from Wuhan PrimeTox Biomedical Technology Co., Ltd. (Wuhan, Hubei, China; pooled Chinese male donors, 20 mg/ml). The standard incubation mixture contained gelsevirine (10  $\mu\text{g}/\text{ml}$ ),  $\text{MgCl}_2$  (5 mmol/L), liver microsomal protein (2-mg protein/ml) and an NADPH system in a final volume of 200  $\mu\text{l}$  in 0.05 M Tris-HCl buffer (pH 7.4). Gelsevirine was dissolved in methanol (final amount in the reaction medium did not exceed 1%). Then, the incubation mixture temperature was set at  $37^{\circ}\text{C}$  for 5 min, and the reaction was started by adding 10- $\mu\text{g}/\text{ml}$  gelsevirine solution. Control incubations were performed in the absence of drug or NADPH. Each reaction was incubated at  $37^{\circ}\text{C}$  for 60 min and terminated with 50  $\mu\text{l}$  of ice cold 15% trichloroacetic acid (TCA). The mixture was then vortexed and centrifuged for 15 min at 12,000 g and  $4^{\circ}\text{C}$ . The clear supernatant was obtained and analyzed by HPLC-QqTOF/MS for the identification of gelsevirine metabolites.

### 2.3 | Instruments and analytical condition

The LC-MS system consisted of an Agilent 1,290 HPLC system (Agilent Technologies, Palo Alto, CA, USA) with a Hypersil GOLD column (150 mm  $\times$  2.1 mm, 5  $\mu\text{m}$ ) and a 6,530 Q-TOF-MS accurate-mass spectrometer (Agilent Technologies) equipped with an electrospray ionization source (ESI+). The LC gradient used mobile phase A containing 0.1% formic acid in water and mobile phase B (acetonitrile). The flow rate was kept constant at 0.3 ml/min, and the injection volume was 5  $\mu\text{l}$ . The gradient elution system began at 10% B for 5 min, 10%–90% B from 5 to 20 min, held at 90% B for 5 min and then returned to the initial conditions for reequilibration. The whole analysis took 30 min. The sample chamber in the autosampler was maintained at  $4^{\circ}\text{C}$ , while the column was set at  $30^{\circ}\text{C}$ . Auto MS/MS acquisition was performed in positive ion mode with the following conditions: scan range,  $m/z$  50–1000; capillary voltage, 4.0 kV; fragmentor voltage, 170 V; collision energy, slope 3.7 V/100 Da per  $m/z$  100; gas temperature,  $300^{\circ}\text{C}$ ; nebulizer gas pressure, 40 psig. Automated calibration was in place during data acquisition. All the acquisition of data was controlled by Agilent Mass Hunter software (version B.01.03 Build 1.3.157.0 2).

### 2.4 | Analysis of metabolites

We assumed that the obtained compounds were metabolites of gelsevirine by comparison of the incubation samples with the control samples (incubation in the absence of drug), as well as the agreement between the accurate mass measurement in the MS spectra and predicted formula calculations within 10 ppm. If a peak appeared in the accurate EIC and the accurately measured mass in the MS spectrum did not agree with the theoretical mass within the maximal inaccuracy of 10 ppm, then the peak was determined to not be a possible metabolite.



**FIGURE 1** Accurate extracted ion chromatograms of gelsevirine and its metabolites produced following human liver microsomes treatment in the presence of the NADPH-generating system: (a) gelsevirine ( $m/z$  353); (b) M1 ( $m/z$  339); (c) M3 ( $m/z$  355); (d) M3 ( $m/z$  369); (e) M4 ( $m/z$  371); (f) M5 ( $m/z$  351); (g) M6 ( $m/z$  367)

### 3 | RESULTS

#### 3.1 | Subsection Identification of gelsevirine metabolites in human liver microsomes

Accurate extracted ion chromatograms (EICs) of unchanged gelsevirine and its metabolites formed in human liver microsomes in the presence of the NADPH-generating system are shown in Figure 1. In addition to the parent drug, a total of six metabolites were detected in human liver microsomes. Gelsevirine had a retention time of 9.6 min and showed a protonated molecule ion ( $[M + H]^+$ ) at  $m/z$  353 (Zhang et al., 2019). The proposed fragmentation pathways of gelsevirine have been proven. Detailed mass spectrometric analysis of the fragmentation pattern of gelsevirine provides a basis for assessing the structural assignment of the metabolites. Table 1 lists the predicted elemental compositions, retention times, observed masses and predicted masses, and mass errors of accurate masses of gelsevirine and its metabolites.

Gelsevirine lost an  $OCH_3$  group (observed 31.0209 Da, predicted 31.0184 Da) to form a fragment ion at  $m/z$  322, followed by the loss of  $OCH_3$  to form a fragment ion at  $m/z$  291 (observed 31.0183 Da, predicted 31.0184 Da). The fragment ion at  $m/z$  260 was obtained by the loss of  $NCH_5$  (observed 31.0420 Da, predicted 31.0422 Da) from  $m/z$  291. The fragment ion at  $m/z$  108 was generated by a ring crack from  $m/z$  353. The formation of the ion at  $m/z$  70 corresponded to the loss of  $C_3H_2$  (observed 38.0159 Da, predicted 38.0156 Da) from  $m/z$  108.

Metabolite M1 eluted at a retention time of 9.2 min and showed that the  $[M + H]^+$  ion at  $m/z$  339 was 14 Da lower than that of gelsevirine, suggesting that it was a demethylated metabolite of gelsevirine. M1 (Table 1) generated the same fragment ion at  $m/z$  260 as gelsevirine. The fragment ion at  $m/z$  96 was generated by ring crack from  $m/z$  339. M1 also generated the fragment ion at  $m/z$  56, which was 14 Da lower than the ion at  $m/z$  70 of gelsevirine, indicating that demethylation had occurred in the position of the methoxy group next to the nitrogen. Based on these observations, M1 was tentatively identified as N-demethyl-gelsevirine.

Metabolite M2 eluted at a retention time of 13.6 min and showed the  $[M + H]^+$  ion at  $m/z$  355. The elemental composition of the protonated ion of M2 at  $m/z$  355.2037 was  $C_{21}H_{27}N_2O_3^+$  (predicted 355.2016 Da), according to the formula predictor software (Agilent Mass Hunter software (version B.01.03 Build 1.3.157.0 2)), suggesting that M2 was a hydrogenated metabolite of gelsevirine. M2 (Table 1) generated fragment ions at  $m/z$  324, which was 2 Da

**TABLE 1** Retention times (Rts), elemental compositions, observed and predicted masses, mass errors and product ions of gelsevirine and its metabolites

Compound	Rt (min)	Elemental composition	Observed mass	Predicated mass	Error (ppm)	Product ions
Gelsevirine	9.6	C <sub>21</sub> H <sub>25</sub> N <sub>2</sub> O <sub>3</sub> <sup>+</sup> ([M + H] <sup>+</sup> )	353.1887	353.1860	-7.75	322.1678(-0.69); 291.1495(-1.07); 260.1075(-1.97); 108.0822(-13.3); 70.0663(-17)
M1	9.2	C <sub>20</sub> H <sub>23</sub> N <sub>2</sub> O <sub>3</sub> <sup>+</sup> ([M + H] <sup>+</sup> )	339.1727	339.1703	-7.04	260.1077(-2.74); 96.0799(9.21); 56.0497(-4.08)
M2	13.6	C <sub>21</sub> H <sub>27</sub> N <sub>2</sub> O <sub>3</sub> <sup>+</sup> ([M + H] <sup>+</sup> )	355.2037	355.2016	-5.87	324.1826(1.95); 178.1226(0.23); 122.0946(15.08)
M3	10.2	C <sub>21</sub> H <sub>25</sub> N <sub>2</sub> O <sub>4</sub> <sup>+</sup> ([M + H] <sup>+</sup> )	369.1836	369.1809	-7.38	260.1508(4.36); 86.0594(7.53)
M4	10.7	C <sub>21</sub> H <sub>27</sub> N <sub>2</sub> O <sub>4</sub> <sup>+</sup> ([M + H] <sup>+</sup> )	371.1987	371.1965	-5.85	322.1654(6.79); 291.1468(8.24); 70.0648(4.72)
M5	10.3	C <sub>21</sub> H <sub>23</sub> N <sub>2</sub> O <sub>3</sub> <sup>+</sup> ([M + H] <sup>+</sup> )	351.1735	351.1703	-9.08	320.1508(3.54); 260.1091(-8.14); 237.1015(3.13)
M6	17.9	C <sub>21</sub> H <sub>23</sub> N <sub>2</sub> O <sub>4</sub> <sup>+</sup> ([M + H] <sup>+</sup> )	367.1683	367.1652	-8.37	336.1453(4.61); 253.0950(8.55)

higher than ion at  $m/z$  322 of gelsevirine. The MS<sup>2</sup> spectrum of M2 showed fragment ions at  $m/z$  178 and 122. According to the retention time of the hydrogenated metabolite of gelsevirine at the C = O double bond from our previous reports, we proposed that M2 was a hydrogenated metabolite of gelsevirine at the C = C double bond.

Metabolite M3 had a retention time of 10.2 min and showed that the [M + H]<sup>+</sup> ion at  $m/z$  369 was 16 Da higher than that of gelsevirine. As seen in Table 1, M3 generated the same fragment ion at  $m/z$  260 as gelsevirine. The fragment ion at  $m/z$  86 was 16 Da higher than the ion at  $m/z$  70 of gelsevirine. These observations suggested that M3 was tentatively identified as the gelsevirine N-oxide on the nitrogen at position 4.

Metabolite M4 had a retention time of 10.7 min and showed that the [M + H]<sup>+</sup> ion at  $m/z$  371 was 18 Da higher than that of gelsevirine. M4 generated the same fragment ions at  $m/z$  322, 291 and 70 as gelsevirine, indicating the position of hydrogenation at the C = O double bond instead of the C = C double bond and hydroxylation of the methoxy group. Therefore, we speculated that M4 was a hydroxylated and hydrogenated metabolite of gelsevirine.

Metabolite M5 had a retention time of 10.3 min and showed that the [M + H]<sup>+</sup> ion at  $m/z$  351 was 2 Da lower than that of gelsevirine. The fragment ion at  $m/z$  320 was 2 Da lower than the ion at  $m/z$  322 of gelsevirine. M5 generated the same fragment ion at  $m/z$  260 as gelsevirine, indicating that dehydrogenation occurred on carbon 21 and nitrogen 4. According to a previous report of gelsevirine metabolism, we speculated that M5 was a dehydrogenated metabolite of gelsevirine at carbon 21 and nitrogen 4.

Metabolite M6 had a retention time of 17.9 min and showed that the [M + H]<sup>+</sup> ion at  $m/z$  367 was 14 Da higher than that of gelsevirine. The elemental composition of the protonated ion of M6 at  $m/z$  367.1687 was C<sub>21</sub>H<sub>23</sub>N<sub>2</sub>O<sub>4</sub><sup>+</sup> (predicted 367.1652 Da). M6 lost an OCH<sub>3</sub> group (observed 31.0234 Da, predicted 31.0184 Da) to form fragment ion at  $m/z$  336. M6 generated fragment ions at  $m/z$  336 and 253 that were 16 Da higher than ions at  $m/z$  320 and 237 of M5,

**TABLE 2** Summary of gelsevirine metabolites detected in human, pig, goat and rat liver microsomes

Compound	Human	Pig	Goat	Rat
M1	4.1 × 10 <sup>5</sup>	3.9 × 10 <sup>6</sup>	3.3 × 10 <sup>5</sup>	3.0 × 10 <sup>5</sup>
M2	2.0 × 10 <sup>5</sup>	ND	1.3 × 10 <sup>5</sup>	1.6 × 10 <sup>5</sup>
M3	4.1 × 10 <sup>6</sup>	8.2 × 10 <sup>6</sup>	8.1 × 10 <sup>5</sup>	4.2 × 10 <sup>5</sup>
M4	1.3 × 10 <sup>6</sup>	1.6 × 10 <sup>6</sup>	1.3 × 10 <sup>6</sup>	1.6 × 10 <sup>6</sup>
M5	1.8 × 10 <sup>6</sup>	5.2 × 10 <sup>6</sup>	ND	ND
M6	2.7 × 10 <sup>5</sup>	6.5 × 10 <sup>6</sup>	ND	ND

Note: Abbreviation: ND, not detected.

indicating that M6 was a hydroxylated metabolite of M5. Therefore, it is speculated that M6 is a hydroxylated metabolite of M5.

### 3.2 | Metabolism of gelsevirine in human, pig, goat and rat liver microsomes

Table 2 lists the metabolites of gelsevirine detected in human, pig, goat and rat liver microsomes. The results showed the same number of gelsevirine metabolites (M1–M4) after incubation with rat and goat liver microsomes. In addition to the four metabolites that were detected in both rat and goat liver microsomes, metabolites M5 and M6 were also observed in human microsomes. A total of five gelsevirine metabolites (M1, M3–M6) were identified in pig liver microsomes. The representative EICs of gelsevirine metabolites formed in the four species are shown in Figures S1–S3, and the peak intensity was observed for semi-quantification. In the pig liver microsomes, the peak intensity of M1 was approximately 10 times that of the other three species. The peak concentration of M3 in pig and goat liver microsomes was twice that in human and rat liver microsomes. These results showed that M1 was identified in liver microsomes of all four species in the following order: pig > human > goat > rat. M2

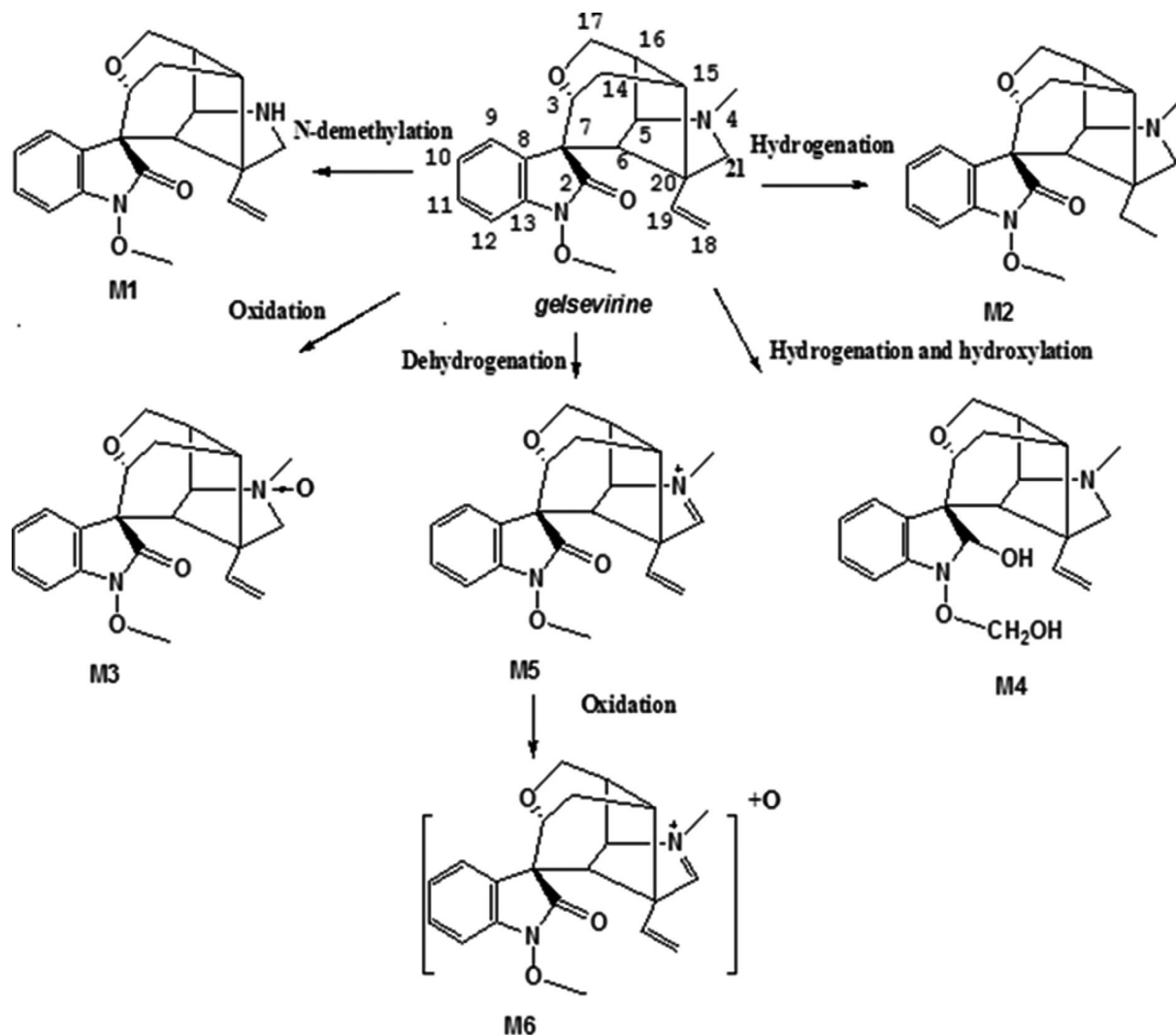


FIGURE 2 Proposed metabolic pathways of gelsevirine in liver microsomes in vitro

was not detected in pig liver microsomes, but in the other three species, the M2 content was identified in this order: human > rat > goat. M3 was detected to the greatest extent in pig liver microsomes and the least in rat liver microsomes. The amounts of M4 detected in the pig, goat, and rat liver microsomes were similar, and the amount detected in human liver microsomes was slightly higher. M5 and M6 were detected in only pig and human liver microsomes, and was observed to be more abundant in pig liver microsomes. Based on these results, the possible metabolic pathways of gelsevirine in liver microsomes were proposed and are shown in Figure 2.

#### 4 | DISCUSSION

We explored the preliminary metabolite identification of gelsevirine using the rat liver S9 fraction in our previous report (Zhang et al., 2019). In the present work, we further examined the species differences in the metabolism of gelsevirine in depth by using human, pig, goat and rat liver microsomes. Our previous results identified four metabolites

of gelsevirine in the rat liver S9 fraction. In this study, a total of six metabolites were identified in liver microsomes, of which N-demethyl-gelsevirine and N-oxide-gelsevirine, which were detected in the liver S9 fraction, were also generated in liver microsomes, and four other metabolites were found for the first time in liver microsomes. Except for metabolite M2, which was not found in pig liver microsomes, three metabolites (M1, M3, M4) were observed in rat, goat, pig and human liver microsomes. In addition to the four metabolites M1-M4, metabolites M5 and M6 were also identified in pig and human liver microsomes. These observations showed that the metabolism of gelsevirine in liver microsomes had almost certain qualitative species differences.

The present results also suggested that hydrogenation and subsequent hydroxylation were the main metabolic pathways of gelsevirine metabolism in rats and goats. N-dioxide and dehydrogenation were also the common main metabolic pathways of gelsevirine in pig and human liver microsomes. Moreover, dehydrogenation, subsequent hydroxylation and hydrogenation followed by hydroxylation were the main metabolic pathways of gelsevirine in pig and human liver microsomes, respectively. Quantitative species differences in the metabolism of gelsevirine among



the three species were observed, and different metabolites were detected in different species of liver microsomes in different amounts. The results indicate that the methylation ability of gelsevirine in pigs was the highest among the four species. The abilities of N-dioxide, dehydrogenation and subsequent hydroxylation of gelsevirine in pigs and humans were higher than those in goats and rats. The quantitative differences in the formation of gelsevirine metabolites found among the four animal species may be related to the levels of the enzymes involved in the liver.

## 5 | CONCLUSIONS

In conclusion, HPLC-QqTOF/MS is a rapid and accurate means to compare the metabolism of gelsevirine in human, pig, goat and rat liver microsomes. Except for metabolite M2, which was not found in pig liver microsomes, three metabolites (M1, M3, M4) were observed in rat, goat, pig and human liver microsomes. In addition to the four metabolites M1–M4, metabolites M5 and M6 were also identified in pig and human liver microsomes. There were qualitative and quantitative species differences in the metabolism of gelsevirine among the four species. The proposed metabolic pathway of gelsevirine in liver microsomes will provide a basis for further studies of the in vivo metabolism of this drug and will improve the toxicological assessment of gelsevirine in animals. However, the enzymes responsible for the metabolism of gelsevirine by microsomes of different species require further study.

## AUTHOR CONTRIBUTION

**Huahai Zhang:** Data curation; Formal analysis; Investigation; Methodology; Software; Writing-original draft; Writing-review & editing. **Wenjia Yang:** Formal analysis; Investigation; Methodology; Software; Writing-review & editing. **Yajun Huang:** Formal analysis; Methodology; Software; Writing-original draft; Writing-review & editing. **Wenjing Li:** Formal analysis; Methodology; Writing-review & editing. **Shuoxin Zhang:** Conceptualization; Funding acquisition; Resources; Supervision. **Zhao-Ying Liu:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Project administration; Resources; Supervision; Writing-review & editing.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.499>.

## DATA AVAILABILITY STATEMENT

All data used during the study appear in the submitted article.

## ORCID

Wen-Jing Li  <https://orcid.org/0000-0002-2222-4609>

Zhao-Ying Liu  <https://orcid.org/0000-0002-9050-2287>

## REFERENCES

Chen, C.-J., Zhong, Z.-F., Xin, Z.-M., Hong, L.-H., Su, Y.-P., & Yu, C.-X. (2017). Koumine exhibits anxiolytic properties without inducing

adverse neurological effects on functional observation battery, open-field and Vogel conflict tests in rodents. *Journal of Natural Medicines*, 71(2), 397–408. <https://doi.org/10.1007/s11418-017-1070-0>

- Jin, G.-L., Su, Y.-P., Liu, M., Xu, Y., Yang, J., Liao, K.-J., & Yu, C.-X. (2014). Medicinal plants of the genus *Gelsemium* (Gelsemiaceae, Gentianales): A review of their phytochemistry, pharmacology, toxicology and traditional use. *Journal of Ethnopharmacology*, 152(1), 33–52. <https://doi.org/10.1016/j.jep.2014.01.003>
- Kitajima, M. (2006). Chemical studies on monoterpenoidindole alkaloids from medicinal plant resources *Gelsemium* and *Ophiorrhiza*. *Journal of Natural Medicines*, 61, 14–23. <https://doi.org/10.1007/s11418-06-0101-z>
- Kitajima, M., Kogure, N., Yamaguchi, K., Takayama, H., & Aimi, N. (2003). Structure reinvestigation of gelsemoxonine, a constituent of *Gelsemium elegans*, reveals a novel, azetidone-containing indole alkaloid. *Organic Letters*, 5(12), 2075–2078. <https://doi.org/10.1021/ol0344725>
- Liu, M., Huang, H.-H., Yang, J., Su, Y.-P., Lin, H.-W., Lin, L.-Q., Liao, W.-J., & Yu, C.-X. (2012). The active alkaloids of *Gelsemium elegans* Benth. are potent anxiolytics. *Psychopharmacology (Berl)*, 225, 839–851. <https://doi.org/10.1007/s00213-012-2867-x>
- Liu, Y.-C., Xiao, S., Yang, K., Ling, L., Sun, Z.-L., & Liu, Z.-Y. (2017). Comprehensive identification and structural characterization of target components from *Gelsemium elegans* by high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry based on accurate mass databases combined with MS/M. *Journal of Mass Spectrometry*, 52(6), 378–396. <https://doi.org/10.1002/jms.3937>
- Su, Y.-P., Shen, J., Xu, Y., Zheng, M., & Yu, C.-X. (2011). Preparative separation of alkaloids from *Gelsemium elegans* Benth. using pH-zone-refining counter-current chromatography. *Journal of Chromatography*, 1218(23), 3695–3698. <https://doi.org/10.1002/jssc.200900561>
- Wang, Y., Wang, H., Wu, S., Li, D., & Chen, S. (2017). Effect of *Gelsemium elegans* and *Mussaenda pubescens*, the components of a detoxification herbal formula, on disturbance of the intestinal absorptions of indole alkaloids in Caco-2 cells. *Evidence-Based Complementary and Alternative Medicine*, 2017, 1–10. <https://doi.org/10.1155/2017/6947948>
- Xiao, S., Huang, Y.-J., Sun, Z.-L., & Liu, Z.-Y. (2017). Structural elucidation of koumine metabolites by accurate mass measurements using high-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, 31, 309–314. <https://doi.org/10.1002/rcm.7797>
- Xu, Y.-K., Yang, S.-P., Liao, S.-G., Zhang, H., Lin, L.-P., Ding, J., & Yue, J.-M. (2006). Alkaloids from *Gelsemium elegans*. *Journal of Natural Products*, 69(9), 1347–1350. <https://doi.org/10.1021/np060156y>
- Yang, K., Huang, Y.-J., Xiao, S., Liu, Y.-C., Sun, Z.-L., Liu, Y.-S., Tang, Q., & Liu, Z.-Y. (2017). Identification of gelsemine metabolites in rat liver S9 by high-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, 32, 19–22. <https://doi.org/10.1002/rcm.8012>
- Zhang, H.-H., Huang, Y.-J., Liu, Y.-C., Jiang, X.-Y., Zhang, S.-X., & Liu, Z. Y. (2019). Characterization of gelsevirine metabolites in rat liver S9 by accurate mass measurements using high-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, 33, 1179–1184. <https://doi.org/10.1002/rcm.8457>
- Zhang, H.-H., Wu, Y., Sun, Z.-L., & Liu, Z.-Y. (2013). Identification of sanguinarine metabolites in pig liver preparations by accurate mass measurements using electrospray ionization hybrid ion trap/time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, 27, 978–984. <https://doi.org/10.1002/rcm.6538>
- Zhang, J.-Y., & Wang, Y.-X. (2015). *Gelsemium* analgesia and the spinal glycine receptor/alloprengnanolone pathway. *Fitoterapia*, 100, 35–43. <https://doi.org/10.1016/j.fitote.2014.11.002>

Zhang, Z., Di, Y.-T., Wang, Y.-H., Zhang, Z., Mu, S.-Z., Fang, X., Zhang, Y., Tan, C.-J., Zhang, Q., Yan, X.-H., Guo, J., Li, C.-S., & Hao, X.-J. (2009). Gelegamines A-E: Five new oxindole alkaloids from *Gelsemium elegans*. *Tetrahedron*, *65*(23), 4551–4556. <https://doi.org/10.1016/j.tet.2009.03.086>

**How to cite this article:** Zhang H-H, Yang W-J, Huang Y-J, Li W-J, Zhang S-X, Liu Z-Y. The metabolism of gelsevirine in human, pig, goat and rat liver microsomes. *Vet Med Sci*. 2021;7:2086–2092. <https://doi.org/10.1002/vms3.499>

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.