Excretion, Metabolism, and Tissue Distribution of *Gelsemium elegans* (Gardn. & Champ.) Benth in Pigs

Xiao Ma 1,2,†, Zi-Yuan Wang 1,2,*, Meng-Ting Zuo 1,2, Kun Yang 1,2, Zhi-Liang Sun 1,2, Yong Wu 1,3,* and Zhao-Ying Liu 1,2,†

1 College of Veterinary Medicine, Hunan Agricultural University, 1 Nongda Rd, District Furong, Changsha 410128, China; maxiaoad489@163.com (X.M.); 15874802528@163.com (Z.-Y.W.);
zuomengting@aliyun.com (M.-T.Z.); yangkun409@163.com (K.Y.); sunzhiliang1965@aliyun.com (Z.-L.S.)
2 Hunan Engineering Technology Research Center of Veterinary Drugs, Hunan Agricultural University, 1 Nongda Rd, District Furong, Changsha 410128, China
3 Hunan Canzho Biological Technology Co., Ltd., 321 Kangning Road, District Economic and Technological Development, Liuyang 410329, China
* Correspondence: wuyong712@126.com (Y.W.); liu_zhaoying@hunau.edu.cn (Z.-Y.L.)
† These authors contributed equally to this work.

Abstract: *Gelsemium elegans* (Gardn. & Champ.) Benth is a toxic flowering plant in the family Loganiaceae used to treat skin diseases, neuralgia and acute pain. The high toxicity of *G. elegans* restricts its development and clinical applications, but in veterinary applications, *G. elegans* has been fed to pigs as a feed additive without poisoning. However, until now, the in vivo processes of the multiple components of *G. elegans* have not been studied. This study investigates the excretion, metabolism and tissue distribution of the multiple components of *G. elegans* after feeding it to pigs in medicated feed. Pigs were fed 2% *G. elegans* powder in feed for 45 days. The plasma, urine, bile, feces and tissues (heart, liver, lung, spleen, brain, spinal cord, adrenal gland, testis, thigh muscle, abdominal muscle and back muscle) were collected 6 h after the last feeding and analyzed using high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Five natural products in plasma, twelve natural products and five metabolites in urine, and three natural products in feces were characterized, suggesting that multiple components from *G. elegans* were excreted in the urine. However, ten natural products and four metabolites were detected in bile samples, which suggested that *G. elegans* is involved in enterohepatic circulation in pigs. A total of seven of these metabolites were characterized, and four metabolites were glucuronidated metabolites. Ten natural products and six metabolites were detected in the tissues, which indicates that *G. elegans* is widely distributed in tissues and can cross the blood-brain barrier. Among the characterized compounds, a highly toxic gelseidine-type alkaloid from *G. elegans* was the main compound detected in all biological samples. This is the first study of the excretion, metabolism and tissue distribution of multiple components from *G. elegans* in pigs. These data can provide an important reference to explain the efficacy and toxicity of *G. elegans*. Additionally, the results of the tissue distribution of *G. elegans* are of great value for further residue depletion studies and safety evaluations of products of animals fed *G. elegans*.

Keywords: absorption; excretion; tissue distribution; metabolic profile; *Gelsemium*

1. Introduction

*Gelsemium* is a genus of flowering plants in the Loganiaceae family [1]. There are four species in this genus: the Asian species, *Gelsemium elegans* (Gardn. & Champ.) Benth (*G. elegans*) and G. rankii Small and two North American species (*Gelsemium sempervirens* (L.) J.St.-Hil. and *Gelsemium rankinii Small*) [2]. The use of *Gelsemium sempervirens* traces back to the 19th century as a homeopathic agent to reduce anticipatory anxiety [3]. Pharmacological reports of *Gelsemium rankinii Small* are scarce because it is a rare species from the southeastern United States [4]. In China, *G. elegans* has been reported for its analgesic and...
anti-inflammatory effects and has been used to treat skin diseases, neuralgia and acute pain [1]. However, the high toxicity of G. elegans is the main limiting factor for its clinical applications. Generally, limb weakness, vomiting, arrhythmia, coma and other symptoms will occur after intoxication, and severe poisoning could lead to death [5]. However, G. elegans has growth-promoting effects in pigs in traditional Chinese medicine, so G. elegans could be added to feed [6]. However, this will cause G. elegans residue in products of animal origin and pose a threat to the safety of consumers, so it is necessary to study the excretion, metabolism and tissue distribution of G. elegans.

There are many compounds in G. elegans. Up to now, a total of 121 alkaloids, 25 iridoids, and several other compounds from a wide spectrum of secondary metabolite classes have been found in G. elegans. Based on the chemical structures of the alkaloids, they have been classified into the following six types: gelsedine-type, gelsemine-type, humantene-type, koumine-type, sarpagine-type and yohimbane-type [7–9]. Among them, the contents of koumine and gelsemine are high, and these types can be rapidly absorbed and widely distributed in tissues and can pass through the blood-brain barrier in rats. Moreover, their toxicity is low, with LD50 values of 99 mg/kg and 56.2 mg/kg [1], respectively. The toxicity of gelsenicne is high, with an LD50 of 0.185 mg/kg [1], and it also has the characteristics of fast absorption, wide distribution, and the ability to cross the blood-brain barrier in rats and mice [10]. A previous study characterized CYP3A4/5 as the main metabolic enzyme of G. elegans alkaloids, which can reduce the toxicity of G. elegans alkaloids through metabolism [11]. In addition, our previous studies investigated the pharmacokinetics of a G. elegans extract in pigs and found that G. elegans alkaloids were characterized by fast absorption and wide distribution, which are similar characteristics to single alkaloids in rats and mice [12]. However, the metabolism and tissue distribution of G. elegans in pigs have not been studied.

Significant differences have been observed between species with respect to the toxicity of G. elegans. According to the existing studies, it has been speculated that the differences in G. elegans toxicity may be closely related to metabolism [11,13]. Many studies have found differences in the metabolism of koumine and gelsemine in liver microsomes of pigs and rats in vitro. However, these previous studies have all been based on the metabolism of single alkaloids in vitro, which cannot completely reflect the metabolism and distribution of multiple components of G. elegans in vivo. Only one study by our team has reported the metabolic profile of multiple components of G. elegans in goats. Therefore, it is important to investigate the in vivo excretion, tissue distribution, and metabolic profile of G. elegans in pigs.

The present study characterized the multiple components of G. elegans in pig plasma, urine, bile, feces, and tissues by using high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (HPLC/QqTOF-MS). This study explored the excretion, metabolism and tissue distribution of G. elegans in pigs. These results may play an important role in explaining the toxicological and pharmacological effects of G. elegans. In addition, the tissue distribution data may provide a reference for further study on the elimination of tissue residues of G. elegans and the safety of products of animal origin, ensuring the safety of consumers.

2. Results

Recently, metabolism studies of G. elegans in goats were reported by our team [14]. The current study’s naming and analysis strategies of the characterized natural products and metabolites were consistent with the previously reported publication. As a result, twenty natural products and seven metabolites were characterized in plasma, urine, bile, feces and tissues. Information on these compounds is shown in Table 1. The main structures of the natural products are shown in Figure 1.
| Compound | RT (min) | Molecular Formula | [M + H]⁺ Error (ppm) | Fragment Ions (m/z) | Compound Class | Name | Proposed Metabolism | Source |
|----------|---------|-------------------|---------------------|------------------|------------------|------|---------------------|--------|
| H1       | 5.0     | C₁₈H₂₄N₂O₁₀      | 309.1579 6.02       | 236.1023, 219.0609, 91.0333, 56.0469 | Alkaloid, Gelsemine-type | Unknown | B                   |
| H2       | 5.2     | C₂₀H₂₂N₂O₉       | 323.1750 1.26       | 236.1076, 115.0551, 77.0367, 70.0641 | Alkaloid, Gelsemine-type | Gelsemine | B                   |
| H6       | 13.4    | C₂₀H₂₂N₂O₉       | 337.1565 −5.45      | 236.1052, 91.0501 | Alkaloid, Gelsemine-type | 21-Oxogelsemine | B, U |
| H6-M1    | 6.0     | C₂₀H₂₂N₂O₉       | 339.1667 10.7       | 206.0973, 77.0389 | Alkaloid, Gelsemine-type | +2H | B                   |
| H6-M2    | 9.8     | C₂₀H₂₂N₂O₉       | 353.1500 −1.18      | 218.0893, 180.0933, 91.0356, 73.0355 | Alkaloid, Gelsemine-type | +O | U                   |
| H6-M3    | 13.4    | C₂₀H₂₂N₂O₉       | 513.1845 4.41       | 337.1425, 91.0490, 73.0355 | Alkaloid, Gelsemine-type | +GlcA | B, U |
| H10      | 5.4     | C₁₈H₂₄N₂O₁₀      | 359.1694 0.7        | 311.1170, 80.0467, 68.0465 | Alkaloid, Gelsemine-type | 11,14-Dihydroxygelsemine | B, U, P |
| H11      | 5.6     | C₁₈H₂₄N₂O₁₀      | 343.1634 5.36       | 312.1356, 281.1184, 108.0689 | Alkaloid, Gelsemine-type | 11-Hydroxygelsemine | B, U, P |
| H11-M1   | 2.4     | C₁₈H₂₄N₂O₁₀      | 519.1978 −0.92      | 312.1504, 281.1258, 108.0389, 80.0476 | Alkaloid, Gelsemine-type | 14-Hydroxygelsemine | B, U, P |
| H12      | 6.6     | C₁₈H₂₄N₂O₁₀      | 343.1640 3.61       | 312.1428, 80.0491 | Alkaloid, Gelsemine-type | 15-Hydroxyhumatenosxenine | B, U, P |
| H14      | 9.7     | C₂₀H₂₂N₂O₉       | 327.1703 0.06       | 265.1240, 108.0703, 95.0869 | Alkaloid, Gelsemine-type | Gelsemine | P                   |
| H15      | 10.9    | C₂₀H₂₂N₂O₉       | 385.1758 0          | 354.1357, 309.1307, 180.0876, 134.0581 | Alkaloid, Gelsemine-type | 11,14-Dihydroxygelsemine | B, U, P |
| H16      | 11.6    | C₂₀H₂₂N₂O₉       | 343.1631 6.24       | 238.0790, 128.0943, 118.0639 | Alkaloid, Gelsemine-type | Hydroyxyl of gelsemine | B, U |
| H18      | 11.4    | C₂₀H₂₂N₂O₉       | 371.1571 8.24       | 340.1368, 295.1354, 120.0412, 91.0508 | Alkaloid, Gelsemine-type | 11,14-Dihydroxygelsemine | B, U |
| H22      | 7.6     | C₁₄H₂₄N₂O₂       | 309.1511 11.53      | 194.0904, 167.0698, 115.0490 | Alkaloid, Sarpagine-type | Oxokoumine | U |
| H23      | 7.9     | C₁₄H₂₄N₂O₂       | 293.1600 −0.55      | 218.0984, 194.0917, 167.0721, 115.0486, 91.0548 | Alkaloid, Sarpagine-type | Dehydrokoumine | B, U, F, P |
| H23-M1   | 6.3     | C₁₄H₂₄N₂O₂       | 485.1920 −0.33      | 309.1506, 194.0918, 167.0697, 154.0607, 115.0543 | Alkaloid, Gelsemine-type | +GlcA | B, U |
| H23-M2   | 6.5     | C₁₄H₂₄N₂O₂       | 309.1586 3.75       | 291.1457, 234.1227, 194.0946, 133.0890 | Alkaloid, Gelsemine-type | +O | Only detected in tissue samples |
| H30      | 11.5    | C₁₈H₂₄N₂O₁₀      | 357.1804 1.36       | 311.1888, 178.1097, 108.0794, 77.0377 | Alkaloid, Humatenosxenine-type | Gelsemine | B, U, F |
| H30-M1   | 7.3     | C₁₈H₂₄N₂O₁₀      | 533.2136 −1.18      | 326.1654, 311.1906, 164.1064, 148.0401 | Alkaloid, Humatenosxenine-type | 14-Hydroxykoumine | B, U |
| H31      | 13.6    | C₂₁H₂₄N₂O₁₀      | 355.2006 2.88       | 309.1609, 122.0968 | Alkaloid, Humatenosxenine-type | Humatenosxenine | +GlcA | Only detected in tissue samples |
| H32      | 7.2     | C₁₄H₂₄N₂O₂       | 307.1786 6.17       | 277.1684, 220.1106, 238.1197 | Alkaloid, Koumine-type | Koumine | U |
| H35      | 4.7     | C₁₄H₂₄N₂O₂       | 377.1422 5.38       | 165.0797, 119.0545, 105.0684, 73.0710 | Alkaloid, Iridoids | 9-Hydroxyisepersamide | U |
| H39      | 4.6     | C₁₄H₂₄N₂O₂       | 201.3119 1.18       | 119.0859, 103.0541, 91.0356, 77.0391 | Alkaloid, Iridoids | Gelsemine | U |
| H44      | 17.5    | C₂₀H₂₄O₃         | 453.3410 −10.78     | 209.1656, 114.0907, 96.0787, 69.0680 | Alkaloid, Sarpagine-type | Triptol | B, U, F, F |
| H45      | 17.7    | C₂₀H₂₄O₃         | 373.1758 −2.15      | 260.0920, 214.0844, 130.1252 | Alkaloid, Gelsemine-type | 13β-Hydroxygelsemine | B, U, F, F |
| H46      | 8.3     | C₁₄H₂₄N₂O₂       | 295.1801 1.33       | 277.1667, 156.0797, 144.0781, 138.0900, 108.0778 | Alkaloid, Sarpagine-type | Koumine | U |

Note: “H” means natural products; “M” means metabolites (M1 to M3 means the natural product has three metabolites identified); “U” means urine sample; “P” means plasma sample; “F” means fecal sample; “B” means bile sample; and “()” means low concentration.
Figure 1. Main structures of the natural products identified in G. elegans: Gelsemine-type alkaloids (A), gelseidine-type alkaloids (B), sarpagine-type alkaloids (C), humantenine-type alkaloids (D), iridoids (E), koumine-type alkaloids (F), and triterpenes (G).

2.1. Characterization of Compounds in Plasma

Five natural products from G. elegans were characterized in plasma samples, and the accurate EICs of these compounds are shown in Figure 2. No metabolites of the natural products were found in plasma samples.

Figure 2. Accurate EICs of natural products and metabolites in plasma.
These five natural products were gelsemine-type alkaloids (compounds H11, H12, H14), a sarpagine-type alkaloid (compound H23), and a triterpene (compound H44). These five natural products were not found in blank samples. All of them were characterized based on their characteristic accurate MS2 fragment (Table 1). H12 and H14 were unique to plasma and were not detected in bile, urine or fecal samples. Compound H11 in the plasma sample was also detected in bile and urine samples. The MS2 spectrum of H14 exhibited a product ion at m/z 296, formed by the loss of CH3O from m/z 327. The ion at m/z 296 could further lose CH3O to produce an ion at m/z 265. The product ion at m/z 225 was formed by the loss of C3H4 from m/z 265; after comparison with the reference data, H14 was characterized as gelsenicine. The MS2 spectrum of H11 generated product ions at m/z 312 and m/z 281, which were 16 Da higher than the m/z values of 296 and 265 of the product ions of gelsenicine, respectively. The product ion at m/z 108 was the same as that in gelsenicine, so H11 is presumably 11-hydroxygelsemine.

2.2. Characterization of Compounds in Urine

Compared with the blank group, in all the biological samples of the experimental group, the highest number of compounds was detected in the urine samples, which shows that the metabolism of G. elegans is fast. Twelve natural products and five metabolites were characterized, and their EICs are shown in Figure 3.

2.2.1. Gelsemine-Type Alkaloids (H6) and Its Metabolic Products

Gelsemine-type natural products (H6) and two related metabolites (H6-M2 and H6-M3) were detected and identified. H6 was characterized as either gelsemine or 21-oxogelsemine based on comparing the data with the reference compounds. Metabolite H6-M2 was characterized as an oxidation metabolite of H6. Additionally, a phase II metabolite conjugated with glucuronic acid was detected and named H6-M3.

The retention time of H6 is 13.469 min, and the [M + H]+ was observed at m/z 337.1565. First, the MS2 spectrum of compound H6 generated a product ion at m/z 236, formed by the loss of C3H6O2 from m/z 337, and the product ion at m/z 204 was due to the loss of C6H7NO from m/z 337. Then, the product ion at m/z 77 may have been formed by the loss of C6H4 from m/z 236. Therefore, we speculate that compound H6 is 21-oxogelsemine.

Metabolite H6-M2 presented an m/z of 353, which was 16 Da greater than that of natural product H6. The product ion at m/z 222 of H6-M2 was also 16 Da higher than the product ion at m/z 206 of H6, suggesting that H6-M2 is an oxidation metabolite of H6. Glucuronic acid conjugates could produce the [M + H – 176] fragment in the MS2 spectrum. The ion produced by the loss of C4H8O6 from metabolite H6-M3 was the same as the m/z of compound H6. Therefore, metabolite H6-M3 was characterized as a phase II glucuronic acid conjugated metabolite of compound H6.

2.2.2. Gelsemine-Type Alkaloids (H10, H11, H15, H16, H18) and Their Metabolic Products

Five gelsemine-type natural products (compounds H10, H11, H15, H16, H18) and one related metabolite (H11-M1) were detected in urine samples. Compounds H10, H11, H15, H16 and H18 were characterized as 11,14-dihydroxygelsemine, 11-hydroxygelsemine, 15-hydroxyhumantenoxenine, hydroxygelsemine and gelsemolenine B, respectively. Metabolite H11-M1 may be a phase II metabolite conjugated with glucuronic acid.

The retention time of compound H15 was 10.9 min, which was used as an example for the characterization of gelsemine-type compounds. The product ion at m/z 354 was formed by the loss of OCH3 from m/z 385, and further, the product ion at m/z 309 was formed by the loss of CHO2 from m/z 354. The product ion at m/z 134 may have been formed by loss of CH2NO from m/z 180. According to this fragmentation information, natural product H15 was suggested to be 15-hydroxyhumantenoxenine.

Metabolite H11-M1 may be a phase II glucuronic acid conjugated metabolite because the [M + H]+ of H11-M1 was observed at m/z 519, 176 Da higher than the m/z of H11.
Figure 3. Accurate EICs of natural products and metabolites in urine.
2.2.3. Sarpagine-Type Alkaloids (H23)

There was only one compound (compound H23) belonging to the sarpagine type in bile samples, and no metabolites were found. In our previous study, the compound koumidine was found in the crude extract of *G. elegans*. The MS2 spectrum of H23 was compared with the MS2 spectrum of koumidine, and the product ion at \( m/z \) 154 from H23 was 2 Da lower than that of the product ion at \( m/z \) 156 of koumidine. Combined with the other product ions, H23 was characterized as dehydrokoumidine.

2.2.4. Humantenine-Type Alkaloids (H30) and Its Metabolic Products

Compound (H30) was classified as a humantenine-type alkaloid. The phase II metabolite of H30 was characterized and named H30-M1.

The fragment ions of compound H30 showed that the product ion at \( m/z \) 326 was formed by the loss of OCH\(_3\) from \( m/z \) 357. The product ion at \( m/z \) 311 was formed by the loss of CH\(_3\) from \( m/z \) 326, and the further loss of CH\(_2\) from the ion at \( m/z \) 311 followed to generate the \( m/z \) of 297. Compound H30 was tentatively characterized as 14-hydroxyrankinidine.

Metabolite H30-M1 displayed an \([M + H]^+\) ion at \( m/z \) 533, which is consistent with the molecular formula of C\(_{26}\)H\(_{32}\)N\(_2\)O\(_{10}\). The MS2 spectrum revealed that the \([M + H]^+\) ion could further lose a glucuronic acid moiety (176 Da) to produce the fragment ion at \( m/z \) 357. The fragment ion at \( m/z \) 357 then fragmented into an ion at \( m/z \) 311, which was consistent with the fragmentation of H30. Therefore, we deduced that metabolite H30-M1 was a glucuronidated metabolite of 14-hydroxyrankinidine.

2.2.5. Non-Alkaloids (H37, H39, H44)

Two iridoid compounds (H37 and H39) and one triterpene compound (H44) were found in urine samples. H37 and H39 were characterized as 9-hydroxysemperoside and gelsemiol. H44 was characterized by triterpene 3-keto-urs-11-en-13β(28)-olidevia database matching.

2.3. Characterization of Compounds in Bile

A total of ten natural products and four metabolites were detected in bile samples. These ten natural products and four metabolites were not found in the blank samples. Accurate EICs of the natural products and metabolites in bile samples are shown in Figure 4.

Three of these natural products were determined to be gelsemine-type alkaloids (compounds H1, H2, H6), four were determined as gelseidine-type alkaloids (compounds H10, H11, H15, H16), and the other three were a sarpagine-type alkaloid (compound H23), a humantenine-type alkaloid (compound H30) and a triterpene (compound H44). Based on the MS2 data and accurate mass analysis, the structures of these compounds were characterized. The protonated molecular ion ([M + H]\(^+\)) of H2 was observed at \( m/z \) 323.1750 (C\(_{20}\)H\(_{23}\)N\(_2\)O\(_3\)^+\), 1.26 ppm). As observed from the MS2 spectrum, the more abundant product ion at \( m/z \) 236.1076 was formed by the neutral losses of CH\(_3\)O (30 Da) and C3H\(_7\)N (57 Da). H2 was characterized as gelsemine by comparison with the reference standard.

A total of four metabolites (H6-M1, H6-M3, H11-M1, H30-M1) were detected in the bile samples. H6-M3, H11-M1 and H30-M1 may be phase II glucuronic acid conjugated metabolites of compounds because their \([M + H]^+\) values were observed at \( m/z \) 519, 513 and 533, which were all 176 Da greater than H6, H11 and H30, respectively. When the natural products (H6, H11, H30) were combined with glucuronic acid, the water solubility increased, followed by entry into the bile and participation in enterohepatic circulation.
Figure 4. Accurate EICs of natural products and metabolites in bile.

2.4. Compounds Identification in Feces

Three compounds were tentatively identified via comparisons of the retention times and observed masses in feces samples, including one sarpagine-type alkaloids (H23), one humantanine-type alkaloids (H30), and a triterpene (H44). Only three natural products were detected in the feces of the experimental group, and no metabolites were detected.
Many compounds were detected in bile but not in feces, suggesting that these compounds may have been reabsorbed. The accurate EICs of those compounds are shown in Figure 5.

![Figure 5. Accurate EICs of natural products and metabolites in feces.](image)

### 2.5. Characterization of Compounds in Tissue

As a result, nine natural products were detected in the brain, which suggested that *G. elegans* could cross the blood-brain barrier. All compounds were a gelsermine-type alkaloid (*H2*), gelsedine-type alkaloids (*H11, H45*), sarpagine-type alkaloids (*H23, H46*), humantenine-type alkaloids (*H30, H31*), a koumine-type alkaloid (*H32*) and a triterpene (*H44*). *H46* and *H32* were selected as examples to characterize the fragmentation patterns for the other compounds. *H46* was characterized as koumidine, the major fragment ion at *m/z* 277 was due to the loss of H$_2$O from the ion at *m/z* 295, and compared with the reference data, the structure was determined. Compound *H32* was characterized as koumine because the fragment ion at *m/z* 277 was formed by loss of CH$_2$ from the ion at *m/z* 307 and the further loss of C$_3$H$_7$N to form an *m/z* of 220. Moreover, the ion at *m/z* 307 was formed by *m/z* 238 + *m/z* 70. Only three compounds were detected in the spinal cord, including gelsermine-type alkaloids (*H2, H45*) and a sarpagine-type alkaloid (*H23*), which were also detected in the brain. All compounds detected in the tissues are shown in Table 2.

A total of eight compounds were tentatively characterized in the heart, including *H2, H11, H14, H23, H30, H31, H32* and *H44*. Only compound *H23* was detected in the liver and adrenal gland, and their content was very low. Five natural products were characterized in the lung, including *H2, H14, H23, H46, H30*, and *H23-M2* were also detected. Compounds *H2, H11, H14, H23, H30, H32* and *H44* were also detected in the spleen and testis, compound *H46* was only found in the spleen, and *H31, H44* and *H30-M1* were only detected in the genitals.

Finally, compounds *H2, H11, H14, H23, H30* and *H32* were detected in the thigh, abdominal, and back muscle samples. However, *H31* was only found in abdominal and back muscles, and *H44* was only detected in back muscles.

Dehydrokoumidine (*H23*) was detected in all tissues, and its content in the lung was the highest. Gelsermine (*H2*) was also detected in most tissues except the lungs and adrenal gland and was highest in the brain. Two metabolites (*H23-M1, H30-M1*) were detected only in the lungs, spleen, and testis, indicating that most metabolites are highly polar and excreted through the kidneys and bile.
| Tissues          | Gelsemine (H2) | 11-Hydroxygelsenicene (H11) | Gelsenicene (H14) | Dehydrokoumidine (H23) | 14-Hydroxyrankinidine (H130) | Humantennine (H31) | Koumine (H32) | 3-keto-urs-11-en-13β (28)-olide (H44) | GS-2(11-Methoxy-14-Hydroxygelsenicene) | Koumidine (H46) | H23-M2 | H30-M1 |
|-----------------|----------------|-----------------------------|-------------------|------------------------|-------------------------------|-------------------|-------------|-------------------------------------|---------------------------------------|----------------|--------|--------|
| Brain           | 8 × 10⁶        | 2.9 × 10⁷                   | -                 | 0.9 × 10⁹              | 2.1 × 10⁶                     | 2 × 10⁵           | 4 × 10⁶      | 2.9 × 10⁷                           | 2.2 × 10⁵                             | 3.1 × 10⁶       | -       | -       |
| Spinal cord     | 3 × 10³        | -                           | -                 | 1 × 10⁹                | -                             | -                 | -           | -                                   | -                                | -              | -       | -       |
| Heart           | 8.2 × 10⁵      | 8 × 10⁵                     | 2.9 × 10⁵         | 2.9 × 10⁵              | 6.1 × 10⁵                     | 1.1 × 10⁵         | 2.6 × 10⁵    | 3.9 × 10⁷                           | -                                    | -              | -       | -       |
| Liver           | -              | -                           | -                 | 2.9 × 10⁵              | -                             | -                 | -           | -                                   | -                                    | -              | -       | -       |
| Lung            | 2.0 × 10⁶      | -                           | 1.9 × 10⁶         | 3.1 × 10⁶              | 2.9 × 10⁵                     | -                 | -           | -                                   | -                                    | -              | -       | -       |
| Spleen          | 2.5 × 10⁴      | 5.8 × 10⁵                   | 4.9 × 10⁵         | 6.1 × 10⁶              | 1.2 × 10⁵                     | -                 | 3.2 × 10³    | -                                   | -                                    | -              | 5.9 × 10⁶ | 3.1 × 10⁵ |
| Adrenal gland   | -              | -                           | 0.7 × 10⁹         | -                      | -                             | -                 | -           | -                                   | -                                    | -              | -       | -       |
| Testis          | 6.1 × 10⁵      | 1.3 × 10⁶                   | 2 × 10⁵           | 2 × 10⁵                | 4.2 × 10⁵                     | 1.0 × 10⁵         | 3.9 × 10⁵    | 3 × 10⁷                           | -                                    | -              | 5.6 × 10⁴ | 3.7 × 10⁵ |
| Thigh muscle    | 2.0 × 10⁶      | 1.4 × 10⁶                   | 3 × 10⁵           | 4.4 × 10⁶              | 0.75 × 10⁵                    | 0.9 × 10⁵         | 0.5 × 10⁵    | -                                  | -                                    | -              | -       | -       |
| Abdominal muscle| 1.25 × 10⁶     | 1 × 10⁶                     | 4 × 10⁵           | 2.1 × 10⁵              | 3.9 × 10⁵                     | -                 | 6 × 10⁵      | -                                  | -                                    | -              | -       | -       |
| Back muscle     | 7.9 × 10⁵      | 5 × 10⁵                     | 1.8 × 10⁵         | 1.9 × 10⁵              | 3.1 × 10⁵                     | 8 × 10⁵           | 2.5 × 10⁵    | 4.1 × 10⁷                           | -                                    | -              | -       | -       |

"-" means that it was not detected in the tissue sample.
3. Discussion

Previously, there have been studies on the pharmacokinetics of koumine, gelsemine, and gelsenicine in rats or mice [10]. These three single alkaloids have rapid absorption and are widely distributed and rapidly eliminated. Subsequently, our study explored the multicomponent pharmacokinetics of *G. elegans* in pigs [15,16]. The results showed that *G. elegans* alkaloids were rapidly absorbed in pigs but eliminated more slowly (the value of T1/2 was 8 to 12 h) than in rats and mice. However, multiple components’ metabolism and tissue distribution have not yet been studied. This study is the first to study the metabolism and tissue distribution of multiple components of *G. elegans* in pigs. This study detected ten natural products and four metabolites in bile, but only three natural products were detected in feces. Therefore, it is speculated that the slow elimination of *G. elegans* in pigs may be because *G. elegans* is involved in enterohepatic circulation. In addition, this study did not detect as many compounds because the pharmacokinetics study used the HPLC/QqQ-MS method. Because the detected concentration was low in the pig biological samples, many compounds were not detected by HPLC/QqTOF-MS. Although it is not possible to detect all compounds, metabolism and distribution studies can be performed on the compounds with a high content, which are active substances with *G. elegans* effects.

We used rat and pig liver S9 to investigate the in vitro metabolism of koumine and gelsemin [17,18]. The results show that the main metabolic pathways of koumine and gelsemine were dehydrogenation, hydrogenation, demethylation and oxidation, which was consistent with previous research results. However, these studies have not found phase II metabolites of these two alkaloids. Our previous study on goats used a single oral administration [14], but this study used long-term feeding. The method of administration was different, so the compounds detected in the biological samples of pigs were less than those observed in goats. In previous in vivo experiments in sheep, only plasma, urine and fecal samples were analyzed and determined, and a total of 44 absorbed natural products and 27 related metabolites were preliminarily characterized. Including gelsdine type, sarpagine type and gelsemine type alkaloids are the compounds with the highest amount of metabolites. Most natural products are metabolized by glucuronidation and oxidation. In addition, hydrogenation, dehydrogenation and demethylation reactions also occur. However, this study analyzed and characterized the metabolites in plasma, urine, feces, bile and various tissues. This study first investigated the metabolic profile of multiple components of *G. elegans* in pigs. The metabolic pathways of 21-oxogelsemine (H6), 11-hydroxygelsenicine (H11), dehydrokoumidine (H23) and 14-hydroxyrankinidine (H30) in pigs are mainly reduced by hydrogenation, oxidation and glucuronidation. Many compounds were detected in bile in this study, especially phase II metabolites, indicating the existence of enterohepatic circulation in pigs, which may be the reason for the slow elimination of *G. elegans*. In addition, glucuronidation was the major metabolic pathway in pigs, accounting for 42.9%, as diagrammed in Figure 6D.

Compounds from *G. elegans* can be detected in all tissues, indicating that *G. elegans* is widely distributed in pigs. Figure 6A,B shows that most natural products were detected in the brain from all tissues, including koumine (H32) and gelsemine (H2). Some studies have shown that koumine and gelsemine have antianxiety and analgesic effects. These two alkaloids have low toxicity and potential for development, indicating that the overall administration of *G. elegans* was a multicomponent multitargeted mechanism. Moreover, it is shown in Figure 6C that the highly toxic alkaloids, the gelsedine type, were present in the highest concentrations in all biological samples, which may be the main reason for human poisoning after eating *G. elegans*. *G. elegans* was not toxic to pigs [19], possibly because of different pharmacokinetics and pharmacodynamics between the receptors and gelsedine-type alkaloids within the different species, but this needs further study. Additionally, gelsedine-type alkaloids have been characterized and detected in the lung, heart, and other tissues, indicating that *G. elegans* poisoning, respiratory failure, and rapid heartbeat may be caused by nerves’ direct and indirect effects. The study also found that compounds from *G. elegans* were distributed in the muscles and liver, which may threaten the safety of...
consumers of these animal products. Therefore, the withdrawal time required for *G. elegans* alkaloids to be eliminated from edible tissues needs further residue depletion studies.

![Figure 6](image)

**Figure 6.** The number of natural products distributed in different biological samples (A). The number of metabolites distributed in different biological samples (B). The types of natural products and their metabolites in different biological samples (C). The types of metabolites formed by different metabolic pathways in different biological samples (D).

In conclusion, high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry was used in this study to characterize multiple components of *G. elegans* in pig plasma, urine, feces, bile and different tissues. This is the first study to provide comprehensive information on the absorption, excretion, metabolism and tissue distribution of multiple components of *G. elegans* in pigs. These findings may help determine gelsedine-type alkaloids from *G. elegans* for further toxicity and residue studies, laying the foundation for further safety evaluations of products of animals fed *G. elegans* and promoting the development and utilization of *G. elegans* in livestock farming.

### 4. Materials and Methods

#### 4.1. Chemicals and Reagents

Gelsemine (CAS#: 509-15-9, purity: 99.28%), koumine (CAS#: 1358-76-5, purity: 99.53%) and humantenmine (CAS#: 354-38-9, purity: 99.84%) were purchased from Shanghai Kang Biao Chemicals Co., Ltd. (Minhang District, Shanghai, China). Methanol, acetonitrile and formic acid were obtained from Merck Chemicals Co. (Darmstadt, Germany). Water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

#### 4.2. Plants Material

The raw material of *G. elegans* plants was cultivated in Longyan City, Fujian Province, China (N 24°43′12″, E 116°43′48″). Vegetative whole plants of *G. elegans* were collected in the area in 2016. Crude samples of *G. elegans* were dried in the sunlight for 2–3 days and milled into a powder. Then, the powder was passed through a 100-mesh sieve to obtain the *G. elegans* powder. Associate Professor Qi Tang at Hunan Agricultural University authenticated the samples. The samples were stored in our laboratory, and the voucher number is 1537201809.

#### 4.3. Animal Experiments

Nine male ternary hybrid pigs approximately 60 days old (20 ± 2 kg) were obtained from Hunan New Wellful Co., Ltd. (Changsha, China). Four were fed a complete diet as the control group, and the other five were fed *G. elegans* (a complete diet supplemented...
with 2% *G. elegans* powder) as the experimental group. After 45 days of continuous feeding, administration of *G. elegans* was stopped, and all pigs were fasted for 12 h [15]. The experimental protocol was performed in accordance with Animal Care and Use of China. Then, all the pigs were slaughtered (before slaughter, blood was collected from the common artery using vacuum blood collection vessels) 6 h after the last feeding. Bile and 11 tissue samples were obtained from each pig, including the heart, liver, lung, spleen, brain, spinal cord, adrenal gland, genitals, thigh muscle, abdominal muscle and back muscle. Instantly, anticoagulated blood was collected and centrifuged at 1200 rpm for 15 min. Since the defecation time of pigs is uncontrollable, all urine and feces excreted within six hours after stopping feeding were collected and stored at −80 °C.

4.3.1. Plasma Samples

Before analysis, pig plasma was warmed to room temperature, and 200 µL of each plasma sample was mixed with 1 mL of 1% formic acid-acetonitrile to precipitate the proteins, followed by centrifugation at 10,000 rpm for 10 min. The supernatant was filtered through a 0.22-µm microbore cellulose membrane for HPLC/QqTOF-MS analysis. Treatment of the plasma samples was based on our previous study [16].

4.3.2. Urine Samples

Urine (200 µL) was added to 1 mL of 1% formic acid-acetonitrile, vortexed 2–3 times for 1–2 min each time and centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through a 0.22-µm microbore cellulose membrane for HPLC/QqTOF-MS analysis. Treatment of the urine samples was similar to the treatment of the plasma samples.

4.3.3. Feces Samples

Two grams of feces were accurately weighed and mixed with 4 mL of 1% formic acid-acetonitrile and then centrifuged at 10,000 rpm for 10 min. One milliliter of supernatant was collected and filtered through a 0.22-µm microbore cellulose membrane for HPLC/QqTOF-MS analysis. Treatment of the fecal samples was similar to the treatment of the plasma samples.

4.3.4. Bile Samples

A 200 µL aliquot of bile was mixed with 1 mL of 1% formic acid-acetonitrile to precipitate the proteins and then centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through a 0.22-µm microbore cellulose membrane for HPLC/QqTOF-MS analysis. The treatment of bile samples was similar to the treatment of the plasma samples.

4.3.5. Tissue Samples

Tissue samples (2 g) were accurately weighed and mixed with 4 mL of 1% formic acid-acetonitrile. The samples were vortexed 2–3 times for 1–2 min each time and centrifuged at 10,000 rpm for 10 min. The supernatant was poured into a clean 10.0-mL centrifuge tube, the liquid was dried completely with nitrogen, and then the samples were dissolved in acetonitrile (200 µL) and 0.1% formic acid (800 µL). After all the solids had dissolved, 1 mL of the supernatant was filtered through a 0.22-µm microbore cellulose membrane for HPLC/QqTOF-MS analysis.

4.3.6. HPLC/QqTOF-MS Analysis Conditions

An Agilent 6530 Q-TOF mass spectrometer coupled with an Agilent 1290 HPLC system was used (Agilent Technologies, Palo Alto, CA, USA). The HPLC chromatographic system was equipped with an autosampler, a rapid-resolution binary pump, a vacuum degasser, a thermostatic column compartment and a UV detector. A Thermo-C18 column (2.1 mm × 150 mm i.d.; particle size, 3.5 µm) was used for the separation. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The gradient was as follows: 0–5 min, 10% B; 5–20 min, 10–90% B; 20–25 min, 90% B; and 25–30 min, 10% B. The
injection volume was 5 µL, and the flow rate was 0.3 mL/min. The column temperature was maintained at 30 °C.

The mass spectrometer was equipped with an electrospray ionization source and was operated in positive mode. Mass spectrometric analyses were carried out in full-scan MS mode with a mass range of m/z 50–1000 and auto MS/MS acquisition. The operating parameters were as follows: gas temperature, 300 °C; capillary voltage, 4.0 kV; nebulizer pressure, 35 psi; sheath gas temperature, 350 °C; sheath gas flow rate, 11 L/min; skimmer voltage, 65 V; and fragmentor voltage, 175 V. Nitrogen was used as the nebulizing gas at a flow rate of 9 L/min. Accurate mass measurements of each peak from the total ion chromatograms (TIC) were obtained using an automated calibrant delivery system to provide the mass corrections. The calibration solution contained internal reference masses at m/z 121.0508 and 922.0098 in positive ion mode. All data acquisition was controlled by Agilent Mass Hunter software (version B.01.03, build 1.3.157.0 2).

Author Contributions: Conceptualization, Z.-Y.L. and Z.-L.S.; methodology, Z.-Y.W., M.-T.Z. and Z.-Y.L.; investigation, Y.W. and K.Y.; data curation, X.M., Z.-Y.W. and M.-T.Z.; writing—original draft preparation, X.M. and Z.-Y.W.; writing—review and editing, Y.W. and Z.-Y.L.; project administration, Z.-Y.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (Grant No.31972737) and the Double first-class construction project of Hunan Agricultural University (No.kxk20180108).

Institutional Review Board Statement: This study was carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals of China and was approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences (IACUC# 201302).

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during the present study are included in this published article.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

References
1. Jin, G.L.; Su, Y.P.; Liu, M.; Xu, Y.; Yang, J.; Liao, K.J.; Yu, C.X. Medicinal plants of the genus Gelsemium (Gelsemiaceae, Gentianales)—A review of their phytochemistry, pharmacology, toxicology and traditional use. *J. Ethnopharmacol.* 2014, 152, 33–52. [CrossRef] [PubMed]
2. Xu, Y.K.; Yang, L.; Liao, S.G.; Cao, P.; Wu, B.; Hu, H.B.; Guo, J.; Zhang, P. Koumine, Humantenine, and Yohimbane Alkaloids from Gelsemium elegans. *J. Nat. Prod.* 2015, 78, 1511–1517. [CrossRef] [PubMed]
3. Paris, A.; Schmidlin, S.; Mouret, S.; Hodaj, E.; Marijnen, P.; Boujedaini, N.; Polosan, M.; Cracowski, J.L. Effect of Gelsemium 5CH and 15CH on anticipatory anxiety: A phase III, single-centre, randomized, placebo-controlled study. *Fundam. Clin. Pharmacol.* 2012, 26, 751–760. [CrossRef] [PubMed]
4. Pascarella, J.B. Mechanisms of prezygotic reproductive isolation between two sympatric species, Gelsemium rankinji and G. sempervirens (gelsemiaceae), in the southeastern united states. *Am. J. Bot.* 2007, 94, 468–476. [CrossRef] [PubMed]
5. Xue, L.J.; Han, X.F. Mechanism, clinical characteristics and management of Gelsemium elegans poisoning. *Advers. Drug React. J.* 2006, 8, 202–204.
6. Wang, J.; Sun, Z.L. Effect and mechanism of feeding hook-kiss dry powder on growth performance of piglets. *Chin. J. Tradit. Vet. Sci.* 2019, 2, 3–4.
7. Liu, M.; Huang, H.H.; Yang, J.; Su, Y.P.; Lin, H.W.; Lin, L.Q.; Liao, W.J.; Yu, C.X. The active alkaloids of Gelsemium elegans Benth. are potent anxiolytics. *Psychopharmacology* 2013, 225, 839–851. [CrossRef] [PubMed]
8. Liu, Y.C.; Xiao, S.; Yang, K.; Ling, L.; Sun, Z.L.; Liu, Z.-Y. 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/MS spectra. *J. Mass Spectrom.* 2017, 52, 378–396. [CrossRef] [PubMed]
9. Zhang, W.; Zhang, S.Y.; Wang, G.Y.; Li, N.P.; Chen, M.F.; Gu, J.H.; Zhang, D.M.; Wang, L.; Ye, W.C. Five new koumine-type alkaloids from the roots of Gelsemium elegans. *Fitoterapia* 2017, 118, 112–117. [CrossRef] [PubMed]
10. Wang, Z.Y.; Zuo, M.T.; Liu, Z.Y. The Metabolism and Disposition of Koumine, Gelsemine and Humantenmine from Gelsemium. *Curr. Drug Metab.* 2019, 20, 583–591. [CrossRef] [PubMed]

11. Sun, R.J.; Chen, M.H.; Hu, Y.X.; Lan, Y.; Gan, L.L.; You, G.Q.; Yue, M.; Wang, H.M.; Xia, B.J.; Zhao, J.; et al. CYP3A4/5 mediates the metabolic detoxification of humantenmine, a highly toxic alkaloid from Gelsemium elegans Benth. *J. Appl. Toxicol.* 2019, 39, 1283–1292. [CrossRef] [PubMed]

12. Yang, K.; Long, X.M.; Cao, J.J.; Li, Y.J.; Wu, Y.; Bai, X.; Sun, Z.L.; Liu, Z.Y. An analytical strategy to explore the multicomponent pharmacokinetics of herbal medicine independently of standards: Application in Gelsemium elegans extracts. *J. Pharm. Biomed. Anal.* 2019, 176, 112833. [CrossRef] [PubMed]

13. Wang, L.; Sun, Q.; Zhao, N.; Wen, Y.Q.; Song, Y.; Meng, F.H. Ultra-Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS)-Based Pharmacokinetics and Tissue Distribution Study of Koumine and the Detoxification Mechanism of Glycyrrhiza uralensis Fisch on Gelsemium elegans Benth. *Molecules* 2018, 23, 1693. [CrossRef] [PubMed]

14. Zuo, M.T.; Wang, Z.Y.; Yang, K.; Li, Y.J.; Huang, C.Y.; Liu, Y.C.; Yu, H.; Zhao, X.J.; Liu, Z.Y. Characterization of absorbed and produced constituents in goat plasma urine and faeces from the herbal medicine Gelsemium elegans by using high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *J. Ethnopharmacol.* 2020, 252, 112617. [CrossRef] [PubMed]

15. Yang, K. *Study on Pharmacokinetics and Elimination Rules of Gelsemium Alkaloids in Pigs*, Hunan Agricultural University: Changsha, China, 2019.

16. Yang, K.; Long, X.M.; Liu, Y.C.; Chen, F.H.; Liu, X.F.; Sun, Z.L.; Liu, Z.Y. Development and in-house validation of a sensitive LC–MS/MS method for simultaneous quantification of gelsemine and humantenmine in porcine plasma. *J. Chromatogr. B* 2018, 1076, 54–60. [CrossRef] [PubMed]

17. Xiao, S.; Huang, Y.J.; Liu, Y.C.; Sun, Z.L.; Liu, Z.Y. In vitro metabolism of koumine in pig. *Chin. J. Vet. Sci.* 2018, 38, 1568–1572. [CrossRef]

18. Yang, K.; Huang, Y.J.; Xiao, S.; Liu, Y.C.; Sun, Z.L.; Liu, Y.S.; Tang, Q.; Liu, Z.Y. Identification of gelsemine metabolites in rat liver S9 by high-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* 2017, 32, 19–22. [CrossRef] [PubMed]

19. Huang, C.Y.; Yang, K.; Cao, J.J.; Wang, Z.Y.; Wu, Y.; Sun, Z.L.; Liu, Z.Y. Integration of metabolomics and transcriptomics to comprehensively evaluate the metabolic effects of *Gelsemium elegans* on pigs. *Animals* 2021, 11, 1192. [CrossRef] [PubMed]