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Pharmacokinetics of *Gelsemium elegans* in female rats

Xiao-Tong Zhang[1], Jun-Jie Cao[1], Meng-Ting Zuo[1], Zhao-Ying Liu[1*]

[1]School of Veterinary Medicine, Hunan Agricultural University, Changsha 410128, China.

[*]**Correspondence to:** Zhao-Ying Liu, School of Veterinary Medicine, Hunan Agricultural University, No.1 Nongda Road, Furong District, Changsha 410128, China. E-mail: liu_zhaoying@hunau.edu.cn.

Author contributions

The experiments were conceived and designed by Zuo MT. The experiments were performed by Cao JJ. The manuscript was drafted by Zhang XT and critically edited by Zuo MT and Liu ZY.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

G. elegans, *Gelsemium elegans Benth*; bw, bodyweight.

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Abstract

Background: *Gelsemium elegans Benth (G. elegans)* is a poisonous perennial evergreen vine plant that has been applied in livestock production and veterinary clinical practice. Early studies found that the toxicity of *G. elegans* showed significant gender differences in rats, but the underlying reasons for this difference are still not well understood. **Methods:** In order to explore whether the gender differences in the toxicity of *G. elegans* are related to pharmacokinetic differences, based on the previous pharmacokinetic study of multiple components of *G. elegans* in male rats, this study used HPLC-MS/MS method established in the laboratory to conduct a pharmacokinetic study of multiple alkaloids in the plasma of female rats after a single gavage administration of *G. elegans* (dose of 0.1 g/kg). **Results:** Through detection, 17 alkaloid components in the plasma of female rats were identified, and the pharmacokinetic parameters of 11 of these alkaloids were calculated. We find that in female rats. The T_{max} values were generally less than 0.5 h, and the $T_{1/2}$ values exceeded 3 h, with the longest reaching up to 32.80 h half elimination time. Additionally, the C_{max} and AUC results indicated that female rats had generally higher absorption and exposure levels for most alkaloids. **Conclusion:** These results suggest that the reason for the differences in the toxicology of *G. elegans* may be related to the absorption and exposure of gelsemidine-type alkaloids in animals.

Keywords: *Gelsemium elegans*; alkaloids; pharmacokinetics; female rats

Introduction

Gelsemium elegans, is a perennial evergreen climbing plant that belongs to the genus *Gelsemium* of the family Loganiaceae, a highly toxic plant [1]. *Gelsemium elegans*, has been recorded in ancient Chinese medical texts, such as the Shennong Bencao Jing, as a medicinal herb with a variety of uses. In recent years, with increasing attention from experts and scholars both domestically and internationally, modern pharmacological studies have revealed that *G. elegans* possesses a range of pharmacological activities, including analgesic and anti-inflammatory effects, anti-anxiety effects, and anti-tumor effects [2–4]. The herb is characterized by its bitter and pungent taste and warm nature, with a high level of toxicity. The entire plant can be used in traditional Chinese medicine, and is classified into three types based on its origin. Two of these types are North American *Gelsemium* (*G. sempervirens Ait.*), found in the Americas, and Asian *Gelsemium* (*G. elegans Benth.*), which is mainly found in Southeast Asia and southern China, including Yunnan, Guangxi, and Fujian. *G. elegans* has been used as a traditional Chinese herb for centuries, with a wide range of pharmacological activities, including the functions of dispersing wind and stasis, relieving swelling and pain, eliminating toxins, and killing parasites [5]. However, due to its high toxicity, it has traditionally been used primarily for external applications.

So far, more than 120 alkaloids belonging to six major classes, including gelsemine-type, gelsedine-type, humantenine-type, koumine-type, sarpagine-type, and yohimbane-type, have been isolated from *Gelsemium* sempervirens [6–8]. Among them, the humantenine-type and gelsedine-type alkaloids are the two classes with higher toxicity.

Because of its insecticidal and growth-promoting properties, *Gelsemium* has been widely used in livestock breeding [5]. However, the safety risks of *Gelsemium* to animals and humans also limit its use. The early research team established a high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) method, that can simultaneously quantify three alkaloids in plasma (gelsemine, koumine and gelsenicine), and semi-quantitatively determine 27 *Gelsemium* alkaloids in plasma [9]. Through the study of multi-component pharmacokinetics in male rats and pigs, it was found that multi-component alkaloids were rapidly absorbed in male rats after oral *Gelsemium* [10]. The results of pharmacokinetic characterization also showed differences in toxicity among different species [11]. In addition, in the previous study, the team also found that there were significant sex differences in the metabolism of multi-component alkaloids in female and male rats, and the differences in animal metabolism between different genders were one of the reasons for the pharmacokinetic differences, and also had an important impact on the toxicological effects of drugs [12–14].

Therefore, based on the previous pharmacokinetic studies on male rats, this study aims to investigate the pharmacokinetic characteristics of multi-component alkaloids in female rats by studying the pharmacokinetic characterization of multi-component alkaloids in female rats, in order to explore whether the pharmacokinetic difference of *G. elegans* in rats is one of the toxic causes of gender differences, and to provide a scientific basis for explaining the toxic differences of *G. elegans* in animals of different genders.

Materials and methods

Experimental instrument and reagent

The QTRAP 4500 mass spectrometer from AB Sciex (Framingham, MA, USA); Waters BEH C18 chromatographic column (2.1 mm × 100 mm, 1.7 μm); KS600DE ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); Weighing balance (Sartorius Scientific Instrument Co., Ltd.); Milli-Q system (Millipore Corporation, USA); Methanol, formic acid and acetonitrile were chromatographic grade and obtained from Merck Chemical Company (Darmstadt, Germany). The water was prepared using the Millipore-q water purification system (Millipore, Bedford, MA, USA). Other reagents used in the experiment were all analytical grade reagents. The whole grass of *G. elegans* was purchased from Fujian Province; Gelsemine, koumine and gelsenicine (purity > 98%, HPLC) were purchased from Chengdu Man Si Te Biotechnology Co., Ltd (Wuhou District, Chengdu, China). Ultra-pure water was obtained from the Milli-Q ultra-pure water purification system, Millipore Corporation, USA. Disposable vacuum blood collection heparin sodium tubes (Batch No. 20180504, Huabo Medical Devices Co., Ltd., Chengwu County, Shandong Province); Disposable blood collection needles (Batch No. 20180507, Huabo Medical Devices Co., Ltd., Chengwu County, Shandong Province).

Experimental animals

All animal studies were performed following the national legislation and were approved by the Institutional Animal Care and Use Committee at the Center for Laboratory Animals, Hunan Agricultural University (approval number: 2020-43). Adult female Sprague-Dawley rats (200 ± 20 g) were purchased from Hunan Si Laikejingda Experimental animal Co., Ltd. (Changsha, China). The animal production approval number was SCXK-2016-0002. All rats were housed at a temperature of 24 ± 1 ˚C with a 24 h of light. They were fed the basic diet for 5–7 days, and given clean drinking water during this period. The experimental animals were divided into 2 groups, namely the blank control group and the experimental group, with 5 animals in each group (0.1 g/kg of the whole grass powder of *G. elegans* bw).

Experimental methods

Preparation of administration solution. The whole grass powder of *G. elegans* was accurately weighed to 500.0 g and was formulated into a suspension with a concentration of 0.1 g/mL with normal saline, and was freshly prepared for immediate use.

Animal experiment. Rats were given 1.0 mL of 0.1 g/mL *G. elegans* powder by gavage. The rats were fasted for 12 h before administration and had free access to water. Blood was collected from the orbital venous plexus using a glass capillary at 0.083, 0.25, 0.5, 2, 4, 6, 8, 12, 24, 48 and 72 h after administration, approximately 0.5 mL each time, and placed in a 3.0 mL anticoagulation tube containing heparin sodium. The sample was centrifuged at 3500 rpm on a centrifuge for 10 min, and the supernatant was taken into a 2.0 mL centrifuge tube and stored at −80 ˚C.

Sample detection. Each time point plasma sample of each rat stored at −80 ˚C was thawed at room temperature. 100.0 μL was transferred to a 2.0 mL centrifuge tube, 500.0 μL of pure methanol was added, vortexed for 30 s, and then 1.0 mL of 1% formic acid-acetonitrile was added, vortexed for 10 s, sonicated for 30 min, centrifuged at 14000 rpm for 10 min, the supernatant was transferred to a 10.0 mL glass centrifuge tube, blown dry with N2 at 30 ˚C, and re-dissolved with 400.00 μL of 10% methanol −0.1% formic acid water. The supernatant was taken, filtered through a 0.22 μm filter membrane, and placed in an injection vial for injection analysis.

UPLC-MS/MS analysis. The collected plasma samples were carried out by two previously published validated LC-MS/MS analytical method, one was the precise quantitative, and other was the semi-quantitative method) [9, 15]. determination of *Gelsemium* alkaloids was performed with a UPLC-MS/MS system consisting of a Shimadzu chromatography-30A system (Shimadzu Co, Kyoto, Japan) and an AB Sciex QTRAP 4500 mass spectrometer (AB SCIEX, Framingham, USA). Separation was achieved on a Waters ACQUITY BEH C18 column (2.1 × 100 mm, 1.7 μm) with a constant flow rate of 0.3 mL/min at 35 ˚C Desirable chromatographic separation was achieved by use of mobile phase A (0.1% formic acid-water) and mobile phase B (acetonitrile). The gradient elution of three alkaloids (gelsemine, koumine and gelsenicine) with reference standards was as follows: 0–2.0 min, 10% B; 2.0–6.0 min, 65% B; 6.0–8.0 min, 65% B; 8.0–10.0 min, 90% B, and the gradient elution of *Gelsemium* alkaloids was as follows: 0–1.5 min, 8% B; 1.5–10 min, 8% B to 40% B; 10.1–12.0 min, 95% B; and 12.1–14.0 min, 8% B. The sample injection volumes were 20.0 μL and 5.0 μL, respectively.

Mass spectrometry detection was performed in positive electrospray ionization (ESI) mode with multiple reactions monitoring (MRM) and derived multiple reactions monitoring (DeMRM). The MS/MS analytical conditions were as follows: the Turbo Spray® source voltage and temperature were set to 5.5 kV and 500 ˚C, respectively; the nebulizer gas (GS1) and heater gas (GS2) were maintained at 35 psi and 60 psi, respectively; and the curtain gas and collision gas were set to 30 psi and 10 psi, respectively.

Data analysis. Sample data analysis was processed using the quantitative analysis data software AnalystSoftware1.6.3 that came with the QTRAP 4500 mass spectrometer from AB Sciex, and the resulting blood drug concentrations were analyzed using the non-compartment model of the pharmacokinetic software WinNonlin5.2.1 to obtain the main pharmacokinetic parameters. These parameters include the time to maximum concentration (T_{max}), maximum concentration (C_{max}), area under the concentration time curve (AUC), half elimination time ($T_{1/2}$). Considering the individual differences of the experimental animals, the average blood drug concentration of rats at each time point was used to plot the drug-time curve respectively.

Pharmacokinetic results

A total of 17 alkaloid compounds were detected in the plasma samples of female rats. Figure 1 shows the blood drug concentration-time curves of 17 alkaloids. Among them, six alkaloids, namely gelsemine, Na-desmethoxyhumantenine, 19-Hydroxdihyogelsemine, koumine, 19-Akuammidine, and 6-hydroxyhumantenirine, could not calculate the pharmacokinetic parameters due to too few effective concentrations time points. The pharmacokinetic parameters of the other 11 alkaloids were calculated, and the results are shown in Supplementary Table S1. The characterization of these alkaloids found that one type of serpentine alkaloids was detected, two types of gelsemine -type, one type of koumicine, and four types of humantenirine -type belonged to nine types of gelsedine. All the 11 alkaloids reached the peak concentration between 0.083 and 0.8 h, and their elimination half-lives were between 1.58 and 32.80 h, and the absorption rate of the alkaloids was fast.

According to the results in Supplementary Table S1, the *G. elegans* alkaloids obtained for kinetics parameters mainly included three types, namely gelsemine-type, humantenirine-type and gelsedine alkaloids. Among the gelsemine-type alkaloids, the T_{max} and $T_{1/2}$ values of gelsemine (GA-4) were 0.81 h and 3.51 h, respectively; for humantenine -type, the T_{max} and $T_{1/2}$ values of humantenine (GA-15) were 0.083 h and 29.03 h respectively, and the T_{max} value of humantenoxenine (GA-19) was 0.14 h and the $T_{1/2}$ value was 32.80 h; for the gelsedine-type, the T_{max} values of humantenine(GA-6), Nb-Methylgelsedilam (GA-7), 14-hydroxygelsenicine (GA-10), 11-hydroxygelsenicine (GA-11), gelsemicine (GA-16), gelsemoxonine (GA-17), gelselegine (GA-18) and GS-2 (11-Methoxy-14-Hydroxygelsenicine) (GA-23) were 0.17 h, 0.19 h, 0.65 h, 0.35 h, 0.27 h, 0.70 h, 0.32 h and 0.14 h, respectively, and their $T_{1/2}$ values were 4.10 h, 3.46 h, 11.39 h, 4.95 h, 10.08 h, 5.49 h, 0.32 h and 1.58 h respectively. The above results indicated that the 11 alkaloids detected in the plasma of female rats could be rapidly absorbed within 1 h, and among these 11 alkaloids, gelselegine and GS-2 of gelsedine alkaloids showed a faster elimination rate and could be eliminated rapidly within 2 h, while the four alkaloids of humantenoxenine and humantenine (humantenirine-type), 14-hydroxygelsenicine and Gelsemicine (gelsedine) showed a slower elimination rate, and the elimination time was more than 10 h, and the half-elimination time of humantenoxenine exceeded 30 h.

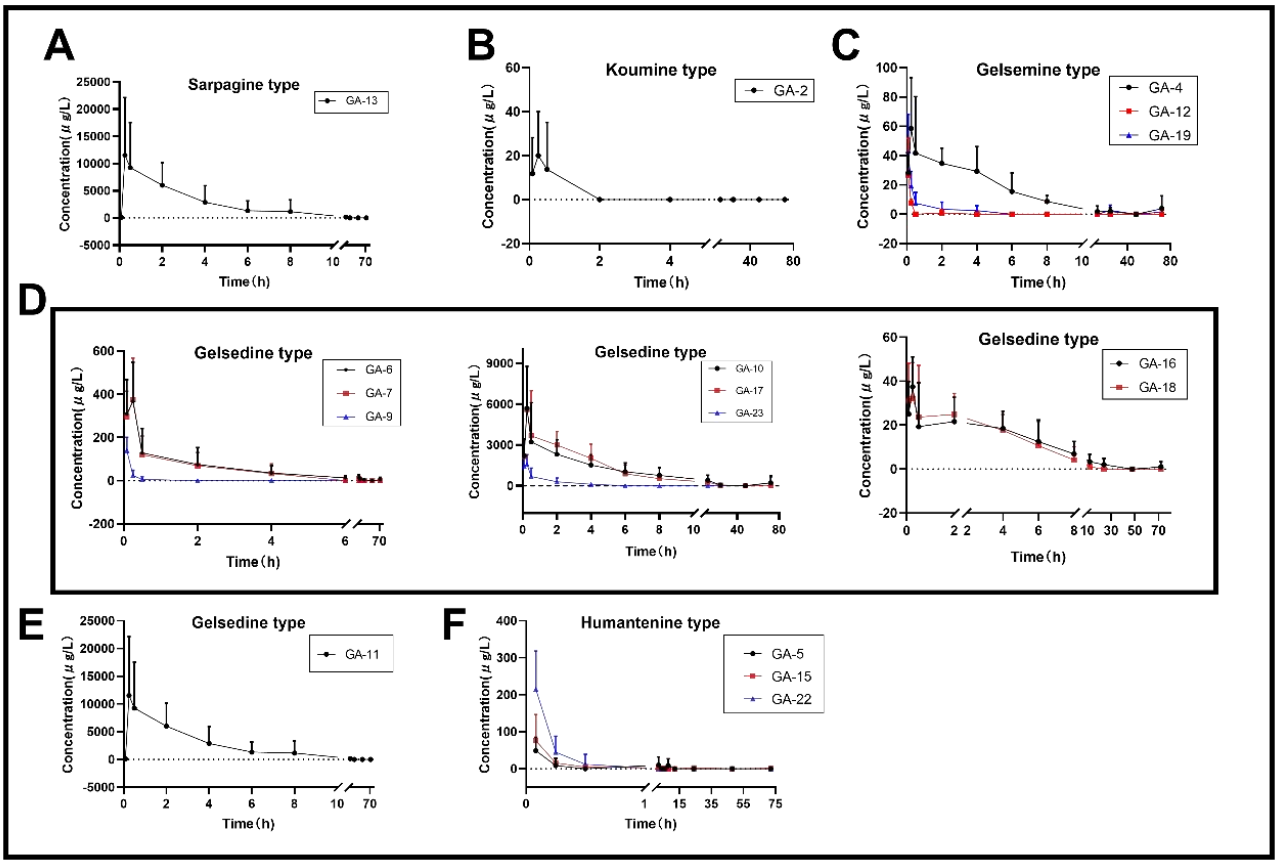

Figure 1 Average drug-time curve of a single oral administration of *G.elegans* alkaloids in the plasma of female rat. (A) Sarpagine-type alkaloids. (B) Koumine-type alkaloids. (C) Gelsemine type alkaloids. (D–E) Gelsedine type alkaloids. (F) Humantenine type alkaloids.

Discussion

This study focused on the pharmacokinetics of multi-component alkaloids of *G. elegans* in female rats. In the earlier toxicity studies of *G. elegans*, the acute toxicity experiments of crude alkaloid extracts extracted from *G. elegans* were evaluated by determining the 50% lethal dose (LD50). The results showed that the LD50 of intraperitoneal injection of crude alkaloids in male rats was 1.2 mg/kg, while the LD50 of intramuscular injection of crude alkaloids in female mice was 1.5 mg/kg; in the acute toxicity study of koumine, the results indicated that female rats were more sensitive to koumine than male rats, and their LD50 were 0.520 mg/kg and 0.996 mg/kg, respectively [16, 17]. These results clearly demonstrated that the toxicity of *G. elegans* showed obvious gender differences in rats. In the previous studies, we conducted a comprehensive and systematic analysis of the metabolism and processes of multi-components of *Gelsemium elegans* in rats, and found that compared with male rats, female rats were more active in the absorption, distribution and excretion of *G. elegans* alkaloids, indicating that gender differences might be related to the *in vivo* processes of active ingredients. Although the pharmacokinetic study of *G. elegans* (0.1 g/kg) in male rats has been conducted previously, there has been no detailed research on the pharmacokinetic data of female rats at the same dose. Therefore, this study aimed to further investigate the correlation between gender differences in toxicity and pharmacokinetic differences of *G. elegans* by conducting a pharmacokinetic study of single gavage administration of the same dose of *G. elegans* powder to female rats.

Compared with the 13 alkaloids detected in male rat plasma using a semi-quantitative method in the previous study and the 6 kinetic parameters calculated, it was found that the drug concentration peak time of *G. elegans* alkaloids in male and female rats was both < 1 h [10]. Specifically, female rats showed shorter T_{max} for gelsedine alkaloids compared to male rats, including Humantenmine, Gelsemicine, and GS-2 (11-Methoxy-14-Hydroxygelsenicine). This result indicated that the absorption rate of these three *G. elegans* alkaloids was faster in female bodies. However, by comparing the $T_{1/2}$ of these three alkaloids, we found that the half-elimination period of these alkaloids was shorter in male rats. This meant that although Humantenmine, Gelsemicine, and GS-2 (11-Methoxy-14-Hydroxygelsenicine) were rapidly absorbed in female rats, their residence time in the body was shorter. In addition, the peak times of two gelsedine alkaloids, 14-hydroxygelsenicine and gelsemoxonine, in female rats were slightly slower than those in male rats, but their elimination rates were much slower than those in male rats, resulting in their elimination half-lives being twice as long as those in male rats. At the same time, by comparing the results of C_{max} and AUC, we found that although the absorption rates of the four alkaloids, 14-hydroxygelsenicine, koumine, gelsemoxonine, and GS-2 (11-Methoxy-14-Hydroxygelsenicine), were lower in female rats, their exposure levels were higher. Meanwhile, five alkaloids, Nb-Methylgelsedilam, 11-hydroxygelsenicine, humantenine, Gelselegine, and Humantenoxenine, were also detected in the plasma of female rats. These alkaloids showed rapid absorption but slow elimination in the rat body. Among them, Nb-Methylgelsedilam, 11-hydroxygelsenicine, and Gelselegine belong to the gelsedine alkaloids, and 11-hydroxygelsenicine (gelsedine) alkaloid showed higher absorption and exposure levels. Additionally, Humantenoxenine belongs to the humantenine -type alkaloids. The previous literature indicated that the LD50 of humantenine-type alkaloids was 0.21 mg/kg, belonging to a more toxic category [18]. And the experimental data showed that the peak time of this alkaloid in female rats could reach 0.14 h, but its half elimination time was up to 32.80 h. This further indicated that female rats were more sensitive to *G. elegans* alkaloids.

Conclusion

This study systematically investigated the pharmacokinetic characteristics of *Gelsemium elegans* alkaloids in female rats. The results indicated that the T_{max} values were generally low and the $T_{1/2}$ values exceeded 3 h, with the half elimination time of humantenoxenine reaching up to 32 h, indicating rapid absorption but slow half elimination time elimination of *Gelsemium elegans* alkaloids in female rats. Additionally, the C_{max} and AUC results demonstrated significant absorption and high exposure levels of most gelsedine-type alkaloids in female rats. This study provides reliable data for understanding the sex-specific toxicity of *Gelsemium*. Further toxicological research is needed to explore the mechanisms underlying the sex differences in *Gelsemium* toxicity, offering valuable insights for its development and application.

References

1. Struwe L, Albert VA, Bremer B. Cladistics and Family Level Classification of the Gentianales. *Cladistics.* 1994;10(2):175–206. Available at: http://doi.org/10.1111/j.1096-0031.1994.tb00171.x
2. Shoaib RM, Zhang JY, Mao XF, Wang YX. Gelsemine and koumine, principal active ingredients of *Gelsemium*, exhibit mechanical antiallodynia via spinal glycine receptor activation-induced allopregnanolone biosynthesis. *Biochem Pharmacol.* 2019;161:136–148. Available at: http://doi.org/10.1016/j.bcp.2019.01.014
3. Shoaib RM, Zhang JY, Mao XF, Wang YX. Gelsemine and koumine, principal active ingredients of *Gelsemium*, exhibit mechanical antiallodynia via spinal glycine receptor activation-induced allopregnanolone biosynthesis. *Biochem Pharmacol.* 2019;161:136–148. Available at: http://doi.org/10.1016/j.bcp.2019.01.014
4. Liu M, Huang HH, Yang J, et al. The active alkaloids of *Gelsemium elegans* Benth. are potent anxiolytics. *Psychopharmacology (Berl)*. 2013;225(4):839–851. Available at: https://doi.org/10.1007/s00213-012-2867-x
5. Dutt V, Thakur S, Dhar VJ, Sharma A. The genus *Gelsemium*: An update. *Pharmacogn Rev*. 2010;4(8):185–194. Available at: https://doi.org/10.4103/0973-7847.70916
6. Kitajima M, Kogure N, Yamaguchi K, Takayama H, Aimi N. Structure Reinvestigation of Gelsemoxonine, a Constituent of *Gelsemium elegans*, Reveals a Novel, Azetidine-Containing Indole Alkaloid. *Org Lett.* 2003;5(12):2075–2078. Available at: http://doi.org/10.1021/ol0344725
7. Jin GL, Su YP, Liu M, et al. Medicinal plants of the genus *Gelsemium* (Gelsemiaceae, Gentianales)—A review of their phytochemistry, pharmacology, toxicology and traditional use. *J Ethnopharmacol.* 2014;152(1):33–52. Available at: http://doi.org/10.1016/j.jep.2014.01.003
8. Kogure N, Ishii N, Kitajima M, Wongseripipatana S, Takayama H. Four Novel Gelsenicine-Related Oxindole Alkaloids from the Leaves of *Gelsemium elegans* Benth. *Org Lett.* 2006;8(14):3085–3088. Available at: http://doi.org/10.1021/ol061062i
9. Yang K, Long XM, Liu YC, et al. Development and in-house validation of a sensitive LC–MS/MS method for simultaneous quantification of gelsemine, koumine and humantenmine in porcine plasma. *J Chromatogr B.* 2018;1076:54–60. Available at: http://doi.org/10.1016/j.jchromb.2018.01.019
10. Cao JJ, Yang K, Yu H, et al. Comparative toxicokinetic profiles of multiple-components of *Gelsemium elegans* in pigs and rats after a single oral administration. *Toxicon.* 2020;181:28–35. Available at: http://doi.org/10.1016/j.toxicon.2020.04.093
11. Cao JJ. Pharmacokinetics studies of *Gelsemium elegans* alkaloids in goats and rats. *Hunan Agric Univ*. 2020;05:72. Available at: https://link.cnki.net/doi/10.27136/d.cnki.ghunu.2020.000747
12. Zuo MT, Gong MD, Ma X, et al. Sex Differences in the *In Vivo* Exposure Process of Multiple Components of *Gelsemium elegans* in Rats. *Metabolites.* 2022;13(1):33. Available at:

http://doi.org/10.3390/metabo13010033
13. Zucker I, Prendergast BJ. Sex differences in pharmacokinetics predict adverse drug reactions in women. *Biol Sex Differ.* 2020;11(1):32. Available at: http://doi.org/10.1186/s13293-020-00308-5
14. Rowland M, Peck C, Tucker G. Physiologically-Based Pharmacokinetics in Drug Development and Regulatory Science. *Annu Rev Pharmacol Toxicol.* 2011;51(1):45–73. Available at: http://doi.org/10.1146/annurev-pharmtox-010510-100540
15. Yang K, Long XM, Cao JJ, et al. 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. Available at: http://doi.org/10.1016/j.jpba.2019.112833
16. Rujjanawate C, Kanjanapothi D, Panthong A. Pharmacological effect and toxicity of alkaloids from *Gelsemium elegans* Benth. *J Ethnopharmacol.* 2003;89(1):91–95. Available at: http://doi.org/10.1016/S0378-8741(03)00267-8
17. Li YJ, Yang K, Long XM, et al. Toxicity assessment of gelsenicine and the search for effective antidotes. *Hum Exp Toxicol.* 2022;41:096032712110628. Available at: http://doi.org/10.1177/09603271211062857
18. Jin GL, Su YP, Liu M, et al. Medicinal plants of the genus *Gelsemium* (Gelsemiaceae, Gentianales)—A review of their phytochemistry, pharmacology, toxicology and traditional use. *J Ethnopharmacol.* 2014;152(1):33–52. Available at: http://doi.org/10.1016/j.jep.2014.01.003