Acta Pharmaceutica Sinica B 2025;15(9):4872−4885

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

ORIGINAL ARTICLE

The toxic components, toxicological mechanism and effective antidote for *Gelsemium elegans* poisoning

Niping Li[a,b,†], Yaorong Yang[a,b,†], Shengyuan Zhang[b,e,†], Bin Jiang[c], Wei Zhang[a,b], Haibo Wang[d], Lixin Chen[d], Liwei Wang[d], Yiyi Li[a,b], Lei Shi[a,b,*], Wencai Ye[a,b,*], Lei Wang[a,b,*]

[a]*State Key Laboratory of Bioactive Molecules and Druggability Assessment, Jinan University, Guangzhou 510632, China*
[b]*Center for Bioactive Natural Molecules and Innovative Drugs Research, Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, China*
[c]*Guangdong Province Key Laboratory of Brain Function and Disease, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China*
[d]*Department of Pharmacology, Medical College, Jinan University, Guangzhou 510632, China*
[e]*Guangdong Provincial Key Laboratory of Conservation and Precision Utilization of Characteristic Agricultural Resources in Mountainous Areas, Jiaying University, Meizhou 514015, China*

Received 30 December 2024; received in revised form 14 May 2025; accepted 3 June 2025

KEY WORDS

Gelsemium elegans poisoning;
Toxic components;
Alkaloids;
Structure−toxicity relationship;
Toxicological mechanism;
$GABA_A$ receptor;

Abstract *Gelsemium elegans* (*G. elegans*) is an extremely poisonous plant that is widely distributed in southern China and southeastern Asia. *G. elegans* poisoning events occur frequently in southern China, and are therefore an urgent public health problem requiring multidisciplinary action. However, the toxic components and toxicological mechanisms remain unclear. Here, we describe a systematic investigation on the toxic components of *G. elegans*, resulting in the isolation and identification of 120 alkaloids. Based on acute toxicity screening, the structure−toxicity relationship of *Gelsemium* alkaloids was proposed for the first time. Moreover, gelsedine- and humantenine-type alkaloids were detected in the clinical blood sample, and were confirmed to be causative in the poisoning. The most toxic compound, gelsenicine (**1**), had selective inhibitory effects toward ventral respiratory group (VRG) neurons in the medulla, which

*Corresponding authors.
E-mail addresses: t_shilei@jnu.edu.cn (Lei Shi), chywc@aliyun.com (Wencai Ye), cpuwanglei@126.com (Lei Wang).
†These authors made equal contributions to this work.
Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2025.07.016

Antidote;
Respiratory failure

is the main brain region controlling respiration in the central nervous system. Gelsenicine (**1**) strongly inhibited the firing of action potentials in VRG neurons through its ability to stimulate $GABA_A$ receptors, the main receptors involved in inhibitory neurotransmission. Application of $GABA_A$ receptor antagonists successively reversed action potential firing in gelsenicine (**1**)-treated VRG neurons. Importantly, the $GABA_A$ receptor antagonists securinine and flumazenil significantly increased the survival of poisoned animals. Our findings provide insight into the components and mechanisms of *G. elegans* toxicity, and should assist the development of effective emergency treatments for *G. elegans* poisoning.

1. Introduction

Ingestion of the highly toxic plant *Gelsemium elegans* (*G. elegans*), which is widely distributed in southern China and southeastern Asia, causes severe poisoning and can result in death[1]. The high toxicity of *G. elegans* was described in Shen Nong Herbal (Shen Nong Ben Cao Jing) more than 2000 years ago[2]. Because the whole *G. elegans* plant is highly poisonous[3], and its flowers and roots are often confused with those of plants widely used in herbal medicines, such as *Lonicera japonica* and *Ficus hirta*[4,5], poisonings caused by misidentification and improper use occur frequently in southern China[1,6−8]. Over the past 20 years, more than 678 cases of *G. elegans* poisoning have been reported, with 141 mortalities (incomplete statistics, Supporting Information Table S1). The frequent occurrence and high mortality rates of *G. elegans* poisoning make it a serious public health concern in China.

Previous studies suggest that *Gelsemium* alkaloids may be the leading cause of *G. elegans* poisoning[3]. Phytochemical investigations on the leaves and roots of *G. elegans* have led to the isolation of six types of monoterpenoid indole alkaloids[9]. Despite the presence of numerous structurally diverse alkaloids in *G. elegans*, only a few compounds (gelsenicine, 14-hydroxygelsenicine, gelsemicine, and humantenirine) have so far been reported to be toxic[3,10−13]. Among the six types of alkaloids, those responsible for the toxicity of *G. elegans* are still unclear. The clinical symptoms of *G. elegans* poisoning are complex, and include respiratory and nervous system symptoms, such as convulsions, chest tightness, continuous irregular breathing, arrhythmia and respiratory arrest, among which respiratory failure is the primary cause of fatality[14,15]. Because of a lack of research, there is yet no effective antidote for *G. elegans* poisoning, and the main clinical treatment approach is symptomatic supportive therapy[16].

Studies show that the toxic effects of *Gelsemium* alkaloids are related to altered function of $GABA_A$ receptors, the main receptors in inhibitory neurotransmission. One study reported that the gelsedine-type alkaloid gelsepyrrodine B decreases the chloride ion current evoked by GABA in rat neurons[17]. However, as respiratory distress is normally the main cause of death by *G. elegans* poisoning, the toxic effects of *Gelsemium* alkaloids cannot be fully explained by convulsion-related mechanisms[17]. In contrast, another study reported that 14-hydroxygelsenicine enhances the GABA-induced current in hippocampal neurons, although the hippocampus does not play a major role in respiration[11]. Other proposed mechanisms include the modulation of NMDA receptors[18]. Thus, the toxicological mechanisms by which *Gelsemium* alkaloids cause respiratory arrest and death remain unclear. Ventral respiratory group (VRG), located in the ventrolateral part of medulla, is an important group of neurons in the respiratory center which fires action potentials in phase with respiration[19−21]. When VRG neuronal function is perturbed, they fail to fire normal action potentials, resulting in abnormal respiration. Because of the critical role of VRG neurons in respiration, we hypothesized that *Gelsemium* alkaloids may directly act on VRG neurons to inhibit respiration.

In the present study, we performed phytochemical investigation of all parts (roots, leaves and stems, flowers, and fruits) of the plant *G. elegans*, leading to the isolation and identification of 120 alkaloids belonging to eight structural types, including gelsedine-, humantenine-, gelsemine-, koumine-, sarpagine-, yohimbine-, bisindole- and other-type. Among these, 54 representative compounds with adequate amounts (>20 mg) were selected for acute toxicity screening in mice. Only gelsedine- and humantenine-type alkaloids exhibited high toxicity (LD_{50} < 10 mg/kg). The structure−toxicity relationship of *Gelsemium* alkaloids was proposed for the first time. Furthermore, in the blood of *G. elegans* poisoning victim, four gelsedine- and humantenine-type toxic alkaloids (**1**, **2**, **17**, **18**) were detected, providing direct evidence that these two types of toxic alkaloids are the cause of *G. elegans* poisoning. The most toxic compound, gelsenicine (**1**), was selected for further investigation. We found that the VRG neurons were more sensitive to gelsenicine (**1**) than the hippocampus, and $GABA_A$ receptor antagonists were able to prevent the toxic effects and restore action potential firing, suggesting that the inhibitory effect of gelsenicine (**1**) on VRG neurons is mediated by the activation of $GABA_A$ receptors. Furthermore, the clinically approved $GABA_A$ receptor antagonists securinine and flumazenil significantly improved the survival of poisoned animals.

2. Results

2.1. Chemical investigation of the toxic fraction of G. elegans

All parts of *G. elegans* are highly poisonous[3,11]. Previous phytochemical investigations show that *G. elegans* is rich in alkaloids, triterpenoids and iridoids, as well as other secondary metabolites[3]. Furthermore, the total alkaloid fraction was reported to be responsible for *G. elegans* toxicity[9], which was confirmed by the results of acute toxicity test (Supporting Information Table S2). To identify the toxic constituents, the total alkaloid fractions of roots, leaves and stems, flowers and fruits of *G. elegans* (Fig. 1A) were prepared using a standard procedure[22]. Each alkaloid fraction was subjected to column chromatography using silica gel, ODS and Sephadex LH-20, followed by preparative HPLC, to yield

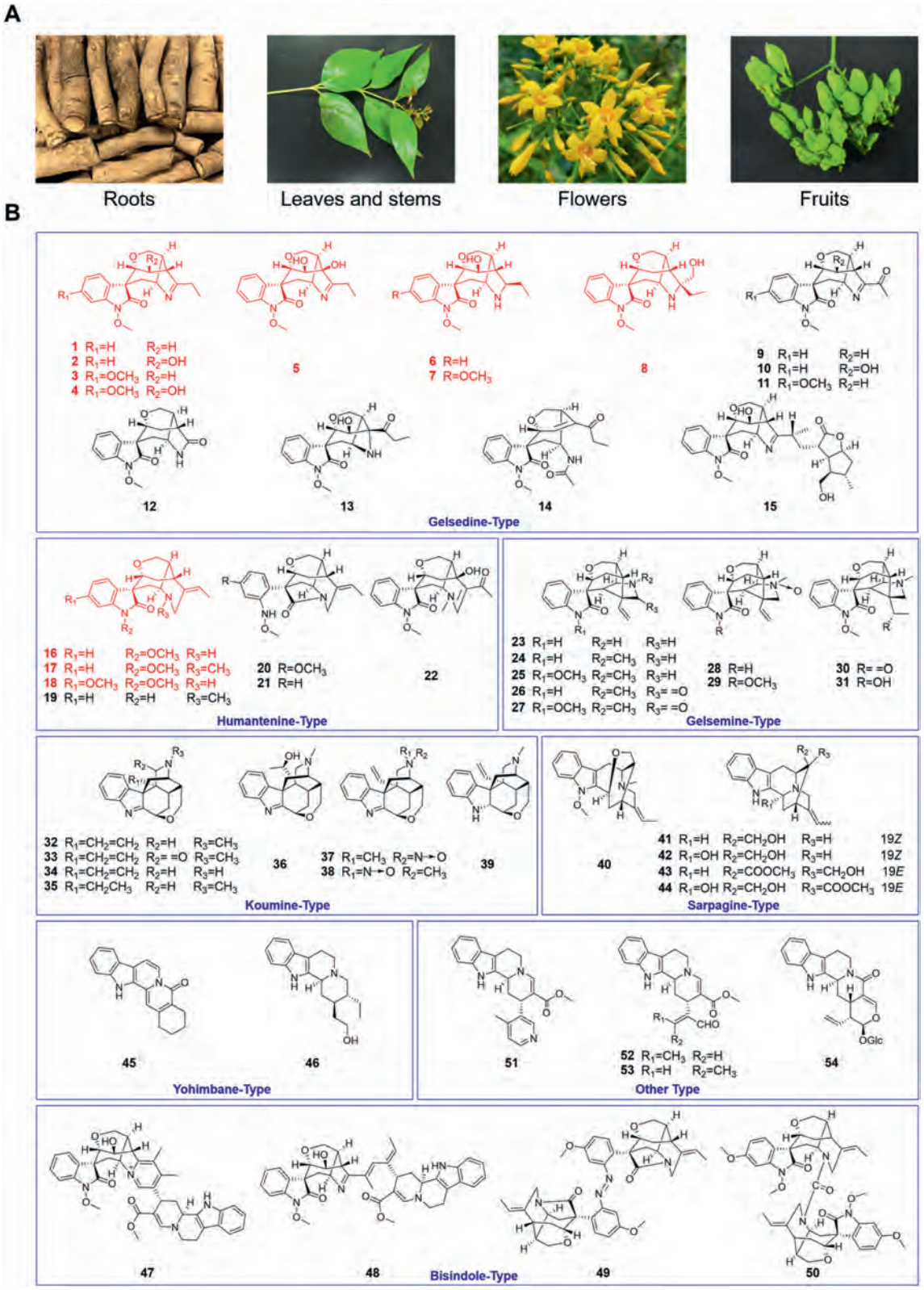

Figure 1 Chemical investigation of *G. elegans*. (A) Different parts of the plant. (B) Chemical structures of representative *Gelsemium* alkaloids. The red chemical structures indicate toxic alkaloids.

compounds **1**−**120** (Supporting Information Figs. S1−S43, Supporting Information Tables S3−S11). Their structures and absolute configurations were elucidated by NMR spectroscopy, single-crystal X-ray diffraction, and electronic circular dichroism data analyses. The structural features of these alkaloids permitted their classification into eight types, including gelsedine-, humantenine-, gelsemine-, koumine-, sarpagine-, yohimbine-, bisindole- and other-type (Fig. 1B). Several new alkaloids (gelsekoumidines A and B, gelsecorydines A−E, and gelserancines A−E) were previously published by our group for their structural novelty[23−27].

2.2. Structure−toxicity relationship of Gelsemium alkaloids

To investigate the toxicity of *Gelsemium* alkaloids, we conducted *in vitro* cytotoxicity evaluations on various alkaloids. The results demonstrated that these compounds did not exhibit significant cytotoxicity (hippocampus-derived HT22 cells and renal epithelium-derived Vero cells), corroborating previously reported results[3] (Supporting Information Figs. S44 and S45). Subsequently, 54 representative alkaloids with isolated amounts of more than 20 mg were selected for acute toxicity screening in mice (Fig. 1B). These compounds covered all eight types of alkaloids isolated, including monomeric and dimeric *Gelsemium* alkaloids. The screen identified 11 monomeric alkaloids exhibiting high toxicity, including gelsenicine (**1**), 14-hydroxygelsenicine (**2**), 4,20-dehydrogelsemicine (**3**), 11-methoxyl-14-hydroxygelsenicine (**4**), 14,15-dihydroxygelsenicine (**5**), 14-hydroxygelsedine (**6**), 14-hydroxygelsemicine (**7**), gelselegine (**8**), rankinidine (**16**), humantenine (**17**) and humantenirine (**18**), with LD_{50} values of 0.14−7.11 mg/kg (Table 1, Supporting Information Table S12). As published findings of *Gelsemium* alkaloids[3,11,12,28], mice experienced severe respiratory distress shortly after the intraperitoneal administration of the toxic alkaloids, and the symptoms included abdominal breathing, gasping, cyanosis, and convulsions before dead. Notably, all toxic alkaloids could be attributed to two types (gelsedine- and humantenine-type) (Table 1). From a structure−toxicity relationship perspective, the skeletons of both types of alkaloids are very similar. Both possess an indolin-2-one unit and a unique tricyclic fused monoterpene core (Fig. 1B), indicating this complex and specific unit is essential for the toxicity. Furthermore, all toxins have a methoxy (OMe) group at the N_1 position and a C2 unit at the C_{20} position. If the OMe was missing [such as in *N*-demethoxyhumantenine (**19**)], or if the C2 unit was oxidized [such as in 19-oxogelsenicine (**9**), 14-hydroxyl-19-oxogelsenicine (**10**), gelegamine E (**11**) and 15-hydroxyhumantenoxenine (**22**)] or missing [such as in gelsedilam (**12**)], toxicity was significantly reduced. Thus, the OMe group at the N_1 position and the C2 unit at the C_{20} position are important for the toxicity of gelsedine- and humantenine-type alkaloids (Fig. 1B).

2.3. Analysis of toxic alkaloids responsible for G. elegans poisoning

With an aim to identify which toxin is the arch-criminal of *G. elegans* poisoning, the identification of main alkaloid components in the plant was performed by high performance liquid chromatography (HPLC)-diode array detection (DAD) based on authentic standards. In the whole plant of *G. elegans*, the main alkaloid components were gelsenicine (**1**), 14-hydroxygelsenicine (**2**), humantenine (**17**), humantenirine (**18**), gelsemine (**24**), gelsevirine (**25**) and koumine (**32**) (Fig. 2A, Supporting Information Fig. S46), among which gelsenicine (**1**), 14-hydroxygelsenicine (**2**), humantenine (**17**) and humantenirine (**18**) exhibited high toxicity. Next, we examined whether these toxins could be detected in blood using UPLC-Q Exactive Plus MS. After oral administration of *G. elegans* extract in mice, 4 toxins [gelsenicine (**1**), 14-hydroxygelsenicine (**2**), humantenine (**17**), and humantenirine (**18**)] were detected in the plasma by comparing the retention times and the primary and secondary ion fragments with authentic standards (Supporting Information Fig. S47). This result suggested that gelsedine and humantenine types toxins could be orally absorbed and entered into bloodstream.

Unfortunately, two 6-year-olds died of accidental ingestion of the stems of *G. elegans* on March 16, 2022 in Puning People's Hospital, Puning city, Guangdong province. The blood sample of one victim was analyzed using the UPLC-Q Exactive MS/MS. The above four toxic *Gelsemium* alkaloids detected in mice plasma (**1**, **2**, **17**, and **18**) were also clearly detected in the clinical blood sample (Supporting Information Fig. S48). The levels of these toxic alkaloids in serum of the victim range from 9.90 to 192.40 ng/mL (Fig. 2B and Supporting Information Table S13). In addition, some non-toxic alkaloids (**24**, **25**, and **32**) could also be detected Fig. S48. Unexpectedly, the level of 14-hydroxygelsenicine (**2**) was much higher than that of gelsenicine (**1**), which was inconsistent with the observation in the mis-ingested stems (Fig. 2A). Because the serum sample was collected at least 12 h after the patient experienced symptoms, these results led us to speculate whether gelsenicine (**1**) could be alleviated by

Table 1 The acute toxicity of *Gelsemium* alkaloids in mice (intraperitoneal injection).

Structural types	Compounds	LD_{50} (mg/kg)[a]	95% CI (mg/kg)[b]	Structural types	Compounds	LD_{50} (mg/kg)[a]	95% CI (mg/kg)[b]
Gelsedine	**1**	0.14	0.13−0.16	Humantenine	**16**	0.21	0.19−0.23
	2	0.46	0.45−0.47		**17**	7.11	6.45−7.88
	3	0.56	0.51−0.63		**18**	0.15	0.14−0.17
	4	0.41	0.36−0.47		**19**−**22**	>30	ND
	5	3.59	3.17−4.07	Gelsemine	**23**−**31**	>30	ND
	6	0.17	0.13−0.22	Koumine	**32**−**39**	>30	ND
	7	0.17	0.14−0.21	Sarpagine	**40**−**44**	>30	ND
	8	0.54	0.47−0.61	Yohimbane	**45**−**46**	>30	ND
	9−**15**	>30	ND	Bisindole	**47**−**50**	>30	ND
				Other	**51**−**54**	>30	ND

ND: not detected.
[a]LD_{50}: median lethal dose.
[b]CI: confidence interval.

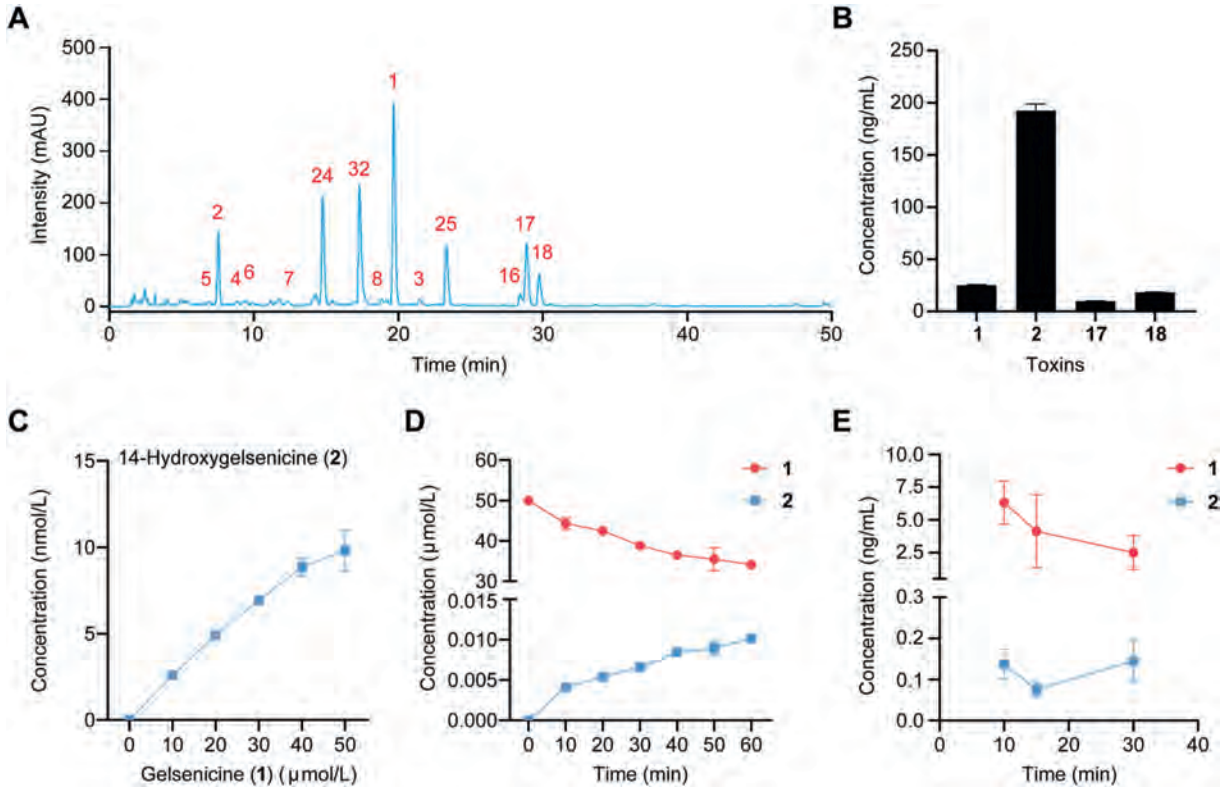

Figure 2 The qualitative and quantitative analysis of toxins. (A) HPLC profiling (recorded at 254 nm) of the total alkaloid fraction of *G. elegans* (stems). (B) Quantitative analysis of toxins in the clinical blood sample. (C) The production of 14-hydroxygelsenicine (**2**) from different concentrations of gelsenicine (**1**) in human liver microsomes. (D) The concentration of the substrate gelsenicine (**1**) and the metabolite 14-hydroxygelsenicine (**2**) at different time points in human liver microsomes. (E) The concentration of the substrate gelsenicine (**1**) and the metabolite 14-hydroxygelsenicine (**2**) at different time points in rats ($n = 6$). Data are shown as mean ± SD.

converting into the less toxic 14-hydroxygelsenicine (**2**) *in vivo*. To test this hypothesis, the *in vitro* and *in vivo* metabolism of gelsenicine (**1**) was investigated. The results showed that 14-hydroxygelsenicine (**2**) was the metabolite of gelsenicine (**1**) in human liver microsomes and rats, firstly confirmed the metabolic transformation of gelsenicine (**1**) (Fig. 2C−E). Together, these results demonstrate that gelsedine- and humantenine-type toxic alkaloids, gelsenicine (**1**), 14-hydroxygelsenicine (**2**), humantenine (**17**), and humantenirine (**18**), are the major toxic components responsible for *G. elegans* poisoning. Among them, gelsenicine (**1**) is the most toxic one and abundant in the plant.

2.4. *The toxicological mechanism of gelsenicine*

To directly evaluate the effect of gelsenicine (**1**) on respiration, we examined key parameters of respiratory function using whole-body plethysmography[29]. Within 5−10 min after receiving intraperitoneal injection of gelsenicine (**1**, 0.22 mg/kg), mice experienced severe respiratory distress, exhibiting symptoms including abdominal breathing, gasping, cyanosis, and convulsions. Whole-body plethysmography showed that the respiratory flow rate sharply decreased in mice 10 min post gelsenicine (**1**) injection, when compared to those injected with saline (Fig. 3A and B). Statistical analyses revealed a significant reduction of peak expiratory flow, peak inspiratory flow, frequency, tidal volume, and minute ventilation in mice administered with gelsenicine (**1**) (Fig. 3C−G). These data provide direct evidence that gelsenicine (**1**) led to respiratory failure.

To investigate whether gelsenicine (**1**) has an inhibitory effect on respiratory neurons in the central nervous system (CNS), we examined gelsenicine (**1**)-mediated excitability changes in VRG neurons in the medulla, the principal neurons for regulating respiration. First, to confirm whether gelsenicine (**1**) is distributed to medulla, we collected medulla tissues from C57BL/6 mice following intraperitoneal injections of 1 mg/kg gelsenicine (**1**) at 5 and 10 min post-administration. Further time points were not examined as most mice died around 10 min. Gelsenicine (**1**) was quickly distributed to medulla at 5 min, and sustained until 10 min (Supporting Information Fig. S49).

We then measured activity of VRG neurons using whole-cell electrophysiological recordings. For comparison, we also examined activity changes in CA1 pyramidal neurons in the hippocampus as a representative neuronal population involved in higher-order brain functions such as cognition. Various concentrations of gelsenicine (**1**) were applied to VRG neurons in medullary slices or to CA1 pyramidal neurons in hippocampal slices (Fig. 4A). The firing of action potentials was recorded to compare the effects of gelsenicine (**1**) on neuronal excitability in the two brain regions. The action potentials of VRG neurons before gelsenicine (**1**) application were firing rhythmically, in phase with the respiratory rhythm, showing clustered events in each episode (Fig. 4B−D). Gelsenicine (**1**) was applied at a concentration range of 0.3−2 μmol/L. The 0.3 and 0.6 μmol/L concentrations did not have an inhibitory effect on VRG neurons, whereas the 1 and 2 μmol/L concentrations significantly reduced the firing rates of action potentials. Specifically, 1 μmol/L gelsenicine (**1**) decreased

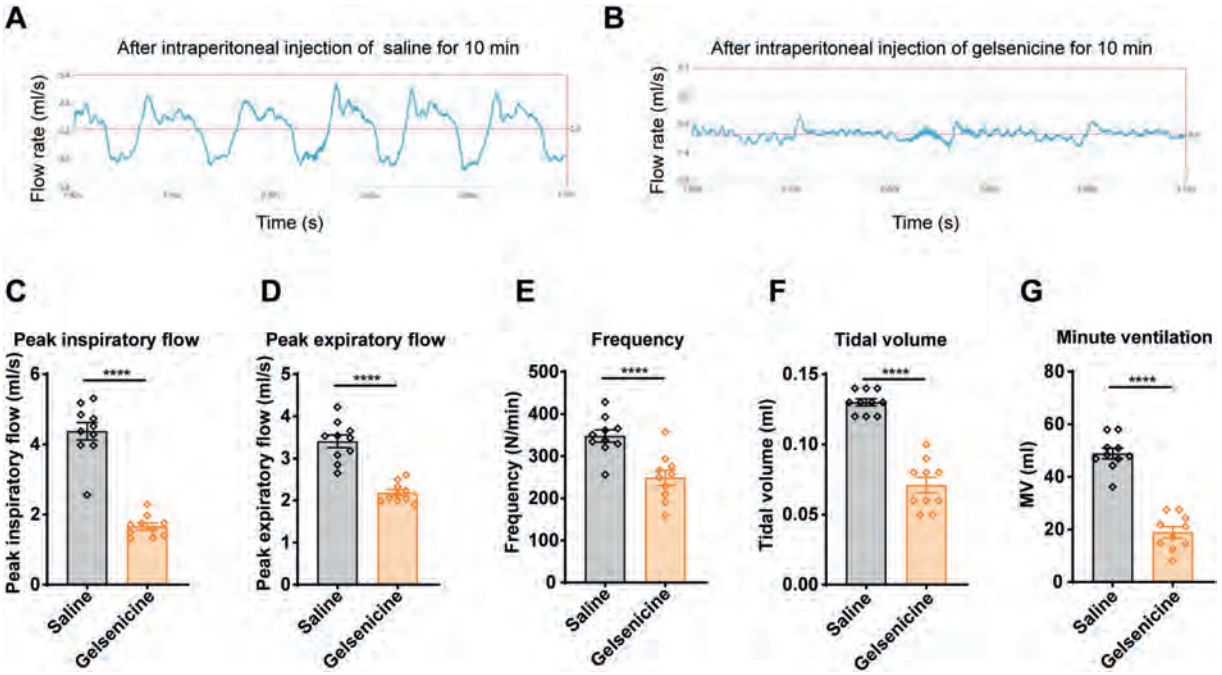

Figure 3 Gelsenicine (**1**) led to severe respiratory failure. (A, B) Breathing curves at 10 min after intraperitoneal injection of saline (A) or gelsenicine (**1**) at a dose of 0.22 mg/kg body weight (B). (C−G) Respiratory function indices were detected by the whole-body plethysmography, including peak inspiratory flow (C), peak expiratory flow (D), frequency (E), tidal volume (F), and minute ventilation (G). Ten mice per group were used for the experiments. Two-tailed unpaired *t*-test, ****$P < 0.0001$.

the firing rate by about 60%, and 2 μmol/L decreased it by about 70% (Fig. 4D). In contrast, 1 and 2 μmol/L gelsenicine (**1**) failed to show an inhibitory effect on CA1 pyramidal neurons in the hippocampus, and the action potentials were only suppressed when the concentration of gelsenicine (**1**) was increased to 5 μmol/L or more (Fig. 4C−E). In addition, 5 μmol/L of gelsenicine (**1**) increased the amplitude of miniature inhibitory synaptic currents (mIPSCs) in hippocampus CA1 region (Supporting Information Fig. S50). Therefore, low concentrations of gelsenicine (**1**) selectively inhibit central respiratory neurons without affecting neurons in other brain regions such as the hippocampus. The selective inhibition of respiratory center neurons may thus underlie respiratory failure, the primary cause of death by gelsenicine (**1**).

Neuronal activity is balanced by excitatory input and inhibitory input. It has been suggested that gelsenicine (**1**) modulates $GABA_A$ receptors[11], which are the main inhibitory receptors in neurons. However, it is unclear whether gelsenicine (**1**) directly stimulates $GABA_A$ receptors to inhibit VRG neurons. To address this, picrotoxin, a non-competitive $GABA_A$ receptor antagonist, was applied to block $GABA_A$ receptors before addition of gelsenicine (**1**). As the activation site is occupied by picrotoxin, the receptors cannot be stimulated by other drugs. VRG neurons incubated with picrotoxin (50 μmol/L) showed a robust increase in activity caused by the blockade of $GABA_A$ receptor-mediated inhibitory input, shown by the significantly higher action potential firing rates compared with baseline (Fig. 5A and B). Gelsenicine (**1**) (1 μmol/L) was then added to VRG neurons in the presence of picrotoxin. Gelsenicine (**1**) did not affect the firing rate of picrotoxin-treated VRG neurons, which remained high (Fig. 5A and B). These results suggest that gelsenicine (**1**) inhibits VRG neurons by stimulating $GABA_A$ receptors.

We then tested whether the inhibition of VRG neurons by gelsenicine (**1**) could be reversed by $GABA_A$ receptor antagonism. Gelsenicine (**1**) (1 μmol/L) was first added to VRG neurons to inhibit their activity, and then a competitive $GABA_A$ receptor antagonist, bicuculline (20 μmol/L), was added. Notably, bicuculline blocked the inhibitory effect of gelsenicine (**1**), restoring the normal firing rate (Fig. 5C and D). The rescue of VRG neuronal activity by bicuculline suggests that $GABA_A$ receptor inhibitors may be effective antidotes for the respiratory failure caused by gelsenicine (**1**).

2.5. *Drugs for treating G. elegans poisoning*

We have shown that *Gelsemium* toxic alkaloids are highly selective for the $GABA_A$ receptor in the VRG, resulting in severe respiratory depression. Because of the widespread occurrence and high mortality of *G. elegans* poisoning, the development of effective antidotes is of great importance. To this end, four clinically used $GABA_A$ antagonists—suramin, securinine, flumazenil and bemegride—were tested for their effect on gelsenicine (**1**) poisoning in mice. All animals died around 15 min after administration of gelsenicine (**1**). Suramin, an antiparasitic drug, has been reported to be a selective competitive antagonist of $GABA_A$[30], showed weak effect on overall survival. A previous study suggested that flumazenil increases the survival of 14-hydroxygelsenicine-poisoned mice[11]. We also confirmed the antidote effect of flumazenil against gelsenicine (**1**), increasing the survival rate to 50%. Securinine also increased the survival of

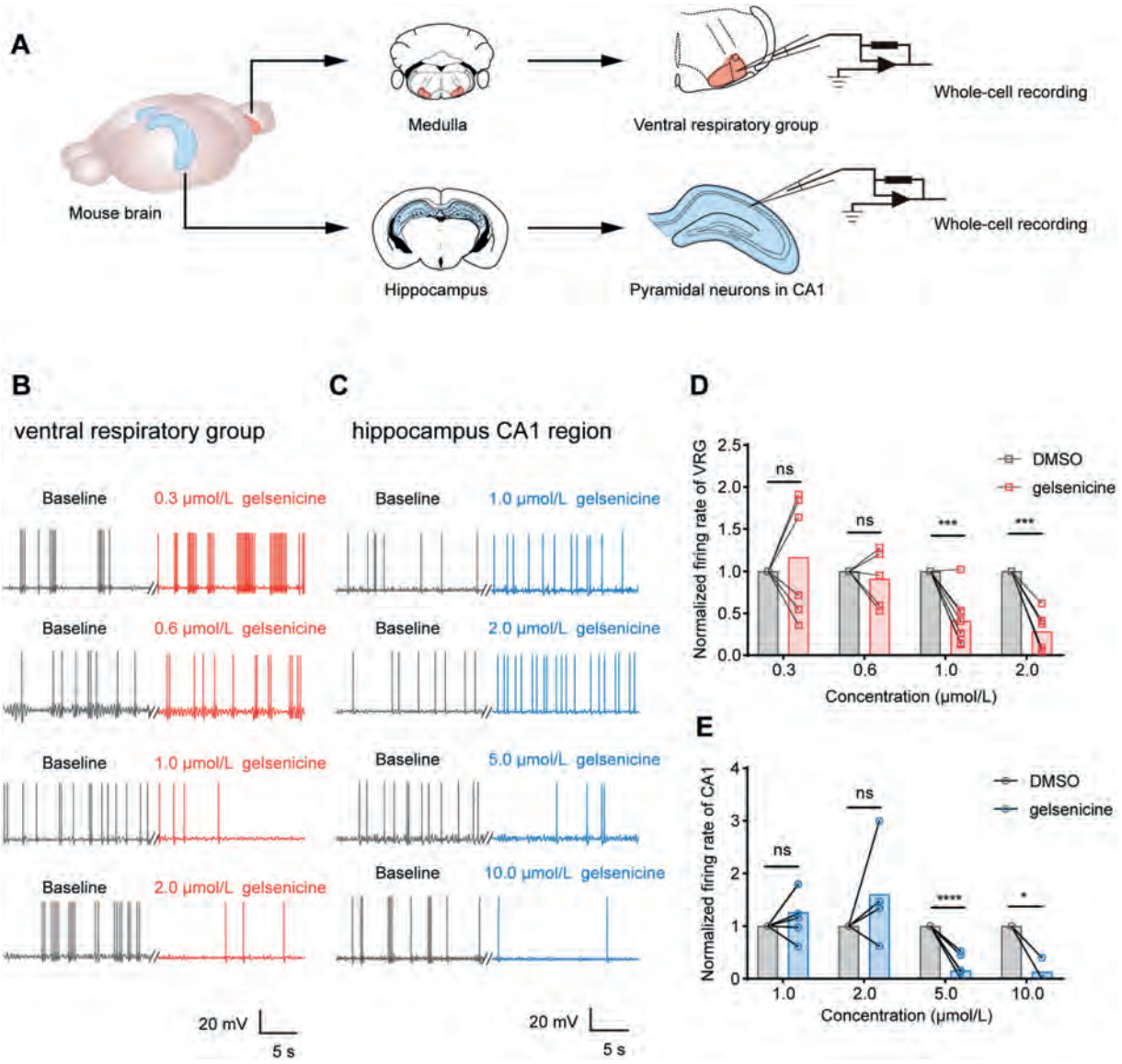

Figure 4 The inhibitory effects of gelsenicine (**1**) on the ventral respiratory group (VRG) and hippocampal CA1 region. (A) Schematic diagram of spontaneous action potentials recorded in CA1 pyramidal neurons and VRG neurons. (B) Representative action potential traces of VRG neurons before (black traces; baseline) and after (red traces) the administration of gelsenicine (**1**) at concentrations of 0.3, 0.6, 1 and 2 μmol/L. (C) Representative action potential traces of hippocampus CA1 region neurons before (black traces; baseline) and after (blue traces) the administration of gelsenicine (**1**) at concentrations of 1, 2, 5 and 10 μmol/L; (D) 0.3 μmol/L (n = 7 cells) and 0.6 μmol/L (n = 5 cells) gelsenicine (**1**) did not have inhibitory effects, while 1 μmol/L (n = 8 cells) and 2 μmol/L (n = 7 cells) gelsenicine (**1**) markedly reduced the firing rate of neurons in the VRG. (E) 1 μmol/L (n = 6 cells) and 2 μmol/L (n = 4 cells) gelsenicine (**1**) did not show inhibitory effects, whereas 5 μmol/L (n = 8 cells) and 10 μmol/L (n = 3 cells) gelsenicine (**1**) markedly reduced the firing rate of CA1 neurons. Data are presented as mean ± SEM. Two-tailed paired t-test, *P < 0.05, ***P < 0.001, ****P < 0.0001; ns, not significant.

poisoned animals to 50% at a dose of 3 mg/kg, and bemegride increased the survival to 30% at 6.5 mg/kg (Fig. 6). Securinine has long been used to treat CNS diseases such as amyotrophic lateral sclerosis in the clinic[31]. Bemegride has exhibited remarkable efficacy in the treatment of barbiturates overdose poisoning[32]. Therefore, among the four drugs tested, flumazenil and securinine produced the best effects on the inhibition of gelsenicine (**1**) poisoning, showing effects starting from 10 to 13 min. This prompt relief effects resulted in long-lasting survival until 24 h, suggesting that fast-acting $GABA_AR$ antagonization is a promising resuscitating method for *G. elegans* poisoning.

3. Discussion

G. elegans poisoning remains an important public health issue, as it has no effective treatment. Although *G. elegans* poisoning events are frequently reported from southern China and always be warned by government, studies on the toxic components, toxicological mechanisms and treatment methods are still lacking. In the present study, based on a toxicity-guided phytochemical investigation of *G. elegans*, we elucidated the structure−toxicity relationship of *Gelsemium* alkaloids. Four highly toxic alkaloids were detected in the clinical blood sample. Given the high toxicities and

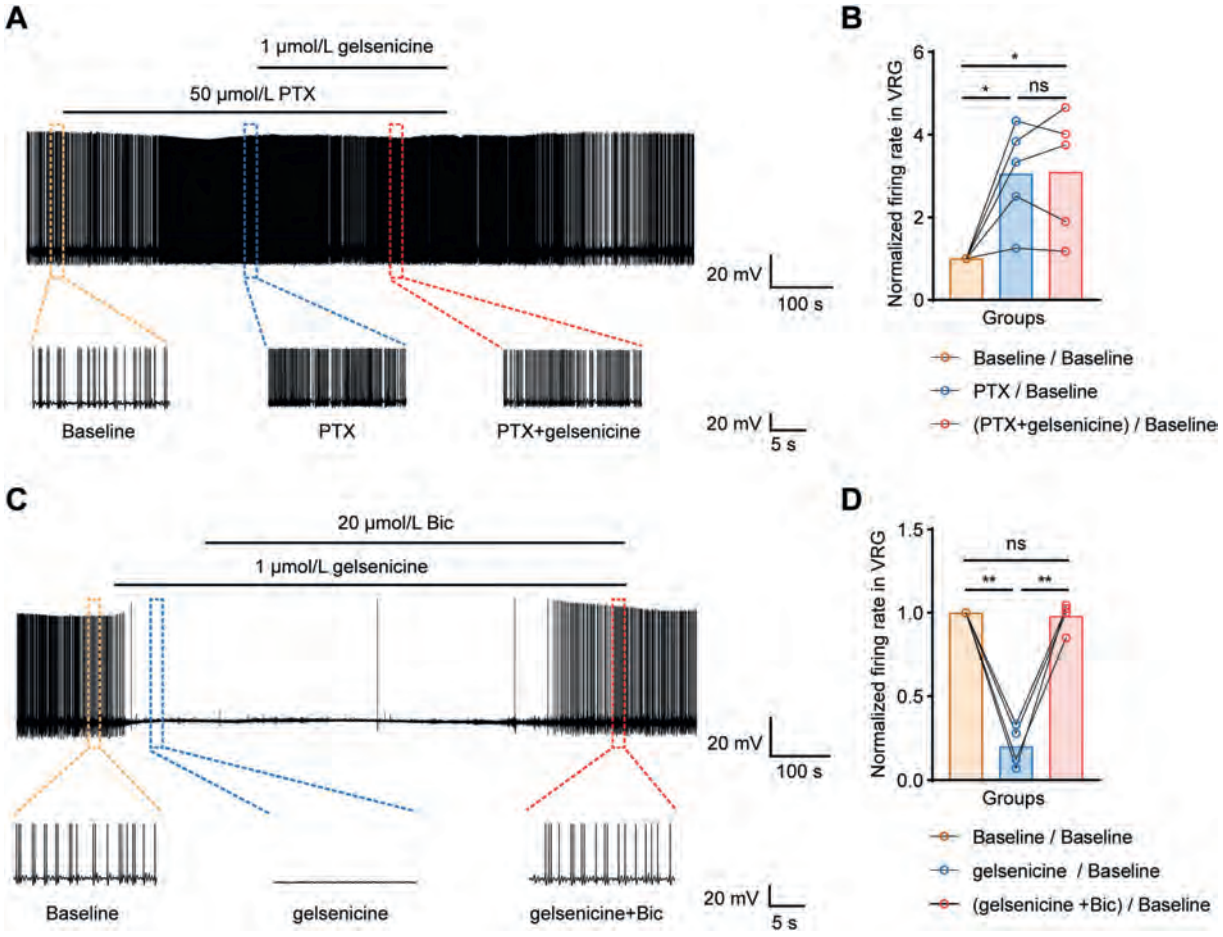

Figure 5 The inhibitory effect of gelsenicine (**1**) on VRG neurons is mediated by activation of $GABA_A$ receptors. (A) Representative action potential traces of VRG neurons treated with picrotoxin (PTX; 50 μmol/L) for 5 min and gelsenicine (**1**) (1 μmol/L) for 5 min. (B) Picrotoxin blocked the inhibitory effect of gelsenicine (**1**) on action potentials ($n = 5$ cells). (C) Representative action potential traces of VRG neurons treated with gelsenicine (**1**) (1 μmol/L) for 2 min and bicuculline (Bic; 20 μmol/L) for 12 min. (D) Bicuculline restored the action potential firing of VRG neurons exposed to gelsenicine (**1**) ($n = 4$ cells). Data are presented as mean ± SEM. Two-tailed paired *t*-test, $^*P < 0.05$, $^{**}P < 0.01$; ns, not significant.

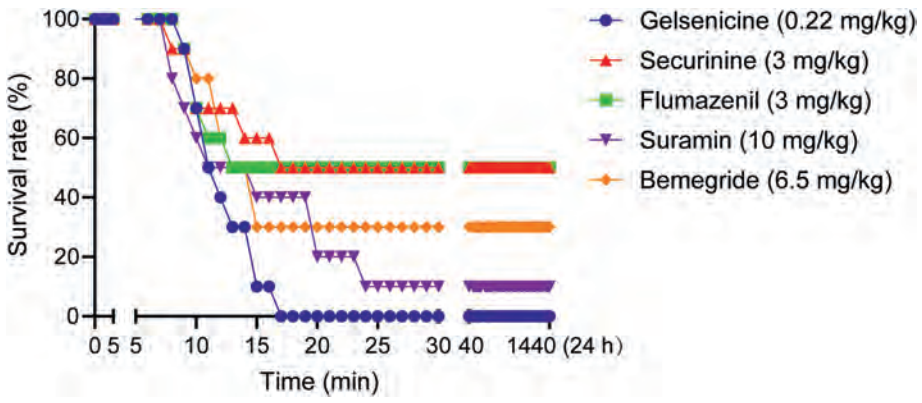

Figure 6 Survival rate curves of mice with different treatments ($n = 10$ of each group).

high levels in *G. elegans* and in the blood of the poisoning victim, gelsenicine (**1**), 14-hydroxygelsenicine (**2**), humantenine (**17**) and humantenirine (**18**) are believed to be the main toxins in *G. elegans* poisoning.

Recent studies show that gelsedine-type alkaloids can influence $GABA_A$ receptor function in cultured neurons, but the findings have been inconsistent, with the alkaloids either increasing or inhibiting receptor activity[17,33]. We speculate that

the discrepancy may be caused by differences in experimental protocol and duration of treatment, as *in vitro* cultured neurons lack the *in vivo* spatial configurations and functional connections. Moreover, the neurons in these studies were from brain regions other than the VRG, and thus, the effects on respiratory neurons remained unknown. Here, in contrast to these studies, we used acute medullary slices to examine the direct effects of gelsenicine (**1**) on respiratory neurons. Gelsenicine (**1**) strongly inhibited VRG neurons by activating $GABA_A$ receptors, and treatment with $GABA_A$ receptor antagonists rescued both action potential firing and survival. Notably, the inhibitory effect of gelsenicine (**1**) was highly selective for VRG neurons. This suggests that gelsenicine (**1**) mainly affects the respiratory center, accounting for the primary cause of death by *G. elegans* poisoning—respiratory arrest. We also confirmed that gelsenicine (**1**) showed minimal cytotoxic effects ($IC_{50} > 100$ μmol/L), suggesting that the poisoning effect of gelsenicine (**1**) was not attributable to cytotoxicity, but rather the inhibition of excitation of VRG neurons in the respiratory center through $GABA_A$ receptor activation.

The differential effects of gelsenicine (**1**) on different types of neurons might be due to differences in $GABA_A$ receptor subunit composition. $GABA_A$ receptors are heteropentamers composed of a combination of at least 19 subunits, including six α (α1−6), three β (β1−3), three γ (γ1−3), three ρ (ρ1−3) and one each of the δ, ε, π and θ subunits[34]. The majority of $GABA_A$ receptors in the cortex and hippocampus are formed by two α subunits, two β subunits and one γ subunit. It has been revealed that α1-and α2-containing $GABA_AR$s are reported to mediate the effects of benzodiazepines on the shortening of expiratory and inspiratory phase of resting breathing respectively[35]. Moreover, tramadol intoxication death involves a mechanism of respiratory depression induced by over-expression of α1 subunit in medulla oblongata solitary nucleus and ambiguous nucleus[36]. However, there are limited studies that address the specific roles of different $GABA_AR$ subtypes in respiratory toxicity. In this study, we observed higher potency of $GABA_AR$ inhibition in the VRG than in hippocampus by gelsenicine (**1**). This difference may be due to the different composition of $GABA_AR$ subunits in different brain regions.

Hippocampus is a brain region associated with higher functions including cognition, emotion, and social function. On the other hand, the medulla is part of the brainstem which regulates autonomic, involuntary functions essential for survival, including respiration, cardiovascular activities such as heart rate and blood pressure, and reflexes such as vomiting and swallowing. The hippocampus and medulla play distinct physiological roles, and they also express different compositions of $GABA_AR$s. In hippocampus, the predominant $GABA_AR$ subunits are α1, α2, α5, β2/3, and γ2, whereas in the medulla are α2, α3, and β1[37,38]. Here, we showed that the expression of GAB_AR α1 is lower, while β2 and α5 subunit are higher in the medulla when compared to hippocampus (Supporting Information Fig. S51). In addition, the δ and ε subunits also play major roles in controlling VRG neuronal excitability and respiratory behavior[39,40]. In hibernating squirrels who have significant reduction of heart rate and respiratory function, the mRNA and protein expression of the δ and ε subunits showed a notable increase in the medulla[39]. Therefore, the differential effects of gelsenicine (**1**) toward VRG and hippocampal neurons may be related to the binding affinity of gelsenicine (**1**) for different $GABA_A$ receptor subtypes. Moreover, it was reported that the NMDA receptors, the main receptors in excitatory synaptic transmission, may also contribute to the effect of gelsedine-type alkaloids[41]. NMDA receptors are heterotetramers that differ in composition in various brain regions. Therefore, further investigations are required to dissect the molecular action of gelsenicine (**1**) toward the different subunits, individually, as well as the whole receptor.

Our results showed that securinine and flumazenil increased the survival rate of poisoned animals from 0% to 50%, and bemegride to 30%. We believe that all of these three drugs exhibit protective effects in the poisoned animals. Suramin only increased the survival rate to 10%, suggesting poor antidote potential. The varied effects may depend on the different pharmacological pharmacokinetic properties of these drugs.

The GABA binding sites are located at the interfaces between the principal face (+) of β and complementary face (−) of α subunits. $GABA_AR$ also possesses multiple allosteric modulation sites, including the benzodiazepine site at $\alpha+/\gamma-$ interface, and the barbiturate site at $\alpha+/\beta-$ and $\gamma+/\beta-$ interfaces[34,42]. Flumazenil is a potent $GABA_AR$ antagonist that targets the benzodiazepines site. It exhibits an IC_{50} value of 2 nmol/L in a radioligand binding assay utilizing rat cortical synaptosomes, which reflects a high efficiency on $GABA_AR$ inhibition[43]. According to clinical and animal studies[44,45], flumazenil can be rapidly absorbed, reaching the peak level within 5 min and taking effect quickly whether administered orally or intravenously. Securinine is a $GABA_AR$ antagonist that competes the receptor-binding with GABA with an IC_{50} of 57 μmol/L[46]. It reaches peak concentration within 9 min in plasma and 30 min in the brain following intraperitoneal injection[47]. Bemegride, with an IC_{50} of 200 μmol/L, inhibits $GABA_AR$ in a barbiturate-like manner[48,49]. Bemegride reaches to peak concentration and readily passes in the brain within 30 min[50]. However, bemegride has relatively short action period which may require repeated administration. This may explain the weaker effects of bemegride than flumazenil and securinine. Regarding suramin, it is a multi-targeted agent exhibiting antagonism on $GABA_AR$, purinergic receptors, and many enzymes. The wide range of targets and complex mechanism may cause various adverse reactions. Additionally, suramin has poor ability for passing through the blood−brain barrier. Although it may reach to the brainstem, the relatively slow peak time in plasma (>30 min) makes it not a suitable antidote for central nervous system $GABA_AR$ hyperfunction poisoning[51,52].

The clinical approaches to *G. elegans* poisoning mainly include clearance of toxic substances and respiratory support. We show here that the representative toxic component gelsenicine (**1**) exerts a strong inhibitory effect on VRG neurons by stimulating $GABA_A$ receptors. This key finding should provide a foundation for guiding clinical treatment of *G. elegans* poisoning. Importantly, securinine and flumazenil significantly increased the survival of gelsenicine (**1**)-poisoned mice. As securinine and flumazenil are both clinically approved drugs, they may have potential in the emergency treatment of *G. elegans* poisoning.

4. Conclusions

In summary, we performed a systematic phytochemical investigation of *G. elegans*, resulting in the isolation and identification of 120 alkaloids. Acute toxicity screening enabled the first elucidation of the structure−toxicity relationship of *Gelsemium* alkaloids. Among them, gelsedine- and humantenine-type alkaloids were confirmed as the major toxic components through toxicity assays and clinical sample validation. The most toxic compound, gelsenicine (**1**), selectively inhibited the firing of action potentials in

ventral respiratory group neurons in the medulla by stimulating $GABA_A$ receptors. Importantly, the clinically approved $GABA_A$ receptor antagonists securinine and flumazenil significantly improved the survival of poisoned animals. These findings provide critical insight into the toxicological mechanisms of *G. elegans* poisoning and offer a basis for developing effective emergency treatments.

5. Experimental

5.1. Materials

Pure alkaloids (purity >95%), 95% ethanolic extract, non-alkaloidal extract and total alkaloid extract of *G. elegans* were made in our laboratory. Human liver microsomes were acquired from PrimrTox Technology Inc. (Wuhan, China). Carbamazepine was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Suramin (purity ≥98.0%) was obtained from Meryer Chemical Technology Co., Ltd. (Shanghai, China). Securinine (purity ≥98.0%) was purchased from Mreda Technology Inc. (Fair lawn, NJ, USA). Flumazenil (purity >99.0%), NaCl, $NaHCO_3$, sucrose and dextrose were obtained from Aladdin Reagent Ltd. (Shanghai, China). Bemegride (purity 99.6%) was bought from Bide Pharmatech Co., Ltd. (Shanghai, China). KCl, NaH_2PO_4, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, K-gluconate, HEPES, Na_3GTP, MgATP and Na-phosphocreatine were purchased from Sigma−Aldrich (St. Louis, MO, USA).

5.1.1. Plant material

The leaves and stems (40.0 kg) and fruits (26.5 kg) of *G. elegans* were collected from Shaoguan city, Guangdong province, and Zhangzhou city, Fujian province, China, in October, 2011 and February, 2016, respectively. The roots (18.0 kg) and flowers (11.5 kg) of *G. elegans* were collected from Conghua city, Guangdong province, China, in October, 2014. Due to a large demand for plant material, they were collected from different habitats. All materials were authenticated by Prof. Guang-Xiong Zhou (Jinan University, Guangzhou, China). The voucher specimens have been deposited in the Center for Bioactive Natural Molecules and Innovative Drugs Research, Jinan University, Guangzhou, China.

5.1.2. Clinical blood specimens

The blood sample of a 6-year-old *G. elegans* poisoning fatality was obtained from Puning People's Hospital, Puning city, Guangdong province, China. Human serum was obtained from consenting healthy human volunteers. The clinical blood specimen used in this study was approved by the Institutional Review Board of Jinan University (approval number: JNUKY-2022-001), and written informed consent was obtained from the patient's guardian prior to inclusion in the current study.

5.2. Animals

Adult Sprague−Dawley (SD) rats (male, 7−8 weeks of age, 180−220 g) and Kunming mice (male and female, 3 weeks of age, 16−22 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). C57BL/6J mice (male and female, 3 weeks of age, 20−25 g) were purchased from SPF Biotechnology Co., Ltd. (Beijing, China). All animals were maintained in animal rooms at standardized temperature (25−28 °C), under a 12/12-h light/dark cycle, with free access to standard rodent diet and water. The animals were allowed to adapt to the environment for 1 week before the experiments. Prior to treatment, the animals were fasted for 12 h, and free access to water and standard diet was given thereafter. The animal experiments were approved by the Laboratory Animal Ethics Committee of Jinan University (approval numbers: 20220607-09 and 20220708-01).

5.3. Extraction, isolation, and structural characterization

A systematic chemical investigation on the total alkaloid fractions of roots, leaves and stems, flowers and fruits of *G. elegans* resulted in the isolation and identification of 120 alkaloids. The extraction, isolation, and structural characterization methods were described in the Supporting Information Their structures with absolute configurations were determined by extensive spectroscopic analyses, single-crystal X-ray diffraction, and quantum chemical calculations.

5.4. Acute toxicity study

The Kunming mice were randomly assigned to groups of 10 mice each (five males and five females). The extracts were dissolved in DMSO, PEG400 and sterile water to the required concentration, and then administered by oral gavage. The pure alkaloids were dissolved in DMSO, PEG400 and sterile water, and administered by intraperitoneal injection at a dose of 30 mg/kg body weight. After treatment, the mice were allowed free access to food and water, and behavioral changes were assessed for 7 days. Furthermore, symptoms, including time of onset and severity, were monitored, and time of death was recorded.

Compounds causing death at 30 mg/kg body weight were selected to determine the dose causing 50% lethality (LD_{50}) using five dosages, each randomly assigned to a group of 10 mice, as above. After administration, mice were given food and water *ad libitum*, and were continuously observed for behavioral changes, symptoms and mortality for 7 days. Finally, the LD_{50} values were calculated using the Bliss method[53].

5.5. Detection and identification of toxic alkaloids in plasma

Adult male Kunming mice were administered the 95% ethanolic extract of *G. elegans* (200 mg/kg body weight) by intragastric administration. Retro-orbital blood samples were rapidly collected 5 min after gavage, and plasma samples were obtained by centrifugation (4000×*g*, 10 min, 4 °C). Acetonitrile (2:1 ratio) was added and vortexed for 1 min to precipitate plasma proteins, and then centrifuged at 14,000×*g* for 10 min. The supernatant was dried with N_2 at room temperature, re-dissolved in methanol, vortexed, and centrifuged (14,000×*g*, 10 min, 4 °C). The resulting supernatant was transferred to UPLC/MS vials, and 5 μL was used as the injection volume for analysis.

UPLC−MS/MS analysis was performed using a Q-Exactive Plus MS instrument coupled to a Dionex Ultimate 3000 UPLC system (Thermo Fisher Scientific, Bremen, Germany)[54]. Separation was performed using a Waters ACQUITY BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm, Waters Corp., Milford, MA, USA) and a binary solvent system composed of mobile phase A (water with 0.1% formic acid) and mobile phase B (acetonitrile with 0.1% formic acid). The gradient elution conditions were as follows: 0−8 min, 10%−20% B; 8−12 min, 20%−80% B. The flow rate was set to

0.3 mL/min, and the injection volume was 5 μL. The MS parameters were as follows: spray voltage of 3500/2500 V (+/−), capillary temperature of 320 °C, sheath gas (nitrogen) flow rate of 25 arb, capillary voltage of 25 V, and radio frequency lens voltage of 50 V. Analytes were detected using full-scan MS analysis in a mass range of *m/z* 50−750 at a resolving power of 70,000. A Thermo Xcalibur 2.1 workstation was used for data acquisition and processing.

5.6. Detection and quantitative analysis of toxic alkaloids in G. elegans poisoning victim serum

The whole blood of the *G. elegans* poisoning victim was centrifuged at 4000×*g* for 10 min to obtain serum. A 300-μL aliquot of the serum was added to 600 μL acetonitrile and vortexed for 2 min, and then centrifuged at 14,000×*g* for 10 min at 4 °C. The resulting supernatant was dried under a stream of nitrogen and resuspended in 100 μL methanol. After vortexing the mixture for 1 min and centrifuging at 14,000×*g* for 10 min at 4 °C, 5 μL of the supernatant was analyzed by UPLC−MS/MS. The chromatography and MS conditions were as described above for the detection of toxic alkaloids in mouse plasma, and the MS instrument used was a Q-Exactive (Thermo Fisher Scientific, Bremen, Germany).

For quantitative analysis, the samples were processed as described above after addition of internal standard (carbamazepine). The prepared samples were analyzed on a Thermo Ultimate LC 3000 (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an AB Sciex Triple Quad 4500 MS instrument (AB SCIEX, MA, USA). The liquid chromatography conditions were as described above. The analytes were analyzed using MRM mode, and the detailed MRM conditions for each compound are given in Supporting Information Table S14. The LOQ ranged from 6.2 to 1683.2 ng/mL for gelsenicine (**1**), 14-hydroxygelsenicine (**2**), humantenine (**17**) and humantenirine (**18**) (described in Supporting Information Table S15). The recovery was 86.66%−95.40%, with a coefficient of variation (CV) of <7.58% for the four compounds (Supporting Information Table S16), suggesting that the employed method can be used for the quantification of alkaloids in blood samples[55,56].

5.7. In vitro metabolism of gelsenicine (1) by human liver microsomes

The incubation mixture consisted of 50 mmol/L Tris−HCl buffer solution (pH 7.4), $MgCl_2$ (5 mmol/L), human liver microsomes (0.5 mg/mL), and different concentrations of gelsenicine (**1**)[57,58]. A sample lacking gelsenicine (**1**) was used as the blank control, and incubation without NADPH served as negative control. After preincubation at 37 °C for 5 min, NADPH was added to a final volume of 200 μL to initiate the reaction, and then incubated for 10, 20, 30, 40, 50 or 60 min. The reactions were terminated by adding 400 μL of ice-cold acetonitrile (containing carbamazepine as an internal standard). The mixtures were vortexed and centrifuged at 14,000×*g* for 10 min. The supernatant was dried with N_2 and re-suspended with 100 μL of methanol for centrifugation again. Finally, 5 μL supernatant was injected into the UPLC−MS/MS system for analysis.

5.8. In vivo metabolism of gelsenicine (1) in rats

Twenty-four SD rats were randomly divided into the following four groups: control group ($n = 6$, one each for blank plasma) and three drug groups ($n = 6$, one each for dosed plasma). The administration groups were given gelsenicine (**1**) (0.8 mg/kg) by oral gavage, and the control group received the same volume of saline. Blood samples were collected from abdominal vein at 10, 15, and 30 min post-administration, and were processed as described above for quantitative analysis by UPLC−MS/MS[59].

5.9. Evaluation of respiratory function

Twenty C57BL/6J mice were randomly divided into two groups, the treatment group and the control group, each consisting of ten mice. Mice were first placed in the plethysmography chambers of a whole-body plethysmograph (WBP-8WR, TOW) for 30 min to acclimate the environment. The mice were then received intraperitoneal injection of 0.22 mg/kg gelsenicine (**1**; treatment group) or equivalent volume of saline (control group), and immediately monitored for respiratory function in the plethysmography chambers for 5−10 min. Respiratory parameters including peak inspiratory flow, peak expiratory flow, respiration frequency, tidal volume, and minute ventilation were determined by ResMass version 1.4.2 (TOW)[29].

5.10. In vitro cytotoxicity evaluation

HT22 cells (Jennio Biotech, Guangzhou, China) and Vero cells (National Collection of Authenticated Cell Cultures, Shanghai, China) were seeded into 384-well plates (Corning, Cat#15140163, USA) at densities of 800 and 1000 cells per well, respectively. Additionally, different *Gelsemium* alkaloid was transferred to 384-well plates with a concentration range of 0.2−100 μmol/L in 1:2 gradient dilutions. Both blank (empty wells) and control groups received the same volume of DMSO. The plates were then incubated in a 37 °C CO_2 incubator (ESCO, CCL-170B-8, Singapore) for 24 h. Subsequently, 3 μL of CCK-8 (Bimake, B34306, USA) solution was added to each well. After an additional 2−3 h, the absorbance at 450 nm was measured. The cell viability was calculated as Eq. (1):

$$\text{Cell viability} = (A_{\text{compound}}\text{-}A_{\text{blank}})/(A_{\text{control}}\text{-}A_{\text{blank}}) \times 100\% \quad (1)$$

where A stands for absorbance.

5.11. Western blot analysis

After sacrifice, hippocampus and medulla were quickly dissected out. Tissue samples were homogenized in cold RIPA lysis buffer (1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS) containing protease inhibitors (TargetMol, USA, C0001), and phosphatase inhibitors (TargetMol, USA, C0002/C0003), and lysed for 30 min. Lysates were cleared by centrifugation at 12,000 rpm (15,294×*g*) for 30 min at 4 °C. Protein concentration was determined using bicinchoninic acid assay (Beyotime Biotechnology, P0012, Shanghai, China), and the total protein for each sample was separated by SDS-PAGE followed by Western blot analysis. The primary antibodies used were as follows: anti-$GABA_A$ receptor α1 (Abcam, ab252439), anti-$GABA_A$ receptor α5 (Abcam, ab259880), anti-$GABA_A$ receptor β2 (Abcam, ab156000).

5.12. Electrophysiology

Whole-cell recordings were performed as described before[41,60,61]. Coronal slices (300-μm-thick) of hippocampus were prepared

from 2-week-old C57BL/6J mice, and 200-μm coronal slices of the medulla were prepared from 8 to 11-day-old C57BL/6J mice on a tissue slicer (Vibratome 300; Vibratome) in ice-cold dissection buffer (213 mmol/L sucrose, 2.6 mmol/L KCl, 1.6 mmol/L NaH_2PO_4, 10 mmol/L $MgCl_2$, 0.5 mmol/L $CaCl_2$, 26 mmol/L $NaHCO_3$ and 9 mmol/L dextrose, bubbled with 95% O_2/5% CO_2). The slices were immediately transferred to isotonic artificial cerebrospinal fluid (ACSF; 126 mmol/L NaCl, 3 mmol/L KCl, 1.25 mmol/L NaH_2PO_4, 1 mmol/L $MgCl_2$, 2 mmol/L $CaCl_2$, 26 mmol/L $NaHCO_3$ and 10 mmol/L dextrose, bubbled with 95% O_2/5% CO_2) at 32 °C for 30 min before recordings. All recordings were performed in modified ACSF (126 mmol/L NaCl, 3.5 mmol/L KCl, 1 mmol/L NaH_2PO_4, 0.5 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 26 mmol/L $NaHCO_3$ and 25 mmol/L dextrose, bubbled with 95% O_2/5% CO_2) at 28−30 °C[62]. Pyramidal cells in CA1 areas were identified visually under infrared differential interference contrast microscopy on the basis of their pyramidal somata and prominent apical dendrites, and excitatory neurons in the VRG were identified based on their triangular-shaped somata.

The excitability of CA1 and VRG neurons was measured by whole-cell recordings in current-clamp mode at a voltage range of −48 to −55 mV using a B700 amplifier (Axon Instruments). Patch pipettes (4−6 mΩ) were filled with internal solution consisting of the following components (in mmol/L): 130 K-gluconate, 10 KCl, 10 HEPES, 0.5 Na_3GTP, 4 MgATP, 10 Na-phosphocreatine, pH 7.2−7.4. Osmolarity ranged from 290 to 300 mOsm. After recording basal spontaneous action potentials for 10 min, different concentrations of gelsenicine (**1**) were bath-applied to VRG neurons (0.3−2 μmol/L) or CA1 pyramidal neurons (1−10 μmol/L) for 10 min to record drug-evoked spontaneous action potentials. To investigate the participation of $GABA_A$ receptors in the effect of gelsenicine (**1**), the $GABA_A$ receptor antagonist picrotoxin (50 μmol/L) was applied to VRG neurons for 5 min before application of gelsenicine (**1**; 1 μmol/L) for 5 min. Alternatively, gelsenicine (**1**; 1 μmol/L) alone was given for 2 min to inhibit the neurons first, and then the $GABA_A$ receptor antagonist bicuculline (20 μmol/L) was applied in the presence of gelsenicine (**1**; 1 μmol/L) for 12 min. Electrical signals were digitized at 2 kHz by a data acquisition board (National Instruments) and were quantified with Igor ProTM software (WaveMetrics, Portland, OR, USA).

5.13. Quantitative analysis of gelsenicine (*1*) in central nervous system

Twelve C57BL/6J mice were randomly divided into two groups ($n = 6$ per group) and administered gelsenicine (**1**) (1 mg/kg) *via* intraperitoneal injection. The mice were sacrificed at 5 min (group 1) and 10 min (group 2) post-administration, and their medullas were dissected out. Tissue samples (∼50 mg) were homogenized in 500 μL of deionized water and spiked with 20 μL of carbamazepine. Subsequently, the samples underwent processing as previously described for quantitative analysis using UPLC−MS/MS as applied to plasma samples.

5.14. Screening of effective antidotes for G. elegans poisoning

Fifty male Kunming mice were randomly divided into five groups ($n = 10$ for each group), including control and four treatment groups. Based on the acute toxicity test results, the mice in the control group were injected intraperitoneally with a lethal dose of gelsenicine (**1**) (0.22 mg/kg), while those in the treated groups were intraperitoneally administered different doses of therapeutic agents after injection of gelsenicine (**1**). Four $GABA_A$ antagonist drugs—suramin (10 mg/kg), securinine (3 mg/kg), flumazenil (3 mg/kg) and bemegride (6.5 mg/kg)—and their doses were selected as previously published with slight modification or converted from the human clinical dose[11,47,63].

5.15. Statistical analysis

All statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, La Jolla, USA) and Igor Pro (WaveMetrics, Portland, OR, USA). Data for clinical samples and metabolic experiments were expressed as mean ± standard deviation (SD), and those for electrophysiological data were expressed as mean ± standard error of mean (SEM). Differences between the firing rates were analyzed using two-tailed paired *t*-test, and were considered significant at values of $P < 0.05$.

Acknowledgments

This work was supported by the Guangdong Basic and Applied Basic Research Foundation (Nos. 2024B1515040014, 2022B1515130007, 2023B1515040015, and 2023A1515030012, China), the National Natural Science Foundation of China (Nos. 82373750, 82293681 (82293680), 82204236, and 82371175), the Science and Technology Key Project of Guangdong Province (Nos. 2020B1111110004 and 2023A0505050121, China), and the Science and Technology Project in Guangzhou (Nos. 2024B03J1286 and 202102070001, China).

Author contributions

Niping Li: Validation, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization, Writing − original draft. Yaorong Yang: Writing − original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. Shengyuan Zhang: Validation, Methodology, Formal analysis, Data curation. Bin Jiang: Supervision, Methodology, Conceptualization. Wei Zhang: Methodology, Investigation, Data curation. Haibo Wang: Data curation, Methodology, Investigation. Lixin Chen: Supervision, Methodology, Conceptualization. Liwei Wang: Supervision, Methodology, Conceptualization. Yiyi Li: Visualization, Investigation, Data curation. Lei Shi: Funding acquisition, Conceptualization, Writing − review & editing, Supervision. Wencai Ye: Writing − review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Lei Wang:** Writing − review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting Information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2025.07.016.

References

1. Cheung WL, Law CY, Lee HCH, Tang CO, Lam YH, Ng SW, et al. *Gelsemium* poisoning mediated by the non-toxic plant *Cassytha filiformis* parasitizing *Gelsemium elegans*. *Toxicon* 2018;**154**:42−9.
2. Shen N. *Materia medica. Beijing: ancient books of traditional Chinese*. Medicine publishing house; 1982. p. 274−5.
3. Jin GL, Su YP, Liu M, Xu Y, Yang J, Liao KJ, et al. Medicinal plants of the genus *Gelsemium* (Gelsemiaceae, Gentianales)—a review of their phytochemistry, pharmacology, toxicology and traditional use. *J Ethnopharmacol* 2014;**152**:33−52.
4. Zhang H, Li Z, Chen T, Liu JJ. Detection of poisonous herbs by terahertz time-domain spectroscopy. *J Appl Spectrosc* 2018;**85**:197−202.
5. Zheng X, An W, Yao H, Xu J, Chen S. Rapid authentication of the poisonous plant *Gelsemium elegans* by combining filter-paper-based DNA extraction and RPA−LFD detection. *Engineering* 2021;**7**:14−6.
6. Qu D, Qiao DF, Chen XC, Feng CQ, Luo QZ, Tan XH, et al. Fatal poisoning by accidental ingestion of the "heartbreak grass" (*Gelsemium elegans*) verified by toxicological and medico-legal analyses. *Forensic Sci Int* 2021;**321**:110745.
7. Wen HZ, Zhu HC, Gao SP, Li ZM. Incident of gelsemine poisoning caused by migrant workers drinking self-made medicinal wine. *Shanghai J Prev Med* 2018;**30**:484−6.
8. Chen C, He J, Wei J, Chen Y, Qin N, He J, et al. Literature analysis of 1034 cases of poisoning events induced by *Gelsemium*. *J Wuzhou Univ* 2020;**30**:11−9.
9. Lin H, Qiu H, Cheng Y, Liu M, Chen M, Que Y, et al. *Gelsemium elegans* Benth: chemical components, pharmacological effects, and toxicity mechanisms. *Molecules* 2021;**26**:7145.
10. Liu M, Shen J, Liu H, Xu Y, Su YP, Yang J, et al. Gelsenicine from *Gelsemium elegans* attenuates neuropathic and inflammatory pain in mice. *Biol Pharm Bull* 2011;**34**:1877−80.
11. Yang S, Liu Y, Sun F, Zhang J, Jin Y, Li Y, et al. Gelsedine-type alkaloids: discovery of natural neurotoxins presented in toxic honey. *J Hazard Mater* 2020;**381**:120999.
12. Li YJ, Yang K, Long XM, Xiao G, Huang SJ, Zeng ZY, et al. Toxicity assessment of gelsenicine and the search for effective antidotes. *Hum Exp Toxicol* 2022;**41**:1−13.
13. Qi XJ, Huang CY, Zuo MT, Gong MD, Huang SJ, Tang MH, et al. Network pharmacology and experimental verification to unveil the mechanism of *N*-methyl-D-aspartic acid rescue humantenirine-induced excitotoxicity. *Metabolites* 2023;**13**:195.
14. Yang H, Xiang P, Yu M, Zou D, Fan X, Wang X, et al. Confirmation of *Gelsemium elegans* poisoning by UHPLC−MS/MS analysis of koumine, gelsemine, and gelsenicine in hair. *J Pharm Biomed Anal* 2022; **210**:114546.
15. Xiang H, Zhou YJ, Huang PL, Yu CN, Liu J, Liu LY, et al. Lethal poisoning with *Gelsemium elegans* in Guizhou, China. *Public health* 2016;**136**:185−7.
16. Zhou Z, Wu L, Zhong Y, Fang X, Liu Y, Chen H, et al. *Gelsemium elegans* poisoning: a case with 8 months of follow-up and review of the literature. *Front Neurol* 2017;**8**:204.
17. Sun MX, Gao HH, Zhao J, Zhang L, Xiao K. New oxindole alkaloids from *Gelsemium elegans*. *Tetrahedron Lett* 2015;**56**:6194−7.
18. Huang SJ, Zuo MT, Qi XJ, Huang CY, Liu ZY. Phosphoproteomics reveals NMDA receptor-mediated excitotoxicity as a key signaling pathway in the toxicity of gelsenicine. *Food Chem Toxicol* 2021;**156**: 112507.
19. Neubauer JA. NEUROPHYSIOLOGY|Neuroanatomy. In: Laurent GJ, Shapiro SD, editors. *Encyclopedia of respiratory medicine*. Oxford: Academic Press; 2006. p. 145−9.
20. Powell FL. VENTILATION|Control. In: Laurent GJ, Shapiro SD, editors. *Encyclopedia of respiratory medicine*. Oxford: Academic Press; 2006. p. 438−46.
21. Ikeda K, Kawakami K, Onimaru H, Okada Y, Yokota S, Koshiya N, et al. The respiratory control mechanisms in the brainstem and spinal cord: integrative views of the neuroanatomy and neurophysiology. *J Physiol Sci* 2017;**67**:45−62.
22. He QF, Wu ZL, Li L, Sun WY, Wang GY, Jiang RW, et al. Discovery of neuritogenic securinega alkaloids from *flueggea suffruticosa* by a building blocks-based molecular network strategy. *Angew Chem Int Ed* 2021;**60**:19609−13.
23. Gu JH, Zhang W, Cai WY, Fu XX, Zhou HL, Li NP, et al. Gelserancines A-E, monoterpenoid indole alkaloids with unusual skeletons from *Gelsemium elegans*. *Org Chem Front* 2021;**8**:1918−25.
24. Li NP, Liu JS, Liu JW, Tian HY, Zhou HL, Zheng YR, et al. Monoterpenoid indole alkaloids from the fruits of *Gelsemium elegans* and their anti-inflammatory activities. *Bioorg Chem* 2021;**107**:104624.
25. Li NP, Liu M, Huang XJ, Gong XY, Zhang W, Cheng MJ, et al. Gelsecorydines A−E, five gelsedine-corynanthe-type bisindole alkaloids from the fruits of *Gelsemium elegans*. *J Org Chem* 2018;**83**: 5707−14.
26. Zhang W, Xu W, Wang GY, Gong XY, Li NP, Wang L, et al. Gelsekoumidines A and B: two pairs of atropisomeric bisindole alkaloids from the roots of *Gelsemium elegans*. *Org Lett* 2017;**19**:5194−7.
27. Zhang W, Huang XJ, Zhang SY, Zhang DM, Jiang RW, Hu JY, et al. Geleganidines A-C, unusual monoterpenoid indole alkaloids from *Gelsemium elegans*. *J Nat Prod* 2015;**78**:2036−44.
28. Rujjanawate C, Kanjanapothi D, Panthong A. Pharmacological effect and toxicity of alkaloids from *Gelsemium elegans* Benth. *J Ethnopharmacol* 2003;**89**:91−5.
29. Xiong J, Zhuang T, Ma Y, Xu J, Ye J, Ma R, et al. Optimization of bifunctional piperidinamide derivatives as σ1R antagonists/MOR agonists for treating neuropathic pain. *Eur J Med Chem* 2021;**226**: 113879.
30. Luo H, Wood K, Shi FD, Gao F, Chang Y. Suramin is a novel competitive antagonist selective to $\alpha1\beta2\gamma2$ $GABA_A$ over $\rho1$ $GABA_C$ receptors. *Neuropharmacology* 2018;**141**:148−57.
31. Klochkov S, Neganova M. Unique indolizidine alkaloid securinine is a promising scaffold for the development of neuroprotective and antitumor drugs. *RSC Adv* 2021;**11**:19185−95.
32. Gershon S, Shaw FH. Effects of bemegride on barbiturate overdosage in humans. *Br Med J* 1957;**2**:1509−14.
33. Zuo MT, Wu Y, Wang ZY, Wang N, Huang SJ, Yu H, et al. A comprehensive toxicity evaluation in rats after long-term oral *Gelsemium elegans* exposure. *Biomed Pharmacother* 2021;**137**:111284.
34. Ghit A, Assal D, Al-Shami AS, Hussein DEE. $GABA_A$ receptors: structure, function, pharmacology, and related disorders. *J Genet Eng Biotechnol* 2021;**19**:123.
35. Masneuf S, Buetler J, Koester C, Crestani F. Role of α1-and α2-$GABA_A$ receptors in mediating the respiratory changes associated with benzodiazepine sedation. *Br J Pharmacol* 2012;**166**:339−48.
36. Zhang S, Guan DW, Wang L, Wang HX, Zhang GH, Zhao R, et al. The expression of $GABA_A$ receptor alpha1 and $GABA_B$ receptor 1 in medulla oblongata solitary nucleus and ambiguous nucleus in the cases of tramadol intoxication. *J Forensic Med* 2011;**27**:401−4.
37. Speigel I, Bichler EK, García PS. The influence of regional distribution and pharmacologic specificity of $GABA_AR$ subtype expression on anesthesia and emergence. *Front Syst Neurosci* 2017;**11**:58.
38. Waldvogel HJ, Baer K, Eady E, Allen KL, Gilbert RT, Mohler H, et al. Differential localization of γ-aminobutyric acid type A and glycine receptor subunits and gephyrin in the human pons, medulla oblongata and uppermost cervical segment of the spinal cord: an immunohistochemical study. *J Comp Neurol* 2010;**518**:305−28.
39. Hengen KB, Gomez TM, Stang KM, Johnson SM, Behan M. Changes in ventral respiratory column GABAaR ε- and δ-subunits during hibernation mediate resistance to depression by EtOH and pentobarbital. *Am J Physiol Regul Integr Comp Physiol* 2011;**300**:R272−83.
40. Hengen KB, Nelson NR, Stang KM, Johnson SM, Crader SM, Watters JJ, et al. Increased $GABA_A$ receptor ε-subunit expression on ventral respiratory column neurons protects breathing during pregnancy. *PLoS One* 2012;**7**:e30608.
41. Guo D, Peng Y, Wang L, Sun X, Wang X, Liang C, et al. Autism-like social deficit generated by Dock4 deficiency is rescued by restoration of Rac1 activity and NMDA receptor function. *Mol Psychiatr* 2021; **26**:1505−19.

42. Zhu S, Noviello CM, Teng J, Walsh Jr RM, Kim JJ, Hibbs RE. Structure of a human synaptic $GABA_A$ receptor. *Nature* 2018;**559**: 67−72.
43. Möhler H, Burkard WP, Keller HH, Richards JG, Haefely W. Benzodiazepine antagonist Ro 15-1788: binding characteristics and interaction with drug-induced changes in dopamine turnover and cerebellar cGMP levels. *J Neurochem* 1981;**37**:714−22.
44. Mandema JW, Gubbens-Stibbe JM, Danhof M. Stability and pharmacokinetics of flumazenil in the rat. *Psychopharmacology (Berl)* 1991;**103**:384−7.
45. Klotz U, Kanto J. Pharmacokinetics and clinical use of flumazenil (Ro 15-1788). *Clin Pharmacokinet* 1988;**14**:1−12.
46. Beutler JA, Karbon EW, Brubaker AN, Malik R, Curtis DR, Enna SJ. Securinine alkaloids: a new class of GABA receptor antagonist. *Brain Res* 1985;**330**:135−40.
47. Xiao H, Zhang Q, Zhong P, Tang G, Tao L, Huang Z, et al. Securinine promotes neuronal development and exhibits antidepressant-like effects *via* mTOR activation. *ACS Chem Neurosci* 2021;**12**:3650−61.
48. Gonzalez LA, Gatch MB, Taylor CM, Bell-Horner CL, Forster MJ, Dillon GH. Carisoprodol-mediated modulation of $GABA_A$ receptors: *in vitro* and *in vivo* studies. *J Pharmacol Exp Therapeut* 2009;**329**: 827−37.
49. Ramanjaneyulu R, Ticku MK. Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex. *Eur J Pharmacol* 1984;**98**:337−45.
50. Soto Otero R, Mendez Alvarez E, Sierra Paredes G, Galan Valiente J, Aguilar Veiga E, Sierra Marcuño G. Reversed-phase high-performance liquid chromatography method for the determination of bemegride in serum and brain tissue: pharmacokinetics and brain distribution of an intraperitoneal subconvulsive dose in rats. *J Pharm Biomed Anal* 1991;**9**:177−82.
51. Wu G, Zhou H, Lv D, Zheng R, Wu L, Yu S, et al. Phase I, single-dose study to assess the pharmacokinetics and safety of suramin in healthy Chinese volunteers. *Drug Des Dev Ther* 2023;**17**:2051−61.
52. Naviaux JC, Schuchbauer MA, Li K, Wang L, Risbrough VB, Powell SB, et al. Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy. *Transl Psychiatry* 2014;**4**:e400.
53. Zhang L, Li T, Wang R, Xu J, Zhou L, Yan L, et al. Evaluation of long-time decoction-detoxicated *Hei-shun-pian* (processed *Aconitum carmichaeli* debeaux lateral root with peel) for its acute toxicity and therapeutic effect on mono-iodoacetate induced osteoarthritis. *Front Pharmacol* 2020;**11**:1053.
54. Xu H, Li S, Liu J, Cheng J, Kang L, Li W, et al. Bioactive compounds from Huashi Baidu decoction possess both antiviral and anti-inflammatory effects against COVID-19. *Proc Natl Acad Sci U S A* 2023;**120**:e2301775120.
55. Geng Y, Chen S, Yang Y, Miao H, Li X, Li G, et al. Long-term exposure to genistein inhibits the proliferation of gallbladder cancer by downregulating the MCM complex. *Sci Bull* 2022;**67**: 813−24.
56. Hartung HP, Steinman L, Goodin DS, Comi G, Cook S, Filippi M, et al. Interleukin 17F level and interferon beta response in patients with multiple sclerosis. *JAMA Neurol* 2013;**70**:1017−21.
57. Zhang Y, Yan T, Wang T, Liu X, Hamada K, Sun D, et al. Crosstalk between CYP2E1 and PPARα substrates and agonists modulate adipose browning and obesity. *Acta Pharm Sin B* 2022;**12**:2224−38.
58. Garnar Wortzel L, Bishop TR, Kitamura S, Milosevich N, Asiaban JN, Zhang X, et al. Chemical inhibition of ENL/AF9 YEATS domains in acute leukemia. *ACS Cent Sci* 2021;**7**:815−30.
59. Sun D, Gao X, Wang Q, Krausz KW, Fang Z, Zhang Y, et al. Metabolic map of the antiviral drug podophyllotoxin provides insights into hepatotoxicity. *Xenobiotica* 2021;**51**:1047−59.
60. Ma X, Li L, Li Z, Huang Z, Yang Y, Liu P, et al. eEF2 in the prefrontal cortex promotes excitatory synaptic transmission and social novelty behavior. *EMBO Rep* 2022;**23**:e54543.
61. Li S, Wang L, Tie X, Sohya K, Lin X, Kirkwood A, et al. Brief novel visual experience fundamentally changes synaptic plasticity in the mouse visual cortex. *J Neurosci* 2017;**37**:9353−60.
62. Dani VS, Chang Q, Maffei A, Turrigiano GG, Jaenisch R, Nelson SB. Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc Natl Acad Sci U S A* 2005;**102**:12560−5.
63. Chyła G, Sałaga Zaleska K, Dąbkowski K, Kuchta A, Jankowski M. Suramin enhances the urinary excretion of VEGF-A in normoglycemic and streptozotocin-induced diabetic rats. *Pharmacol Rep* 2021;**73**: 841−6.