Central inhibition prevents the in vivo acute toxicity of harmine in mice

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ABSTRACT — Background: Harmine is a β-carboline alkaloid that displays antidepressant, antitumor and other pharmacological effects. However, the strong toxic effects limit its clinical application, and should be first considered. Purpose: To evaluate the in vivo toxicity of harmine and explore intervention strategies against its toxicity. Methods: The in vivo toxicity of harmine was assessed from the symptoms, biochemical indices, and cardiovascular effects in mice. The intervention experiments were performed by using anesthetics, central drugs, and peripheral anticholinergics. Results: The acute toxicity of harmine is significantly dose-dependent and the median lethal dose is 26.9 mg/kg in vivo. The typical symptoms include convulsion, tremor, jumping, restlessness, ataxia, opisthotonos, and death; it also changes cardiovascular function. The anesthetics improved the survival rate and abolished the symptoms after harmine poisoning. Two central inhibitors, benzhexol and phenytoin sodium, uniformly improved the survival rates of mice poisoned with harmine. The peripheral anticholinergics didn’t show any effects. Conclusion: Harmine exposure leads to central neurological symptoms, cardiovascular effects and even death through direct inhibition of the central AChE activity, where the death primarily comes from central neurological symptoms and is cooperated by the secondary cardiovascular collapse. Central inhibition prevents the acute toxicity of harmine, and especially rapid gaseous anesthetics such as isoflurane, might have potential application in the treatment of harmine poisoning.

Key words: Harmine, In vivo acute toxicity, Anesthetics, Central inhibitors, Anticholinergics

INTRODUCTION

Harmine is an active alkaloid of tricyclic β-carboline, which was first isolated from the seeds and roots of Peganum harmala in 1847 (Moloudizargari et al., 2013). Peganum harmala is named Adelasimanwureke by Uyghurs, and the seeds of it have the functions of relieving depression and nourishing the brain, relieving cough and asthma, killing insects and dysentery, and promoting the flow of body fluids and menstruation. Uyghurs use it to treat mental depression, forgetfulness, paralysis, epilepsy, cough and asthma, enteritis and dysentery, and amenorrhea in women. The herbal medicine has been collected into the Uyghur Medicine Booklet and Uyghur Medicines (Nila and Ziguli, 2004). In the northwestern region of China, harmine is commonly used by ethnic minorities as one of the important traditional medicines for treating pain with chronic strain and aseptic inflammation (Talidao, 2016). As a native component of the Uyghur medicine compound Munizi, it is also used clinically as an adjuvant for tumor therapy (Wei et al., 2017). In addition, the alkaloid in vitro has a broad-spectrum antibacterial effect (Xu et al., 2003).

Several targets of harmine, including monoamine oxidase-A (MAO-A), dual-specificity tyrosine-phosphorylation-regulated kinase1A (DYRK1A) (Bálint et al., 2017), serotonin 5-HT2A receptor (Grella et al., 1998), benzodiazepine/γ-aminobutyric acid type A (GABAA)
receptor, glutamate transporter 1 (GLT-1), reactive oxygen species (ROS) and neurotrophic factor (Moloudizargarri et al., 2013; Grella et al., 1998; Dos Santos et al., 2016; Glennon et al., 2000; Nunes et al., 2016), have been confirmed to produce a wide range of pharmacological effects, such as antitumor (Ruan et al., 2017; Gao et al., 2017; Li et al., 2017), antidepressant (Liu et al., 2018, 2017), and anti-Alzheimer disease (Frost et al., 2011). However, the noticeable toxicities on the nervous system and heart limit its clinical application. In 2015, Li and his colleagues synthesized two harmine derivatives 2DG-Har-01 and MET-Har-02 by modifying the substituents at positions-2, -7 and -9 of the harmine ring, which displayed much higher therapeutic effects for targeting cancer but lower systemic toxicity when compared to that of the native harmine (Li et al., 2015). Using structure-based design, a collection of harmine analogues with tunable selectivity toward its two inhibitory targets DYRK1A and MAO-A were synthesized by Balint and his colleagues in 2017, where modifications at position 7 decrease the affinity for DYRK1A, while substitutions at position-9 display a similar effect on MAO-A inhibition, but DYRK1A inhibition was maintained (Bálint et al., 2017). In the field of pharmaceuticals, Chen et al. (2016) reported that the N-trimethyl chitosan (TMC)-coated harmine liposome prolongs the retention time in the gastrointestinal tract (GIT), protects the harmine from enzymatic degradation, improves the transport across the Caco-2 cell monolayer, and thereby improves the oral bioavailability of harmine. Similarly, Bei and his colleagues synthesized an amphiphilic self-assembling polymer micelle of lactose-palmitoyl-trimethyl-chitosan (Lac-TPCS) with harmine for the purpose of increasing its solubility and reducing its adverse reactions (Bei et al., 2013).

All of the above efforts were carried out with the aim of maximizing the pharmacological effects of harmine while reducing its toxic effects through chemical modification or alteration of the dosage form to improve its feasibility for clinical application. So, it is very important to investigate the toxic effects of harmine and the intervention strategies for harmine poisoning in mice in this study.

**MATERIALS AND METHODS**

**Reagents**

Harmine hydrochloride (≥ 98% purity) was purchased from Chengdu Ruifensi Biotechnology (Chengdu, Sichuan, China) and stored at 4°C. The positive control drug DDVP (O,O-dimethyl-O-2,2-dichlorovinylphosphate, 80% purity) was purchased from Shanghai Yuelian Chemical (Shanghai, China). The intervention reagents used in this study are urethane (> 98% purity, BBI Life Sciences, Shanghai, China), chloral hydrate (≥ 99.5%, Shanghai Zhanyun Chemical, Shanghai, China), isoflurane (100% purity, Shenzhen Ruiwode Biological Technology, Shenzhen, Guangdong, China), benzhexol hydrochloride (98% purity, PERFEMIKER, Shanghai, China), phenytoin sodium, magnesium sulfate, levodopa, atropine sulfate (98% purity, Beijing Bailingwei Technology, Beijing, China), and pralidoxime chloride (98% purity, Huaxia Reagent, Chengdu, Sichuan, China). The acetylcholinesterase (AChE) test kit was bought from the Nanjing Institute of Bioengineering (Nanjing, Jiangsu, China).

**Animals**

The male ICR mice (24 ± 2 g, provided by the Laboratory Animal Center of Second Military Medical University, Shanghai, China) in the experiment were 6 weeks old and had been fed adaptively in the laboratory for a week, then were randomized into groups for treatment with the harmine hydrochloride to assess the acute toxicity, blood biochemical indices, pathological changes, cardiovascular toxicity and drug intervention. During the experiments, the temperature was controlled at 22 ± 2°C, and the relative humidity was 70%. The animals were free to eat and drink. All the animal experiments were approved by the Ethics Committee of the Second Military Medical University and met the relevant regulations.

**Acute toxicity evaluation**

The mice were randomly divided into 8 groups (n = 10) that were injected with different doses of harmine hydrochloride (250 mg/kg, 150 mg/kg, 75 mg/kg, 25 mg/kg, 15 mg/kg, 7.5 mg/kg, 2.5 mg/kg and 0 mg/kg, i.v.) via tail vein. The ratio of drug injection volume to mouse weight was 0.1 mL/10 g, and the injection speed was 0.2 mL/min. Mice were fasted for 12 hr before administration. After harmine administration, the acute toxicity symptoms, including tremors, convulsions, jumping, restlessness, ataxia, opisthotonos and death, were observed until the symptoms completely disappeared. Additionally, we scored the symptoms, where the normal state is 0 and the opisthotonos and death are 10. Since all mice death occurred within 30 min and the symptoms almost completely diminished within 60 min, we also calculated the median lethal dose (LD₅₀) at 60 min after harmine administration.
Central inhibition prevents harmine toxicity in vivo

Blood biochemical and histopathological analysis

The mice were randomly divided into 2 groups (n = 10), where the experimental group was treated with harmine hydrochloride (20 mg/kg, i.v.), and the control group was administered with pure water. After 60 min, blood was drawn from the inner canthus of the eye and placed in anticoagulation centrifuge tubes. After centrifugation at 3000 × g for 5 min (Peres et al., 2014), the supernatant was extracted, where the biochemical indicators, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and creatinine (Cr), were measured by the Shanghai Qianmaibomile Medical Laboratory Co., Ltd. (Shanghai, China). The heart, liver, lungs and kidneys were taken out and fixed with 4% paraformaldehyde universal tissue fixative (Taguchi et al., 2018). Routine hematoxylin-eosin (HE) staining was performed for histopathological examination.

Cardiovascular toxicity assay

The mice were randomly divided into 4 groups that were first anesthetized with urethane (ethyl carbamate, 2,000 mg/kg, i.p.), and the electrode needle for measuring the electrocardiogram was inserted into the subcutaneous tissue of the mouse limbs (Xiao et al., 2011). When everything was ready, the harmine (75 mg/kg, 25 mg/kg and 0 mg/kg, n = 6, i.v. and 50 mg/kg, n = 10, i.v.) was injected through a catheter of the jugular vein to assay its cardiovascular toxicity for a period of 30 min. The initial blood pressure and heart rate of each mouse without harmine injection to 100%, and the changes in blood pressure and heart rate of each mouse afterwards are percentages of its own initial value.

Effects of intervention drugs

A series of intervention drugs were selected to test their effects on harmine poisoning. They are the anesthetics urethane (2,000 mg/kg, i.p.), chloral hydrate (400 mg/kg, i.p.), and isoflurane, central drugs benzhexol hydrochloride (1 mg/kg, i.v.), phenytoin sodium (100 mg/kg, i.v.), magnesium sulfate (10 mg/kg, i.v.), and levodopa (10 mg/kg, i.v.), and peripheral anticholinergic drugs atropine sulfate (3 mg/kg, i.v.), atracurium besilate (3 mg/kg, i.v.), and pralidoxime chloride (10 mg/kg, i.v.). In each drug test, the mice were randomly divided into 2 groups (n = 10), the mice in the control group were only given harmine (25 mg/kg or 50 mg/kg, i.v.), while in the other group, all of the intervention drugs except the gaseous isoflurane were injected 5 min before harmine administration. The toxic symptoms of the mice were scored, and the survival curves were constructed using GraphPad prism 7.00. For the gas anesthetic isoflurane, a short period of 5 min of anesthesia was performed before and after harmine administration in two independent groups to test the effect of anesthesia on harmine poisoning as well as the compliance of neurological symptoms to anesthesia. The acute toxicity of the positive control drug DDVP was first tested, after that, the drug intervention experimental studies were performed in the same way as above.

Acetylcholinesterase activity test

Mouse blood drawn from the inner canthus of the eye was directly diluted 100-fold with pure water (blood:pure water was 1:99) and tested for its AChE activity. The effect of harmine hydrochloride in the dose range of 0-10 mg/mL on in vitro AChE activity was detected by an AChE activity test kit (Nanjing Jiancheng, Nanjing, Jiangsu, China). The in vivo AChE activity of blood influenced by harmine (25 mg/kg, i.v., n = 6) was tested using the above method at 0 min, 5 min, 10 min, and 15 min after administration. The effect of the positive control drug DDVP on AChE activity in vitro and in vivo were tested with the above methods.

Statistics

All data were expressed as the mean ± standard deviation (SD). Statistically significant differences between groups were determined by one-way ANOVA. The statistical method used to compare the experimental group and the control group in the intervention experiments is the Wilcoxon’s rank sum test. All results were considered to be statistically significant at P < 0.05.

RESULTS

Acute toxicity assessment

Harmine is an insoluble small β-carboline alkaloid with strong lipid solubility, and we therefore used the soluble compound harmine hydrochloride (Fig. 1A) to assess its toxicity through tail vein injection (Fig. 1B-D, Tables 1-2). In the dose range of 2.5-250 mg/kg i.v., both the survival rates and symptoms are significantly dose-dependent in mice, and the median lethal dose (LD50) is 26.9 mg/kg (Fig. 1C). The typical symptoms of harmine poisoning include restlessness, tremor, tic, convulsion, jumping, ataxia, opisthotonos, and final death. A scoring table between 0-10 was specially developed to evaluate the symptoms of harmine poisoning (Table 1). No mice died with the low doses of 2.5-7.5 mg/kg i.v. but exhibited a series of symptoms, including restlessness, tremor, tic, convulsion, jumping and ataxia (Table 2). The surviv-
al rates were from 10% to 90% for the median doses of 15-50 mg/kg \textit{i.v.}, with the poisoning symptoms of restlessness, tremor, tic, convulsion, ataxia and opisthotonos. If the mice did not die within 30 min, they would gradually recover to normal (Table 2). All the mice receiving the high dose range of 75-250 mg/kg, \textit{i.v.} immediately demonstrated severe irreversible opisthotonos and died quickly within 2 min (Table 2). A strong relationship between the dose of harmine and the neurological symptom score was observed, where the scores of the low, medium and high dose groups are 2.0 ± 0.0, 4.4 ± 2.2, and 9.3 ± 0.9, respectively (Fig. 1D). All these results indicated that harmine possesses a strong toxicity \textit{in vivo} with a series of central neurological symptoms that both appear and disappear quickly.

### Table 1. Neurological symptom score after harmine administration in intact mice.

| Poisoning symptoms                              | Score |
|-------------------------------------------------|-------|
| No poisoning symptom                            | 0     |
| Restlessness, tremor                            | 2     |
| Restlessness, tremor, tic                       | 4     |
| Restlessness, tremor, tic, convolution, jumping, ataxia | 6     |
| Restlessness, tremor, opisthotonos             | 8     |
| Death                                           | 10    |

Biochemical and histopathological examinations

We then tested biochemical indices (Fig. 2A-F) and performed histopathological examinations (Fig. 2G) to further evaluate the \textit{in vivo} toxicity of harmine as well as to search for injury sites other than the neurological system. The blood and organs were extracted from the live mice with a harmine dose of 20 mg/kg \textit{i.v.} at 60 min after injection when the neurological symptoms almost disappeared and the mice were still flagging due to the harmine poisoning. No significant changes were seen in the indices ALT (Fig. 2A) and AST (Fig. 2B), implying normal liver function after harmine injection. Except that AST did not increase significantly, the other heart indices including CK (Fig. 2C), CK-MB (Fig. 2D) and LDH (Fig. 2E) in the harmine group were significantly higher than those in the control group, indicating that harmine can cause rapid cardiac organ damage. Since the value of CK is much higher than that of CK-MB, it is probable that the serious tremor might lead to the increase of skeletal CK (CK-MM) thereby contributing to the high CK value. Although the index Cr significantly increased (Fig. 2F) after harmine injection, we maintain a cautious view that the increase of Cr is more likely a byproduct of neurological symptoms of harmine poisoning due to the muscle damage caused by harmine-induced tremor rather than acute kidney failure that generally does not occur within 60 min by poisoning. No obvious pathological

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**Fig. 1.** \textit{In vivo} toxicity of harmine in intact mice. (A) Molecular structure of harmine hydrochloride. (B) Survival curves of harmine poisoning in a dose range of 0-250 mg/kg \textit{i.v.} \textit{n} = 10. (C) The median lethal dose (LD50) of acute toxicity of harmine at 60 min after injection. (D) Scoring statistics based on the severity of the neurological symptoms induced by harmine in intact mice. \textit{n} = 10, **P < 0.01.
changes were seen within 60 min after harmine injection in the heart, liver, kidneys, and lungs (Fig. 2G). In summary, it is probable that harmine has strong cardiovascular toxicity in addition to neurological toxicity.

**Cardiovascular toxicity**

In the following, we naturally tested the cardiovascular toxicity of harmine using the various doses of 0, 25, 50, and 75 mg/kg \textit{i.v.}, and a dose-dependent loss of cardiac function was observed as expected (Fig. 3). At a lower dose of 25 mg/kg \textit{i.v.}, both the heart rate and blood pressure sharply decreased within the first minute after injection, following which the heart rate partially recovered and maintained at 50\% of the original value without any change in the ECG waves, while the blood pressure quickly recovered to almost 100\% of the original value within 30 min after injection. When we increased the harmine dose to 50 mg/kg \textit{i.v.}, two different kinds of manifestations appeared. The first was that the mice died immediately with the collapse of blood pressure as well as the sharp decline of the heart rate and the inversion of QRS waves within the first minute after harmine injection. However, if the mice did not die, the decreased heart rate and blood pressure would partially recover and maintain at approximately 40\% and 70\% of their original values, respectively. Although the QRS waves were inverted

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**Table 2. Toxicity symptoms after harmine administration in intact mice.**

| Harmine  | Symptoms                              | Time to death         |
|---------|---------------------------------------|-----------------------|
| Control | Normal                                | All alive             |
| 2.5-7.5 mg/kg | Restlessness, tremor, jumping, convulsion, ataxia, opisthotonos, gradually recovered within 30 min | All alive             |
| 15-50 mg/kg | Restlessness, tremor, convulsion, ataxia, opisthotonos, and death, or gradual recovery within 30 min | Some dead within 30 min |
| 75-250 mg/kg | Immediate stiffness, opisthotonos and death | Dead within 2 min     |

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![Fig. 2. Effects of harmine on blood biochemical parameters and pathological sections at 60 min after administration.](image-url)

(A) Alanine aminotransferase (ALT). (B) Aspartate aminotransferase (AST). (C) Creatine kinase (CK). (D) Creatine kinase-MB (CK-MB). (E) Lactate dehydrogenase (LDH). (F) Creatinine (Cr). \(n = 6\) in A-F, *\(P < 0.05\), **\(P < 0.01\). (G) Histopathological examination of the heart, liver, lungs and kidneys at 60 min after harmine (20 mg/kg, \textit{i.v.}) administration (400 ×, \(n = 3\)).
in the first minute, they soon returned to normal. At the
dose of 75 mg/kg, i.v., all of the mice died with the sharp
collapses of both the heart rate and blood pressure accom-
panied by the inversion of the main QRS waves within
the first minute after harmine injection. Interestingly, it is
notable that all of the anesthetized mice did not appear to
have any of the neurological symptoms such as tremor,
convulsion, jumping, ataxia, and opisthotonos. All these
results not only supported the strong cardiovascular tox-
icty of harmine but also indicated that the central neuro-
logical symptoms are feasibly blocked by anesthesia.

Effect of anesthetics on harmine poisoning

We therefore chose three typical anesthetics, includ-
ing the long-acting urethane, short-acting chloral hydrate
and gaseous isoflurane, to confirm the hypothesis that
the in vivo toxicity and all of the neurological symptoms
induced by harmine can be suppressed under anesthesia.
When measuring cardiac function, we found that the anes-
thetic urethane is able to well eliminate the toxicity symp-
toms of mice administered with harmine 25 mg/kg and
improve the survival rate of mice. We increased the dose
of harmine to 50 mg/kg in order to better show the signif-
icant intervention effect of anesthetics on the toxic reac-
tion of harmine (Fig. 4). Only approximately three out of
ten (30%) anesthetized mice died without any neurologi-
cal symptoms by preinjection with the short-acting anes-
thetic chloral hydrate (400 mg/kg, i.p.), while the awake
mice all died accompanied by typical tremor, convulsion,
jumping, ataxia and opisthotonos at 50 mg/kg i.v. within
60 min after harmine injection (Fig. 4A and B). An even
better result was obtained for urethane (2,000 mg/kg,
 i.p.), where nine out of ten (90%) anesthetized mice sur-
vived without neurological symptoms within 60 min after
harmine injection (Fig. 4A and B).

One detail observed during the chloral hydrate inter-
vention is that the anesthetized mice displayed slight
tremor in the toes of their lower limbs when woken up,
implying that the anesthetic intervention to harmine poi-
soning might be reversible. To further confirm the good
compliance of neurological symptoms to anesthesia, we
then delayed the death by decreasing the harmine dose
from 50 mg/kg i.v. to 25 mg/kg i.v., and we selected gas-
eous isoflurane as the interventional reagent, with which
the anesthetic effect can both appear (30 sec) and dis-
appear (1-3 min) very rapidly. The mice in the isoflu-
rane-harmine group were preanesthetized with isoflurane
before harmine administration. After 5 min, the anes-
thesia was relieved by removing the isoflurane and the
symptoms were continuously observed for a period of
25 min. In the harmine-isoflurane group, the mice were
first injected with harmine to induce the neurological
symptoms for a period of 5 min, followed by quick anes-
thesia with isoflurane for another period of 5 min. Then,
the anesthetic was relieved, and the symptoms were continuously observed for the rest of the 20 min. As expected, only one mouse out of ten (10%) in the harmine-isoflurane group and no mice in the isoflurane-harmine group died, which is much lower than the 50% mortality in the control group (Fig. 4C). Both groups displayed perfect anesthetic compliance in that the neurological symptoms quickly disappeared when the anesthetic was given, while the symptoms reappeared and the mice woke up when the isoflurane was removed (Fig. 4D). All these results indicated that central inhibition by anesthetics are able to prevent against the in vivo toxicity by reversing the neurological symptoms of harmine poisoning.

A positive drug O,O-dimethyl-O-2,2-dichlorovinylphosphate (DDVP) was introduced here to comparatively analyze the poisoning mechanism of harmine since they have similar tremor symptoms. In the dose range of 3.53-118 mg/kg, i.v., both the survival rates and poisoning symptoms are significantly dose-dependent in mice, and the median lethal dose (LD$_{50}$) is 8.49 mg/kg (Fig. 5A). The toxic symptoms include restlessness, tremor, nausea, drooling, corestenoma, uroclepsia, ataxia and even death (Fig. 5B). We speculated that the tremor of the harmine-poisoned mice radiated from the head to the whole body caused by central toxicity, which is different from the systemic intermittent tremor of DDVP caused by the accumulation of peripheral choline. We conducted drug interventions for DDVP (8.5 mg/kg, i.v.) according to the above methods. Compared with the control group, the urethane (2,000 mg/kg, i.p.) anesthetized mice all died, whereas for those treated with chloral hydrate (400 mg/kg, i.p.) only half died (Fig. 5C). Two out of ten (20%) mice in the DDVP-isoflurane group (Fig. 5E) and three out of ten (30%) mice in the isoflurane-DDVP group died (Fig. 5E), which is slightly lower than the 40% mortality of the control group. When the isoflurane was removed and the mice woke up, tremor reappeared (Fig. 5F). In summary, anesthetics have stronger antagonistic effect against the poisoning of harmine than DDVP induced by peripheral choline accumulation, suggesting that harmine has a stronger toxic effect on the central nervous system.

**Effect of central drugs on harmine poisoning**

Since the general anesthetics can improve the survival rate of harmine poisoning and its typical neurological symptoms in mice, central inhibitors, including the
anticholinergic benzhexol hydrochloride, antiepileptic phenytoin sodium, and sedative magnesium sulfate, were selected to further investigate the potential mechanism of harmine. Since the tremor caused by harmine poisoning are very similar to those of Parkinson’s disease, the routine therapeutic medicine dopamine precursor levodopa was also selected. In contrast with the 50% survival rate in the harmine control group at 25 mg/kg i.v., phenytoin sodium at 100 mg/kg i.v. was the most effective, with a 100% survival rate, followed by benzhexol hydrochloride at 1 mg/kg i.v. and magnesium sulfate at 10 mg/kg i.v., with survival rates of 90% and 80%, respectively. However, levodopa (10 mg/kg, i.v.) only slightly delayed the death instead of improving the survival rate (Fig. 6A). All of the central inhibitors as well as levodopa were unable to improve the neurological symptoms (Fig. 6B). As for the interventions for DDVP poisoning, benzhexol hydrochloride at 0.3 mg/kg i.v. was the most effective, with a 100% survival rate, followed by phenytoin sodium at 30 mg/kg i.v. and levodopa at 10 mg/kg i.v., with survival rates of 80% and 60%, respectively. Unfortunately, the survival rate of the sedative magnesium sulfate at 10 mg/kg i.v. decreased to 30% (Fig. 6C). All of central inhibitors failed to eliminate the toxic symptoms of DDVP (Fig. 6D). The benzhexol hydrochloride and phenytoin sodium have direct or indirect anticholinergic effects which showed stronger antagonistic effects on both harmine and DDVP poisoning than other central inhibitory drugs, indicating that harmine may have a similar
mechanism to DDVP by inhibiting the activity of AChE.

**AChE activity test and effect of peripheral anticholinergics**

Naturally, we detected the effect of harmine and DDVP on the activity of acetyl cholinesterase (AChE). By using the whole blood as the samples, two dose-dependent curves were successfully constructed in vitro for harmine and DDVP concentrations of 0.1-10 mg/mL, and the half inhibitory concentrations were 1.16 mg/mL and 0.06 mg/mL, respectively (Fig. 7A). However, we only detected that DDVP (8.5 mg/kg, i.v.) has an inhibition on the activity of AChE in vivo (Fig. 7B). Unfortunately, three peripheral anticholinergic drugs, including the M-receptor blocker atropine sulfate, the nonselective N-receptor blocker atracurium besilate, and the AChE reactivator pralidoxime chloride did not improve the survival rates and the neurological symptoms of harmine poisoning, and the pralidoxime chloride even greatly increased the death of mice (Fig. 7C-D), indicating that the peripheral anticholinergic drugs can’t prevent against the in vivo acute toxicity of harmine. The interventions for DDVP poisoning, atropine sulfate was the most effective, with an 100% survival rate, followed by pralidoxime chloride, which can slightly increase the survival rate of mice to 70%, and the atracurium besilate has the same mortality (50%) as that of the control group (Fig. 7E). Except the atropine, the other two drugs did not improve the toxic symptoms of DDVP in mice (Fig. 7F). In summary, harmine has the ability to inhibit AChE activity, which may occur in the central nervous system.

**DISCUSSION**

**Toxic effects of harmine**

Harmine is a tricyclic β-carboline alkaloid extracted...
from the genus *P. harmala*, and it is widely used in northwestern China. Although harmine has a wide range of pharmacological effects (Li et al., 2015; Nie et al., 2016; Dos Santos and Hallak, 2017; Khan et al., 2017), its toxic effects and poor solubility limit its clinical application, which have been greatly improved by chemical modification and changing its of dosage form. However, harmine itself is a native toxic compound in *P. harmala* and other organisms, and its toxic effects as well as the intervention strategies should always be considered a priority.

In this study, we found that the half lethal dose of harmine is 26.9 mg/kg, and the typical neurological symptoms include systemic muscle tremor, convulsion and even opisthotonos, which is very similar to the toxic symptoms caused by organophosphorus pesticides, e.g., DDVP (Ren et al., 2015). The acute death of harmine poisoning occurs within 30 min. If there is no death within 30 min, the neurological symptoms of mice will grad-

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**Fig. 7.** Effects of peripheral anticholinergics on the *in vivo* toxicity of harmine and DDVP in intact mice. (A) Effects of harmine and DDVP on the *in vitro* AChE activity (*n* = 4). (B) Effects of harmine and DDVP on the *in vivo* blood AChE activity (*n* = 6). (C) Survival curves of harmine poisoning (25 mg/kg, *i.v.* after the injection with atropine sulfate (3 mg/kg, *i.v.*, *n* = 10), atracurium besilate (0.3 mg/kg, *i.v.*, *n* = 10) and pralidoxime chlorhexidine (10 mg/kg, *i.v.*, *n* = 10). (D) Symptom scores of harmine poisoning after injection with atropine sulfate, atracurium besilate and pralidoxime chloride. (E) Survival curves of DDVP poisoning (8.5 mg/kg, *i.v.*) after the injection with atropine sulfate (3 mg/kg, *i.v.*, *n* = 10), atracurium besilate (0.3 mg/kg, *i.v.*, *n* = 10) and pralidoxime chlorhexidine (30 mg/kg, *i.v.*, *n* = 10). (F) Symptom scores of DDVP poisoning after the injection with atropine sulfate, atracurium besilate and pralidoxime chloride. The scoring criteria are shown in Table 2. *n* = 10, *P* < 0.05, **P** < 0.01.
ually relieve and return to normal within 4 hr. In addition, we also found from the hematological indicators and functional studies that harmine can cause acute cardiovascular toxicity, including increases of the myocardial enzymes LDH, CK, and CK-MB, decreases in the blood pressure and heart rate, and changes in the ECG waves. These findings are basically consistent with previous studies that tremors induced by harmine were used as a model for preclinical drug tests of Parkinson’s disease (Slotkin and Distefano, 1970), in addition, the hypotensive effect of harmine was used to develop therapeutic drug for hypertension (Musgrave and Badoer, 2000). A number of studies have shown that harmine can simultaneously inhibit the activity of MAO-A and AChE, thereby increasing the production of monoamine neurotransmitters and the concentration of acetylcholine, which is probably the reason for the excitatory stimulating effects of harmine on the central nervous system. At low concentrations, it can exhibit neuroprotective effects such as antidepressant and anti-senile dementia, and it exerts the potential to treat inhibitory psychotic diseases. When the concentration is high, it leads to severe acute poisoning symptoms of the neurological system such as muscle tremor, convulsion, opisthotonos and even death. Meanwhile, for peripheral cardiovascular effects, although harmine inhibits AChE and possibly increases the concentration of acetylcholine that directly displays the negative effect on the heart, we are still cautious to speculate that the cardiovascular collapse and bradycardia induced by harmine might be from the inhibition of the central nervous system, e.g., the circulatory center, because all of the selected peripheral anticholinergics, including atropine, atracurium and pralidoxime chloride, display no or even a worse effect on harmine poisoning. Moreover, isotopically labeled $^{11}$C-harmine and other studies have confirmed that the brain is one of the main organs of harmine distribution (Murthy et al., 2007), further supporting that the neurological symptoms such as tremors and convulsions are from the central nervous system.

Central inhibition prevents the acute toxicity of harmine

In the studies of drug intervention, we found that urethane anesthesia is able to abolish the neurological symptoms and improve the survival rate of harmine poisoning. Similar results were obtained by anesthesia using chloral hydrate and isoflurane. Urethane is a reversible cholinesterase inhibitor, which can act on multiple channels and exert a wide range of anesthesia effects. Its inhibitory effect on voltage-gated sodium channels may be one of the anesthetic (central inhibitory) mechanisms. Although urethane can increase acetylcholine, its anesthetic effect can still inhibit the toxic effect of harmine (Hara and Harris, 2002). The second short-acting anesthetic chloral hydrate is metabolized to trichloroethanol in the liver, which has a high lipid-water partition coefficient and penetrates the blood-brain barrier, thus leading to anesthesia (Sourkes, 1992). Meanwhile, the inhaled fast-acting anesthetic isoflurane dissolves into the cell membrane after the alveolar blood reaches the brain tissue, leading to the disorder of the membrane lipid arrangement, the conformational and functional changes of membrane proteins such as sodium and potassium channels, depolarization of the nerve cells, and finally inhibition of the transmission of nerve impulses, thus causing general anesthesia (Ng et al., 2018). In contrast, the anesthetics have a much weaker effect on the poisoning by DDVP, which can probably be attributed to the fact that the poisoning by DDVP tends to occur in the periphery. In summary, the anesthetics induced by three different types of anesthetics can uniformly improve the survival rates and diminish the neurological symptoms after harmine exposure, indicating that the central inhibition by anesthetics can prevent against the in vivo acute toxicity of harmine in mice.

Benzhexol hydrochloride is an anticholinergic drug used to treat Parkinson’s disease that helps to restore the balance of dopamine and acetylcholine in the brain and therefore eliminates the symptoms of Parkinson’s disease in patients (Magnus, 1980). In addition to the sedative effect of phenytoin sodium, it also has an indirect anticholinergic effect by significantly inhibiting calmodulin kinase activity and the release of Ca$^{2+}$-dependent excitatory transmitters. These central inhibitors uniformly improved the survival rates of mice poisoned by harmine and DDVP, and we speculated that harmine has the same mechanism of inhibiting AChE as DDVP. The M-type anticholinergic atropine and N-type anticholinergic atracurium as well as the AChE reactivator drug pralidoxime chloride did not show any effect on either the survival rate or neurological symptoms of harmine poisoning, further favoring that harmine may have the ability to inhibit the activity of AChE in the central nervous system.

In conclusion, in this study, we assessed the in vivo acute toxicity of harmine in mice, of which the neurological symptoms of harmine poisoning, such as tremor, jumping, and opisthotonos, are mainly exhibited in a dose-dependent manner within 30 min, followed by a gradual recovery within 4 hr after administration. Meanwhile, harmine also increases the myocardial enzymes, causes the collapse of blood pressure, decreases the heart rate and changes the ECG waves, indicating its strong acute cardiovascular...
lar toxicity that probably also contributes to the death in mice in addition to its neurological toxicity. Three different types of anesthetics, urethane, chloral hydrate and isoflurane, uniformly improved the survival rates as well as abolished the neurological symptoms after harmine poisoning. In contrast, they had a much weaker effect on DDVP poisoning. The two central inhibitors benzhexol and phenytoin sodium uniformly improved the survival rates of mice poisoned with harmine and DDVP, indicating that harmine has a similar effect of inhibiting AChE activity to DDVP. In addition, we detected that harmine inhibited AChE activity in vitro and that peripheral anticholinergics did not improve mortality or neurological symptoms in harmine-poisoned mice.

In summary, harmine exposure leads to central neurological symptoms, cardiovascular effects and even death through direct inhibition of the central AChE activity, where the death primarily comes from central neurological symptoms and cooperated by the secondary cardiovascular collapse. Central inhibition prevents against the acute toxicity of harmine in intact mice. Anesthetics, especially rapid gaseous anesthetics, e.g., isofluorane, have potential applications in the treatment of harmine poisoning.

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Conflict of interest—— The authors declare that there is no conflict of interest.

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