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REVIEW

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Research progress of aconitine toxicity and forensic analysis of aconitine poisoning

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ABSTRACT

Chinese herbal medicines have been extensively used in China and other countries for centuries. Aconitine, a diterpenoid alkaloid extracted from *Aconitum* plants, has anti-inflammatory and analgesic activities, but can also induce severe arrhythmia and neurotoxicity. Aconitine poisoning accidents caused by misuse, suicide, or homicide have been reported in recent years. In China, fatal aconitine poisoning can occasionally happen on account of accidental ingestion of some wild plants or consumption of herbal decoction made from the roots of *Aconitum* plants. However, it is rather difficult for forensic experts to find the specific results in present forensic autopsy of aconitine-induced death. To further clarify its potential risk following the widespread application of aconitine, toxicological characteristics and pharmacokinetics of aconitine are reviewed. Moreover, gastrointestinal, neurological, and cardiovascular symptoms were observed frequently in aconitine poisoning cases. In addition, the review also aims at providing some convincing evidences for forensic experts to identify unexplained death with postmortem examination.

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Introduction

Herbal medicines are commonly used in China and other countries to treat various diseases due to its natural, harmless and less adverse effects for thousands of years. Traditional Chinese medicines are processed by soaking or boiling before consumption to reduce the toxicity [1]. In general, herbal poisoning may frequently occur because of inadequate processing and preparation, overdose, contamination, misidentification, and even in some suicidal or homicidal cases. *Aconitum* plants have been extensively applied to treat multiple diseases in China and some other Asian countries [2–4]. Previous studies [5,6] on *Aconitum* plants have showed its pharmacological properties such as anti-inflammation, analgesia, and anti-rheumatism. Aconitine is one of the major bioactive alkaloids extracted from Monkshood (*Aconitum napellus*), a plant getting its name for its blue to purple coloured flowers that resembles a monk's hood [7]. Aconitine belongs to the *Aconitum* genus of Ranunculaceae family and is frequently employed in herbal medicines for its anti-inflammatory, analgesic, anti-rheumatic, and cardiotonic actions [8–10]. In addition, aconitine could also suppress tumour growth and induce cell apoptosis by NF-κB signalling pathway in human pancreatic cancer, indicating that aconitine may serve as a potent therapeutic strategy for the treatment of several cancers [11]. However, the application of aconitine has been limited in clinical practice due to its toxic effects on the heart and nervous system.

With the increasing popularity of herbal drugs, herb-induced fatal poisoning cases have frequently happened as a result of inappropriate use of herbs in recent years. Aconitine, one of the abundant and high bioactive diterpenoid alkaloids, has a narrow therapeutic index, and it also brings great challenges to identify its appropriate dosage. As far as we know, aconitine poisoning cases occasionally happen in China and some other parts of Asia. For example, people are accustomed to making medicinal liquor or herbal decoctions containing aconitine for treating illness and enhancing health in some rural areas of China [12]. Thus, it is of great concern that improper process and use can lead to severe poisonous cases or even unexpected death. In addition, aconitine poisoning can also happen in some accidental ingestions, suicide or homicide cases. Previous studies [13,14] revealed the half-maximally lethal dose (LD50) aconitine for mice is 1.8 mg/kg by oral administration, and the minimum lethal dose of oral administration in humans is evaluated to be 1–2 mg. Monoester diterpenoid alkaloids (MDAs) are considered as hydrolysed products of

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diester diterpenoid alkaloids (DDAs). The LD50 of aconitine for mice by intravenous injection is approximately 38 times of its hydrolysates [15,16]. Symptoms of aconitine poisoning frequently appear within 2 h or even several minutes via oral ingestion, indicating that it can be easily absorbed with oral administration. It has been demonstrated that aconitine can also be absorbed into systemic circulation through the human skin, leading to fatal and non-fatal poisoning [17]. Furthermore, aconitine poisoning may present with abnormities symptoms of gastrointestinal, neurologic, and cardiovascular system [18]. However, there is no specific antidote and the current treatment is mainly based on the supporting therapies. Nevertheless, it was reported that there were some effective treatment methods or medicines to improve cardiac arrhythmias in aconitine-induced poisoning accidents including charcoal hemoperfusion, amiodarone, magnesium, and lidocaine [19–21].

On the one hand, aconitine is very unstable and decomposed easily in the human body. On the other hand, it is not detected routinely for common toxicology analysis in present forensic practice. Meanwhile, little attention is paid to aconitine poisoning in current clinical practice and medicolegal expertise. Consequently, it is worth noting that the history of use of herbal medication or wild plants should be taken into consideration in exploring unexplained death.

In this paper, we review the toxicological properties and pharmacokinetics of aconitine. We also attempt to describe the autopsy results so as to provide some references for dealing with unexplained death in present forensic practice.

The pharmacokinetic studies and determination method of aconitine

The ester groups combining with C8 and C14 are considered to be primarily responsible for the high toxicity of aconitine, while hydrolysis of esters can reduce its toxicity dramatically [22]. Aconitine is well known for its pharmacology actions, but also recognized as a toxic ingredient that needs to be processed properly in order to use it safely for humans. Aconitine can be hydro-lysed to less toxic benzyl aconine and aconine derivatives [23]. Considering that the therapeutic dose and the toxic dose of aconitine is close, it is crucial to use safely as a medicine in clinical applications. Indeed, establishment of the pharmacokinetic parameters of aconitine would be essential to make it play better pharmacological effects in clinics. Cytochrome P450 (CYP450) enzymes, belonging to membrane-bound hemoproteins, are involved in approximately 80% of phase I metabolism of drugs and play important roles in oxidative metabolism of drugs and exogenous substances [24–26]. CYP3A has been found to be the dominant CYP450 expressed in both liver and intestine, and it principally catalyses phase I metabolism of various alkaloids in human intestine and liver microsomes [27]. Whether CYP450 enzymes are involved in aconitine metabolism has also been further investigated. It has been reported that aconitine is mainly metabolized by CYP3A and CYP1A1/2 into less toxicity derivatives in rat liver microsomes [28]. In addition, further studies showed that aconitine can be converted into several CYP-mediated metabolites in human liver microsomes, and isoforms of CYP including CYP 3A4/5 and 2D6 were primarily responsible for the metabolism of aconitine [29].

It should be noted that the detection of aconitine concentrations in body fluids plays a vital role in clinic and forensic toxicology analysis of suspected poisoning incidents. As aconitine can decompose or metabolize rapidly, it is also difficult to detect aconitine contents in human body fluids. In previous studies, some methods have been employed for detecting aconitine such as capillary electrophoresis, gas chromatography-mass spectrometry, and high performance liquid chromatography [30,31]. However, low selectivity and sensitivity have restricted the application of these methods to some extent. At present, liquid chromatography tandem-mass spectrometry (LC-MS/MS) has developed a valid and precise method to analyse aconitine contents in blood and urine samples [32–34]. Taking into account that aconitine is metabolized fast into several derivatives in animal and human models, accordingly, the findings of aconitine and primary metabolites in blood, urine, or in herb medicine samples together with the important clinical manifestations, will provide some indispensable evidences of aconitine poisoning in present forensic practice.

Symptoms of aconitine poisoning and pathological changes in postmortem examination

Aconitine, which has a narrow therapeutic window, is considered to be the principal highly toxic DDA in *Aconitum* alkaloids. The incubation period between ingestion of aconitine and the onset of symptoms may be as short as several minutes, indicating that aconitine can be absorbed rapidly after oral administration by the upper gastrointestinal tract. It has been reported that aconitine can reach relatively high concentrations in the liver, kidney, and heart following oral intake of aconitine [35]. Patients with aconitine poisoning generally present

with a series of gastrointestinal, cardiovascular, and neurologic symptoms [36,37]. The gastrointestinal features can be nausea, vomiting, diarrhoea, and abdominal pain. Neurologic symptoms include numbness in mouth and limbs, paraesthesia, central nervous system depression, respiratory muscle depression, convulsions, and seizures. Cardiovascular manifestations predominantly include hypotension, palpitations, chest pain, bradycardia, sinus tachycardia, ventricular tachycardia, and ventricular fibrillation. Furthermore, the severity of symptoms appears to be related to the doses and time of exposure to aconitine. In particular, aconitine is also quickly decomposed or eliminated with a short half-life [38]. Thus, it is difficult for forensic specialists to identify death caused by aconitine poisoning with postmortem examination.

The signs of asphyxia are observed at autopsy including facial and nail bed cyanosis. Foamy liquid can be seen in the endotracheal cavity. The postmortem examination may find haemorrhagic points distributed on the surface of the heart and lungs [39,40]. Moreover, the microscopic examination has been reported to reveal bilateral intrapulmonary haemorrhage and oedema, congestion of multiple organs, and bilateral pleural effusions in different degrees [41]. It is worth noting that eosinophilic granulocytes can also be observed in the lungs, liver, pancreas, spleen, and gastrointestinal mucosal tissue [42].

The results of toxicological analysis are rather important for forensic identification of aconitine poisoning. So far, LC-MS/MS is a sensitive and validated method to identify toxic plant alkaloids [43]. It is necessary for forensic pathologists to exclude the presence of physical injury and fatal diseases. Meanwhile, the results of toxicological analysis showed that aconitine and its derivatives were positive and other toxic substances and illegal drugs were negative in blood, gastric content, or urine samples of victims. In this situation, death was generally considered to be caused by aconitine poisoning.

Toxic effects of aconitine

It is rather difficult to define precisely the therapeutic dose and toxic dose of aconitine because of its narrow therapeutic index. The cardiotoxicity and neurotoxicity caused by inadequate consumption of aconitine have been occasionally reported in recent years [42,44]. Therefore, the public should be warned of the risk of aconitine poisoning.

Toxic mechanisms of aconitine in cardiac system

Previous studies have revealed that aconitine could induce various types of life-threatening arrhythmias such as bradyarrhythmia, nodal tachycardia, cardiac fibrillation, bidirectional ventricular tachycardia, and intraventricular block [45–47]. *In vivo* experiment using murine model has confirmed that aconitine could suppress the progression of systemic lupus erythematosus and attenuate the pathologic impairment [48]. Aconitine-induced arrhythmias were observed in isolated rabbit atria, and results showed that aconitine might directly stimulate sinus node and inhibit the propagation of impulses in some degree [49]. Emerging evidences indicated that aconitine could block the inactivation of voltage-dependent sodium channels at the resting membrane potential causing sustained Na^+ influx, therefore leading to arrhythmia [50,51]. The aconitine-induced blockade of HERG and Kv1.5 in Xenopus laevis oocytes is considered to be one of the mechanisms of cardiac arrhythmias [52]. Moreover, in H9c2 cells and cultured neonatal rat cardiomyocytes, aconitine could produce the inhibition effect on ultrarapid-delayed rectifier K^+ current in a time- and dose-dependent manner [53].

Ca^{2+} is one of the important second messengers and plays crucial roles in the process of cells signal transduction and electrical activity of myocardial cells [54–57]. Meanwhile, disruption of the intracellular Ca^{2+} homeostasis is an important mechanism of arrhythmic toxicity of aconitine [51]. It is well known that aconitine can induce abnormities of spontaneous beating rate, amplitude of spontaneous oscillations, intracellular Ca^{2+} signals, and increase the relative intracellular Ca^{2+} concentration in cultured primary cardiomyocytes, indicating that disruption of intracellular Ca^{2+} homeostasis may contribute to arrhythmic toxicity in aconitine-treated cardiomyocytes [58]. Calcium regulatory proteins, including NCX1, RyR2, DHPR, and SERCA2a in sarcoplasmic reticulum, are capable of maintaining intracellular calcium homeostasis in myocardial cells [59]. Previous publications have described that aconitine could damage myocardial cells, increase the expression of RyR2 and decrease the expression of NCX1, DHPR-α1 significantly, thus leading to the unbalance of intracellular calcium homeostasis [60]. The results of current research provided strong evidences that aconitine-induced arrhythmia was associated with intracellular Ca^{2+} signals and pre-treatment with aconitine reduced the sarcoplasmic reticulum Ca^{2+}-ATPase expression, increased the expression of Na^+/Ca^{2+} exchange in rat ventricular myocytes, which is consistent with the previous studies [61,62].

Connexin 43 (Cx43) is the principal gap junction protein in heart [63]. Previous studies have demonstrated that Ser368, a protein kinase C site, is involved in the regulation of gap junction function.

It is well known that the Ser368 remains phosphorylation status under normal circumstances [64,65]. Aconitine could induce Cx43 and protein kinase C-a dephosphorylation and alter Ca^{2+} oscillation frequency, which probably is associated with cellular signal transduction, finally leading to cardiac toxicity in cultured neonatal rats cardiomyocytes [66].

More importantly, aconitine could also shorten action potential duration, reduce L-type calcium currents, thereby contributing to the proarrhythmic effects in human-induced pluripotent stem cell-derived cardiomyocytes. The effects of aconitine-caused arrhythmia and underlying mechanisms were further studied in human cardiomyocytes model [67]. In addition, it is noteworthy that the Na^{+}–Ca^{2+} exchange (NCX) system plays a vital role in regulating cardiac contractility and electrical activity in different animal modes. KB-R9743 (KBR), a selective inhibitor of NCX in cardiac muscle, is capable of inhibiting aconitine-induced arrhythmias in guinea pigs and isolated ventricular myocytes [62,68].

Drug–DNA interaction is regarded as one of the reasons of the DNA damage of some drugs [69]. The cytotoxicities of aconitine were further investigated in rat myocardial cells H9c2. The results showed that aconitine could exhibit cytotoxic activities including promotion of the apoptotic rate, inhibition of the growth of myocardial cells, and interaction with DNA by intercalation and electrostatic binding, implying that the aconitine-induced cardiac toxicity and DNA injury are correlated in a certain degree [70]. Our study showed that aconitine could induce apoptosis of H9c2 cells at least in part via mitochondria-dependent apoptotic pathway [71]. However, the specific mechanism remains unknown and still needs further study.

Toxic effects of aconitine on nervous system

The neurotoxic effects of aconitine have been researched in different cell types. For example, it was reported that aconitine could block neuromuscular transmission and cause depolarization in mice and frog skeletal muscles [72,73]. More importantly, aconitine was reported to decrease the amplitude and block end-plate potentials and nerve action potentials, which contributes to neuromuscular blockade accompanied by excessive presynaptic depolarization in the isolated phrenic nerve-diaphragm muscles of rats [74]. Interestingly, a piece of published report demonstrated that aconitine could suppress delayed rectifier K^{+} current in differentiated NG108-15 neuronal cells and alterations in action potentials caused by aconitine might be concerned with abnormal neuronal excitability [75]. Moreover, it has been confirmed that aconitine can induce epileptiform activity in rat neocortical and hippocampal slices with acute and extended excitatory effects [76,77].

Other toxic effects of aconitine

Published studies have indicated that aconitine has evident toxic effects on the growth of embryos and morphogenesis in rat embryos cultured model. It has been reported that aconitine could cause cardiac defect, irregular somites, and brain malformation during the period of organ formation, suggesting that teratogenesis may be induced by aconitine in the process of embryonic development [78]. The long-term physiological effects of aconitine were also further investigated in mice by detecting the rectal temperature, body weight, and by electrocardiogram, and the results revealed that the toxicity of aconitine might be decreased accompanied by chronic administrations and evaluated metabolic activity of aconitine [79,80]. P-glycoprotein, encoded by the *MDR1* gene, is one of the important transporters in the apical member of mucosal cells in the intestine and excretes toxic substances into the intestinal lumen [81]. It should be stressed that aconitine could dramatically increase the P-glycoprotein levels in mice and LS174T and Caco-2 cells, concomitantly reducing acute toxicity of aconitine and other drugs toxicity by drug–drug interactions [82].

Conclusions and future prospects

With the widespread use of Chinese herb medicines, herbs-induced poisoning may be frequently encountered in the world. *Aconitum* plants are used in China and some Asian countries for treatment of various common medical problems such as pains, rheumatoid arthritis, and cardiac disorders [83]. *Aconitum* alkaloids, mainly containing aconitine, hypaconitine, and mesaconitine, are important representatives derived from the roots of plants in *Aconitum* genus. Aconitine, a high bioactive diterpenoid alkaloid derived from *Aconitum* plants, has great medicinal value, but also can cause serious poisonous cases. Despite this, based on the accumulation of traditional processing experiences and new techniques, aconitine can also be adequately processed to reduce its toxic effects and play better pharmacological effects. In our present review, we describe the toxicological effects of aconitine and further analyse aconitine poisoning cases in forensic practice. Further assessment of the underlying pharmacological properties and its safety profile are

also required for better evaluation of its potential for clinical applications in future.

Authors' contributions

Xiangting Gao participated in the data analysis, and drafted the manuscript; Jun Hu, Xincai Zhang, Yuanyi Zuo, and Yun Wang collected the data, and Shaohua Zhu conceived the idea, analysed the data, and revised the manuscript. All authors contributed to the final text and approved it.

Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of authors.

Disclosure statement

The authors declare that they have no conflict of interest.

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References

[1] Singhuber J, Zhu M, Prinz S, et al. *Aconitum* in traditional Chinese medicine: a valuable drug or an unpredictable risk? J Ethnopharmacol. 2009; 126:18–30.

[2] Chen JH, Lee CY, Liau BC, et al. Determination of aconitine-type alkaloids as markers in *fuzi* (*Aconitum carmichaeli*) by LC/(+)ESI/MS^3. J Pharm Biomed Anal. 2008;48:1105–1111.

[3] Dai PM, Wang Y, Ye L, et al. Pharmacokinetic comparisons of benzoylmesaconine in rats using ultra-performance liquid chromatography-tandem mass spectrometry after administration of pure benzoylmesaconine and Wutou decoction. Molecules. 2014;19: 16757–16769.

[4] Yan Y, Zhang A, Dong H, et al. Toxicity and detoxification effects of herbal *caowu* via ultra performance liquid chromatography/mass spectrometry metabolomics analyzed using pattern recognition method. Pharmacogn Mag. 2017;13: 683–692.

[5] Wang CF, Gerner P, Wang SY, et al. Bulleyaconitine A isolated from *Aconitum* plant displays long-acting local anesthetic properties *in vitro* and *in vivo*. Anesthesiology. 2007;107:82–90.

[6] Guo Q, Xia H, Shi G, et al. Aconicarmisulfonine A, a sulfonated C20-diterpenoid alkaloid from the lateral roots of *Aconitum carmichaelii*. Org Lett. 2018;20:816–819.

[7] Dyer S. Plant exposures: wilderness medicine. Emerg Med Clin North Am. 2004;22:299–313.

[8] Arlt EM, Keller T, Wittmann H, et al. Fatal aconitine intoxication or thyroid storm? A case report. Leg Med (Tokyo). 2012;14:154–156.

[9] Pullela R, Young L, Gallagher B, et al. A case of fatal aconitine poisoning by Monkshood ingestion. J Forensic Sci. 2008;53:491–494.

[10] Chan TY, Tomlinson B, Critchley JA, et al. Herb-induced aconitine poisoning presenting as tetraplegia. Vet Hum Toxicol. 1994;36:133–134.

[11] Ji BL, Xia LP, Zhou FX, et al. Aconitine induces cell apoptosis in human pancreatic cancer via NF-κB signaling pathway. Eur Rev Med Pharmacol Sci. 2016;20:4955–4964.

[12] Lin CC, Chan TY, Deng JF. Clinical features and management of herb-induced aconitine poisoning. Ann Emerg Med. 2004;43:574–579.

[13] Sato H, Yamada C, Konno C, et al. Pharmacological actions of aconitine alkaloids. Tohoku J Exp Med. 1979;128:175–187.

[14] Fujita Y, Terui K, Fujita M, et al. Five cases of aconite poisoning: toxicokinetics of aconitines. J Anal Toxicol. 2007;31:132–137.

[15] Zhou YP, Liu WH, Zeng GY, et al. [The toxicity of aconitine and its analogs and their effects on cardiac contractile function]. Yao Xue Xue Bao. 1984;19:641–646. Chinese.

[16] Zhang M, Peng CS, Li XB. Human intestine and liver microsomal metabolic differences between C19-diester and monoester diterpenoid alkaloids from the roots of *Aconitum carmichaelii* Debx. Toxicol In Vitro. 2017;45:318–333.

[17] Chan TY. Aconite poisoning following the percutaneous absorption of *Aconitum* alkaloids. Forensic Sci Int. 2012;223:25–27.

[18] Chan TY. *Aconitum* alkaloid poisoning related to the culinary uses of aconite roots. Toxins (Basel). 2014;6:2605–2611.

[19] Chen X, Wu R, Jin H, et al. Successful rescue of a patient with acute aconitine poisoning complicated by polycystic renal hemorrhage. J Nippon Med Sch. 2015;82:257–261.

[20] Yeih DF, Chiang FT, Huang SK. Successful treatment of aconitine induced life threatening ventricular tachyarrhythmia with amiodarone. Heart. 2000;84:E8.

[21] Lin CC, Chou HL, Lin JL. Acute aconitine poisoned patients with ventricular arrhythmias successfully reversed by charcoal hemoperfusion. Am J Emerg Med. 2002;20:66–67.

[22] Li TF, Gong N, Wang YX. Ester hydrolysis differentially reduces aconitine-induced anti-hypersensitivity and acute neurotoxicity: involvement of spinal micro-glial dynorphin expression and implications for *Aconitum* processing. Front Pharmacol. 2016;7:367.

[23] Chan TY, Critchley JA. Usage and adverse effects of Chinese herbal medicines. Hum Exp Toxicol. 1996;15: 5–12.

[24] Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat Rev Cancer. 2006;6: 947–960.

[25] Omura T. Forty years of cytochrome P450. Biochem Biophys Res Commun. 1999;266:690–698.

[26] Wilkinson GR. Cytochrome P4503A (CYP3A) metabolism: prediction of *in vivo* activity in humans. J Pharmacokinet Biopharm. 1996;24:475–490.

[27] Galetin A, Gertz M, Houston JB. Potential role of intestinal first-pass metabolism in the prediction of

drug-drug interactions. Expert Opin Drug Metab Toxicol. 2008;4:909–922.
[28] Wang Y, Wang S, Liu Y, et al. Characterization of metabolites and cytochrome P450 isoforms involved in the microsomal metabolism of aconitine. J Chromatogr B Analyt Technol Biomed Life Sci. 2006;844:292–300.
[29] Tang L, Ye L, Lv C, et al. Involvement of CYP3A4/5 and CYP2D6 in the metabolism of aconitine using human liver microsomes and recombinant CYP450 enzymes. Toxicol Lett. 2011; 202:47–54.
[30] Sun A, Chen D, Bi P. [Determination of the aconitine alkaloids in traditional Chinese medicine chuanwu and caowu by high performance capillary electrophoresis (HPCE)]. Se Pu. 1999;17:67–69. Chinese.
[31] Xie Y, Jiang ZH, Zhou H, et al. Simultaneous determination of six *Aconitum* alkaloids in proprietary Chinese medicines by high-performance liquid chromatography. J Chromatogr A. 2005; 1093:195–203.
[32] Plaisance P, Lurie KG, Vicaut E, et al. A comparison of standard cardiopulmonary resuscitation and active compression-decompression resuscitation for out-of-hospital cardiac arrest. French Active Compression-Decompression Cardiopulmonary Resuscitation Study Group. N Engl J Med. 1999; 341:569–575.
[33] Usui K, Hayashizaki Y, Hashiyada M, et al. Simultaneous determination of 11 *aconitum* alkaloids in human serum and urine using liquid chromatography-tandem mass spectrometry. Leg Med (Tokyo). 2012;14: 126–133.
[34] He H, Yan F. Relative quantification of the metabolite of aconitine in rat urine by LC-ESI-MS/MS and its application to pharmacokinetics. Anal Sci. 2012;28: 1203–1205.
[35] Wang Z, Ye M, Xing J, et al. [Determination of aconitine distribution in acute toxic rats by HPLC-MS]. Se Pu. 2005;23:316. Chinese.
[36] Chan TY, Tomlinson B, Tse LK, et al. Aconitine poisoning due to Chinese herbal medicines: a review. Vet Hum Toxicol. 1994;36:452–455.
[37] Ohuchi S, Izumoto H, Kamata J, et al. [A case of aconitine poisoning saved with cardiopulmonary bypass]. Kyobu Geka. 2000;53:541–544. Japanese.
[38] Tang L, Gong Y, Lv C, et al. Pharmacokinetics of aconitine as the targeted marker of fuzi (*Aconitum carmichaeli*) following single and multiple oral administrations of *Fuzi* extracts in rat by UPLC/MS/MS. J Ethnopharmacol. 2012;141:736–741.
[39] Pullela R, Young L, Gallagher B, et al. A case of fatal aconitine poisoning by Monkshood ingestion. J Forensic Sci. 2008;53:491–494.
[40] Dickens P, Tai YT, But PP, et al. Fatal accidental aconitine poisoning following ingestion of Chinese herbal medicine: a report of two cases. Forensic Sci Int. 1994;67:55–58.
[41] Mori A, Mukaida M, Ishiyama I, et al. [Homicidal poisoning by aconite: report of a case from the viewpoint of clinical forensic medicine]. Nihon Hoigaku Zasshi. 1990;44:352–357. Japanese.
[42] Li H, Liu L, Zhu S, et al. Case reports of aconite poisoning in mainland China from 2004 to 2015: a retrospective analysis. J Forensic Leg Med. 2016; 42: 68–73.
[43] Ng SW, Ching CK, Chan AY, et al. Simultaneous detection of 22 toxic plant alkaloids (*aconitum* alkaloids, solanaceous tropane alkaloids, sophora alkaloids, strychnos alkaloids and colchicine) in human urine and herbal samples using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2013;942–943:63–69.
[44] Chan TY. *Aconitum* alkaloid poisoning related to the culinary uses of aconite roots. Toxins (Basel). 2014;6: 2605–2611.
[45] Chan TY. Aconite poisoning presenting as hypotension and bradycardia. Hum Exp Toxicol. 2009; 28:795– 797.
[46] Smith SW, Shah RR, Hunt JL, et al. Bidirectional ventricular tachycardia resulting from herbal aconite poisoning. Ann Emerg Med. 2005;45:100–101.
[47] Yim KM, Tse ML, Lau FL. Reversible intraventricular conduction defect in aconitine poisoning. Singapore Med J. 2009;50:e302–e305.
[48] Li X, Gu L, Yang L, et al. Aconitine: a potential novel treatment for systemic lupus erythematosus. J Pharmacol Sci. 2017;133:115–121.
[49] Yelnosky J, Clark BB. The response of isolated rabbit atria to aconitine. Br J Pharmacol Chemother. 1960;15:448–453.
[50] Wang SY, Wang GK. Voltage-gated sodium channels as primary targets of diverse lipid-soluble neurotoxins. Cell Signal. 2003;15:151–159.
[51] Wright SN. Comparison of aconitine-modified human heart (hH1) and rat skeletal (μ1) muscle Na^+ channels: an important role for external Na^+ ions. J Physiol. 2002;538:759–771.
[52] Li Y, Tu D, Xiao H, et al. Aconitine blocks HERG and Kv1.5 potassium channels. J Ethnopharmacol. 2010; 131:187–195.
[53] Wang YJ, Chen BS, Lin MW, et al. Time-dependent block of ultrarapid-delayed rectifier K^+ currents by aconitine, a potent cardiotoxin, in heart-derived H9c2 myoblasts and in neonatal rat ventricular myocytes. Toxicol Sci. 2008;106:454–463.
[54] Bagur R, Hajnoczky G. Intracellular Ca^{2+} sensing: its role in calcium homeostasis and signaling. Mol Cell. 2017;66:780–788.
[55] Fang YC, Chou CT, Liang WZ, et al. Effect of tetramethylpyrazine (TMP) on Ca^{2+} signal transduction and cell viability in a model of renal tubular cells. J Biochem Mol Toxicol. 2017. doi:10.1002/jbt.21952
[56] Morgan AJ, Platt FM, Lloyd-Evans E, et al. Molecular mechanisms of endolysosomal Ca^{2+} signalling in health and disease. Biochem J. 2011;439: 349–374.
[57] Fleig A, Parekh AB. New insights into Ca^{2+} channel function in health and disease. J Physiol. 2017; 595: 2997–2998.
[58] Fu M, Wu M, Wang JF, et al. Disruption of the intra-cellular Ca^{2+} homeostasis in the cardiac excitation-contraction coupling is a crucial mechanism of arrhythmic toxicity in aconitine-induced cardiomyocytes. Biochem Biophys Res Commun. 2007; 354:929–936.
[59] Ketzer LA, Arruda AP, Carvalho DP, et al. Cardiac sarcoplasmic reticulum Ca^{2+}-ATPase: heat production and phospholamban alterations promoted by cold exposure and thyroid hormone. Am J Physiol Heart Circ Physiol. 2009;297:H556–H563.

[60] Zhang Y, Yu L, Jin W, et al. Reducing toxicity and increasing efficiency: aconitine with liquiritin and glycyrrhetinic acid regulate calcium regulatory proteins in rat myocardial cell. Afr J Tradit Complement Altern Med. 2017;14:69–79.
[61] Zhou YH, Piao XM, Liu X, et al. Arrhythmogenesis toxicity of aconitine is related to intracellular Ca^{2+} signals. Int J Med Sci. 2013;10:1242–1249.
[62] Amran MS, Hashimoto K, Homma N. Effects of sodium-calcium exchange inhibitors, KB-R7943 and SEA0400, on aconitine-induced arrhythmias in guinea pigs *in vivo*, *in vitro*, and in computer simulation studies. J Pharmacol Exp Ther. 2004; 310:83–89.
[63] Dhein S. Gap junction channels in the cardiovascular system: pharmacological and physiological modulation. Trends Pharmacol Sci. 1998;19:229–241.
[64] Bao X, Reuss L, Altenberg GA. Regulation of purified and reconstituted connexin 43 hemichannels by protein kinase C-mediated phosphorylation of Serine 368. J Biol Chem. 2004;279:20058–20066.
[65] Lampe PD, TenBroek EM, Burt JM, et al. Phosphorylation of connexion 43 on serine 368 by protein kinase C regulates gap junctional communication. J Cell Biol. 2000;149:1503–1512.
[66] Zhang SW, Liu Y, Huang GZ, et al. Aconitine alters connexin43 phosphorylation status and $[Ca^{2+}]$ oscillation patterns in cultured ventricular myocytes of neonatal rats. Toxicol In Vitro. 2007; 21:1476–1485.
[67] Wu J, Wang X, Chung YY, et al. L-type calcium channel inhibition contributes to the proarrhythmic effects of aconitine in human cardiomyocytes. PLoS One. 2017;12:e168435.
[68] MacDonald AC, Howlett SE. Differential effects of the sodium calcium exchange inhibitor, KB-R7943, on ischemia and reperfusion injury in isolated guinea pig ventricular myocytes. Eur J Pharmacol. 2008;580:214–223.
[69] Nitzsche D, Melzig MF, Arlt VM. Evaluation of the cytotoxicity and genotoxicity of aristolochic acid I — a component of *Aristolochiaceae* plant extracts used in homeopathy. Environ Toxicol Pharmacol. 2013;35: 325–334.
[70] Liu F, Tan X, Han X, et al. Cytotoxicity of *Aconitum* alkaloid and its interaction with calf thymus DNA by multi-spectroscopic techniques. Sci Rep. 2017; 7:14509.
[71] Gao X, Zhang X, Hu J, et al. Aconitine induces apoptosis in H9c2 cardiac cells via mitochondria-mediated pathway. Mol Med Rep. 2018;17:284–292.
[72] Nanasi PP, Kiss T, Danko M, et al. Different actions of aconitine and veratrum alkaloids on frog skeletal muscle. Gen Pharmacol. 1990;21: 863–868.
[73] Muroi M, Kimura I, Kimura M. Blocking effects of hypaconitine and aconitine on nerve action potentials in phrenic nerve-diaphragm muscles of mice. Neuropharmacology. 1990;29:567–572.
[74] Onur R, Bozdagi O, Ayata C. Effects of aconitine on neurotransmitter release in the rat neuromuscular junction. Neuropharmacology. 1995;34: 1139–1145.
[75] Lin MW, Wang YJ, Liu SI, et al. Characterization of aconitine-induced block of delayed rectifier K^+ current in differentiated NG108-15 neuronal cells. Neuropharmacology. 2008;54:912–923.
[76] Voss LJ, Voss JM, McLeay L, et al. Aconitine induces prolonged seizure-like events in rat neocortical brain slices. Eur J Pharmacol. 2008;584:291–296.
[77] Ameri A, Shi Q, Aschoff J, et al. Electrophysiological effects of aconitine in rat hippocampal slices. Neuropharmacology. 1996;35:13–22.
[78] Xiao K, Wang L, Liu Y, et al. Study of aconitine toxicity in rat embryos *in vitro*. Birth Defects Res B Dev Reprod Toxicol. 2007;80:208–212.
[79] Wada K, Nihira M, Ohno Y. Effects of chronic administrations of aconitine on body weight and rectal temperature in mice. J Ethnopharmacol. 2006;105:89–94.
[80] Wada K, Nihira M, Hayakawa H, et al. Effects of long-term administrations of aconitine on electrocardiogram and tissue concentrations of aconitine and its metabolites in mice. Forensic Sci Int. 2005; 148:21–29.
[81] Su CY. [Role of P-glycoprotein in pharmacokinetics and its clinical implications]. Yao Xue Xue Bao. 2005; 40:673–679. Chinese.
[82] Wu J, Lin N, Li F, et al. Induction of P-glycoprotein expression and activity by *Aconitum* alkaloids: implication for clinical drug-drug interactions. Sci Rep. 2016;6:25343.
[83] Zhang P, Zhang F, Wang Z, et al. [Simultaneous determination of four trace *aconitum* alkaloids in urine using ultra performance liquid chromatography-mass spectrometry]. Se Pu. 2013;31:211–217. Chinese.