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Review

A review on phytochemistry, pharmacology and toxicology studies of *Aconitum*

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Abstract

Objectives A number of species belonging to herbal genus Aconitum are well-known and popular for their medicinal benefits in Indian, Vietnamese, Korean, Japanese, Tibetan and Chinese systems of medicine. It is a valuable drug as well as an unpredictable toxic material. It is therefore imperative to understand and control the toxic potential of herbs from this genus. In this review, the ethnomedicinal, phytochemistry, pharmacology, structure activity relationship and toxicology studies of Aconitum were presented to add to knowledge for their safe application.

Key findings A total of about 76 of all aconite species growing in China and surrounding far-east and Asian countries are used for various medical purposes. The main ingredients of aconite species are alkaloids, flavonoids, free fatty acids and polysaccharides. The tuberous roots of genus Aconitum are commonly applied for various diseases such as rheumatic fever, painful joints and some endocrinal disorders. It stimulates the tip of sensory nerve fibres. These tubers of Aconitum are used in the herbal medicines only after processing. There remain high toxicological risks of the improper medicinal applications of Aconitum. The cardio and neurotoxicities of this herb are potentially lethal. Many analytical methods have been reported for quantitatively and qualitatively characterization of Aconitum.

Summary Aconitum is a plant of great importance both in traditional medicine in general and in TCM in particular. Much attention should be put on Aconitum because of its narrow therapeutic range. However, Aconitum's toxicity can be reduced using different techniques and then benefit from its pharmacological activities. New methods, approaches and techniques should be developed for chemical and toxicological analysis to improve its quality and safety.

Introduction

For centuries, the history of pharmacy has been interlinked with the history of pharmacognosy, or the study of material medica, which is obtained from natural sources – mostly plants.[1]

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries. Over the past decade, interest in drugs derived from higher plants, especially the phytotherapeutic ones, has increased expressively. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants.[2–5] According to the World Health Organization,[6] because of poverty and lack of access to modern medicine, about 65–80% of the world's population that lives in developing countries depends essentially on plants for primary health care. Herbal medicinal preparations are normally very popular in developing countries with a long tradition in the use of medicinal plants and also in some developed countries such as Germany, France, Italy and the USA where appropriate guidelines for registration of such medicines exist.[7–16]

Traditional Chinese medicine (TCM) has gained increasing acceptance worldwide in recent years and is generally considered as being natural and harmless.[17–20] With the increasing popularity of TCM in the world, it is necessary to find out the proper use of herbal medicines by clinical physicians and practitioners, as some side effects may occur with TCM and can sometimes be serious. Systematic research on TCMs has centred on identification of chemical components, pharmaceutical activity, processing methods and quality control. The accumulated knowledge about TCMs makes their use reasonable and safe, but many problems are not resolved. Providing a safe application of those medicines to patients is necessary, and a method for standardization of those medicines is in demand. In other words, the quality control of TCM is an urgent problem to be solved, especially for herbs that contain high toxic compounds such as *Aconitum*.[21]

As a widely used Chinese herbal medicine, the tubers and roots of *Aconitum* (Ranunculaceae) are commonly applied for various diseases, such as collapse, syncope, rheumatic fever, painful joints, gastroenteritis, diarrhoea, oedema, bronchial asthma, various tumours, and some endocrinal disorders like irregular menstruation. However, the cardio and neurotoxicities of this drug is potentially lethal, and the improper use of *Aconitum* in China, India, Japan and some other countries still results in a high risk of severe intoxications. Based upon the regulations stipulated by the State Food and Drug Administration of China, only the processed, detoxified tubers and roots of *Aconitum* are allowed to be administered orally, used in clinical decoctions and adopted as raw materials for pharmaceutical manufacturing. To date, more than 70 traditional and modern techniques are applied for processing *Aconitum* roots for medicinal use. In recent years, a large number of studies have investigated the toxicological and pharmacological characteristics of *Aconitum*, their main alkaloids and their derivatives. The study of TCM clinical use does not only provide an insight into how the field has developed, but is also an interesting example of our ability to develop intercultural practices. Furthermore, with the rapid progress of phytochemical detection and quantification methods, the two above-mentioned aspects of the drug, as well as its quality assurance and quality control, can be handled more precisely and steadily.[22]

In this review, we present an overview and critical assessment of published data concerning *Aconitum*: ethnomedicinal, phytochemistry of *Aconitum* that includes classification of isolated compounds, pharmacology, structure activity relationship and toxicology of *Aconitum* that includes the detoxification process and the analytical methods for *Aconitum* chemical toxicity assessment with emphasis and discussion on the application of the analytical techniques.

Ethnomedicinal uses of *Aconitum*

Aconitum L. is a large genus of the Ranunculaceae family, which consists of over 300 species distributed all over the world. Most of them grow naturally in high altitudes in the northern hemisphere. More than 200 species grow naturally in various parts of China[23] where they are famously used in medicinal healing. The Chinese Pharmacopeia (CP) 2005 has records of only two species; *Aconitum kusnezoffii Reichb* (Caowu) and *Aconitum carmichaeli Debx.* with its dried mother root; *Radix Aconiti praeparata* (Chuanwu) and processed daughter root; *Radix Aconiti lateralis praeparata* (Fuzi).[24]

Other common species for various medicinal purposes include *Aconitum coreanum (Levl.) Rapaics* (Guanbaifu), *Aconitum bullatifolium Levl. var. homotrichum* W. T. Wang., *Aconitum japonica Thunb.*, *Aconitum alboviolaceum Kom.* (Baihuawutou), *Aconitum paniculigerum var. wulingense* (Nakai) W. T. Wang (Wulingwutou), *Aconitum brachypodum Diels.* (Xueshangyizhihao), *Aconitum pendulum Busch.* (Tiebangchui), *Aconitum subrosulatum Hand.-Mazz* (Xuanweiwutou) and *Aconitum lycoctonum* L. (Langduwutou).

A total of about 76 of all aconite species growing in China and surrounding far-east and Asian countries are used for various medical purposes. They are frequently applied in Oriental medicine, primarily as painkillers, antirheumatic agents and less commonly as cardiotonics.[21] They are characteristically taken after cautious processing to reduce their toxicity. The unprocessed roots are too toxic for internal use and as such are used externally as anaesthetics.[23,25,26] In Japanese traditional medicine, *bushi* prepared from processed roots of *Aconitum* species (particularly *A. carmichaeli*) is commonly applied to relieve muscular pain.[27] In Korea, the roots of *A. koreanum* are used for similar indications.[28] Interestingly, in Vietnam the processing methods are not familiar, and hence the tuberous roots of *A. fortunei* are indicated as an analgesic only for external application.[29] In Ayurvedic medicine, *Vatsnabhi* is a herbal product containing processed tuberous roots of various *Aconitum* species applied as a diaphoretic, diuretic, antiperiodic anodyne, antidiabetic, antiphlogistic and antipyretic.[30]

Aconitum heterophyllum, a less toxic species is applied in both Ayurvedic and Tibetan medicine as a bitter tonic for the treatment of abdominal pain, fever and cough.[31]

According to legends of TCM history, *Aconitum* species are noted as one of 'The Four Pillars' of ancient herbs used in Chinese medicinal healing. *Aconitum* species by TCM theory possess hot and dry nature and have the potential to resist cold environments. Therefore, they are important in treating cold diseases.[32] *A. brachypodum Diels.* (Xueshangyizhihao) is well known for its antirheu-

matic and analgesic properties. It mainly grows in Yunnan and Sichuan provinces. It is applied locally at low doses for the treatment of abrasions and wounds. In Jiangyou-Sichuan, *Aconitum* is used as a dietetic vegetable. However, slices of its roots have to be boiled for several hours before eating.[33,34]

More than 90% of the marketed *Aconitum* products in China are derived from the two main species *A. kusnezoffii Reichb* and *A. carmichaeli Debx.* Some common herbal products on the market include 'Fuzi Lizhong Borus', 'Xiao Huoluo Dan', 'Jingui Shenqi Borus' and 'Shenfu Injection'.[32] In Europe, *Aconitum* species have mainly been used as poisons. Extracts of the plants were earlier applied externally to kill lice and parasites.[35,36]

Aconitum napellus L., whose morphological appearance is very similar to *A. carmichaeli*, is still been used in Europe in homeopathic preparations.[37] Due to its poisonous nature, its internal application was not typical.

Phytochemistry

The medicinal plant species of *Aconitum* are a rich source of alkaloids and flavanoids, many of which exhibit broad spectrum of activity. Also isolated and identified are various polysaccharides and free fatty acids (FFAs).

Alkaloids

Alkaloids are a class of natural products described by Pelletier[38] as naturally occurring cyclic organic compounds containing nitrogen in a negative oxidation state. The first alkaloid identified from *Aconitum* species was aconitine (AC), which was isolated by Geiger *et al.* in 1833.[39] Systematic investigation of the *Aconitum* alkaloids was initiated in 1936 by Jacobs,[40] who published more than 20 articles on the subject. The alkaloids benzoylmesaconine, mesaconitine (MA), AC, hypaconitine (HA), heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, hetsinone and benzoylheteratisine have been isolated from tuberous roots of genus *Aconitum*.[41,42]

New derivatives of different alkaloids have also been isolated from different species of *Aconitum*. Dzhakhangirov and Bessonova isolated 11 diterpene alkaloids.[43] Isoatisine and coryphine were found to be most active possibly because of the presence of oxazolidine rings. Investigation on alkaloidal constituents of *A. jaulense* led to the isolation of seven C-19 norditerpenoids and C-20 diterpene alkaloids.[44]

8-O-Azeloyl-14-benzoylaconine, a new alkaloid from roots of *A. karacolicum Rapcs.*, has been reported by Chodoeva and his team in 2005.[45]

In 2008, Ahmad and his friends[46] isolated two new AC-type norditerpenoid alkaloids namely 6-dehydroacetylsepaconitine and 13-hydroxylappaconitine along with three known norditerpenoid alkaloids, while Nisar and colleagues[47] isolated two new diterpene alkaloids heterophylline A and heterophylline B along with two known alkaloids from the roots of *A. heterophyllum* wall. In a 2006 isolation study by Gao and his team, diterpene alkaloids were reported for the first time in *A. spicatum Stapf*. Thirteen norditerpenoids were isolated from the chloroform fraction of 90% ethanol extract of roots, of which two were new, namely spicatine A and spicatine B.[48] In 1990, Bessonova and his colleagues isolated a new alkaloid from the epigeal parts of *A. coreanum (Levl.) Rapaics*. This new alkaloid has the structure of 14-hydroxy-2-isobutrylhetisine N-oxide deduced on the basis of spectral data and chemical transformation. New norditerpenoid alkaloid, swatinine and benzene derivative 4-(2-(methoxycarbonyl)-anilino)-4oxobutanoic acid along with four known alkaloids, delphatine, lappaconitine, puberanine and N-acetylsepaconitine were isolated from the aerial parts of *A. leave Royle*[49] (Figure 1).

Flavonoids

Flavonoids are a class of low molecular weight phenolic compounds widely distributed in the plant kingdom. They are mainly involved in photo-protection from sunlight ultraviolet involved in scavenging reactive oxygen species to prevent lipid peroxidation.[50] Only little information is available on the flavonoids composition of the *Aconitum* sp. Research is being done on this aspect of genus *Aconitum* as well.

The few flavonoids studied in the last 10 years have been rather used as chemotaxonomic markers.[51] Some common *Aconitum* flavonoids including quercetin 7-*O*-(6-*trans*-caffeoyl)-β-glucopyranosyl-(1→3)-α-rhamnopyranoside-3-*O*-β-glucopyranoside, kaempferol 7-*O*-(6-*trans*-caffeoyl)-β-glucopyranosyl-(1→3)-α-rhamnopyranoside-3-*O*-β-glucopyranoside and kaempferol 7-*O*-(6-*trans*-*p*-coumaroyl)-β-glucopyranosyl-(1→3)-α-rhamnopyranoside-3-*O*-β-glucopyranoside, together with β-3,4-dihydroxyphenethyl β-glucopyranoside, have been isolated from the flowers of *A. napellus* subsp. *Neomontanum*.[52]

Free Fatty Acids

A number of FFAs have also been reported in *Aconitum* sp. The esterification of the alcoholic extract followed by gas chromatography–mass spectrometry (GC-MS) analysis showed the abundance of three types of FFA predominantly linoleic acid followed by palmitic acid and oleic acid.[53,54]

Polysaccharides

A water-soluble polysaccharide, FPS-1, was isolated from the root of *A. carmichaeli Debx.* by hot-water extraction,

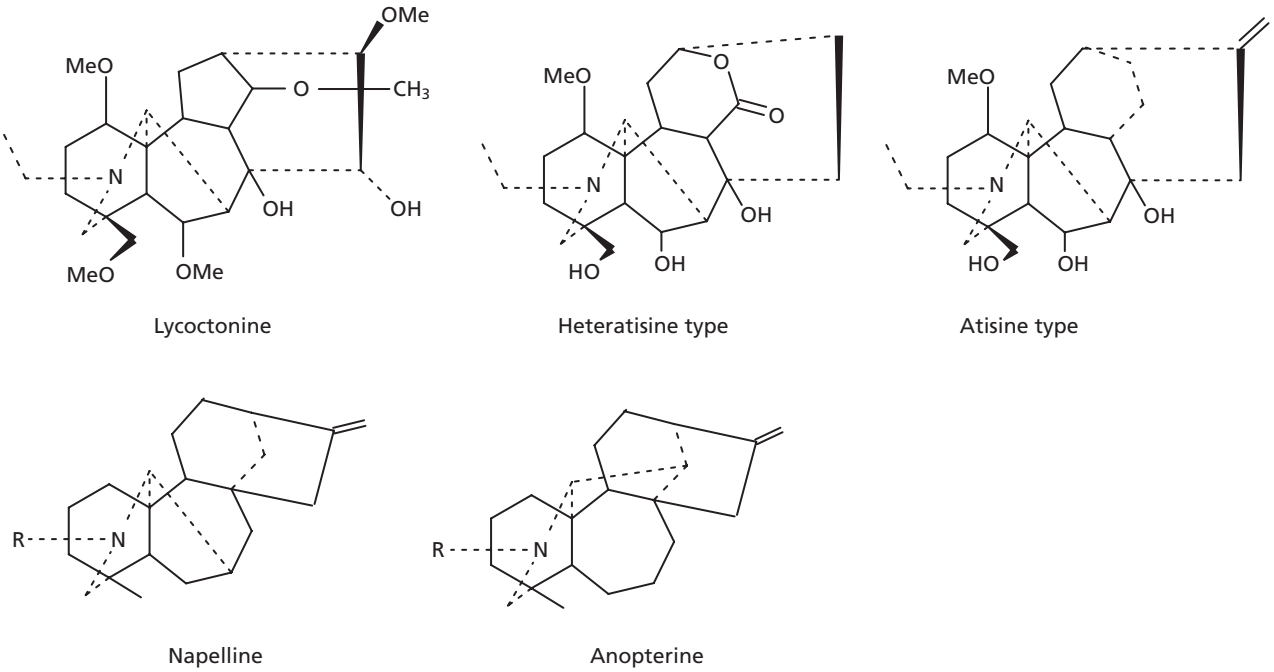


Figure 1 Comparative chemical structure of some *Aconitum* compounds.

anion exchange and gel permeation chromatography technique.[54] Four water-soluble polysaccharide fractions have also been isolated from the tubers of *A. kusnezoffii Reichb*.[55]

Pharmacology of *Aconitum*

Aconitum therapeutic effects

The pharmacological analysis of *Aconitum* species and their compounds have shown various therapeutic effects. The key points of the scientific research have been the effects of the diterpene alkaloids on the central nervous system and the heart. Their antimicrobial and cytotoxic effects have also been studied.

Cardiac effects

The initial research focused on the cardiovascular (arrhythmogenic) toxicity of *Aconitum* alkaloids and especially AC. The marked cardiac activity of diterpene alkaloids is mainly due to their effect on the voltage-gated Na^+ channels.[56] Depending on their mechanism of action, the *Aconitum* alkaloids action on cardiac function can be subdivided into arrhythmogenic and anti-arrhythmic alkaloids.

Alkaloids with arrhythmogenic activity

Arrhythmogenic alkaloids induce their effect by delaying the final repolarization phase of action potential in cardiac cells, which initiates premature or triggered excitations. The final inexcitability of the cells may cause heart arrest.[57]

One of the most active arrhythmogenic alkaloids is AC.[56] These alkaloids are highly toxic, and several fatal intoxications have been attributed to arrhythmogenic alkaloids, because AC and related compounds are the main alkaloids of certain *Aconitum* species used in traditional medicine.[58] They have high affinity for the open state of Na^+ channels at neurotoxin binding site-2.

They activate voltage-dependent Na^+ channels at the resting potential and inhibit their inactivation, resulting in the final inexcitability of the cells. The alkaloids by structure possess an AC skeleton. The qualitative directivity of their cardiorrhythmic action is dependent on the substituents; β-OH on C-13, α-aroyl on C-14, β-acetate on C-8 and a positively charged nitrogen atom. Destruction of the integrity of this system of three substituents leads to the loss of the specific arrhythmogenic activity and to the acquisition of qualitatively opposite properties.

Alkaloids with anti-arrhythmic activity

Anti-arrhythmic compounds are based on a variety of diterpene skeletons. Regarding the mechanism of action, inhibition of the voltage-dependent Na^+ channels and blocking of the delayed rectifier K^+ current are the key components. Na^+ channel-blocking diterpene alkaloids are competitive antagonists of the arrhythmogenic Na^+ channel activating alkaloids.

These compounds block the voltage-dependent Na^+ channels. The high-frequency discharge of action potentials that can occur, for instance, during arrhythmia

could be a prerequisite for the blocking action of these alkaloids.[57]

The most active are the C18 diterpene alkaloids. Common structural elements of these compounds are the presence of a residue of acetylanthranilic or anthranilic acid on C-4, methoxy groups on C-1, C-14 and C-16, and an OH on C-8.[56] Lappaconitine, a member of this group, irreversibly blocks open human heart Na^+ channels, which is in accordance with its anti-arrhythmic activity.[59] It has been shown that lappaconitine and *N*-deacetyllappaconitine decrease the spontaneous beating frequency and exert a significant negative inotropic action in a use-dependent manner in isolated guinea pig hearts.[60] Anti-arrhythmic AC-type and lycoctonine-type compounds possess an aromatic ester on one of C-1, C-6 or C-14, and the basicity of the nitrogen atom is also important. An unsubstituted C-7 does not seem to be crucial, although AC type alkaloids are more potent anti-arrhythmics.

Alkaloids with a benzoyl group on C-14 (e.g. 14-benzoyltalatisamine) are highly active anti-arrhythmic agents. It was recently reported that 14 benzoyltalatisamine is a potent and selective blocker of the delayed rectifier K^+ channels. This activity may play a role in the anti-arrhythmic effect because voltage-gated K^+ channels have a crucial role in regulation of the heart rate.

Alkaloids with aromatic ester substituents on C-1 and C-6 are considerably less active anti-arrhythmic agents than those considered above. Among heteratisine type alkaloids, the most powerful anti-arrhythmic action was observed in substances with an aromatic residue on C-6.

6-Benzoylheteratisine is equal in activity and therapeutic action to lappaconitine. Compounds of the napelline-type exhibit pronounced anti-arrhythmic activity. From this group, napelline, songorine and their 1-benzoyl derivatives are the most active. Mono-aromatic ester substitution enhances the effects of napelline and songorine, but free hydroxy groups are essential for the anti-arrhythmic action. Benzoylation of the hydroxyl groups on C-1, C-12 and C-15 leads to the loss of the effect.[56]

Hetisine-type members of the 'guanfu' base series possess an anti-arrhythmic effect. 'Guanfu' base A exhibits a strong anti-arrhythmic effect in rats, reducing the incidence of ventricular fibrillation induced by $CaCl_2$.[61]

A very extensive pharmacological examination of *Aconitum* alkaloids led to the discovery of a new group of anti-arrhythmic drugs.

The ED50 and breadth of therapeutic action (LD50/ED50) of several alkaloids such as lappaconitine, *N*-deacetyllappaconitine and 6-benzoylheteratisine proved to be similar or better than those of certain anti-arrhythmic drugs used in medicine (e.g. procainamide and etmozin).[56] Following the discovery of the action of lappaconitine hydrobromide (allapinin),[62] the drug was investigated extensively in clinical trials and proved to be efficacious as class I C anti-arrhythmic drug. Allapinin is especially effective in the prevention of paroxysmal atrial fibrillations. With a view to increasing the therapeutic activity, experiments were carried out to complex lappaconitine.[63] Industrial methods have been developed to obtain lappaconitine from *A. leucostomum* and *A. septentrionale*. 'Guanfu' base A and 6-benzoylheteratisine have been shown to have potent anti-arrhythmic activity with low toxicity in animal experiments and are currently undergoing clinical trials.[64]

Effects on the nervous system

Recent experimental findings indicate that some diterpene alkaloids act as selective antagonists on the bungarotoxin-sensitive nicotinic acetylcholine receptors (nAChR) or inhibit the delayed rectifier K^+ current.

Several diterpene alkaloids also have either anti-epileptic or epileptiform effects, which are also related to the effect on the Na^+ channels. The analgesic and anti-epileptiform action of the *Aconitum* alkaloids have been the most studied.

Analgesic effect

Alkaloids that activate voltage-dependent Na^+ channels are antinociceptive and have the potential to depolarize neurons permanently and hence block the neuronal conduction.[63] Na^+ channel blockers possess antinociceptive activity by inhibiting neuronal activity.[63,65]

Although certain lipophilic alkaloids can integrate into the cellular membrane, the antinociceptive effect is likely to be a result of interaction with channel proteins because the use-dependent inhibition of evoked potentials was observed in rat hippocampal slices.

Moreover, alkaloids with low affinity for the neurotoxin receptor site 2 of Na^+ channels lack antinociceptive action.[63] The analgesic effect of *Aconitum* preparations was earlier thought to be due to AC.

Numerous other diterpene alkaloids have also been demonstrated to have peripheral analgesic and antinociceptive properties in different test models.[57] In animal experiments, intracerebrally administered MA (*N*-methyl-*N*-deethylaconitine) exhibited dose-dependent analgesic action via the central nervous system. The effect was not mediated by stimulation of the opioid receptors because the opioid antagonist levallorphan did not affect the analgesic activity.[66] However, a more recent study indicated that the antinociception caused by the crude and the processed roots of *A. carmichaeli* was attenuated but not totally blocked by naloxone at doses sufficient to block opioid μ-receptors, and the analgesic effectiveness was decreased in opioid μ-receptor knockout mice.[67] The mode of inhibition of noradrenaline uptake is thought to be similar to

centrally acting analgesics. Na^+ channel activators inhibit the reuptake of noradrenaline, which can be a result of the increase of the intracellular Na^+ concentration, a decrease of the electrochemical gradient and consequently a decrease of Na^+-noradrenaline co-transport.[68]

Several studies have indicated that β-adrenoreceptor antagonists reduce while agonists enhance the central analgesic effect of diterpene alkaloids.[57] MA, the main alkaloid of several *Aconitum* species used in Japan, exerted inhibitory action on acute inflammation in different animal experiments.

The anti-inflammatory activity was not elicited by glucocorticoids because the effect was observed in sham-operated mice and also in adrenalectomized mice. In contrast with nonsteroidal anti-inflammatory agents, MA did not inhibit the biosynthesis of prostaglandins. In analgesic doses, MA injected into the lateral ventricle of the rat brain dose dependently inhibited hind-paw oedema. Thus, the anti-inflammatory activity of MA may involve the central nervous system, although the exact mode of action is unknown.[69] The analgesic activity of diterpene alkaloids is supported by several animal experiments. *Aconitum* preparations and pure diterpene alkaloids exhibit analgesia both peripherally and centrally.

The effectiveness of traditional *Aconitum* drugs as analgesics is beyond question, although the low LD50/ED50 ratios of the diterpene alkaloids pose a severe risk. Although the toxicity of the processed *Aconitum* products is milder, the analgesic effect, too, is decreased, and the risk of poisoning is therefore not significantly lower.[67]

Anti-epileptiform effects

The inhibition of the neuronal activity and anti-epileptiform activity of several diterpene alkaloids have been studied most exhaustively by Ameri *et al.* on rat hippocampal slices *in vitro*. The anti-epileptiform activity of diterpene alkaloids is in line with the blockade of the Na^+ channels because Na^+ channels are known to be involved in the genesis of abnormal activity in epilepsy. Na^+ channel-blocking compounds (e.g. lappaconitine) inhibit experimentally induced epileptiform activity frequency dependently by sparing the normal neuronal activity.[57] An important implication of the frequency-dependent mode of action is the suppression of aberrant neuronal activity that occurs in the pathophysiological state of epileptic seizures. Anti-epileptic studies revealed that the presence of an aromatic substituent is essential for anti-epileptic activity. *Aconitum* compounds with aromatic substituent 6-benzoylheteratisine, 1-benzoylnapelline, lappaconitine and 14 benzoyltalatisamine inhibited rat hippocampal excitability more potently than heteratisine, napelline, lappaconidine and talatisamine, respectively.[70–73]

This also further confirms the strong association of anti-epileptic activity of the diterpene alkaloids with the inhibition of Na^+ channels. Studies have shown that apart from the Na^+ channel antagonist compounds, Na^+ channel activator alkaloids also exert an anti-epileptic effect.

After a transient hyperexcitability, AC and 3-acetylaconitine completely suppressed epileptiform activity and normal neuronal activity.[74,75] The complete blockade of neuronal activity can be partly antagonized by the Na^+ channel inhibitor 6-benzoylheteratisine.[76]

The involvement of α-adrenoreceptor activation and the enhanced neuronal release of noradrenaline may play a role in the inhibitory action of MA on experimentally induced epileptiform activity and spontaneously occurring epileptiform discharges in the rat hippocampus. The α-adrenoreceptor antagonist yohimbine antagonized the anti-epileptiform activity of MA, whereas the effect was not affected by the β-adrenoreceptor antagonist timolol.[57,77]

In contrast with alkaloids with anti-epileptiform activity, songorine enhanced the excitability of neurons in the rat hippocampus. It was speculated that this effect stemmed from the agonistic action at D_2 receptors because the activity could be antagonized by the selective D_2 antagonist sulpiride and also by the D_1/D_2 receptor antagonist haloperidol.[78] However, more recent results indicate that songorine noncompetitively inhibits the γ-amino butyric acid (GABA) A receptors, and the alkaloid acts at a site distinct from the GABA recognition site.[79] Diterpene alkaloids acting on the GABA receptors may play an important role in the investigation of receptors and GABA agonist drugs. Alkaloids with anti-epileptiform activity are important prospects for anti-epileptic drug development.

Effects on nicotinic acetylcholine receptors

Methyllycaconitine, a diterpene alkaloid isolated first from *Delphinium brownii*, competitively inhibits the binding of [125I]-α-bungarotoxin to rat brain membranes and significantly decreases the channel openings;[80] the compound exerts specific, concentration-dependent, reversible and voltage-independent blockade of nicotinic currents in rat hippocampal neurons. Methyllycaconitine is one of the most selective antagonists of the brain α_7-type nAChRs so far identified.[81] Further research has revealed that the preference for the neuronal [125I]-α-bungarotoxin binding sites rather than to the [3H]-nicotine binding sites of the brain or muscle [125I]-α-bungarotoxin binding sites is characteristic of several C19 diterpene alkaloids.[82]

However, 2-(methylsuccinimido)benzoyl substitution on C-18 is essential for nicotinic potency because only compounds bearing this side chain are potent antagonists of the α_7 nAChRs, with IC50 values in the nanomolar range.

The anthranoyl-substituted compounds lacking the methylsuccinyl ring (e.g. 18 anthranoyllycoctonine) or

lacking anthranoyl substitution on C-18 (e.g. lycoctonine) are at least two orders magnitude less active at rat brain α-bungarotoxin binding sites.[82,83] NAChRs have been targeted for the development of drugs for cognitive function and for the treatment of Tourette's syndrome, anxiety, depression, smoking cessation and irritable bowel syndrome.[84–87] NAChR ligands that are selective for the central nervous system may be useful agents in the discovery of the functioning of acetylcholine receptors and are lead compounds for the design of novel drugs. Such molecules are particularly relevant as imaging agents because reduced numbers of nAChRs have been observed in Parkinson's and Alzheimer's diseases and in schizophrenia.

In contrast with α-bungarotoxin, methyllycaconitine is a small molecule that crosses the blood–brain barrier, and α_7 nAChR-selective diterpene alkaloids are therefore unique tools in neurobiological research.[84] It has been suggested that the neuroprotective actions of α_7 nicotinic agonists arise from the activation of receptors and not from the extensive desensitization, which rapidly follows activation. However, subsequent studies have demonstrated that methyllycaconitine itself has neuroprotective activity.

The compound protected against β-amyloid induced neurotoxicity *in vitro*, which demonstrates that α_7 nAChRs are involved in modulating neuronal viability and suggests that α_7-selective antagonists might be useful therapeutics in treating neurodegenerative disorders such as Alzheimer's disease.[88]

Antimicrobial activity

On the basis of the in-vitro antiproliferative effects of several atisine-type diterpene alkaloids against *Leishmania infantum*, the antiprotozoal activity of 43 C-19 diterpene alkaloids were tested on the extracellular and intracellular stages of the parasite. From among the tested compounds, three atisine-type *Aconitum* alkaloids inhibited the growth of *L. infantum* similarly to the reference drug, without being toxic to the host cells.[89]

In an in-vitro culture system infected with *Trypanosoma cruzi*, 64 C-19 and C-20 alkaloids were tested. Two atisine-type C-20 diterpene alkaloids inhibited the growth of *T. cruzi* epimastigotes with activity levels similar to that of the reference drug, with no toxicity to the host cells.[90]

Cytotoxic activity

The cytotoxic activity of diterpene alkaloids on normal cells were first studied in experiments aimed at the insect-repellent effects of the compounds. A few of the tested 64 alkaloids, apparently randomly distributed among chemical classes, had a selective cytotoxic effect against insect-derived Sf9 cells; only 13-oxocardiopetamine was cytotoxic to mammalian CHO cells.[91,92] Fractions of the root extract of *A. karakolicum*, a plant traditionally used against cancer in Kyrgyzstan, exhibited antiproliferative effects of different degrees. Activity-oriented phytochemical experiments resulted in the isolation of 8-*O*-azeloyl-14-benzoylaconine, a diterpene alkaloid with marked cytotoxic activity on HCT-15, A549 and MCF-7 tumour cell lines.[45] The most comprehensive examination of the cytotoxic activity of diterpene alkaloids was carried out by de Ines *et al.*,[93] who evaluated the cytotoxic effects of 43 C-19 diterpene alkaloids on the tumour cell lines CT26, SW480, HeLa, SkMel25 and SkMel28 and the non-tumour cell line CHO. Twelve compounds with various structures exhibited selective cytotoxicity to cancerous cell lines.

Structure activity relationship

The general structural properties of the alkaloids in relation to their biological activity have been studied. Alkaloids with an aroyl or an aroyloxy group at R14 exhibit an analgesic potency approximately 30 times higher than alkaloids with an aroyloxy group at R4. Although the hydroxyl or acetyl groups can contribute to differences in biological activity between the two groups, the position of the aroyl/aroyloxy group is the major determinant.[57,94] Several mechanisms of action of *Aconitum* alkaloids have been demonstrated and comprise a combination of both peripheral and central effects as mentioned by Friese *et al.*[65] and Gutser *et al.*[95] (Figures 2 and 3; Tables 1 and 2).

Furthermore, the molecular weight of *Aconitum* alkaloids is inversely related to the ED50 and therefore directly related to potency, and finally the group with the highest analgesic potency exhibits the highest molecular weight (Table 3). This is suggestive of the fact that the effect is not limited to the central nervous system. In fact, other pathways of action of *Aconitum* alkaloids are inhibition of prostaglandin synthesis, activation of voltage-dependent sodium channels and inhibition of noradrenaline reuptake, as summarized by Ameri.[57]

In accordance with total energy, heat of formation and electronic energy, relatively more stable compounds exhibit more analgesic potency.

Furthermore, the reactivity index R(I) of three of the six carbons of the aryl group was also different between the groups of *Aconitum* alkaloids. The R(I), introduced by Nakayama *et al.*,[97] is an index that shows the electron density of highest occupied molecular orbital at an atom that may be an active site in the molecule having an important role in the possible chemical reactions. According to Bello-Ramirez *et al.*,[94] both R(I)-C3' and R(I)-C5' were lower in more active compounds and higher in weaker alkaloids, while R(I)-C2' showed an opposite pattern, indicating

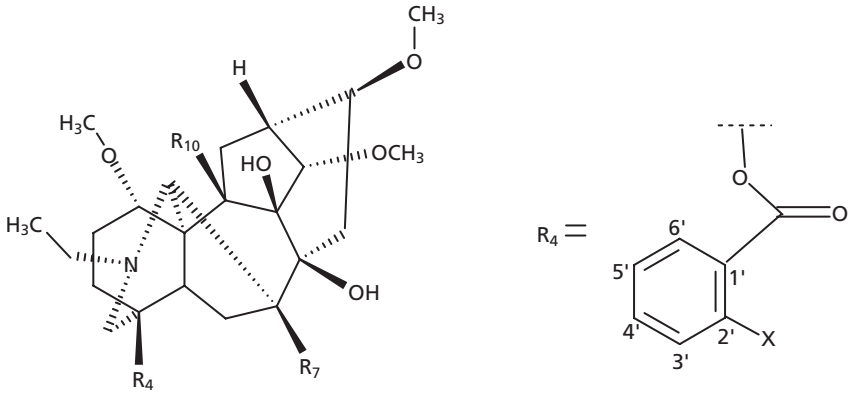


Figure 2 Alkaloids with the aroyloxy group at R4 position. The analgesic ED50, obtained after subcutaneous administration of alkaloids to mice with acetic acid-induced writhing, were obtained from Wang and Xie.[96]

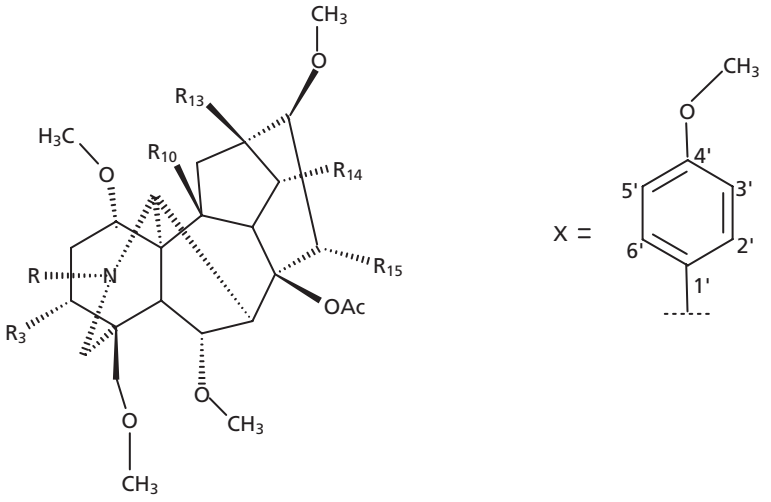


Figure 3 Alkaloids with an aroyl/aroyloxy group at R14. The analgesic ED50, obtained after subcutaneous administration of alkaloids to mice with acetic acid-induced writhing, were again obtained from Wang and Xie.[96]

Table 1 Alkaloids with the aroyloxy group at R4 position

Alkaloid	ED50 (μmol/kg)	Molecular weight	R_7	R_{10}	X
N-deacetyllappaconitine	4.24	542.6	H-	H-	NH_2-
Lappaconitine	5.99	584.7	H-	H-	AcNH-
Ranaconitine	6.99	600.1	HO-	H-	AcNH-
N-deacetylfinaconitine	14.71	574.6	HO-	HO-	NH_2-
N-deacetylranaconitine	15.39	558.6	HO-	H-	NH_2-

The analgesic ED50, obtained after subcutaneous administration of alkaloids to mice with acetic acid-induced writhing, were obtained from Wang and Xie.[96]

that these three atoms mainly the C5' of the aromatic ring play an important role in the analgesic potency of these compounds.

With respect to the findings obtained with electrophysiological methods, it is intriguing that those *Aconitum* alkaloids, which lack the benzoyl ester side chain in the steroid skeleton of the molecule, differ markedly from the alkaloids bearing this group: first, their inhibitory potency was lower. Second, and more important, they diminished only the orthodromic population spike but

Table 2 Alkaloids with an aroyl/aroyloxy group at R14

Alkaloid	ED50 (μmol/kg)	Molecular weight	R	R_3	R_{10}	R_{13}	R_{15}	R_{14}
Yunaconitine	0.061	659.8	CH_3CH_2-	HO-	H-	HO-	H-	OCOX
Bulleyaconitine	0.078	627.8	CH_3CH_2-	H-	H-	HO-	H-	COX
Aconitine	0.085	645.7	CH_3CH_2-	HO-	H-	HO-	OH-	OCOPh
Beiwutine	0.093	647.9	CH_3-	HO-	HO-	HO-	HO-	OCOPh
Nagarine	0.21	661.7	CH_3CH_2-	HO-	HO-	HO-	HO-	OCOPh
3-Acetylaconitine	0.23	687.6	CH_3CH_2-	OAC	H-	HO-	OH-	OCOPh
Penduline	1.45	613.7	CH_3CH_2-	H-	H-	H-	OH-	OCOPh

The analgesic ED50, obtained after subcutaneous administration of alkaloids to mice with acetic acid-induced writhing, were again obtained from Wang and Xie.[96]

Table 3 Different compounds isolated from *Aconitum*

Compounds	Exact mass	R_1	R_2	R_3	R_4
Aconitine	645.3	Et[a]	OH	Ac[b]	Bz[c]
Mesaconitine	631.3	Me[d]	OH	Ac	Bz
Hypaconitine	615.3	Me	H	Ac	Bz
Jesaconitine	675.3	Et	OH	Ac	As[e]
Benzoylaconine	603.3	Et	OH	H	Bz
Benzoylmesaconine	589.3	Me	OH	H	Bz
Benzoylhypaconine	573.3	Me	H	H	Bz
14-O-Anisoylaconine	633.3	Et	OH	H	As
Aconine	499.3	Et	OH	H	H
Mesaconine	485.3	Me	OH	H	H
Hypaconine	469.3	Me	H	H	H
D5-aconitine (I.S.)	650.3	Et	OH	Ac	Bz

completely failed to alter the size of the antidromic spike. Third, the inhibition was independent from stimulation frequency. These differences underline the importance of the benzoylester as an essential group for interaction with the binding site at voltage-dependent Na^+ channels.

Furthermore, AC and 3-acetyllaconitine that are known to suppress inactivation of Na^+ channel and to activate the current already at resting potential have a benzoylester side chain in C-14 position. Thus, it seems to be the benzoylester group and its position that determine the interaction with the binding site on the α-subunit of the Na^+ channel protein. Considering the chemical structure of the *Aconitum* alkaloids reported as well as their mode of action, a subdivision in three groups becomes obvious:

The first group comprises the alkaloids AC, MA and 3-acetylaconitine.

The common structural properties of these alkaloids primarily responsible for the high toxicity are the ester bonding at C8 and C14. Removal of these ester groups by hydrolysis is known to cause sharp decrease in toxicity.[98–102]

These compounds are reported to evoke arrhythmia and to be potent analgesics. At least AC and 3-acetylaconitine have been reported to be Na^+ channel activators.

Activation of Na^+ channels and in consequence excessive depolarization with final inexcitability and suppression of pain transmission may account for the antinociceptive properties of this group of alkaloids.

The second group comprises the less toxic alkaloids lappaconitine, N-deacetyllappaconitine, 6-benzoylheteratisine and 1-benzoylnapelline. The most striking difference in the chemical structure of these alkaloids and group I-alkaloids is the lack of ester bonding at C8 and C14, whereas they possess a benzoylester side chain at C4, C6 and C1, respectively. While AC and 3-acetylaconitine completely put an end to both normal neuronal activity and epileptiform activity, these compounds attenuate epileptiform activity in the hippocampal slice stronger than normal neuronal activity. Because of its Na^+ channel-blocking properties, the antinociceptive activity of lappaconitine, demonstrated previously with various models such as tail pinch, hot plate and acetic acid-induced writhing assay,[103] and its anti-arrhythmic activity in animals[104,105] and humans[106,107] may be explained by a mechanism of action similar to local anaesthetics and class I-anti-arrhytmics, respectively. Despite the lipophilic nature, an integration of lappaconitine into the cell membranes as primary mechanism of action seems to be unlikely. This assumption is supported by the recent findings that lappaconitine evoked a use-dependent inhibition on population spikes in rat hippocampal slices.[108] This implies a specific interaction of lappaconitine to channel proteins rather than an unspecific membrane intercalation.

Furthermore, a number of antidepressant and anticonvulsant drugs, including amitriptyline, desipramine and carbamazepine, have been useful in treating central pain.[109] These drugs block voltage-dependent Na^+ channels and are likely to block pain-generating ectopic activity in the brain.[110]

The third group is represented by lappaconitine, heteratisine and napelline. It is intriguing that these are such alkaloids that are lacking a benzoylester side chain in the molecule, underlining the physiological importance of

this group for the interaction with the binding site at the Na^+ channel protein.

It is also intriguing that napelline and heteratisine have been reported to have an anti-arrhythmic effect,[64,111] although there was no activity-dependent inhibition of neuronal activity in the hippocampus slice.[74,112] These findings imply that at least napelline and heteratisine might have different affinities to various subtypes of the α-subunit of the Na^+ channel in brain and heart.

Toxicology of *Aconitum*

In recent years, a large number of studies have investigated the toxicological characteristics of *Aconitum*, its main alkaloids and their derivatives.[22] It has been observed that the whole plant of *Aconitum* is highly toxic, with the concentration of toxic compounds higher in roots and flowers than in leaves and stems.[113] The symptoms of toxicity affect mainly the central nervous system and the heart, with concomitant gastrointestinal signs. The cause of death is the development of ventricular tachyarrhythmia and heart arrest. No specific therapy exists for *Aconitum* poisoning; cardiovascular supportive treatment is usually applied.[114]

Accidental human poisoning may occur if *Aconitum* roots are used as cooking material instead of horseradish or parsley. As herbal medicine is becoming increasingly popular, *Aconitum* toxicosis are becoming more prevalent. In Europe, poisonings with home-made *Aconitum* preparations have recently been reported.[115,116] Poisoning with processed *Aconitum* preparations of Oriental medicine are common in Asia.[117] Although processing reduces the toxicity of the preparations, AC-like alkaloids are detectable in different TCMs.[118] Because both activation of Na^+ channels and inhibition of nAChRs are characteristic of esterified alkaloids, it is assumed that reduction of the total alkaloid amount or hydrolysis of the ester groups decreases the toxicity.

Boiling markedly reduces the alkaloid content: the initial 1.10–1.56% alkaloid content of the raw tubers of *A. carmichaeli* decreased to 0.14–0.29% during the preparation of *Fu tzu*. The Japanese method of processing significantly decreases the proportion of AC-type alkaloids (from 10.89–17.27% of the overall alkaloids to 3.87–4.59%).[23]

As a consequence of the high diversity of the processing methods, the variability of the alkaloid content of the processed drugs and the narrow therapeutic index of the alkaloids, poisoning may still occur after the consumption of processed aconite roots. Accordingly, processed *Aconitum* preparations are relatively unsafe products of the Oriental materia medica.

Different *Aconitum* species, together with plants of the *Delphinium* genus, frequently cause cattle poisoning in the flowering period.[119] To reduce the losses attributed to larkspur poisoning, there has been a great effort to develop vaccination against diterpene alkaloids. The vaccination of mice with lycoctonine conjugated to protein slightly increased the LD50 for intravenous methyllycaconitine toxicity. This result suggests that vaccination may be useful in decreasing the toxic effects of diterpene alkaloids in animals.[120]

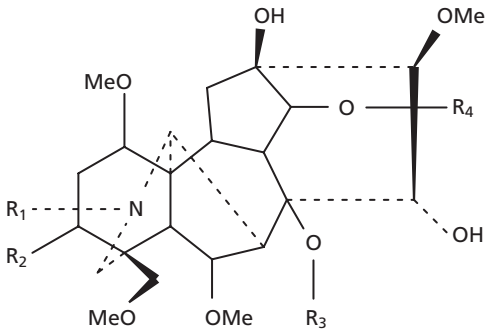


Figure 4 General structure of *Aconitum*.

The toxicity of *Aconitum* is mainly due to the diester diterpene alkaloids (DDAs) and monoester diterpene alkaloids (MDA) such as AC, deoxyaconitine, MA, benzoylmesaconitine, jesaconitine, benzoylhypaconine, 14-O-anisoylaconine, benzoylaconine and HA[121,122] (Figure 4 and Table 3).

Some of the alkaloids may be highly toxic; even 2 mg of AC can cause fatal human toxicosis. The acute oral LD50 of AC in mice is 1.8 mg/kg, while for benzoylhypaconine the LD50 is 830 mg/kg. Moreover, different species possess diverse sensitivities. The intravenous LD50 dose of AC was found to be 0.07–0.13 mg/kg in cats and 0.5 mg/kg in dogs.

The toxicity of *Aconitum* diterpene alkaloids is closely related to their effects on the Na^+ channels and nAChRs.[123] Most of the cardiovascular and neurologic features of poisoning with *Aconitum* can be explained on the basis of activation of the Na^+ channels in excitable tissues, including the myocardium, nerve and muscle, which results in the final inexcitability of the cells. Quantitative structure-activity relationship analysis of C-19 diterpene alkaloids has revealed that, apart from the activators of Na^+ channels, compounds esterified with *N*-(methylsuccinyl) anthranilic acid on C-18 belong to the most toxic diterpene alkaloids. This is connected with the selective nAChR inhibitory activity of such compounds.[124]

Aconitum poisoning and traditional detoxification processes

The knowledge of *Aconitum* toxicity was well noted in various areas of its medicinal application. This resulted in the development of various traditional processing methods before its application.

In settings where knowledge of processing is limited, it is administered in low doses or applied for external purposes only. In China where it is famously used as a drug and food, several processing methods are available for its preparation and detoxification. In south-western China where *Aconitum* is used as a vegetable, the roots are cut into slices and boiled for several hours to a couple of days depending on the amount of *Aconitum* used.[33] Traditionally, the boiled roots will only be ready for use when the tongue or the buccal part of the mouth does not feel numb after tasting the decoction.[34] In Yunnan and Sichuan provinces of China, powdered unprocessed crude herbs of *A. brachypodum Diels.* (Xueshangyizhihao) is given orally at low doses of about 0.06–0.12 g as antirheumatic and analgesic agents.

For external use, about 0.08 g are mixed with two to five drops of alcohol and applied directly onto the wound. *Ts'ao wu* is usually produced from the tubers of *A. kusnezoffii* by boiling in water with the addition of lime or alum or a soup made of liquorice and black beans. *Fu tzu* is the daughter tuber of *A. carmichaeli* processed by soaking or boiling the herbal material in brine or alum. The effectiveness of processing is checked by tasting the tubers: processing is carried on until the tubers no longer cause a tingling or numbing sensation on tasting. Some other traditional processing methods also include the preparation of *Baifupian* or *Heishunpian*. The herbs are usually roasted along with ginger to give *Paofupian*. If *Yanfuzi* is decocted with *Radix Glycyrrhizae* and black beans, the obtained product is called *Danfupian* (Figure 5).

There is also a classical method for the general processing Chinese herbal medicine known commonly as *Paozhi*. Below are a list of some *Paozhi* products and preparation methods of *Aconitum* species.

Yanfuzi is a salted product from the daughter root of *Aconitum*. It is prepared in a detoxification process by soaking *Nifuzi* in a solution of mineral salt overnight.

To achieve the characteristic crystals of sodium chloride on the surface of the drug, it is dried and soaked again over a couple of days.

The time of dryness is prolonged gradually each day during the processing period until enough sodium chloride crystallizes on the surface of the roots.

Heishunpian is translated as black slices product of *Aconitum*. Like *Yanfuzi*, it is soaked in salt solution. After it is thoroughly boiled, it is rinsed with water, cut into longitudinal slices, soaked in salt solution and rinsed with water again.

According to CP 2005, the slices get stained until they turn dark. Finally, the pieces are baked to half dryness and then completely dried in the sun. This procedure gives an oily and lustrous looking root surface with the colour of black tea.

Baifupian is the white slices of *Aconitum*. *Nifuzi* is boiled in mineral salt solution. In contrast to *Heishunpian*, the bark is removed before it gets cut into slices. It is steamed thoroughly and dried in the sun. It is sometimes dried over sulfur vapour to obtain the characteristic yellowish-white colour.

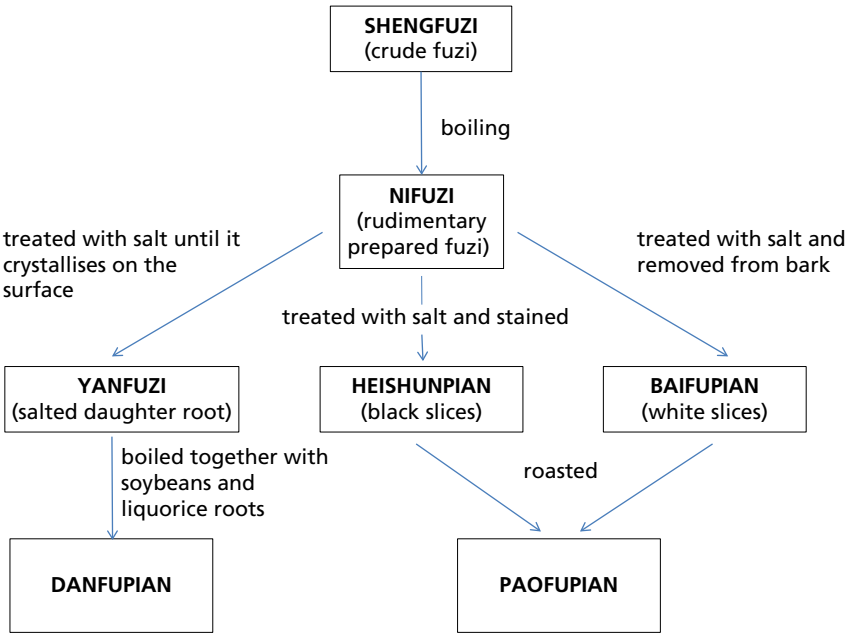
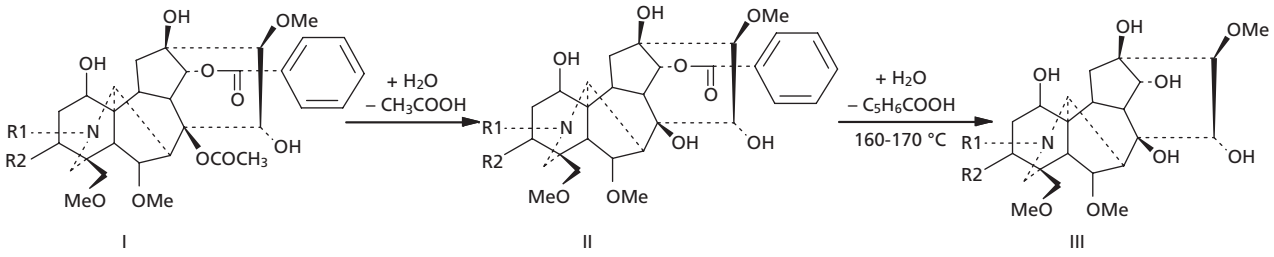


Figure 5 The basic forms of traditional processing of Fuzi in Chinese Pharmacopoeia.



I II III

I	II	III	R_1	R_2
Mesaconitine	Benzoylmesaconine	Mesaconine	CH_3	OH
Aconitine	Benzoylaconine	Aconine	CH_3CH_2	OH
Hypaconitine	Benzoylhypaconine	Hypaconine	CH_3	H

Figure 6 Detoxification reaction of poisonous alkaloids of *Aconitum* species.

Detoxification processes

Through various physical and chemical methods of treatment, highly toxic *Aconitum* alkaloids could be transformed into less toxic derivatives. Modern Chinese medicine has developed techniques such as pressure steam processing for the preparation of Chinese herbal medicine. Its application to *Aconitum* species results in the decomposition of poisonous DDA to the less toxic MDA. After optimization, the pressure-steaming technique is now carried out as follows: crude herbs and pure water are put into an autoclave and then the drug is steamed at 127°C under high pressure (0.15 MPa).

Based on optimal technological research and clinical investigations, the best steaming time for reducing the *Aconitum* DDA content while maintaining the drug's pharmacological efficacies was determined to be 60–90 min. Online supervision of the changes in DDA and MDA contents was performed by HPLC throughout the whole processing time. During the suggested optimum time period, the total amount of both MDAs and DDAs can be maintained with little variation; while the DDA content decreases to a minimum, the monoester formation increases.[125] The optimum decomposition was found to be independent of the method herbal preparations. Nevertheless, an appropriate minimal DDA content is still required to achieve its pharmacological effects while reducing its high toxicity.[73,126]

The research project of the pressure-steaming technique method has been accelerated during the past decade to ensure a safety of *Aconitum*.

Using HPLC online supervision, this method seems to be a key technology, which enables the manufacturers to watch dynamic changes of DDA content during the processing of the drug.[125]

The heating or alkaline treatment through deacetylation, debenzoylation or oxidation of AC results in less toxic benzylaconine, aconine and pyraconine.[127] Processing reduces toxicity because of hydrolysis of the ester groups of AC-type alkaloids.[24] First, the acetyl group is hydrolysed, and in the second step the benzoyl group is hydrolysed[24,33] as shown by Figure 6.

Analytical methods for *Aconitum* chemical toxicity

The high cases of aconite toxicity and difficulties in treating aconite poisoning makes it of great importance to develop accurate qualitative and quantitative analytical methods for the assessment of DDAs as an important index in quality evaluation of these crude drugs.[22,128–130]

A reliable and precise HPLC method coupled with photodiode array detection (HPLC-DAD) has been developed for the identification and quantification of three major AC-type alkaloids (AC, MA and HA) in the roots of *A. carmichaelii Debeaux*.[131] Csupor *et al.*[132] also optimized the extraction and analytical conditions using HPLC-DAD for quantitative and qualitative analysis of AC-type and lipo-alkaloids of *A. carmichaeli* roots. Effects of processing on both types of alkaloids and pure AC were evaluated, which revealed higher levels of alkaloids in the unprocessed one. Various alkaloids are reported as principal components of the processed aconite tubers that manifest pharmacological activity.[127,133,134]

HPLC is broadly used to evaluate the chemical composition of TCMs. A quantitative method developed by HPLC is simple, stable and durable. LC-DAD, LC-MS and other hyphenated techniques can be effectively applied to identify and quantify DDAs and MDAs as mentioned in CP 2005.

Hyphenated techniques are powerful tools for rapid online qualitative analysis of unknown compounds in complex samples.[135] Characterization by hyphenated techniques gives rich information regarding structure. LC-MS profiling is an important approach for the identification and the quantification of the metabolite from complex biological samples. MS analysis provides diagnostic information for structure determination of *Aconitum* alkaloids.

Then the effect of processing on AC-type alkaloids, lipo-alkaloids and pure AC can be studied. The qualitative analysis of the plant material can be carried out by LC-atmosphere pressure chemical ionization-MS.

About 26 lipo-alkaloids have been identified from the roots of *A. carmichaelii* where the product ion spectra of *Aconitum* alkaloids were measured in multiple reaction monitoring-enhanced product ion scan mode for quantitative analysis. Solid-phase extraction (SPE) can be used to extract *Aconitum* alkaloids because it has a high selectivity. Combination of SPE and LC coupled with tandem MS (LC-MS/MS) is a successful method to detect *Aconitum* products and its hydrolysis products.

The novel alkaloids including one MDA, two DDA and 48 lipo-alkaloids were detected. In addition, one DDA, seven lipo-alkaloids and two alkaloids with small molecular weights that possess C-19-norditerpenoid skeleton were reported in *A. carmichaeli* for the first time.[136] Alkaline extraction of DDAs led to decreased alkaloid contents in the aconite samples compared with extraction with aqueous HCl. The acidic extraction conditions are comparable with the ones used for the HPLC analysis of DDAs.[22] DDAs should be analysed rapidly after dissolving in a suitable solvent, keeping in mind the fact that their stability is prolonged when the right extraction method is applied. Aqueous buffer systems with pH 2.00, 3.37, 6.68, 8.00 and 10.00 were investigated for their ability to decompose the compounds. It was found that with increasing pH, the stability of DDAs is decreased. Ultra performance liquid chromatography-quadrupole time-of-flight-high-definition mass spectrometry (UPLC-Q-TOF-HDMS combined with pattern recognition methods and ingenuity pathway analysis are used to investigate comprehensive metabolomic characters of the aconite crude and its processed products where significant difference in metabolic profiles and changes of metabolite biomarkers of interest between the crude and processed preparations were well observed.[137]

UPLC-MS has been used for observing the subtle metabolic changes in some diseases or treatment of diseases and has provided informative data for elucidating the biochemical basis of diseases and addressing the therapeutic effect of the medicines.[138,139]

Seeing the advantages of UPLC – high efficiency of separation and shorter analysis time – it is progressively being applied in the characterization of TCMs. Research has proven that UPLC meets the requirement of fast or rapid, reproducible and sensitive quantitative analysis of TCMs.

HPLC-MS also provides higher selectivity and sensitivity for assaying minor components, isomeric compounds or compounds without chromophore groups. Nuclear magnetic resonance (NMR) methods present advantages of being noninvasive, quick and does not need any sample preclean steps.

They were used to assess *Aconitum* toxic effects in rat urine and plasma samples.[140] Standard compounds are not needed to prepare calibration curves, and NMR also helps to identify at once different components present in herbal preparations in a single measurement.[141] NMR is comparatively less used than chromatographic methods for the quantitative analysis of TCMs, which may be caused by the complexity of TCMs. LC-NMR can produce comprehensive elucidation of the structure of novel compounds.

LC-NMR bypasses the laborious and time-consuming purification process and is therefore particularly suitable for the identification of isomeric pairs and unstable compounds. In most LC-NMR protocols, SPE is used to trap target compounds and substitute solvent with deuterated reagent, and the stopped-flow mode is used to enable a long scan time to record NMR spectra.[135,142–144]

In recent years, online LC-NMR combined with liquid chromatography-ultraviolet/mass spectrometry has attracted increasing attention because of abundant qualitative information, including UV spectra, molecular weight, mass fragments and NMR spectra. LC-NMR method has clear superiority in unambiguous identification of structure, but disadvantages include relatively lower sensitivity and expensive instrumentation.[145] Capillary electrophoresis methods have been used for determination of AC, MA and HA in herbs and Chinese medicinal preparations as well as ion-pair chromatography.[146] This method has the advantage of consuming small quantities of solvent and sample compared with HPLC. Recently, this method has been validated with good separation outcomes and has been extensively applied in TCM analysis.[147–154] Several analytical methods using GC-MS with a derivatization procedure,[155–159] LC-MS[121,160] and LC–MS/MS[161–166] have been proposed for the determination of *Aconitum* alkaloids (including hydrolysate forms) in biological samples like human serum and urine.[167]

GC-MS with derivatization has been reported as the only method for the detection of trace levels of alkaloids in both blood and urine. Volatile oil is one of the major active pharmacological ingredients of TCMs in general and *Aconitum* in particular. The alkaloids are frequently determined by GC or GC-MS, and both qualitative and quantitative analyses are based on the retention index of GC and mass spectra.[168–173]

The quantitative analysis of alkaloids by GC or GC-MS permits high efficiency of separation and lower limitation.

Conclusion and future directions

Aconitum is a plant of great importance both in traditional medicine in general and in TCM in particular. This plant is used to treat a lot of diseases in many countries. According to Paracelsus, everything is toxic at a certain dosage. Much attention should be put on *Aconitum* because it has shown toxicity even with small doses. Although it is toxic, *Aconitum*'s toxicity can be reduced using different techniques and then benefit from its pharmacological activity mentioned such as cardiac, analgesic, anti-epileptiform, anti-microbial and cytotoxic activity.

It is in this context that a lot of studies and researches using different methods, approaches and techniques have been carried out, and they showed successful and excellent outcomes in terms of their application in different domains. Because of the fact that in practice, the diagnosis of herbal toxicity is often based on clinical grounds alone and because sophisticated assays for target toxicology screening are not widely available, many scientists and researchers should carry out a lot of toxicological analysis on *Aconitum* using new methods, approaches and techniques to improve its quality and safety.

Declarations

Acknowledgement

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