

A REVIEW OF RESEARCH PROGRESS ON EFFECTIVE CHEMICAL COMPOUNDS OF *RADIX ACONITI KUSNEZOFFII*

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ABSTRACT

Aconitum species have been used in China as an essential drug in Traditional Chinese Medicine (TCM). Reviewing the effective compounds of *Radix aconiti kusnezoffii*, toxicity, Stability and Quality control methods based upon a wide range of literature investigations serve as a case study to explore the implications of botanicals used in TCM. The toxicity of *Aconitum* mainly derives from the diester diterpene alkaloids (DDAs) including aconitine, mesaconitine and hypaconitine. They can be decomposed into less or non-toxic derivatives through Chinese traditional processing methods. Therefore, a stipulation for a maximum level of DDA content of *Aconitum* is highly desirable in order to guarantee the clinical safety and its low toxicity in decoctions. Newly developed HPLC methods have made the accurate and simultaneous determination and quantification of DDA content interesting.

Keywords: *aconitum*, chemical composition, aconitine alkaloids, toxicity.

INTRODUCTION

Radix aconiti kusnezoffii is dry roots of *Aconitum kusnezoffii* Reichb., Plants of genus *Aconitum* (Ranunculaceae) are widely distributed across North Asia and North America. According to the records of “Flora of China”, there are about 200 species of *Radix aconiti kusnezoffii* in China, which are distributed in northeast China, Hebei, Shanxi and Inner Mongolia. Most of them are wild, growing in Liaoning, Hebei, Shandong, Shanxi, Hubei, Sichuan, Guizhou and other provinces^[i]. “Compendium of Materia Medica” records, *Aconitum* is pungent, warm and strong toxicity. According to the 2015 edition of “Chinese pharmacopoeia”, *Radix aconiti kusnezoffii* has the functions of dispelling wind and eliminating dampness, warming the channels to relieve pain. It is used for wind-cold-dampness arthralgia, arthralgia, cold pain in the heart and abdomen, cold hernia ache and Anesthesia analgesia. Due to strong toxicity, long-term or excessive use will cause mouth and limbs paralysis, nausea, vomiting and so on, in serious cases, it can be life-threatening^[ii]. For a long time, there have been a lot of studies on *Radix aconiti kusnezoffii* at home and abroad, it mainly involves chemical composition, processing, pharmacology, toxicology and clinical aspects. In this paper, in order to provide reference for the further study of *Radix aconiti kusnezoffii*, the chemical composition, toxicity and separation methods were reviewed.

CHEMICAL COMPOSITION OF *RADIX ACONITI KUSNEZOFFII*

The main component in *Radix aconiti kusnezoffii* is alkaloid, and have a few volatile oil, saccharide and so on. In the early 19th century, chemists isolated diterpenoid alkaloids with strong toxicity and activity from aconite plants, subsequently, it was found that diterpene alkaloids were the main chemical constituents in *Radix aconiti kusnezoffii*, can be classified

into C₁₉ (C₁₈) - diterpene alkaloids, C₂₀ - diterpene alkaloids, and non - diterpene alkaloids. C₁₉ (C₁₈) - diterpene alkaloids be classified as lycoctonice, aconitine, lactone. C₂₀ - diterpene alkaloids be classified as veatcheine, atisine, hetidine, hetisine^[iii].

Aconitine, hypoaconitine and mesaconitine in *Radix aconiti kusnezoffii* are the main medicinal constituents. Yonggao Wang et al. had isolated five alkaloids from *Radix aconiti kusnezoffii* (chifeng, Inner Mongolia), one of them is a new compound which was named beiwutine^[iv]. D. Uhrin et al. had isolated two new alkaloids from *Radix aconiti kusnezoffii* Lepenine and Denudatine, respectively^[v]. Zhengbang Li et al. had isolated and qualitative analysis ten new alkaloids from *Radix aconiti kusnezoffii*, they are aconifine, 14-benzoylaconine, 14-benzoylmesaconine, neoline, 15- α -hydroxyneoline, chasmanine, talatizamine, foresticine, lycoctonine, anthranoyllycotonine^[vi]. Later, they had isolated Beiwusine A and Beiwusine B^[vii]. WANG Feng-peng et al. had isolated a new alkaloids, named as Beiwudine^[viii], Zinurova E.G. et al. had isolated and named it acsonine^[ix], Ning Xu et al. had isolated two new alkaloids, named as Beiwudine, respectively^[x].

In addition to alkaloids, *Radix aconiti kusnezoffii* contains a number of other components. Yujun Sun et al. study the isolation and composition of *Aconitum kusnezoffii* Reichb. polysaccharkide (AK), the results that Rhamnose, xylose, mannose, glucose, galactose and arabinose were included in AK with the molar ratio of 1:1.7:203:1.7:59.7:3.5, the content of saccharide was 88%^[xi]. Yingyong Zhao et al. determine the volatile oil in *Radix aconiti kusnezoffii* with GC-MS. They are mainly fatty acids and their esters and amines, the highest content of palmitic acid^[xii]

TOXICITY OF RADIX ACONITI KUSNEZOFFII

The main medicinal constituents in *Radix aconiti kusnezoffii* are Aconitine, hypoaconitine and mesaconitine, they are also highly toxic compounds. Processing can reduce the toxicity of aconitine. The longer the processing time, the less the toxicity. After the processing, the double ester alkaloid hydrolyzed into the single ester alkaloid, the amount of the single ester alkaloid increased greatly, however, the toxicity of the single ester alkaloid was 1/150 ~ 1/500 of that of aconitine^[xiii].

Certain toxicological studies demonstrated that the high toxicity of aconite derives from the diester diterpene alkaloids, and further showed their physiological responses as increased salivation, respiratory paralysis, muscular weakness and convulsions in different animal models^[xiv]. After ingestion of the roots, the patient may present the same signs or other typical symptoms of aconitine poisoning, such as nausea, vomiting, dizziness, palpitation, hypotension, arrhythmia, shock and coma. Moreover, death may occur from ventricular arrhythmia, which is most likely to happen within the first 24 h after intake of *Aconitum*^[xv].

STABILITY OF RADIX ACONITI KUSNEZOFFII

Aconitine alkaloids contain ester moieties in their molecules and are easily hydrolyzed to form more polar compounds. Ohta Hikoto et al. research shows that the aconitine alkaloids were hydrolyzed rapidly in alkaline solution (half lives($t_{1/2}$)<one day), were stable in solutions of acetonitrile, tetrahydrofuran and diluted hydrochloric acid ($t_{1/2}$ >five months) and were unstable in solutions of methanol and ethanol ($t_{1/2}$ <one month) (Table 1)^[xvi]. Hao Yue et al. studied the stability of diester-diterpenoid alkaloids (DDA) from plants of the genus *Aconitum* L. in different solvents and pH buffers. In different solvents, e.g. dichloromethane, ether, methanol

and distilled water, the decomposition pathways of DDA are quite different and their difference in stabilities depends on the difference of their structures. The experimental results demonstrate that the stability of DDA depends on pH values of the buffer. Aconine as hydrolysate has been only found in pH 10.0 buffer, and the other hydrolysates and benzoyleaconine and deacetoxy aconitine, have been observed in all pH aqueous solutions. The decomposition pathways of DDA in buffers are related to the substituent on the C-3 position. The decomposition pathway of aconitine is similar to that of mesaconitine, but different from that of hypaconitine^[xvii].

Table 1 Half-lives of aconitine alkaloids in organic and aqueous solutions

Solvent	Aconitine	Mesaconitine	Hypaconitine
Ammonia (pH 10)	4 h	19 h	10 h
Methanol	16 days	21 days	14 days
Ethanol	16days	16 days	20 days
1 M HCl	5 months	6 months	5 months
Acetonitrile (dry)	> 1 year	> 1 year	> 1 year
Tetrahydrofuran	> 1 year	> 1 year	> 1 year

The half-lives were calculated from the time course curves of the alkaloids remaining in a solution that was spiked originally with 20 mg/mL alkaloids and was subsequently stored at 20°C over several months.

Conclusively, it can be summarized that DDAs should be analyzed rapidly after dissolving in a suitable solvent, keeping in mind the fact that their stability is prolonged when the right extraction method is applied.

QUALITY CONTROL METHODS OF *RADIX ACONITI KUSNEZOFFII*

In order to ensure the safety and effectiveness of medication, a stable, reliable and reproducible quantitative method was established. It is very important to determine the content of aconitine in radix aconiti and compound preparation. HPLC has been widely used in the determination of aconitine^{[xviii][xix][xx]}. In the 2015 edition of the Chinese pharmacopoeia, 6 kinds of aconitine alkaloids can be determined simultaneously by HPLC in medicinal materials and compound preparations, including monoester alkaloids and diester alkaloids. This method has high sensitivity and good reproducibility. Determination of four toxic aconitine alkaloids, in blood and urine samples has been established using high-performance liquid chromatography (HPLC) combined with ultraviolet absorbance detection, solid-phase extraction and mass spectrometry (MS). Calibration curves with UV detection were linear on injection of amounts ranging from 2.5 to 500 ng, and the limit of detection was 1 ng (S/N=3)^[xvi]. By optimizing the extraction, separation and analytical conditions, a reliable and accurate HPLC-DAD was developed for simultaneous quantitative determination of six aconitine alkaloids in aconite roots, and 12 proprietary Chinese medicines containing processed aconite roots. Intra-assay and inter-assay precision of the analytes were less than 2.97%, and the average recovery rates obtained were in the range of 90–103% for all with RSDs below 3.28%. Good linear relationships were showed with correlation coefficients for the analytes exceeded 0.999^[xviii]. Xin Yang et al. simultaneously determined aconitine, hypoaconitine and mesaconitine in *Radix aconiti kusnezoffii*, the three kinds of diester alkaloids showed a good linear relationship in 1~5, 0.35~1.75, 0.5~2.5 µg, respectively^[xxi]. Yingyong Zhao et al. studied the fingerprint of *Radix aconiti kusnezoffii* by HPLC^[xxii]. Above of studies provided a reliable scientific basis for the evaluation of *Radix aconiti kusnezoffii* resources and the quality. It is highly recommended that the determination of these alkaloids is done as a routine measurement. This would provide a

safe application to patients in clinics, and would meet the standards of good manufacturing practice.

CONCLUSION AND PERSPECTIVE

Radix aconiti kusnezoffii has a wide range of resources and has high medicinal value. By analyzing the structure of the isolated compounds and combining pharmacological effects, it helps to clarify the structure-activity relationship and exert its medicinal value. At the same time, the known compounds can be structurally modified to increase efficiency and reduce toxicity. And then, how to maximize its value while avoiding the occurrence of poisoning incidents has always been the focus of researchers.

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