An Efficient High-performance Liquid Chromatography Combined with Electrospray Ionization Tandem Mass Spectrometry Method to Elaborate the Changes of Components Between the Raw and Processed Radix Aconitum kusnezoffii

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ABSTRACT

Background: Crude radix Aconitum kusnezoffii (RAK) has great toxicity. Traditional Chinese medicine practice proved that processing may decrease its toxicity. In our previous study, we had established a new method of RAK processing (Paozhi). However, the mechanism is yet not perfect. Objective: To explore the related mechanism of processing through comparing the chemical contents. Materials and Methods: A new processing method of RAK named stoving (Hong Zhi) was used. In particular, RAK was stored at 110°C for 8 h, and then high performance liquid chromatography combined with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS²) was developed for the detection of the alkaloids of the crude and processed RAK decoction pieces. Results: Thirty components of the crude RAK were discovered, among which, 23 alkaloids were identified. Meanwhile, 23 ingredients were detected in the processed RAK decoction pieces, among which, 20 alkaloids were determined yet. By comparison, eight alkaloids were found in both crude and processed RAK decoction pieces, 15 alkaloids were not found in the crude RAK, however, 10 new constituents yield after processing, which are 10-OH-hypaconine, 10-OH-mesaconine, isomer of bullatine A, 14-benzoyl-10-OH-mesaconine, 14-benzoyl-10-OH-acconine, 14-benzoyl-10-OH-hypaconine, dehydrated aconitine, 14-benzoylaconine, chuanfumine, dehydrated mesaconine. Conclusion: The present study showed that significant change of alkaloids was detected in RAK before and after processing. Among them, the highly toxic diester alkaloids decreased and the less toxic monoester alkaloids increased. Moreover, the concentration changes significantly. HPLC-ESI-MS² are Efficient to elaborate the mechanism of reduction of toxicity and enhancement efficacy after processing.

Key words: Alkaloids, electrospray ionization; mass spectrometry, processing (Paozhi), radix Aconitum kusnezoffii

SUMMARY

• Stoving is a simple and effective method for the processing of radix Aconitum kusnezoffii.

In the positive mode, the characteristic fragmentations of Aconitum alkaloids were obtained.
• The highly toxic alkaloids have decreased, and the new constituents appeared, which has explained successfully the processing mechanism of radix Aconitum kusnezoffii in chemistry.

INTRODUCTION

Radix Aconitum kusnezoffii (RAK), a commonly used Traditional Chinese Medicine (TCM), termed as Caowu, originated from the dried root of Aconitum kusnezoffii Reichb. (Family Ranunculaceae).¹ With the efficiency of dispelling wind and dampness, warming the channels and relieving pain, RAK was found to possess many biological activities, such as, the treatment of wind cold damp impediment symptom, joint pain, abdominal pain with cold sensation, and anesthesia pain.²⁻⁶ Whereas, the crude is with strong toxicity and can generally be used externally.⁷⁺⁸ Chemical compositions of the plant contain C-19 (C-18)-diterpenoidalalkaloids, lycoctonine-type and aconitine-type alkaloids, as well as a small amount of volatile oil, polysaccharides, heavy metals, proteins and other ingredients.⁹⁻¹⁰ Mainly, diester-type alkaloids, including aconitine, hypaconitine, mesaconitine and so on are both the active ingredients and the toxic components, since the therapeutic dose is close to the toxic dose with a small safety range.²⁴⁻²⁵ The processing of Chinese herbal medicine (TCM)²⁶ was strictly required and displayed an efficient effect of efficacy enhancing and toxicity reducing in TCM clinical application in China.²⁷⁻²⁹ Until now, there are varieties of herbal processing methods. In summary, these methods can be divided into three types: Dealing with water (immersion, bubbling, wetting with water or other liquid excipients), drying with heat (stoving, to a sting, fumigating), (moistening and frying).³⁰⁻³⁴ As for RAK, the traditional processing method is immersion, achieving a phenomenon of tasting with a little benumbing, which aims to accelerate hydrolysis of diester-type...
alkaloids, thus reducing its toxicity. However, it is worth noting that many alkaloids are lost by water processing and the RAK efficacy decreases. Steaming and boiling, that is, treatment of RAK with thermal pressure steaming after completely moistening, has varieties of advantages, such as high contents of total alkaloids, lower content of toxic diester-type alkaloids, short production cycle and keep similar analgesic effect with the herb. However, they need high-pressure equipment which is not suitable for large-scale production. In our previous study, we compared seven documented processing methods according to the purpose of RAK processing and proved that the most optimal way is to stoving, which facilitates toxic diester-type alkaloids hydrolysis under proper temperature. It can not only accelerate hydrolysis of aconitine and decrease its toxicity, but also guarantee the amount of total alkaloids.\[30\]

To investigate the mechanism of the new processing method of RAK, termed as stoving, the components change of RAK before and after stoving were detected using high-performance liquid chromatography combined with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS\(^+\)). This is a sensitive, accurate and rapid technique, which can acquire the mass spectrum information of pure compounds from complex system.\[15\]-\[18\] In this paper, a HPLC-ESI-MS\(^+\) method was applied to lay the foundation for the further study of RAK processing.

**MATERIALS AND METHODS**

The HPLC/MS was recorded on an Agilent 1100 HPLC/MSD Trap XCT/plus mass spectrometer (Wilmington, Germany), which equipped with an ESI source, a diode array detector, a binary pump, an auto-sampler, a column compartment and a Chem Station (Agilent, USA). Samples for detection, benzoylaconine, neoline and fuziline (HPLC-ELSD >98\%), were isolated by our lab (School of Chinese Materia Medica, Beijing University of Chinese Medicine). Samples of RAK were collected from (Xinlinhot, China), and were air-dried. All the samples were authenticated by Prof. Shengsang Na. HPLC-grade (MeCN) was purchased from Fisher (USA). Water for HPLC was double distilled and filtered by 0.45 μm filter membrane.

**High-performance liquid chromatography method**

Separation of the samples was performed on an Agilent XDB-C\(_18\) (250 mm x 4.6 mm, 5 μm). Solvent A (water/ammonia, 99.6:0.4 v/v), and solvent B (MeCN) were used as mobile phase (gradient eluting), 33% (B) in 0–15 min, 33–50% (B) in 15–45 min, 50–80% (B) in 45–60 min, with the flowing rate of 1.0 ml/min and an column temperature of 30°C. The detection wavelength was 240 nm.

**Mass spectrometry method**

All experiments were performed on a mass spectrometer equipped with an electro spray source and capable of analyzing ions up to m/z 1000. The ion source temperature was maintained at 350°C. The spray voltage was 40 psi in the positive ion mode. Sheath gas (N\(_2\)) was infused at 10 L/min. The capillary voltage was fixed at 3000 ev.

**Processing of radix Aconitum kusnezoffii**

Herbs of RAK were processed, briefly, removing impurity, then placing in a drying oven at an constant temperature (110°C) for 8 h, and took out. The processed sample was reserved in School of Chinese Materia Medica, Beijing University of Chinese Medicine.

**Preparation of samples**

**Preparation of the standards**

Benzoylaconine, neoline and fuziline were weighed and fully dissolved with 15 ml methanol to conical flasks, finally, diluted to 50 ng/ml for reserving. The result of mass spectrometer was shown in Figure 1.

**Preparation of the samples**

The 0.5 g air-dried power and 0.5 g processed power of RAK, accurately weighted, were fully dissolved with 25 ml methanol to conical flasks, separately. Weighted, after circumscription extraction for 30 min, cooled and weighed again, compensating the losing weight with methanol and amply shaking. Supernatant of the samples was filtrated with a 0.45 μm Nylon filter (Iwaki Glass, Tokyo, Japan) into a HPLC amber sample vial for HPLC-MS\(^+\) analysis. The result of the mass spectrometer was shown in Figure 2.

**RESULTS**

Establishment of mass spectrometry cracking regulation

A sensitive detector of MS was developed for the detection of benzoylaconine, neoline and fuziline, with the MS\(^+\) spectral data shown in Figure 1. After further analysis, regulations were represented as follows: Peaks of [M + H]\(^+\) is prone to appear, easy to lose CH\(_2\)OH, H\(_2\)O, CH\(_3\)COOH, as well as C\(_6\)H\(_5\)COOH, which laid the foundation for rapid identification of alkaloids.

Identification of alkaloids in radix Aconitum kusnezoffii by liquid chromatography-mass spectrometry

In the experiments, 30 alkaloids in RAK were discovered, and 23 compounds were tentatively identified by detailed study of their fragmentation regulations [Table 1]; whereas 23 alkaloids were detected in the processed product, and 20 compounds identified [Table 2], simultaneously. The comparison between the compounds identified from RAK before and after processing on MS\(^+\) shows, 15 alkaloids were not observed in the processed part, including sachaconitine, dehydrated deoxypaconitine, chasmanine, karakolidine, aconitine, neoline, dehydrated hypaconitine, 10-OH-mesaconitine, 10-OH-hypaconitine, dehydrated-3,13-deoxyaconine, mesaconitine, 10-OH-aconitine, 14-acetylneoline, hypaconitine and deoxymesaconitine. However, 10-OH-mesaconitin, 10-OH-hypaconitin, mesaconitine, 10-OH-aconitine, aconitine, hypaconitine, deoxymesaconitine, these seven compounds were diester-type alkaloids with great toxicity and easily hydrolyzed after heating. Ten new compounds were identified in processed RAK, including 10-OH-mesaconine, 14-benzoyl-10-OH-mesaconine, 14-benzoyl-10-OH-hypaconine, 14-benzoyl-10-OH-aconine, 14-benzoylaconine, chasmanine, talatizamine, foresticine, lycoctonine, dehydrated fuziline, beiwusine A, beiwusine B, beiwusine C, acasone, 10-aconine, benzoylaconine, benzoyl mesaconitine, neoline, 15-α-hydroxyneoline, chasmanine, talatizamine, foresticine, lycoctonine, ananthrolycoctonine, lepenine, denudatine.
songorine, 1, 15-dimethoxy-3-hydroxy-14-benzyol-16-ketoneoline, 8-ethoxy-14-benzyol-15-methoxyaconoine, songoramine and karakoline. In our paper, another 16 alkaloids were identified.

| Name                        | Time | MS1 | MS2 | MS3 | MS4 |
|-----------------------------|------|-----|-----|-----|-----|
| Mesaconine                  | 3.1  | 486 | 436 | 404 | 372 |
| Sachaconitine[39]           | 3.5  | 392 | 356 | 338 | -   |
| Unknown                     | 3.7  | 440 | 422 | 390 | 372 |
| Mesaconine                  | 3.8  | 486 | 436 | 404 | 372 |
| 10-OH-aconine               | 4.1  | 516 | 466 | 434 | 402 |
| Aconine                     | 4.3  | 500 | 450 | 418 | 386 |
| Fuziline                    | 4.6  | 454 | 436 | 404 | 372 |
| Unknown                     | 4.7  | 376 | 358 | 340 | 322 |
| Dehydrated deoxyhypaconine  | 5.1  | 436 | 418 | 386 | 360 |
| Karakolidine                | 6.1  | 394 | 376 | 358 | -   |
| Chasmanine[40]              | 6.2  | 452 | 434 | 402 | 370 |
| Neoline                     | 7.2  | 438 | 420 | 388 | 356 |
| Unknown                     | 7.9  | 452 | 434 | 402 | 370 |
| 14-benzoylmesaconine        | 8.2  | 590 | 540 | 508 | 476 |
| Neoline                     | 11.2 | 438 | 420 | 388 | -   |
| Unknown                     | 15.2 | 432 | 372 | 354 | -   |
| Unknown                     | 17.3 | 390 | 358 | 340 | -   |
| Dehydrated aconine          | 17.7 | 482 | 450 | 418 | 386 |
| 10-OH-mesaconitine          | 19.3 | 648 | 588 | 528 | 478 |
| Unknown                     | 20.5 | 416 | 356 | 338 | 310 |
| 10-OH-hypaconitine          | 22.2 | 632 | 572 | 512 | 480 |
| Dehydrated-3,13-deoxyaconine| 22.6 | 450 | 418 | 386 | 342 |
| Dehydrated deoxyaconine     | 28.0 | 466 | 434 | 402 | 370 |
| Mesaconitine                | 30.6 | 632 | 572 | 512 | 480 |
| 10-OH-aconitine             | 33.9 | 662 | 602 | 542 | 492 |
| Dehydrated hypaconitine     | 37.0 | 452 | 388 | 356 | 324 |
| 14-acetylineone             | 43.2 | 480 | 448 | 416 | 384 |
| Aconitine                   | 43.8 | 646 | 586 | 526 | 494 |
| Unknown                     | 43.9 | 466 | 344 | 316 | -   |
| Hypaconitine                | 54.9 | 616 | 556 | 496 | 478 |
| Deoxymesaconitine           | 56.8 | 616 | 556 | 496 | 464 |

MS: Mass spectrometry
**Table 2:** The MS1-4 data and the identification of alkaloids after processing

| Name                        | Time | MS1  | MS2  | MS3  | MS4  |
|-----------------------------|------|------|------|------|------|
| Isomer of bullatine A       | 2.4  | 344  | 298  | 266  | 238  |
| 10-OH-hyponaconine          | 3.3  | 486  | 436  | 404  | 372  |
| Mesaconine                  | 3.7  | 486  | 436  | 404  | 372  |
| 10-OH-mesaconine            | 3.9  | 502  | 452  | 420  | 392  |
| 10-OH-aconine               | 4.2  | 516  | 466  | 434  | 402  |
| Fuzilide                    | 4.6  | 454  | 436  | 404  | 372  |
| Aconine                     | 4.8  | 500  | 450  | 418  | 386  |
| Unknown                     | 4.9  | 376  | 358  | 340  | -    |
| 14-benzoyl-10-OH-mesaconine | 5.8  | 606  | 556  | 524  | -    |
| Chuanfunime[41]             | 6.8  | 394  | 364  | 346  | -    |
| Dehydrated hypaconitine     | 8.0  | 452  | 434  | 402  | 370  |
| 14-benzoylmesaconine        | 8.3  | 590  | 540  | 508  | 476  |
| Unknown                     | 11.8 | 420  | 402  | 370  | 292  |
| 14-benzoyl-10-OH-aconine    | 12.9 | 620  | 570  | 538  | 506  |
| 14-benzoyl-10-OH-hyponaconine | 13.2 | 588  | 538  | 506  | 474  |
| Dehydrated aconine          | 18.1 | 482  | 450  | 418  | 386  |
| 14-benzylaconine            | 19.8 | 604  | 554  | 522  | 490  |
| 14-benzoyl-10-OH-aconine    | 23.8 | 572  | 522  | 490  | 458  |
| Dehydrated mesaconine       | 24.2 | 572  | 522  | 490  | 458  |
| Dehydrated deoxyaconine     | 33.8 | 466  | 434  | 402  | 370  |
| Dehydrated aconitine        | 35.3 | 586  | 536  | 504  | 472  |
| Unknown                     | 44.1 | 466  | 344  | 272  | -    |

MS: Mass spectrometry

in both crude RAK and the processed product for the first time, including mesaconine, sachoaconine, 10-OH-aconine, aconine, fuziline, dehydrated deoxyhypaconine, karakoline, dehydrated aconine, 10-OH-mesaconine, 10-OH-hyponaconine, dehydrated-3, 13-deoxyaconine, dehydrated deoxyaconine, 10-OH-aconine, dehydrated hypaconitine, 14-acetylneoline, and deoxymesaconine.

Chinese medicine processing is the important means to ensure clinical medication safety. In this paper, we demonstrated once again that HPLC-ESI-MS was rapid and effective to clarify the mechanism of processing from the perspective of the chemical compositions. As far as RAK is concerned, both chemical constituents and content change significantly before and after processing. However, it is necessary to obtain all kinds of standard materials, so that the changes of chemical constituents can be analyzed accurately and quantitatively.

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**Conflicts of interest**

There are no conflicts of interest.

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