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Original Research Article

Effect of processing on the alkaloids in *Aconitum* tubers by HPLC-TOF/MS

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ABSTRACT

According to the Chinese Pharmacopoeia 2015, only processed *Aconitum* tubers can be clinically applied, and the effect of processing is unclear. This research aimed to explore the effect of processing on cardiac efficacy of alkaloids in *Aconitum* tubers. First, the chemical ingredients in unprocessed and processed *Aconitum* tubers were identified and compared by using high performance liquid chromatography time-of-flight mass spectrometry (HPLC-TOF/MS) and multivariate pattern recognition methods. Then the representative alkaloids in *Aconitum* tubers, aconitine, benzoylaconine, and aconine, which belong to diester-diterpenoid alkaloids, monoester-diterpenoid alkaloids, and amine-diterpenoid alkaloids, respectively, were selected for further validation of attenuated mechanism. Subsequent pharmacological experiments with aconitine, benzoylaconine, and aconine in SD rats were used to validate the effect of processing on cardiac functions. After processing the *Aconitum* tubers, it was found that the contents of diester-diterpenoid alkaloids were reduced, and those of monoester-diterpenoid alkaloids and amine-diterpenoid alkaloids were increased, suggesting that diester-diterpenoid alkaloids were transformed into monoester-diterpenoid alkaloids and amine-diterpenoid alkaloids. Through further decocting the aconitine in boiling water, it was confirmed that the three alkaloids could be progressively transformed. Pharmacological experiments with aconitine, benzoylaconine, and aconine in SD rats showed that aconitine at a dose of 0.01 mg/kg and aconine at a dose of 10 mg/kg enhanced the cardiac function, while benzoylaconine at a dose of 2 mg/kg weakened the cardiac function. The effect of processing is attributed to the transformation of the most toxic diester-diterpenoid alkaloids into less toxic monoester-diterpenoid alkaloids and amine-diterpenoid alkaloids.

1. Introduction

Aconitum tubers, or Wutou in Chinese, is the root of the genus *Aconitum* of the family Ranunculaceae that has long been used in the practice of traditional Chinese medicine (TCM) for its analgesic, anti-inflammatory and cardiotonic actions [1,2]. *Aconitum*, which dispels cold and relieves pain, is used to treat rheumatic arthritis in single herb or with other herbs. The main chemical ingredients in *Aconitum* are aconitum alkaloids, including diester-diterpenoid alkaloids, monoester-diterpenoid alkaloids, and amine-diterpenoid alkaloids [3–7]. Representative diester-diterpenoid alkaloids include aconitine, mesaconitine and hypaconitine; representative monoester-diterpenoid alkaloids include benzoylaconine, benzoylmesaconine and benzoylhypaconine; and representative amine-diterpenoid alkaloids include aconine, mesaconine and hypaconine [8–10]. Aconitum alkaloids are supposed to be the main toxic ingredients in *Aconitum*, and may cause severe cardio-, neuro- and cyto-toxicities [11,12]. It was reported that the LD_{50} value of intravenous injection of aconitine, mesaconitine and hypaconitine in mice was 0.12, 0.10 and 0.47 mg/kg, respectively [13], that of benzoylaconine, benzoylmesaconine and benzoylhypaconine was 23, 21 and 23 mg/kg, respectively, and that of aconine was 120 mg/kg, indicating the toxicity of the three types of aconitum alkaloids in descending order.

Processing, named *Paozhi* in Chinese, is one of traditional Chinese medicinal processing methods to remove unwanted or toxic substances from Chinese herbal medicines [14,15], in addition to decoction or setting with other Chinese herbs [16]. Only processed *Aconitum* is allowed to be clinically used in TCM practice. According to the Chinese

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Pharmacopoeia 2015, *Aconitum* can be processed by steaming and boiling to reduce the content of toxic diester-diterpenoid alkaloids [17–20]. It was reported that the processing or decoction can attenuate the toxicity of *Aconitum* [21–25]. However, there are few studies reporting the differences in chemical components and their pharmacological actions between the unprocessed and processed *Aconitum*. In addition, it is unclear whether the changes of ingredients after processing help enhance the cardiac efficacy.

There are controversies over the pharmacological activities of diester-diterpenoid alkaloids, monoester-diterpenoid alkaloids and amine-diterpenoid alkaloids [26,27]. The diester-diterpenoid alkaloids were reported to be toxic and manifesting arrhythmia [28]. It has been always recognized that the content of diester-diterpenoid alkaloids in *Aconitum* was reduced and transformed into new alkaloids after processing, so it plays synergistic and attenuated roles eventually. Nowadays some studies showed the effective components in *Aconitum* were the water-soluble fraction which could act on the cardiovascular system [29]. It remains unclear whether the toxic diester-diterpenoid alkaloids are not only the toxic ingredients but also the effective substances.

The aim of the present study was to use HPLC-TOF/MS and multivariate pattern recognition methods to investigate diversification of the chemical ingredients in processed *Aconitum* in an attempt to evaluate the effect of processing on the chemical substances in *Aconitum*, explore the transformation mechanism among the three types of alkaloids during the processing procedure, explain the differences in pharmacological effects between the unprocessed and the processed *Aconitum*, and explore the cardiac efficacy of the three types of alkaloids by using hemodynamic experiments in rats.

2. Materials and methods

2.1. Chemicals and materials

The aconitine, benzoylaconine, aconine and benzoylmesaconine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and benzoylmesaconine was used as an internal standard for HPLC-MS analysis. The compound 2-chloro-L-phenylalanine, which was purchased from Aladdin Reagent Co., Ltd., was used as an internal standard for HPLC-TOF/MS analysis. Their purities are all more than 98%. Acetonitrile and formic acid of HPLC grade were purchased from Burdick & Jackson (USA). Ultrapure water was prepared by Milli-Q System (Millipore, Bedford, MA, USA). All the other reagents were of analytical grade. The herb, *Aconitum carmichaelii* Debx., was purchased from Shanghai Leiyunshang Pharmaceutical Co., Ltd. (Shanghai, China) and authenticated by Professor Lianna Sun from the Department of Pharmacognosy, Second Military Medical University (Shanghai, China).

2.2. Animals

This animal experimental protocol was carried out according to the Guidelines for the Care and Use of Laboratory Animals, and was approved by the Animal Ethics Committee of Second Military Medical University. Male Sprague-Dawley (SD) rats, supplied by Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China), were housed at 22–25 °C with free access to tap water and standard rat chow, and then fasted overnight with free access to water prior to each experiment.

2.3. Processing of Aconitum carmichaelii Debx

According to the Chinese Pharmacopoeia (2015 Edition), the main root of *Aconitum carmichaelii* Debx was soaked in water for 7 days, then steamed for 6 h and dried for 12 h at 40 °C in the oven.

2.4. Preparation of unprocessed and processed Aconitum samples

Both unprocessed and processed *Aconitum* were crushed to powder at a 50 mesh pulverization degree, and 2 g *Aconitum* powder was taken, soaked in 25 mL ethyl ether with 2 mL of ammonia solution for 12 h. The supernatant (1 mL) was transferred into a 1.5 mL of polypropylene tube and dried under a flow of nitrogen gas. The residual was reconstituted in 200 μL of acetonitrile and vortexed for 1 min, followed by centrifuge for 5 min at 12,000 rpm, and the supernatant (200 μL) was taken for HPLC-TOF/MS analysis. Another 20 μL of acetonitrile solution, mixed with 180 μL of acetonitrile solution containing the internal standard (2-chloro-L-phenylalanine, 1 μg/mL), was prepared and injected into the HPLC/MS system for analysis.

2.5. Transformation among aconitine, benzoylaconine and aconine

Aconitine, benzoylaconine, aconine and benzoylmesaconine were dissolved in DMSO to prepare stock solutions. The stock solutions of aconitine, benzoylaconine and aconine were diluted to the concentration of 10 μg/mL. Aconitine, benzoylaconine and aconine (1 mL each) were added to a 1.5 mL polypropylene tube respectively, and each solution was taken for three replicates. The tubes were heated in boiling water, and 100 μL of heated solution was collected at the designated time points of 0, 5, 10, 15, 30, 45 and 60 min. 400 μL acetonitrile, which was iced in advance, containing the IS at the concentration of 50 ng/mL, was added into the solutions immediately and vortexed for 1 min, followed by centrifuge for 3 min at 12,000 rpm, and an aliquot of 5 μL of supernatant was injected into the HPLC/MS system for analysis.

2.6. Hemodynamic evaluation of aconitine, benzoylaconine and aconine

Eighteen male SD rats weighing 250–280 g were equally randomized into three groups: A (*aconitine*), B (*benzoylaconine*), and C (*aconine*). SD rats were anesthetized with an intra-peritoneal injection (i.p.) of 1.4 g/kg urethane. The cardiac function was evaluated on the Power Lab 8/35 (AD instrument, Australia), and the rats were connected to Power Lab through three polyethylene catheters. One was inserted into the right carotid artery and then advanced into the left ventricular cavity to record left ventricular systolic (LVSP) and end-diastolic pressures (LVEDP) and heart rate (HR), while another was inserted into the right femoral artery to record systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP), and the third one was placed in the right femoral vein for drug injection. The HR, LVSP, LVEDP, SBP, DBP, MBP and maximal rate of left ventricular systolic pressure development ($+dp/dt_{max}$) were analyzed by Labchart software. After 30 min recording of the stable ventricular pressure, different concentrations of aconitine, benzoylaconine and aconine solution were injected intravenously (i.v.) into the rats. All the parameters described previously were recorded for 30 min. Paired *t-test* was used for comparison ($p < 0.05$), and all the results were expressed as arithmetic mean ± standard deviation (SD).

2.7. Data acquisition

HPLC-TOF/MS analysis was performed on Agilent 1100 series HPLC coupled to Agilent 6220 Accurate-Mass TOF mass spectrometer (Agilent, USA). Chromatographic separations were performed on an Agilent ZORBAX SB-C_{18} column (3.0 mm×100 mm, 3.5 μm, Agilent, USA). The mobile phase consisted of 0.1% formic acid (A) and ACN (B). The following gradient program was used: 5%–50% B at 0–20 min, followed by 5 min re-equilibration. The column temperature was maintained at 25 °C. The injection volume was 5 μL, which was introduced into the mass spectrometer at a flow rate of 0.8 mL/min and a post-column splitting ratio of 1:1 with a three-way joint.

An electrospray ionization source (ESI) interface was used and set in positive scan mode. The MS instrumental settings were as follows: capillary voltage 3.5 kV, nozzle voltage 500 V, nebulizer gas pressure 45 psig, drying gas flow rate 11 L/min, gas temperature 350 °C, sheath gas temperature 400 °C, and sheath gas flow 11 L/min. Data were collected in a centroid mode and the mass range was set at *m/z* 100–1000 by using an extended dynamic range.

HPLC-MS analysis was performed on the Agilent 1100 series HPLC coupled to Agilent mass spectrometer (Agilent, USA). An Agilent ZORBAX SB-C_{18} column (3.0 mm×100 mm, 3.5 μm, Agilent, USA) was used to separate the analytes. The mobile phase was composed of A (0.1% formic acid) and B (acetonitrile) by using the gradient program as follows: 0–8 min, 20%–60% B; 8–12 min, 60%–60% B; post time 5 min. The column temperature was 25 °C. The injection volume was 5 μL, which was introduced into the mass spectrometer at a flow rate of 1 mL/min and a post-column splitting ratio of 1:2 with a three-way joint. The MS parameters were optimized through FIA to obtain the highest response by using SIM mode, and quantification analysis was performed in a positive ion mode. The $[M-H]^+$ was *m/z* 646.3, 604.4, 500.2 and 590.4 for aconitine, benzoylaconine, aconine and IS, respectively. Agilent MassHunter Workstation Data Acquisition software was used for equipment control and data acquisition, and Agilent Qualitative Analysis software B.04.00 was used for data analysis.

2.8. Data analysis

The acquired HPLC-TOF/MS original data in the instrument specific format (.d) were first converted to a common data format (.mzData) by using Agilent MassHunter Qualitative Analysis B.04.00. The program XCMS was then used for nonlinear alignment of the data in the time domain and automatic integration and extraction of the peak intensities by the software R 2.14.0. XCMS parameters were default settings except for the following ones: fwhm =8, snthersh =5 and bw =10. The output data were imported into MATLAB R2010 software, where data were normalized using the summation of response of all the analytes in one sample. The data pre-processed were introduced to SIMCA-P+11 (demo, Umetrics, Sweden) for partial least squares discriminant analysis (PLS-DA) after mean centering and pare to scaling. The quality of the models was evaluated with the relevant R^2 and Q^2 as discussed elsewhere.

3. Results and discussion

3.1. Ingredients in the processed and unprocessed Aconitum

Fig. 1 shows the total ion chromatogram (TIC) of the processed and unprocessed *Aconitum*. As shown in Fig. 1, Peaks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 increased, while Peaks 13, 14, 15, 16, 17, 18, 19, 20 and 21 decreased after processing, indicating that the ingredients underwent changes during processing.

A partial least squares-discriminate analysis (PLS-DA) model was established to evaluate different ingredients in the unprocessed and processed *Aconitum*. The score scatter plot in Fig. 2A showed the trend that the unprocessed *Aconitum* was away from the processed *Aconitum*, and as shown in the loading scatter plot (Fig. 2B), 21 ingredients were found to be away from the others.

The variable importance list demonstrates that 21 alkaloids were obviously different between the two groups in terms of molecular weight. The detailed information is shown in Table 1, including the retention time (t_R), formulae and variable importance in the projection

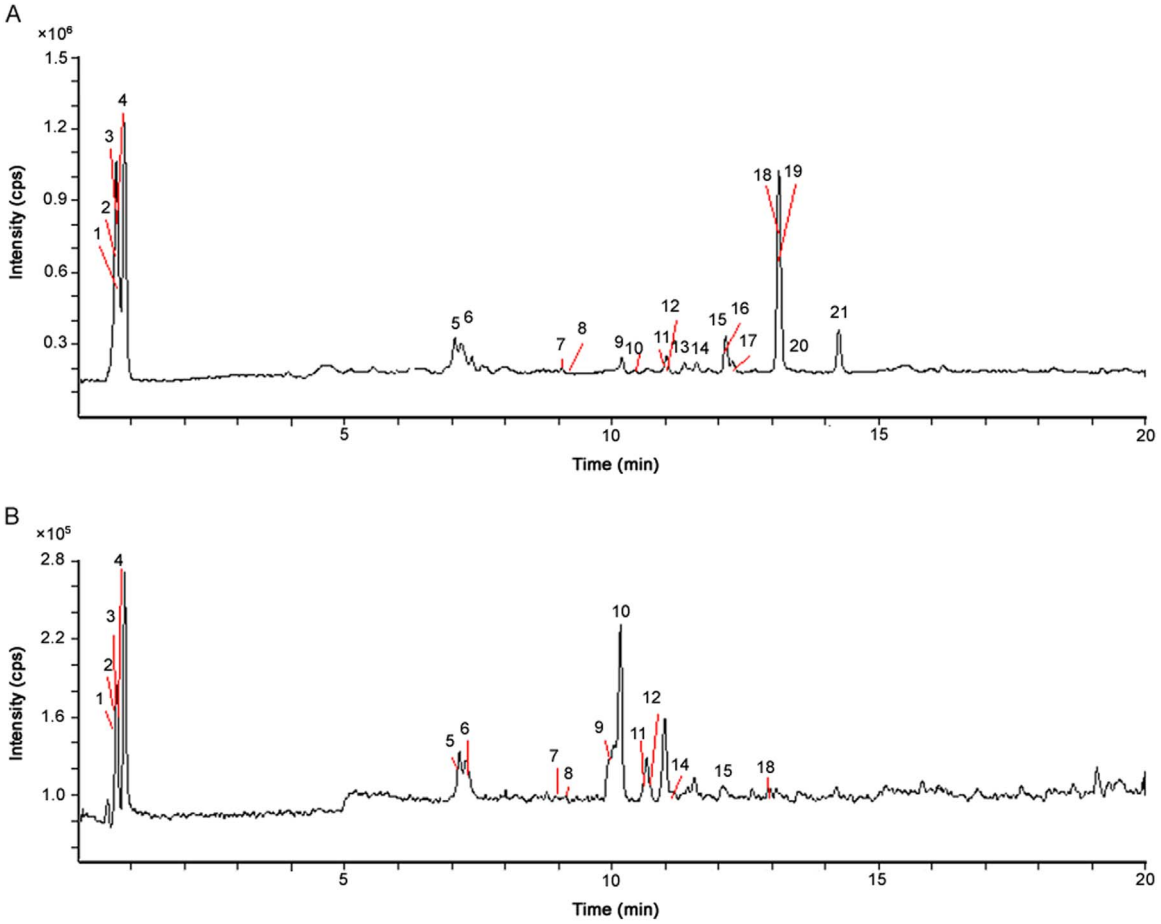

Fig. 1. The total ion chromatogram of the (A) unprocessed (B) processed *Aconitum*.

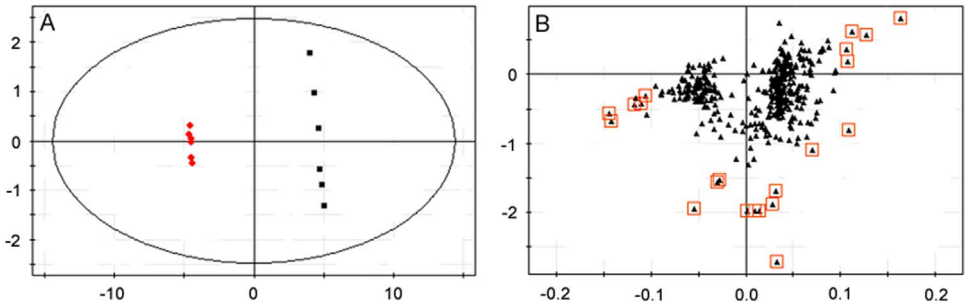

Fig. 2. (A) PLS-DA score plot and (B) loading plot of HPLC-TOF/MS spectra from the unprocessed (red) and processed *Aconitum* (black).

(VIP) value of the alkaloids. Among the 21 alkaloids, six were diester-diterpenoid alkaloids including beiwutine, mesaconitine, 10-OH-aconitine, hypaconitine, aconitine and deoxyaconitine, and eight were monoester-diterpenoid alkaloids including 14-benzoyl-10-OH-mesaconine, benzoylmesaconine, benzoylaconine, benzoylhypaconine, benzoyldeoxyaconine, pyromesaconitine, pyrohypaconine and pyroaconitine, while the other seven were amine-diterpenoid alkaloids including mesaconine, hypaconine, isotalatizidine, aconine, fuziline, talatizamine and 14-acetyltalatizamine (Table 1).

The contents of the seven amine-diterpenoid alkaloids were increased after processing, while the contents of the six diester-diterpenoid alkaloids were sharply decreased. Among the eight monoester-diterpenoid alkaloids, 14-Benzoyl-10-OH-mesaconine, benzoylmesaconine, benzoylaconine, benzoylhypaconine, pyromesaconitine were increased in the processed group, while benzoyldeoxyaconine, pyrohypaconine, pyroaconitine were decreased after processing. The results suggested that diester-diterpenoid alkaloids might be transformed into monoester-diterpenoid alkaloids, and monoester-diterpenoid alkaloids might be converted into amine-diterpenoid alkaloids after processing.

3.2. Contents of aconitine, benzoylaconine and aconine in the unprocessed and processed Aconitum

Because of the different trend among the three forms of alkaloids, the concentrations of the three typical alkaloids (aconitine, benzoylaconine and aconine) were determined by HPLC/MS. The retention time of aconitine, benzoylaconine, aconine and IS was 10.01, 7.84, 2.10 and 7.20 min, respectively. The calibration curve of aconitine, benzoylaconine and aconine showed the satisfactory linearity over the concentration range of 1.35–54 ng/mL for aconitine, 1.21–43.6 ng/mL for benzoylaconine, and 0.815–32.6 ng/mL for aconine, while the regression equation was y=0.409x+0.083 for aconitine, y=0.986x+0.758 for benzoylaconine, and y=0.156x+0.157 for aconine. All the correlation coefficients (r) were >0.99.

Based on the above method, the contents of aconitine, benzoylaconine and aconine were determined as 0.83 ± 0.03, 0.16 ± 0.008 and 0.11 ± 0.006 mg/g in the unprocessed *Aconitum*, and 0.10 ± 0.005, 0.67 ± 0.02 and 0.14 ± 0.003 mg/g in the processed *Aconitum*, respectively.

3.3. Hydrolysis of aconitine, benzoylaconine and aconine

As shown in Fig. 3, the content of aconitine decreased quickly in less than 10 min during the 60-min heating process, while the contents of benzoylaconine and aconine, especially aconine increased. After heating for 45 min, the content of benzoylaconine dropped slowly, while the content of aconine increased simultaneously. These results suggested that aconitine might be converted to benzoylaconine and aconine, and benzoylaconine could be further converted to aconine. As shown in Fig. 4, a carboxyl group of aconitine was taken off and

Table 1
Comparison of the chemical ingredients between unprocessed and processed *Aconitum* tubers.

No.	t_R (min)	Compound name	Formula	$[M+H]^+$			VIP	Trend
				Detected	Expected	Error		
1[c]	0.8746	Mesaconine	$C_{24}H_{39}NO_9$	486.2695	486.2703	−1.6	1.373	↑
2[c]	0.8748	Hypaconine	$C_{24}H_{39}NO_8$	470.2744	470.2754	−2.1	0.6974	↑
3[c]	0.8792	Isotalatizidine	$C_{23}H_{37}NO_5$	408.2747	408.2750	−0.7	1.394	↑
4[c]	0.8880	Aconine	$C_{25}H_{41}NO_9$	500.2846	500.2860	−2.8	0.731	↑
5[c]	7.039	Fuziline	$C_{24}H_{39}NO_7$	454.2811	454.2805	1.3	1.880	↑
6[c]	7.164	Talatizamine	$C_{24}H_{39}NO_5$	422.2908	422.2901	1.7	2.116	↑
7[c]	9.047	14-acetyltalatizamine	$C_{26}H_{41}NO_6$	464.3008	464.3012	−0.9	0.9646	↑
8[b]	9.153	14-Benzoyl–10-OH- mesaconinemesaconine	$C_{31}H_{43}NO_{11}$	606.2906	606.2914	−1.3	1.238	↑
9[b]	10.17	Benzoylmesaconine	$C_{31}H_{43}NO_{10}$	590.2976	590.2965	1.9	2.844	↑
10[b]	10.65	Benzoylaconine	$C_{32}H_{45}NO_{10}$	604.3132	604.3122	1.7	2.072	↑
11[b]	11.00	Benzoylhypaconine	$C_{31}H_{43}NO_9$	574.3021	574.3016	0.9	2.303	↑
12[b]	11.16	Pyromesaconitine	$C_{31}H_{41}NO_9$	572.2855	572.2860	−0.9	1.298	↑
13[a]	11.33	Beiwutine	$C_{33}H_{45}NO_{12}$	648.3029	648.3015	2.2	1.541	↓
14[b]	11.55	Benzoyldeoxyaconine	$C_{32}H_{45}NO_9$	588.3179	588.3173	1.0	1.323	↓
15[b]	12.08	Pyrohypaconine	$C_{31}H_{41}NO_8$	556.2913	556.2910	0.5	1.199	↓
16[a]	12.10	Mesaconitine	$C_{33}H_{45}NO_{11}$	632.3085	632.3065	3.2	2.099	↓
17[a]	12.23	10-OH-Aconitine	$C_{34}H_{47}NO_{12}$	662.3182	662.3171	1.7	1.571	↓
18[a]	13.08	Hypaconitine	$C_{33}H_{45}NO_{10}$	616.3143	616.3122	3.4	3.235	↓
19[a]	13.08	Aconitine	$C_{34}H_{47}NO_{11}$	646.3231	646.3222	1.4	1.607	↓
20[b]	13.23	Pyroaconitine	$C_{32}H_{43}NO_9$	586.3018	586.3011	1.2	0.9038	↓
21[a]	14.20	deoxyaconitine	$C_{34}H_{47}NO_{10}$	630.3293	630.3273	3.2	2.206	↓

[a] : Diester-diterpenoid alkaloid;
[b] : Monoester-diterpenoid alkaloid;
[c] : Amine-diterpenoid alkaloid.

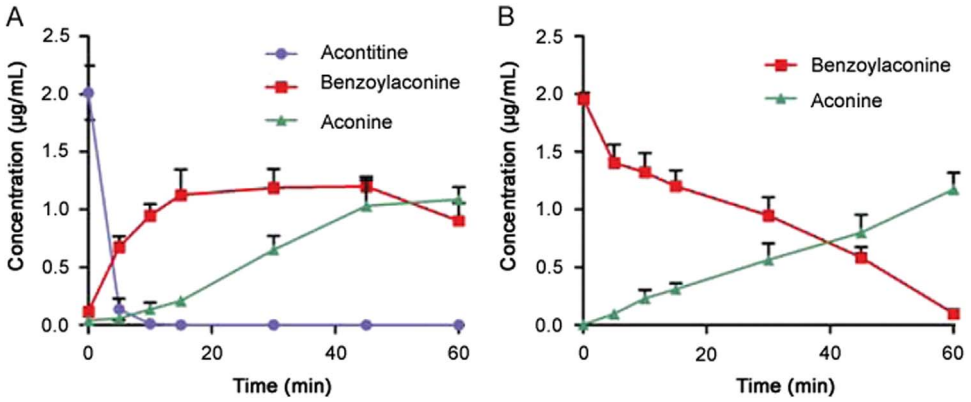

Fig. 3. Concentration changes of (A) aconitine, benzoylaconine and aconine when aconitine was decocted in boiling water, and (B) benzoylaconine and aconine when benzoylaconine was decocted in boiling water.

converted to benzoylaconine, while a phenyl group of benzoylaconine was taken off and transformed to aconine during the heating process.

3.4. Cardiac functions of aconitine, benzoylaconine and aconine

The results of hemodynamic experiments showed that aconitine, benzoylaconine and aconine had different cardiac effects, which are shown in Table 2. SBP, DBP, MBP, HR, LVSP and $+dp/dt_{max}$ increased significantly after intravenous administration of 0.01 mg/kg aconitine, indicating that aconitine could improve the cardiac function of SD rats. Although benzoylaconine could not enhance the heart function, the parameters of ventricular pressure showed the heart function was weakened at the dose of 2 mg/kg, as represented by decreased LVSP and $+dp/dt_{max}$, and increased LVEDP. Aconine also could improve the cardiac function, and the effective dosage of 10 mg/kg was 1000-fold higher than that of aconitine.

3.5. Clarification of the processing mechanism of Aconitum tubers

Aconitum tubers have been considered extremely toxic, and only processed *Aconitum* tubers can be clinically applied in clinic. In this study, the chemical compositions of unprocessed and processed *Aconitum* tubers were analyzed and compared by using HPLC-TOF/MS. It was found that there were significant differences in the identified 21 alkaloids between the unprocessed and processed *Aconitum* tubers. After processing, the contents of all diester-diterpenoid alkaloids were decreased, and all amine-diterpenoid alkaloids were increased. But five monoester-diterpenoid alkaloids were increased and three monoester-diterpenoid alkaloids were decreased. These results suggest that diester-diterpenoid alkaloids may be transformed into monoester-diterpenoid and amine-diterpenoid alkaloids after processing. Aconitine, benzoylaconine and aconine are three representative alkaloids in *Aconitum* tubers, belonging to diester-diterpenoid alkaloids, monoester-diterpenoid alkaloids and amine-diterpenoid alkaloids, respectively. In order to verify the conversion of these alkaloids, they were further decocted in boiling water. The results confirmed that the three types of alkaloids were progressively transformed during the heating process in boiling water, suggesting the conversion of diester-diterpenoid alkaloids into monoester-diterpenoid alkaloids and amine-diterpenoid alkaloids.

There are controversies over whether the alkaloids in *Aconitum* tubers are pharmacologically toxic or effective ingredients. In this study, we performed pharmacological experiments with aconitine, benzoylaconine and aconine to evaluate their pharmacological activities on the cardiac function in SD rats. The results showed that aconitine could improve the cardiac function at the dosage of 0.01 mg/kg, benzoylaconine not only reduced the cardiac function but caused serious arrhythmia, and aconine could play a cardiac effect at the dose of 10 mg/kg intravenously, but its effective dosage was 1000-fold higher than aconitine. The LD_{50} of aconitine, benzoylaconine and aconine was 0.12, 23 and 120 mg/kg as reported previously. We therefore believe that diester-diterpenoid alkaloids are the main effective and toxic ingredients in *Aconitum* tubers, and that transformation of most toxic diester-diterpenoid alkaloids into less toxic monoester-diterpenoid alkaloids and amine-diterpenoid alkaloids may be the attenuated mechanism of processing of *Aconitum* tubers, which does not affect the cardiac effect of *Aconitum* tubers due to the low effective dosage of diester-diterpenoid alkaloids. Based on the above findings, we strongly suggest that the content of diester-diterpenoid alkaloids in *Aconitum* tubers should be strictly controlled in clinical practice.

4. Conclusions

After identification and comparison of the chemical ingredients in unprocessed and processed *Aconitum* tubers by using HPLC-TOF/MS and multivariate pattern recognition methods, it was found that diester-diterpenoid alkaloids can be transformed into monoester-diterpenoid alkaloids and amine-diterpenoid alkaloids during the processing procedures. Through decocting the three representative alkaloids, aconitine, benzoylaconine and aconine, in boiling water, it was further proved that they can be progressively transformed. Subsequent pharmacological experiments with aconitine, benzoylaconine and aconine in SD rats showed that the effect of processing the *Aconitum* tubers was attributed to the transformation of the most toxic diester-diterpenoid alkaloids into less toxic monoester-diterpenoid

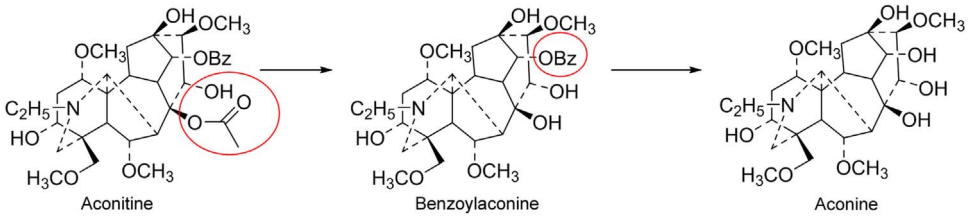

Fig. 4. Conversion among aconitine, benzoylaconine and aconine.

Table 2
Cardiac functions of aconitine, benzoylaconine and aconine before and after injective administration in rats ($n=6$).

Parameters	Aconitine (0.01 mg/kg)		Benzoylaconine (2 mg/kg)		Aconine (10 mg/kg)	
	Before i.v.	After i.v.	Before i.v.	After i.v.	Before i.v.	After i.v.
SBP (mmHg)	96.7 ± 11.5	112.7 ± 10.5**	104.1 ± 11.8	100.6 ± 11.9	105.1 ± 7.3	122.3 ± 11.1**
DBP (mmHg)	64.8 ± 10.4	82.7 ± 10.4**	75.6 ± 8.2	69.8 ± 14.8	74.1 ± 6.9	89.9 ± 7.1**
MBP (mmHg)	80.1 ± 11.1	94.9 ± 10.0**	88.3 ± 9.1	82.6 ± 13.7	86.3 ± 6.3	103.7 ± 7.7**
HR (bpm)	388 ± 47	412 ± 57*	387 ± 34	348 ± 47	380 ± 43	430 ± 54*
LVSP (mmHg)	108.6 ± 6.6	121.5 ± 6.2**	119.8 ± 10.9	112.9 ± 14.2	114.5 ± 3.4	129.0 ± 8.9**
LVEDP (mmHg)	8.9 ± 3.0	7.6 ± 3.0*	9.6 ± 3.1	11.4 ± 2.8*	8.8 ± 0.6	6.7 ± 1.8*
$+dp/dt_{max}$ (mmHg/s)	3473.1 ± 367.4	4078.1 ± 402.0**	4156.7 ± 466.9	3575.6 ± 1085.8	3857.7 ± 338.1	4568.4 ± 291.4**

* $p < 0.05$,
** $p < 0.01$.

alkaloids and amine-diterpenoid alkaloids, which will provide support for processing and clinic application of *Aconitum* tubers.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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