

# Pharmacokinetics of the Couple of Radix Aconiti Lateralis Preparata and Cinnamomum Cassia in Rats with Osteoporosis with Kidney-yang Deficiency by UPLC and Microdialysis Method

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## Research

**Keywords:** Radix Aconiti Lateralis Preparata (Aconite) and Cinnamomum cassiais (Cinnamon), osteoporosis with kidney-yang deficiency, trans-cinnamic acid, hypoaconitine, mesaconitine, material basis, UPLC and microdialysis

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# Abstract

**Background:** To confirm the metabolites of the Aconite and Cinnamon herb couple in rat, and characterize its pharmacokinetics.

**Methods:** Microdialysis probes were inserted into the jugular vein and knee joint of Sprague Dawley rat, and dialysates of different administration groups were collected. Target analytes were separated on a hydrophilic interaction liquid chromatography column (ACQUITY UPLC BEH C8 2.1×100 mm, 1.7 μm) and analyzed with an ultra-performance liquid chromatography (UPLC) under multiple reaction monitoring modes.

**Results:** The experiment shows that the concentrations of quality of the three metabolites in mixing extraction of the herb couple were 0.3701% for trans-cinnamic acid, 0.1249 % for mesaconitine, and 0.0469 % for hypoaconitine, and Cinnamon group was 0.1731 % for trans-cinnamic acid, Aconite group were 0.0017 % for mesaconitine, and 0.0300 % for hypoaconitine. The concentrations of quality of the metabolites in rat plasma of the herb couple group were 0.0028% for trans-cinnamic acid, 0.0947% for mesaconitine, and 0.1124% for hypoaconitine. And the concentrations of quality of Aconite group and Cinnamon group were 0.0019% for trans-cinnamic acid, 0.0307% for mesaconitine, and 0.0220% for hypoaconitine. Pharmacokinetic results showed that the mean half-lives of the microdialysis samples of blood of Cinnamon group was 492.18 min for trans-cinnamic acid, and Aconite group were 102.48 min for mesaconitine and 93.27 min for hypoaconitine, and the herb couple were 181.36 min for trans-cinnamic acid, 103.9 min for mesaconitine and 116.01 min for hypoaconitine, and the microdialysis samples of joint of Cinnamon group was 190.85 min for trans-cinnamic acid, and Aconite group were 48.51 min for mesaconitine and 46.01 min for hypoaconitine, and the herb couple was 131.19 min for trans-cinnamic acid, 49.36 min for mesaconitine and 146.68 min for hypoaconitine.

**Conclusions:** The mixed extraction of aconite and cinnamon can promote the dissolution of the active ingredients. The herb couple can promote the absorption of the active ingredients, improve the distribution of the active ingredients in the joint and blood, prolong the half-lives of the active ingredients. It shows that the compatibility of aconite and cinnamon can increase the bioavailability of the drug and improve the clinical efficacy.

## 1. Introduction

Osteoporosis (OP) is a systemic metabolic bone disease characterized by bone loss and degeneration of bone microstructure<sup>[-1]</sup>. The traditional theory of traditional Chinese medicine holds that OP is closely related to the deficiency of kidney<sup>[-1]</sup>. Studies have shown that kidney-yang deficiency is an important factor in the occurrence of OP<sup>[-1]</sup>. Kidney deficiency can cause hypothalamic-pituitary-gonadal axis hypofunction, decreased sex hormone secretion, hyperabsorption of bone, decreased secretion of calcitonin, and decreased intestinal calcium absorption, resulting in the decrease of osteogenesis and bone tissue mass. The treatment of primary OP is mainly based on western medical methods. These methods have good prevention and treatment effects, but some serious adverse reactions can't be avoided,

limiting their clinical applications<sup>[1]</sup>. Traditional Chinese medicine has fewer side effects, which has been used to treat kidney-yang deficiency. Traditional Chinese medicine therapy is a very promising for OP treatment<sup>[1]</sup>. However, due to the complex mechanism, the development of traditional Chinese medicine therapy is restricted. Therefore, the mechanism of traditional Chinese medicine t needs to be further studied<sup>[1]</sup>.

Aconite and Cinnamon herb were first recorded in "Shennong Herbal Classic"<sup>[1]</sup>. Aconite is the processed product of *Aconitum carmichaelii* Debeaux tuber. The components of Aconiti are mainly diester alkaloids, including aconitine, mesaconitine and hypoaconitine, etc., which have analgesic and anti-inflammatory effects<sup>[1]</sup>. Studies show that it can influence energy metabolism and gene expression spectrum in rats and activate the proliferation of bone marrow mesenchymal stem cells of mouse<sup>[1]</sup>. Cinnamon is the dried bark of *Cinnamomum cassia* Presl<sup>[1]</sup>. The main content of Cinnamon is trans-cinnamic acid<sup>[1]</sup>. According to pharmacological research, trans-cinnamic acid has antibacterial and anti-inflammatory effects<sup>[1]</sup>. Studies have found that trans-cinnamic acid has influence on bone marrow mesenchymal stem cell proliferation and osteoblast differentiation of rat<sup>[1]</sup>. The compatibility of Aconite and Cinnamon can improve kidney deficiency and spleen deficiency<sup>[1]</sup>, and the hormone level of hypothalamus-pituitary-adrenal cortex axis (HPA) in rats with kidney yang deficiency<sup>[1]</sup>.

The herb couple was first recorded in Treatise on Febrile and Miscellaneous Diseases<sup>[1]</sup>. Relevant studies have shown that both Aconite and Cinnamon can be used to treat OP separately, but the mechanism of the herb couple in the treatment of OP with kidney-yang deficiency need to be revealed<sup>[1]</sup>.

In this paper, the PK-PD model and microdialysis sampling coupled with UPLC were used to study the active ingredients of the aconite, cinnamon, and the compatibility of the two. The metabolites of the herb couple were confirmed, its pharmacokinetics was characterized. This work may provide a basis for the clinical application of aconite and cinnamon herb couple to treat OP of kidney-yang deficiency syndrome.

## 2. Materials And Methods

### 2.1 Chemicals

All reagents were of analytical grade unless specified otherwise. Water (18.2 MΩ cm) was produced by a laboratory Milli-Q pure water meter.

trans-cinnamic acid, mesaconitine and hypoaconitine reference substance, Lot number: AA0806LB14, 110799–200505, 110798–201106 purity:≥ 98%). Chromatographic methanol (product batch number: Q/BSYZ01-2006) purchased in Tianjin Siyou Fine Chemicals Co., Ltd. Chromatographic acetonitrile (product batch number: R200813) purchased from Tanmo Quality Inspection Technology Co., Ltd. formic acid, 98% purity ≥, CAS NO. 64-18-6), Purchased from Hangzhou Changzheng Chemical Reagent Co., Ltd. Ammonia, Lot No. 20091022, Purchased from Hangzhou Changzheng Chemical Reagent Co., Ltd). Pure water (pure water produced by laboratory Milli-Q pure water meter). aconite (lot: 200901), cinnamon (lot:

181201), All purchased from Zhejiang University of Traditional Chinese Medicine Chinese Herbal Medicine Co., Ltd. Hydrocortisone injection (batch number: 1904021). Heparin sodium solution (0.1%, 125U/mL, LOT: L16N10G103237). saline (LOT: L12A10G95086). Probe CMA 20 Elite 4mm 3/pkg (REF :8010435, LOT: T54996), CMA 30Linear MD Probe 4/pkg (REF: 8010460, LOT: T55153). ring reagent (NaCl( batch number: 8015031801), KCL( batch number: 20180110), MgSO4( batch number : 2015052501), KH2PO4( batch number: 2015082901), CaCL2( batch number: 2015050501). Pentobarbital Sodium( batch number: DW-PS-05)

## 2.2 Apparatus and software

Detection and quantification of the analytes were performed using a UPLC Acquity system from Waters (Waters, USA) equipped with a binary pump, vacuum membrane degasser, a thermostated column compartment, an autosampler, and an automatic injector. An Acquity ACQUITY UPLC BEH C8 column (100 mm×2.1 mm I.D., 1.7 μm particle size, Part No 186002878) from Waters were tested as chromatographic columns. Auxiliary apparatuses were: HC-3018 High-Speed Centrifuge (Anhui Zhongke Zhongjia Science Instrument Co., Ltd), WH-861 Vortex Mixer (Taicang Hualida Experimental Equipment Co., Ltd), Electronic scales (METTLER TOLEDO 1/100000 electronic scales), KQ-500B ultrasonic cleaner (Kunshan ultrasonic instrument Co., Ltd), Liquid transfer gun (Eppendorf Research plus range 100–1000μl), SHZ-III B circulating water multi-purpose vacuum pump (Linhai Tan vacuum Equipment Co., Ltd).

## 2.3 Animals and microdialysis experiment

### 2.3.1 Animals

Sprague Dawley (SD) rats were purchased from the Animal Experimental Center of Zhejiang Chinese Medicine University. They were raised in the Animal Experimental Center of Zhejiang Chinese Medicine University (license number: 20180006004067). Before experimentation, the rats were allowed a 1-week acclimation period in the animal house using the independently isolated feeding cages with natural illumination. The rats were fed standard food and purified water. The environment temperature was kept at  $25 \pm 2^\circ\text{C}$ , and the humidity was  $\geq 40\%$ .

### 2.3.2 Establishment of pathological model of OP with kidney-yang deficiency

SD rats were randomly grouped according to their body weight. Anesthetized with ip10% chloral hydrate 3mL/kg, then fix the animal on the operating table. Through bilateral oophorectomy, the fallopian tubes and accompanying blood vessels are ligated first, and then the "mulberry-like" ovaries are separated with elbow surgical clamps, ligated, and cut off. The rats were injected with 40,000 U/mL penicillin every day after the operation, each injection was 1 mL/d, and the injection was continued for 3 days, and then the rats were routinely reared. Starting from the second week, the experimental group was injected with hydrocortisone injection (5 mg/mL) 20 mg/kg on the hind limbs every day. From the 4th week to the 8th week, the rats in the experimental group were injected with hydrocortisone injection twice a week to maintain the state of kidney-yang deficiency. Generally based on rat behavior indicators, serum E2, T, ACTH, cAMP, cGMP, etc.

levels, bone density and bone mineral content and other tests. Judge whether the model of OP with kidney-yang deficiency type is successful.

### **2.3.3 microdialysis experiment**

Ringer-HEPES buffer solution (precise weighing NaCl 1.78g, KCl 55.91mg, MgSO<sub>4</sub> 36.11 mg, KH<sub>2</sub>PO<sub>4</sub> mg, NaHCO<sub>3</sub> 13.61 mg CaCl<sub>2</sub> 525.06 mg, and watering to 100 mL volume bottle) was used for the preparation of intermediate solutions and standard solutions for calibration purposes.

The rats were divided into Aconite group, Cinnamon group and the herb couple (1:1). The rats were a successful model and anesthetized. Rat body temperature was maintained at 37°C using a heating pad during the experiment, and their heads were fixed on the brain stereotaxic locator to fix the limbs of the rats. In the first step, to make an incision at the upper left of the left clavicle, then to find the jugular vein, insert the torn tube into the vein, then place the blood probe in the venous blood vessel, and suture the skin wound carefully. In the second step, insert the traction needle into the cartilage of the rat's leg joint cavity, and insert the probe into the overlap of the traction needle and articular cartilage carefully, so that the probe membrane of the joint probe is partially placed subcutaneously.

To use Ringer's solution to prime and balance 30 ~ 60 min. Aconite and Cinnamon 1 mL / 100g were injected into the duodenum according to the weight of each rat. The samples of the jugular vein and joint dialysate were collected by cryopreservation sampler after administration at different times at 10 min □ 20min □ 30min □ 40min □ 50min □ 60min □ 120min □ 180min □ 240min □ 300min □ 360min □ 480min, a total of 8 h.

After all the samples are collected, the last dose, half an hour after administration, to take out rat whole blood, to wait for it to stand and layer, to separate the supernatant and store it in -20°C refrigerator.

## **2.4 Ultra-performance liquid chromatography (UPLC)**

Chromatography was separation was carried out in an ACQUITY UPLC BEH C8 column (100 mm × 2.1 mm i.d., 1.7 μm ) from Waters Corp., USA. The column temperature was maintained at 30°C. The analysis was achieved with gradient elution using (mobile phase A) acetonitrile and (mobile phase B) water (containing 0.1% formic acid). The gradient elution program started with 0–3 min, 21% A; 3–11 min, 21%-25% A; 11–18 min, 25% A; 18-18.1 min, 25%-21% A; 18.1–20 min, 21% A. The total analysis time including the washing and equilibrium steps was 20 min. The flow rate was maintained at 0.3 mL/min. The injection volumes of the samples or standard solutions were 5 μL.

### **2.4.1 Preparation of standard and unmeasured solutions and Determination**

#### **Preparation of standard solutions**

Precision weighing standard mesaconitine 4.60 mg, hypoaconitine 4.70 mg, and trans-cinnamic acid standard 2.26 mg, with Chromatography Acetonitrile and methanol at 10 mL capacity bottle, to dilute ten times and store at 4°C.

## Preparation of blood samples group

To precision absorption the blood 60 uL of different components, precision addition 20 uL ammonia water, vortex 2 min, precision addition 520 uL Chromatography Acetonitrile and methanol to precipitation protein, and vortex 3 min, 15000 r/min centrifuges for 10 minutes. Take supernatant, use 0.22 um organic filter membrane to filter.

And to analyze them according to the chromatographic conditions respectively, and record the peak area data. Calculate the content value of each group.

## 2.4.2 Method validation and Determination

There is the linearity of the standard solution, preparation of reference standard solution method was 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL, 2 mL, 4 mL, 5 mL in 5 mL capacity bottle, Volume to scale with Chromatographic methanol and Chromatography Acetonitrile (1:1) solution, different concentrations of mixed reference solution were obtained and filtered into the injection bottle with a disposable syringe to use 0.22 um organic filter membrane to filter. According to chromatographic conditions to inject the sample, determine the peak area. With the peak area as the ordinate and the concentration of each group of samples as the abscissa to draw a standard curve. The specificity of the method was determined by comparing the chromatograms of blanks with those corresponding to the samples. The precision of the method was determined by taking each group of blood samples, according to the chromatographic conditions for 8 times, and to determine the peak area of blood samples. To take the same batch of the test solution and analyze it at 0, 2, 4, 6, 8, 12, 16, 18, 24 h according to the chromatographic conditions to determine the stability of the method, and record the peak area data. The repeatability of the method was determined by preparing the same batch of blood for 8 groups, then the peak area of the peak plasma sample was determined according to the chromatographic conditions.

## 2.5 Freeze-dried powder production and determination

Preparation of freeze-dried powder of Aconiti or Cinnamon: To use 240g Aconiti or 80g Cinnamon with water as a solvent to condense and reflux for extraction twice respectively. Put it in a freeze dryer to freeze-drying, quickly weigh and transfer the powder to a plastic bag, and store at 4°C in the refrigerator.

To weigh freeze-dried powder of Aconiti 1.0085g, freeze-dried powder of Cinnamon 0.5081g and 0.1096g freeze-dried powder of the herb couple precisely, To draw 20 ul formic acid to add, and fix to 20 mL volumetric flask with methanol, to vortex for 5min, after standing for 1h, to ultrasound for 30min, and to centrifugation (10000r/min, 10min) to separate the supernatant, use 0.22 um organic filter membrane to filter. And analyze them according to the chromatographic conditions respectively, and record the peak area data.

## 2.6 Statistical analysis

All statistical analyses were performed using the SPSS software (Version 13.0). P-values less than 0.05 were considered statistically significant.

## 3. Results And Discussion

### 3.1 validation of UPLC–MD method

Linearity: The calibration equations for trans-cinnamic acid, mesaconitine and hypoaconitine were shown in Table 1. And the respective r of 0.9998, 0.9995, and 0.9993. The linearity of the UPLC method was evaluated by the correlation coefficients (r) of the calibration curves generated using three standard mixtures, this indicated good linearity within the stated ranges<sup>1</sup>.

Table 1  
Calibration data for metabolites of Aconite and Cinnamon

Analyte	Regression equation	Correlation coefficient (r)	Linear range ug / mL
trans-cinnamic acid	$y = 277746x - 142135$	0.9998	0.904–22.6
mesaconitine	$y = 2E + 06x - 30487$	0.9995	36.8–460
hypoaconitine	$y = 1E + 06x + 13311$	0.9993	37.6–470

Selectivity: No interferences from endogenous substances were observed at the retention times of each respective analyte (Fig. 1), trans-cinnamic acid, mesaconitine and hypoaconitine were eluted at 7.5, 11.9 and 15.3 min, respectively.

Precision: The calculated RSD values of the blood samples in each group are 0.4674%, 1.3080% and 1.7328%, and the RSD values are less than 2%, which indicates that the precision of the instrument method is good.

Stability: The calculated RSD values of the peak areas of trans-cinnamic acid, mesaconitine and hypoaconitine are 1.5489%, 1.9260% and 1.9556%, respectively. And their RSD value is less than 2%, the data showed that the three components in the sample solution were stable within 24 hours after preparation.

Repeatability: The RSD values of each component peak area of trans-cinnamic acid, mesaconitine and hypoaconitine were calculated as follows: 1.8659%, 1.4124% and 1.2253%, respectively. And their RSD value is less than 2%, which indicates that the repeatability of the method is good. In short, the precision and accuracy data in Table 1 indicated that the present UPLC method showed good accuracies and reproducibilities.

### 3.2 Powder rate and Determination of Medicinal Materials in Frozen Dry Powder

#### 3.2.1 powder rate of Frozen Dry Powder

Calculate the powder rate separately: [the weight of the dry powder after drying (including the weight of the evaporating dish)-the weight of the evaporating dish (after drying)] / the quality of the pieces. Available as shown in Table 2.

Table 2  
Powder output of each group of medicinal materials after being made into freeze-dried powder

group	Weight of pieces(g)	Vapor dish weight (after drying)(g)	Weight of dry powder after drying (including weight of evaporating dish)(g)	Powder quality(g)	flour yield	
Aconite	240.1	97.3	109.4	12.1	55	0.229071
		86.9	97.8	10.9		
		202.2	234.2	32		
Cinnamon	80.0	86.9	91.3	4.4	0.055	
Aconite and Cinnamon	50.0 + 50.0	51.7	68	16.3	0.163	

### 3.2.2 Determination of Frozen Dry Powder

The peak area external standard method combined with the powder extraction rate was used to calculate the content of each component in each group of freeze-dried powder medicinal materials, as shown in Fig. 2. P-values less than 0.05 were considered statistically significant. The content of trans-cinnamic acid, mesaconitine and hypoaconitine in the herb couple was significantly higher than trans-cinnamic acid of Aconite group and mesaconitine and hypoaconitine of Cinnamon group ( $P < 0.001$ ), indicating that the mixed extraction of Aconite and Cinnamon may exert a synergistic effect and can promote effectiveness. Promoting and inhibiting the dissolution of chemical components by the interaction between chemicals from different herbs, which might explain the mechanism of toxicity reduction and efficacy enhancement of TCM by formulation<sup>[-]</sup>. The mixed extraction of aconite and cinnamon can promote the dissolution of the active ingredients. It can provides great help for the subsequent absorption and utilization of the drug in the body, which lays a solid foundation for enhancing the absorption of the drug in the body.

### 3.3 Determination of blood content in each group

The peak area external standard method was used to calculate the content of each component in the blood of each group, as shown in Fig. 3. Under the premise that the combination of the herb couple, the content of trans-cinnamic acid, mesaconitine and hypoaconitine of the herb couple were significantly higher than trans-cinnamic acid of Aconite group and mesaconitine and hypoaconitine of Cinnamon group

in the early stage, the combined administration of the herb couple was tested after absorption in the body, and it was concluded that trans-cinnamic acid, mesaconitine and hypoaconitine of the herb couple were higher than trans-cinnamic acid of Aconite group and mesaconitine and hypoaconitine of Cinnamon group ( $P < 0.001$ ). Drug metabolism contributes substantially to interindividual differences in drug response and is also often involved in drug interactions, resulting in either therapeutic failure or adverse effects<sup>1</sup>. Pharmaceutical interactions are caused by the chemical, physical or physicochemical incompatibility of drugs or adjuvants used. These can even occur outside the body and during concomitant administration via the same route<sup>1</sup>. It can be seen that the combination of the herb couple may exert a synergistic effect, which can promote the absorption of active ingredients, further increase the bioavailability of the drug and thus improve the clinical efficacy, which makes the application of Chinese medicine compatibility particularly important.

### **3.4 Determination of the microdialysis samples of blood and joint**

The peak area external standard method was used to calculate the content of the components of the microdialysis samples at different time points, and then the average content at each time point was calculated. To take the time of the microdialysis samples connected as the abscissa, and the content of the microdialysis samples at each time point as the ordinate, as shown in Fig. 4 and Fig. 5, and the relevant kinetic parameters are shown in Table 3.

Table 3  
Pharmacokinetic parameters of the microdialysis samples

classification	group	ingredient	T <sub>1/2</sub> /min	C <sub>max</sub> /(ng/mL)	T <sub>max</sub> /min	AUC(ng/mL*min)
Parameters of components in hemodialysis fluid	the single couple	trans-cinnamic acid	492.18	12.36	60	2471.80
		mesaconitine	102.48	122.35	120	30586.77
		hypoaconitine	93.27	202.18	120	50546.13
	the herb couple	trans-cinnamic acid	181.36	76.56	30	21436.38
		mesaconitine	103.96	540.56	60	205413.78
		hypoaconitine	116.01	796.15	60	302535.20
Parameters of each component in the articular cavity dialysate	the single couple	trans-cinnamic acid	190.86	10.97	50	1645.56
		mesaconitine	48.51	122.35	120	37927.59
		hypoaconitine	46.01	202.18	120	62677.20
	the herb couple	trans-cinnamic acid	131.19	88.29	30	29135.82
		mesaconitine	49.36	540.56	60	167574.40
		hypoaconitine	146.68	796.15	60	246805.03

The purpose of the article is to clarify the law of drug pair compatibility and reveal the essence of the effect of drug pair through the law of chemical composition change after drug pair compatibility. The time-concentration profiles for Aconite and Cinnamon metabolites were biphasic, suggesting an increasing phase followed by an elimination phase. The mean half-lives of the microdialysis samples of blood of Cinnamon group was 492.18 min for trans-cinnamic acid, and Aconite group were 102.48 min for mesaconitine and 93.27 min for hypoaconitine, and the herb couple were 181.36 min for trans-cinnamic acid, 103.9 min for mesaconitine and 116.01 min for hypoaconitine. And the mean half-lives of the microdialysis samples of joint of Cinnamon group was 190.85 min for trans-cinnamic acid, and Aconite group were 48.51 min for mesaconitine and 46.01 min for hypoaconitine, and the herb couple was 131.19 min for trans-cinnamic acid, 49.36 min for mesaconitine and 146.68 min for hypoaconitine, respectively. Trans-cinnamic acid, mesaconitine and hypoaconitine were rapidly accessed to plasma and Joint cavity, and the concentration peaked of the microdialysis samples of blood of Cinnamon group was 60 min for trans-cinnamic acid, and Aconite group were 120 min for mesaconitine and 120 min for hypoaconitine, and the herb couple were 30 min for trans-cinnamic acid, 60 min for mesaconitine and 60 min for hypoaconitine. And the concentration peaked of the microdialysis samples of joint of Cinnamon group was 50 min for trans-cinnamic acid, and Aconite group were 120 min for mesaconitine and 120 min for

hypoaconitine, and the herb couple was 30 min for trans-cinnamic acid, 60 min for mesaconitine and 60 min for hypoaconitine, respectively. In the microdialysis samples of blood and joints, the action time of trans-cinnamic of the herb couple was 20 minutes earlier than Cinnamon group, and the action time of mesaconitine and hypoaconitine of the herb couple were 10 minutes earlier than Aconite group ( $P < 0.05$ ). In the microdialysis samples of blood and joints, compared with Cinnamon group, the action time of trans-cinnamic of the herb couple can hold on for about 80 min longer ( $P < 0.05$ ). And compared with Aconite group, the action time of mesaconitine and hypoaconitine of the herb couple can hold on for about 130 min longer ( $P < 0.05$ ). The pharmacokinetic study of monomeric trans-cinnamic acid reported that the peak time of trans-cinnamic acid by gavage was about 30 min, and the  $t_{1/2}$  was about 36 min<sup>[1]</sup>. After oral administration of aconitine to SD rats, the blood concentration in the body is eliminated quickly, and individual differences are large. The  $t_{1/2}$  after the percutaneous replacement is about 360 min, and the peak time after oral administration is 15 min<sup>[1]</sup>. To observe the difference in the content of the microdialysis samples at different times and under different administration conditions. It can be seen that the contents of trans-cinnamic acid of the herb couple was significantly higher than Cinnamon group, and mesaconitine and hypoaconitine of the herb couple were significantly higher than Aconite group in the microdialysis samples of blood and joint cavity. From the peak content of trans-cinnamic, mesaconitine and hypoaconitine, the content of the synovial fluid is slightly more than that in the blood as shown in Fig. 4 and Fig. 5. Among them, the highest is that the hypoaconitine of cinnamon group in the microdialysis samples of joints is 0.303% more than that of cinnamon group in the microdialysis samples of blood, and the lowest is that the trans-cinnamic of cinnamon group in the microdialysis samples of joints is 0.01% more than that of cinnamon group in the microdialysis samples of blood. The effective time of trans-cinnamic acid of the herb couple was 150 min, and that of Cinnamon group was 230. Mesaconitine and hypoaconitine of the herb couple were 360 min, and that of Aconite group was 200 min. The effective time of trans-cinnamic acid of the herb couple was significantly longer than Cinnamon group, and mesaconitine and hypoaconitine of the herb couple were significantly higher than Aconite group ( $P < 0.05$ ). It can be seen that the herb couple can improve the distribution of the active ingredients in the joint and blood, the absorption of the active ingredients in the body is improved, and the time for the effective substances to be distributed in the body is prolonged, making the drug action more durable ( $P < 0.05$ ). According to the above, the mixed extraction of aconite and cinnamon can promote the dissolution of the active ingredients. It can provide great help for the subsequent absorption and utilization of the drug in the body. Combined with the change in the content of the drug in the body, it shows that the compatibility of Aconite and Cinnamon may play a synergistic effect, promote dissolution and enhance absorption, further improve the bioavailability of Aconite and Cinnamon and improve the clinical efficacy, which makes the application of Aconite and Cinnamon compatibility particularly important.

## 4. Conclusion

The mixed extraction of aconite and cinnamon can promote the dissolution of the active ingredients. The herb couple can promote the absorption of the active ingredients, improve the distribution of the active ingredients in the joint and blood, prolong the half-lives of the active ingredients. It shows that the

compatibility of aconite and cinnamon can increase the bioavailability of the drug and improve the clinical efficacy.

## 5. Abbreviations

OP: Osteoporosis; HPA: hypothalamus-pituitary-adrenal cortex axis; UPLC: Ultra-performance liquid chromatography; SD: Sprague Dawley; RSD: Relative Standard Deviation; TCM: Traditional Chinese Medicine; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; ACTH: adreno-cortico-tropic-hormone; E2: estradiol; T: temperature.

## 6. Declarations

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### Authors' contributions

YXM, JQG, LPY, XJL, LPQ and LZ contributed to Research and data analyses. YXM, LPQ and LZ contributed to the project design and paper writing. All authors read and approved the final manuscript.

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### Availability of data and materials

All the data used to support the findings of this study are available from the corresponding author upon reasonable request.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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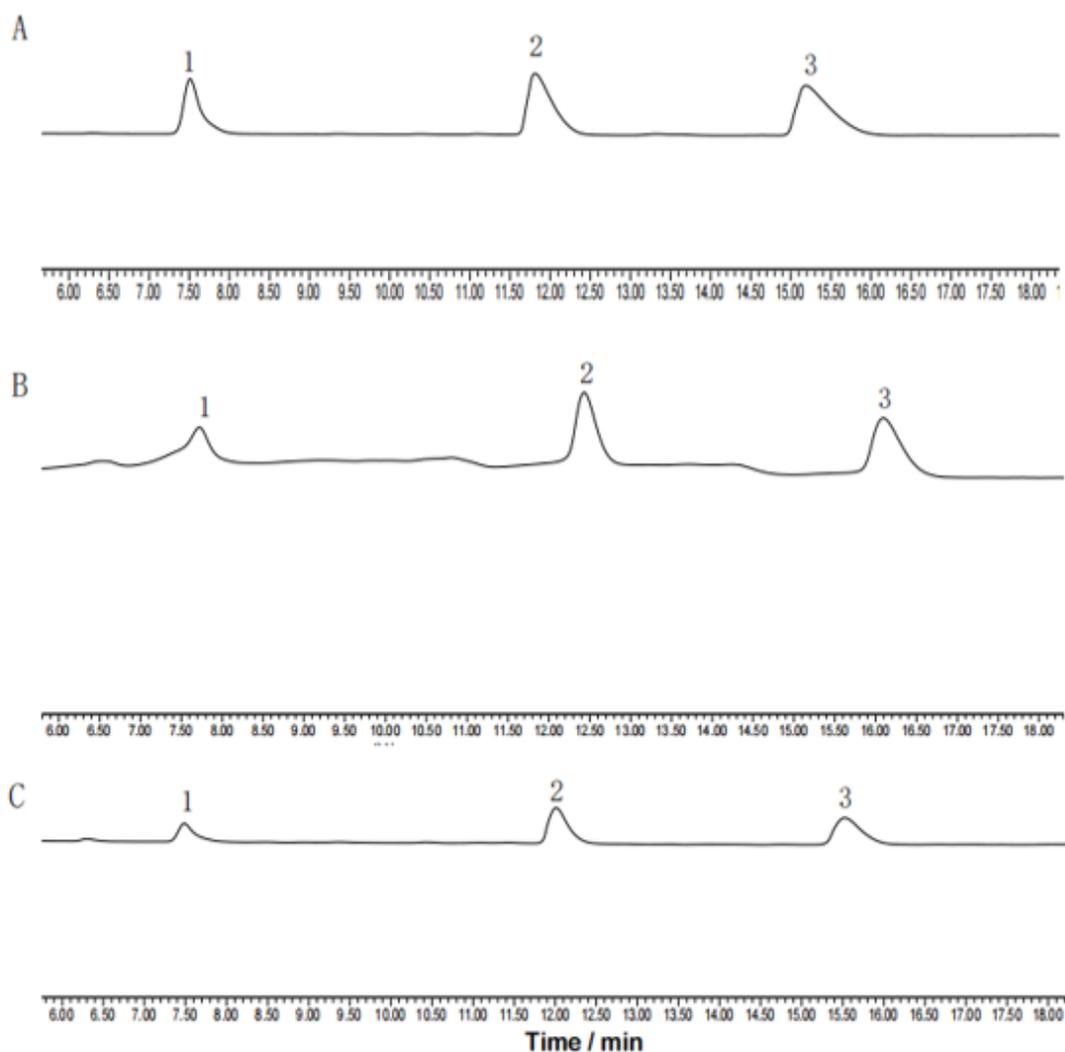
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## Figures



**Figure 1**

Atlas of the three active components 1. trans-cinnamic acid 2.mesaconitine 3.hypoaconitine A. UPLC image acquisition of standard products B. UPLC image acquisition of blood samples C. UPLC image acquisition of the herb couple

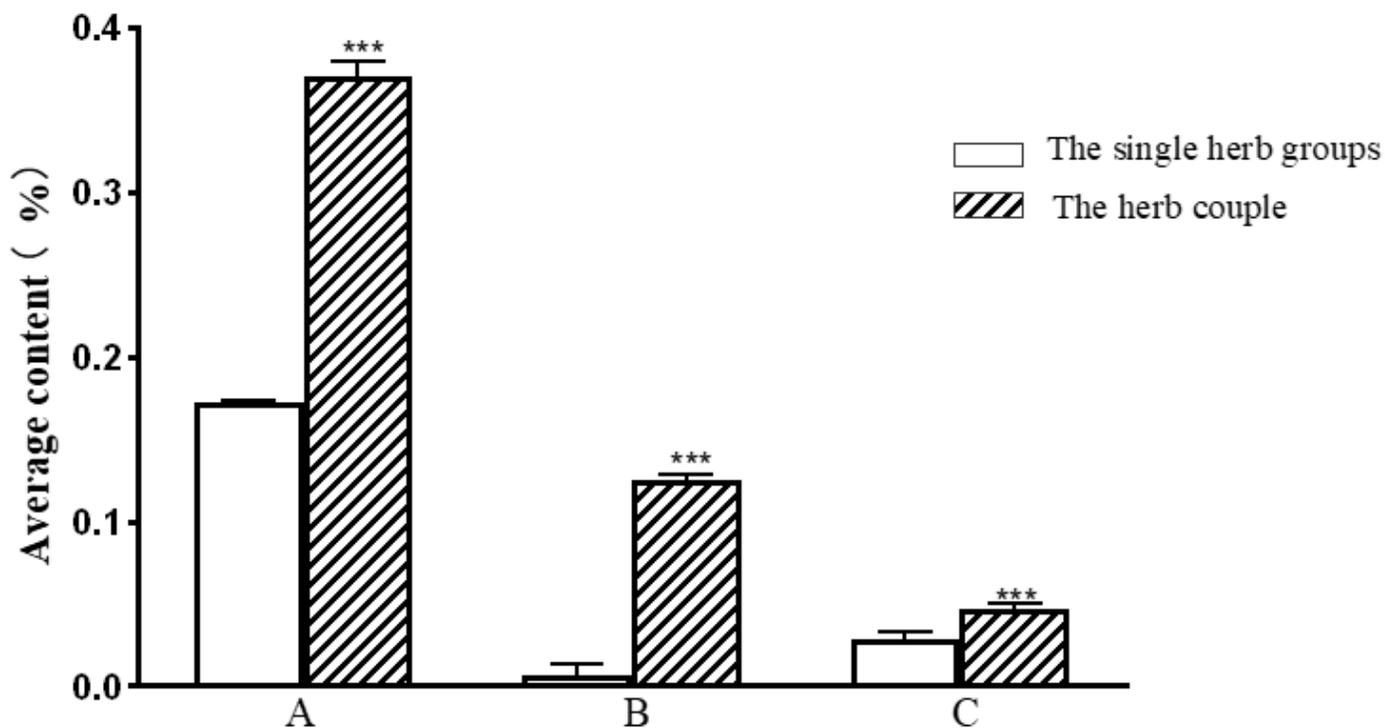


Figure 2

Content of freeze-dried powder in each group A. trans-cinnamic acid B. mesaconitine C. hypoaconitine Compared to the single herb groups, \*\*\*p<0.001.

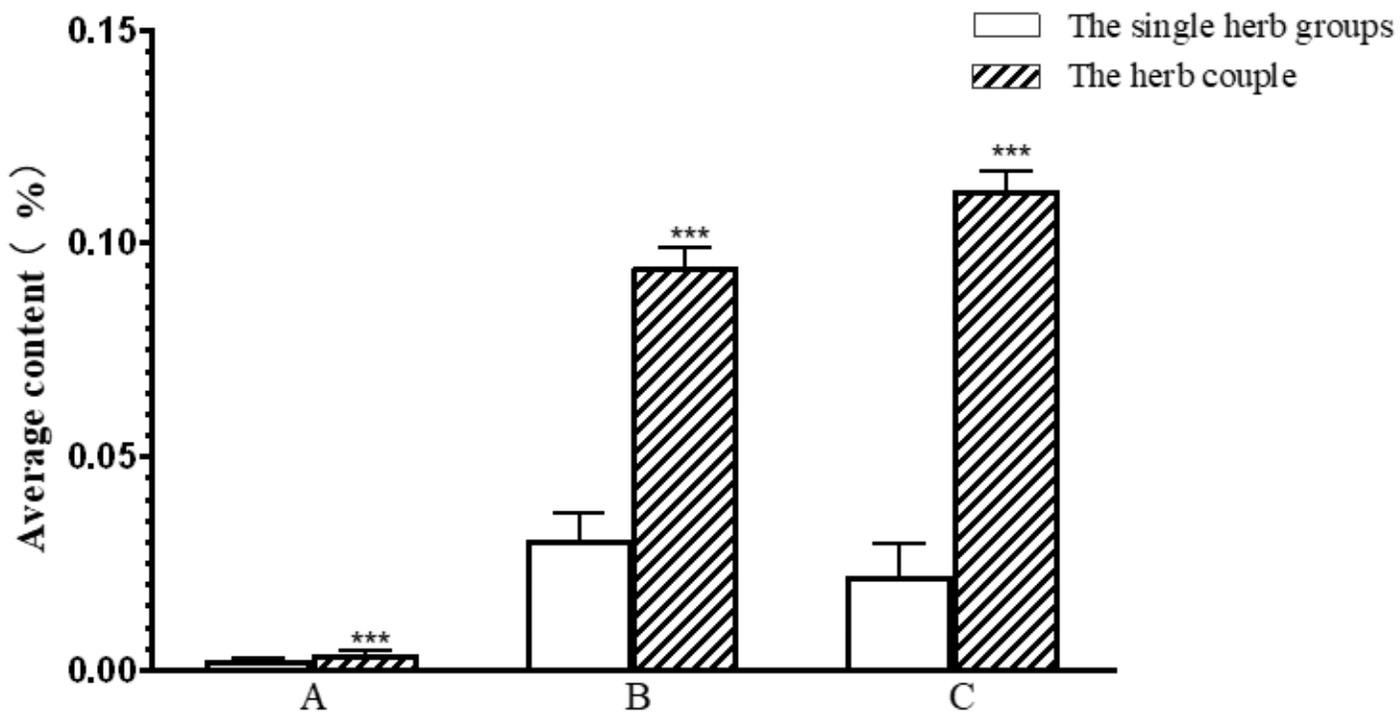
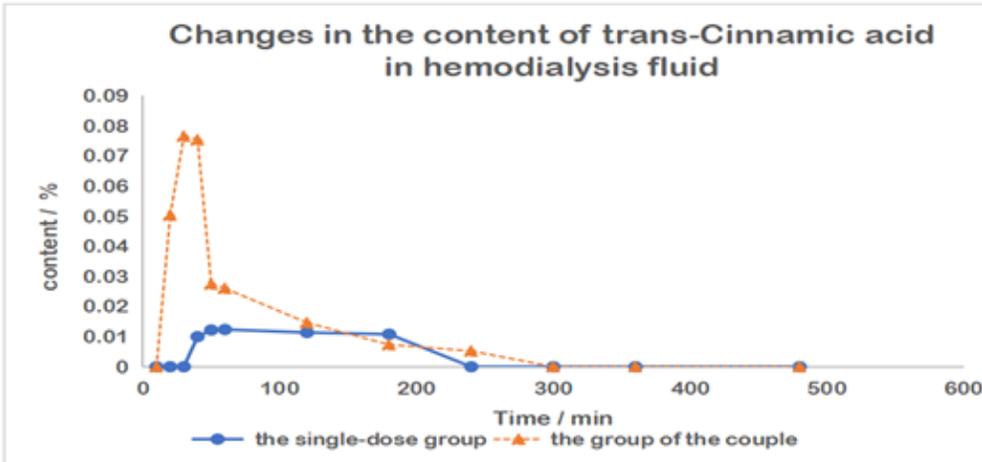


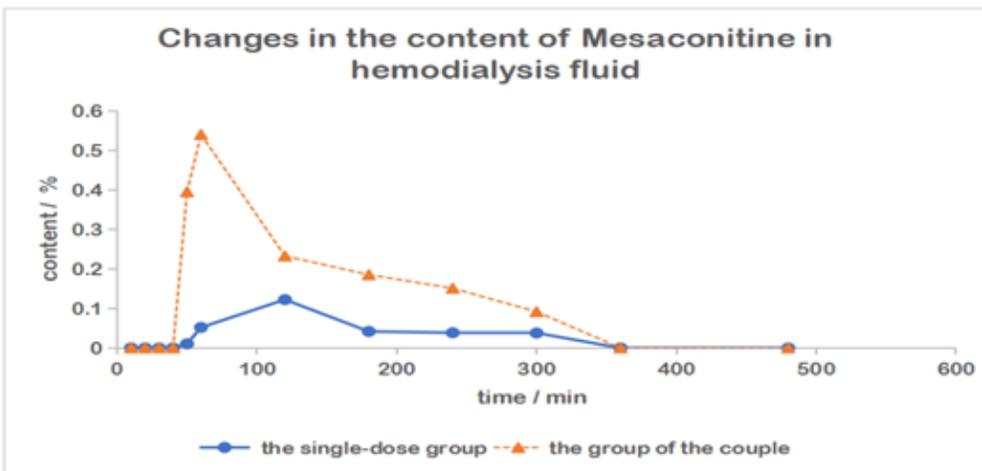
Figure 3

Contents of blood components in each group A. trans-cinnamic acid B. mesaconitine C. hypoaconitine Compared to the single herb groups, \*\*\*p<0.001.

A



B



C

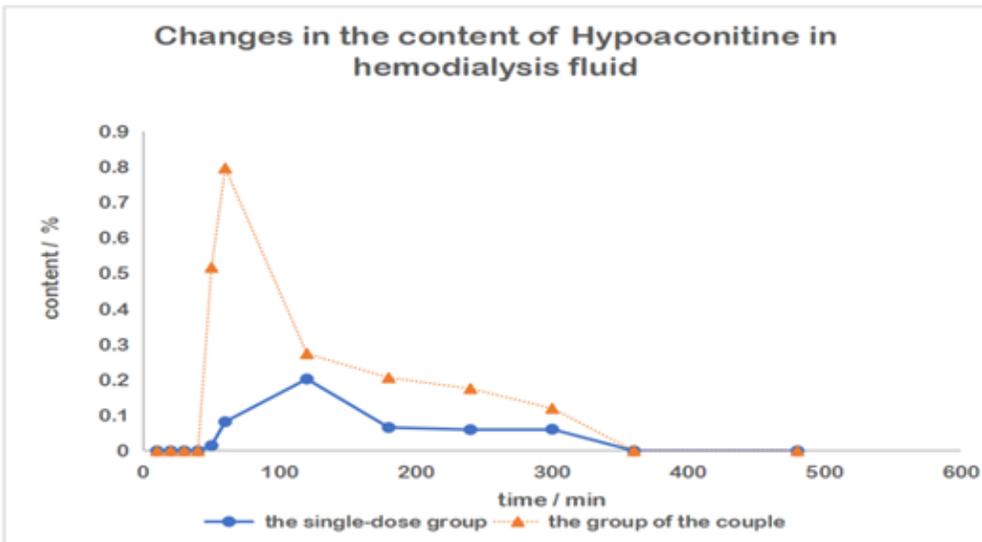
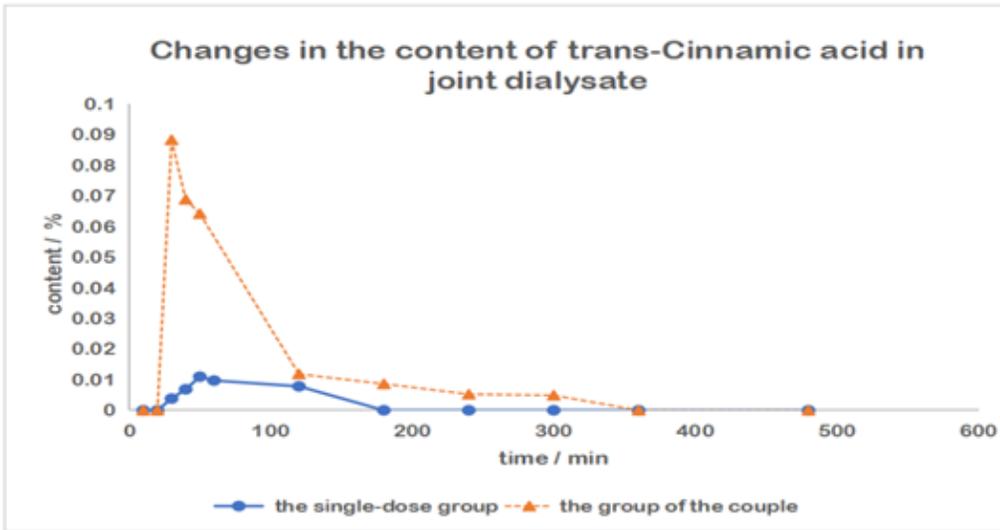


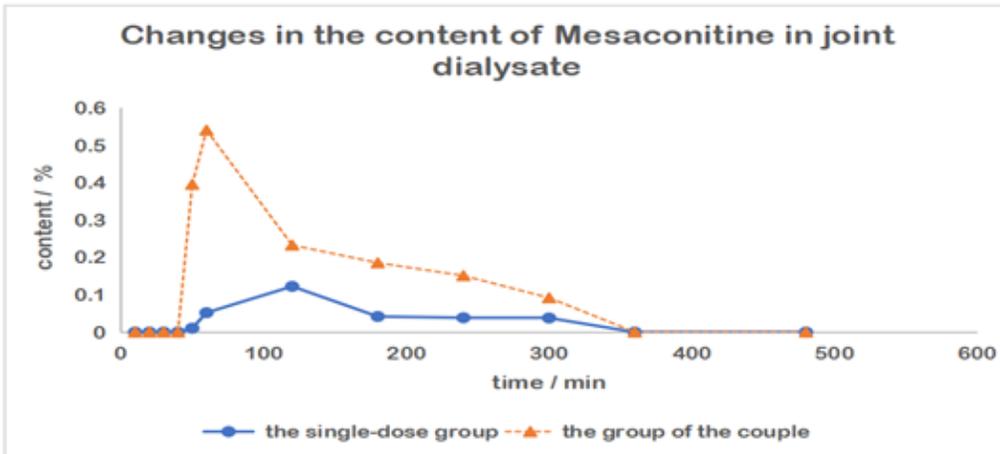
Figure 4

Changes in the contents of the microdialysis samples of blood in different groups

A



B



C

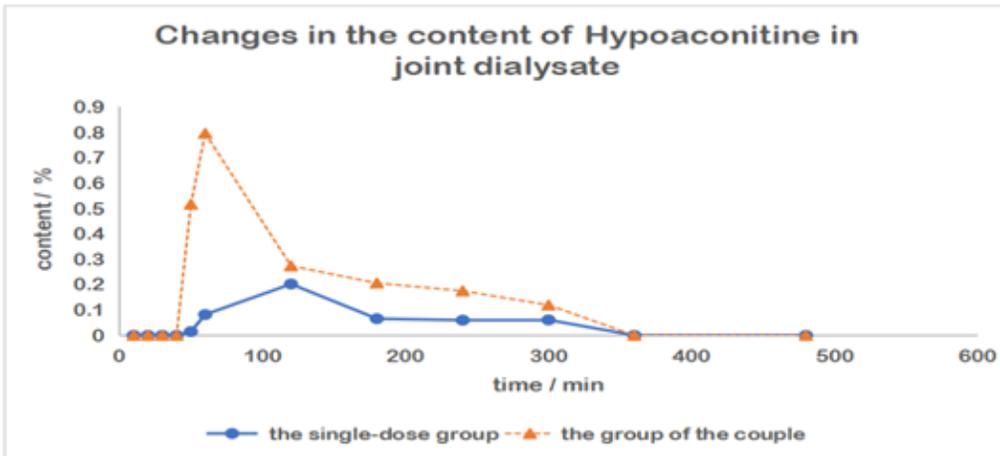


Figure 5

Changes in the contents of the microdialysis samples of joint in different groups