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Review

Metabolomics as an emerging tool for the pharmacological and toxicological studies on *Aconitum* alkaloids

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ABSTRACT

Aconitum (Ranunculaceae) has a long-standing history in traditional Chinese medicine (TCM), where it has been widely used to treat conditions such as rheumatoid arthritis (RA), myocardial infarction, and heart failure. However, the potency of *Aconitum* alkaloids, the primary active components of *Aconitum*, also confers substantial toxicity. Therefore, assessing the efficacy and toxicity of these *Aconitum* alkaloids is crucial for ensuring clinical effectiveness and safety. Metabolomics, a quantitative method for analyzing low-molecular-weight metabolites involved in metabolic pathways, provides a comprehensive view of the metabolic state across multiple systems *in vivo*. This approach has become a vital investigative tool for facilitating the evaluation of their efficacy and toxicity, identifying potential sensitive biomarkers, and offering a promising avenue for elucidating the pharmacological and toxicological mechanisms underlying TCM. This review focuses on the applications of metabolomics in pharmacological and toxicological studies of *Aconitum* alkaloids in recent years and highlights the significant role of metabolomics in exploring compatibility detoxification and the mechanisms of TCM processing, aiming to identify more viable methods for characterizing toxic medicinal plants.

1. Introduction

Aconitum L. (Ranunculaceae family) possesses therapeutic properties for "tonifying yang, dissipating cold, and alleviating pain" and is extensively used in countries such as China, India, Vietnam, Korea, and Japan. It is traditionally employed to address "wind-cold-dampness obstruction, blood stasis, bruise and pain, and heart-kidney yang deficiency pattern", among other conditions. *Aconitum* alkaloids, the primary active components in *Aconitum* plants, demonstrate anti-arrhythmic, analgesic, anti-inflammatory, and local anesthetic effects, as well as capabilities to improve intestinal flora and exhibit anti-tumor properties [1, 2]. Despite the remarkable efficacy of *Aconitum* alkaloids in treating various diseases, severe poisoning and fatalities have resulted from the inappropriate use of *Aconitum* plants [3-6]. Studies have confirmed that *Aconitum* alkaloids can induce neurological, cardiovascular, hepatic, renal, and embryotoxicity, among other adverse effects [7, 8]. Given the narrow therapeutic window of toxic *Aconitum* alkaloids, particular attention must be paid to the application of *Aconitum* L.. Accurate evaluation of the efficacy and toxicity of *Aconitum* alkaloids and their rational utilization to prevent toxic reactions or fatalities is crucial for the safe use of this toxic traditional Chinese medicine (TCM). Consequently, the mechanisms underlying the pharmacological and toxicological activities of *Aconitum* alkaloids have garnered significant attention [9-11], providing valuable guidance for the production, safety evaluation, and clinical application of *Aconitum* plants.

Recent research has revealed limitations in conventional pharmacology and toxicology analysis technologies, which can be time-consuming and less efficient. Characterizing compound toxicity at the histopathological level often requires extended periods, while the vast array of genes and proteins complicates further confirmation and subsequent analysis, particularly when certain functions remain unclear. In contrast, metabolite changes can indicate drug effects at an early stage [12], and metabolomics studies do not require complete genome sequencing or the establishment of expressed sequence tags [13]. Moreover, metabolomics examines the final step in a series of changes following drug administration: alterations at the metabolic level. Many metabolites have known functions and can be detected in body fluids [14]. These advantages position metabolomics as a prominent tool in pharmacological and toxicological studies.

Metabolomics technology can elucidate the syndrome differentiation principles in TCM theory and explain the mechanism of TCM in treating metabolic and cardiovascular diseases by identifying interactions among disease-responsive metabolites of TCM compounds [15-17]. Recently, the concept of functional metabolomics has emerged to discover differential metabolites from large metabolomes, focusing on the function of metabolites and related enzymes, thereby overcoming the limitation of relying on literature for biological interpretation [18]. Functional metabolom-

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ics accurately captures the molecular interaction between biomarkers of disease response and secondary metabolites, making it suitable for revealing the systematic therapeutic characteristics of functional compounds derived from TCM, thus providing better insights into TCM therapeutic mechanisms [19]. According to metabolomics techniques, *Aconitum* alkaloids demonstrate protective effects against lipopolysaccharide-induced inflammation [20]. Regarding the toxicity of *Aconitum* alkaloids, a metabolomic study combining ^{1}H NMR and GC/TOF-MS reveals that aconitine and mesaconitine could elevate the levels of lactate, alanine (Ala), and lipids [21]. Additionally, a GC/TOF-MS study indicated that the damage to heart and muscle function caused by alkaloids might be associated with decreased glutamine and creatinine levels [22].

However, there has been limited discourse regarding the applications of metabolomics in pharmacological and toxicological studies of *Aconitum* alkaloids. This review delineates the natural origins, chemical structures, and effects of *Aconitum* alkaloids, emphasizing the crucial role of metabolomics in researching these compounds. These recent advancements offer a more viable approach for obtaining phenotypic evidence in the study of toxic herbal medicines.

2. Source and chemistry

2.1. Natural origin and traditional use

Aconitum L. is a traditional medicinal genus in the Ranunculaceae family with significant therapeutic value [23]. The commonly utilized TCMs derived from *Aconitum* plants include Aconiti Radix (AR, "Chuanwu" in Chinese), Aconiti Lateralis Radix Praeparata (ALR, "Fuzi" in Chinese), and Aconiti Kusnezoffii Radix (AKR, "Caowu" in Chinese). AR and ALR originate from the main and lateral roots of *Aconitum carmichaelii* Debeaux (Fig. 1A), respectively. AKR is derived from the dried root tubers of *Aconitum kusnezoffii* Rchb. [24]. These three common Chinese herbal medicines are illustrated in Fig. 1.

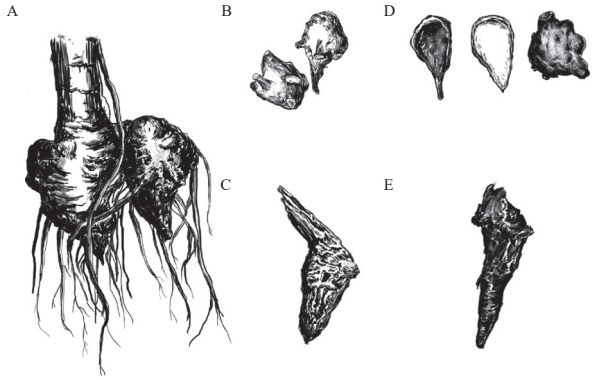

Fig. 1 The morphology of raw and commercial roots of *Aconitum* L.. (A) *Aconitum carmichaelii* Debeaux; (B) Aconiti Lateralis Radix Praeparata (ALR, the lateral roots of Fig. 1A); (C) Aconiti Radix (AR, the main roots of Fig. 1A); (D) three types of ALR processed products, from left to right: heishunpian (HSP), baifupian (BFP), yanfuzi (YFZ); E, AKR. Ink line diagram of medicinal plants drawn by Ms. Yingxi Huang (Shenzhen Xingxun Electronic Technology Co.).

The earliest description of ALR appears in *Shennong's Classic of Materia Medica* (Eastern Han Dynasty), attributing it with pungent and hot medicinal properties capable of dissipating wind-cold, tonifying fire, breaking blood stasis, and treating arthralgia. *Bencao Zhengyi* (1828, by Deyu Zhang) characterizes ALR as "the foremost medicine for pure Yang in twelve meridians, adept at relieving cold exterior and warming source Qi interior". The *Chinese Pharmacopoeia* (CP, 2020) documents ALR's ability to restore yang, addressing collapse syndrome, impotence, abdominal and uterine cold, external contraction, vomiting, diarrhea, edema, and arthralgia due to Yang deficiency. AR and AKR exhibit similar effects, notably in expelling wind, eliminating dampness, warming meridians, and alleviating pain. AR serves as a surgical anesthetic, while AKR strengthens sinew-bone and supplements essence [25]. Both AR and AKR are components in numerous formulas treating joint mobility issues and limb pain. Wutou Decoction (WTD), a classic prescription from *Synopsis of the Golden Chamber*, contains AR and effectively addresses arthralgia and pain in conditions like osteoarthritis [26]. Caowu Powder, documented in *Puji Fang*, employs AKR as its primary ingredient to reduce swelling and pain in treating loose teeth and toothache.

2.2. Chemical structures, bioactivity, and processing detoxification of *Aconitum* alkaloids

The primary alkaloids isolated and identified from AR and ALR are C_{19}-diterpene alkaloids, followed in quantity by C_{20}-diterpene alkaloids, amides, quaternary ammonium salts, and aporphines. While ALR and AKR share similar alkaloid profiles, those of ALR and AR exhibit a low coincidence rate [27]. Talatizamine, isotalatizidine, karacoline, neoline, and fuziline were isolated from AR, whereas isodelphinine, neojiangyouaconitine, and aldohypaconitine were identified from ALR [28]. Li et al. [29] isolated hokbusine A and 8-methoxy-14-benzoyl-beiwutinine from AKR. Among these, the critical bioactive components of *Aconitum* L. are C_{19}-diterpene alkaloids, which constitute the main types of *Aconitum* alkaloids. The structure of C_{19}-diterpenoid alkaloids primarily consists of an aconitine skeleton, typically with oxygen-containing substituents at C_1, C_6, C_8, C_{14}, C_{16}, and C_{18}. Acetic acid or benzoic acid often links to the hydroxyl groups at C_8 and C_{14}, forming esters [30]. Based on various substituents, C_{19}-diterpene alkaloids can be categorized into diester-diterpenoid alkaloids (DDAs), monoester-diterpenoid alkaloids (MDAs), amine diterpenoid alkaloids (ADAs), and lipid diterpene alkaloids (LDAs) [31]. DDAs are representative ingredients of *Aconitum* alkaloids, primarily including aconitine, mesaconitine, and hypaconitine [32]. Figs. 2A and 2B display the C_{19}-diterpene alkaloids aconitine type and chemical structures of major DDAs. Additionally, a water-soluble polysaccharide named FZPS-1 was extracted from ALR, with its antioxidant and immunomodulatory activities confirmed.

DDAs exhibit both therapeutic effects and significant toxicity on the cardiovascular and nervous systems. MDAs, such as benzoylaconine, benzoylmesaconine, and benzoylhypaconine, promote cardiotonic effects while inducing arrhythmias, functioning as double-edged swords [33]. The half-maximal lethal dose (LD_{50}, $mg{\cdot}kg^{-1}$, i.v. mice) of benzoylaconine was approximately 38-fold higher than that of aconitine [34]. Consequently, processing technologies ("Paozhi" in Chinese) are essential to mitigate the toxicity of *Aconitum* plants while preserving their efficacy. Processing represents a unique pharmaceutical method to reduce TCM toxicity and ensure safe clinical use [35]. Fig. 2C illustrates the common understanding that the hydrolysis of DDAs into MDAs and ADAs is responsible for processing detoxification [36]. Processed products of ALR primarily come in three forms and specifications: HSP, BFP, and YFZ, as shown in Fig. 1D.

3. Research strategies of metabolomics on *Aconitum* alkaloids

Metabolomics technology has garnered significant interest as an effective method for quantitative and qualitative analysis of metabolites in complex biological systems. This approach has made substantial contributions to target discovery and validation, assessment of drug efficacy and safety, disease diagnosis, molecular pathology, and precision medicine [37-39].

The conventional metabolomics research approach involves

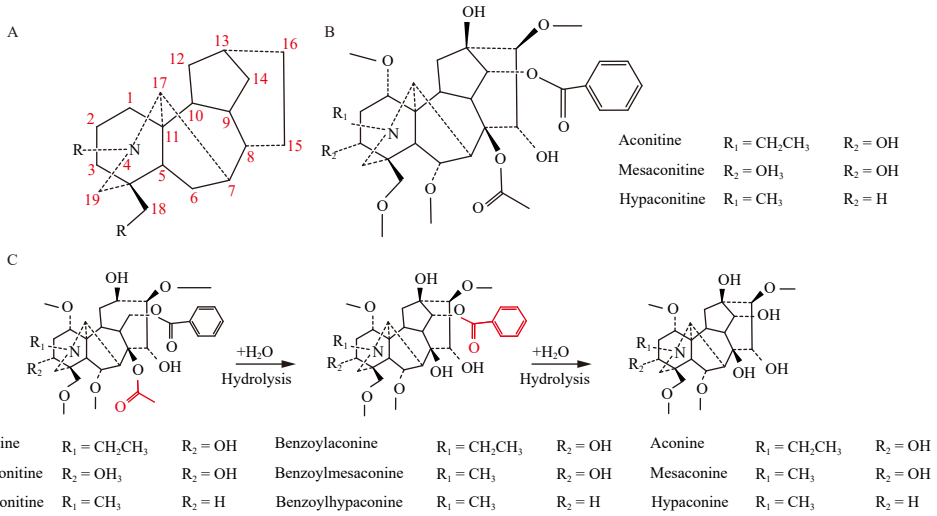

Fig. 2 Structures of major *Aconitum* alkaloids. (A) C_{19}-diterpene alkaloids aconitine type; (B) chemical structures of major DDAs; (C) chemical structures of major *Aconitum* alkaloids and the hydrolysis of processing detoxification.

conducting statistical analysis following sample collection, utilizing databases to elucidate biological phenomena, or further validating potential biomarkers. Fig. 3 illustrates a typical workflow for metabolomics research on *Aconitum*. Wu et al. [40] conducted a comprehensive metabolomics evaluation on the effects of three processed ALR products on rat metabolites. Ultra-high performance liquid chromatography (UHPLC)-quadrupole (Q)-orbitrap tandem mass spectrometry (MS/MS) can be employed for screening metabolites from various parts of *Aconitum* plants [41]. A UHPLC-diode array detection (DAD)-based quantification method for specific *Aconitum* alkaloids has been developed for subsequent metabolomics analysis [42]. Metabolomics findings confirmed that the inhibitory effect of ALR on non-small cell lung cancer may be associated with aminoacyl-tRNA biosynthesis, Ala, aspartate, and glutamate metabolism, and the citrate cycle [43]. In the application of *Aconitum* for rheumatoid arthritis (RA) treatment, D-galactose, glycerol, myoinositol, propionaldehyde, and inositol were identified as differential markers, which are related to galactose metabolism, glycerolipid metabolism, and inositol phosphate metabolism [44].

Metabolomics has facilitated significant advancements in identifying marker metabolites for TCM syndrome types, evaluating TCM efficacy, and elucidating TCM mechanisms. These developments have provided valuable insights into TCM therapeutic practices [45-47]. In essence, metabolomics enables the characterization of metabolic changes induced by medicines, serving as a powerful tool for studying endogenous metabolites. Furthermore, it offers a method to elucidate the mechanisms underlying the pharmacological and toxicological activities of *Aconitum* alkaloids.

4. Metabolomics application on toxicological studies of *Aconitum* alkaloids

While *Aconitum* alkaloids demonstrate significant therapeutic potential, they can also induce toxicity affecting the nervous system, cardiovascular system, liver, and kidneys, following a "dose-time-toxicity" relationship. Metabolomics provides an effective method for elucidating the toxicological profile of *Aconitum* alkaloids.

4.1. Toxicological effects of specific Aconitum alkaloids and single herbs

Numerous studies have demonstrated that AKR and ALR exhibit severe dose-dependent toxic effects on the cardiovascular

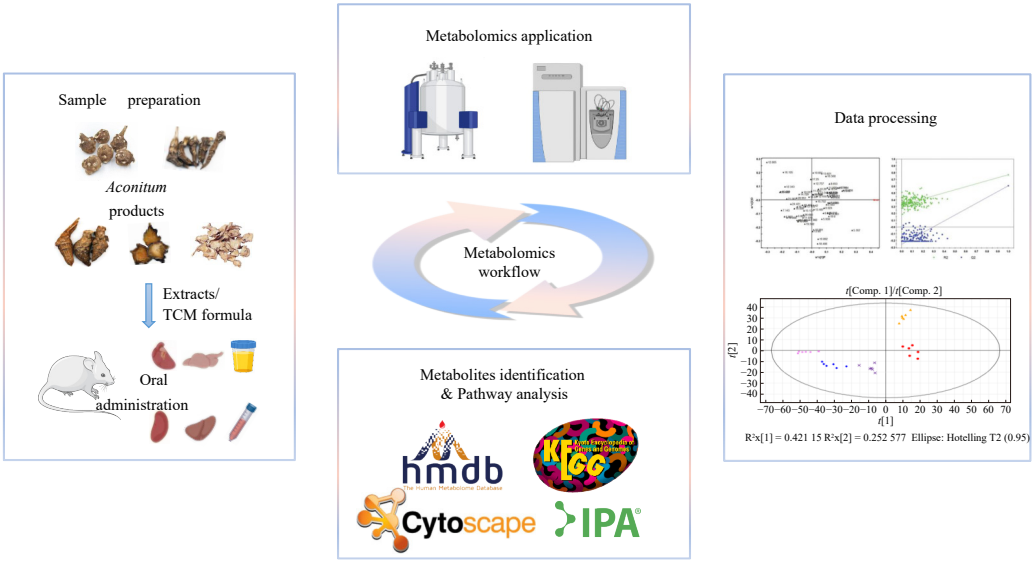

Fig. 3 A typical process of metabolomics research on *Aconitum*.

system, with 7.5 g·kg^{-1} of HSP in rats identified as the highest dose level without substantial adverse effects [48]. Metabolic pathways, including lipid, protein, energy, and glucose metabolism, play a crucial role in myocardial injury induced by aconitine [49]. 5-Ketone proline (Pro) and 1,5-anhydrohexitol in rat blood samples have been preliminarily identified as potential biomarkers of aconitine poisoning [50].

The application of metabolomic technology to elucidate potential indicators of toxic *Aconitum* alkaloids has significant implications for clinical practice. In rats with aconitine-induced myocardial damage, mass spectrometry analysis of myocardial metabolites revealed significant elevations in 17 metabolites associated with FA metabolism, amino acid metabolism, and purine (Pu) metabolism. Palmitic acid emerged as a potential marker for aconitine-induced myocardial injury due to its substantial alteration. The aconitine-induced increase in palmitic acid may exacerbate cardiac damage through the NOD-like receptor (NLR) signaling pathway [51]. *Aconitum* alkaloids are frequently utilized to simulate myocardial channelopathy and establish an LVTA rat model. To investigate the characteristics of myocardial metabolism in rats with aconitine-induced sudden cardiac death (SCD), ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) was employed to identify disturbances in energy and lipid metabolism of the left ventricular myocardium. The altered lipids primarily included phosphatidylcholine (PC), cardiolipin (CL), phosphatidylserine (PS), sphingomyelin (SM), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), ceramide (Cer), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), phosphatidylinositol phosphate (PIP), lysophosphatidylserine (LPS), triglycerides (TG), and diacylglycerol [52]. Furthermore, GC-MS and ^{1}H nuclear magnetic resonance (NMR) analysis of the myocardial metabolic composition in LVTA rats indicated that valine, stearic acid, and Leu might serve as biomarkers in LVTA-SCD events. The primary pathways responsible for metabolic disorders following LVTA include energy overconsumption and deficit, ionic imbalance, oxidative stress, cardiac cytotoxicity, and membrane damage [53].

To investigate whether aconitine-induced LVTA-SCD leads to changes in serum lipid levels compared to heart tissue, a comprehensive analysis of 188 lipid species was conducted. This analysis revealed significant alterations in lipid profiles and the activation of ten metabolic pathways in response to LVTA-SCD. Notably, Cer, SM, PC, PE, and PS were identified as the primary pathways modulated in this condition. Further correlation analysis and hierarchical clustering were employed to identify specific lipid biomarkers, with SM, PC, and PE as key potential blood lipid biomarkers for aconitine-induced LVTA events [54].

Hepatorenal toxicity is a common adverse effect associated with TCM, while neurotoxicity is a key characteristic of *Aconitum* alkaloids. In documented poisoning cases, *Aconitum* alkaloids tend to accumulate in the liver and kidneys [55]. Additionally, nervous system symptoms are primary clinical manifestations in poisoning patients, including perioral numbness, dizziness, parasympathetic excitation, and muscle weakness [56]. However, limited studies have investigated the effects of *Aconitum* alkaloids on the liver, kidneys, and nervous system using metabolomics approaches. Sui et al. [57] observed increases in serum creatinine and blood urea nitrogen, and a decrease in renal tubular filtration rate in the AKR group. In rats administered AKR orally at a dose of 0.9408 g·kg^{-1}·d^{-1}, it was observed significant liquefactive necrosis in the cerebral cortex in the brain section. According to UH-PLC-Q-TOF-MS analysis, there were 19 possible biomarkers for its neurotoxicity and nephrotoxicity, involving SM metabolism, glycerol phospholipid metabolism, Ala, aspartic acid and glutamic acid metabolism, and linoleic acid metabolism. Zhou et al. [58] characterized urine metabolic profiles in a yunaconitine poisoning model using UPLC-Q-TOF-MS. Their analysis revealed that yunaconitine-induced neurotoxicity, cardiotoxicity, and hepatotoxicity were closely associated with disruptions in key metabolic pathways, including amino acid and glucose metabolism, alongside the induction of oxidative stress. Specifically, alterations were observed in the biosynthesis and degradation of valine, leucine, and isoleucine, as well as in the metabolism of pentose, glucuronic acid, propanoate, and amino acids such as alanine, aspartate, glutamate, and tyrosine. In clinical research on AKR poisoning, Zhang et al. [56] conducted a metabonomic analysis of serum samples from poisoning patients and identified Ala, threonine, valine, isoleucine, lysine, Leu, Phe, and malic acid as potential biomarkers. An analysis using UPLC-Q-Exactive Orbitrap-MS revealed that mesaconitine hepatotoxicity is associated with L-phe, retinyl ester, L-pro, and 5-hydroxyindole acetaldehyde. Research indicates that mesaconitine may induce liver toxicity by stimulating oxidative stress, inflammation, and apoptosis [59]. Upregulation of the bile acid pathway may also be one of the metabolic pathways through which *Aconitum* alkaloids cause liver damage [48].

4.2. Metabolomics analysis for Aconitum processing detoxification

In CP 2020, traditional processing methods for *Aconitum* plants involve boiling, steaming, rinsing, or drying after continuous salt soaking, a practice known as “Paozhi” in Chinese. An analysis of alkaloid quality and quantity from raw and prepared *Aconitum* plants revealed changes in alkaloid types, quantities, and contents during processing. Some DDAs were hydrolyzed to MDAs, thereby reducing toxicity [20]. Notably, the increase in MDAs exceeded the decrease in DDAs, suggesting that MDAs’ increase is not solely attributable to DDA hydrolysis [60]. The specific mechanism of processing detoxification warrants further investigation. Moreover, the ethanol extract of ALR exhibits higher toxicity than the water extract, as evidenced by significant variations in rat metabolic pathways. The cardiotoxicity signal transduction pathways of these two extracts also differ [61]. A UPLC-Q-TOF-HDMS metabolomics approach was employed to examine the distinct effects of AR, ALR, and their processed products (based on CP 2010) on rat metabolism. The study posited that SM and glycerophospholipid (GPL) metabolism constituted their detoxifying mechanism [62]. In conclusion, processing and extraction techniques significantly influence *Aconitum* alkaloid toxicity. Further research is necessary to elucidate the specific processing detoxification mechanism and metabolic differences among products processed by various techniques.

Advanced metabolomics techniques, such as RPLC-Q-TOF/MS, are increasingly utilized in research on processing detoxification mechanisms. A study examining prepared AKR samples, boiled at 100 °C for 8 h and subsequently dried, identified 22 metabolic biomarkers distinguishing processed products from raw AKR. These processed samples exhibited lower concentrations of DDAs and higher levels of MDAs and lipid alkaloids compared to raw *Aconitum* plants [63]. A subsequent UPLC-Q-TOF-HDMS investigation aimed to compare substantial metabolites of ALR with its processed products. Findings revealed that DDAs, including aconitine, hypaconitine, and mesaconitine, decreased in HSP and BFP but increased in YFZ. Certain MDAs, such as 8-acetyl-15-hydroxyneoline, 14-acetylkarakoline, and chasmanine, showed elevated levels in YFZ but reduced concentrations in HSP and BFP. Conversely, other MDAs like benzoylmesaconine increased in HSP and BFP but decreased in YFZ [31]. This research highlights the variability in toxic substance profiles among TCM preparations processed using different methods.

The utilization of desorption electrospray ionization mass spectrometry imaging (DESI-MSI) enables the visualization of metabolic markers and facilitates research on the effects of boiling and steaming for varying durations on the toxicity of *Acon-*

itum alkaloids. In conjunction with UPLC and multivariate statistical analysis, the findings revealed a distinction between raw and processed ALR samples based on steaming times, with 4 h identified as the optimal duration for reducing toxicity while maximizing effectiveness [64]. Furthermore, different boiling times (30, 60, 120 min) had minimal impact on the therapeutic efficacy of BFP in treating adjuvant arthritis. BFP subjected to 120-min decoction demonstrated both therapeutic effects and non-toxicity in adjuvant arthritis rats [65]. This independent relationship between the toxicity and efficacy of *Aconitum* alkaloids ensures that the objective of attenuating toxicity while preserving efficacy can be achieved through processing.

Developing a more effective processing technique is crucial to reduce toxicity while preserving efficacy for safe clinical use. Current research indicates that the traditional Danba pretreatment processing solution is relatively inefficient. Soaking and washing in Danba salts are not essential to ensure the safety of *Aconitum* alkaloids in clinical applications. Metabolomics emerges as a valuable tool for monitoring the safety of TCM and developing more efficient processing techniques. UPLC-MS/MS results demonstrated that boiling or steaming for over 2 h can reduce the overall concentrations of DDAs to below 0.02%, with boiling exhibiting superior detoxification effects compared to steaming. Roasting at 105 °C proved more effective than roasting at 60 °C in degrading DDAs and increasing MDAs, while also being more time-efficient [60]. Electrospray ionization-mass spectrometry (ESI-MS) was used to monitor the dynamic boiling detoxification process of hypaconitine in real time, revealing that fatty acids (FAs) could effectively catalyze the decomposition of DDAs into MDAs. Furthermore, the co-decoction of *Aconitum* alkaloids with FA-rich substances may serve as a viable new processing method [66].

4.3. Detoxification of Aconitum in TCM compatibility and prescriptions

The combination of HSP and Fritillariae Thunbergii Bulbus (FTB) in "Eighteen Incompatible Medicaments" alters the distribution patterns of *Aconitum* alkaloids, as confirmed by a metabolomics study using UHPLC-MS/MS [67]. Additionally, *Veratrilla baillonii* Franch. (Gentianaceae) demonstrates hepatoprotective effects against severe liver injury induced by *Aconitum brachypodum* Diels [68]. Consequently, the compatibility of TCM or compounds in prescriptions plays a critical role in modulating *Aconitum* alkaloid toxicity. Metabolomics offers an effective approach to analyzing the principles of *Aconitum* alkaloid compatibility for detoxification.

According to *Jingyue's Complete Works*, ALR is characterized as potent and toxic; however, when combined with Glycyrrhizae Radix et Rhizoma (GR, "Gancao" in Chinese), it becomes milder and less harmful. Research has shown that administering glycyrrhetinic acid, the primary metabolite of GR's main constituent, to rats with mesaconitine-induced metabolic disorders can mitigate disruptions in amino acid biosynthesis and metabolism [69]. Furthermore, studies have identified Phe, Trp biosynthesis, TCA cycle, and the synthesis and degradation of ketone bodies as significant factors in GR's detoxification process [70]. Additionally, the combined detoxification effect of GR and ALR has been attributed to the regulation of various biological metabolic pathways, including glycolysis, lipid metabolism, TCA cycle, and amino acid metabolism [71,72].

UPLC-Q-TOF-HDMS analysis of urine metabolites can elucidate the phenotypic biochemical disturbances and potential toxic mechanisms of *Aconitum* alkaloids. Research has confirmed the combined detoxifying effects of GR, Zingiberis Rhizoma (ZR, "Ganjiang" in Chinese), and Paeoniae Radix Alba (PRA, "Baishao" in Chinese) on AR. These components effectively modulated toxicity biomarkers related to pentose and glucuronic acid interconversion, as well as Ala, aspartate, and glutamate metabolism, bringing them within normal ranges [73]. Differential metabolites of GR, PRA, and Ginseng Radix et Rhizoma (RS, "Renshen" in Chinese) mitigated cardiotoxicity and hepatotoxicity induced by AKR. These effects involved the conversion of pentose and glucuronic acid, Trp metabolism, amino sugar and nucleotide sugar metabolism, Tau and hypotaurine metabolism, fructose and mannose metabolism, and starch and sucrose metabolism [74]. Additionally, a metabolomics study observed a reduction in mesaconitine and an increase in active MDAs in RS-ALR decoction, likely achieved through a nucleophilic substitution reaction mechanism catalyzing DDAs hydrolysis [75].

Terminalia chebula Retz. (TCR) demonstrates the ability to reverse metabolic processes associated with cardiotoxicity induced by AKR. The detoxification mechanism of TCR may be related to several metabolic pathways, including aminoacyl-tRNA synthesis, glycine and histidine metabolism, serine and threonine metabolism, alanine, acetaldehyde, and dicarboxylic acid metabolism, as well as aspartic acid and glutamic acid metabolism [76]. Additionally, YNBY, which contains AKR, is widely utilized clinically, although the mechanism by which YNBY attenuates AKR toxicity remains elusive. Studies have shown that when AKR is co-administered with YNBY, the concentrations of 17 endogenous serum metabolites associated with GPL metabolism, tyrosine metabolism, and primary bile acid biosynthesis metabolic pathways are normalized [77]. Furthermore, research suggests that the detoxification process of YNBY primarily involves the lysine degradation metabolic pathway [78].

5. Metabolomics applications on pharmacological studies of *Aconitum* alkaloids

Researchers have dedicated their efforts to elucidating the therapeutic effects of *Aconitum* alkaloids. These effects include alleviating myocardial ischemia, providing hepatorenal protection, and offering anti-inflammatory and analgesic properties. The researchers have employed metabolomics techniques to identify sensitive biomarkers and metabolic pathways.

5.1. Cardioprotection

Aconitum alkaloids in ALR and associated prescriptions are crucial components in the treatment of cardiovascular diseases. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) results indicated that *Aconitum* alkaloids may be the primary constituents of ALR in treating cardiovascular disorders. These alkaloids demonstrated the ability to decrease glucose and potassium levels in myocardial infarction areas while increasing glutamine concentrations, thereby offering myocardial infarction protection [79]. UPLC-MS/MS analysis revealed that arachidonic acid (AA) metabolism, LA metabolism, niacin and nicotinamide metabolism, starch and sucrose metabolism, and inositol phosphate metabolism might serve as the metabolic pathways through which *Aconitum* alkaloids in ALR enhance energy metabolism and exert therapeutic effects on the cardiovascular system [80].

A serum metabolomics study utilizing UHPLC-Q-TOF-MS was conducted to validate the efficacy of *Aconitum* alkaloids in SND for treating cardiac failure [81]. SND, a traditional prescription comprising ALR, ZR, and GR, originating from *Treatise on Febrile Diseases*, exemplifies the essence of TCM compatibility of *Aconitum* plants to a considerable degree [82]. The combination of ZR–ALR significantly enhances hemodynamic parameters and mitigates cardiac tissue damage, demonstrating superior therapeutic effects compared to their individual use. This therapeutic improvement is linked to the modulation of energy metabolism pathways,

which are critical for maintaining cardiac function in chronic heart failure (CHF) rats [83]. GC/LC-MS analysis integrated with network pharmacology revealed that *Aconitum* alkaloids were not only the primary therapeutic components of SND, but other active ingredients such as gingerols, flavonoids, and saponins also synergistically enhanced the cardiomyopathy-alleviating effects of *Aconitum* alkaloids [84]. Additionally, Tan et al. [85] identified 12 active ingredients of SND effective in alleviating myocardial ischemia, including fuziline, neoline, benzoylaconine, benzoylmesaconine, benzoylhypaconine, 14-acetyltalatizamine, isotalatizidine, songrine, and talatizamine using UHPLC-Q-TOF-MS.

Furthermore, ALR water-soluble alkaloids significantly enhance hemodynamic parameters and neuroendocrine cytokine levels in acute heart failure (AHF) rat models. The combination of ALR water-soluble alkaloids with total ginsenosides resulted in marked improvements in hemodynamic indices and neuroendocrine cytokines. This study identified six distinct metabolites involved in various metabolic pathways, including lysine degradation, Arg and Pro metabolism, Pu metabolism, sphingolipid (SL) metabolism, D-Arg and D-ornithine metabolism, Phe metabolism, and aminoacyl-tRNA biosynthesis [86].

5.2. Liver and kidney protection

Aconitum alkaloids demonstrate synergistic hepatoprotective effects with GR. A UPLC-MS/MS study indicated that the synthesis of primary bile acid was a crucial pathway for CCl_4-induced hepatotoxicity in a rat model. The accumulation of bile acids can stimulate the release of various proinflammatory factors, indirectly causing hepatocyte injury [87]. ALR–GR administration reversed the toxic responses induced by CCl_4, enhancing the combination of free bile acid with glycine and Tau to reduce its accumulation in the liver. This administration also regulated Tau compensation levels, promoted the uptake of free hydrophobic bile acid, improved hepato-intestinal circulation, and facilitated liver injury repair [88].

Betaine has the potential to serve as a biomarker for the kidney-protective effects of BFP in clinical trials. LC-Q-TOF-MS analysis has confirmed that BFP can reverse certain metabolic alterations in a dose-dependent manner in kidney-yang deficiency syndrome (KYDS) rats. A network analysis revealed four primary pathways associated with BFP in the treatment of KYDS: the superpathway of methionine degradation, Pu nucleotides *de novo* biosynthesis II, tyrosine synthesis and serotonin receptor signaling [89]. Furthermore, RhoA signaling, choline metabolism, and disruptions in free radical scavenging may contribute to the dose-dependent changes in metabolites such as betaine and PC induced by BFP [90].

5.3. Anti-inflammation and analgesia

Yang et al. [91] identified LA, daidzein, and daidzin as responsible for the anti-RA properties of Gastrodiae Rhizoma ("Tianma" in Chinese)−ALR. An untargeted metabolomic study revealed that WTD could modulate AA levels in RA rat serum [92]. The primary active components in Gancao Fuzi Decoction (GFD) are *Aconitum* alkaloids, flavonoids, and saponins. Analysis of urinary metabolites from RA model rats using UHPLC-Q-TOF-MS demonstrated that these three components in GFD exerted comprehensive anti-inflammatory and analgesic effects. This involved the regulation of Tau and hypotaurine metabolism, Phe metabolism, TCA cycle, Trp metabolism, FA metabolism, vitamin B6 metabolism, Arg and Pro metabolism, and Pu metabolism [93].

6. Conclusions and future perspectives

Metabolomics enables the quantitative determination of small-molecular-weight metabolites involved in metabolic processes, facilitating the observation of comprehensive metabolic states across multiple systems *in vivo*. Over recent decades, numerous metabolomic studies have been conducted to demonstrate the therapeutic and toxic effects of drugs, evaluate the efficacy and safety of TCM, explore potential mechanisms, and identify potential biomarkers. These efforts have yielded significant progress [94, 95].

This review examines the application of metabolomics, an emerging tool in pharmacological and toxicological studies of *Aconitum* alkaloids, and summarizes recent research on their symbolic metabolites. It details the significant role of metabolomics in understanding the effects of *Aconitum* alkaloids on cardiovascular diseases, hepatorenal function, and rheumatic conditions while also considering the mechanisms of processing and compatibility detoxification. Metabolomics has emerged as a crucial method for investigating drug-induced metabolic disturbances, evaluating drug toxicity and efficacy, and identifying potential characteristic biomarkers. This approach offers a promising avenue for elucidating the pharmacological and toxicological mechanisms of TCM [96, 97].

The ongoing exploration of metabolic markers with diagnostic and therapeutic potential will enhance the clinical application of *Aconitum* alkaloids and contribute to their development prospects. However, previous metabolomics studies primarily focused on *Aconitum* crude plants and extracts, as well as drug pairs or prescriptions containing *Aconitum* alkaloids, where the alkaloids lack specificity. Their precise pharmacological mechanisms and associated metabolic pathways require extensive experimental validation. Research on *Aconitum* alkaloids in metabolomics lacks standardized protocols for sample collection, analysis, and data interpretation, reducing the comparability of different study results. Moreover, few clinical metabolomics studies on *Aconitum* alkaloids have been conducted. It is crucial to collect serum and urine samples from patients with *Aconitum* overdose to further elucidate the mechanism of *Aconitum* alkaloid poisoning. In the future, metabolomics can be employed to assess efficacy, predict side effects, and investigate the mechanism in patients using *Aconitum* alkaloids for treating cardiovascular diseases, metabolic disorders, and immune system diseases. Additionally, there are limited reports on their hepatorenal toxicity mechanism, despite it being the most common adverse reaction in TCM. While metabolomics technology identifies numerous differential compounds, further research is necessary to determine their reliability as disease markers. Currently, certain metabolites serve as dependable diagnostic markers in fields such as diabetes and cancer. However, in the realm of *Aconitum* alkaloids, only preliminary advancements have been achieved. Future progress necessitates the amalgamation of multiple omics and conducting a number of experiments to validate these compounds as reliable diagnostic markers. In addition, in order to ensure their safety and effectiveness, more efforts are still needed to develop strict quality control methods and standardized processing measures [98].

Metabolomics offers crucial insights into the metabolic alterations induced by *Aconitum* alkaloids. However, integrating this information with multi-omics data, including genomics and proteomics, is essential for a comprehensive understanding [99, 100]. Multi-omics approaches enable the identification of genetic variations that may influence responses to *Aconitum* alkaloids and detect protein expression changes induced by these compounds. This integration will elucidate genetic factors, molecular pathways, metabolic processes, and their complex interactions. Furthermore, while traditional metabolomics primarily focuses on metabolites at specific times or conditions, it is important to note that metabolic changes can be both causes and consequences of drug toxicity. Future research related to *Aconitum* alkaloids

would benefit from incorporating other analytical methods, such as spatiotemporal metabolomics analysis. This approach can reveal metabolite change patterns in different tissues, organs, or cell types, distinguish key metabolites at various time points and spatial locations, and enhance the accuracy and reliability of identifying potential pathogenic factors, thus addressing limitations of traditional metabolomics [101, 102].

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Supporting information

Supporting information for this work can be obtained by contacting the corresponding authors *via* E-mail.

Declaration of competing interest

These authors have no conflict of interest to declare.

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