REVIEW
Toxicology Advances 2022;4(4):18. https://doi.org/10.53388/20220202018

Study on hepatotoxicity of *Aconitum brachypodum* based on network pharmacology and molecular docking

Teng-Da Li[1], An-Lan Zhao[2], Rui Gong[3], Xue-Feng Li[1], Cheng Chen[3], Xin-Ju Li[1*]

[1]School of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China. [2]Graduate school, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China. [3]School of Acupuncture Moxibustion and Tuina, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China.

[*]**Corresponding to:** Xin-Ju Li, Tianjin University of Traditional Chinese Medicine, No. 10, Poyang Lake Road, Tuanbo Xincheng West District, Jinghai District, Tianjin 301617, China. E-mail: mars402498971@126.com.

Author contributions

Teng-Da Li, Xin-Ju Li designed and performed research and wrote the paper; An-Lan Zhao, Rui Gong analyzed the data; Xue-Feng Li, Cheng Chen revised the paper.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Abbreviations

PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; STAT3, signal transducer and activator of transcription 3; EGFR, epidermal growth factor receptor; MAPK8, mitogen-activated protein kinase 8; HIF-1, hypoxia-inducible factor 1; BP, biological process; CC, cellular component; MF, molecular function; TCM, traditional Chinese medicine; DILI, drug induced liver injury; PPI, protein-protein interaction; DC, degree centrality; BC, betweenness centrality; CC, closeness centrality; DISC, death-inducing signaling complex; MAPK, mitogen-activated protein kinase; LPS, lipopolysaccharide; PI3K, phosphoinositide 3-kinase; NF-κB, nuclear factor-kappa B; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; Th17, T helper 17.

Peer review information

Toxicology Advances thanks Raghu Ramanathan and other anonymous reviewers for their contribution to the peer review of this paper.

Citation

Li TD, Zhao AL, Gong R, Li XF, Chen C, Li XJ. Study on hepatotoxicity of *Aconitum brachypodum* based on network pharmacology and molecular docking. *Toxicol Adv*. 2022;4(4):18. doi: 10.53388/20220202018.

Executive editor: Hong-Xiang Li.

Received: 25 October 2022; **Accepted:** 30 October 2022;

Available online: 31 October 2022.

Abstract

Objective: To explore the potential mechanism of hepatotoxicity induced by *Aconitum brachypodum* through network toxicology. **Methods:** The active components and targets of *Aconitum brachypodum* were identified and screened by CNKI, PubChem database, Swiss Target Prediction database. Genecards, pharmGKB and DisGeNET databases were used to collect hepatotoxicity related targets. The intersection targets were obtained by matching the active component targets with the hepatotoxic targets of *Aconitum brachypodum*. Cytoscape software was used to construct the "*Aconitum brachypodum*-potential active components-potential targets-hepatotoxicity" network. The STRING database was used to construct the protein-protein interaction (PPI) network of the targets and to screen out the core targets. In addition, Gene Ontology (GO) function enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted by R software. The toxic components in *Aconitum brachypodum* were docked with the core targets. **Results:** In this study, 26 chemical components were screened via SwissADME, 297 targets for the active components of *Aconitum brachypodum* were obtained. There were 1,096 hepatotoxicity-related targets, 73 potential targets for hepatotoxicity caused by *Aconitum brachypodum*, and 15 potential active components, among which Penduline, Songoramine, Sitosterol, Daucosterol and Bullatine A were the key active components for hepatotoxicity caused by *Aconitum brachypodum*, and signal transducer and activator of transcription 3 (STAT3), epidermal growth factor receptor (EGFR), mitogen-activated protein kinase 8 (MAPK8) and tyrosine-protein kinase JAK2 (JAK2) were the potential targets for hepatotoxicity caused by *Aconitum brachypodum*. There were 1,133 GO entries ($P < 0.05$), including 1,045 entries of biological process (BP), 19 entries of cellular component (CC), and 69 entries of molecular function (MF). KEGG enrichment analysis revealed 115 pathways ($P < 0.05$), of which EGFR tyrosine kinase inhibitor resistance, hypoxia-inducible factor 1 (HIF-1) signaling pathway, PI3K-Akt signaling pathway, calcium signaling pathway, T helper 17 (Th17) cell differentiation was strongly correlated with the hepatotoxicity caused by *Aconitum brachypodum*. Molecular docking results showed that the binding activity was good. **Conclusion:** Through network toxicology analysis, it was found that the active ingredients in *Aconitum brachypodum* may act on multiple targets and signaling pathways, thereby participating in the activation of an excessive inflammatory response, oxidative stress, apoptosis and other pathways on the whole, thus resulting in hepatotoxicity.

Keywords: network pharmacology; *Aconitum brachypodum*; hepatotoxicity; apoptosis; inflammatory response; oxidative stress

Toxicology Advances 2022;4(4):18. https://doi.org/10.53388/20220202018

Background

Aconitum brachypodum is a dried tuberous root of *Aconitum brachypodum* of the Buttercup family. It is warm, bitter and pungent, and has great toxicity. It belongs to the liver and kidney meridian. It has the functions of dispelling wind and eliminating dampness, dispersing blood stasis and healing wounds, promoting blood circulation and alleviating pain [1]. The modern pharmacological experiment has proved that it has significant analgesic and anti-inflammatory effects [2]. It is one of the essential drugs for the treatment of rheumatic pain, arthritis and fall injury [3], and is used in many pharmaceutical preparations, such as *Aconitum brachypodum* tablet, *Aconitum brachypodum* instant analgesic Liniment, etc [4].

Aconitum brachypodum contains various alkaloids such as aconitine, hypoaconitine and bullatine, and non-alkaloid components such as β-sitosterol and carotenoside. The diterpenoid alkaloids contained in the chemical constituents of these drugs exert both pharmacological and toxic effects [5]. However, the therapeutic dose of *Aconitum brachypodum* is very close to the toxic dose. If the dosage is improper, severe toxic reactions may occur and even death may occur [6]. It has been proved that the related toxic components of *Aconitum brachypodum* can cause liver injury in mice [7]. At present, the established chemical composition library of *Aconitum brachypodum* has become increasingly mature, but the mechanism of hepatotoxicity is mostly based on the exploration of single toxic components. The molecular mechanism of hepatotoxicity induced by this herb has not been fully elucidated.

Network pharmacology can use network and pathway analysis and a variety of databases to establish a multi-level network model of "multi-component, multi-target and multi-pathway" to predict the potential active components and targets of traditional Chinese medicine (TCM) intervention and explore the mechanism of TCM treatment of diseases [8]. With the help of this research method, we can simultaneously study the regulatory effect of *Aconitum brachypodum* on multiple targets and signaling pathways, aiming to elucidate the molecular mechanism of hepatotoxicity and provide some reference value for the safe clinical use and scientific research of this drug.

Materials and methods

Collection the active components and targets of *Aconitum brachypodum*

In this study, the chemical constituents of *Aconitum brachypodum* were obtained by searching the key words "雪上一枝蒿" in CNKI [1, 9, 10]. The SMILES of the chemical composition was downloaded using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and then imported into the SwissADME database (http://www.swissadme.ch/) for drug prediction. Because Lipinski's rule of 5 can screen compounds with poor oral absorption according to their partitioning, molecular weight and hydrogen bonding [11], compounds that meet Lipinski's rule of 5 in the SwissADME database were used as active ingredients of *Aconitum brachypodum* in this study. SMILES of these active ingredients was imported into the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) and the species' Homo sapiens' was selected to predict the corresponding target. Finally, all obtained targets were entered into the UniProt database (https://www.uniprot.org/) for target annotation, and the Gene source was set as "Homo sapiens". Thus, the official name of the human gene of each target was obtained. The obtained genes constitute the gene pool of *Aconitum brachypodum*.

Collection of hepatotoxicity targets

Through the Genecards database (https://www.genecards.org/), the PharmGKB database (https://www.pharmgkb.org/) and the DisGeNet Database (https://www.disgenet.org/), the related gene targets of hepatotoxicity were searched with the keyword "hepatotoxicity" and "drug induced liver injury (DILI)". The targets collected from the above three databases were collated and combined to obtain the targets related to hepatotoxicity after merging and deduplication.

Intersection targets of hepatotoxicity induced by *Aconitum brachypodum*

Venny 2.1 online tool (https://bioinfogp.cnb.csic.es/tools/venny/) was used to intersect the related targets of the active components of *Aconitum brachypodum* and hepatotoxic target, and the Venn diagram was drawn. The intersection targets were obtained.

Construction of protein-protein Interaction network and screening of the potential targets for hepatotoxicity caused by *Aconitum brachypodum*

The STRING database (https://string-db.org/) aims to integrate all known and predicted associations between proteins, including both physical interactions as well as functional associations [12]. The intersection targets were input into the STRING database, and the organisms were restricted as "Homo sapiens", and the disconnected nodes were hidden. Protein-protein interaction (PPI) network information was obtained with a confidence score > 0.7 and saved as a tsv file. The tsv file is opened by Cytoscape software, and the PPI network is further constructed. The PPI network was topologically analyzed by using the plug-in CytoNCA, and the degree centrality (DC), betweenness centrality (BC) and closeness centrality (CC) were calculated to obtain the topological characteristics of each node. According to the calculated topological coefficients, the potential targets for hepatotoxicity caused by *Aconitum brachypodum* were screened.

Constructing a network of "*Aconitum brachypodum*-potential active components-potential targets-Hepatotoxicity"

The active components of *Aconitum brachypodum* and their corresponding active targets were reversely screened and correlated by using hepatotoxicity-related targets. The correlation information was input into Cytoscape software to construct "*Aconitum brachypodum*-potential active components-potential targets-hepatotoxicity" network. Topological analysis of the network was conducted using the plug-in CytoNCA. According to the value of DC, the key active components were screened.

GO function enrichment analysis and KEGG pathway enrichment analysis of potential target protein

In this study, R software was used for GO functional enrichment analysis and KEGG pathway enrichment analysis($P < 0.05$). GO functional enrichment analysis included biological process (BP), cellular component (CC) and molecular function (MF). After the enrichment analysis, the top 10 results of the *P*-value ranking of the enrichment analysis results of the three GO categories were selected to draw a histogram, and the top 20 results of the *P*-value ranking of the enrichment analysis results of KEGG were selected to draw a histogram.

Molecular docking

The key active components of *Aconitum brachypodum* causing hepatotoxicity were docked with the potential targets for hepatotoxicity caused by *Aconitum brachypodum* to predict their binding capacity. The pdb file of the core target protein structure was obtained using the RCSB PDB database (https://www.rcsb.org/), the mol2 file of the key active ingredient was obtained using Chem3D, and AutoDock Tools was used to convert the above files simultaneously to obtain the corresponding pdbqt file. Finally, the vina program was used for molecular docking, and the binding energy of each model was counted. The PyMol software was used to perform 2D and 3D processing on the docking model with higher activity.

Results

Active components and targets of *Aconitum brachypodum*

A total of 36 chemical constituents of *Aconitum brachypodum* were obtained by searching the CNKI platform with "*Aconitum*

brachypodum" as the key word. Among them, 26 chemical constituents were included in the PubChem platform. After screening with Lipinski's rule of 5, 15 active components met the criteria. The SMILES of the above active components were input into the Swiss Target Prediction database platform to predict the targets of the active ingredients. After merging and deduplication, a total of 297 targets were predicted (Table 1).

Targets related to hepatotoxicity

The targets related to hepatotoxicity were integrated from GeneCards database, PharmGKB database, and DisGeNET database, and 1,096 hepatotoxicity-related targets were obtained after eliminating duplicates.

Intersection targets of hepatotoxicity induced by *Aconitum brachypodum*

The 1,096 hepatotoxicity-related targets were matched with the 297 active component targets of Aconitum short, and 73 intersection targets were obtained (Figure 1).

PPI network and the potential targets for hepatotoxicity caused by *Aconitum brachypodum*

The intersection targets were imported into the STRING database to construct a PPI network of potential targets (Figure 2). The network has 56 nodes and 132 edges (16 nodes are hidden, with nodes representing all predicted targets and edges representing interactions between targets). The resulting network data were imported into Cytoscape software to construct the network (Figure 3A). The topological characteristics of 56 targets in the network were calculated by CytoNCA. According to the topological characteristics of network nodes, 23 targets with DC ≥ 3, BC ≥ 8.379, CC ≥ 0.094 (median) were selected to create a sub-network (Figure 3B). The sub-network has 23 nodes and 74 edges. Then, a second screening was performed for the sub-network, and 7 nodes with "DC ≥ 6, BC ≥ 5.431 and CC ≥ 0.536" were selected to form the sub-network (Figure 3C). These seven target proteins may be important target proteins for hepatotoxicity induced by *Aconitum brachypodum* (Table 2). Based on DC, BC and CC sorting, we predicted that STAT3, EGFR and MAPK8 may be the potential targets for hepatotoxicity caused by *Aconitum brachypodum*.

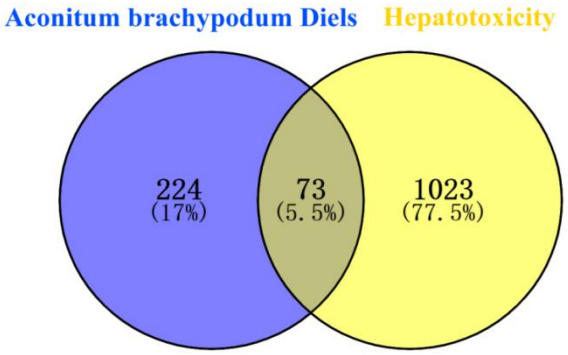

Figure 1 Venn diagram of *Aconitum brachypodum* targets and hepatotoxicity targets

Table 1 Components and target number of *Aconitum brachypodum*

No.	Components	Lipinski's rule of 5	Target number
1	penduline	Yes; 0 violation	100
2	songoramine	Yes; 0 violation	100
3	brachyaconitine A	No; 2 violations: MW > 500, NorO > 10	75
4	bullatine A	Yes; 0 violation	73
5	denudatine	Yes; 0 violation	73
6	songorine	Yes; 0 violation	69
7	sitosterol	Yes; 0 violation	44
8	12-epi-napelline	Yes; 0 violation	39
9	hypaconitine	No; 2 violations: MW > 500, NorO > 10	36
10	3-acetylaconitine	No; 2 violations: MW > 500, NorO > 10	36
11	lepenine	Yes; 0 violation	33
12	3-deoxyaconitine	No; 2 violations: MW > 500, NorO > 10	28
13	daucosterol	Yes; 0 violation	26
14	N-deethylaconitine	No; 2 violations: MW > 500, NorO > 10	22
15	aconitine	No; 2 violations: MW > 500, NorO > 10	20
16	virescenine	Yes; 0 violation	20
17	talatisamine	Yes; 0 violation	18
18	mesaconitine	No; 2 violations: MW > 500, NorO > 10	17
19	brachyaconitine D	No; 2 violations: MW > 500, NorO > 10	15
20	aconifine	No; 2 violations: MW > 500, NorO > 10	14
21	bullatine B	Yes; 0 violation	12
22	neoline	Yes; 0 violation	12
23	aconine	Yes; 0 violation	9
24	senbusine C	Yes; 0 violation	9
25	brachyaconitine B	No; 2 violations: MW > 500, NorO > 10	0
26	brachyaconitine C	No; 2 violations: MW > 500, NorO > 10	0

MW, molecular weight ; NorO, number of hydrogen bonds acceptors (N and O).

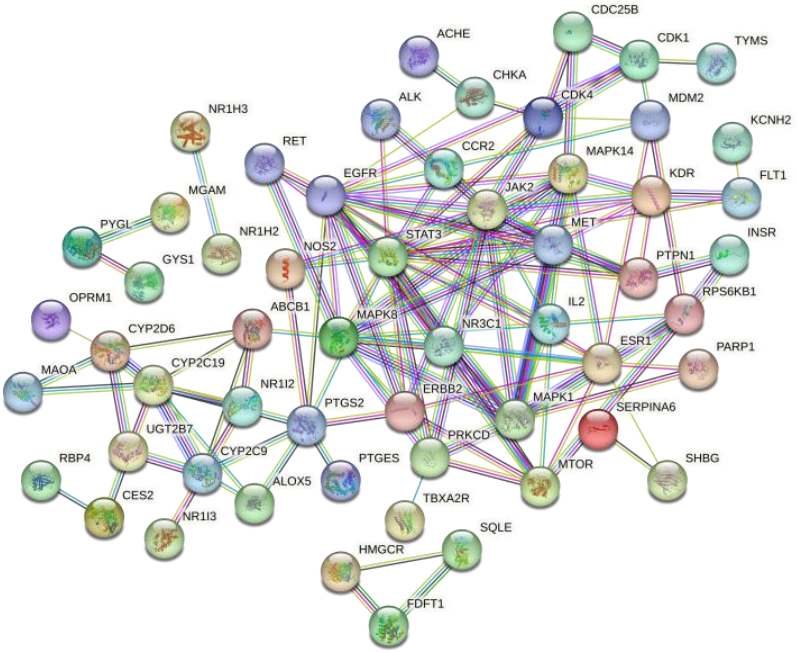

Figure 2 PPI network diagram of the intersection targets

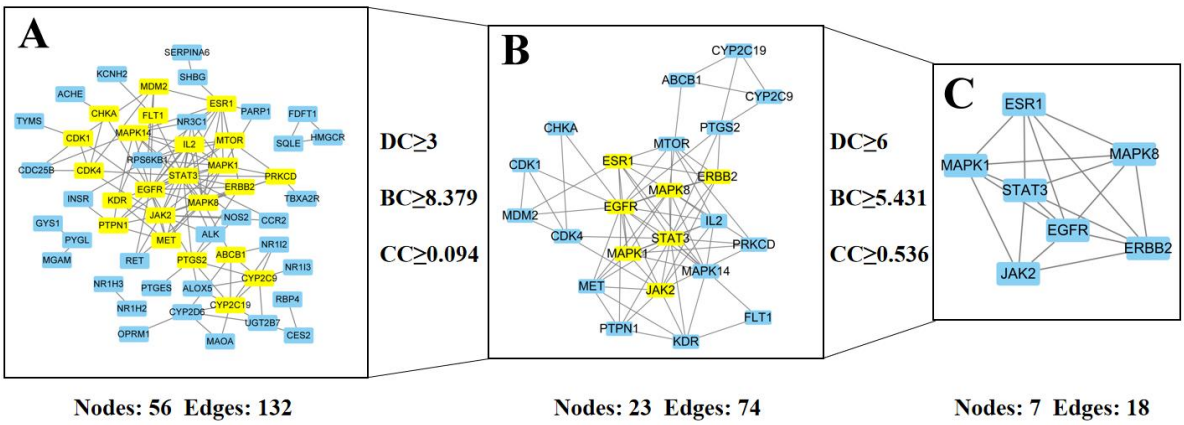

Figure 3 Screening process of potential targets of the hepatotoxicity induced by *Aconitum brachypodum*. (A) PPI original network; (B) The sub-network obtained after the first screnning; (C) The network of important targets of hepatotoxicity of Aconitum brachycephalum was obtained after the second screening.

Table 2 Information of core target proteins in hepatotoxicity of *Aconitum brachypodum*

No.	Degree	Betweenness	Closeness	Name
1	16	116.135	0.785	STAT3
2	15	119.439	0.758	EGFR
3	10	51.668	0.647	MAPK8
4	10	20.393	0.594	JAK2
5	9	6.506	0.594	MAPK1
6	8	10.971	0.578	ESR1
7	7	8.471	0.594	ERBB2

"*Aconitum brachypodum*-potential active components-potential targets-hepatotoxicity" network analysis

The "*Aconitum brachypodum*-potential active components-potential targets-hepatotoxicity" network was constructed (Figure 4). The network contains 90 nodes (15 potential active ingredients, 73 potential targets, 1 drug name, 1 disease name) and 121 edges. The DC values of the potential active ingredients in the network were calculated. The DC values of Penduline, Songoramine, Sitosterol, Daucosterol and Bullatine A were 24, 24, 22, 12 and 8, respectively, which played an important role in the network. Therefore, these five compounds were selected as key active compounds.

GO functional enrichment and KEGG pathway analysis

In this study, 73 potential therapeutic target genes were analyzed by GO based on BP, CC and MF to confirm the biological process of target proteins. The specific results were shown in Figure 5. GO analysis results showed that a total of 1,133 items were obtained with *P*-value < 0.05 as the screening criterion. BP enrichment analysis obtained 1,045 items, including response to xenobiotic stimulus, regulation of protein transport, steroid metabolic process, etc. In the CC enrichment analysis, there were 19 items involving nuclear envelope lumen, nuclear envelope, membrane raft, etc. MF enrichment analysis obtained 69 items, including nuclear receptor activity, ligand-activated transcription factor activity, protein serine/threonine/tyrosine kinase activity, etc. KEGG pathway analysis showed that these targets were enriched in 115 pathways with *P* values < 0.05. The top 20 KEGG pathways of enrichment analysis were shown in Figure 6. Among these pathways, the top 3 results ranked by the number of enriched targets were PI3K-Akt signaling pathway, calcium signaling pathway, Chemical carcinogenesis - receptor activation.

Molecular docking

According to the chemical experiment, aconitine, hypaconitine and bullatine A and other active bases contained in *Aconitum brachypodum* are both active components and highly toxic components. They have the value of molecular docking to judge whether they have good binding activity with hepatotoxic targets [9]. Therefore, in this study, the AutoDock Vina program was used to dock the important chemical components of aconitine, hypaconitine and bullatine A with all core targets. The lower the binding energy, the stronger the binding force between the target and the chemical components. It is generally believed that binding energy less than –5.0 kJ/mol represents good binding activity, while less than –7.0 kJ/mol represents strong binding activity [13]. In this study, 21 docking results between chemical components and core targets were all $\leq$ –5.0 kJ/mol, and 15 molecules had docking binding energies $\leq$ –7.0 kJ/mol (Table 3). The 6 results with optimal molecular docking binding energy were visualized (Figure 7).

Discussion

Related studies have found that the chemical constituents of *Aconitum brachypodum* were mainly diterpenoid alkaloids, including C19-diterpenoid alkaloids and C20-diterpenoid alkaloids, such as aconine, bullatine B, bullatine A and lepenine, and non-alkaloid compounds, such as daucosterol, etc. Because the efficacy of the treatment of rheumatic pain and traumatic injury is definite, its safety should be paid attention to [10]. Therefore, in this study, the mechanism of hepatotoxicity of *Aconitum brachypodum* was investigated by network toxicology, and after data mining and screening, 15 active components of *Aconitum brachypodum* and 73 potential targets of hepatotoxicity of *Aconitum brachypodum* were obtained.

The PPI network topology analysis predicted that STAT3, EGFR, MAPK8 and JAK2 are the potential targets for hepatotoxicity caused by *Aconitum brachypodum*. Signal transducer and activator of transcription 3 (STAT3), as a member of STAT3 protein family, plays a crucial role in cell differentiation, proliferation, inflammation, apoptosis and other processes [14]. One study showed that increasing the expression of STAT3, which led to Bcl-xL up-regulation. The longer form Bcl-x (Bcl-xL) becomes a repressor of apoptosis. Over-expression of STAT3 can be inhibited, which reduces the apoptosis, and play a role in antagonizing liver injury [15]. Epidermal growth factor receptor (EGFR) plays an important role in hepatocyte proliferation and liver regeneration. EGFR is involved in the impairment of mitochondrial oxidative phosphorylation and respiratory capacity, leading to mitochondrial oxidative damage, and ultimately the release of cell death mediators (including apoptosis inducers) from the mitochondrial intermembrane space, leading to nuclear DNA damage and hepatocyte death. Various toxins in these hepatocytes can activate EGFR through ligand-independent oxidative stress. This activated EGFR is associated with the cell death receptor FAS, resulting in its phosphorylation, oligomerization, and stabilization of the death-inducing signaling complex (DISC), leading to apoptotic cell death [16]. It has been reported that mitogen-activated protein kinase (MAPK) signaling pathway regulates various cellular processes such as apoptosis, mitochondrial dysfunction and endoplasmic reticulum stress during chemical toxicity. One study predicted that up-regulation of MAPK8 may be a potential mechanism for inducing hepatotoxicity [17]. At the same time, the up-regulation of tyrosine-protein kinase JAK2 (JAK2) can lead to hepatotoxicity. At present, natural flavonoids used to ofreverse the changes of JAK2, Bcl-2 and SATA3 have been widely used in the treatment of hepatotoxicity [18].

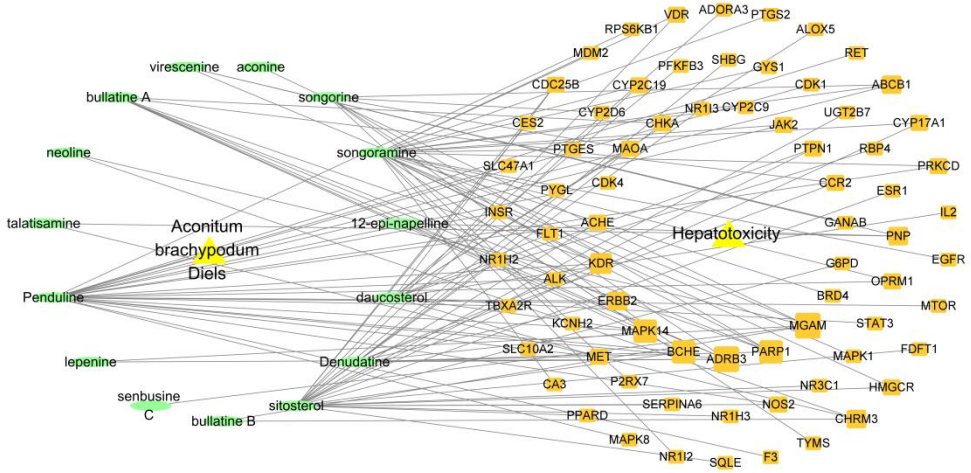

Figure 4 *Aconitum brachypodum*-potential active components-potential targets-hepatotoxicity network

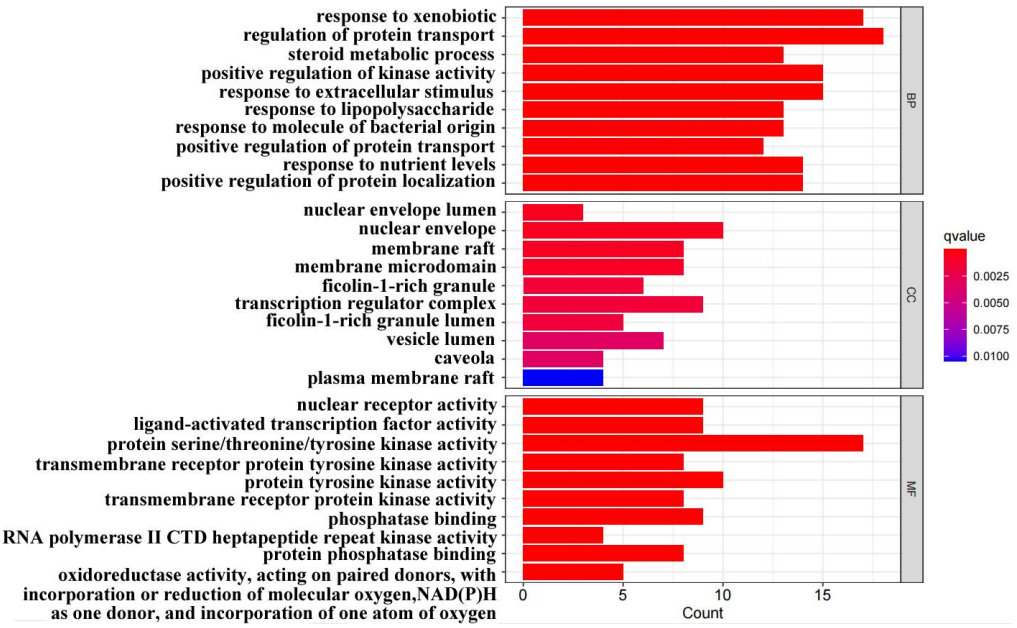

Figure 5 GO functional enrichment analysis

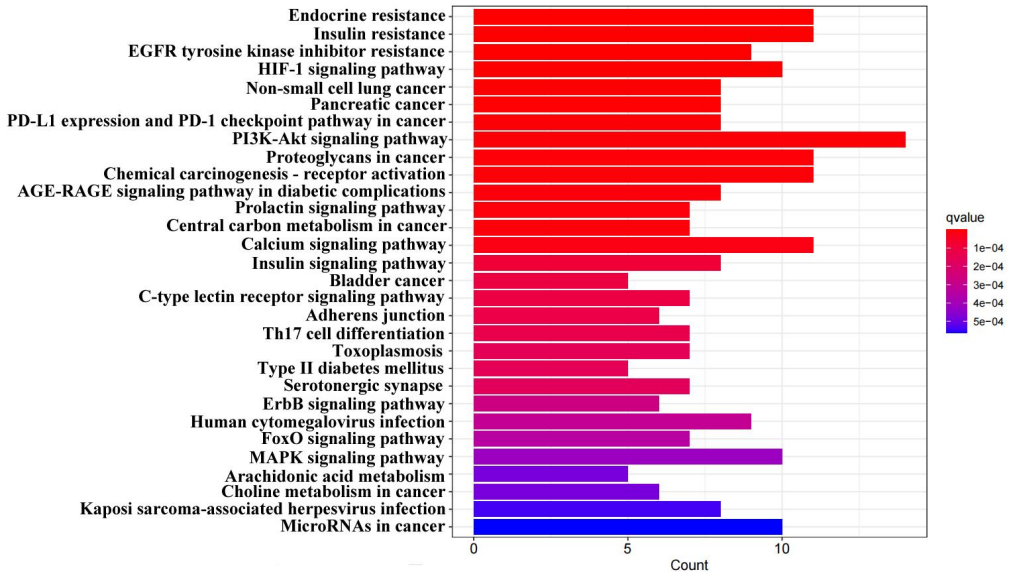

Figure 6 KEGG pathway analysis

Table 3 Molecular docking results

The core target	Lowest binding energy (kJ/mol)		
	Aconitine	Hypaconitine	Bullatine A
EGFR	–6.9	–6.8	–7.5
ERBB2	–6.7	–7.1	–7.4
ESR1	–6.9	–7.2	–7.5
JAK2	–7.2	–7.7	–7.1
MAPK1	–7.0	–7.1	–7.5
MAPK8	–7.7	–8.0	–8.3
STAT3	–6.7	–6.7	–7.1

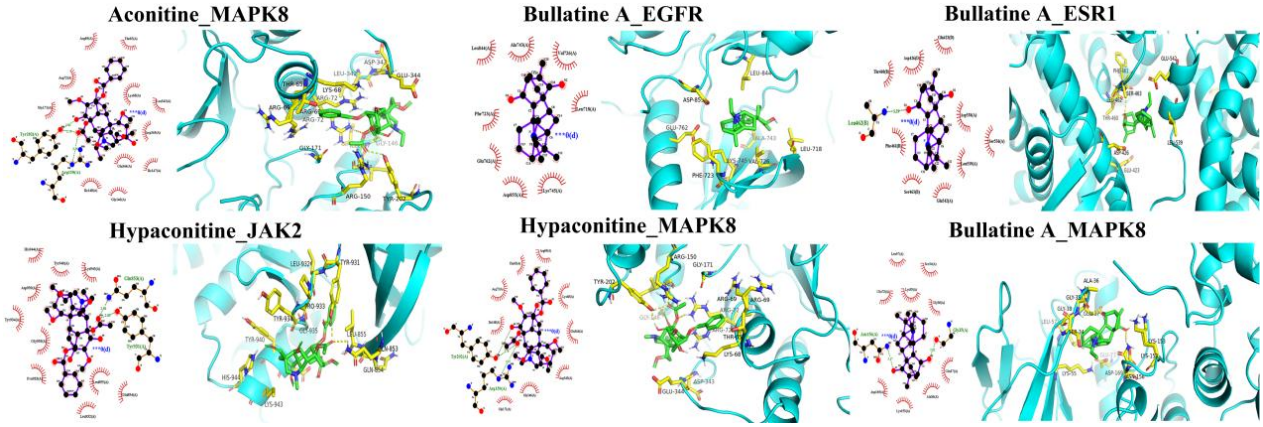

Figure 7 Molecular docking picture

GO function enrichment analysis revealed that the pathways primarily involved in toxicity-related reactions include response to lipopolysaccharide, protein serine/threonine/tyrosine kinase activity, and protein tyrosine kinase activity, etc. Lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, is an important inducer of inflammation in the body. After LPS enters the blood circulation, it can activate various inflammatory cells and induce them to release inflammatory mediators, causing inflammatory response. This was thought to be one of the causes of liver dysfunction [19, 20].

The results of the KEGG pathway analysis show that it mainly involves EGFR tyrosine kinase inhibitor resistance, HIF-1 signaling pathway, PI3K-Akt signaling pathway, calcium signaling pathway, Th17 cell differentiation, etc. Study demonstrated that T cell-specific deletion of HIF-1α increases neutrophil infiltration and abnormal γδt cell entry into the liver, which ultimately favors acute liver inflammation. The result suggested that down-regulation of HIF-1α may aggravate liver injury [21]. In the PI3K-Akt signaling pathway, activated RAC serine/threonine-protein kinase (AKT) is a downstream effector of phosphoinositide 3-kinase (PI3K), which inhibits inflammation and apoptosis by regulating multiple target proteins such as nuclear factor-kappa B (NF-κB) and Bcl-2 family [22]. In addition, increased expression of levels of PI3K and phosphorylated protein kinase B (PBK/Akt) in liver inhibited pro-apoptotic signaling events [23]. Mitochondrial calcium levels play a crucial role in maintaining mitochondrial function. Mitochondrial dysfunction can lead to liver fibrosis by increasing mitochondrial reactive oxygen species (ROS) production [24]. Th17 cells are a subtype of $CD4^+$ T helper cells that mainly produce interleukin 17 (IL-17) and interleukin-22 (IL-22). Studies have found that Th17/IL-17 axis is involved not only in the activation of astrocytes, increased expression of profibrotic factors as transforming growth factor-β (TGF-β), but also in the promotion of myofibroblast or epithelial-mesenchymal cell transformation and stimulation of the synthesis of collagen. In addition, the activated IL-17 signaling pathway can cause apoptosis and autophagy induced by NaF in liver cells [25]. Thus, Th17 cells and IL-17 play an important role in the mechanism of hepatotoxicity.

Based on network toxicology and molecular docking studies, it was found that the active components of *Aconitum brachypodum* may cause hepatotoxicity through various pathways such as apoptosis, inflammatory response and oxidative stress. The results of this study provide a certain reference for the molecular mechanism of hepatotoxicity induced by *Aconitum brachypodum*. However, since the selected compounds are only related to the hepatotoxicity targets, it is not clear whether they have therapeutic or toxic effects, which still needs to be further verified by subsequent experiments.

References

1. Han DT. Survey on chemical constituents and pharmacological effects of *Aconitum Brachypodum* Diel. *J Med Sci Yanbian Univ* 2007;(3):223–224. Available at: http://dx.doi.org/10.16068/j.1000-1824.2007.03.009
2. Wei WZ, Wu YM, Tang HY, et al. The Analgesic Effect Study on Therapeutic Window of *Aconitum brachypodum* and its Preparation. *J Guiyang Coll Tradit Chin Med* 2016;38(3):23–28. Available at: http://dx.doi.org/10.16588/j.cnki.issn.1002-1108.2016.03.006
3. Luo CY, Chen XL, Yang JH, et al. Study on the mechanism of *Aconitum brachypodum* alkaloids on arthritis in rats. *Guizhou Sci* 2020;38(1):51–53. Available at: http://dx.doi.org/10.3969/j.issn.1003-6563.2020.01.010
4. Xie W, Zhou K, Liu Y, et al. Advances and strategy in studies on toxicity of *Aconitum brachypodum*. *Chin J Ethnomed Ethnopharmacy* 2018;27(23):65–68. Available at: https://www.nstl.gov.cn/paper_detail.html?id=990889dc7bbf75d4491fb415f0ef4617
5. Cao HY, He YT, Niu YF, et al. Simultaneous Determination of Six Aconitum Alkaloids in *Aconitum brachypodum* by HPLC. *Guangzhou Chem Ind* 2021;49(8):93–96. Available at: http://dx.doi.org/10.3969/j.issn.1001-9677.2021.08.031
6. Huang XJ, Zhou H, Jiang Y, et al. Investigation of the Toxicity of *Aconitum brachypodum* in vitro and in vivo. *J South-Central Univ Nationalities (Nat Sci Ed)* 2013;32(4):50–68. Available at: https://www.nstl.gov.cn/paper_detail.html?id=6af407e3206d4e1de8622ec8ac46b151
7. Li J, Liu G, Ihsan A, et al. Effects of Veratrilla baillonii Extract on Hepatic Gene Expression Profiles in Response to *Aconitum brachypodum*-Induced Liver Toxicity in Mice. *Front Pharmacol* 2019;10:568. Available at: http://dx.doi.org/10.3389/fphar.2019.00568
8. Li X, Yang H, Xiao J, et al. Network pharmacology based investigation into the bioactive compounds and molecular mechanisms of Schisandrae Chinensis Fructus against drug-induced liver injury. *Bioorg Chem* 2020;96:103553. Available at: http://dx.doi.org/10.1016/j.bioorg.2019.103553
9. Zou DJ, Huang XJ, Gao RX, et al. HPLC Determination of Three Alkaloids in *Aconitum Brachypodum*. *Chin J Exp Tradit Med Formul* 2014;20(8):41–43. Available at: http://en.cnki.com.cn/Article_en/CJFDTOTAL-ZSFX201408016.htm
10. Yang LG, Xia WJ, Tang MY, et al. Reseach Progress on Chemical Constituents and Pharmacological Activities of *Aconitum Brachypodum*. *J Yunnan Univ Tradit Chin Med* 2016;39(6):95–102. Available at: http://dx.doi.org/10.19288/j.cnki.issn.1000-2723.2016.06.023
11. Nendza M, Müller M. Screening for low aquatic bioaccumulation. 1. Lipinski's 'Rule of 5' and molecular size. *SAR and QSAR in Environ Res* 2010;21:495–512. Available at: http://dx.doi.org/10.1080/1062936X.2010.502295

12. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 2020;49:D605–D612. Available at: http://dx.doi.org/10.1093/nar/gkaa1074
13. Hsin K-Y, Ghosh S, Kitano H. Combining Machine Learning Systems and Multiple Docking Simulation Packages to Improve Docking Prediction Reliability for Network Pharmacology. *PLoS ONE* 2013;8:e83922. Available at: http://dx.doi.org/10.1371/journal.pone.0083922
14. Fouad A, Hafez H, Hamouda A. Hydrogen sulfide modulates IL-6/STAT3 pathway and inhibits oxidative stress, inflammation, and apoptosis in rat model of methotrexate hepatotoxicity. *Hum Exp Toxicol* 2019;39:77–85. Available at: http://dx.doi.org/10.1177/0960327119877437
15. Hafez MM, Al-Harbi NO, Al-Hoshani AR, et al. Hepato-protective effect of rutin via IL-6/STAT3 pathway in CCl4-induced hepatotoxicity in rats. *Biol Res* 2015;48(1):30. Available at: http://dx.doi.org/10.1186/s40659-015-0022-y
16. Bhushan B, Michalopoulos GK. Role of epidermal growth factor receptor in liver injury and lipid metabolism: Emerging new roles for an old receptor. *Chem Biol Interact* 2020;324:109090. Available at: http://dx.doi.org/10.1016/j.cbi.2020.109090
17. Öztaş E, Kara M, Boran T, et al. Cellular Stress Pathways Are Linked to Acetamiprid-Induced Apoptosis in SH-SY5Y Neural Cells. *Biology* 2021;10:820. Available at: http://dx.doi.org/10.3390/biology10090820
18. Ghosh A, Sarmah P, Patel H, et al. Nonlinear molecular dynamics of quercetin in Gynocardia odorata and Diospyros malabarica fruits: Its mechanistic role in hepatoprotection. *PLoS ONE* 2022;17:e0263917. Available at: http://dx.doi.org/10.1371/journal.pone.0263917
19. Zhang Y, Cen J, Jia Z, et al. Hepatotoxicity Induced by Isoniazid-Lipopolysaccharide through Endoplasmic Reticulum Stress, Autophagy, and Apoptosis Pathways in Zebrafish. *Antimicrob Agents Chemother* 2019;63(5):e01639-18. Available at: http://dx.doi.org/10.1128/AAC.01639-18
20. Beheshti F, Hosseini M, Taheri Sarvtin M, et al. Protective effect of aminoguanidine against lipopolysaccharide-induced hepatotoxicity and liver dysfunction in rat. *Drug Chem Toxicol* 2019;44:215–221. Available at: http://dx.doi.org/10.1080/01480545.2018.1561712
21. Corrado C, Fontana S. Hypoxia and HIF Signaling: One Axis with Divergent Effects. *Int J Mol Sci* 2020;21:5611. Available at: http://dx.doi.org/10.3390/ijms21165611
22. Zhou YD, Hou JG, Liu W, et al. 20(R)-ginsenoside Rg3, a rare saponin from red ginseng, ameliorates acetaminophen-induced hepatotoxicity by suppressing PI3K/AKT pathway-mediated inflammation and apoptosis. *Int Immunopharmacol* 2018;59:21–30. Available at: http://dx.doi.org/10.1016/j.intimp.2018.03.030
23. Liu CM, Zheng GH, Ming QL, et al. Sesamin Protects Mouse Liver against Nickel-Induced Oxidative DNA Damage and Apoptosis by the PI3K-Akt Pathway. *J Agric Food Chem* 2013;61:1146–1154. Available at: http://dx.doi.org/10.1021/jf304562b
24. Dong Z, Yang X, Qiu T, et al. Exosomal miR-181a-2-3p derived from citreoviridin-treated hepatocytes activates hepatic stellate cells trough inducing mitochondrial calcium overload. *Chem Biol Interact* 2022;358:109899. Available at: http://dx.doi.org/10.1016/j.cbi.2022.109899
25. Chen Y, Wang YF, Song SS, et al. Potential shared therapeutic and hepatotoxic mechanisms of Tripterygium wilfordii polyglycosides treating three kinds of autoimmune skin diseases by regulating IL-17 signaling pathway and Th17 cell differentiation. *J Ethnopharmacol* 2022;296:115496. Available at: http://dx.doi.org/10.1016/j.jep.2022.115496