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Network toxicology combined with molecular docking technology to explore the molecular mechanism of amatoxin causing liver injury

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As one of the most toxic molecules in the fungal kingdom, amatoxin exhibit exceptional thermal stability and acid resistance. Once ingested, these compounds are rapidly absorbed and transported unimpeded to vital organs. They disrupt cellular metabolism by inhibiting nucleic acid and protein synthesis in target organs, ultimately causing hepatic and renal necrosis. Without prompt intervention, this molecular sabotage can progress to multiorgan failure and death. Early diagnosis combined with aggressive therapeutic measures is crucial for mitigating acute hepatic damage and significantly improving survival outcomes. This study aims to elucidate the molecular mechanisms underlying amatoxin-induced hepatic injury and establish a theoretical framework for targeted therapeutic interventions. Computational toxicology approaches utilizing ProTox-3.0 and ADMETlab 2.0 platforms were employed to characterize amatoxin's toxicological profile. Target prediction was performed through STITCH and SwissTargetPrediction databases, while liver injury-associated targets were identified from GeneCards, OMIM, and TTD repositories. The intersectional targets underwent systematic bioinformatics analysis, including protein-protein interaction (PPI) network construction, Gene Ontology (GO) annotation, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. Molecular docking simulations were subsequently conducted to characterize three-dimensional binding conformations between amatoxin and core target proteins. Computational screening identified 11 potential amatoxin targets using STITCH and SwissTargetPrediction databases. Parallel interrogation of GeneCards, OMIM, and TTD repositories yielded 1,730 liver injury-related genes. Venn diagram analysis pinpointed SP1 and CNR1 as consensus molecular targets at the amatoxin-hepatic injury interface. PPI network topology revealed critical nodal connections, while functional enrichment analyses delineated key biological processes and signaling pathways associated with these targets. Molecular docking simulations demonstrated high-affinity binding between amatoxin and both SP1 and CNR1, suggesting direct mechanistic interactions. Amatoxin likely exerts hepatotoxic effects through direct binding to the core molecular targets SP1 and CNR1, thereby perturbing downstream transcriptional regulation and disrupting critical signaling cascades, ultimately culminating in hepatic necrosis.

Keywords Network toxicology, Molecular Docking, Amatoxin, Liver injury, SP1, CNR1

Amanitin poisoning represents one of the most lethal forms of mushroom intoxication, predominantly caused by Amanita species[1]. These bicyclic octapeptides exhibit exceptional chemical stability, with molecular weights ranging from 973 to 990 Da. Structural variability arises from position-specific substitutions, generating nine characterized variants including α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, amanin, amaninamide, amanullin, amanullinic acid, and proamanullin. Notably, α-amanitin and β-amanitin demonstrate the highest toxic potency[2,3]. The liver serves as the principal target organ, manifesting histopathological hallmarks of diffuse hepatic necrosis characterized by architectural distortion (loss of hepatocyte cord organization, obliterated lobular boundaries, and portal vein dilatation) accompanied by hemorrhagic infiltration (extravasated erythrocytes) and mixed inflammatory cell exudates. Human exposure typically occurs through accidental ingestion of hepatotoxic mushrooms from genera Amanita, Lepiota, and Galerina. The clinical manifestations

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in patients are categorized into four stages: the incubation period, the acute gastroenteritis period, the pseudo-recovery period, and the fulminant hepatic failure period. These stages primarily involve symptoms related to the digestive tract system, liver function impairment, and liver failure, potentially culminating in multi-organ failure and death. Additionally, over 90% of mushroom-related fatalities are attributed to amatoxin[4]. The lethal dose of amatoxin is extremely low, with adults potentially dying from as little as 0.1 mg/kg of body weight, and a single mushroom ingestion could reach this dose. The fatality rate of amanita poisoning ranges from 10 to 20%, accounting for 90% of the total deaths from mushroom poisoning[5,6]. The mechanism of amatoxin is complex, primarily involving the inhibition of RNA polymerase II (RNAP II) activity, thereby disrupting protein synthesis, inducing cell apoptosis through oxidative stress and autophagy pathways[7]and causing mitochondrial dysfunction[8]. Early diagnosis of amatoxin poisoning relies on medical record and clinical manifestations, levels of amatoxin in urine (ATOu), and biochemical tests[9]. Current treatment strategies primarily involve preventing toxin absorption, eliminating absorbed toxins, potential antidote therapy, and liver transplantation. However, the high mortality rate and poor prognosis associated with amatoxin poisoning necessitate continued in-depth exploration and research to discover specific treatments, such as targeted therapy against specific biomarkers, especially in the absence of liver transplantation[6,10–12].

Contemporary toxicological research has witnessed the emergence of network toxicology as a pivotal methodology, demonstrating robust applications in Traditional Chinese Medicine (TCM) safety evaluation[13]and environmental toxicant assessment[14]. It can elucidate the mode of action of compounds and identify biomarkers of toxicological mechanisms[15]highlighting its systematic analysis of complex toxicological mechanisms. Complementing these advances, molecular docking simulations provide atomic-resolution characterization of ligand-target interactions, particularly effective for validating amatoxin's binding modalities with hepatic macromolecular targets. Our investigation employs this dual approach - integrating network toxicology with computational structural biology - to systematically unravel the molecular pathogenesis underlying amatoxin-induced hepatotoxicity. The methodological architecture, encompassing target prediction, pathway mapping, and binding affinity validation, is schematically detailed in Fig. 1.

Methods

Acquisition of amatoxin molecular structure

PubChem (https://pubchem.ncbi.nlm.nih.gov/), a publicly accessible chemical repository hosted by the National Institutes of Health (NIH), integrates three core modules: Substance, Compound, and BioAssay. This platform provides comprehensive coverage of chemical entities spanning small organic molecules, nucleic acid derivatives, carbohydrate polymers, lipid species, bioactive peptides, and engineered macromolecular conjugates[16,17]. For this investigation, the canonical amatoxin entry was retrieved from PubChem, with its two-dimensional (2D) structural coordinates and Simplified Molecular Input Line Entry System (SMILES) notation exported for downstream computational modeling.

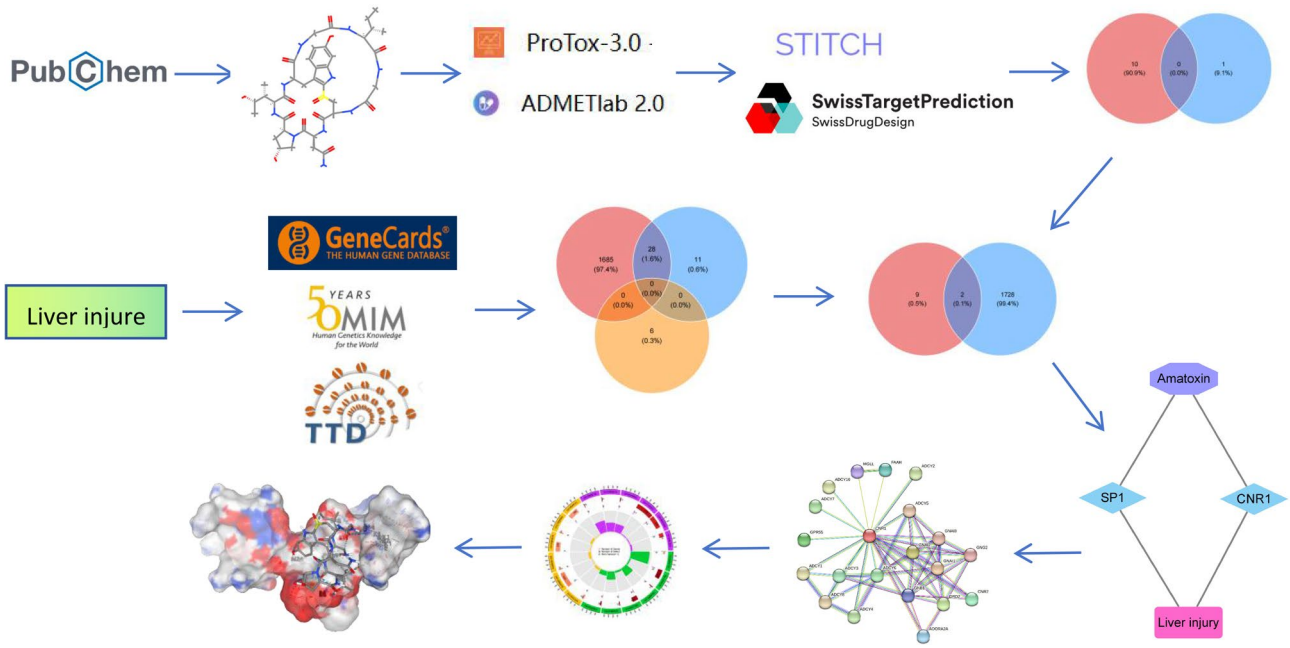

Fig. 1. Research flowchart. The molecular structure of amatoxin was retrieved using the PubChem database, and the toxicity of amatoxin was predicted by ProTox-3.0 and ADMETlab 2.0. STITCH and SwissTargetPrediction mined the targets of amatoxin. GeneCards, OMIM, and TTD mined targets related to liver injury. Cytoscape constructs a regulatory network of hepatic injury caused by amatoxin. PPI, GO, and KEGG were used for relevant analysis of targets. Finally, molecular docking technology was used to demonstrate the binding conformation between amatoxin and the core targets.

Toxicity prediction of amatoxin

ProTox (https://tox.charite.de/protox3/), a machine learning-driven predictive algorithm leveraging molecular similarity, enables systematic toxicity profiling across 61 endpoints including: acute toxicity, organ-specific toxicity, clinical toxicity biomarkers, molecular initiating events (MIEs), and Tox21-compliant adverse outcome pathways[18]. The ADMETlab platform (http://admetmesh.scbdd.com/) can be used to evaluate the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of compounds, as well as basic physicochemical endpoints[19]. SMILES notation of amanitin was computationally interrogated through both platforms to predict its toxicodynamic patterns and prioritize target organ susceptibility.

Target prediction of amatoxin

STITCH (http://stitch.embl.de), a comprehensive database featuring genomic data encompassing single nucleotide polymorphism (SNP), copy number variation(CNV), structural variant (SV), and gene expression profiles across diverse biological populations including homo sapiens, mice, rats, etc., serves as a rich resource for researchers aiming to explore and visualize biomolecular interactions[20]. SwissTargetPrediction (http://www.swisstargetprediction.ch) is an online platform designed to predict compound targets based on 2D and 3D structural similarities[21]. By inputting the SMILES structure of amatoxin into the STITCH and SwissTargetPrediction databases, setting homo sapiens as the target organism, and then downloading the resulting targets and finally the duplicate targets in the two databases were removed and combined.

Screening of targets related to liver injury

GeneCards (https://www.genecards.org/) is a comprehensive database of the human genome, transcriptome, and proteome, as well as information on the function of all known and presumed human genes[22]. OMIM (https://omim.org/) is a comprehensive and authoritative database of phenotypic and genotypic relationships in humans, including information on all known Mendelian diseases and more than 16,000 genes[23]. TTD (https://db.idrblab.net/ttd/) stands as a pivotal data plat form in the realm of drug target discovery and novel drug development. Users can search using disease, drugs, or targets, which are interconnected, facilitating the identification of therapeutic drugs and their corresponding targets relevant to specific diseases[24]. To systematically identify liver injury-associated genes, the following cross-database strategy was implemented: (1) In the GeneCards database, a keyword search for "liver injury" was performed, followed by applying a relevance score filter (> 10) to extract and download high-confidence candidate genes; (2) Within the OMIM database, the "Gene Map" module was accessed, where the keyword "liver injury" was queried, and the corresponding gene list was retrieved via the download function; (3) For the Therapeutic Target Database (TTD), a targeted search using the keyword "liver injury" was conducted to identify and download pharmacologically relevant target genes. This tripartite approach ensures comprehensive coverage of both mechanistic and therapeutic gene candidates associated with liver injury. After the duplication genes in the three databases were removed, all target genes were unionized.

Selecting targets of liver injury caused by amatoxin

Bioinformatic analysis using the ggvenn package in R 4.4.2 systematically identified intersectional targets between amatoxin's molecular targets and liver injury-associated genes. These consensus targets were computationally validated as core molecular drivers of amatoxin-induced hepatotoxicity through subsequent network pharmacology investigations.

Regulatory network of amatoxin

Cytoscape (v3.8.0), an open-source bioinformatics platform, enables systematic interrogation of biomolecular networks through advanced topological analysis, functional annotation prediction, and pathway architecture reconstruction[25]. In this study, we employed its network visualization capabilities to construct a tripartite interaction network elucidating the mechanistic relationships between amatoxin exposure, intersection targets, and liver injury.

PPI analysis

The STRING database (https://string-db.org/) provides comprehensive protein-protein interaction (PPI) data encompassing experimentally validated and computationally predicted associations across multiple species, with integrated confidence metrics (0–1 scale) quantifying interaction reliability through evidence-based scoring[26]. Our analytical pipeline imported the consensus hepatic injury targets into STRING, applying a stringent minimum confidence threshold of 0.9 to prioritize the 20 most topologically significant hub genes. Subsequent network topology analysis generated a PPI network map using the Fruchterman-Reingold layout algorithm, enabling visualization of molecular interplay between core hepatotoxic targets.

Enrichment analysis

Enrichment analysis is a method used to analyze high-throughput experimental data, often used to understand the degree of enrichment of gene collections or biological entities' functions, pathways, or specific biological processes under given experimental conditions[27]. GO analysis defines the categories used to describe gene functions and the relationships between these concepts, and it classifies functions according to three aspects: MF (Molecular Function), CC (Cellular Component), BP (Biological Process). KEGG analysis is a collection of molecular interaction and response network pathway diagrams that cover a wide range of biochemical processes in seven categories: Metabolism, Genetic information processing, Environmental information processing, Cellular processes, Organismal systems, Human diseases and Drug development. R language was used for GO enrichment analysis, and Xiantao Academic (https://www.xiantaozi.com/) was used for GO-KEGG joint analysis.

Molecular docking

The Protein Data Bank (PDB) (https://www.rcsb.org/) is currently the most important database for collecting the 2.5-dimensional (three-dimensional data in the form of two-dimensional representation) structures of biological macromolecules (proteins, nucleic acids, and sugars)[28]. DOCK is a classical molecular docking procedure for identifying potential structural conformations and interactions between molecular and targets[29]. The molecular structure of the target protein was obtained using the PDB website, and the SMILES structure formula of amatoxin was obtained by Pubchem. Molecular docking was performed using the online website CB-Dock2 (https://cadd.labshare.cn/cb-dock2/php/index.php) to explore the form of spatial binding between amatoxin and the core targets. The binding energy thresholds are generally defined as follows: (1) Values ≤ -4.25 kcal/mol indicate weak binding activity, (2) ≤ -5.0 kcal/mol suggest moderate binding affinity, (3) ≤ -7.0 kcal/mol demonstrate strong molecular interactions, based on empirical criteria for ligand-receptor docking[30].

Results

Molecular structure of amatoxin

Enter "amatoxin" on the Pubchem website to get the two-dimensional structure of amatoxin as well as the SMILES structural formula: CC[C@H](C)[C@H]1 C(=O)NCC(=O)N[C@H]2 C[S@@](=O)C3=C(C[C@@H](C(=O)NCC(=O)N1)NC(=O)[C@@H](NC(=O)[C@@H]4 C[C@H](CN4C(=O)[C@@H](NC2=O)CC(=O)N)O)[C@@H](C)[C@H](C)O)C5=C(N3)C=C(C=C5)O. The physicochemical properties of Amatoxin are shown in Table 1.

Toxicity prediction of amatoxin

ProTox toxicity analysis showed that the predicted LD50 of amatoxin was 73 mg/kg, and the predicted toxicity class was 3, representing high toxicity, with an average similarity of 49.45% and a prediction accuracy of 54.26% (Fig. 2A-B). ADMETlab toxicity analysis showed that the main damages of amatoxin were Human Hepatotoxicity (H-HT), Drug Induced Liver Injury (DILI), Maximum Recommended Daily Dose (FDAMDD), and Respiratory Toxicity (Fig. 2C).

Target prediction of amatoxin

A total of 10 amatoxin targets (POLR2A, TCEA2, TCEA3, POLR2G, TCEA1, GTF2B, POLR2J, PAFAH1B1, POLR2D, and SP1) were identified from the STITCH database. An additional target, CNR1, was predicted through the SwissTargetPrediction platform. By integrating results from both databases, a consolidated set of 11 amatoxin targets was established (Fig. 3A).

Screening of targets related to liver injury

Using "liver injury" as the search term, 1713 related genes (e.g., PIK3CA, STAT3, ABCB11) were retrieved from the GeneCards database; 39 genes were identified in OMIM, and 6 genes were extracted from the TTD database. After removing duplicates, the integrated results from these three databases yielded a non-redundant set of 1730 liver injury-associated genes (Fig. 3B).

Selecting targets of liver injury caused by amatoxin

The Venn diagram revealed two overlapping targets between amatoxin and liver injury-associated genes: SP1 and CNR1. These shared targets were designated as the core mediators of amatoxin-induced liver injury pathogenesis (Fig. 4A).

Regulatory network of amatoxin

The regulatory network delineated the functional connections among amatoxin, core targets (SP1 and CNR1), and liver injury pathogenesis. Furthermore, it elucidated the mechanistic pathway through which amatoxin modulates SP1 and CNR1 activity to mediate hepatic damage (Fig. 4B).

Name	Amatoxin
Molweight	902.97
Number of hydrogen bond acceptors	22
Number of hydrogen bond donors	12
Number of atoms	63
Number of bonds	67
Number of rotable bonds	6
Molecular refractivity	251.42
Topological polar surface area	379.86
Octanol/water partition coefficient (logP)	-1.09

Table 1. Physicochemical properties of Amatoxin. Properties of Amatoxin include Molweight, Number of hydrogen bond acceptors, Number of hydrogen bond donors, Number of atoms, Number of bonds, etc.

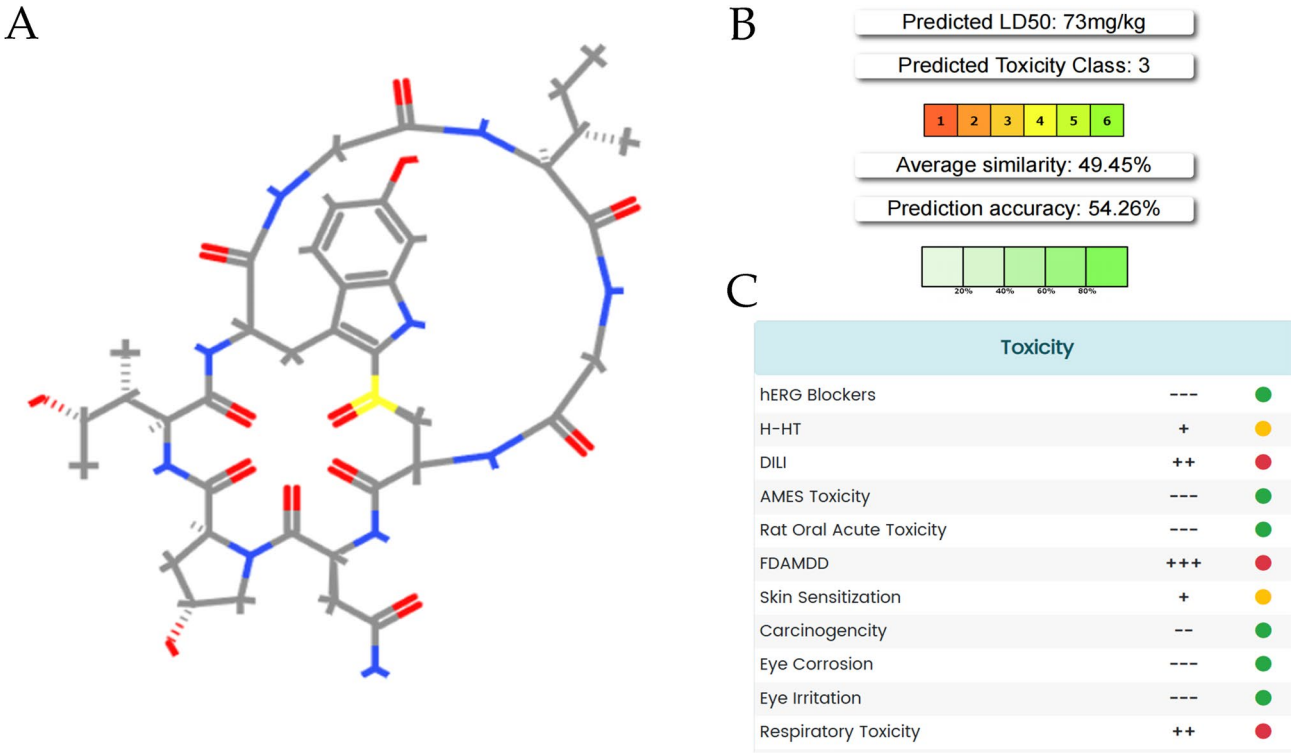

Fig. 2. Toxicity analysis of amatoxin. (**A**) Two-dimensional structure of amatoxin. (**B**) The Predicted LD50 of amatoxin was 73 mg/kg, the Predicted Toxicity Class was 3, the Average similarity was 49.45%, and the Prediction accuracy was 54.26%. (**C**) Amatoxin can cause strong human hepatotoxicity as well as strong drug-induced liver injury and respiratory toxicity.

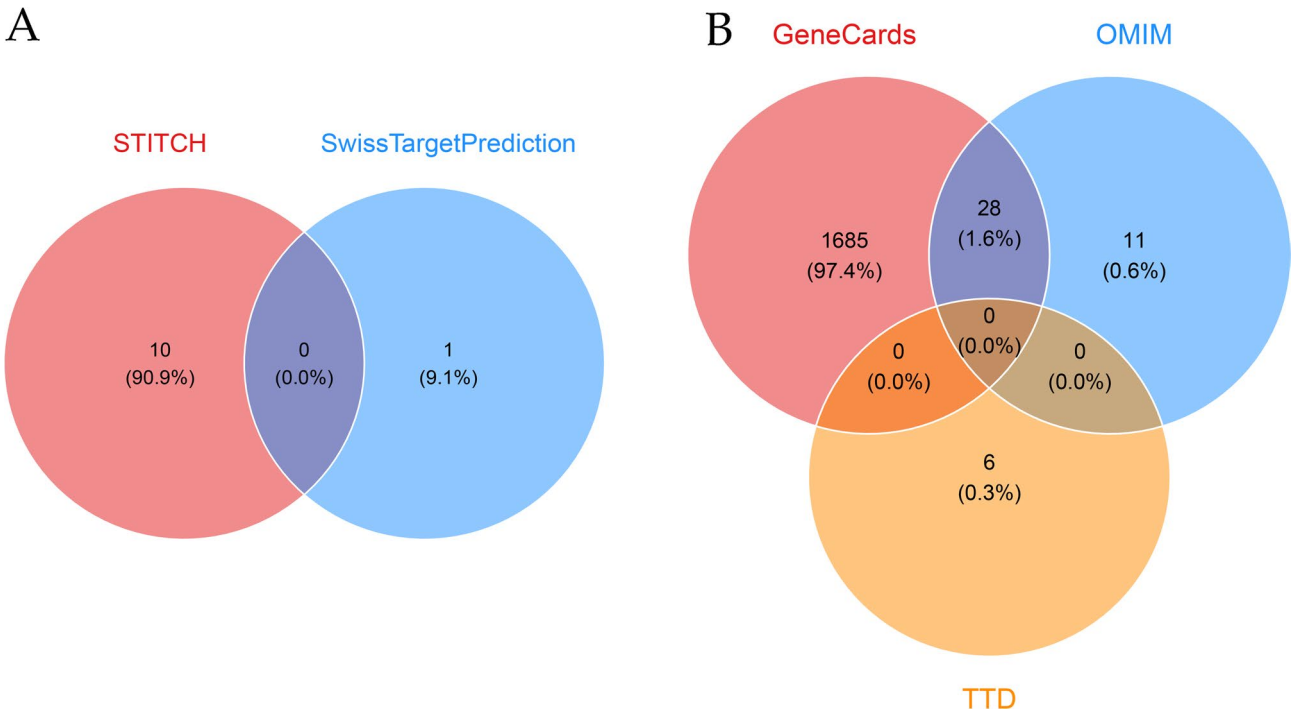

Fig. 3. Target analysis of amatoxin and liver injury. (**A**) 11 targets of amatoxin were predicted by STITCH and SwissTargetPrediction. (**B**) A total of 1730 genes related to liver injury were screened by GeneCards, OMIM, and TTD.

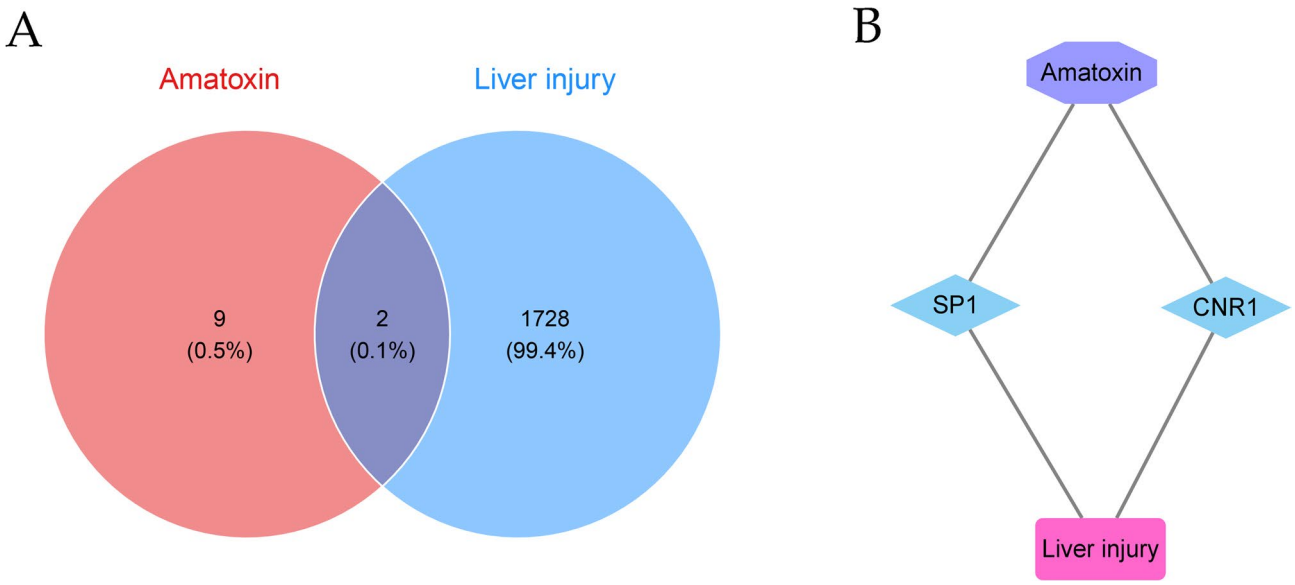

Fig. 4. Regulatory network of amatoxin-induced liver injury. (**A**) 2 intersecting targets were obtained by intersecting the targets of amatoxin and liver injury. (**B**) Amatoxin regulates the expression of SP1 and CNR1, which in turn leads to liver injury.

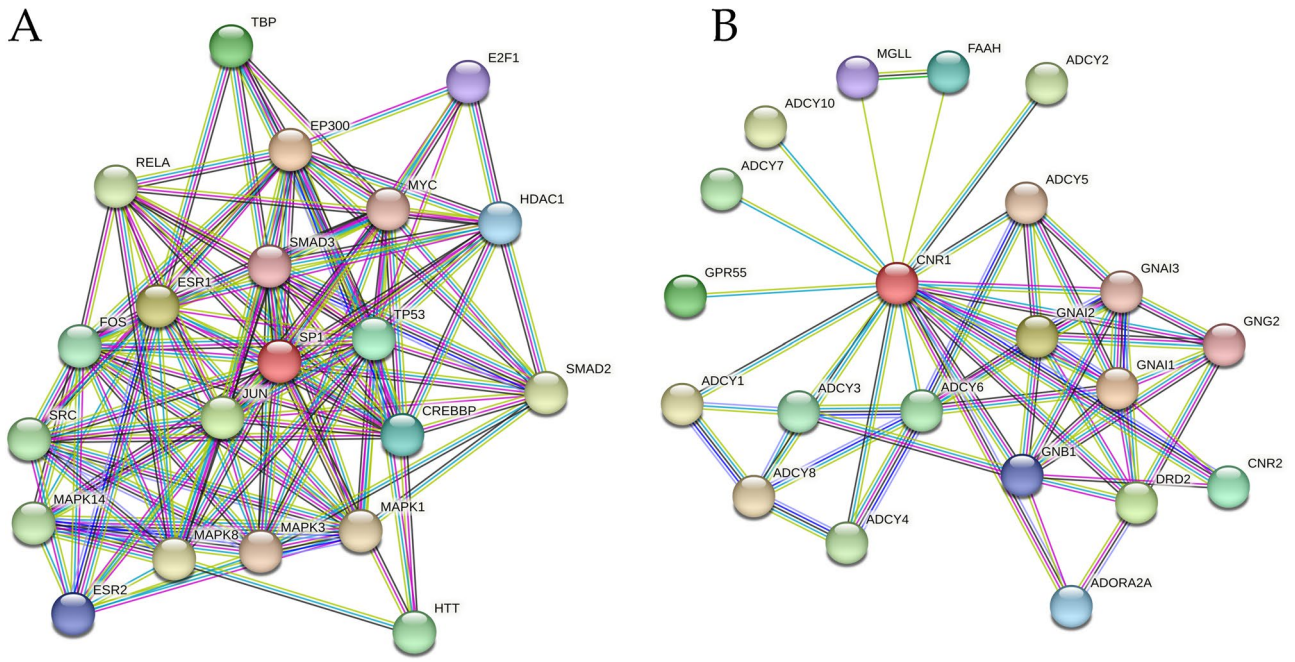

Fig. 5. PPI analysis. (**A**) Interaction of SP1 with its top 20 most closely associated proteins. (**B**) Interaction of CNR1 with its top 20 most closely associated proteins.

PPI analysis

In the two PPI networks centered on SP1 and CNR1, it can be observed that SP1 is closely related to SMAD3, TP53 and other targets, and the average node degree is 11.8, and the PPI enrichment p-value is observed < 1.0e-16 (Fig. 5A). CNR1 was closely related to ADCY6, GNAI2 and other targets, with an average node degree of 5.24 and a PPI enrichment p-value of 1.25e-09 (Fig. 5B).

Enrichment analysis

GO analysis showed that these targets were mainly involved in biological processes such as adenylate cyclase-modulating G protein-coupled receptor signaling pathway, cellular response to keton, cAMP biosynthetic process. They were mainly involved in glutamatergic synapse, RNA polymerase II transcription regulator comple,

membrance raft and other cellular components. They were mainly involved in DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding and other molecular function. (Fig. 6A). The first six BPs, CCs, and MFs with the most significant enrichment are shown separately (Fig. 6B). GO-KEGG analysis showed that the research targets were involved in the signaling pathways of Endocrine resistance, Relaxin signaling pathway, Growth hormone synthesis, secretion and action (Fig. 7).

Molecular docking

After retrieving the three-dimensional structures of SP1 and CNR1 from the PDB, molecular docking was performed to investigate the binding modes of amatoxin to these targets. The results demonstrated strong binding affinities: (1) For the amatoxin-SP1 complex, the Vina score was − 44.0 kcal/mol with a cavity volume of 58 Å^3 (Fig. 8A-B); (2) In the amatoxin-CNR1 interaction, the Vina score reached − 9.0 kcal/mol accompanied by a significantly larger cavity volume of 1737 Å^3 (Fig. 8C-D). These findings indicate that amatoxin exhibits robust binding activity with both SP1 and CNR1.

Discussion

Amatoxin is a bicyclic octapeptide, which is mainly found in some toxic varieties of Amanita phalloides, such as the famous death cap mushroom. It includes 9 types, including α-amanitin, β-amanitin, and γ-amanitin, and their molecular weight is about 920 Da. These three toxins were the most distributed and most toxic in species within the genus amanita, and were the main toxins causing hepatic damage poisoning. Amatoxin can bind tightly to DNA-dependent RNA polymerase II (RNAP II) and inhibit its activity, strongly hindering protein synthesis and can lead to hepatocyte necrosis and even death in poisoned individuals[3,31,32]. Amatoxin has the biological properties of being heat-resistant, cold-tolerant, and acid-resistant, and cannot be inactivated by ordinary heating and cooking methods[3]. Patients typically present with severe gastrointestinal symptoms such as nausea, vomiting, and diarrhea during the initial phase following poisonous mushroom ingestion. This acute phase is followed by a transient period of pseudo-recovery, after which patients develop progressive liver failure characterized by a triad of biochemical markers: Dramatic elevation of aminotransferases; Hyperbilirubinemia; Coagulopathy evidenced by prolonged prothrombin time. The clinical trajectory may culminate in acute hepatic failure accompanied by multi-organ dysfunction syndrome (MODS). This toxidrome carries an estimated mortality rate of 20%, primarily attributable to fulminant hepatic necrosis[33,34]. The diagnosis of acute liver injury due to amatoxin on early recognition of medical record, clinical manifestations, and results of laboratory tests and toxin testing, supplemented by medical imaging if necessary. Currently, treatments for amatoxin are limited, and commonly used antidotes include OATP1B3 transporter inhibitors, antioxidants, free radical scavengers, or anti-inflammatory drugs such as silybinin, N-acetylcysteine, penicillin G, polymyxin B, and prednisolone[35,36]. In conclusion, elucidating the pathogenic mechanisms of amatoxin-induced hepatotoxicity and developing targeted therapeutic strategies are critical to enhancing survival outcomes in poisoned patients. To address this unmet clinical need, our study employed an integrated approach combining network toxicology with molecular docking simulations to systematically investigate the molecular pathways underlying amatoxin-mediated liver injury. This methodology enables the identification of novel druggable targets for translational clinical applications.

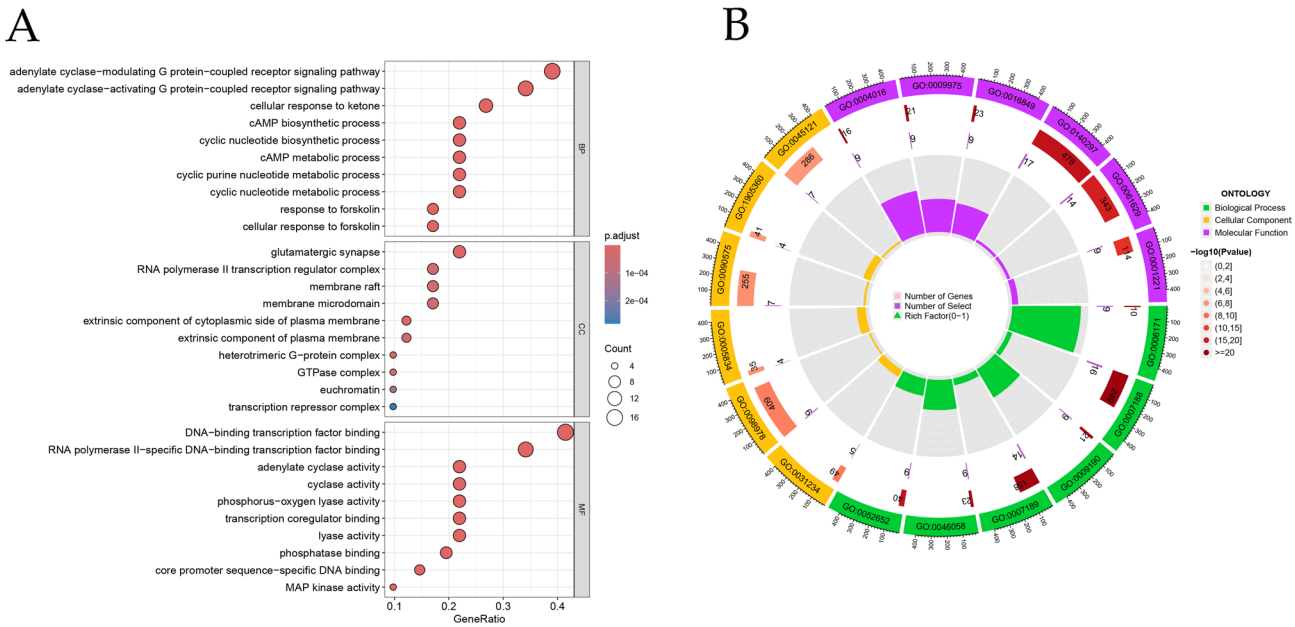

Fig. 6. GO analysis. (**A**) The BP, CC and MF in which the core genes are mainly involved. (**B**) The top six BP, CC, MF with the most significant, such as BP: GO: 0052652, GO: 0007189. CC: GO: 0098978, GO: 0045121. MF: GO: 0140297, GO: 0061629.

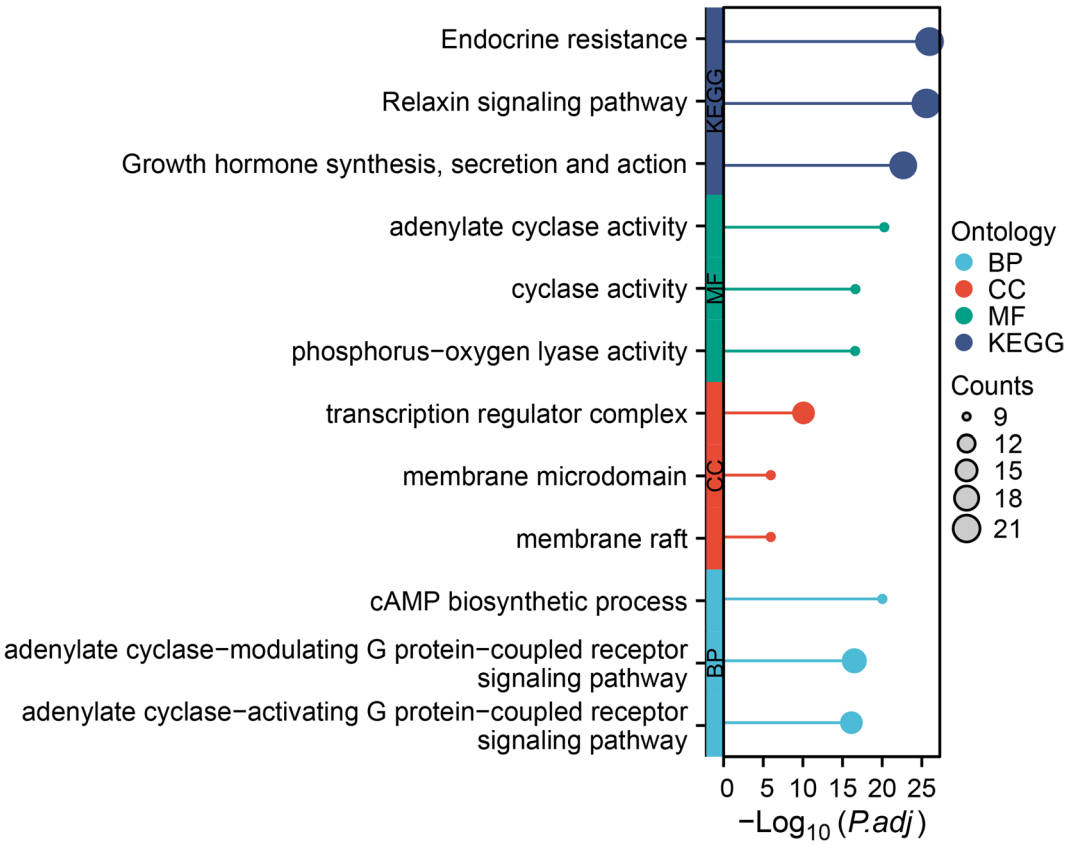

Fig. 7. GO-KEGG analysis. The core genes are mainly related to Endocrine resistance, Relaxin signaling pathway, Growth hormone synthesis, secretion and action.

The experimental workflow commenced with structural validation of amatoxin using using SMILES-based toxicity profiling via ProTox and ADMETlab, which confirmed its Class I hepatotoxicity. Subsequent target identification employed STITCH and SwissTargetPrediction for human proteome-wide screening, yielding 11 putative targets. Cross-referencing these with 1,730 liver injury-associated genes from GeneCards, OMIM and TTD databases identified two hub targets: SP1 (Specificity Protein 1) and CNR1 (Cannabinoid Receptor 1). PPI network analysis using STRING DB delineated their protein interactome, while functional enrichment revealed predominant involvement in Endocrine resistance, Relaxin signaling pathway, Growth hormone synthesis, cAMP biosynthetic process. Molecular docking with CB-Dock2 demonstrated high-affinity binding of amatoxin to SP1 (-44.0 kcal/mol) and CNR1 (-9.0 kcal/mol). Our study identified SP1 and CNR1 as hub targets in amatoxin-induced hepatotoxicity, which expands the current understanding of amatoxin pathogenesis. While the classical mechanism of amatoxin toxicity involves direct inhibition of RNAP II through trigger-loop disruption, leading to transcriptional arrest and hepatocyte necrosis[37]our findings suggest additional pathways may synergistically contribute to liver injury. Specifically: SP1 could regulates apoptosis-related genes (e.g., BCL2, CASP3)[38]. Our findings demonstrate that the binding of amatoxin to SP1 may amplify apoptotic signaling beyond RNAP II inhibition, which provides a novel mechanistic explanation for the clinically observed delayed hepatocyte demise (48–72 h post-ingestion). Concurrently, CNR1 modulates hepatic lipid metabolism and inflammatory responses. Its interaction with amatoxin potentially disrupts endocannabinoid homeostasis, exacerbating mitochondrial dysfunction—a mechanism not previously documented in amatoxin studies but consistent with oxidative stress marker alterations observed in rodent models.

SP1 is a transcription factor that is ubiquitous in many important organs of the human body, belonging to the zinc-finger transcription factors family, which is closely related to GC-rich promoter sequences[39]. Studies have shown that SP1 can regulate the expression of a variety of related factors in the liver, such as TGF-β1, and then regulate the activation of hepatic stellate cells (HSCs), thereby regulating the occurrence and development of liver fibrosis. Targeted inhibition of SP1 can effectively block extracellular matrix expression, thereby attenuating liver fibrosis[40]. SP1 is involved in the occurrence of liver cancer and affects the prognosis of patients by regulating PNPT1 expression[41]. SP1 exacerbates acetaminophen (APAP)-induced liver injury in mice by promoting CYP2E1 expression[42]. Therefore, SP1 is gradually recognized as a key mediator of liver lesions. In this study, the core gene SP1 of amatoxin-caused liver injury was screened by network toxicological analysis. The main mechanism of amatoxin is inhibiting the activity of RNAP II, which interferes with protein synthesis and causes apoptosis through oxidative stress and autophagy pathway, which is consistent with the results of our GO enrichment analysis. Therefore, we speculate that the amatoxin in patients with poisonous mushroom poisoning exerts biological activity by binding to SP1, and may mediate liver injury by regulating RNA polymerase II

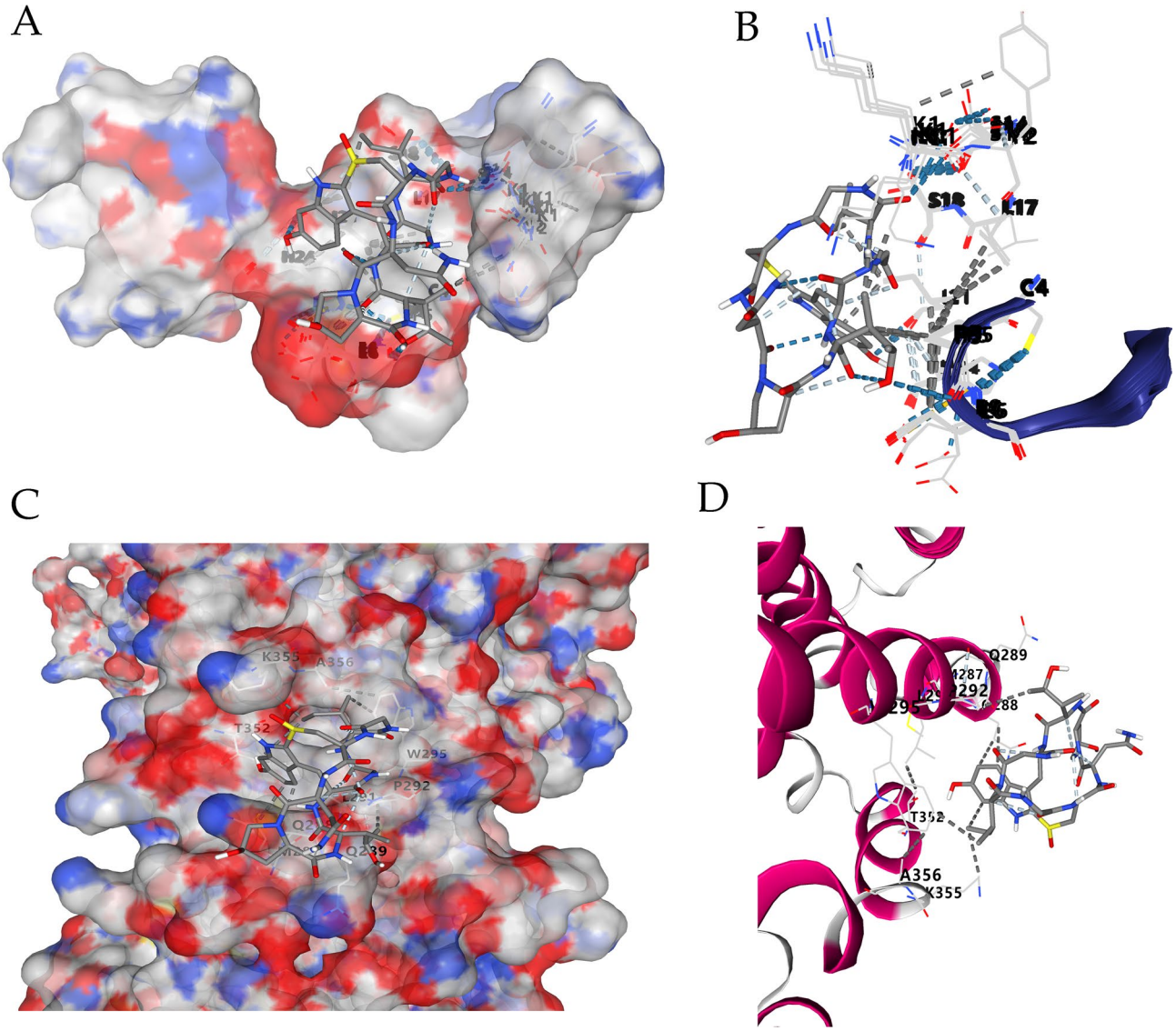

Fig. 8. Molecular docking. (**A**) the overall plot of the docking between amatoxin and SP1, with a Vina score of − 44.0. (**B**) Local enlarged view of the docking of amatoxin and SP1. (**C**) the overview depiction of the docking between amatoxin and CNR1, with a Vina score of -9.0. D, Local magnified perspective of the docking of amatoxin with CNR1.

transcription regulator comple, RNA polymerase II-specific DNA-binding transcription factor binding, etc. Therapy targeting SP1 may be a new way to treat amatoxin poisoning. SP1 Inhibition: May attenuate apoptosis via BCL2 upregulation, complementing RNAP II-targeted therapies.

CNR1 is a G protein-coupled receptor that is mainly distributed in the central nervous system and peripheral nervous system, especially in the liver. CNR1 inhibits adenylate cyclase and reduces cAMP levels, while stimulating MAP kinases to activate ERK, FAK, JNK, or PI3K/AKT downstream of the pathway to control cell fate[43]. Inhibition of CNR1 and CNR2 disrupts liver development and metabolic function in zebrafish, affecting liver differentiation[44]. The CB1 receptor encoded by CNR1 has profibrotic properties, and deletion of the CB1 gene or mice treated with specific CB1 antagonists reduce liver fibrosis in different animal models of acute and chronic liver injury[45]. CB1 receptors are highly induced in human cirrhosis samples and in liver fibrotic cells. Genetic or pharmacological inactivation of the CB1 receptor reduces the expression of TGF-β1 and the formation of liver fibrosis[46]. While CNR1 emerges as a critical mediator of amatoxin-induced hepatotoxicity, its biological functions exhibit paradoxical roles across liver disease models. In hepatic stellate cells (HSCs), CNR1 activation drives fibrogenesis through Gαi-cAMP signaling-mediated lipolysis suppression and TGF-β/Smad2/3-dependent collagen deposition[47]. Paradoxically, CNR1 demonstrates hepatoprotective potential in metabolic contexts: it maintains endocannabinoid system-regulated lipid homeostasis, where pharmacological antagonism ameliorates insulin resistance and steatosis in metabolic dysfunction-associated steatotic liver disease (MASLD)[48]. Furthermore, CNR1 activation attenuates acute inflammation via NF-κB inhibition during macrophage polarization, potentially decelerating early fibrotic progression[49]. This functional

dichotomy necessitates precision therapeutic strategies—selective CNR1 inhibition for acute toxin-induced injury versus activity modulation in chronic metabolic disorders—requiring integration of patient-specific genetic polymorphisms and microenvironmental profiling to optimize target engagement while balancing antifibrotic efficacy with metabolic homeostasis preservation. Combined with functional enrichment analysis, we speculated that CNR1 may promote hepatocyte fibrosis by regulating biological processes such as adenylate cyclase-modulating G protein-coupled receptor signaling pathway, cAMP biosynthetic process, etc. CNR1, as a specific target for the liver injury caused by amatoxin, is a promising biomarker for the diagnosis, treatment and prognosis of amatoxin poisoning. CNR1 Antagonism: Could mitigate metabolic derangements, as evidenced by glycyrrhizin's hepatoprotective effects through similar pathways.

While our computational strategy provides mechanistic insights into amatoxin hepatotoxicity, several constraints merit consideration. First, network pharmacology relies on curated databases that may incompletely capture tissue-specific protein interactions—for example, SP1's isoform expression patterns in human hepatocytes remain poorly annotated. Second, molecular docking scores prioritize binding affinity but neglect pharmacokinetic factors like hepatocyte membrane permeability. Future studies employing CRISPR-interference in primary hepatocytes and patient-derived organoids will be essential to validate these targets' therapeutic utility.

Given the critical need to improve clinical outcomes in amatoxin-containing mushroom poisoning, this study employs an integrative computational approach combining network toxicology with molecular docking simulations to systematically elucidate the pathogenic mechanisms underlying amatoxin-induced hepatotoxicity. Our multidisciplinary strategy aims to identify mechanism-driven therapeutic targets while establishing a robust theoretical framework for (1) developing precision antidotes and (2) guiding rational biomarker discovery for early diagnosis and treatment monitoring.

Conclusion

Emerging as hub regulatory targets in amatoxin-induced hepatotoxicity, SP1 and CNR1 are postulated to exert pivotal regulatory control over the pathophysiological cascade of hepatic damage. Targeted pharmacological intervention against these dual molecular switches represents a promising therapeutic strategy, potentially revolutionizing clinical management through mechanism-based antidote development for Amanita poisoning.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Author contributions

Chenglin Wang and Xin Wang drafted the manuscript and performed statistical analyses. Yaxing Deng and Yingchun Hu were responsible for data acquisition and curation. Li Hu conceived the study design and provided scientific supervision throughout the research process. All authors critically reviewed the manuscript for intellectual content and approved the final version prior to submission.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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