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Investigation of the Toxicity and Mechanisms of Acetyl Tributyl Citrate in Chronic Liver Injury Using Network Toxicology and Molecular Docking Techniques

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Abstract: Acetyl tributyl citrate (ATBC) is a commonly used plasticizing agent. Recent studies have shown that long-term exposure to ATBC may cause liver injury, but the mechanism of injury still needs to be further studied. The aim of this study is to investigate the hepatotoxicity and potential mechanism of ATBC using network toxicology strategy. Two online sites, ADMETlab and ProTex, were used to search the toxic effects of acetyl-tributyl citrate. Then six databases, ChEMBL, STITCH, SwissTargetPrediction, GeneCards, TTD, OMIM, were used to search the toxic effects of acetyl-tributyl citrate. We identified 43 potential biomarkers associated with ATBC exposure and liver injury. Using comprehensive analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, we found that the targets of ATBC induced liver injury were mainly enriched in metabolic disorders, oxidative stress, inflammatory response, organelle dysfunction, abnormal enzyme activity, and protein kinase signaling pathway disorders. Then we used Cytoscape software and STRING website optimization to highlight five core targets. Finally, we used molecular docking technology to combine these five core targets, and confirmed the high binding affinity of ATBC with the core targets. In summary, these results suggest that ATBC may induce liver injury through a closely related cascade of metabolism imbalance, organelle dysfunction, oxidative stress, inflammation, and fibrosis. On the basis of previous findings, this study confirmed that ATBC causes chronic liver injury, explored its injury mechanism, and provided a systematic and effective framework for researchers to evaluate the potential toxicity of various chemical products and medical devices.

Keywords: Acetyl Tributyl Citrate (ATBC); Network Toxicology; Liver Injury; Molecular Docking.

1. Introduction

The widespread production and use of industrial chemicals have generated numerous environmental pollutants, yet their toxicological mechanisms remain poorly understood[1]. Conventional toxicity assessments, focused on single-target analyses, fail to capture the multi-pathway cascade effects of pollutants. Emerging strategies integrating network toxicology and molecular docking offer a holistic approach, mapping compound-target-phenotype interactions to decode complex toxicity mechanisms[2]. Network toxicology combines multi-omics data and bioinformatics to construct dynamic networks linking pollutants to biological disruptions, while molecular docking quantifies ligand-target binding affinities (such as vina score < 0 indicates stable interactions). This synergy enables systematic identification of toxicity pathways and prioritization of high-risk targets.

Acetyl tributyl citrate (ATBC), a phthalate substitute in food packaging and medical devices, exhibits environmental persistence due to hydrolysis, photodegradation, and biodegradation. Its non-covalent structure facilitates migration into ecosystems and human bodies via dietary intake, inhalation, and dermal absorption[3]. Despite its "green" label, structural similarities to hepatotoxic phthalates raise concerns. Studies show phthalates induce liver injury via oxidative stress and metabolic interference[4,5], suggesting ATBC may share analogous risks. However, ATBC's hepatotoxic mechanisms remain unclear, necessitating urgent evaluation given its rising global use.

Network toxicology combined with molecular docking was used to explore the targets of ATBC on liver injury. Our analysis process included obtaining ATBC from ChEMBL, STITCH and SwissTargetPrediction. GeneCards, TTD and OMIM databases were used to obtain the targets related to liver injury. We used network toxicology methods to integrate multi-source heterogeneous data to construct a multi-dimensional interaction network model of ATBC-liver injury, identify and analyze the toxic pathways related to liver injury caused by ATBC, in order to clarify the potential toxicological characteristics of ATBC and predict its toxicity and potential molecular mechanisms. This study establishes a framework for rapid toxicity screening of environmental pollutants, linking computational predictions with mechanistic insights to safeguard public health.

2. Material and Methods

2.1. ATBC Toxicity Analysis

A standard structural arrangement of the ATBC and its Simplified Molecular Input Line Entry System (SMILES) nodes was established by searching "acetyl tributyl citrate" in the PubChem database. We used two online databases, ADMETlab and ProTex, to retrieve the physicochemical properties, pharmaceutical chemistry, metabolism and toxicity target prediction results of ATBC[6], which provided relatively reliable predictive value for our analysis of the toxic effects of ATBC on the liver.

2.2. Acquisition of ATBC Targets

Potential ATBC targets were retrieved from ChEMBL[7], STITCH, and SwissTargetPrediction databases using "acetyl tributyl citrate" (species: Homo sapiens) and its SMILES

identifier. Merged results were deduplicated, visualized via Venn diagram. These merged targets were then used to construct the target library of ATBC.

2.3. Collection of Liver Injury Targets

Liver injury-related targets were identified in GeneCards[8], TTD, and OMIM databases using keywords "liver injury" and "chronic liver injury", with median thresholds to filter high-confidence targets. Merged results from the three databases were integrated via Venn diagram to construct a liver injury target library.

2.4. Construction of Compound Regulatory Network and Protein Interaction Network

A venn diagram was constructed by ggvenn package in R (version 4.3.3) to identify common targets of ATBC and liver injury. The network relationship data and node attribute data of intersection targets were imported into Cytoscape software (version 3.10.2) to visualize regulatory interactions[9]. Protein-protein interactions (PPI) were analyzed using STRING database[10]. The analysis was conducted by restricting the species to "Homo sapiens", "Minimum required interaction score" to "High Confidence > 0.7", and hide which proteins are not connected. The network relationship files were saved online, and the Degree of nodes was calculated by topological analysis. Cytoscape software was used to draw concentric circles according to the degree score, and the complex network was transformed into a hierarchical visual structure by spatial stratification to display their interactions, and the core network targets were screened. Degree algorithm refers to the evaluation of the Degree Centrality of nodes in the protein interaction network, which is used to measure the degree of protein connectivity in the network and help quickly identify key nodes in the network[11].

2.5. Functional Annotation of Potential Targets of ATBC-induced Liver Injury

The "clusterProfiler" package in R was used to perform GO enrichment analysis based on molecular function (MF), biological process (BP), and cellular component (CC) and KEGG pathway enrichment analysis[12]. P-value < 0.05 was used as the threshold for significant enrichment[13,14]. Results were visualized to interpret mechanisms linking ATBC to liver injury.

2.6. Molecular Docking of ATBC with Core Targets

The top core targets from the concentric circle analysis were docked with ATBC using CB-Dock2[15]. Crystal structures of targets from Protein Data Bank (PDB) database and ATBC's 2D structure from PubChem database were uploaded to CB-Dock2 website, a deep learning-enhanced tool combining traditional docking with AI for accurate binding mode and affinity prediction. It improves docking efficiency and accuracy by combining traditional molecular docking methods with deep learning techniques[16].

3. Results

3.1. Preliminary Confirmation of ATBC Toxicity

ADMETlab predicted a high risk of ATBC-induced liver injury (DILI probability = 0.806), which is consistent with the recently published literature reports of ATBC-mediated toxicity in humans. It is necessary to pay attention to the risk of liver toxicity with long-term use or high dose exposure. ATBC also showed ocular corrosion potential (0.865) but low skin sensitization, cardiotoxicity, and mutagenicity (Table 1). ProTex indicated ATBC's BBB penetration (Table 2). Previous relevant literature has concluded that the core targets of ATBC induced brain toxicity are mainly enriched in cancer signaling and neuroactive ligand receptor interaction pathways through network toxicology. These insights guided exploration of liver injury mechanisms.

Table 1. ATBC toxicity targets obtained from ADMETlab

Property	Value
hERG Blockers	0.125
DILI	**0.806**
Ames Toxicity	0.014
ROA	0.104
FDAMDD	0.013
Skin Sensitization	0.166
Carcinogenicity	0.102
EC	**0.865**
EI	0.6
Respiratory Toxicity	0.02

Abbreviations: hERG, Human ether-a-go-go-related gene; DILI, drug induced liver injury; Ames, the mutagenicity of a chemical or drug as detected by the Ames Test; ROA, rat oral acute toxicity; FDAMDD, U.S food and drug administration maximum recommended daily dose; EC, Eye Corrosion; EI, Eye irritation;

Table 2. ATBC toxicity targets obtained from ProTex

Target	Shorthand	Prediction	Probability
Hepatotoxicity	dili	Inactive	0.89
Neurotoxicity	neuro	Inactive	0.93
Nephrotoxicity	nephro	Inactive	0.59
Respiratory toxicity	respi	Inactive	0.96
Cardiotoxicity	cardio	Inactive	0.91
Carcinogenicity	carcino	Active	0.64
Immunotoxicity	immuno	Inactive	0.99
Mutagenicity	mutagen	Inactive	0.78
Cytotoxicity	cyto	Inactive	0.83
BBB-barrier	**bbb**	**Active**	**0.92**
Ecotoxicity	eco	Inactive	0.52
Clinical toxicity	clinical	Inactive	0.77
Nutritional toxicity	nutri	Inactive	0.91

3.2. Identification of the Targets of ATBC-induced Liver Injury

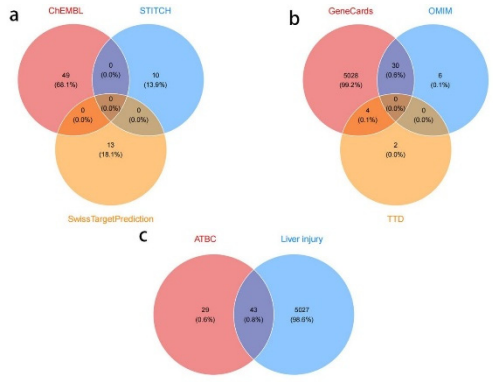


Figure 1. Screening of ATBC related targets, liver injury related targets, and potential targets for ATBC induced liver injury. **(a)** The Venn diagram showed the identification of 72 ATBC related targets from three databases: ChEMBL, STITCH, and SwissTarget Prediction. **(b)** The Venn diagram showed 5070 identified targets with high correlation to liver injury from three databases: GeneCards, TTD, and OMIM. **(c)** The venn diagram showed 43 potential targets of ATBC induced chronic liver injury.

Seventy-two ATBC-related targets were identified from ChEMBL, STITCH, and SwissTargetPrediction, while 5,070 liver injury-associated targets were retrieved from GeneCards, TTD, and OMIM (Figures 1a – b). Merging and deduplicating these datasets yielded 43 key targets for ATBC-induced chronic liver injury (Figure 1c). Cytoscape software mapped regulatory networks linking ATBC, targets, and disease progression (Figure 2). Through a compound regulatory network, it could indicate that ATBC can influence liver damage through 43 potential targets of action.

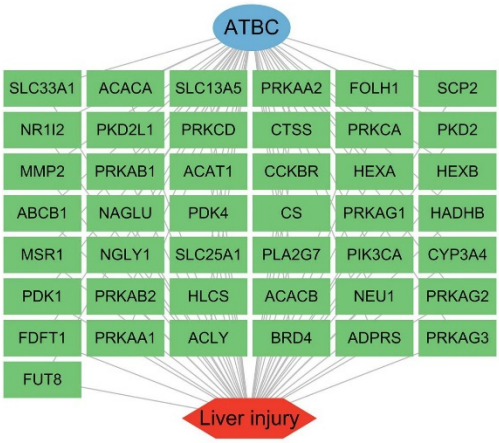


Figure 2. The compound regulatory network of ATBC and liver injury constructed by Cytoscape software.

3.3. Interaction Network of Potential Targets and Acquisition of Core Targets

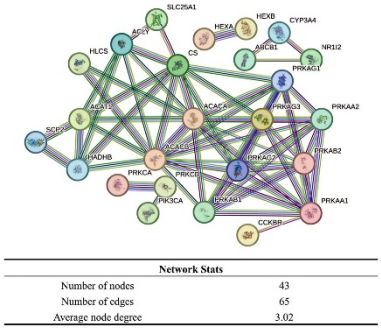


Figure 3. The PPI network of 43 potential targets.

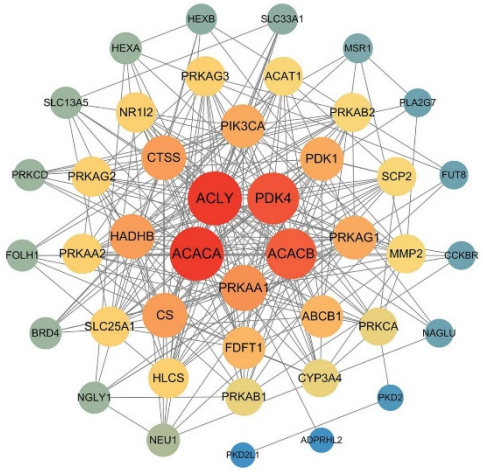


Figure 4. The concentric circle chart of potential targets based on degree scores.

The ATBC-induced liver injury PPI network (43 nodes, 65 edges; average node degree = 3.02) was constructed via STRING (Figure 3). According to the results of network analysis, we defined the five targets with the highest degree scores as core regulatory targets and visualized them using concentric circle plots (Figure 4). Node sizes and colors correspond to their respective degree values, with larger and more active nodes representing higher degree values. Edge thickness and blackness are proportional to their connection scores, suggesting that thicker and darker edges predict higher connection scores. Proteins with a Degree value greater than 10 May play the role of "core coordinator" in the PPI network (Table 3). The five core targets were: Acetyl-CoA Carboxylase Alpha (ACACA), ATP Citrate Lyase (ACLY), Pyruvate Dehydrogenase Kinase 4 (PDK4), Acetyl-CoA Carboxylase Beta (ACACB) and Protein Kinase AMP-Activated Catalytic Subunit Alpha 1 (PRKAA1). This indicates that their node sizes are larger and the colors in the graph are redder. A large number of studies have shown that the proteins encoded by these genes form the core of the cellular metabolic regulatory network. For example, PRKAA1 acts as a central regulator to coordinate the metabolic state of cells when energy is sufficient or insufficient.

Table 3. Potential targets with a degree score greater than 10 screened from PPI network

Gene	Degree	Closeness Centrality	Betweenness Centrality	Topological Coefficient
ACLY	26	0.62687	0.05350	0.42967
ACACA	26	0.67742	0.07547	0.39152
PDK4	24	0.62687	0.05350	0.44643
ACACB	23	0.64615	0.04552	0.40022
PRKAA1	19	0.61765	0.06123	0.39136
CTSS	18	0.62687	0.17857	0.28726
HADHB	18	0.59155	0.01451	0.45760
CS	18	0.59155	0.02128	0.43713
PIK3CA	17	0.60000	0.02815	0.39065
PDK1	17	0.55263	0.01576	0.49412
PRKAG1	17	0.57534	0.03014	0.44356
ABCB1	16	0.58333	0.02888	0.41118
FDFT1	16	0.57534	0.01019	0.46546
NR1I2	14	0.56000	0.01596	0.45753
SLC25A1	14	0.54545	0.01095	0.47421
PRKAG2	14	0.52500	0.00213	0.53571
PRKAA2	14	0.53846	0.00917	0.51224
HLCS	14	0.52500	0.01214	0.50866
PRKAG3	14	0.52500	0.00296	0.52521
MMP2	13	0.56000	0.02268	0.32794
ACAT1	13	0.51220	0.00520	0.54079
PRKAB2	13	0.51852	0.00471	0.52262
SCP2	13	0.51852	0.00921	0.54779
PRKCA	12	0.56000	0.10132	0.36842
CYP3A4	12	0.52500	0.00866	0.49524
PRKAB1	12	0.53846	0.01534	0.45270

3.4. Functional Analysis and Pathway Enrichment Analysis of Potential Targets

GO/KEGG enrichment analysis of 43 targets revealed 325 significant GO terms (280 biological processes; 17 cellular components; 27 molecular functions) and 35 KEGG pathways. The enriched terms were sorted by Count value and GeneRatio. Top terms were visualized (Figures 5 – 6). The detailed GO and KEGG enrichment analyses results are provided in the Tables 4-5.

The enrichment results showed that the main pathways enriched for potential genes related to ATBC-induced liver injury were closely related to metabolic disorders, oxidative stress, inflammatory response, organelle dysfunction,

abnormal enzyme activity and protein kinase signaling pathway dysregulation.

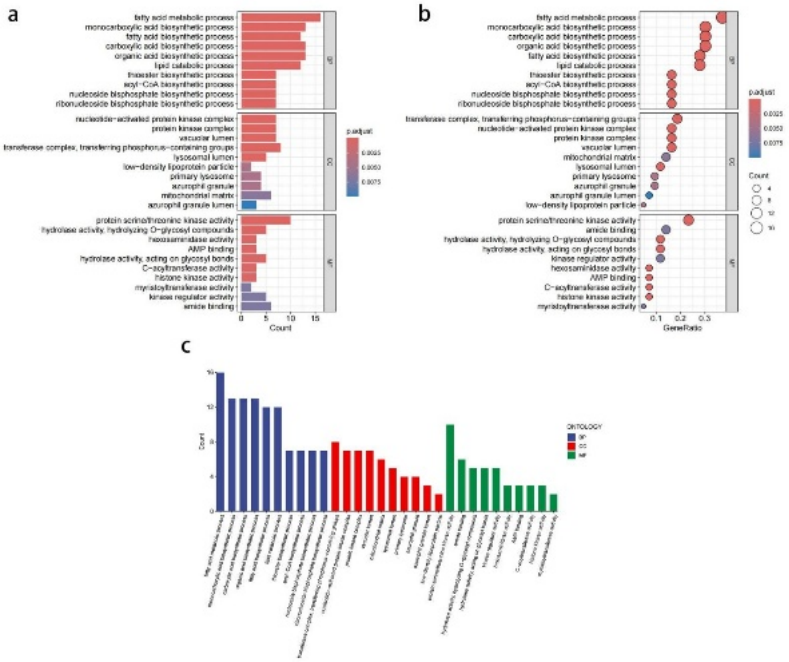


Figure 5. GO enrichment analysis of potential targets. **(a)** The bar chart illustrated GO functional enrichment analysis sorted in descending order based on Count value, showing the top ten items in biological processes (BP), cellular components (CC), and molecular functions (MF). **(b)** The bubble plot illustrated GO functional enrichment analysis ranked in descending order according to GeneRatio value, showing the top ten terms in biological processes (BP), cellular components (CC), and molecular functions (MF). **(c)** The classification bar chart illustrated GO functional enrichment analysis sorted in descending order based on Count value, showing the top ten items in biological processes (BP), cellular components (CC), and molecular functions (MF).

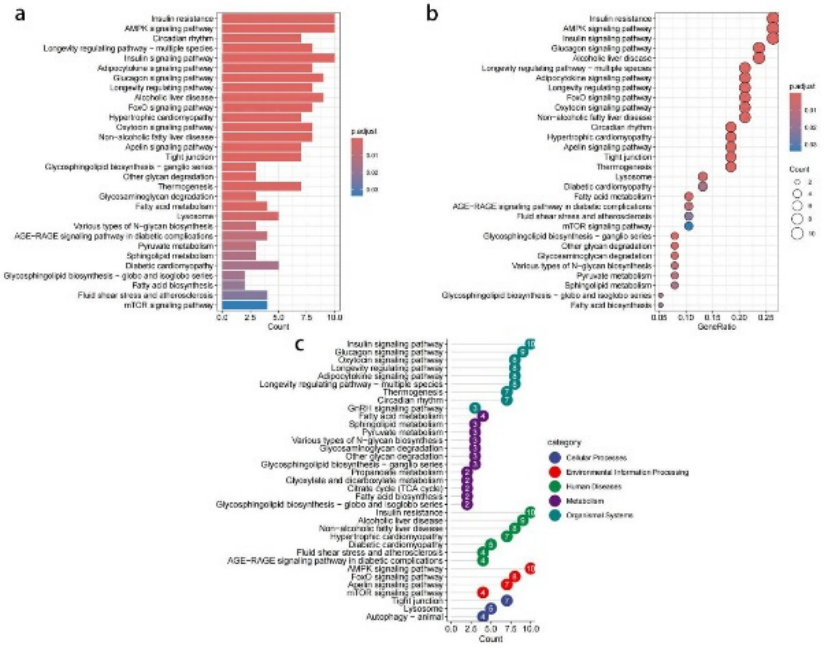


Figure 6. KEGG enrichment analysis of potential targets. **(a)** The bar chart illustrated the top 30 KEGG signaling pathways in descending order, enriched to the Count value. **(b)** The bubble chart illustrated the top 30 KEGG signaling pathways in descending order, enriched to the GeneRatio value. **(c)** The lollipop chart illustrated a visual representation of the KEGG signaling pathway based on enrichment category and Count value

3.5. Molecular Docking of ATBC and Core Target Proteins of Liver Injury

Molecular docking (CB-Dock2) revealed strong binding (vina score< 0) between ATBC and five core targets (ACACA, ACLY, PDK4, ACACB, PRKAA1), confirming spontaneous, high-affinity interactions. We used the conformation with the strongest binding energy of the molecule for visualization (Figure 7). Furthermore, through three-dimensional interaction visualization analysis, it was found that there were strong binding modes of key amino acid residues in the minimum binding energy conformation of ATBC and each target protein, such as the precise matching of hydrogen bond network and hydrophobic cavity.

Table 4. The top 10 terms in BP, CC and MF

ONTOLOGY	Description	p.adjust
BP	fatty acid metabolic process	2.81E-13
BP	monocarboxylic acid biosynthetic process	9.33E-13
BP	fatty acid biosynthetic process	9.33E-13
BP	carboxylic acid biosynthetic process	9.38E-11
BP	organic acid biosynthetic process	9.38E-11
BP	lipid catabolic process	1.69E-09
BP	thioester biosynthetic process	1.95E-09
BP	acyl-CoA biosynthetic process	1.95E-09
BP	nucleoside bisphosphate biosynthetic process	6.66E-09
BP	ribonucleoside bisphosphate biosynthetic process	6.66E-09
CC	nucleotide-activated protein kinase complex	8.32E-14
CC	protein kinase complex	1.40E-06
CC	vacuolar lumen	3.07E-06
CC	transferase complex, transferring phosphorus-containing groups	6.45E-06
CC	lysosomal lumen	4.31E-05
CC	low-density lipoprotein particle	0.00428
CC	primary lysosome	0.00428
CC	azurophil granule	0.00428
CC	mitochondrial matrix	0.00650
CC	azurophil granule lumen	0.00992
MF	protein serine/threonine kinase activity	8.68E-06
MF	hydrolase activity, hydrolyzing O-glycosyl compounds	0.00028
MF	hexosaminidase activity	0.00030
MF	AMP binding	0.00030
MF	hydrolase activity, acting on glycosyl bonds	0.00043
MF	C-acyltransferase activity	0.00043
MF	histone kinase activity	0.00043
MF	myristoyltransferase activity	0.00721
MF	kinase regulator activity	0.00721
MF	amide binding	0.00721

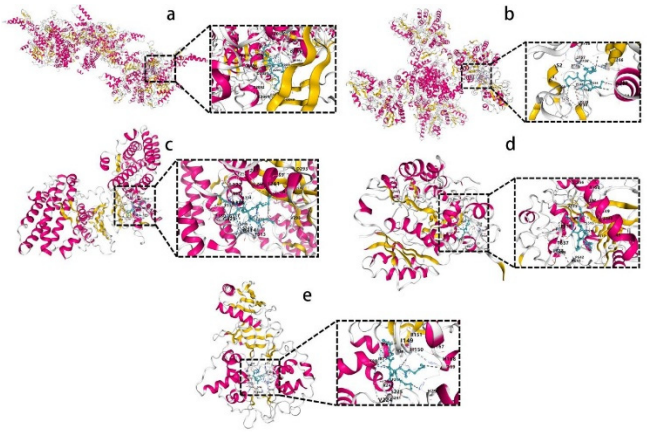


Figure 7. The molecular docking results of the lowest binding energy between each core target protein and ATBC. (a) ATBC and ACACA. (b) ATBC and ACLY. (c) ATBC and PDK4. (d) ATBC and ACACB. (e) ATBC and PRKAA1.

4. Discussion

In this study, ADMETlab and ProTex identified ATBC's hepatotoxic potential, supported by literature. Multi-database screening (ChEMBL, STITCH, SwissTargetPrediction for ATBC targets; GeneCards, TTD, OMIM for liver injury targets) yielded 43 key targets. PPI network analysis used by STRING database prioritized five core targets (ACACA, ACLY, PDK4, ACACB, PRKAA1) driving ATBC-induced liver injury.

Table 5. The top 30 KEGG signaling pathways

ID	Description	p.adjust
hsa04931	Insulin resistance	5.27E-09
hsa04152	AMPK signaling pathway	6.30E-09
hsa04710	Circadian rhythm	6.30E-09
hsa04213	Longevity regulating pathway - multiple species	9.93E-09
hsa04910	Insulin signaling pathway	1.12E-08
hsa04920	Adipocytokine signaling pathway	1.80E-08
hsa04922	Glucagon signaling pathway	1.85E-08
hsa04211	Longevity regulating pathway	1.04E-07
hsa04936	Alcoholic liver disease	2.04E-07
hsa04068	FoxO signaling pathway	1.84E-06
hsa05410	Hypertrophic cardiomyopathy	3.86E-06
hsa04921	Oxytocin signaling pathway	5.02E-06
hsa04932	Non-alcoholic fatty liver disease	5.11E-06
hsa04371	Apelin signaling pathway	3.21E-05
hsa04530	Tight junction	0.00011
hsa00604	Glycosphingolipid biosynthesis - ganglio series	0.00044
hsa00511	Other glycan degradation	0.00073
hsa04714	Thermogenesis	0.00073
hsa00531	Glycosaminoglycan degradation	0.00078
hsa01212	Fatty acid metabolism	0.00110
hsa04142	Lysosome	0.00267
hsa00513	Various types of N-glycan biosynthesis	0.00746
hsa04933	AGE-RAGE signaling pathway in diabetic complications	0.00862
hsa00620	Pyruvate metabolism	0.00952
hsa00600	Sphingolipid metabolism	0.01368
hsa05415	Diabetic cardiomyopathy	0.01527
hsa00603	Glycosphingolipid biosynthesis - globo and isoglobo series	0.01633
hsa00061	Fatty acid biosynthesis	0.01997
hsa05418	Fluid shear stress and atherosclerosis	0.02326
hsa04150	mTOR signaling pathway	0.03375

As for ACACA, as the rate-limiting enzyme of fatty acid synthesis, it mainly promotes lipogenesis in liver and adipose tissue. Current studies have shown that Acetyl CoA carboxylase alpha is involved in the progression of liver diseases mainly by regulating fatty acid metabolism, and the literature supports the involvement of ACACA in the development of nonalcoholic fatty liver disease (NFLD) by promoting fatty acid synthesis, which is an important cause of liver injury[17]. Similarly, it has been shown that ACC inhibitors improve liver fat content with a good safety profile[18]. Our pathway analysis results were consistent with the results of previous studies, indicating that ACACA, as a key gene in the regulation of metabolism, plays a crucial role in the regulation of fat metabolism in terms of fatty acid biosynthesis, fatty acid metabolic process and adipokine signaling pathway regulation. Additionally, ACACA and ACACB have been shown to have increased de novo lipogenesis (DNL) and liver lesions in mice carrying targeted inactivation point mutations in the AMP-activated protein kinase (AMPK) phosphorylation sites on them. The same mutation in ACACA also increased DNL and human hepatocellular carcinoma (HCC) cell proliferation[19].

As for ACLY, its main function is to cleave mitochondrial-derived citrate to acetyl-coa and oxaloacetate in the cytoplasm, which plays a central role in the stage of cellular lipid metabolism and has important effects on the occurrence of nonalcoholic fatty liver disease and liver oxidative stress and inflammation[20]. It has been shown that inhibition of ACLY can ameliorate nonalcoholic steatohepatitis (NASH) and

dyslipidemia[21,22]. It has been reported in the literature that it is often highly expressed in cancer cells, supporting membrane lipid synthesis required for rapid proliferation[23]. ACLY inhibitors have also been shown to have promising potential in the treatment of tumors, including HCC[24,25]. Our pathway analysis results were consistent with the previous results, indicating that ACLY also acts as a key metabolic gene, which plays a crucial role in promoting lipid synthesis in fatty acid metabolism/biosynthesis, glycolipid metabolism (such as sphingolipid, glycosphingolipid) pathway regulation.

PDK4 is upregulated in insulin resistance and diabetes, which may reduce glucose oxidation and promote fatty acid oxidation by inhibiting pyruvate dehydrogenase (PDH). Excessive activation of PDK4 may lead to lipid accumulation and oxidative stress in the liver, which aggravates NAFLD progression[26]. Whereas PDK4 deficiency reprograms intrahepatic glucose and lipid metabolism to promote liver regeneration in mice[27]. Experiments have shown drug-induced liver injury in PDK4-deficient mice, which have constitutively active PDH, resulting in increased flux through the mitochondrial electron transport chain. PDH is critical for mitochondrial bioenergetics because it controls the rate of production of reducing equivalents that drive respiration, and excessive activation of PDK4, by inhibiting PDH through phosphorylation, may lead to mitochondrial dysfunction and oxidative stress[28]. Similarly, we found that this gene was consistent with our pathway enrichment analysis results, indicating that PDK4 plays a crucial role in the interaction between pyruvate metabolismg, lucose metabolic reprogramming and lipid accumulation, mitochondrial dysfunction and oxidative stress, inflammatory signal activation. The roles and underlying mechanisms of other PDK family members, namely PDK1, PDK2, and PDK3, in tumor progression have been extensively studied and are well understood[29]. Studies have shown that the loss of PDK4 expression contributes to HCC malignant progression[30].

In the case of ACACB, it is homologous to ACACA, but mainly catalyzes the production of acetyl CoA outside the mitochondria to acetyl CoA. Studies have shown that, like ACACA, ACACB may also inhibit fatty acid β-oxidation and promote lipid synthesis[31], leading to abnormal accumulation of triglyceride in hepatocytes, lipotoxicity, and mitochondrial oxidative stress, while inhibiting autophagy and activating inflammatory.

For PRKAA1, whose main function is the core catalytic subunit of AMPK that senses cellular energy status. Down-regulation of PRKAA1 expression can lead to inhibition of AMPK signaling pathway activity, release its inhibition of acetyl-coa carboxylase (ACC) phosphorylation, and promote malonyl-coa production. Inhibition of CPT1A-mediated fatty acid β-oxidation (lipid metabolism pathway disorder) leads to lipid accumulation and lipotoxicity in hepatocytes. At the same time, AMPK inactivation may activate the mTOR signaling pathway to block the removal of damaged mitochondria and lipid droplets by autophagy, leading to oxidative stress (excessive accumulation of ROS). In addition, impaired AMPK function weakens the inhibitory effect on inflammatory pathways, promotes the release of proinflammatory factors, activates hepatic stellate cells, and drives collagen deposition and fibrosis[32]. In animal experiments, PRKAA1 knockout showed more severe liver damage in trauma models characterized by neutrophil infiltration, edema, and necrotic areas, while in sepsis models, PRKAA1 knockout mice showed higher levels of TNF-α and IL-6 and showed more severe liver damage than wild mice[33,34]. Our pathway analysis results were consistent with previous findings, suggesting that PRKAA1 is closely related to AMPK signaling pathway, lipid metabolism pathway disorders, oxidative stress and inflammation.

When the enriched pathways were investigated, the expression of AMPK signaling pathway is often reduced in metabolic disorders and NAFLD[35,36]. Insulin resistance and AGE-RAGE signaling in diabetic complications could induce proinflammatory cytokines and chemokines through the activation of growth factor cascades such as TGF-β/Smad3 and PDGF. Ultimately upregulating fibrotic stromal cell proteins (key drivers of liver fibrosis)[37]. Enrichment analysis further suggested that Apelin signaling (APLN) is a prognostic marker and therapeutic target for HCC[38]. In addition, enrichment analysis indicated that sphingolipid metabolism also affected ATBC-induced liver injury. There is increasing evidence for the role of sphingolipids in stress-induced and death ligand-induced hepatocyte death, which can promote the progression of several liver diseases, including steatohepatitis, ischemia-reperfusion liver injury, or liver cancer[39].

It is worth noting that five core targets mainly induce liver injury through metabolism disorders, mitochondrial dysfunction and oxidative stress, activation of inflammatory signals and fibrosis. The functional analysis and pathway enrichment analysis of potential targets revealed that the pathways closely related to liver injury, namely insulin resistance/signaling pathway, AMPK signaling pathway, adipokine signaling pathway, etc, indicating that ATBC mainly induces liver metabolism disorders and oxidative stress. These processes trigger inflammation and fibrosis, which may eventually lead to the development of cancer, highlighting ATBC's hepatotoxic potential. Molecular docking results confirmed that all the five core target proteins stably bound to the active pocket of ATBC with vina score less than zero kcal/mol, which played an important role in ATBC-induced liver injury. Although there was no direct evidence that ATBC could cause liver fibrosis and HCC in the current study, there was still a potential fibrogenic and carcinogenic risk. Combined with the existing literature and the expression imbalance of enriched pathways and key genes, we can speculate that the progression of liver injury caused by ATBC is often driven by a vicious cycle of metabolic imbalance, lipotoxicity, oxidative stress, inflammation and fibrosis.

This study suggests that compared with the potential risks revealed by network toxicology studies, the failure of animal models to clearly confirm the correlation between ATBC exposure and metabolic abnormalities may be due to the double differences in experimental design and realistic exposure conditions. In particular, the high-intensity acute exposure model used in routine animal studies cannot realistically simulate the characteristics of long-term low-dose exposure experienced by humans through environmental contaminants and consumer products. Short-term high-dose intervention cannot reflect the bioaccumulation effect of chronic ATBC exposure, nor can it reproduce the dose-response dynamic relationship in the actual exposure scenario. Of note, the delayed nature of hepatic metabolic disorders suggests that short-term experiments (usually <6 months) in rodents are difficult to fully assess the progressive effects of long-term cumulative ATBC exposure on hepatic metabolic

homeostasis over the life span of humans.

This study pioneers the integration of network toxicology and molecular docking to assess environmental pollutant toxicity, overcoming limitations of traditional animal models (high cost, species-specific biases, and fragmented endpoint analysis). By mapping multi-level molecular interactions, this approach identifies conserved toxicity mechanisms and prioritizes key targets (such as ATBC-induced hepatotoxicity pathways), this framework enables rapid, systematic screening of emerging pollutants, bridging gaps between high-throughput data and mechanistic toxicology. In the future, epidemiological cohorts or translational models are needed to verify the targets and pathways of ATBC-induced liver injury.

5. Conclusion

In conclusion, our study conducted a comprehensive investigation of the potential hepatotoxicity of ATBC using the strategies of network toxicology and molecular docking analysis. We identified 43 potential targets related to ATBC induced liver injury and further extracted five core targets: ACACA, ACLY, PDK4, ACACB and PRKAA1, which may play an important role in mediating the toxic effects of ATBC on the liver. Integrating the key enriched pathways such as inflammation-related signaling pathways and lipid metabolism pathways, our results suggest that ATBC exposure may induce liver injury through metabolism disorders, mitochondrial dysfunction and oxidative stress, and activation of inflammatory signaling and fibrosis.

This study not only elucidates the underlying molecular mechanism of ATBC induced liver injury, but also promotes the strategy of network toxicology and molecular docking for efficient analysis of the toxicity and molecular biological mechanism of potential environmental pollutants.

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