Network Pharmacology Study on the Neurotoxic Mechanism of *Acorus tatarinowii*

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Abstract. *Acorus tatarinowii* was a traditional Chinese medicine used to treat neurological diseases such as epilepsy with significant effects. When the oral dosage is too large, it has potential neurotoxic effects. However, the toxicity mechanism is still unclear. In this study network pharmacology methods were used to explore the mechanism of neurotoxicity of *Acorus tatarinowi*i. The results showed that 5 toxic components of Acorus tatarinowii were identified, involving 73 neurotoxic targets. Among them, AKT1, CASP3, PTGS2, ESR1, EGFR, HIF1A, GSK3B, MMP9, SRC, and CCND1 may be key targets for *Acorus tatarinowii* to exert neurotoxic effects; KEGG pathway analysis found that the neurotoxicity caused by *Acorus tatarinow*ii may be mainly related to Pathways in cancer, Endocrine resistance, and Chemical carcinogenesis-receptor activation. It was found that *Acorus tatarinowii* may exert neurotoxic effects through multiple components and targets.

Keywords: *Acorus tatarinowii*; network pharmacology; neurotoxic; mechanism.

1. Introduction

Acorus tatarinowii, as a perennial herbaceous plant in the Araceae family, was first recorded in the "Shennong Materia Medica Classic". It has significant therapeutic effects on neurological diseases such as epilepsy[1,2]. As the oral dose of *Acorus tatarinowii* gradually increases, the concentration of active ingredients passing through the blood-brain barrier continues to increase, and the possibility of neurotoxicity continues to increase[3,4]. However, the mechanism of potential neurotoxicity of *Acorus tatarinowii* is still unclear. Traditional Chinese medicine has the characteristics of multiple components, targets, and pathways, and the study of drug molecules acting on a single target or signaling pathway is difficult to reveal its scientific connotation. Network pharmacology is an emerging discipline that utilizes information technology to study the relationship between drugs and diseases, and can better reflect the overall and systematic interactions between drugs, targets, and diseases. This study aims to explore the neurotoxic mechanism of *Acorus tatarinowii* using network pharmacology methods, and provide a basis for its clinical application.

2. Methods

2.1 Collection and screening of components from *Acorus tatarinowii*

In the analysis platform of the TCMSP, the chemical components contained in *Acorus tatarinowi*i were obtained using the search term "Acorus tatarinowii" and screening conditions of bioavailability greater than or equal to 30% and drug like properties greater than or equal to 0.18. CTD database was further used for toxicity query, and obtain the toxic compounds of *Acorus tatarinowi*i[5].

2.2 Prediction of toxicity targets of *Acorus tatarinowii*

Using the SMILES chemical formula of the toxic components of *Acorus tatarinowi*i,Set the "Human" attribute on the SwissTargetPrediction platform to predict the potential targets of active compounds, and finally use the UniProt platform to standardize gene correction.

2.3 Collection of neurotoxicity related targets

In the GeneCards and OMIN databases, search for genes related to neurotoxicity using the keywords "toxicity" and "nerve toxicity", and merge and delete duplicate target genes. Intersect the target genes of *Acorus tatarinowii* with neurotoxicity related genes, and ultimately obtain potential neurotoxicity targets for the chemical components of *Acorus tatarinowii*.

2.4 Construction of *Acorus tatarinowii*-Component-Neurotoxic Target Network

Using Cytoscape software, establish a "*Acorus tatarinowii*-Component-Neurotoxic Target" network to predict the relationship between the components and target network that cause neurotoxic reactions in *Acorus tatarinowii*.

2.5 Construction of Protein Interaction Network (PPI) Relationships

The intersection targets were import into the string database, and 0.4 was set as the lowest interaction score to obtain the interaction relationship between the targets. The genes ranked high in the degree are used as core targets.

2.6 Enrichment analysis of GO and KEGG signaling pathways

Utilize the GO biological function annotation and KEGG enrichment analysis of the DAVID database to process data, pay attention to important neurotoxic related functions and pathways, and plot them.

3. Results

3.1 Screening of neurotoxic components from *Acorus tatarinowii*

By querying two databases, TCMSP and CTD, five potential toxic components were ultimately identified. These components are eudesmin, cycloartenol, kaempferol, α-Asarone and β-Asarone, respectively (Table 1).

Table 1. Active Ingredients of Eucommia ulmoides

CAS No.	Molecule Name	MW	OB（%）	DL
526-06-7	eudesmin	386.48	52.35	0.62
469-38-5	cycloartenol	426.8	38.69	0.78
520-18-3	kaempferol	286.25	41.88	0.24
2883-98-9	α-Asarone	208.28	35.61	0.06
5273-86-9	β-Asarone	208.28	35.61	0.06

3.2 Establishment of Neurotoxic Target Network for the Components of *Acorus tatarinowii*

Using 5 toxic components and 73 intersecting targets of Acorus tatarinowii, a "*Acorus tatarinowii*-Component-Neurotoxic Target " network diagram was constructed, as shown in Fig. 1. *Acorus tatarinowii* may exert neurotoxic effects through multiple components and targets.

3.3 Establishment of PPI relationship and screening results of core targets

The top 10 genes with a degree value were used as core targets for neurotoxicity, including AKT1, CASP3, PTGS2, ESR1, EGFR, HIF1A, GSK3B, MMP9, SRC, and CCND1. The results are shown in Fig. 2. These targets play important roles in the PPI network and may be the core targets for the neurotoxic effects of *Acorus tatarinowii*.

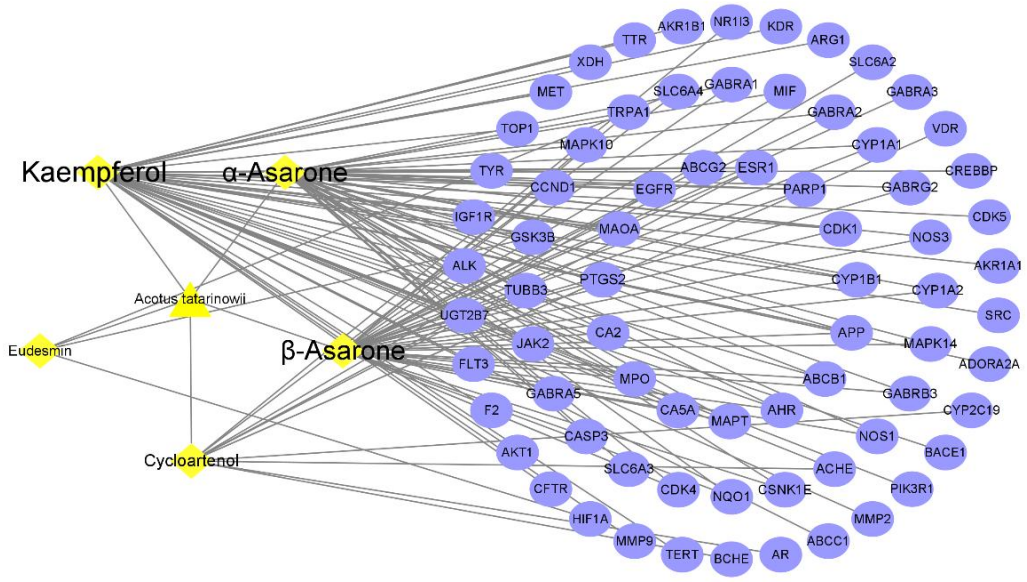

Fig. 1 *Acorus tatarinow*ii-component-neurotoxic target network diagram

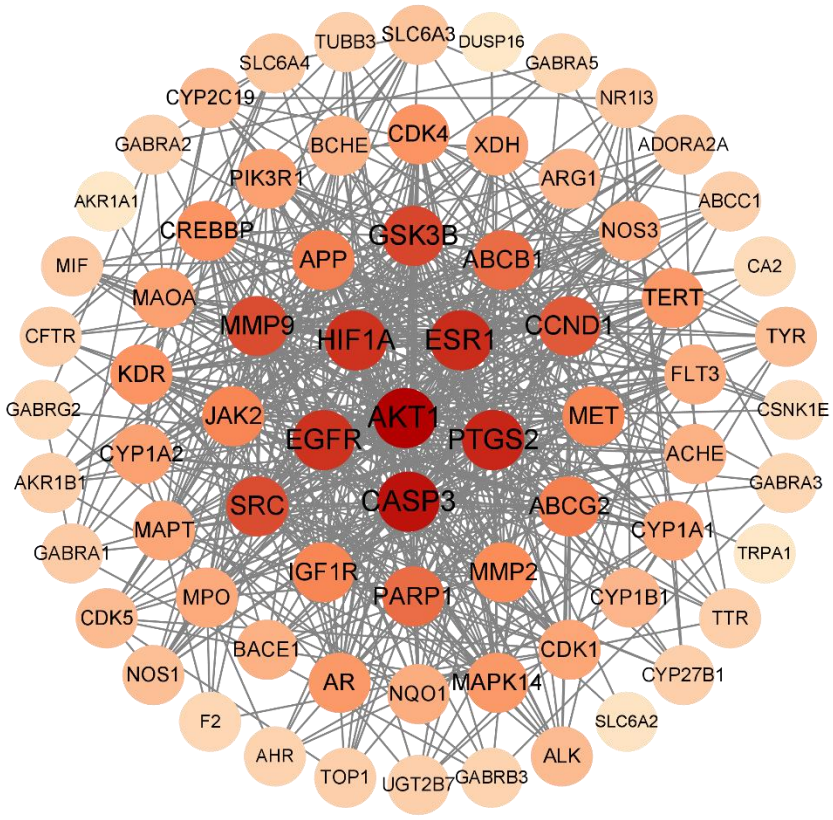

Fig. 2 PPI network diagram of neurotoxicity of *Acorus tatarinowii*

3.4 Results of GO enrichment analysis

By using the DAVID database analysis, 328 biological processes (BP) were obtained, with 76 processes (P<0.01), mainly including response to xenobiotic stimuli, signal transformation,

xenobiotic metabolic processes, etc. There are 68 cellular components (CC) and 29 with P<0.01, mainly including membrane raft, neuron projection, GABA-A receptor complex, etc. There are 105 molecular functions (MF) and 32 with P<0.01, mainly including enzyme binding, GABA gated chloride ion channel activity, protein serine/threonine/tyrosine kinase activity, etc.The top 10 ranked items was shown in Fig. 3.

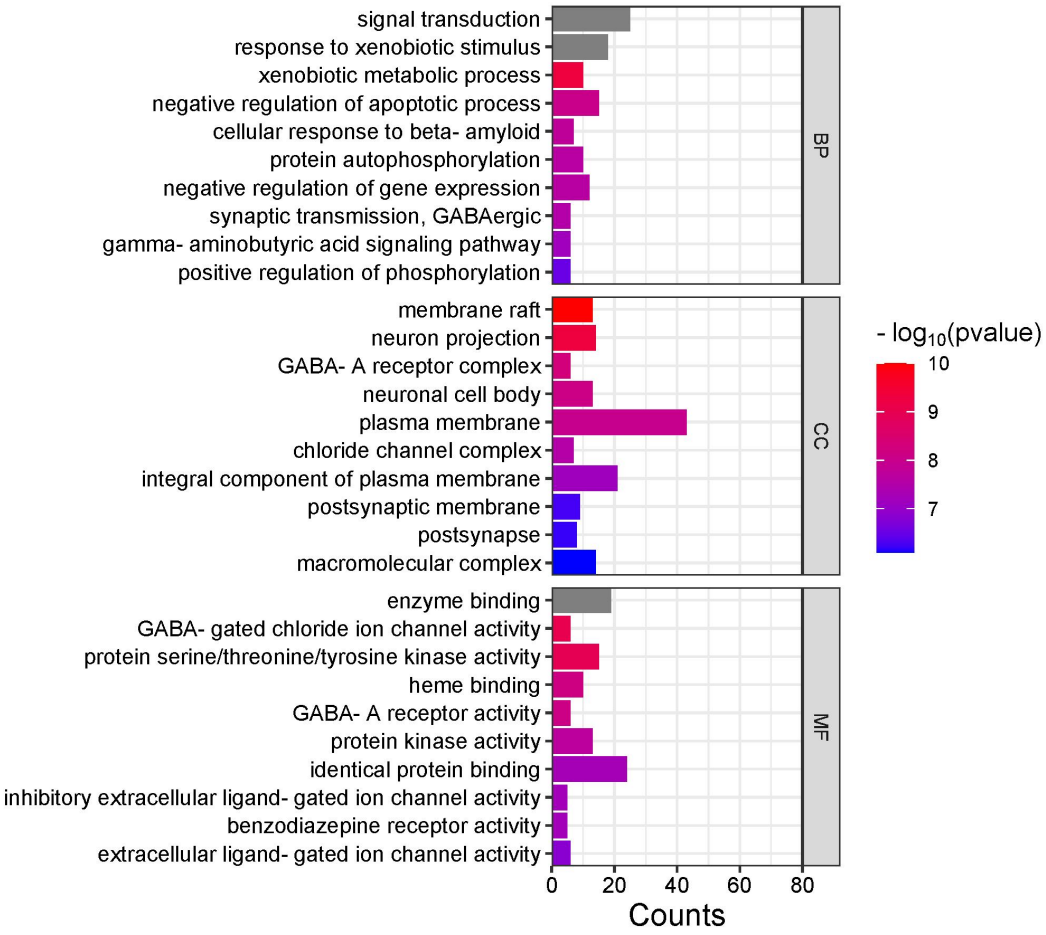

Fig. 3 GO enrichment analysis of neurotoxicity of *Acorus tatarinowii*

3.5 Results of KEGG signaling pathway enrichment analysis

A total of 130 related pathways were identified through KEGG pathway enrichment analysis, of which 75 pathways were found to be P<0.01. The main pathways include Pathways in cancer, Endocrine resistance, Chemical carcinogenesis-receptor activation, etc. The top 10 related pathways was shown in Figure 4.

However, due to the limitations of bioinformatics research, although the use of network pharmacology can intuitively predict the binding of chemical components and action targets in Acorus tatarinowii, the relationship between the upregulation and downregulation of key gene expression by chemical components has not been clarified, and the drug's status in the organism cannot be fully revealed[6,7]. Therefore, it is necessary to further study the toxicity of chemical components of *Acorus tatarinowii* in vivo.

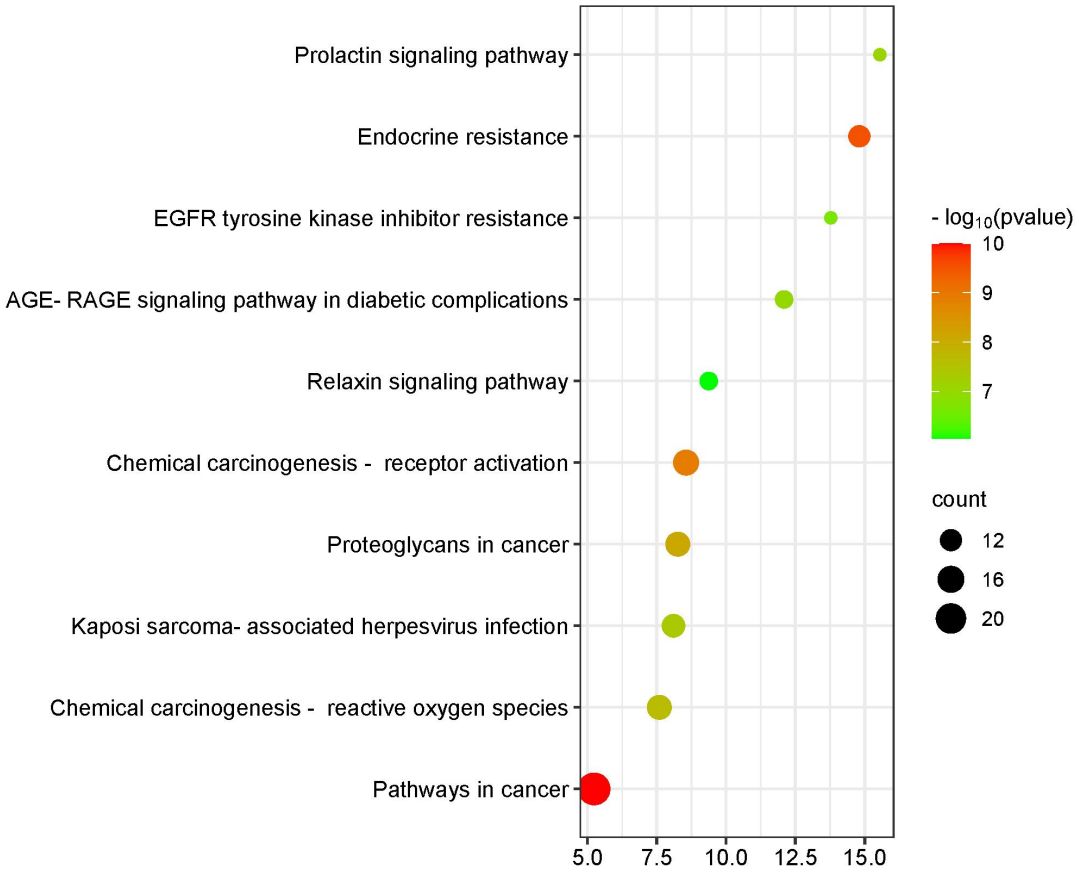

Fig. 4 Enrichment analysis of KEGG pathway in neurotoxicity of *Acorus tatarinowii*

4. Conclusion

In summary, the method of network pharmacology was used in this study to explore the mechanism of neurotoxicity of the anti epileptic drug *Acorus tatarinowii*. The potential toxic components in *Acorus tatarinowii* mainly exert toxic effects by affecting the Pathways in cancer, Endocrine resistance, Chemical carcinogenesis - receptor activation signaling pathways, as well as targets such as AKT1, CASP3, PTGS2, ESR1, etc. The neurotoxicity of *Acorus tatarinowi*i is a complex network structure mechanism with multiple targets, pathways, and functions.

5. Acknowledgments

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References

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