Inhibitory Effect of *Acorus tatarinowii* Nasal in Situ Gel on Acetylcholinesterase

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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 is a traditional Chinese medicine widely used in neurological diseases[1]. It is a perennial herbaceous plant in the shape of grass, with its roots and stems having an odor. Modern research indicates that volatile oil is the main chemical component of *Acorus tatarinowii. Acorus tatarinowii* and its active ingredients can regulate the permeability of the blood-brain barrier and promote the passage of drugs through the blood-brain barrier. It can exert anti vascular dementia effects by reducing cell apoptosis and protecting hippocampal neurons[2,3].

However, traditional Chinese medicine needs to be boiled and used, which brings inconvenience to patients. Nasal in situ gel is a new drug delivery method developed in recent years. Patients who receive nasal administration have better compliance. In situ nasal gel has the advantages of higher bioavailability and better therapeutic effect[4,5]. Therefore, we prepared *Acorus tatarinowii* nasal in situ gel in the early stage, laying the foundation for the development of new drugs for nervous system diseases.

Alzheimer's disease (AD) is a progressive degenerative disease of the central nervous system, belonging to the category of cognitive impairment. Its clinical features include progressive degradation of memory and cognitive function, accompanied by psychobehavioral abnormalities and decreased social function[6]. The pathogenesis of senile dementia is complex, and there is no specific drug for clinical treatment, which seriously affects human life and health. Acetylcholinesterase is an important target for Alzheimer's disease. Acetylcholinesterase (AChE) has the activity of carboxypeptidase and aminopeptidase, which can degrade the neurotransmitter acetylcholine and weaken its effect on the postsynaptic membrane. Acetylcholinesterase inhibitors (AChEIs), also known as anticholinesterase drugs, mainly increase synaptic ACh levels by inhibiting AChE activity, resulting in significant pseudo ACh effects[7]. Acetylcholinesterase hydrolyzes the substrate acetylcholine to produce thiocholine, which reacts directly with dithiodinitrobenzoic acid to produce a yellow product. The Ellman method is easy to operate, cost-effective, and fast to analyze, and has been widely used in research such as AChE detection and inhibitor screening. In order to verify the therapeutic effect of in situ gel for nasal use of *Acorus*

tatarinowii, we studied its inhibitory effect on acetylcholinesterase and the factors affecting the determination of acetylcholinesterase activity, so as to provide a basis for the further development of in situ gel for nasal use of *Acorus tatarinowii*.

2. Methods

2.1 Instruments and Materials

Thioacholine iodide (ATCI) (Shanghai Ruji Biotechnology Development Co., Ltd.); Sodium chloride, potassium dihydrogen phosphate, and disodium hydrogen phosphate (analytical grade, Tianjin Damao Chemical Reagent Factory); Potassium chloride (analytical pure, Xilong Chemical Co., Ltd.); Re distilled water (self-made in the laboratory).

2.2 Preparation of volatile oil from *Acorus tatarinowii*

Extract volatile oil from *Acorus tatarinowii* according to the method of extracting volatile oil in the Chinese Pharmacopoeia.

2.3 Determination of acetylcholinesterase activity

The activity of AChE was measured using the classical Ellman method. The Ellman method is a quantitative method for evaluating the activity of AChE, which is based on the principle that the substrate thioacetylcholine iodide (ATCI) rapidly decomposes under the action of AChE, generating thiocholine. thiocholine can react with the chromogenic agent 5,5-mercapto-2,2-dinitrobenzoic acid (DTNB) to produce a yellow substance 5-mercapto-2-nitrobenzoate that can be absorbed under certain conditions. The AChE activity was determined by Ellman method and divided into three groups: Acorus tatarinowii nasal in situ gel group, blank experimental group and positive control group. Specific operation process of in situ gel group for nose use of Acorus tatarinowii: take AChE 100 μL. Join 400 μL PBS buffer containing a series of concentrations of Acorus tatarinowii nasal in situ gel. After pre incubation at 37 ℃ for 25 minutes, add 250 to the incubation system. Incubate the ATCI solution with a concentration of 3 mM for another 1 minute, and terminate the reaction in an ice bath. Transfer the above incubation system solution, add DTNB solution for color development, dilute and measure absorbance at 405 nm. (Analysis of 3 samples per concentration).

2.4 GC-MS analysis

The measuring instrument adopts HP6890/5975C GC/MS combination instrument (Agilent, USA). The chromatographic column is HP-5MS (60m x 0.25mm x 0.25) μ m) Elastic quartz capillary column, initial temperature 50 ℃ (retained for 2 minutes), heated at 3.5 ℃/min to 183 ℃, then heated at 10 ℃/min to 310 ℃ (retained for 10 minutes), operating time: 62.7 minutes; The temperature of the vaporization chamber is 250 ℃; The carrier gas is high-purity He (99.999%); Column front pressure of 16.58psi, carrier gas flow rate of 1.0 mL/min, split ratio of 50:1, solvent delay time of 6 minutes.

3. Results

3.1 Analysis of volatile oil main components in *Acorus tatarinowii*

The volatile chemical components were determined by searching and verifying the Nist20 and Wiley275 standard mass spectra of each peak in the total ion flow chart using a mass spectrometry computer data system.The analysis results of the main components of volatile oil from *Acorus tatarinowii* by gas chromatography are shown in Table 1. From the results, it can be seen that the main chemical components of the volatile oil of *Acorus tatarinowii* are volatile compounds. The larger the peak area, the higher the content of the chemical component.

Table 1. Active Ingredients of *Acorus tatarinowii*

Retention time (min)	Compound	Molecular formula	Peak area
14.999	.alpha.-Thujene	C10H16	84438624
15.413	.alpha.-Pinene	C10H16	404172410
15.948	.alpha.-Fenchene	C10H16	23239636
16.056	Camphene	C10H16	158969638
17.12	sabinene	C10H16	317862928
17.33	.beta.-Pinene	C10H16	234023338
17.463	5-Hepten-2-one, 6-methyl-	C8H14O	24521396
17.704	.beta.-Myrcene	C10H16	123560546
18.236	(+)-4-Carene	C10H16	4742124
18.406	.alpha.-Phellandrene	C10H16	18080250
18.954	.alpha.-terpipene	C10H16	16835592
19.72	D-Limonene	C10H16	630181291
19.841	Eucalyptol	C10H18O	449028103
20.204	.beta.-Ocimene	C10H16	3856901
20.816	.gamma.-Terpinene	C10H16	93510123
21.18	trans-sabinene hydrate	C10H18O	9268498
22.114	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	C10H16	23586543
22.193	Fenchone	C10H16O	6650896
22.56	Linalool	C10H18O	90678567
24.966	(+)-2-Bornanone	C10H16O	944403943
25.229	Bicyclo[3.1.0]hexan-2-one, 5-(1-methylethyl)-	C9H14O	1220501
25.484	Pinocarvone	C10H14O	786785
25.595	endo-Borneol	C10H18O	57300340
26.091	Terpinen-4-ol	C10H18O	151370413
26.313	P-cymen-8-ol	C10H14O	9453273
26.454	2-Cyclohexen-1-one, 4-(1-methylethyl)-	C9H14O	3427191
26.647	.alpha.-Terpineol	C10H18O	231452687
30.843	Safrole	C10H10O2	906106277
33.171	Eugenol	C10H12O2	9901384
33.915	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	C12H20O2	5115642
34.111	Copaene	C15H24	5128049
34.344	Phenol, 2-methoxy-3-(2-propenyl)-	C10H12O2	678947
34.631	Beta.elemene	C15H24	4986285
34.829	Methyleugenol	C11H14O2	92302549
35.68	Santalen	C15H24	83198532
36.183	trans-.alpha.-Bergamotene	C15H24	24285641
36.685	epi-.beta.-Santalene	C15H24	7187983
37.132	.beta.-Santalene	C15H24	45203871
37.801	.gamma.-Muurolene	C15H24	2092651
38.051	Germacrene D	C15H24	10677378
38.183	Benzene, 1,2-dimethoxy-4-(1-propenyl)-	C11H14O2	3110213
38.291	.beta.-Selinene	C15H24	2786412
38.721	.beta.-Bisabolene	C15H24	3923483
39.223	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	C11H12O3	18790479
39.387	.delta.-Selinene	C15H24	6984916
40.524	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-,	C15H26O	161495575

	(E)-		
41.833	Guaiol	C15H26O	10643246
44.417	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	C15H26O	2385314
48.168	m-Camphorene	C20H32	8495202
53.53	Octan-2-yl palmitate	C24H48O2	12732927

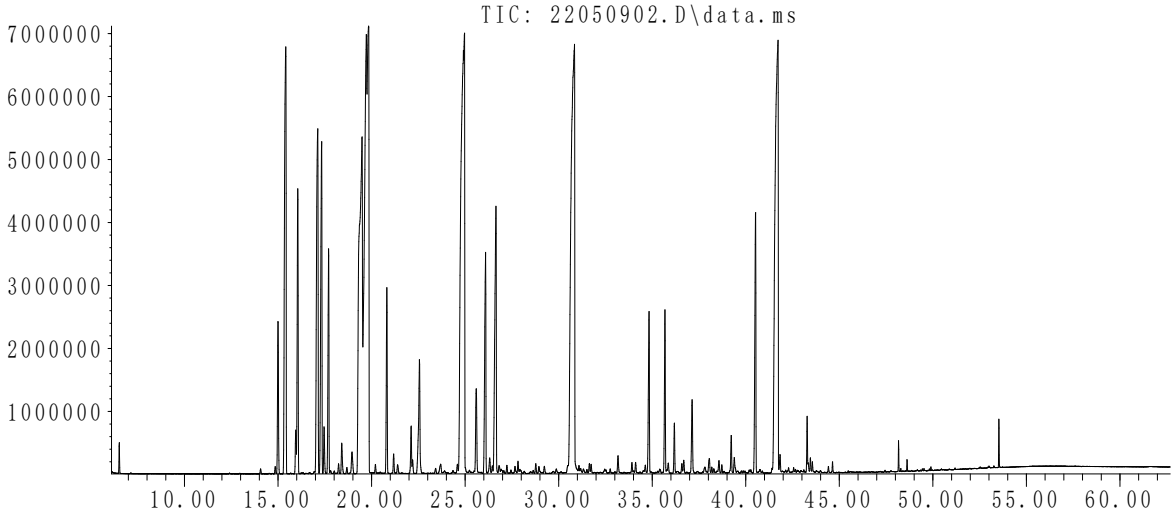

Fig. 1 GC-MS chromatogram of volatile oil from *Acorus tatarinowii*

The GC-MS chromatogram of the volatile oil from Acorus tatarinowii is shown in Fig. 1. The ion source is an EI source; Ion source temperature 230 ℃; Quadrupole temperature of 150 ℃; Electronic energy of 70eV; Emission current 34.6 μA; Multiplier voltage 1729V; Interface temperature of 280 ℃; The quality range is 29~500amu. The representative mass spectrum is shown in Fig. 2.

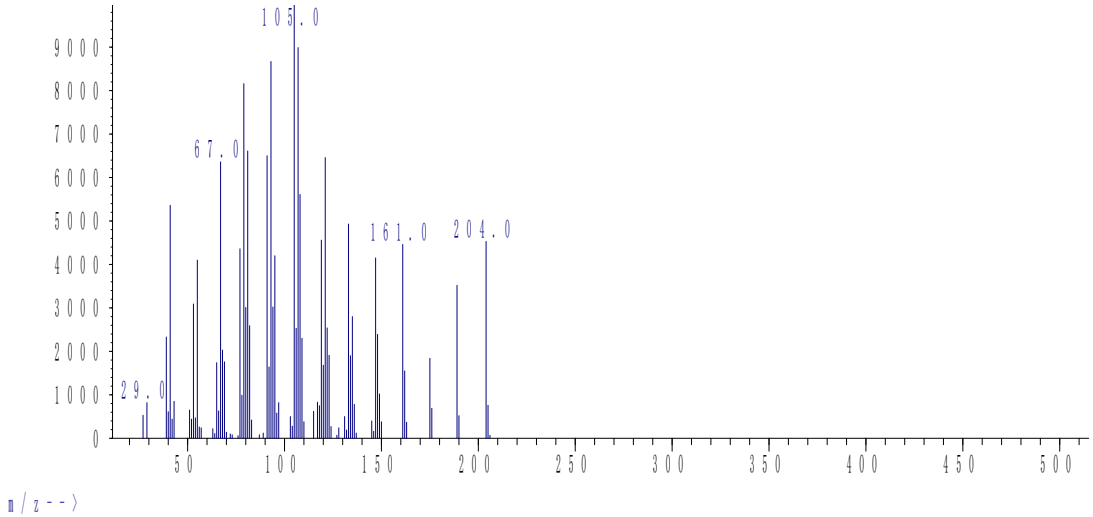

Fig. 2 The representative mass spectrum

3.2 Inhibitory effect of *Acorus tatarinowii* nasal in situ gel on acetylcholinesterase

The results of inhibition of Acorus tatarinowii nasal in situ gel on acetylcholinesterase are shown in Fig. 3. The results showed that the inhibitory activity of *Acorus tatarinowii* nasal in situ gel on AChE was concentration dependent, and the inhibitory rate on AChE gradually increased with the increase of the concentration of *Acorus tatarinowii* nasal in situ gel. This shows that in vitro experiment, *Acorus tatarinowii* nasal in situ gel can effectively inhibit the activity of AChE.

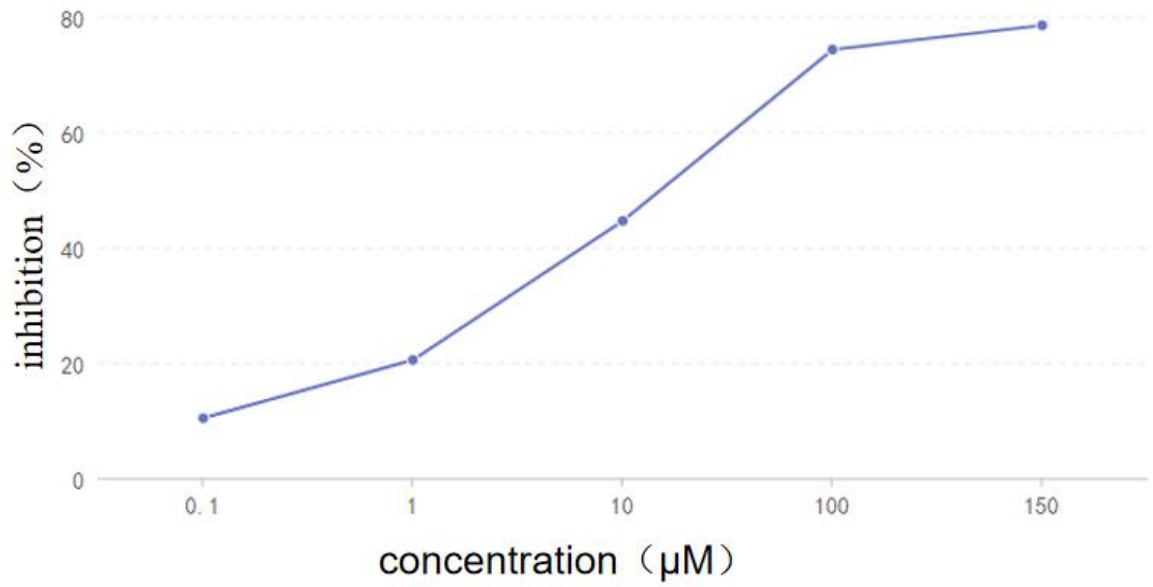

Fig. 3 Inhibitory effect of *Acorus tatarinowii* nasal in situ gel on acetylcholinesterase

4. Conclusion

In summary, the components of *Acorus tatarinowii* are mainly volatile compounds. The inhibitory activity of *Acorus tatarinowii* nasal in situ gel on AChE was concentration dependent. The results provide a theoretical basis for the further development and application of *Acorus tatarinowii* nasal in situ gel.

Acknowledgments

This work was supported by the College Student Innovation and Entrepreneurship Training Program Project (Gui Zhong Yi Da Chuang He Zi [2022]-13), the National Natural Science Foundation of China (No. 82160803) and High level innovative talents in Guizhou Province (Guizhou Science and Technology Cooperation Platform Talents-GCC [2023] 077).

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