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Protective Effects of Acorus Tatarinowii Schott Water Decoction on Cognitive Function in Alzheimer's Disease Model Mice and Preliminary Exploration of Its Multi-target Mechanism

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Abstract: *This study investigated the protective effects of Acorus tatarinowii Schott water decoction on cognitive function and its multi-target mechanism in APP/PS1 transgenic Alzheimer's disease (AD) model mice. Behavioral performance was evaluated using the Morris water maze and Y maze tests. Combined with analysis of hippocampal oxidative stress indicators (SOD, MDA, GSH), inflammatory factors (TNF-α, IL-6), and neuronal morphology, the results showed that Acorus tatarinowii Schott water decoction significantly improved spatial learning and memory ability in model mice, upregulated antioxidant enzyme activity (SOD), reduced lipid peroxidation (MDA), restored antioxidant reserves (GSH), and inhibited the expression of inflammatory factors. Further mechanistic studies revealed that Acorus tatarinowii Schott exerts cognitive protective effects by alleviating oxidative stress, inhibiting neuroinflammation, and promoting neuronal plasticity (dendritic spine regeneration, synaptic protein expression), with synergistic effects between oxidative stress and inflammation. This study provides experimental evidence for the multi-target intervention of AD with traditional Chinese medicine compounds and expands the clinical application of Acorus tatarinowii Schott.*

Keywords: *Acorus tatarinowii Schott water decoction; Alzheimer's disease; Oxidative stress; Neuroinflammation; multi-target mechanism; Neuronal plasticity*

1. Introduction

As a chronic, multifactorial neurodegenerative disease, Alzheimer's disease (AD) has become a significant threat to the health of the elderly worldwide. According to the latest report from the WHO, there are 55 million dementia patients globally, with AD accounting for 60%-70%, and the annual social cost exceeding $1 trillion [1]. Its clinical features are mainly manifested as progressive memory impairment, cognitive decline, and behavioral abnormalities, with pathological hallmarks of β-amyloid protein (Aβ) deposits forming senile plaques and neurofibrillary tangles [2]. Current FDA-approved AD drugs can only alleviate some symptoms and lack disease-modifying effects, while traditional Chinese medicine (TCM) compounds exhibit unique advantages due to their multi-target characteristics.

TCM has unique advantages in the treatment of neurodegenerative diseases. Acorus tatarinowii Schott, as a representative of TCM for opening orifices, has been used since ancient times to improve cognitive dysfunction [3]. The "Shen Nong's Herbal Classic" records its efficacy in "opening the heart orifice, tonifying the five viscera, and unblocking the nine orifices." Modern pharmacological studies have further revealed its multi-component, multi-target pharmacological effects. Acorus tatarinowii Schott water decoction, a commonly used clinical dosage form, is rich in active ingredients such as β-asarone, α-asarone, and eugenol, which have been shown to have multiple biological effects including regulating neurotransmitters, antioxidant stress, anti-inflammatory, and neuroprotective effects [4].

In recent years, research on the improvement of AD cognitive function by Acorus tatarinowii Schott has gradually deepened. Tian et al. [5] found through experiments on AD model mice that Acorus tatarinowii Schott water decoction can significantly improve the learning and memory ability of

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animals, and its mechanism is closely related to reducing nitric oxide synthase (NOS) activity in the hippocampus. A review by Lu et al. [6] indicated that Acorus tatarinowii Schott exerts neuroprotective effects through multi-target mechanisms such as inhibiting Aβ deposition, regulating glutamate (Glu) transport, and reducing neuroinflammation. Studies by Gao et al. [7] showed that Acorus tatarinowii Schott volatile oil can promote hippocampal neuron regeneration and improve cognitive function in AD model mice.

This study utilized APP/PS1 transgenic AD model mice, which express a fusion of mutant human presenilin (DeltaE9) and amyloid precursor protein (APPswe), mimicking the core pathological features of AD patients in terms of amyloid plaque formation, cognitive decline, and neuronal degeneration. Compared with other models, APP/PS1 mice develop cortical amyloid plaques at 4 months of age, with significantly increased Aβ deposits in the hippocampus at 6 months, and exhibit memory impairments highly similar to human AD at 10-12 months, showing stronger predictability for clinical translation. This study explored the protective effects and mechanisms of water decoction from behavioral, oxidative stress indicators, inflammatory factors, and neuronal morphology dimensions, aiming to provide experimental evidence for TCM intervention in AD and expand new ideas for the clinical application of Acorus tatarinowii Schott.

2. Materials and Methods

2.1. Experimental Animals and Grouping

(1) Animal Selection and Housing

Six-month-old male APP/PS1 double transgenic mice (purchased from Beijing HFK Bioscience Co., Ltd., License No.: SCXK-2023-0001), weighing 25-30g, were selected. This model mimics Alzheimer's disease (AD) pathologies including Aβ deposition, neurofibrillary tangles, and cognitive impairments by expressing DeltaE9-mutated presenilin and APPswe fusion proteins, offering advantages over traditional Aβ injection models in replicating clinical pathological progression. Animals were housed under SPF conditions with constant temperature (22±2℃), humidity (55±5%), and 12h light/dark cycles, with free access to food and water.

(2) Grouping and Intervention

① Experimental Groups (n=10 per group):

② Control Group: Wild-type C57BL/6J mice, administered saline via oral gavage.

③ Model Group: APP/PS1 mice, administered saline via oral gavage.

④ Treatment Group: APP/PS1 mice, administered Acorus tatarinowii decoction via oral gavage (dose: 0.2g crude drug/10g body weight, freshly prepared daily).

Administration Details:

Custom-made 12-gauge stainless steel gavage needles (length: 4.5cm, ball-end diameter: 2mm) were used.

Dosage volume: 0.1ml/10g body weight.

Administration time: Daily between 9:00-11:00 AM for 8 weeks.

Acorus tatarinowii was authenticated against Chinese Pharmacopoeia standards, sourced from Sichuan, and prepared by triple decoction (8x water volume each time, boiled for 1.5h). The combined filtrate was concentrated to 1g crude drug/ml and stored at 4℃.

2.2. Cognitive Function Assessment

(1) Morris Water Maze Test

Apparatus: Circular pool (diameter: 120cm), water depth: 30cm (temperature: 25±1℃), hidden platform (diameter: 10cm, submerged 1cm below water surface). Four distinct geometric cues were affixed to pool walls.

Tracking System: Smart 3.0 Behavioral Analysis System (Shanghai Xinruan Information

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Technology Co., Ltd., Model: XR-XM101), resolution: 0.1cm, sampling rate: 30Hz. System calibrated by China National Metrology Institute, spatial positioning error <0.5cm.

(2) Place Navigation Test

Training Phase (Days 1-5): Mice were released from 4 different quadrants daily. Escape latency (time to find platform within 60s) was recorded. If unsuccessful, mice were guided to the platform for 15s, with latency recorded as 60s.

Data Analysis: Daily average escape latency was calculated to evaluate spatial learning ability.

(3) Spatial Probe Test

Day 6: Platform removed. Mice were released from a random entry point, and time spent in target quadrant, platform crossings, and swimming paths were recorded over 60s.

Data Analysis: Quadrant retention time and crossings reflected spatial memory retention.

(4) Y-Maze Spontaneous Alternation Test

① Apparatus: 3-arm Y-maze (arm length: 30cm, width: 10cm, height: 15cm), with distinct color cues at each arm end.

② Tracking System: Gene&I Video Analysis System (Beijing Ji'an De'er Technology Co., Ltd., Model: Y-MAZE-101), resolution: 1mm. System calibrated by China National Metrology Institute (Certificate No.: CNAS-2024-0125), body entry recognition accuracy >98%.

③ Procedure: Mice were placed in the start arm, and arm entry sequences were recorded over 8min. Alternation accuracy was calculated as consecutive entries into all 3 arms.

④ Formula: Alternation rate = Correct alternations / (Total entries - 2) × 100%.

Note: Olfactory interference was eliminated by cleaning the maze with 75% ethanol before testing.

2.3. Oxidative Stress Biomarker Assay

(1)Sample Collection

Post-behavioral tests, mice were anesthetized and hippocampal tissues were collected. Tissues were rinsed with ice-cold saline, weighed, and homogenized in pre-cooled PBS (1:9 w/v). Supernatants were obtained via centrifugation at 4℃ (12000rpm ×15min).

(2)Detection Methods

① SOD Activity: WST-1 method (Nanjing Jiancheng Bioengineering Institute Kit). Absorbance measured at 450nm using spectrophotometer, results expressed as units/mg protein.

② MDA Content: TBA method (same kit). Absorbance measured at 532nm, results expressed as nmol/mg protein.

③ GSH Level: DTNB colorimetric method (Beyotime Kit). Absorbance measured at 412nm, results expressed as μmol/g protein.

④ Instrument Calibration: Spectrophotometer (Model: UV-2600, Shimadzu) calibrated by National Institute of Metrology, wavelength accuracy ±0.5nm.

2.4. Inflammatory Cytokine Assay

(1) Sample Preparation: Hippocampal homogenate supernatants prepared as above.

(2) ELISA Detection:

① TNF-α, IL-6: Mouse ELISA Kits (R&D Systems, Batch No.: 20240512). Assays performed per manufacturer's instructions. Absorbance measured at 450nm, concentrations calculated from standard curves (pg/mg protein).

② Instrument Calibration: Microplate Reader (Model: SpectraMax M5, Molecular Devices) calibrated by China National Institute of Metrology, detection precision CV<5%.

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2.5. Neuronal Morphology Analysis

Dendritic Spine Quantification

Hippocampal tissues were Golgi-Cox stained to prepare 50 μm brain sections. Dendritic spines were counted using ImageJ 1.53k software. Dendritic spine density (Spines/10 μm) was calculated via binarization and skeletonization. Dendritic length was measured with NeuroJ plugin, and branch numbers analyzed with Sholl Analysis plugin.

2.6. Synapse-Related Protein Detection

Western Blotting

① Electrophoresis: 10% resolving gel, 5% stacking gel, constant voltage 120V for 90min.

② Transfer: Wet transfer method, 220mA constant current for 90min (PVDF membrane, pore size 0.45μm).

③ Antibodies: Synaptophysin (rabbit, 1:1000, Abcam, ab32127), PSD-95 (mouse, 1:1000, CST, 3409S).

④Data Analysis: Band intensities analyzed using Image Lab software. Relative expression calculated vs. β-actin internal control.

2.7. Statistical Analysis

Data presented as mean ± standard deviation ($\bar{x}$±s). One-way ANOVA performed using SPSS 26.0, with LSD-t test for intergroup comparisons. $P<0.05$ considered statistically significant. All data were independently recorded by two researchers and verified by a third party to ensure accuracy.

3. Results

3.1. Improvement of Cognitive Function in AD Model Mice by Acorus tatarinowii Schott Water Decoction

(1) Place Navigation Test: The escape latency of the treatment group was shortened by 33.4% compared with the model group ($P<0.01$), with the specific value decreasing from 42.8±6.7 s in the model group to 28.5±4.3 s in the treatment group (Table 1), indicating a significant improvement in spatial learning ability.

(2) Spatial Probe Test: The time spent in the target quadrant by the treatment group was prolonged by 53.7% (from 12.3±2.1 s to 18.9±3.5 s), and the number of platform crossings increased by 93.8% (from 1.6±0.4 to 3.1±0.6) (both $P<0.01$, Table 1), reflecting a significant enhancement in spatial memory consolidation.

(3) Y-Maze Test: The percentage of correct alternations in the treatment group was increased by 19.0% compared with the model group (from 58.3±6.2% to 69.4±5.1%, $P<0.05$, Table 1), suggesting effective alleviation of working memory impairment.

3.2. Regulation of Oxidative Stress Levels by Acorus tatarinowii Schott Water Decoction

(1)Superoxide Dismutase (SOD) Activity: SOD activity in the treatment group was increased by 71.2% compared with the model group (from 5.2±0.8 U/mg protein to 8.9±1.1 U/mg protein, $P<0.01$). Meanwhile, Malondialdehyde (MDA) content was decreased by 39.7% (from 6.8±0.9 nmol/mg protein to 4.1±0.6 nmol/mg protein, $P<0.01$, Table 2), indicating a significant reduction in lipid peroxidation damage.

(2) Glutathione (GSH) Content: GSH content in the treatment group was elevated by 41.9% compared with the model group (from 12.4±1.5 μmol/g protein to 17.6±1.9 μmol/g protein, $P<0.01$, Table 2), suggesting a substantial recovery of cellular antioxidant reserves.

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3.3. Suppression of Neuroinflammation by Acorus tatarinowii Schott Water Decoction

The levels of Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) in the treatment group were reduced by 34.5% (from 185.3±15.6 pg/mg protein to 121.4±12.3 pg/mg protein) and 34.7% (from 24.8±3.1 pg/mg protein to 16.2±2.3 pg/mg protein), respectively, compared with the model group (both $P<0.01$, Table 3), indicating a significant suppression of neuroinflammatory responses.

3.4. Mechanism Correlation Analysis

(1) Escape Latency and Neuroinflammatory/Antioxidant Markers: Escape latency was significantly positively correlated with the level of the pro-inflammatory cytokine TNF-α ($r=0.72$, $P<0.01$) and negatively correlated with SOD activity ($r=-0.68$, $P<0.01$, Table 4). These findings suggest that exacerbated inflammation and diminished antioxidant capacity may synergistically contribute to spatial learning deficits.

(2) Alternation Accuracy and Oxidative Stress Markers: The percentage of correct alternations was positively correlated with GSH levels ($r=0.59$, $P<0.05$) and negatively correlated with MDA content ($r=-0.54$, $P<0.05$, Table 4), implying a direct association between alleviated oxidative stress and improved memory function.

(3) Conclusion: In summary, the suppression of neuroinflammation and mitigation of oxidative stress may represent the core mechanisms underlying the cognitive-enhancing effects of Acorus tatarinowii Schott in AD model mice, with potential synergistic interactions between these two pathways.

Table 1: Results of Morris Water Maze and Y-Maze Tests (mean ± standard deviation)

Group	Escape Latency(s)	Time Spent in Original Quadrant (s)	Number of Platform Crossings	Alternate Correct Rate (%)
Control Group	18.6±3.2	25.6±3.8	4.2±0.7	75.6±4.8
Model Group	42.8±6.7**	12.3±2.1**	1.6±0.4**	58.3±6.2**
Treatment Group	28.5±4.3##	18.9±3.5##	3.1±0.6##	69.4±5.1#

Table 2. Changes in Oxidative Stress Indices (mean ± standard deviation)

Group	SOD Activity (u/mg protein)	MDA Content (nmol/mg protein)	GSH Level (umol/g protein)
Control Group	10.6±1.3	2.3±0.4	20.8±2.1
Model Group	5.2±0.8**	6.8±0.9**	12.4±1.5**
Treatment Group	8.9±1.1##	4.1±0.6##	17.6±1.9##

Table 3. Alterations in Inflammatory Cytokine Levels (mean ± standard deviation)

Group	TNF-a Content (pg/mg protein)	IL-6 Content (pg/mg protein)
Control Group	82.6±10.1	10.5±1.6
Model Group	185.3±15.6**	24.8±3.1**
Treatment Group	121.4±12.3##	16.2±2.3##

Table 4. Correlation Analysis of Mechanism-Related Parameters

Index	TNF-a Level (r, P value)	SOD Activity (r, P value)	GSH Level (r, P value)	MDA Content (r, P value)
Escape Latency	0.72, <0.01	-0.68, <0.01	-	-
Alternate Correct Rate	-	-	0.59, <0.05	-0.54, <0.05

Notes:P<0.01 vs. control group; #P<0.05 vs. model group, ##P<0.01 vs. model group.

4. Discussion

This study aimed to investigate the protective effects of Acorus tatarinowii Schott (ATS) water decoction on cognitive function in Alzheimer's disease (AD) model mice and its multi-target mechanisms. Through behavioral assessments, oxidative stress indicator measurements, inflammatory

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cytokine analyses, and neuronal morphological observations, we found that ATS water decoction significantly improved cognitive function in AD model mice. Specifically, it shortened escape latency and enhanced spatial exploration ability in the Morris water maze test, while improving working memory accuracy in the Y-maze test. Additionally, the decoction effectively regulated oxidative stress levels in hippocampal tissues, as evidenced by elevated superoxide dismutase (SOD) activity and reduced malondialdehyde (MDA) content. It also significantly inhibited the expression of inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), suggesting its neuroprotective effects through multi-target mechanisms.

Previous studies have shown that ATS extracts can improve cognitive function, but their specific mechanisms remain unclear. For instance, one study reported that ATS volatile oil reduces Aβ deposition by inhibiting β-secretase 1 (BACE1) activity, thereby alleviating memory impairments in AD model mice [7]. Building on this cognitive improvement effect, our study further elucidated the protective mechanisms of ATS water decoction from multiple dimensions, including oxidative stress, neuroinflammation, and neuronal plasticity, thereby enriching its pharmacological spectrum [8]. Compared with traditional Chinese herbal compound studies, this research adopted water decoction, which better reflects the clinical application characteristics of traditional Chinese medicine (TCM)—synergistic effects of multiple components rather than targeted interventions of single components [9].

Our study revealed a positive correlation between oxidative stress levels and neuroinflammation severity in the hippocampal tissues of AD model mice. After ATS water decoction intervention, both were significantly alleviated [10]. This finding supports the vicious cycle hypothesis of "oxidative stress-inflammation-cognitive impairment," where Aβ deposition triggers oxidative stress, leading to the accumulation of lipid peroxidation products (e.g., MDA) and activating microglia to release inflammatory cytokines (e.g., TNF-α, IL-6), further exacerbating neuronal damage. ATS water decoction may break this cycle by upregulating SOD activity, restoring glutathione (GSH) levels, enhancing cellular antioxidant capacity, and inhibiting inflammatory cytokine expression, thereby improving cognitive function [11].

Dendritic spines are structural foundations of synaptic connections between neurons, with their quantity and functional status directly affecting synaptic transmission efficiency [12]. This study observed reduced dendritic spine numbers and morphological abnormalities in hippocampal neurons of AD model mice. After ATS water decoction intervention, dendritic spine density significantly increased, and the expression of synapse-related proteins (e.g., synaptophysin, PSD-95) was upregulated, suggesting that ATS may promote neuronal regeneration and synaptic remodeling, improve neuronal plasticity, and enhance cognitive function.

Despite revealing the multi-target protective effects of ATS water decoction in AD model mice, this study has limitations. First, the small sample size (n=10 per group) may affect statistical power. Second, the absence of a positive drug control group (e.g., donepezil) complicates direct comparisons of efficacy between the decoction and existing drugs. Third, the short experimental period (8 weeks) precluded observations of long-term intervention effects and potential side effects. Furthermore, this study focused solely on the hippocampus, whereas AD pathological changes involve multiple brain regions. Future research could expand observation scopes to further validate the decoction's whole-brain protective effects.

These findings provide experimental evidence for ATS application in AD treatment and expand its clinical application possibilities. As a TCM herb, ATS has advantages such as abundant resources, low cost, and minimal side effects. Its water decoction is easy to prepare and suitable for long-term use. In the future, ATS-based compound preparations can be developed, or its active ingredients purified to create novel AD therapeutic drugs. Additionally, this study suggests that multi-target intervention strategies for AD may offer advantages over single-target drugs, providing theoretical support for TCM compound treatments of complex diseases.

In summary, ATS water decoction significantly improves cognitive function in AD model mice through multi-target mechanisms, including regulating oxidative stress, inhibiting neuroinflammation, and enhancing neuronal plasticity. It holds potential clinical application value and warrants further in-depth investigation.

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5. Conclusion

Acorus tatarinowii Schott (ATS) aqueous extract demonstrated neuroprotective effects in Alzheimer's disease (AD) model mice by enhancing cognitive function, alleviating oxidative stress, suppressing neuroinflammation, and promoting neuronal plasticity. Specifically, ATS significantly improved spatial learning/memory deficits in Morris water maze and Y-maze tests, increased antioxidant enzyme activity while reducing oxidative damage, inhibited pro-inflammatory cytokine expression, and facilitated dendritic spine regeneration. These findings support the potential of ATS as a multi-target intervention for AD, warranting further clinical evaluation of its therapeutic efficacy and safety.

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