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A Comprehensive Approach to Preventing Tauopathy and Enhancing Learning and Memory through Neurotrophic Factor Induction with *Cistanche tubulosa* Extract

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Abstract

The neuroprotective efficacy and safety of *Cistanche tubulosa* extract (CTE) in reducing tauopathy levels were studied. However, evidence-based insights into how CTE enhances memory ability remain unclear. A total of 85 healthy volunteers (with MMSE scores ≥ 24, which is Normal/No impairment) participated in a double-blind, randomized, placebo-controlled study. Participants were assigned to one of three groups: Group A (600 mg CTE), Group B (300 mg CTE), or Group C (placebo), administered once daily for six weeks. Efficacy was assessed using the clinical memory scale, along with measurements of brain-derived neurotrophic factor (BDNF) and acetylcholinesterase (AChE) concentrations. Results showed statistically significant changes in BDNF and AChE levels in both treatment groups (Groups A and B) compared to baseline and the placebo group. Memory tests also demonstrated significant improvements ($p < 0.01$) in summary test scores in Groups A and B compared to Group C after six weeks. Additionally, we utilized the Tol2 transposon-mediated gene transfer method to generate transgenic zebrafish expressing tau-GFP (green fluorescent protein), and employed CTE compounds (echinacoside, acteoside, and isoacteoside) to screen for anti-secretase enzyme activity. Results from transgenic fluorescent triple-transgenic (3xTg-AD) zebrafish demonstrated that CTE exhibits anti-tauopathy, anti-secretase, and neuroprotective properties. These findings, combined with the above evidence, confirm that CTE has potential neuroprotective effects and may improve memory function.

Subject Areas

Biotechnology and Neuroscience

Keywords

Neurotrophic, Cistanche Tubulosa, Memory, BDNF, Transgenic Zebrafish, Anti-Secretase

1. Introduction

The rapid growth of the global aging population has led to a significant rise in the number of older individuals living with dementia in recent years. In 2019, an estimated 51.6 million people worldwide were affected by dementia, with over a quarter (13.1 million) residing in China [1]. WHO collaborating centers support dementia research and promote evidence-based biomedical practices aimed at preventing declines in physical and mental health, including those linked to unhealthy lifestyles [2]. Tau, a microtubule-associated protein abundantly expressed in central nervous system (CNS) neurons, plays a critical role in maintaining microtubule stability within neuronal axons [3]. Hyperphosphorylation of tau causes abnormal aggregation, impairing neuronal function by destabilizing microtubules. While acetylcholinesterase inhibitors (AChEIs), such as donepezil and rivastigmine, help alleviate symptoms by increasing intracerebral acetylcholine levels, abnormally phosphorylated tau proteins accumulate to form neurofibrillary tangles [4] [5]. They disrupt synaptic communication by damaging neuronal connections, particularly in brain regions associated with memory, underscoring the urgent need for early diagnosis and prevention of memory impairment.

Cistanche tubulosa extract (CTE) are botanical prescription drug for vascular dementia in Chinese mainland. CTE of active ingredients extracted from the desert plant *Cistanche tubulosa* (Schenk) R. Wight. These are rich in phenylethanoid glycosides, primarily echinacoside, acteoside, and isoacteoside. Over the past few decades, CTE has demonstrated diverse pharmacological activities, including neuroprotection, memory and learning enhancement, cardiac function improvement, reduction of hyperlipidemia and hyperglycemia, and prevention of obesity-induced diabetes and metabolic syndrome [6].

Among its key components, echinacoside exhibits neuroprotective effects in Parkinson's disease models, suggesting therapeutic potential for AD [7]. Acteoside shows protective effects against amyloid-beta-induced memory impairment, glutamate-induced neurotoxicity, and neuronal damage in cultured neurons and rat cortical cells, primarily due to its potent antioxidant properties [8]. Isoacteoside has been shown to promote exploratory behavior and restore cortical and hippocampal dopamine levels in rats. Collectively, these findings suggest that CTE may enhance learning and memory by inducing nerve growth factor [9] [10].

Neurotrophic factors, particularly brain-derived neurotrophic factor (BDNF)

and glial cell line-derived neurotrophic factor (GDNF), play key roles in neuroplasticity [11]. BDNF supports the differentiation, maturation, and survival of neurons and has been a focus of research as a potential blood-based biomarker for AD. However, studies on plasma BDNF levels in AD patients have yielded conflicting results. GDNF, on the other hand, has emerged as a potent neurotrophic factor with therapeutic potential against neurodegenerative diseases, including AD. Previous studies have utilized plasmid DNA (Tol2 transposon-donor plasmid) transgenic zebrafish models, such as the triple-transgenic (3xTg-AD) zebrafish expressing PS1M146V, APPSwe, and tauP301L transgenes, to investigate AD pathology [12]. Lentiviral vector-mediated overexpression of the GDNF gene in hippocampal astrocytes of 3xTg-AD mice demonstrated neuroprotective effects in experimental AD models [13]. Furthermore, zebrafish embryos expressing Green fluorescent protein (GFP) fusion proteins of zebrafish and human tau under the control of a neuron-specific HuC promoter have been developed to identify anti-tauopathy drugs and treatments for AD [14].

Despite significant advancements in AD research, the disease remains incurable. To develop effective therapeutic agents for treating and preventing AD progression, it is essential to investigate the underlying mechanisms of tauopathy. We hypothesized that CTE may exert beneficial effects on memory ability in healthy individuals, supported by its evidence-based neuroprotective properties.

This randomized, double-blind, placebo-controlled preliminary observational study was conducted to evaluate the effects of CTE on memory function in a healthy population, with assessments of brain-derived neurotrophic factor (BDNF) and acetylcholinesterase (AChE) levels. Additionally, we employed previously developed zebrafish tauopathy models, including the 3xTg-AD zebrafish model, to screen CTE isolates (echinacoside, acteoside, and isoacteoside) for anti-secretase enzyme activity and neurotrophic support.

2. Materials and Methods

2.1. Preparation of CTE Materials

The stem of *Cistanche tubulosa* (CT) was cut into small pieces and extracted by refluxing with water, after which the resulting filtrate was collected. The filtrate was concentrated. Ethanol was then added to the concentrate, and the supernatant was collected. This supernatant was further purified using macro-porous absorption resin and subsequently spray-dried to obtain the aqueous extract (CTE). The yield of the CTE is 10% from the dried stem of CT. This extract was produced by Sinphar Tian-Li Pharmaceutical Co., Ltd.

For this study, zebrafish embryos were treated with CTE and assigned to four treatment groups: 1) QT1: CTE, 2) QT2: Echinacoside, 3) QT3: Acteoside, and 4) QT4: Isoacteoside.

2.1.1. Zebrafish Care

Zebrafish embryos were raised at 28.5˚C, and their developmental stages were de-

termined based on the criteria described in *The Zebrafish Book*. All animal procedures were approved by the Academia Sinica Institutional Animal Care and Utilization Committee (ASIACUC) (protocol #10-12-114).

2.1.2. Microinjection of Zebrafish Embryos

Plasmid DNA (Tol2 transposon-donor plasmid) was injected into one-cell zygotes using a microinjection system consisting of a SZX9 stereomicroscope (Olympus, Tokyo, Japan) and an IM300 Microinjector (Narishige, Tokyo, Japan). The concentration of all plasmid DNA used to do the microinjection is about 500 ng/μl and the amount of all plasmid DNA injected into zebrafish embryos is about 0.2 ng. The working concentration of CTE (QT1 - 4) is 400 μM. Embryos at 48 and 72 hours post-fertilization (hpf) were observed under an Olympus IX70-FLA inverted fluorescence microscope. Images were taken using the SPOT system (Diagnostic Instruments, Sterling Heights, MI).

2.2. Screening for Anti-Tauopathy and Neurite Outgrowth Effects

2.2.1. Screening for Anti-Tauopathy Effects

Truncated human tau proteins in zebrafish were tagged with green fluorescent protein (GFP). Zebrafish embryos injected with tau-GFP were treated with QT1 - QT4 at 48 and 72 hours post-fertilization. High-content immunofluorescence live imaging analysis was performed to assess anti-tauopathy effects.

2.2.2. Screening for Human-APPswe Anti-Secretase Effects

To investigate anti-secretase activity, zebrafish embryos co-expressing Bcl-2 and tau-GFP in spinal cord neurons were used. Embryos co-injected with pHuC-APPswe and pHuC-APPswe-2A-PS1 (M146V) were treated with QT1 - QT4 at 48- or 72-hours post-injection. Anti-secretase effects were evaluated using high-content direct imaging and time-lapse recording analysis.

2.2.3. Screening for Neurite Outgrowth Effects

To express GFP fusion proteins in neurons, GFP genes were driven by the zebrafish neuron-specific HuC promoter. Zebrafish neurons expressing GFP fusion proteins were treated with QT1 - QT4 for 72 hours post-fertilization (hpf), and neurite outgrowth was observed and analyzed.

2.3. Preliminary Observational Study Procedure in a Healthy Population

A double-blind, randomized, placebo-controlled preliminary observational study was conducted to evaluate the effects of CTE on learning and memory. Eligible participants were assigned to one of three groups: Group A (600 mg CTE), Group B (300 mg CTE), or Group C (placebo).

Participants were instructed to take the assigned treatment once daily for six weeks. Healthy volunteers with Mini-Mental State Examination (MMSE) scores ≥ 24 were recruited. The study was conducted from August 8, 2008, to December

31, 2008.

2.4. Efficacy and Safety Evaluation

The efficacy endpoint was assessed using the Clinical *Memory Scale* developed by the Psychological Institute of the Chinese Academy of Sciences. The scale comprises five components: 1) directed memory, 2) associative learning, 3) picture-free recall, 4) meaningless figure recollection, and 5) associative memory of portrait characteristics.

The raw scores were converted to an equivalent scale score and memory quotient. Memory ability assessments were conducted at baseline and at the end of the study.

Blood and urine samples were collected at baseline and at the end of the study for biochemical analysis, including white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, creatinine, blood urea nitrogen (BUN), albumin, total protein, alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

The concentrations of brain-derived neurotrophic factor (BDNF) and acetylcholinesterase (AChE) were also measured.

2.5. Statistical Analysis

Anti-secretase enzyme activity and neurotrophic effects of CTE in zebrafish embryos were evaluated using direct imaging and time-lapse recording, including neurite outgrowth measurements.

For the preliminary observational study, clinical Memory Scale efficacy endpoints were analyzed for participants who completed the six-week treatment. A two-sided *t*-test was used to assess changes from baseline, and between groups by one-way ANOVA test. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Anti-Tauopathy and Neurite Outgrowth Effects

This study identified three key findings demonstrating that CTE exhibits antioxidative and neurotrophic properties, contributing to neuroprotection and maintaining human tau-GFP protein in its phosphorylated state, which is essential for preserving microtubule stability within neuronal axons.

3.1.1. Anti-Tauopathy Effects

Treatment with QT1 and QT3 demonstrated antioxidative and neurotrophic effects, leading to a protective outcome and maintaining human tau-GFP protein in a phosphorylated state. Truncated human tau proteins in zebrafish were tagged with green fluorescent protein (GFP) and designated as pHuC-hTau-ΔC50-GFP (**Figure 1(a)**). Viable GFP-positive neurons were counted in individual embryos. QT1 and QT3 effectively suppressed neurotoxicity caused by hyperphosphorylated and aggregated tau (**Figure 1(b)**), whereas QT2 and QT4 exhibited neurotoxic effects (**Figure 1(c)**).

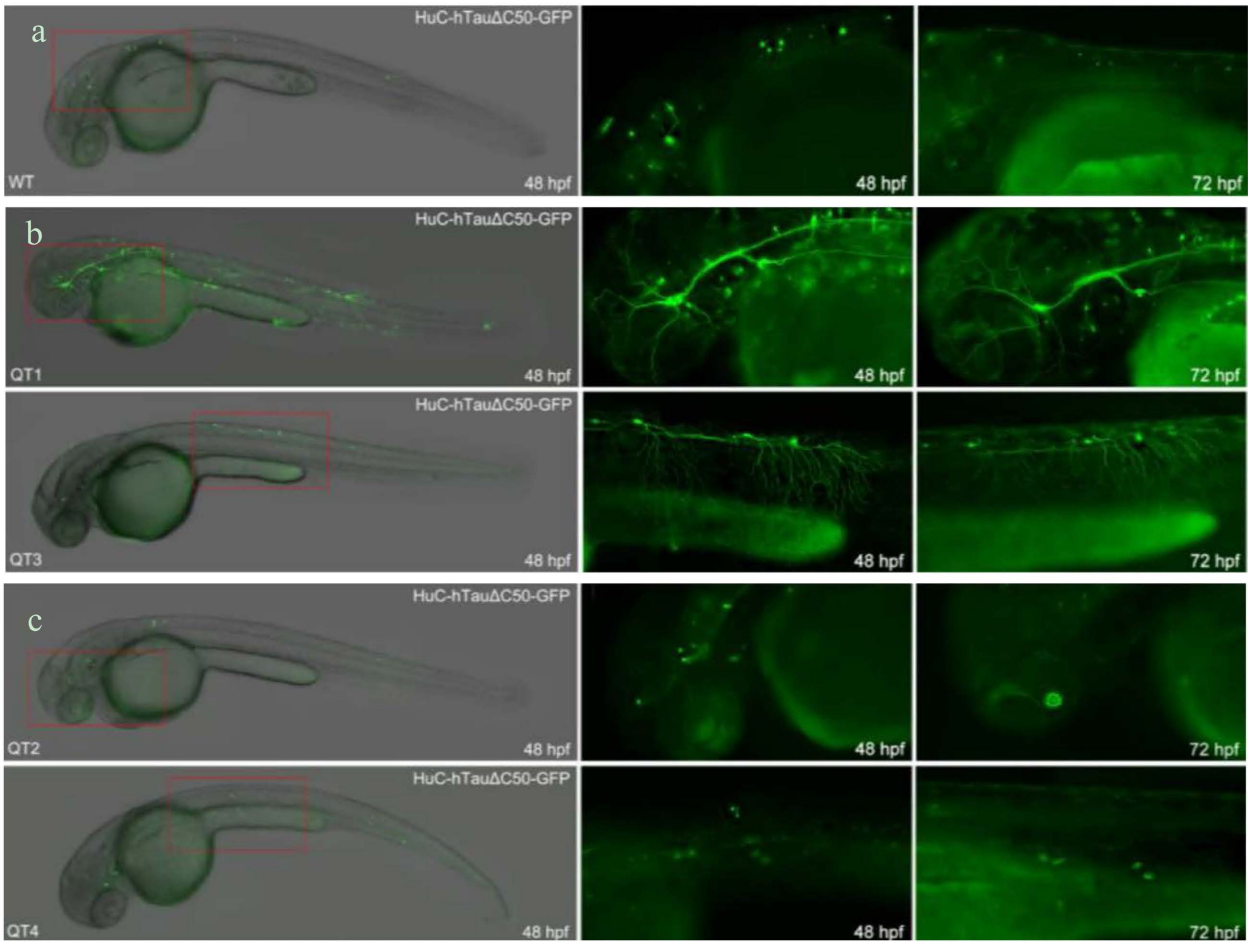


Figure 1. Zebrafish embryos GFP-labeled neuronal cells and axons were observed at 48 and 72 hpf in embryos. (a) Truncated human tau proteins in zebrafish were tagged with green fluorescent protein (GFP) and designated as pHuC-hTau-ΔC50-GFP; (b) QT1 and QT3 effectively suppressed neurotoxicity caused by hyperphosphorylated and aggregated tau; (c) QT2 and QT4 exhibited neurotoxic effects.

3.1.2. Human-APPswe Anti-Secretase Effects

Truncated human tau proteins in zebrafish co-expressing Bcl-2 and tau-GFP in spinal cord neurons were designated as pHuC-hTau-V3-GFP-IRES-Bcl2, tagged with green fluorescent protein, and were observable. Treatment with QT1 - QT4 at 48 or 72 hours post-injection resulted in the presence of GFP-tagged neuronal cells and axons in embryos injected with pHuC-APPswe. Additionally, QT1, QT3, and QT4 effectively suppressed neurotoxicity induced by pHuC-APPswe injection. These effects are shown in **Figure 2**.

3.1.3. Human Neurite Outgrowth Effects

Zebrafish embryos injected with ITR-HuC-GFP and treated with QT1, QT3, or QT4 exhibited neurite outgrowth at 72 hours post-fertilization (**Figure 3**). Statistical analysis indicated that embryos treated with QT1, QT3, and QT4 had a significantly higher percentage of neuronal cells compared to controls. It is noted that QT4 promoted neurite outgrowth at 72 hours post-fertilization due to its antioxidative and neurotrophic properties.

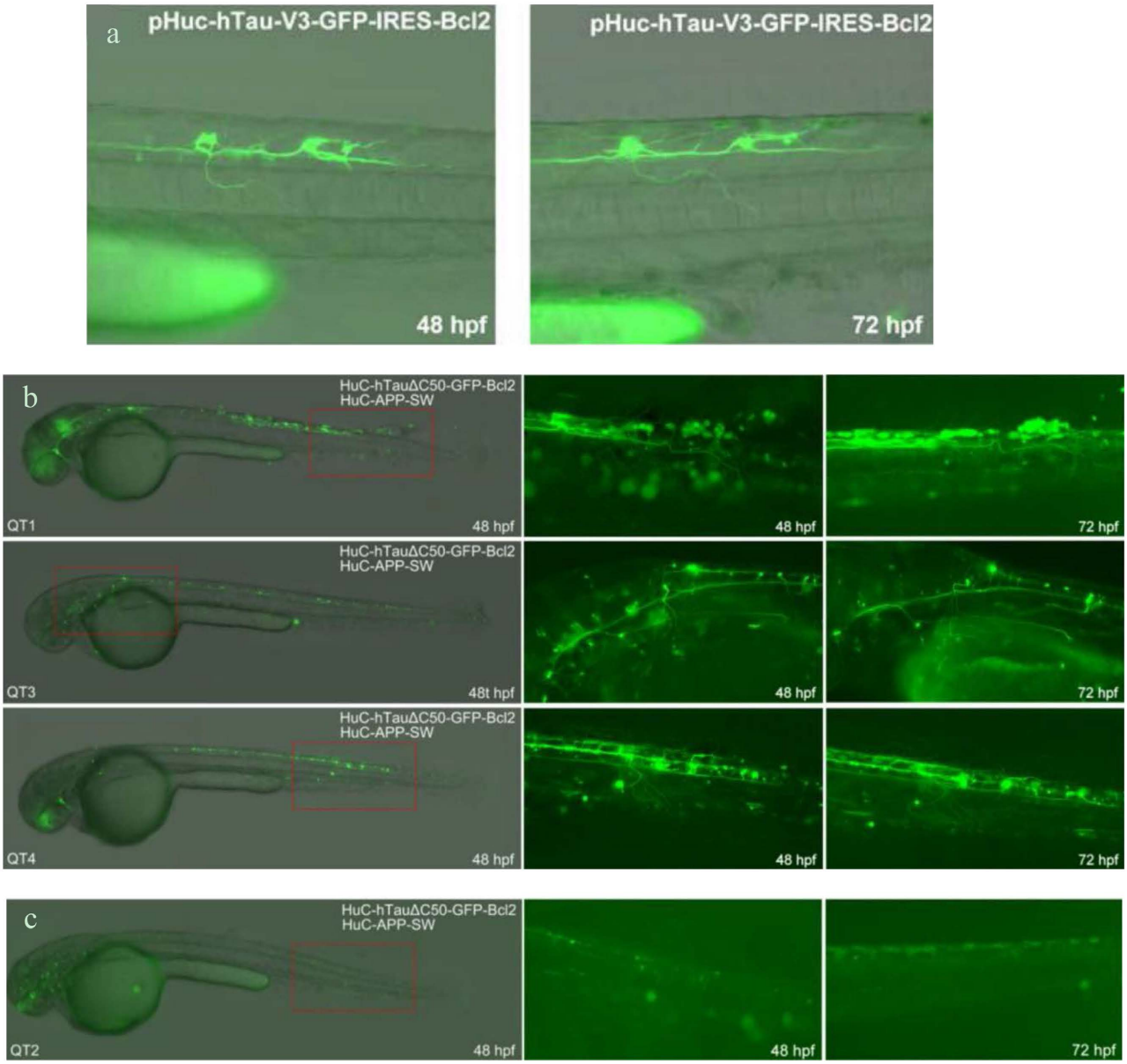
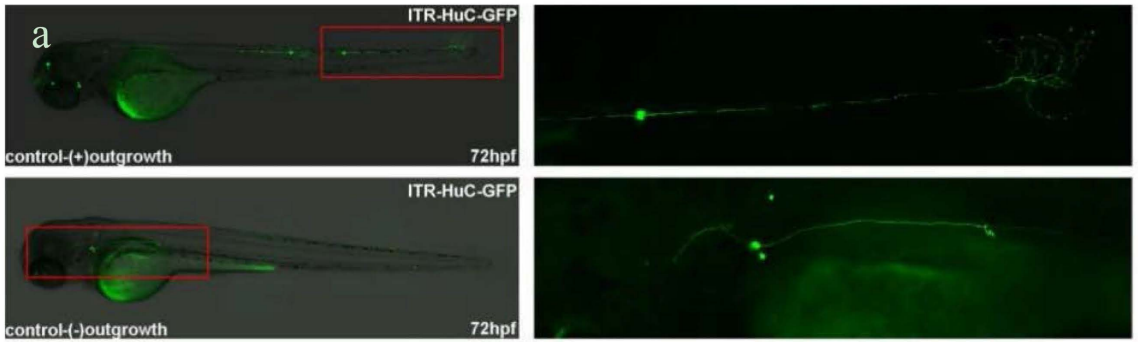
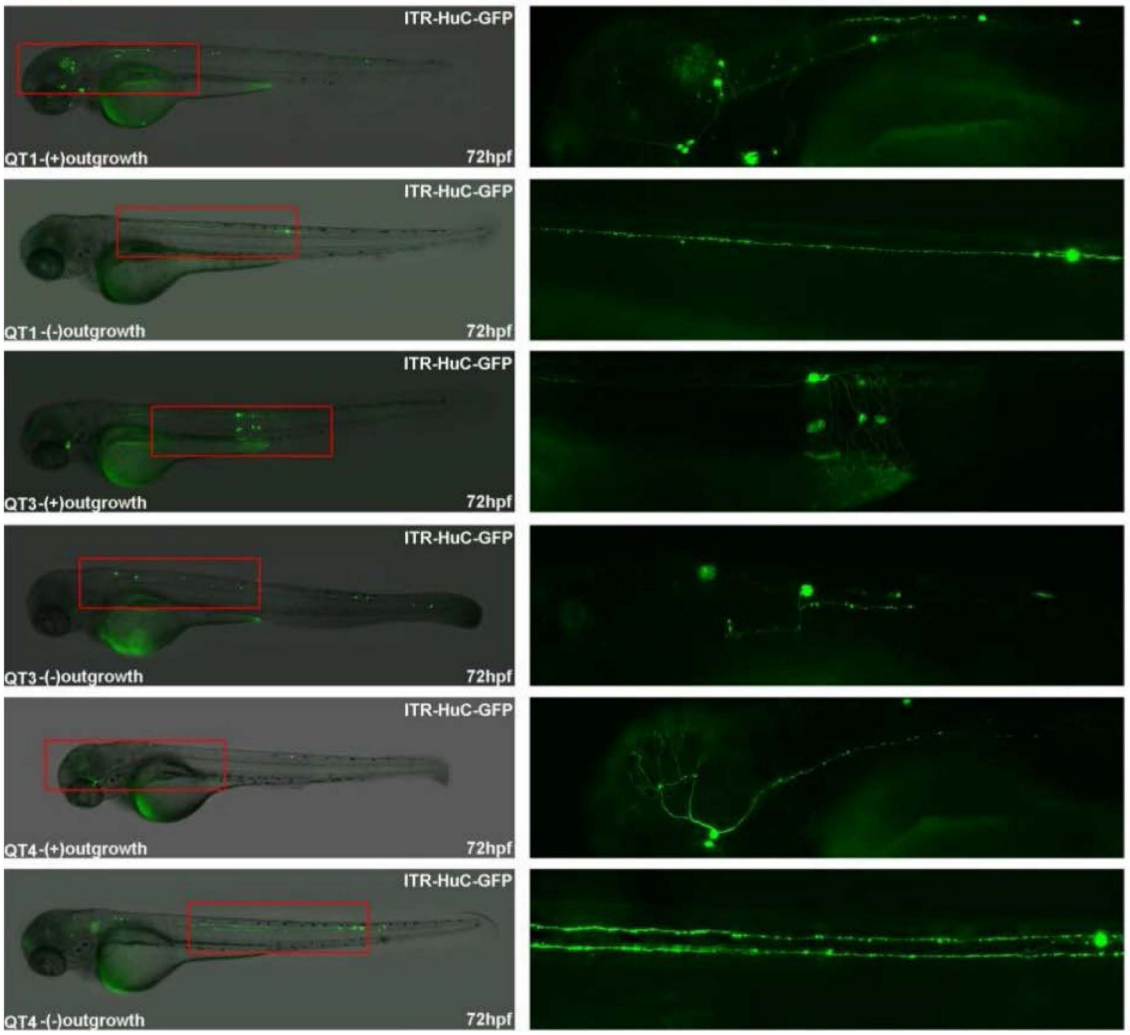


Figure 2. Zebrafish embryos co-expressing Bcl-2 and tau-GFP in spinal cord neurons were observed at 48 and 72 hpf in embryos. (a) Truncated human tau proteins in zebrafish were tagged with green fluorescent protein (GFP) and designated as pHuC-hTau-V3-GFP-IRES-Bcl2; (b) QT1, QT3 and QT4 effectively suppressed neurotoxicity caused by injected with pHuC-APPswe; (c) QT2 exhibited neurotoxic effects.





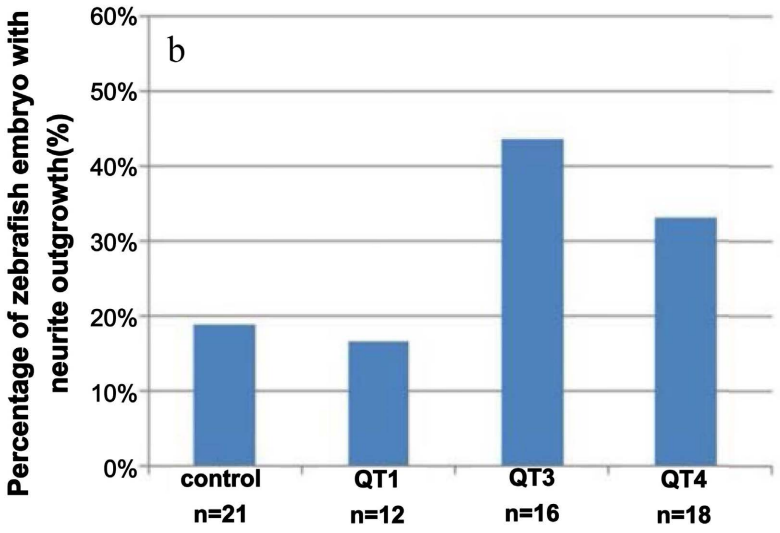


Figure 3. Zebrafish embryos neurons expressing GFP fusion proteins were treated with QT1 - QT4 for 72 hours. (a) Zebrafish embryos injected with ITR-HuC-GFP and treated with QT1, QT3, or QT4 exhibited neurite outgrowth at 72 hours post-fertilization; (b) Statistical analysis indicated that embryos treated with QT1, QT3, and QT4 had a significantly higher percentage of neuronal cells compared to controls.

3.2. Preliminary Observational Study

3.2.1. Demographic Analysis of Healthy Subjects

The demographic characteristics of participants who completed the study are summarized in Table 1. A total of 93 participants were enrolled, with 85 subjects (47 males and 38 females) completing the study. Participants were randomly assigned to one of three groups: Group A (n = 27), Group B (n = 32), or Group C (n = 26).

Table 1. Demographic analysis of healthy subjects.

Group	600 mg (Group A)	300 mg (Group B)	Placebo (Group C)	Total	P value
Case Number (%)	27 (31.8)	32 (37.6)	26 (30.6)	85 (100)	
Age (Mean ± SD)	44.8 ± 10.7	44.0 ± 11.0	42.1 ± 8.8	43.7 ± 10.2	0.618
<40	11 (40.7)	15 (46.9)	11 (42.3)	37 (43.5)	
≥40	16 (59.3)	17 (53.1)	15 (57.7)	48 (56.5)	
Gender					
Male	15 (17.6)	17 (20.0)	15 (17.6)	47 (55.3)	
Female	12 (14.1)	15 (17.6))	11 (13.0)	38 (44.7)	
Education (Mean ± SD)	10.9 ± 4.7	12.0 ± 3.4	11.8 ± 3.2	11.6 ± 3.8	0.524
Memory Test (MQ)					
Baseline (Mean ± SD)	106.0 ± 14.6	108.7 ± 16.9	107.4 ± 14.7	107.4 ± 15.2	0.7657

One-way ANOVA Test.

3.2.2. Results of Memory Tests

No significant differences in memory test outcomes were observed between treatment groups. Improvements in memory tests from baseline were noted in all three groups. Pairwise comparisons of change-from-baseline scores showed statistically significant improvements (from P-value < 0.05 to P-value < 0.01) in Groups A (600 mg) and B (300 mg) compared to Group C (placebo). While Group A exhibited greater improvements than Group B, the difference was not statistically significant (P = 0.273) (Table 2).

Table 2. Memory test of study participants (N = 85).

	600 mg (Group A) N = 27	300 mg (Group B) N = 32	Placebo (Group C) N = 26
Memory Test	*Mean* (*SD*)	*Mean* (*SD*)	*Mean* (*SD*)
1. Summary Test at Baseline	**106.0 (14.6)**	**108.7 (16.9)**	**107.4 (14.7)**
1.1 Directed memory	25.0 (8.9)	25.6 (7.7)	25.9 (7.7)
1.2 Association study	20.4 (6.6)	19.9 (6.0)	21.7 (7.2)
1.3 Pictures freely memory	22.6 (4.6)	23.4 (5.3)	23.2 (6.3)
1.4 Meaningless figures recollection	24.6 (6.9)	24.8 (5.5)	25.3 (6.7)

Continued

1.5 Associational memory of portrait's characteristics	18.3 (5.0)	20.4 (3.5)	21.2 (6.1)
2. Summary Test at End of Study	**124.6 (12.6)**	**123.3 (10.1)**	**116.5 (14.5)**
2.1 Directed memory	28.6 (6.4)	28.7 (4.7)	27.7 (6.4)
2.2 Association study	26.3 (4.9)	26.5 (5.5)	26.3 (6.5)
2.3 Pictures freely memory	25.5 (4.8)	26.6(4.6)	25.0 (6.0)
2.4 Meaningless figures recollection	27.8 (4.5)	27.9 (4.3)	25.2 (4.0)
2.5 Associational memory of portrait's characteristics	27.3 (3.5)	25.6 (4.0)	24.5 (6.6)
3. Summary Test at Change from Baseline	**21.3 (14.2)****	**18.6 (11.7)****	**6.8 (11.2)****
3.1 Directed memory	3.7 (3.2)*	2.7 (3.3)*	1.2 (2.4)
3.2 Association study	5.4 (4.0)**	6.4 (3.8)**	3.8 (6.2)**
3.3 Pictures freely memory	2.3 (3.3)**	2.3 (3.2)**	1.1 (3.4)
3.4 Meaningless figures recollection	3.9 (9.3)*	2.8 (6.6)**	−1.3 (6.3)
3.5 Associational memory of portrait's characteristics	6.1 (4.3)**	4.3 (4.0)**	2.0 (3.0)**

Comparison of within group difference (change-from-baseline) is conducted by paired t-test; Pairwise comparisons of between group difference is conducted by two sample t-test. *P-value < 0.05; **P-value < 0.01.

3.2.3. Results of Safety Evaluation

Analysis of safety endpoints (Table 3) revealed no significant changes in biochemical parameters from baseline, and no treatment-related adverse reactions were observed during the study period.

Table 3. Safety endpoints of biochemical analysis in study participants (N = 85).

	600 mg (Group A) N = 27		300 mg (Group B) N = 32		Placebo (Group C) N = 26	
	Baseline	End of Study	Baseline	End of Study	Baseline	End of Study
Variables	*Mean* (*SD*)	*Mean* (*SD*)	*Mean* (*SD*)	*Mean* (*SD*)	*Mean* (*SD*)	*Mean* (*SD*)
WBC (10^9/L)	4.8	4.7	4.8	4.7	4.5	4.5
RBC (10^{12}/L)	5.7	5.9	6.3	6.3	6.2	6.1
Hemoglobin (g/L)	137.6	138.4	135.2	137.9	129.3	132.0
Creatinine (μmol/L)	62.6	62.8	59.1	62.4	54.7	61.1
BUN (mmol/L)	5.0	5.2	4.6	4.6	4.8	4.3
Albumin (g/L)	46.9	48.9	48.4	49.0	47.3	49.2
Total Protein (g/L)	76.7	76.2	78.1	76.4	77.6	78.1
ALT (U/L)	22.0	20.0	23.1	23.1	21.8	16.4
AST (U/L)	25.0	23.8	23.4	24.1	24.8	23.6

Comparison of within group difference in change-from-baseline is conducted by paired t-test. *P-value < 0.05; **P-value < 0.01.

3.2.4. Results of Serums BDNF and AchE Levels

Biomarker analysis (Table 4) indicated statistically significant changes-from-baseline in BDNF and AChE levels in Groups A and B, both of which received CTE treatment. These changes were also significantly greater compared to the placebo group.

Table 4. Biomarker analysis of BDNF and AChE in selected study participants.

BDNF (pg/mL)	Baseline *Mean* (*SD*)	End of Study *Mean* (*SD*)	P-value[a]	P-value[b]
Group A: 600 mg	335.8 (277.3)	842.5 (513.3)	0.02	<0.01
Group B: 300 mg	371.1 (246.3)	754.2 (380.5)	0.02	<0.01
Group C: Placebo	343.2 (194.4)	235.6 (155.8)	0.23	-
AChE (U/mL)	Baseline *Mean* (*SD*)	End of Study *Mean* (*SD*)	P-value[a]	P-value[b]
Group A: 600 mg	27.2 (6.9)	13.9 (3.7)	<0.01	<0.01
Group B: 300 mg	29.1 (14.4)	16.2 (8.5)	0.04	0.03
Group C: Placebo	28.5 (19.0)	27.2 (10.6)	0.87	-

[a]Comparison of within-group difference (change-from-baseline) is conducted by paired t-test; [b]Pairwise comparisons of between-group difference are conducted by two-sample t-test.

4. Discussion

Preventing *Alzheimer's* disease (AD) has become a major focus for scientists worldwide [15]. This study demonstrates that CTE exhibits therapeutic potential in mitigating tau-induced neuronal death, supported by preliminary observational study evidence highlighting neuroprotection and enhancements in memory and learning. Previous studies have shown that intracisternal infusion of $A\beta$ 1-42 in rats results in its deposition in the frontal cortex and hippocampus, causing memory deficits in behavioral tasks such as the inhibitory avoidance task and the *Morris* water maze [13].

We analyzed the relative anti-secretase enzyme activity of CTE, specifically its APPswe effects, in zebrafish embryos. Acteoside and isoacteoside exerted protective effects against Aβ 1-42, a peptide known to compromise learning and memory by reducing BDNF levels. These protective effects are attributed to the compounds' antioxidative and neurotrophic properties. Our findings indicated that embryos treated with the categories of CTE, acteoside, or isoacteoside exhibited significantly higher neuronal cell differentiation, neurite outgrowth, and presynaptic formation compared to controls. These results support the hypothesis that the cognitive benefits of *Cistanche tubulosa* extracts are partly attributable to their ability to promote neuronal differentiation, neurite outgrowth, and presynaptic formation [7]. *Alzheimer's* disease (AD) is also associated with the loss of cholinergic neurons in the brain and decreased levels of acetylcholine (ACh) [16]. Therefore, one of the major therapeutic targets of *Cistanche tubulosa* extracts in AD treatment strategies is the reduction of acetylcholinesterase (AChE) levels.

Preliminary observational study utilizing the Clinical Memory Scale, developed by the Psychological Institute of the Chinese Academy of Sciences, demonstrated

memory test improvements from baseline in both CTE-treated and placebo groups. However, one-way ANOVA analysis among the three groups showed no statistically significant differences, suggesting that treatment duration may need to exceed six weeks in future studies. Safety evaluations conducted during the treatment period confirmed the absence of treatment-related adverse reactions.

Neurodegenerative diseases such as AD have a complex pathogenesis influenced by numerous factors. Currently, few effective drugs target the root cause of AD, with most offering only symptomatic relief. This highlights the urgent need for novel therapeutic approaches. Our previous research [7] demonstrated that *Cistanche tubulosa* extracts enhance learning and memory by inducing nerve growth factor. This study further revealed that changes in BDNF and AChE levels from baseline were statistically significant. In animal models, oral administration of acetylcholinesterase inhibitors (AChEIs) increased BDNF levels in the hippocampus and cortex [17].

While FDA-approved drugs for AD, such as AChEIs and aducanumab, primarily provide symptomatic relief without halting disease progression [18], this study presents the first evidence that *Cistanche tubulosa* extracts offer a multi-target therapeutic approach to improving memory function [19]. These findings represent a significant advancement in AD research and treatment.

However, this study has certain limitations. First, the mechanism by which CTE treats AD differs from its effects observed in zebrafish embryos, where it exhibits anti-secretase enzyme activity and neurotrophic effects. Additionally, its mechanism differs from that of other traditional Chinese medicines.

5. Conclusion

The findings of this study demonstrate that CTE exhibits anti-tauopathy, anti-secretase, and neuroprotective properties, as evidenced in transgenic fluorescent zebrafish. These results support the hypothesis that the cognitive benefits of *Cistanche tubulosa* extracts are linked to enhanced learning and memory abilities through the induction of nerve growth factor. Collectively, the evidence suggests that CTE has potential neuroprotective effects and may contribute to improved memory function.

Institutional Review Board Statement

This study was approved by Academia Sinica Institutional Animal Care and Utilization Committee (ASIACUC) (protocol #10-12-114).

Data Availability Statement

The data presented in this study are available on request from the corresponding authors. The data are not publicly available.

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natural product (*C. tubulosa* extract (Sinphar AIE2)).

Author Contributions

Conceptualization, C.-T.C.; methodology, C.-J.H.; formal analysis, C.-J.W.; investigation, B.-K.W.; writing—original draft preparation, C.-T.C.; writing—review and editing, C.-J.H. and M.-H.S. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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