Effects of *Cistanche* Polysaccharide on Learning and Cholinergic System of Brain Tissue in Alzheimer's Disease Rats

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To investigate the effect of *Cistanche deserticola* polysacchrides on learning and memory ability, and cholinergic system in rats; replicate Alzheimer's disease rat model using amyloid beta1-40 lateral ventricular injection, randomly divided into sham group, model group, low, medium and high polysaccharide dose (50, 100, 200 mg/kg) group. For 28 d, the effect of *Cistanche* polysaccharide on learning and memory in rats was measured by Morris water maze and escape latency was calculated. Escape latency significantly decreased compared with preoperative ($p<0.01$), after lateral ventricular injection in each group significantly decreased compared with normal and sham group ($p<0.01$). The incubation period of rats in the administration group decreased significantly compared with 7 d, but the *Cistanche deserticola* group was significantly increased compared with normal and sham group ($p<0.01$). Compared with the sham group, acetylcholinesterase vitality was significantly increased and acetylcholine transferase vitality was significantly decreased in the model group ($p<0.05$), indicating that amyloid beta$_{1-40}$ impaired acetylcholinesterase and acetylcholine transferase in rat cortex and hippocampus. *Cistanche deserticola* polysaccharides significantly increased acetylcholine transferase activity ($p<0.05$). Acetylcholinesterase viability was significantly suppressed in the *Cistanche deserticola* polysaccharides high-dose group. The learning and memory ability of Alzheimer disease rats is improved, and the mechanism is related to the effect on the cholinergic system. *Cistanche deserticola* polysaccharides not only enhances acetylcholine transferase activity but also decreases acetylcholinesterase activity, showing that it may inhibit amyloid beta$_{1-40}$ by increasing acetylcholine activity in the brain leading to learning and memory dysfunction in rats.

Key words: *Cistanche* polysaccharide, Alzheimer's disease, acetylcholinesterase, norepinephrine, beta-amyloid

Alzheimer's Disease (AD) is a neurodegenerative disorder of age-related progressive memory loss and higher cognitive decline[1]. The pathological changes are mainly manifested by senile plaques formed by extracellular aggregated Amyloid Beta (Aβ) protein, deposition, Neuronal Fibrous Tangles (NFT) caused by highly intracellular phosphorylated Tau, and significant loss of cholinergic neurons in the forebrain[2]. The incidence of AD accounts for 8 % to 10 % of people over 65 y old, and it is increasing by twice every 5 y[3]. The exact mechanism of AD is still not clear, most scholars believe that the cholinergic system in the brain is mainly damaged, and put forward the "cholinergic hypothesis". The degeneration of cholinergic neurons in the forebrain and the loss of cholinergic transmitters in the cerebral cortex and other regions are the main causes of cognitive dysfunction in AD patients[4]. The severity of dementia in AD was found to have a positive correlation with the extent of the cholinergic loss[5,6], and animals with cholinergic lesions and resultant learning impairments were characterized as models of AD[7]. These developments culminated in the demonstration that inhibitors of Acetylcholinesterase (AChE) could lead to symptomatic improvement in patients with AD[8]. It was subsequently found that neurofibrillary degeneration of the cholinergic neurons, designated Methane (CH4), was already

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present at the very early stages of AD, and that the extent of this neurofibrillary degeneration, even at a stage before cell death, is correlated with cognitive deficits[9,10]. Additional lines of research have also raised the possibility that this cholinergic lesion may influence AD pathogenesis through complex and poorly understood effects on amyloid genesis, tau phosphorylation, and neuroplasticity[11]. Although changes in other cortical neurotransmitters such a dopamine, norepinephrine and histamine were also reported in AD, a review of the extensive literature led Geula *et al.*[12] to conclude that the cholinergic lesion is earlier more widespread and more consistent than the pathologic alterations of other neurotransmitter systems[13,14]. To this day, Cholinesterase Inhibitors (ChEIs) remain the main approved pharmacologic therapies for cognitive deficits in AD. Currently, three ChEIs—donepezil, rivastigmine and galantamine are widely used as standard of care for the pharmacologic treatment of clinical symptomatic stage AD. The efficacy of ChEIs has been demonstrated in multiple large-scale studies, although the effect size is considered modest and ChEIs are traditionally conceptualized as "symptomatic" treatment. *Cistanche* polysaccharide has a long history as a medicinal plant in China because of its wide spectrum of pharmacological activities. It is commonly called Rou Cong Rong in Chinese, and it was first listed medicinal use as a tonic agent in the Chinese Materia Medica Shen Nong's Herbal Classic (Estern Han Dynasty) 2000 y ago, and later recorded in Yaoxing Lun in 1590. The Compendium of Materia Medica (Ben Cao Gang Mu, 1619) documented that *Cistanche* herba invigorated the kidney to treat kidney deficiencies and geriatric constipation strengthened and nourished marrow and essence, protected semen, and moistened dryness to relax the bowels. These properties were also written in Ben Cao Hui Yan in 1619. A total of 200 medicinal books recorded the pharmacodynamics and use of *Cistanche* herba in Chinese history. *Cistanche* herba ranks first in Chinese traditional medicine to strengthen prescriptions, which ranks 2nd in anti-aging prescriptions at the same time, behind *Panax ginseng* in past dynasties. Modern pharmacological investigations demonstrated that *Cistanche* herba was used as a kidney yang reinforcing Chinese medicinal tonic, but it is also exhibits anti-aging, improves memory, and enhances immunity effects , which indicate that extracts or constituents from *Cistanche* herba have a promising future for the treatment of diseases, particularly nervous system disorders. However, systematic data on the pharmacological activities of this agent is lacking[14]. It is urgent and important to study the pharmacological effects and mechanisms of *Cistanche* herba deeply in the future. Studies show that the chemical components of *Cistanche* include benzylethanosides, cyclic terpenids, lignin's, polysaccharide and alkaloids, among which polysaccharides are the main active components of *Cistanche*[15]. Modern pharmacological studies show that *Cistanche* has the functions of regulating immunity, liver, radiation and neuroprotection[16,17]. In this experiment, AD rat model was induced by $A\beta_{1-40}$. After the administration of AChE and Acetylcholine Transferase (ChAT) in AD rats, the possible mechanisms of improving the learning and memory ability of AD rats were investigated. $A\beta_{1-40}$ (Sigma Corporation, United States of America (USA)), 1 mg dissolve in 100 µl of sterile 0.9 % sodium chloride solution to make 5 µg/µl suspension and incubate in a 37° incubator for 7 d to become Aβ in the aggregated state $_{1-40}$ set in 4° refrigerator. ChAT and AchE test kits were purchased from Nanjing Jiancheng Bioengineering Institute. 1 kg raw tablets were soaked in hot ethanol for 3 h, filtered with gauze and the filtrate was discarded. Cook the residue with water for 3 times, 1 h~2 h each time, filter, and combine the filtrate (brown red). After evaporation and concentration, the precipitation was removed by centrifugation, and the supernatant was precipitated with 2-3 times the volume of 95 % ethanol and let it set at 4° for 24 h. The next day, the precipitate was collected by centrifugation at 4° for 20 min (6000 r/min). The precipitate was reconstituted in water, deproteinized and freeze-dried by crude polysaccharide (gray brown red). Wistar 60 rats (experimental animal center of Huazhong university of science and technology) weight 180-220 g, intraperitoneal injection of chloral hydrate (0.35 g/kg) anesthesia, fixed on the brain stereotactic machine, head hair, disinfection skin, head middle incision, anterior fontanelle, after the anterior fontanelle, midline right, micro syringe from brain surface vertical injection, slowly injected 2 µl Aβ into the lateral ventricle $_{1-40}$ the needle was kept to ensure full diffusion of the injection solution, and the needle was slowly removed later. All steps were aseptic, and the skin incision was made with penicillin and the wound was closed. The sham group received an equivalent dose of normal saline.

Experimental animals group and specimen acquisition experimental rats randomly divided into normal control group, model group and drug intervention group, each group 10, model preparation with low to high dose daily, normal group and model group rats with equal amount of corn oil, continuous 7 d observation after all animals killed by cervical dislocation method, rats bilateral hippocampus, wash with ice normal saline preservation, detection hippocampus ChAT, AchE, etc. Morris water maze experiment was conducted in rats at 7 d before, 7 d after and 28 d after surgery. Test mainly complete positioning navigation test, before 2 d for adaptation period, twice a day, afternoon, respectively, the rats into the water by four clockwise in training, record the time needed to find and climb the platform (escape incubation period), if the rat find platform time within 1 min, record the actual escape incubation period, if find the platform more than 1 min clock, the operator on the platform and stay for 15 s, and escape the incubation period recorded for 1 min. Choline acetyltransferase and AChE activity of hippocampal tissue after the water maze experiment, cervical dislocation method, cortex and hippocampus were isolated, quickly frozen at -20° refrigerator, pre-cooled normal saline was added, and 10 % homogenate was made in ice bath. The ChAT assay uses acetyl-coenzyme and choline as substrates. Under the action of ChAT, the product of the reaction was combined with the color developer, and the absorbance was measured at 324 nm in order to calculate the vitality of ChAT. The specific operation was carried out according to the assay kit. AchE hydrolysis of acetylcholine to produce choline and acetic acid, choline can react with sulfhydryl chromo agent to produce symmetrical Three Nitro Benzene (TNB) yellow compound, according to the color depth, the number of hydrolysis products of choline can react to cholinesterase activity specific operation according to the test kit (purchased from Nanjing bioengineering institute). Statistical methods, the experimental data are represented as mean±standard deviation ($\bar{x}$±s), and the data were analyzed by Analysis of Variance (ANOVA) and t-test using Statistical Package for the Social Sciences (SPSS) 13.0 statistical software. Effect of *Cistanche deserticola* Polysacchrides (CDPS) on learning and memory in AD rats significantly decreased latency compared with preoperative ($p<0.01$), significant in each group compared with normal and sham group ($p<0.01$). The incubation period of rats in the administration group decreased significantly compared with 7 d, and the operation was significant ($p<0.01$) as shown in Table 1. After the behavioral experiment, the brain was severed, and the cerebral cortex and hippocampus were dissected for AchE and ChAT. The activity was detected in the cortex and hippocampus tissue. Compared with the sham group, AchE vitality was significantly increased and ChAT vitality was significantly decreased in the model group ($p<0.05$), indicating that $A\beta_{1-40}$ impaired AchE and ChAT in rat cortex and hippocampus. CDPS significantly increased ChAT activity ($p<0.05$). AchE viability was significantly suppressed in the CDPS high-dose group ($p<0.05$), as shown in Table 2. The cholinergic hypothesis of AD pathogenesis is that the basal cholinergic neurons at the base of the brain develop degeneration during the pathological process of AD, and the activity of ChAT and AChE is changed, which reduces the transport of ACh, leading to the loss of learning and memory[18]. Studies have shown that ACh synthesis secretion decreases in the brain of AD, and its synthase ChAT activity decreases, which is closely related to the severity of dementia. The synapses, synaptic nicotinic receptors and presynaptic M receptors are reduced in hippocampus and cortical pyramidal cells[19].

TABLE 1: EFFECTS OF CDPS ON LEARNING AND MEMORY IN AD RATS ($\bar{x}$±s)

Group	Escape latency (s)		
	Pre-operation	7 d after surgery	28 d after surgery
Normal	17.69±0.84	16.41±0.94	16.57±0.69
Sham	17.43±0.83	16.82±0.61	16.92±0.38
Model	18.15±0.65	46.18±1.04*	45.52±0.93*
Low dose	18.44±0.45	44.64±1.53*	25.39±1.22#
Middle dose	18.23±0.23	48.35 ±1.41*	19.32±0.81#
High dose	17.43±0.36	46.21±1.52*	15.42±0.19#

Note: *$p<0.01$ with the normal and sham groups and #$p<0.01$ with the model group

TABLE 2: EFFECT OF CDPS ON AchE AND ChAT ACTIVITY IN HIPPOCAMPAL AND CORTICAL TISSUES OF AD RATS

Group	Dose/mg.kg^{-1}	AchE/ug.l^{-1}	ChAT/ug.l^{-1}
Normal	-	2.28±0.21	8.96±1.10
Sham	-	2.16±0.18	8.65±1.15
Model	-	1.23±0.14	3.65±0.68
Low dose	50	1.89±0.22$^{\#}$	5.63±0.43$^{\#}$
Middle dose	100	2.45±0.41$^{\#}$	7.86±0.61$^{\#}$
High dose	100	3.21±0.22$^{\#}$	8.82±0.59$^{\#}$

Note: $^{\#}$p<0.01 compared with the model group

Therefore, the decrease of learning and memory is most closely related to the cholinergic dysfunction. A lateral ventricle stereotaxic meter was used to inject $A\beta_{1-40}$. An AD animal model was made and the results suggested injection of condensed $A\beta_{1-40}$ into the lateral ventricle $A\beta_{1-40}$ after 7 d, the rats showed a decrease in learning and memory function. Cui *et al.*[20] reported that a lateral ventricle stereotaxer injected $A\beta_{1-40}$ the escape latency was significantly prolonged and the number of platforms decreased on d 5, showing bilateral lateral ventricle injection of $A\beta_{1-40}$ both can reduce the rat learning and memory ability, consistent with the results of this experiment. In the water maze experiment, CDPS significantly reduced the latency and shortened the time to reach the platform, and the results suggested that CDPS could improve the cognitive function of rats. Study found that rat lateral ventricles injected $A\beta_{1-40}$. Later, ChAT was decreased in the cortex and hippocampus, consistent with the pathological features of AD. CDPS can significantly improve the ChAT viability of rat hippocampal tissue, and can inhibit AchE in cortical and hippocampal tissues. ACh is an important neurotransmitter in the brain, and the cholinergic system plays an important function for the normal cognitive function of the body. Loss of acetylcholine in the cortex and hippocampus will result in a significant reduction in learning and memory cognitive function[21]. The hippocampus is the most important part of the brain to complete learning and memory, and plays an important role in the acquisition and retrieval of spatial memory[22]. We found that both cognitive and spatial memory function were improved after increasing hippocampal acetylcholine concentrations in patients with cognitive impairment[23], The key enzyme of Ach synthesis is ChAT, and the main synthesis site is in the cholinergic cell body of neurons. ChAT activity directly reflects the cholinergic system function of the brain, and the improvement of ChAT activity can improve the neuronal area and cognitive function[24]. AchE can accelerate axonal growth, promote neuronal cell interactions, promote axonal growth, improve dopaminergic neuronal function, and promote β-amyloid peptide precipitation formation[25]. CDPS not only enhances ChAT activity but also decreases AchE activity, showing that it may inhibit Aβ by increasing acetylcholine activity in the brain $A\beta_{1-40}$ leading to learning and memory dysfunction in rats. CDPS could improve the cognitive function of rats. And CDPS not only enhances ChAT activity but also decreases AchE activity, by increasing acetylcholine activity in the brain $A\beta_{1-40}$ leading to learning and memory dysfunction in rats.

Conflict of interests:

The authors declared no conflict of interests.

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