Yuan-zhi-san inhibits tau protein aggregation in an $A\beta_{1-40}$-induced Alzheimer's disease rat model via the ubiquitin-proteasome system

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Abstract. Yuan-zhi-san (YZS) is a classic type of Traditional Chinese Medicine, which has been reported to aid in the treatment of Alzheimer's disease (AD). The present study aimed to investigate the effects of YZS on tau protein aggregation, a hallmark of AD pathology, and its possible mechanisms. The results demonstrated that YZS improved learning and memory abilities, and decreased the severity of AD pathology in β-amyloid ($A\beta_{1-40}$)-induced AD rats. Moreover, YZS administration inhibited the hyperphosphorylation of tau protein at Ser199 and Thr231 sites. Several vital enzymes in the ubiquitin-proteasome system (UPS), including ubiquitin-activating enzyme E1a/b, ubiquitin-conjugating enzyme E2a, carboxyl terminus of Hsc70-interacting protein, ubiquitin C-236 terminal hydrolase L1 and 26S proteasome, were all significantly downregulated in AD rats, which indicated an impaired enzymatic cascade in the UPS. In addition, it was identified that YZS treatment partly increased the expression levels of these enzymes in the brains of AD rats. In conclusion, the present results suggested that YZS could effectively suppress the hyperphosphorylation of tau proteins, which may be partially associated with its beneficial role in restoring functionality of the UPS.

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Introduction

Alzheimer's disease (AD) is the most common form of dementia present in the aging population worldwide and the number of patients with AD is predicted to rise (1). According to the World Alzheimer Report 2018 (2), of the 50 million individuals in the world with dementia, two thirds were diagnosed with AD. While there have been numerous attempts to develop an effective drug for treating AD, only two types of drugs are currently available. One such type is cholinesterase inhibitors, such as donepezil, which function to prevent acetylcholinesterase from breaking down acetylcholine (3); therefore, signals are able to be transmitted between nerve cells. The other type of drug is an N-methyl-D-aspartic acid receptor antagonist (memantine), which acts to relieve the damage to nerve cells caused by excessive amounts of released glutamate (4). However, the currently available drugs are only able to provide symptomatic relief (5). Due to the high socioeconomic costs associated with patients with AD worldwide (6,7), the development of effective therapies to treat AD is of great significance.

Although the fundamental mechanisms underlying AD remain unknown, two classic pathological proteins, β-amyloid (Aβ) and tau, are reported to be involved (8). Aβ accumulates and forms amyloid plaques, which causes damage to the synapses (9). In addition to Aβ, hyperphosphorylated tau forms neurofibrillary tangles (NFT) inside neurons, and these tau aggregations block the neuronal transport system (10). The ubiquitin (Ub)-proteasome system (UPS) is a sophisticated mechanism for intracellular protein degradation and turnover (11). Ub is a polypeptide that can label substrate proteins via the covalent attachment of multiple Ub molecules, such as Ub-activating enzyme E1 (UbE1) and Ub-conjugating enzyme E2 (UbE2). In addition, the 26S proteasome serves a major role in the degradation of Ub-conjugated proteins (12). Dysregulation of the UPS is of particular interest in the pathogenesis of AD, and emerging evidence has suggested that aberrant UPS activity may contribute to the disorder of Aβ and tau degradation (13,14).

Yuan-zhi-san (YZS) is an herbal drug in Traditional Chinese Medicine that has been clinically applied to treat dementia. Previous studies have revealed that YZS and its components are

effective and safe in relieving some symptoms and improving cognitive impairment in patients with AD (15-17). Our previous study reported that YZS could improve learning and memory abilities in a D-galactose-induced aging mouse model, which was achieved, in part, by the attenuation of oxidative stress (18). A similar result was also reported by Jin *et al* (19). Moreover, our previous study using another scopolamine-induced mouse model of dysmnesia further validated the memory-improving ability of YZS (20). Qiang *et al* (21) observed that YZS relieved AD by regulating acetylcholine receptor activity and by binding to Aβ. Furthermore, nearly 180 active ingredients of YZS demonstrating multi-component and multi-target characteristics have been screened, and a specific database of the active ingredients has been established (21). However, whether YZS has an impact on UPS-mediated tau degradation remains to be elucidated.

The present study aimed to investigate the effects of YZS on learning and memory abilities, as well as AD pathology, in an Aβ-induced AD rat model. Moreover, it was determined whether YZS affected the phosphorylation of tau protein, and whether its potential therapeutic effects were achieved by modulating the UPS.

Materials and methods

Animals. Sprague-Dawley rats (n=40; equal number of males and females; weight, 200±20 g; age, 7 weeks) were obtained from Chengdu Dashuo Experimental Animal Co., Ltd. (animal license no. SYXK-2017-179). Animals were housed under controlled conditions of 22±2˚C and 50±10% humidity, with a 12-h light/dark cycle and *ad libitum* access to standard rodent chow and water. The present study was approved by the Medical Ethics Committee of Chengdu University of Traditional Chinese Medicine (Chengdu, China; approval no. 2017-02).

Drugs. $A\beta_{1-40}$ was purchased from Sigma-Aldrich; Merck KGaA (cat. no. A1075); donepezil was obtained from Shanxi Aark Pha. Ltd. (drug approval. no. H20030583; https://www.800pharm.com/shop/groupId_101169.html).

YZS was composed of 12 g Radix polygala (Yuan Zhi), 12 g Rhizomacoptidis (Huang Lian), 15 g Sclerotium poriaecocos (Fu Ling), 9 g Radix ginseng (Ren Shen) and 18 g Acori Tatarinowii Rhizoma (Shi Chang Pu), and was prepared into a standardized granule formula by Sichuan Neo-Green Pharmaceutical Technology Development Co., Ltd.

AD animal model establishment and treatment. Sprague-Dawley rats were randomly divided into four groups: i) Sham group (n=10; treated with vehicle); ii) AD model group (n=10; treated with $A\beta_{1-40}$ and vehicle); iii) AD model + donepezil group (n=10; treated with $A\beta_{1-40}$ and donepezil); and iv) AD model + YZS group (n=10; treated with $A\beta_{1-40}$ and YZS). The Aβ-induced AD rat model was established as described previously (22-24). Following anesthesia with intraperitoneal injection of 0.45% pentobarbital sodium (40 mg/kg), rats were secured on a stereotaxic device. Subsequently, referring to the Paxinos and Watson rat brain atlas (25), the sham-operated rats received a saline injection into the bilateral hippocampus CA1, whereas the other AD rats received 5 μg $A\beta_{1-40}$ solution. The solution was infused at a speed of 0.5 μl/min, and the needle remained in place for 5 min. A total of 200,000 U/ml penicillin-G was administered intramuscularly to rats once daily for 7 days after surgery. Following the procedure, 6 g/kg YZS, 0.45 mg/kg donepezil or vehicle (equivalent dose of saline) were intragastrically administered to the rats once daily for 8 consecutive weeks.

Morris water maze test. Rats were subjected to the Morris water test for the spatial navigation task (escape latency) and a probe trial in the last 5 days of the drug administration period. The training period was performed for the first 3 days, and the orientation navigation experiment was conducted on day 4. A video tracking system was used to monitor the whole procedure, which was assessed by two independent investigators in a blinded manner. A hidden platform was placed 2-cm beneath the surface of the water in one pool quadrant. When the rats had successfully located the hidden platform, they were left on the platform for 10 sec. Rats were manually guided to the platform and remained there for 10 sec if they failed to find the platform within 90 sec, wherein the escape latency was recorded as 90 sec. A probe test was performed on day 5 to evaluate memory consolidation.

Hematoxylin & eosin (H&E) staining. Following anesthetization with 0.45% pentobarbital sodium, rats were sacrificed via transcardial perfusion with 0.9% saline (pH 7.4). Hippocampi were harvested, fixed in 4% paraformaldehyde solution for >24 h at 4˚C, dehydrated in graded alcohol series (30, 50, 70, 95 and 100%) and embedded in paraffin. Samples were cut into 5-μm sections and then stained with H&E (hematoxylin at room temperature for 5 min; eosin at room temperature for 1 min) to examine the pathological alterations. Images were captured under a light microscope (IX71; Olympus Corporation) at a magnification of x400.

Golgi-Cox staining. After transcardial perfusion with 0.9% saline, hippocampi were collected and immersed in a vial containing Golgi-Cox solution (prepared solution from FD Rapid GolgiStain™ kit; cat. no. PK401; FD Neurotechnologies, Inc.). The vial was kept in the dark and the Golgi-Cox solution was replaced every alternate day for 2 weeks. Subsequently, the samples were transferred to a vial containing 30% sucrose solution and stored at 4˚C in the dark for 6 days. The samples were cut into 100-μm sections and then rinsed with distilled water. Subsequently, the sections were treated with 22.5% ammonium hydroxide solution for 30 min and then with 5% thiosulphate solution for 30 min at room temperature. After rinsing with distilled water for 2 min, routine glass slides containing the sections were processed. Images were captured under a light microscope (Eclipse E100; Nikon Corporation) at magnification of x600. The density of the dendritic spines was measured using Image-pro plus 6.0 (Media Cybernetics, Inc.).

Transmission electron microscopy. A small portion of the hippocampus was fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4) at 4˚C for 4 h and were post-fixed in 2% osmium tetroxide at 4˚C for 1 h in the same buffer. Subsequently, the tissues were routinely dehydrated in acetone, infiltrated and embedded in epoxy resin-filled capsules. Finally, 70-nm ultrathin sections were prepared with an LKB ultramicrotome

and counterstained with 2% aqueous uranyl acetate for 10 min and 0.8% lead citrate for 2 min at room temperature. The ultrastructure of nerve cells was observed and imaged under a transmission electron microscope (TEM; magnification, x2,500 and x6,000; H-7650; Hitachi Ltd.).

Immunohistochemistry. Brain tissues were fixed in 4% paraformaldehyde at 4˚C for 24 h, dehydrated and embedded in paraffin. Paraffin-embedded specimens were sliced into 3-μm sections, dewaxed and rehydrated. After routine peroxidase blocking for 10 min and blocking with 5% BSA (cat. no. G5001; Wuhan Servicebio Technology Co., Ltd.) for 30 min at room temperature, the sections were incubated with antibodies against Tau5 (1:100; cat. no. MAB361; EMD Millipore), phosphorylated (p)-Tau (Ser199; 1:100; cat. no. 44-734G; Invitrogen; Thermo Fisher Scientific, Inc.), p-Tau (Thr231; 1:100; cat. no. 11110; Signalway Antibody LLC) and p-Tau (Ser396; 1:100; cat. no. 11102; Signalway Antibody LLC) overnight at 4˚C. The sections were then exposed to horseradish peroxidase (HRP)/Fab polymer-conjugated secondary antibodies (solution from the kit; cat. no. PV-6000-D; OriGene Technologies, Inc.) at room temperature for 30 min, followed by staining with 3,3'-diaminobenzidine solution at room temperature for 5 min and counterstaining with hematoxylin at room temperature for 20 sec. To evaluate the protein expression levels, three visual fields were randomly selected from each slice under a light microscope (magnification, x400). Images were acquired and analyzed using Image Pro Plus 6.0 software (Media Cybernetics, Inc.). Quantification of expression levels was determined using mean integrated optical density (IOD).

Western blotting. Hippocampal sections were homogenized in pre-cooled RIPA buffer (cat. no. G2002; Wuhan Servicebio Technology Co., Ltd.) containing a protease inhibitor cocktail, and homogenates were then incubated on ice for 10 min and centrifuged at 16,128 x g at 4˚C for 15 min. The protein concentration was determined using a commercial BCA kit (cat. no. P0010S; Beyotime Institute of Biotechnology). Proteins (30 μg) were separated by SDS-PAGE on 8-10% gels and transferred onto PVDF membranes. The membranes were blocked with 5% skimmed milk in Tris-buffered saline containing 0.05% Tween-20 (TBST) at room temperature for 1 h and incubated at 4˚C overnight with primary antibodies against UbE1a/b (1:1,000; cat. no. 4891; Cell Signaling Technology, Inc.), UbE2a (1:500; cat. no. 11080-1-AP; ProteinTech Group, Inc.), carboxyl terminus of Hsc70-interacting protein (CHIP; 1:1,000; cat. no. 2080; Cell Signaling Technology, Inc.), Ub C-236 terminal hydrolase L1 (UCH-L1; 1:1,000; cat. no. 14730-1-AP; ProteinTech Group, Inc.), 26S proteasome (1:500; cat. no. sc-65755; Santa Cruz Biotechnology, Inc.) and β-actin (1:4,000; cat. no. 200068-8F10; Chengdu Zen BioScience Co., Ltd.). After washing three times with TBST, the membranes were incubated at room temperature for 1 h with HRP-conjugated secondary antibodies (1:3,000; cat. nos. GB23301 and GB23303; Wuhan Servicebio Technology Co., Ltd.). An enhanced chemiluminescence kit (cat. no. NEL103E001EA; PerkinElmer, Inc.) was used to detect the bands using a Molecular Imager PharosFX Plus system (Bio-Rad Laboratories, Inc.), and the bands were analyzed and semi-quantified using Quantity One software system (ver. no. 4.6.9; Bio-Rad Laboratories, Inc.).

Statistical analysis. Statistical analyses were performed using SPSS 17.0 software (SPSS, Inc.). Differences between groups were evaluated using one-way ANOVA followed by Tukey's or Dunnett's T3 post hoc tests. Data are presented as the mean ± SD from three independent experiments. $P<0.05$ was considered to indicate a statistically significant difference.

Results

YZS rescues the memory deficit of AD rats. The memory and learning performances of YZS-treated AD rats were examined using a Morris water maze test. As expected, the mean escape latency of all rats declined during the training period. On day 4, AD rats demonstrated a significantly longer escape latency in the orientation navigation experiment compared with that of sham-operated rats ($P<0.01$). It was demonstrated that, similar to the effects of donepezil administration, YZS administration shortened the escape latency ($P<0.05$; Fig. 1B). In the probe test, YZS-treated AD rats had greater crossing times ($P<0.01$; Fig. 1C), increased time in the target quadrant ($P<0.01$; Fig. 1D) and lengthier swimming routes ($P<0.01$; Fig. 1E) compared with those of AD rats. These results indicated the rescuing effect of YZS on the memory and learning abilities of AD rats.

YZS ameliorates AD histopathology and ultrastructural abnormalities. H&E staining indicated that the number of neurons in the CA1 region was markedly lower in AD rats compared with in the sham-operated rats (Fig. 2A). Both donepezil- and YZS-treated AD rats had notably increased numbers of neurons. These results suggested that YZS could protect against neuronal loss in AD rats.

The ultrastructural examination of nerve cells in the hippocampus using a TEM demonstrated a visible difference between AD and sham-operated rats. In nerve cells from AD rats, the nuclei were swollen and shaped like grapes, along with chromatin condensation and nuclear membrane invagination. The presence of swollen mitochondria with broken cristae and an expanded endoplasmic reticulum was also observed. However, both donepezil and YZS intervention appeared to reverse the aberrant morphological ultrastructure in terms of nuclei, mitochondria and endoplasmic reticulum (Fig. 2B). Taken together, these findings indicated that YZS could effectively restore the number of neurons and reduce ultrastructural abnormalities in the brain of AD rats.

YZS enhances the density of dendritic spines in AD rats. To further examine the neuroprotective effect of YZS against Aβ-induced AD, the density of dendritic spines was measured using Golgi-Cox staining (Fig. 3A). AD rats had a significantly lower density of dendritic spines compared with in sham-operated rats ($P<0.01$; Fig. 3B). Moreover, YZS treatment could protect against the loss of dendritic spines in the brain of AD rats, which was reflected by an increased density of dendritic spines in AD rats treated with YZS or donepezil ($P<0.01$; Fig. 3B). Notably, the protective capability of YZS

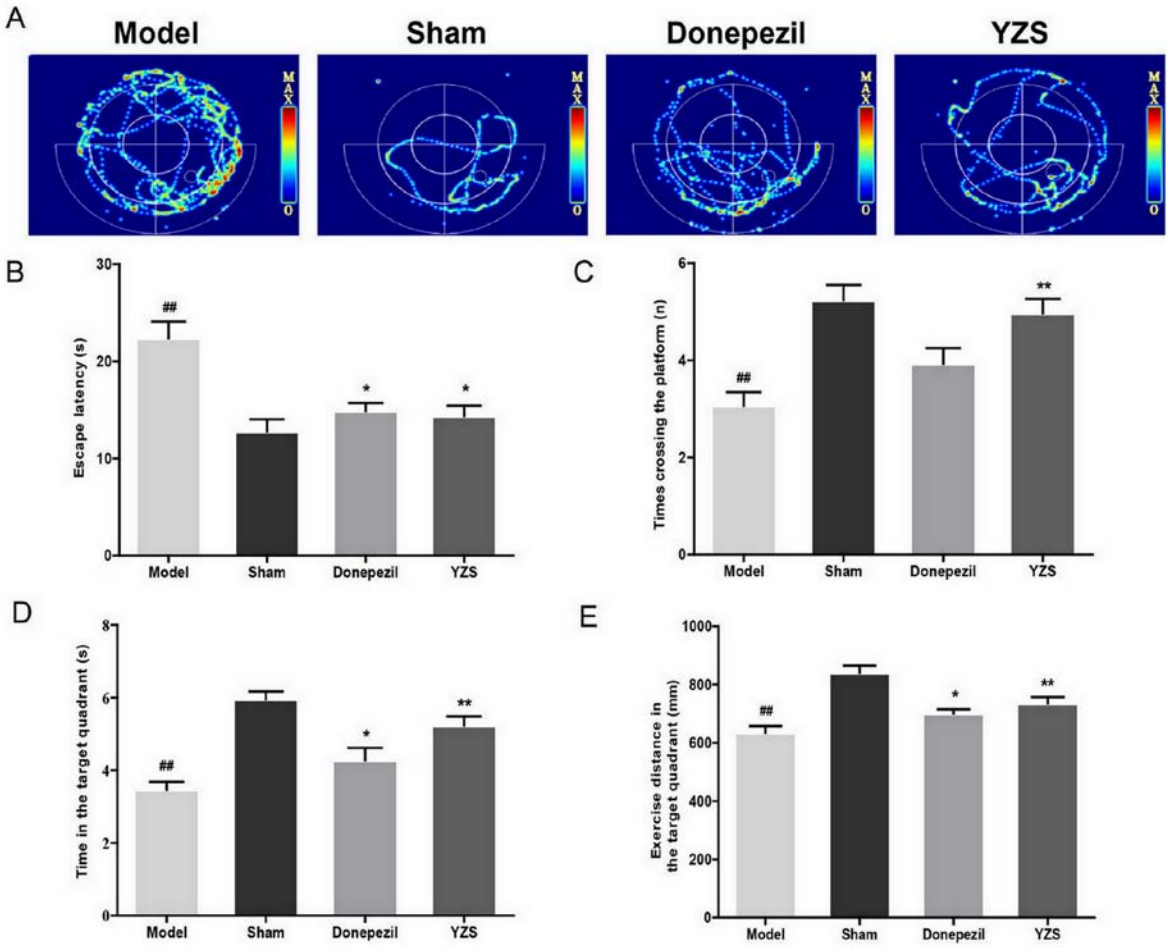

Figure 1. Effects of YZS on the spatial learning and memory abilities of Alzheimer's disease model rats in the Morris water maze test. (A) Representative tracks of the probe trials of rats in different groups. (B) Time of escape latency. (C) Times crossing the target platform. (D) Time in the target quadrant. (E) Exercise distance in the target quadrant. Data are presented as the mean ± SD (n=10). $^{\#\#}$P<0.01 vs. Sham group; *P<0.05, **P<0.01 vs. Model group. YZS, Yuan-zhi-san.

on dendritic spines appeared stronger compared with that of donepezil.

YZS inhibits the hyperphosphorylation of tau protein in AD rats. Tau is a microtubule-associated protein that, in a hyperphosphorylated state, is considered to self-assemble into a paired helical filament, and ultimately contribute to the NFT pathology of AD (10). The results of the present study detected no significant differences in the expression of total tau5 protein among all rats (P>0.05; Fig. 4A and B). Conversely, YZS administration significantly inhibited the hyperphosphorylation of tau protein at Ser199 and Thr231 sites (P<0.01; Fig. 4A, C and E). Although p-Tau Ser396 was identified to be hyperphosphorylated in AD rats, the hyperphosphorylation level of p-Tau Ser396 was barely reduced by YZS treatment (P>0.05 Fig. 4A and D). These results indicated that YZS could suppress hyperphosphorylation of tau protein (Ser199 and Thr231), thereby decreasing its self-assembly into paired helical filaments and reducing the NFT pathology in AD rats.

YZS modulates the expression levels of UPS-related proteins in AD rats. The UPS is essential for degrading misfolded and damaged intracellular proteins, including tau degradation, and it has been reported that an impaired UPS is involved in AD pathogenesis (26). Thus, it was investigated whether YZS could restore functionality of the UPS by impacting several vital enzymes involved in the complex enzymatic cascade of UPS, including UbE1a/b, UbE2a, CHIP, UCH-L1 and 26S proteasome. Western blot analysis identified that the protein expression levels of UbE1a/b, UbE2a, CHIP, UCH-L1 and 26S proteasome were all significantly downregulated in the AD model rats (P<0.01 or P<0.05; Fig. 5A-E), which suggested an impaired enzymatic cascade of the UPS. When compared with the AD model rats, these enzymes were upregulated in the brains of AD rats co-treated with YZS or donepezil (P<0.01; Fig. 5A-E). These findings indicated that YZS could, at least in part, effectively upregulate the protein expression levels of UbE1a/b, UbE2a, CHIP, UCH-L1 and 26S proteasome, which may facilitate restoration of the UPS to remove tau accumulation.

Discussion

AD is mainly characterized by a progressive loss of memory and the development of cognitive deficits, leading to profound dementia. It has been suggested that the two pathological hallmarks of AD include extracellular Aβ plaques and intracellular tau tangles (8). Due to the huge socioeconomic burden caused by AD globally (6,7), discovering potential effective therapies to treat AD is of great importance. Several studies

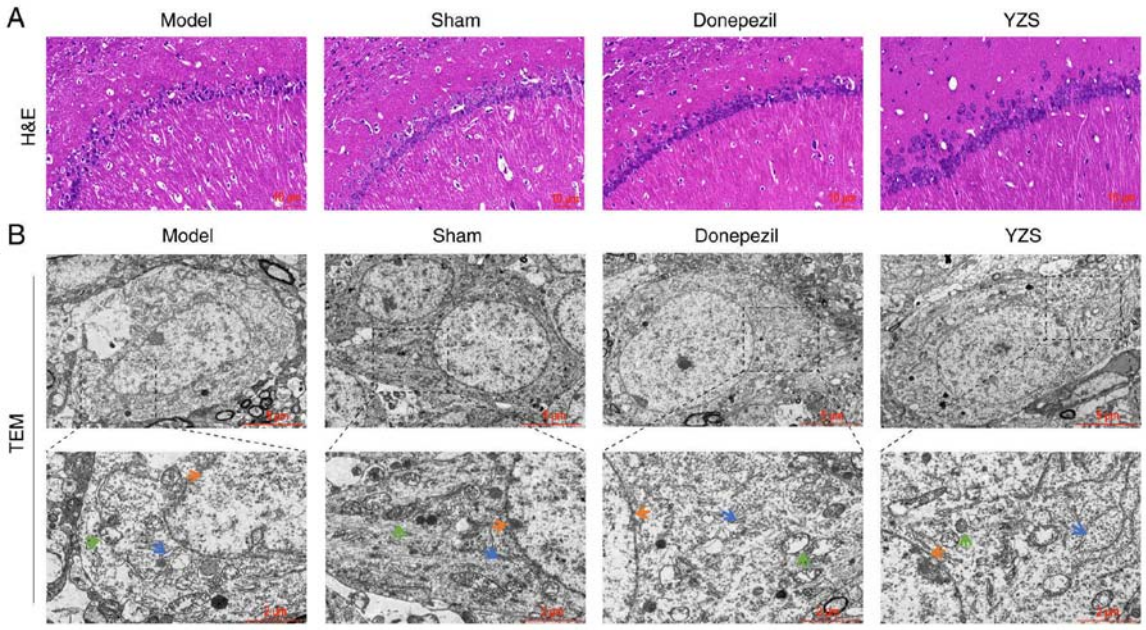

Figure 2. Effects of YZS on the morphology and number of neurons in Alzheimer's disease model rats. (A) Representative images of hippocampal neurons from the CA1 region using hematoxylin and eosin (H&E) staining (magnification, x400). (B) Representative images indicating the ultrastructure of nerve cells using transmission electron microscopy (magnification, x2,500 and x6,000). Orange arrows indicate nuclear membrane; blue arrows indicate endoplasmic reticulum; green arrows indicate mitochondrion. YZS, Yuan-zhi-san.

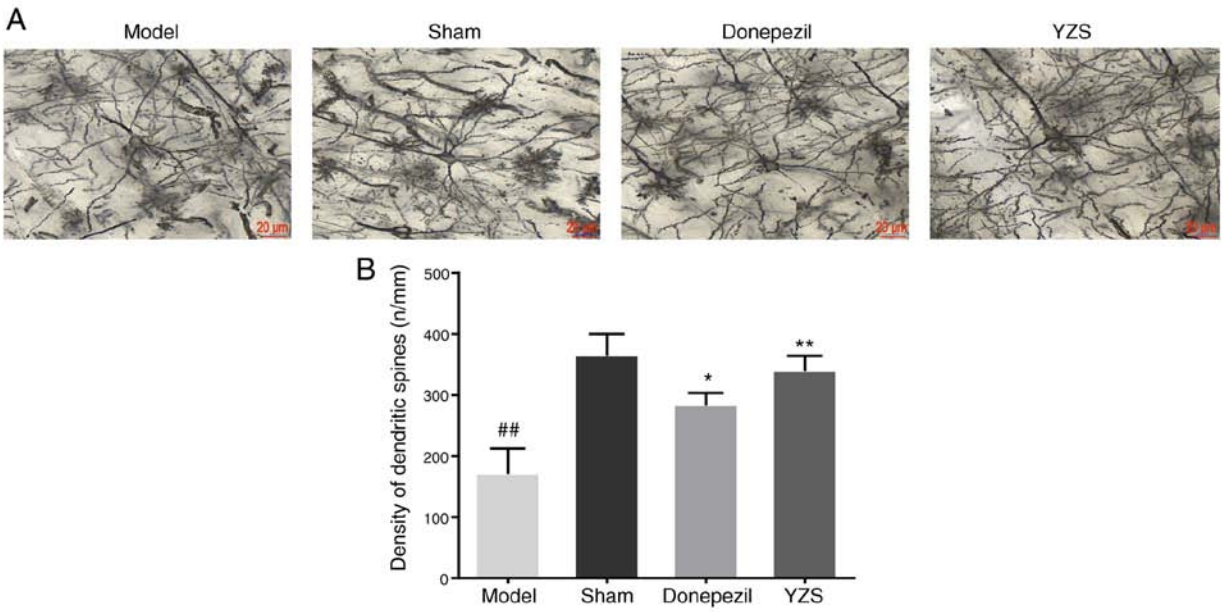

Figure 3. Effects of YZS on the density of dendritic spines in Alzheimer's disease model rats. (A) Representative images of dendritic spines in the brain tissues using Golgi-Cox staining (magnification, x600). (B) Analysis of density of dendritic spines in each group. Data are presented as the mean ± SD (rats n=6). $^{\#\#}$P<0.01 vs. Sham group; *P<0.05, **P<0.01 vs. Model group. YZS, Yuan-zhi-san.

have revealed the neuroprotective effects of YZS, with its multi-component and multi-target characteristics (18-21).

To verify the present hypothesis that YZS may enhance learning and memory abilities partly by restoring UPS-mediated tau degradation, an Aβ-induced AD rat model treated with YZS was established. The present results demonstrated that treatment with YZS, similar to donepezil, improved learning and memory, and restored the number of neurons and reversed ultrastructural abnormalities. Furthermore, YZS could protect against the loss of dendritic spines in the brains of AD rats. These findings suggested that YZS may serve as an effective herbal formula that brings relief to AD rats.

In the past two decades, a positive association has been identified between pathological tau aggregation and the progression of AD (27). Ultrastructurally, when tau, a microtubule-associated protein, is in an abnormally hyper-phosphorylated state it is consequently incapable of binding to microtubules and thus self-assembles into paired helical

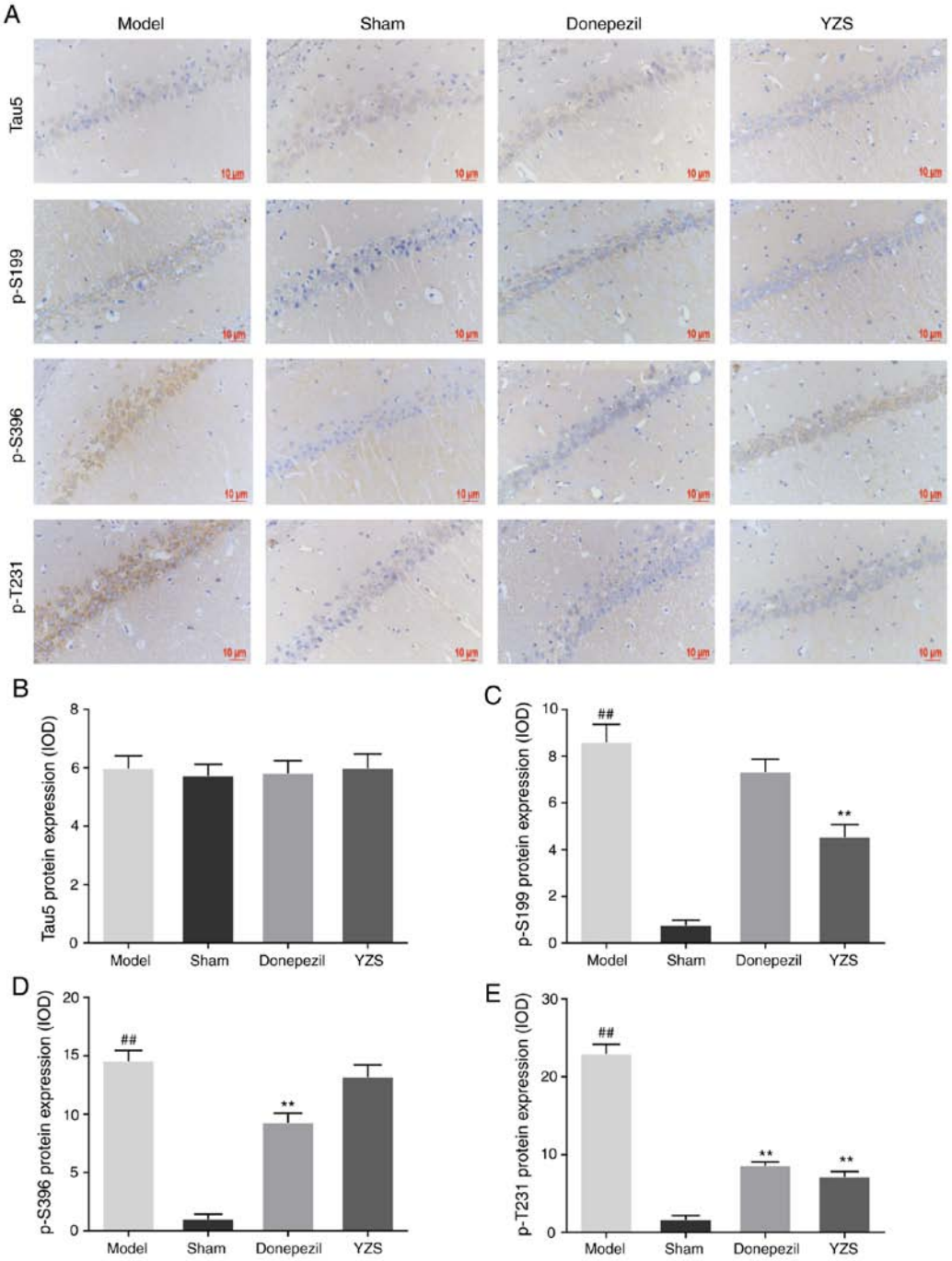

Figure 4. Effects of YZS on the expression levels of Tau5, p-S199, p-S396 and p-T231 in Alzheimer's disease model rats. (A) Representative immunohistochemistry images demonstrating the expression levels of Tau5, p-S199, p-S396 and p-T231 in the CA1 region from each group (magnification, x400). Analysis of (B) Tau5, (C) p-S199, (D) p-S396 and (E) p-T231 protein expression levels in each group. Data are presented as the mean ± SD (n=10). $^{\#\#}$P<0.01 vs. Sham group; *P<0.05, **P<0.01 vs. Model group. p-S199, phosphorylated-Tau (Ser199); p-S396, phosphorylated-Tau (Ser396); p-T231, phosphorylated-Tau (Thr231); YZS, Yuan-zhi-san.

filaments. Studies have reported that paired helical filaments serve as the major fibrous component of NFTs found in cell bodies and apical dendrites (10). Abundant NFTs distribute in the nerve cells that undergo degeneration and their degree of abundance has been shown to be closely associated with the severity of AD (28). In the current study, hyperphosphorylated tau protein at Ser199, Ser396 and Thr231 sites was observed in AD model rats. Tau hyperphosphorylation at Ser199 and Thr231 was significantly decreased following YZS treatment, which may contribute to the remission of AD pathology. However, it was revealed that YZS treatment exhibited little effect on tau hyperphosphorylation at Ser396; a possible reason for this is that p-Tau Ser396 may not be a potential target of YZS.

The UPS is essential for degrading misfolded and damaged intracellular proteins, including tau degradation. Therefore, the present study investigated whether YZS was capable of regulating the expression of UPS-related molecules, which are critical for functionality of the UPS and may contribute to tau degradation. Ub-activating enzymes

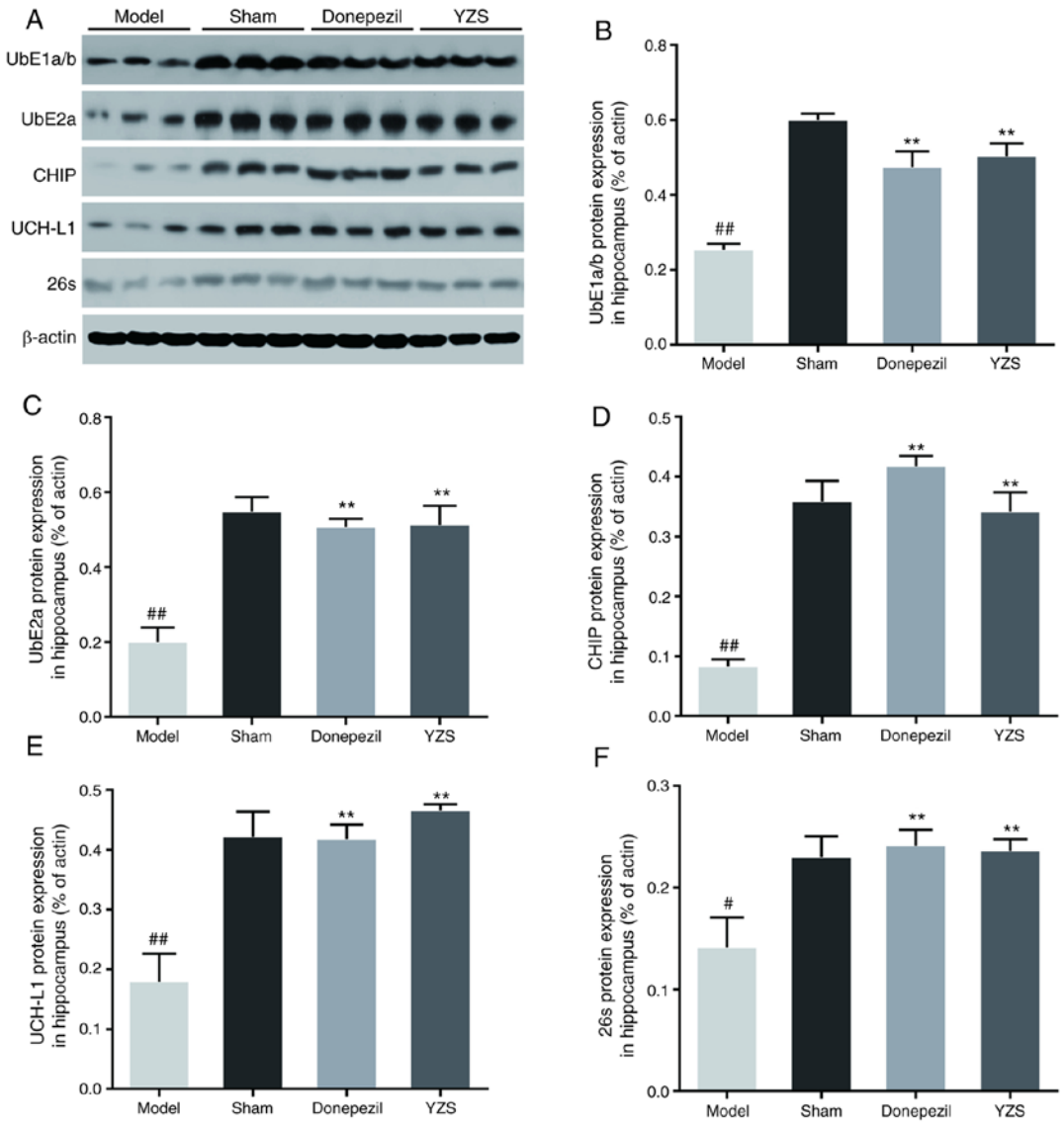

Figure 5. Effects of YZS on the protein expression levels of UbE1a/b, UbE2a, CHIP, UCH-L1 and 26S proteasome in Alzheimer's disease model rats. (A) Representative western blotting images of UbE1a/b, UbE2a, CHIP, UCH-L1 and 26S proteasome in each group. Semi-quantification of (B) UbE1a/b, (C) UbE2a, (D) CHIP, (E) UCH-L1 and (F) 26S proteasome expression levels in each group. Data are presented as the mean ± SD (rats n=3). $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. Sham group; **P<0.01 vs. Model group. UbE1a/b, ubiquitin-activating enzyme E1a/b; UbE2a, ubiquitin-conjugating enzyme E2a; CHIP, carboxyl terminus of Hsc70-interacting protein; UCH-L1, ubiquitin C-236 terminal hydrolase L1; YZS, Yuan-zhi-san.

(UbE1) form a thioester bond with Ub via an ATP-dependent mechanism. Subsequently, Ub is shifted to Ub-conjugating enzymes (UbE2), which function as scaffold proteins that favor the interaction between Ub ligase (E3) and the target substrate protein (11), allowing the ligase to transfer the Ub from E2 to the substrate protein (11). Polyubiquitinated proteins are subsequently recognized and degraded by the 26S proteasome into small peptides (12). It has been reported that the 26S proteasome can degrade phosphorylated and non-phosphorylated tau proteins (29). In the UPS, CHIP, an E3 ligase, facilitates UPS-mediated tau degradation, and the activity of CHIP has been reported to be impaired in the human AD brain (30,31), suggesting that CHIP inhibition may lead to tau accumulation and exacerbation of AD pathology. Moreover, UCH-L1 is a protease belonging to the deubiquitinating enzyme family, which removes Ub from Ub chains or the substrate (32). It has been established that inhibition of UCH-L1 activity may decrease the microtubule-binding ability and increase the phosphorylation of tau protein (33). The present study demonstrated that the expression levels of UbE1a/b, UbE2a, CHIP, UCH-L1 and 26S proteasome were significantly decreased in the brains of AD rats, which indicated an impaired enzymatic cascade of the UPS. Moreover, co-administration with YZS partly increased the expression levels of these enzymes, suggesting that YZS may be beneficial in restoring functionality of the UPS, so as to regain the ability to degrade ubiquitinated and hyperphosphorylated tau protein. Future studies should focus on *in vitro* experiments to further understand the molecular mechanisms underlying the regulatory effect of YZS on the UPS.

In conclusion, the present study demonstrated that YZS may improve the learning and memory abilities, and reduce the severity of AD pathology in an Aβ-induced AD rat model. Furthermore, it was identified that YZS could suppress the

hyperphosphorylation of tau protein, which may be partially associated with its beneficial role in restoring functionality of the UPS.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BL, JHZ and YZ designed the study. JG and PJX carried out the experiments. JRY and YG interpreted the results of the experiments. YWH and DYG organized the database and conducted the statistical analysis. BL and PJX prepared the manuscript. YZ and JHZ confirmed the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of Chengdu University of Traditional Chinese Medicine (approval no. 2017-02).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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