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Tubuloside B Ameliorates Blood-Brain Barrier Disruption and Cognitive Deficits after Cerebral Ischemia by Inhibiting TRIC Protein Ubiquitination and Degradation

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Running Title: Tubuloside B Improves BBB Function

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Abstract

Background and Purpose: Vascular cognitive impairment (VCI) is strongly linked to blood-brain barrier (BBB) dysfunction. This study investigated whether Tubuloside B (Tub-B), a primary bioactive compound of *Cistanche tubulosa*, ameliorates cognitive deficits by protecting BBB integrity through inhibition of tricellulin (TRIC) ubiquitination and degradation.

Methods: The main plasma metabolites of total glycosides of *Cistanche* (TCGs) were identified by UPLC-QQQ-MS. A bilateral common carotid artery occlusion (BCCAO) rat model was used to induce chronic cerebral hypoperfusion (CCH). Cognitive function was assessed using the Morris water maze, and BBB permeability was evaluated by Evans blue extravasation. TRIC expression and ubiquitination were analyzed by Western blot, co-immunoprecipitation, and immunohistochemistry. An in vitro oxygen-glucose deprivation (OGD) model in brain microvascular endothelial cells was used to validate the effects on barrier function.

Results: Tub-B was identified as the major circulating metabolite, accounting for $52.3\% \pm 2.1\%$ of plasma components. In CCH rats, Tub-B (20 mg/kg) significantly improved spatial memory, increasing platform crossings (3.00 ± 0.58 vs. CCH, $P < 0.05$) and reducing Evans blue leakage (0.038 ± 0.007 $\mu\text{g/g}$ tissue vs. 0.15 ± 0.02 $\mu\text{g/g}$ in CCH, $P < 0.001$). It also attenuated the downregulation of TRIC and suppressed its ubiquitination. In OGD-injured endothelial cells, Tub-B enhanced barrier integrity and inhibited TRIC ubiquitination.

Conclusion: Tub-B attenuates CCH-induced BBB disruption and cognitive impairment by inhibiting TRIC ubiquitination and degradation, likely via interference with VEGF signaling. These results highlight its potential as a therapeutic agent for VCI.

Keywords: Vascular cognitive impairment; blood-brain barrier; Tubuloside B; Tricellulin;

ubiquitination; neuroinflammation; chronic cerebral hypoperfusion

1. Introduction

Vascular cognitive impairment (VCI) represents the second most common cause of cognitive dysfunction after Alzheimer's disease, posing a growing clinical and societal challenge amid aging populations worldwide [1–5]. Its pathogenesis involves multifactorial mechanisms, including chronic cerebral hypoperfusion (CCH), neuroinflammation, neuronal damage, and blood-brain barrier (BBB) disruption [6–11]. In particular, BBB dysfunction is increasingly recognized as a pivotal early event triggering neuroinflammatory cascades and cognitive decline [7–9, 11].

The BBB is primarily composed of brain microvascular endothelial cells interconnected via tight junctions (TJs), which include proteins such as claudins, occludin, and tricellulin (TRIC) [12–16]. TRIC is specifically localized at tricellular contacts and is critical for sealing paracellular pathways against macromolecule leakage, thus maintaining CNS homeostasis [21–23]. Under CCH conditions, TJ integrity is compromised, resulting in increased BBB permeability, infiltration of inflammatory mediators, and subsequent neuronal injury [17–20]. Although ubiquitin-mediated degradation has been implicated in the regulation of TJ proteins such as occludin [26–31], the post-translational modulation of TRIC—particularly via ubiquitination—under ischemic conditions remains poorly understood [24, 25].

Cistanche tubulosa and its extract, total glycosides of *Cistanche* (TCGs), have demonstrated neuroprotective and cognitive-enhancing properties [32–34]. However, the specific active compounds responsible for these benefits and their mechanisms of action, especially concerning BBB protection, are not fully elucidated. Tubuloside B (Tub-B) is a key constituent of TCGs, yet its potential role in modulating TRIC stability and BBB function has not been investigated.

In this study, we combined metabolic profiling, network pharmacology, and experimental validation to examine whether Tub-B attenuates CCH-induced BBB disruption and cognitive impairment by inhibiting ubiquitin-dependent degradation of TRIC. Using both *in vivo* and *in*

vitro models of cerebral hypoperfusion, we demonstrate that Tub-B stabilizes TRIC expression, reduces ubiquitination, preserves BBB integrity, and improves cognitive performance. Our findings reveal a novel mechanism by which Tub-B targets the VEGF–ubiquitin–TRIC axis, offering a promising therapeutic strategy for VCI.

2. Methods

2.1. Experimental Animals and Grouping.

Specific pathogen-free male Sprague-Dawley rats (8–10 weeks, 250–300 g; Shanghai SLAC Laboratory Animal Co., Ltd.) were housed under standard conditions. All experimental protocols were approved by the Experimental Animal Ethics Committee of Xuzhou Medical University (XYDW-2023-001). Rats were randomly divided into four groups ($n = 10/\text{group}$): Sham (sham surgery + saline), CCH (BCCAO + saline), CCH + Tub-B-Low (BCCAO + 5 mg/kg Tub-B), and CCH + Tub-B-High (BCCAO + 20 mg/kg Tub-B). Tub-B and TCGs (purity >98% and >50%, respectively; Xi'an Leiberei Biotechnology) were administered by gavage starting 24 h after surgery for 8 weeks.

2.2. Plasma Metabolite Profiling.

Plasma samples from mice orally administered TCGs were analyzed using UPLC-QQQ-MS (Waters ACQUITY UPLC BEH C18 column; 2.1×100 mm, $1.7 \mu\text{m}$) with gradient elution (0.1% formic acid in water and acetonitrile). Detection was performed in negative ion ESI mode. Dexamethasone was used as an internal standard.

2.3. Cell Culture and Drug Treatment.

PC12 cells were maintained in DMEM with 10% FBS and 1% penicillin-streptomycin. $\text{A}\beta_{25-35}$ oligomers were prepared by incubation at 37°C for 7 days. Cells were pretreated with Tub-B (1, 5, 10 μM) for 2 h before $\text{A}\beta_{25-35}$ (20 μM , 24 h) exposure. Cell viability was measured using CCK-8 assay.

2.4. Network Pharmacology.

Potential targets of Tub-B were predicted using SwissTargetPrediction and PharmMapper. Disease-related targets for “vascular cognitive impairment” and “blood-brain barrier dysfunction” were retrieved from GeneCards and OMIM. Protein-protein interaction networks were constructed with STRING and visualized in Cytoscape. GO and KEGG enrichment analyses were performed using DAVID.

2.5. BCCAO Model and Drug Administration.

The CCH model was induced via permanent bilateral common carotid artery occlusion under isoflurane anesthesia. Sham-operated rats underwent identical procedures without ligation. Drug treatments began 24 h post-surgery and continued for 8 weeks.

2.6. Morris Water Maze.

Spatial learning and memory were assessed over 5 training days with a hidden platform. A probe test was conducted on day 6. Escape latency, path length, and time in the target quadrant were recorded.

2.7. Blood-Brain Barrier Permeability.

BBB integrity was evaluated using Evans blue (EB; 2%, 4 mL/kg) extravasation. EB was quantified fluorometrically (620/680 nm) in formamide-extracted brain tissues.

2.8. Western Blot and Co-Immunoprecipitation.

Brain tissues or cells were lysed in RIPA buffer. Proteins were separated by SDS-PAGE, transferred to PVDF membranes, and probed with antibodies against TRIC (1:1000), ubiquitin (1:1000), ZO-1 (1:1000), claudin-5 (1:1000), occludin (1:1000), and β -actin (1:5000). For Co-IP, lysates were immunoprecipitated with anti-TRIC antibody, followed by ubiquitin immunoblotting.

2.9. Immunohistochemistry.

Brain sections (4 μ m) were deparaffinized, subjected to antigen retrieval, and incubated with anti-TRIC antibody (1:200). HRP-conjugated secondary antibody and DAB were used for

detection. Images were acquired with a light microscope.

2.10. In Vitro BBB Model and OGD.

Primary rat brain microvascular endothelial cells (rBMECs) were cultured, and barrier integrity was monitored by transendothelial electrical resistance (TEER). For OGD, cells were placed in glucose-free DMEM in a hypoxic chamber (1% O₂, 5% CO₂, 94% N₂) for 4 h, then reoxygenated with or without Tub-B (10 µM) for 24 h. Permeability was assessed using FITC-dextran (4 kDa).

2.11. Statistical Analysis.

Data are expressed as mean ± SD. Analyses were performed using GraphPad Prism 9.0. One-way or two-way ANOVA followed by Tukey's or Bonferroni's post hoc test was used. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Identification of Tub-B as the Major Circulating Metabolite and Its Neuroprotective Effects

UPLC-QQQ-MS analysis of plasma from mice administered with total glycosides of Cistanche (TCGs) revealed Tubuloside B (Tub-B) as the predominant bioactive metabolite, accounting for $52.3\% \pm 2.1\%$ of the total relative peak area (Supplementary Table S2). In an A β_{25-35} -induced PC12 cell injury model, Tub-B significantly increased cell viability in a dose-dependent manner, with the most potent effect observed at 10 µM ($82.5\% \pm 2.8\%$, $P < 0.001$; Fig. 1). Furthermore, pre-treatment with Tub-B (50 µM) markedly reduced A β -induced apoptosis and caspase-3/7 activation (Figs. 2, 3).

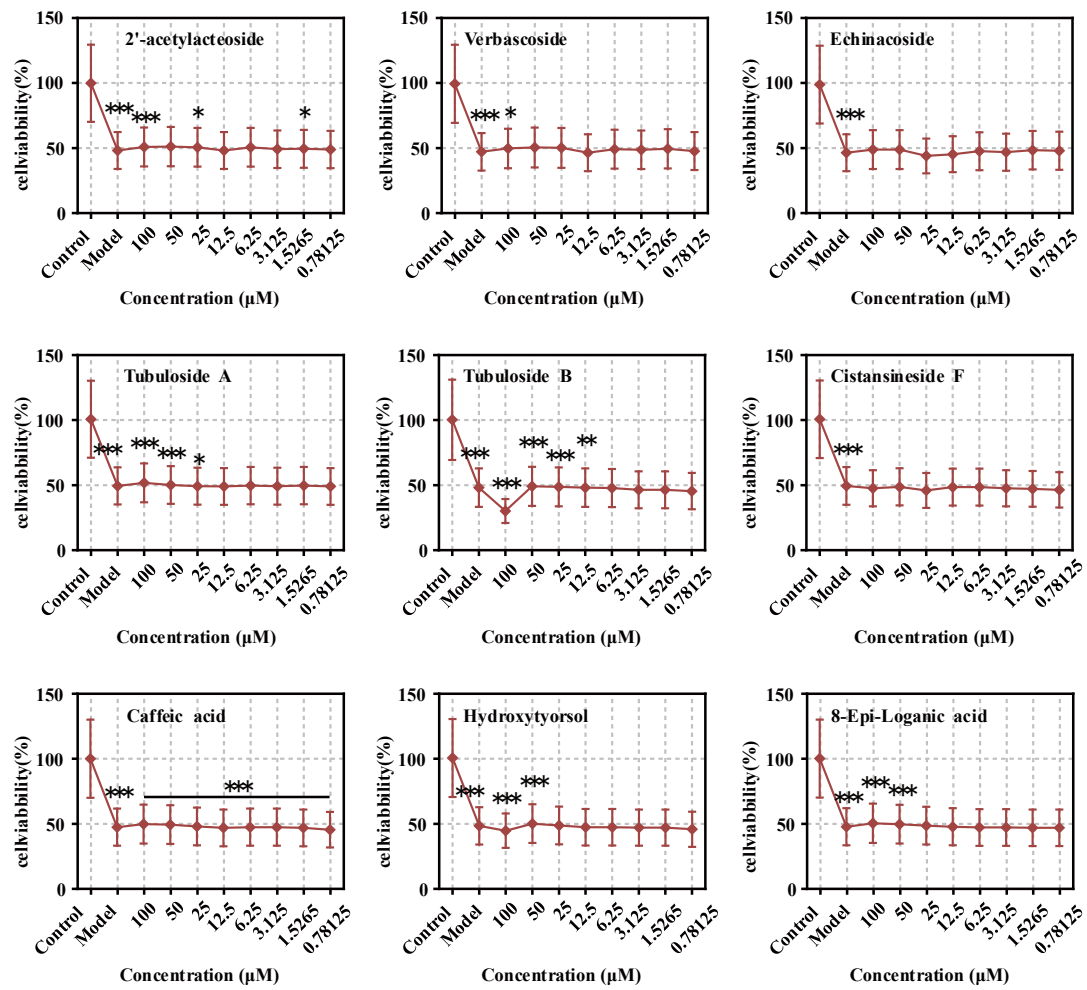


Figure 1. Dose-dependent protective effects of Tubuloside B against $A\beta_{25-35}$ -induced cytotoxicity in PC12 cells. (A) Cell viability was assessed by MTT assay after pretreatment with Tub-B (1, 5, 10 μM) or 8-epi-loganic acid (10, 20, 50 μM) for 24 h, followed by exposure to $A\beta_{25-35}$ (20 μM , 24 h). Data are normalized to control (mean $\pm$ SD, $n \geq 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. $A\beta$ model group; one-way ANOVA with Tukey's post hoc test.

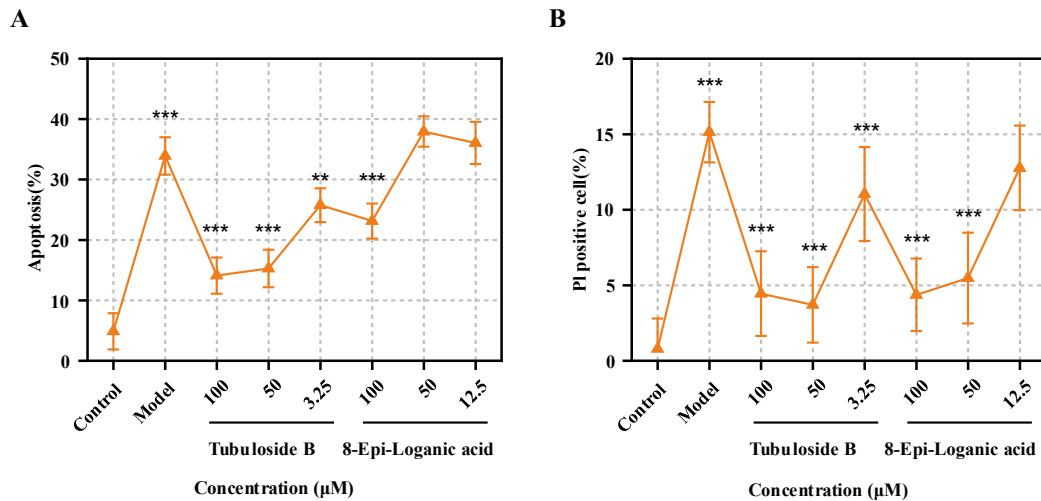


Figure 2. Tub-B reduces Aβ₂₅₋₃₅-induced apoptosis in PC12 cells. (A) Apoptosis was evaluated by Annexin V-FITC/PI staining and flow cytometry. (B) Quantitative analysis of apoptotic rates. Cells were pretreated with Tub-B or 8-epi-loganic acid (50 μM, 24 h) before Aβ₂₅₋₃₅ exposure. Data are mean ± SD (n ≥ 3). ***P* < 0.01, ****P* < 0.001 vs. Aβ model group; one-way ANOVA with Tukey's test.

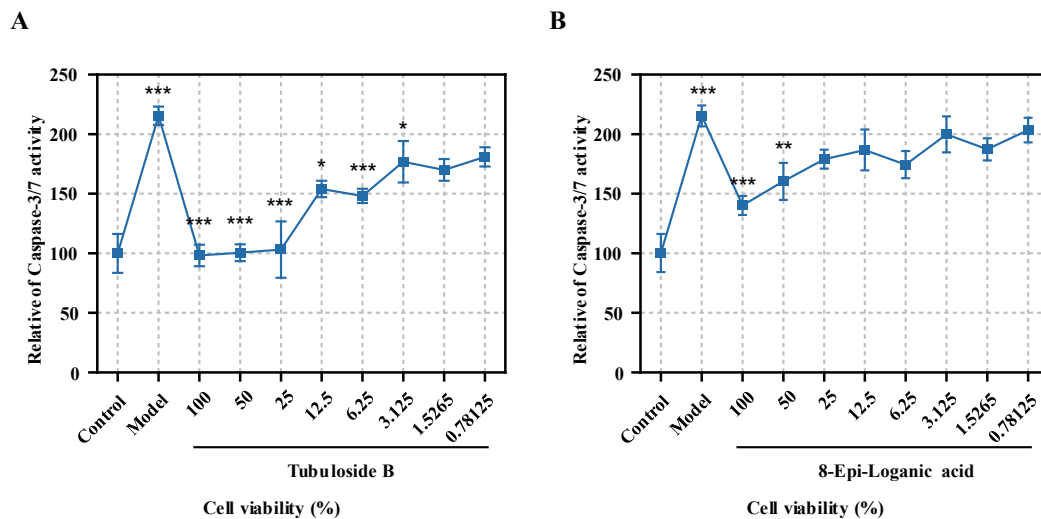


Figure 3. Tub-B inhibits caspase-3/7 activation induced by Aβ₂₅₋₃₅. Caspase-3/7 activity was measured after pretreatment with Tub-B or 8-epi-loganic acid (50 μM, 24 h) and subsequent Aβ₂₅₋₃₅ challenge. Data are expressed as Relative Luminescence Units (RLU; mean ± SD, n ≥ 3). **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. Aβ model group; one-way ANOVA with Tukey's test.

3.2. Network Pharmacology Implicates VEGF and Ubiquitination Pathways

Network pharmacology analysis identified five overlapping targets between Tub-B and chronic cerebral hypoperfusion (CCH), including KDR (VEGFR2) and MARVELD2 (TRIC) (Supplementary Table S3). Protein-protein interaction and KEGG pathway analyses highlighted significant enrichment in VEGF signaling, tight junction, and ubiquitin-mediated proteolysis pathways (Supplementary Fig. 4A–E), suggesting that Tub-B may exert its effects through regulation of endothelial barrier integrity and protein stability.

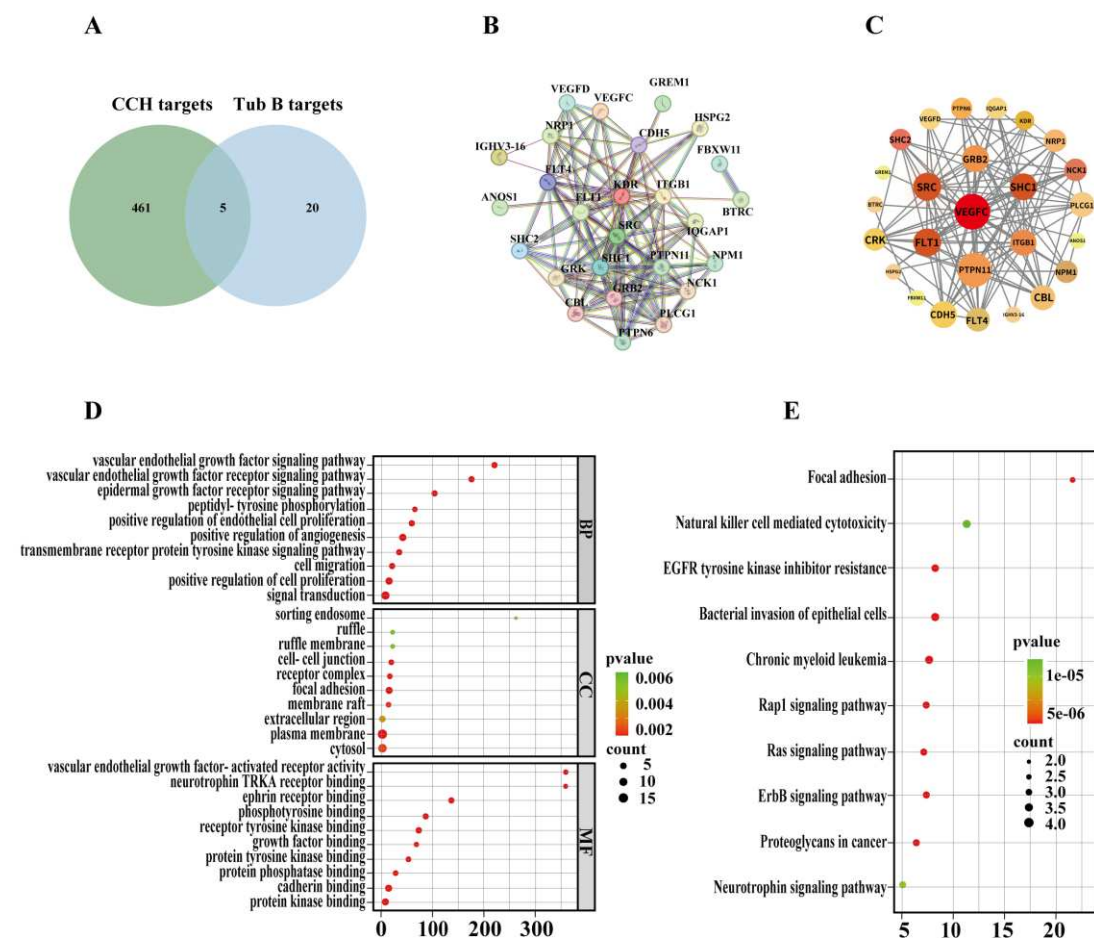


Figure 4. Network pharmacology analysis of Tub-B targets in chronic cerebral hypoperfusion. (A) Venn diagram of overlapping targets between Tub-B and CCH. (B) Protein-protein interaction (PPI) network. (C) Core target subnetwork. (D) Gene Ontology (GO) enrichment analysis. (E) KEGG pathway enrichment analysis.

3.3. Tub-B Improves Cognitive Function and BBB Integrity in CCH Rats

In the Morris water maze test, CCH rats exhibited significantly prolonged escape latency and reduced time in the target quadrant compared to sham controls ($P < 0.05$; Fig. 5). Treatment with high-dose Tub-B (20 mg/kg) significantly improved spatial memory, increasing platform crossings and target quadrant occupancy ($P < 0.05$). Evans blue extravasation assays confirmed that Tub-B dose-dependently reduced BBB leakage, with the high-dose group showing near-normal permeability ($0.038 \pm 0.007 \mu\text{g/g}$ vs. CCH: $0.15 \pm 0.02 \mu\text{g/g}$, $P < 0.001$; Fig. 6).

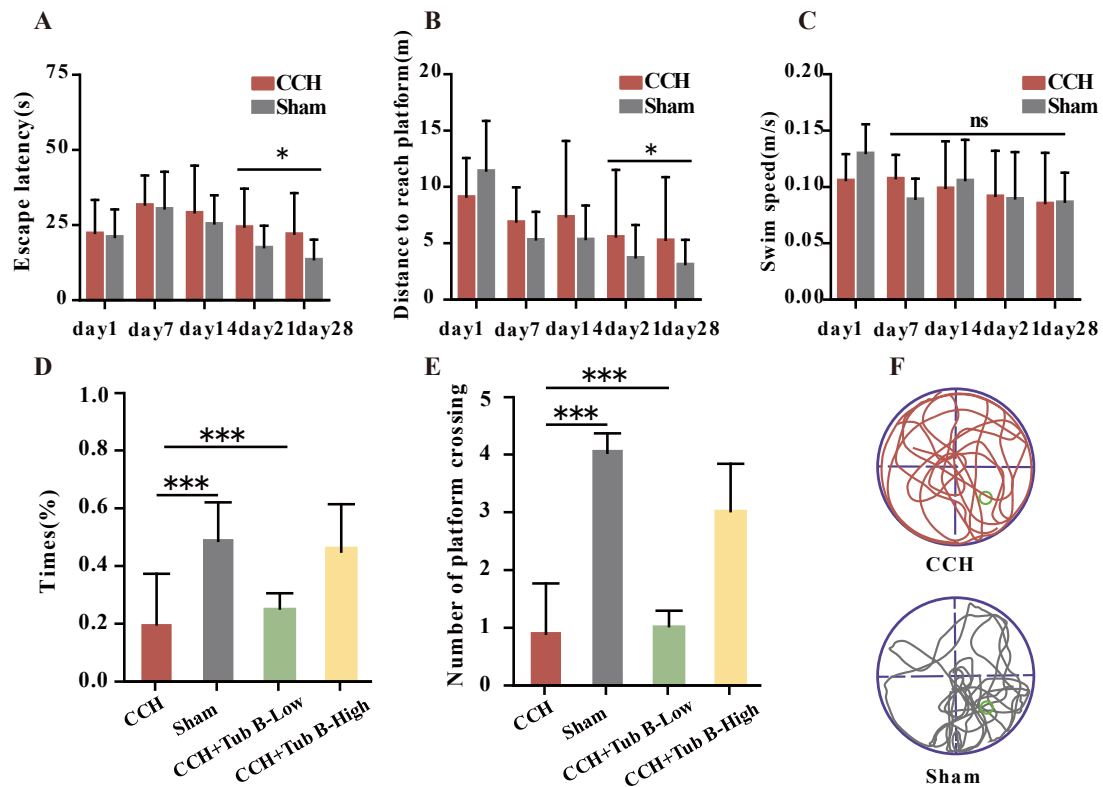


Figure 5. Tub-B improves spatial learning and memory in CCH rats. (A–C) Escape latency during acquisition phase in Morris water maze. (D) Number of platform crossings. (E) Time spent in target quadrant during probe trial. Data are mean $\pm$ SD ($n = 10$). * $P < 0.05$, *** $P < 0.001$ vs. CCH group; two-way repeated measures ANOVA with Tukey's test.

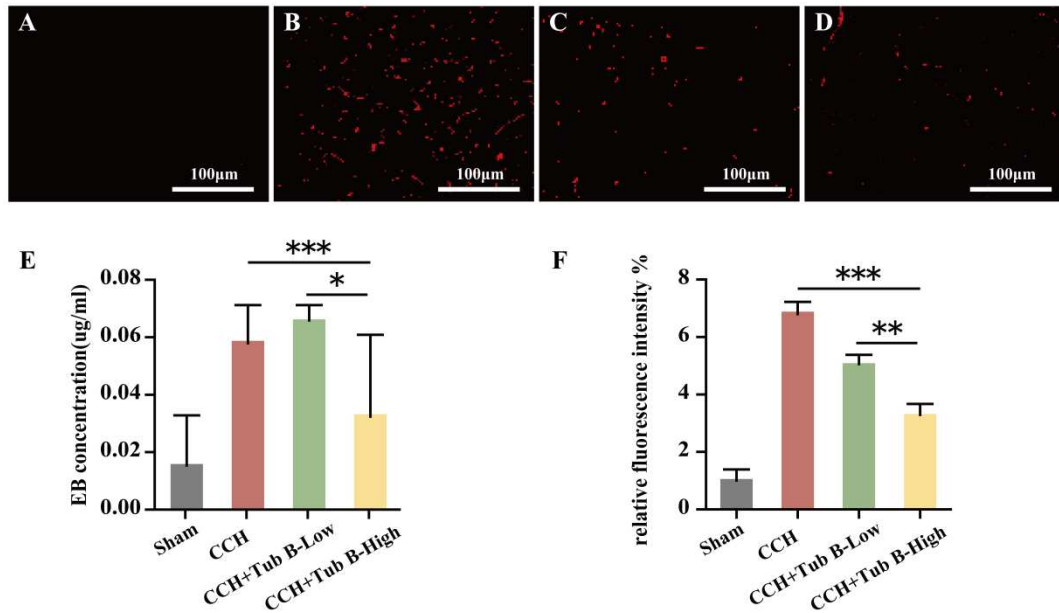


Figure 6. Tub-B alleviates blood-brain barrier disruption in CCH rats. (A–D) Representative images of Evans blue (EB) extravasation. (E) Quantitative analysis of EB content in brain tissue. (F) Quantification of EB fluorescence intensity. Data are mean $\pm$ SD ($n = 10$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. CCH group; one-way ANOVA with Tukey's test. Scale bar: 2 mm.

3.4. Tub-B Preserves TRIC Expression and Suppresses Its Ubiquitination

Immunofluorescence and Western blot analyses revealed that TRIC expression was significantly downregulated in the cortex of CCH rats and in OGD-treated SH-SY5Y cells ($P < 0.05$; Figs. 7, 8A–C). Tub-B treatment effectively restored TRIC protein levels. Co-immunoprecipitation demonstrated increased TRIC ubiquitination under both CCH and OGD conditions, which was markedly inhibited by Tub-B administration (Figs. 8D–F). The effect of Tub-B was comparable to that of the proteasome inhibitor MG-132, confirming its role in suppressing ubiquitin-proteasomal degradation of TRIC.

3.5. Tub-B Enhances Barrier Function in an In Vitro OGD Model

In primary rat brain microvascular endothelial cells (rBMECs) subjected to OGD, Tub-B (10 μ M) significantly increased transendothelial electrical resistance (TEER) and reduced FITC-dextran

permeability, indicating improved barrier integrity. Consistent with in vivo findings, Tub-B treatment also attenuated OGD-induced TRIC ubiquitination.

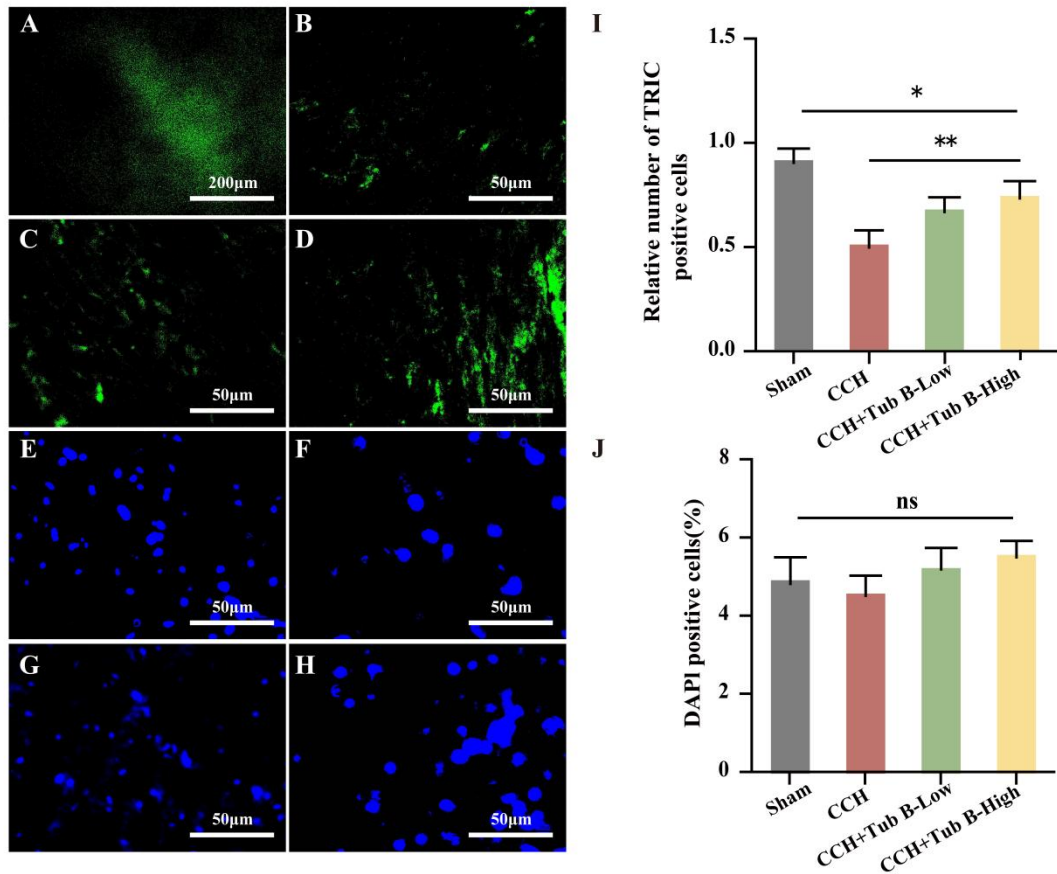


Figure 7. Tub-B attenuates CCH-induced downregulation of endothelial TRIC. (A–H) Representative immunofluorescence images of TRIC (red) and DAPI (blue) in cortical brain sections. (I) Quantification of TRIC-positive cells. (J) Quantification of DAPI-positive nuclei. Data are mean $\pm$ SD ($n = 10$). $*P < 0.05$, $*P < 0.01$ vs. Sham; $*P < 0.05$ vs. CCH; one-way ANOVA with Tukey's test. Scale bar: 50 μ m.

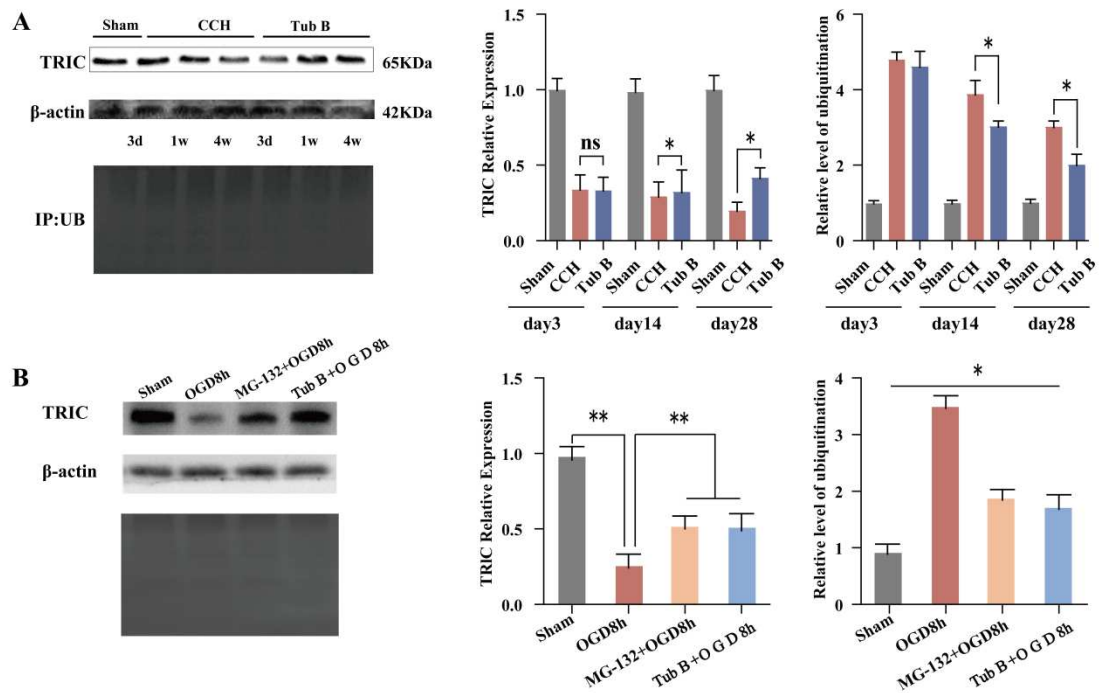


Figure 8. Tub-B inhibits ubiquitination and degradation of TRIC under CCH and OGD conditions. (A–C) Western blot analysis of TRIC protein expression in cortex and SH-SY5Y cells. (D–F) TRIC ubiquitination levels detected by co-immunoprecipitation and immunoblotting. Data are mean $\pm$ SD ($n = 10$). * $P < 0.05$, * $P < 0.01$ vs. Sham/Control; * $P < 0.05$ vs. CCH/OGD group; one-way ANOVA or unpaired t-test.

4. Discussion

This study provides compelling evidence that Tubuloside B (Tub-B), the primary bioactive metabolite of total glycosides of Cistanche, confers significant protection against chronic cerebral hypoperfusion (CCH)-induced cognitive impairment by preserving blood-brain barrier (BBB) integrity through a novel mechanism involving inhibition of tricellulin (TRIC) ubiquitination and degradation.

Our findings establish that Tub-B is not only the major circulating compound after oral

administration of TCGs but also a potent neuroprotective agent. It significantly attenuated A β ₂₅₋₃₅-induced cytotoxicity and apoptosis in PC12 cells, confirming its direct protective effects on neuronal cells. More importantly, through an integrated approach combining network pharmacology and experimental validation, we identified that Tub-B targets key pathways implicated in BBB regulation—specifically VEGF signaling, tight junction assembly, and ubiquitin-mediated proteolysis. Subsequent *in vivo* experiments demonstrated that Tub-B treatment markedly improved spatial memory and reduced BBB leakage in CCH rats, effects that were accompanied by the preservation of TRIC expression and inhibition of its ubiquitination.

The most significant mechanistic insight from this study is the demonstration that Tub-B inhibits ubiquitin-dependent degradation of TRIC, a critical protein localized at tricellular tight junctions that plays a vital role in sealing the paracellular pathway against macromolecule leakage [21-23]. Both *in vivo* and *in vitro* models consistently showed that CCH/OGD conditions promote TRIC ubiquitination and degradation, leading to compromised barrier integrity. Tub-B effectively reversed these changes, restoring TRIC protein levels and reducing its ubiquitination to an extent comparable to the proteasome inhibitor MG-132. These effects translated to functional improvements: Tub-B reduced Evans blue extravasation, enhanced transendothelial electrical resistance, and ultimately improved cognitive performance in CCH rats.

Our data further elucidate the upstream signaling events: under CCH/OGD conditions, VEGF/VEGFR2 activation triggers both PI3K/AKT and MAPK/ERK pathways, resulting in phosphorylation of ZO-1/ZO-2. This phosphorylation disrupts their crucial scaffolding function, impairing association with transmembrane proteins including TRIC. Concurrently, the E3 ubiquitin ligase TICH mediates K48-linked polyubiquitination of TRIC, targeting it for proteasomal degradation. Tub-B competes with VEGF for binding to VEGFR2, thereby inhibiting its activation and subsequent phosphorylation of ZO-1/ZO-2. This action helps stabilize the interaction between ZO proteins and transmembrane junctional proteins. Furthermore, Tub-B specifically blocks TICH-mediated TRIC ubiquitination and degradation. By these mechanisms, Tub-B effectively reduces BBB permeability and protects the neurovascular unit (see Fig S3).

The amelioration of neuroinflammation by Tub-B is likely secondary to BBB stabilization [10, 11]. By preserving TRIC at tricellular junctions, Tub-B limits the paracellular leakage of pro-inflammatory mediators and plasma components into the brain parenchyma, thereby attenuating the activation of innate immune responses in the CNS. This mechanism aligns with growing evidence that BBB breakdown is a critical initiator of neuroinflammation and cognitive decline in vascular cognitive impairment [6-9, 11].

Several limitations should be acknowledged. Although we identified VEGFR2 as a potential upstream target, the direct binding between Tub-B and VEGFR2 warrants further investigation. In addition, future studies using endothelial-specific TRIC knockout models would help to definitively establish the causal role of TRIC in mediating the effects of Tub-B.

In conclusion, our results demonstrate that Tub-B ameliorates CCH-induced cognitive impairment by inhibiting VEGFR2 activation and TICH-mediated ubiquitination of TRIC, thereby preserving BBB integrity. This work not only provides insights into the regulation of tricellular tight junctions under ischemic conditions, but also highlights the therapeutic potential of targeting ubiquitination pathways for the treatment of VCI and other BBB-related neurological disorders. These findings position Tub-B as a promising therapeutic candidate and identify TRIC ubiquitination as a novel target for intervention in cerebrovascular diseases.

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Author Contributions

Professor Mingli He was responsible for experimental design and paper writing; Han Zhang participated in some experiments, data and reference checking, and figure beautification; Ru Liu and Luming Li completed experimental operations and data analysis; Chunyang Zhang and Juan Li participated in the review of the test result data. Liang Li, Hao Yang, and Qiu Liu provided experimental materials and technical support; Xinru Gu reviewed and modified the manuscript. All authors agreed to publish.

Ethical Statement

Experiments complied with international guidelines for the care and use of laboratory animals and were approved by the Ethics Committee of Xuzhou Medical University (Approval No.: XYDW-2023-001).

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Conflict of Interest

The authors declare no conflict of interest.

References

1. O'Brien JT, Erkinjuntti T, Reisberg B, et al. Vascular cognitive impairment. *Lancet Neurol.* 2003;2(2):89-98. doi:10.1016/s1474-4422(03)00305-3
2. Iadecola C. The pathobiology of vascular dementia. *Neuron.* 2013;80(4):844-866. doi:10.1016/j.neuron.2013.10.008
3. Rundek T, Tolea M, Ariko T, Fagerli EA, Camargo CJ. Vascular Cognitive Impairment (VCI).

Neurotherapeutics. 2022;19(1):68-88. doi:10.1007/s13311-021-01170-y

4. Weaver NA, Kuijf HJ, Aben HP, et al. Strategic infarct locations for post-stroke cognitive impairment: a pooled analysis of individual patient data from 12 acute ischaemic stroke cohorts. *Lancet Neurol*. 2021;20(6):448-459. doi:10.1016/S1474-4422(21)00060-0
5. Mok VCT, Cai Y, Markus HS. Vascular cognitive impairment and dementia: Mechanisms, treatment, and future directions. *Int J Stroke*. 2024;19(8):838-856. doi:10.1177/17474930241279888
6. Rost NS, Brodtmann A, Pase MP, et al. Post-Stroke Cognitive Impairment and Dementia. *Circ Res*. 2022;130(8):1252-1271. doi:10.1161/CIRCRESAHA.122.319951
7. Cavanagh L, Paulsen JS. Neuropsychology and Vascular Cognitive Impairment and Dementia. *Neurol Clin*. 2024;42(4):809-820. doi:10.1016/j.ncl.2024.05.006
8. El Hussein N, Katzan IL, Rost NS, et al. Cognitive Impairment After Ischemic and Hemorrhagic Stroke: A Scientific Statement From the American Heart Association/American Stroke Association. *Stroke*. 2023;54(6):e272-e291. doi:10.1161/STR.0000000000000430
9. Rajeev V, Chai YL, Poh L, et al. Chronic cerebral hypoperfusion: a critical feature in unravelling the etiology of vascular cognitive impairment. *Acta Neuropathol Commun*. 2023;11(1):93. doi:10.1186/s40478-023-01590-1
10. Olejnik P, Golenia A. Vascular Cognitive Impairment-The Molecular Basis and Potential Influence of the Gut Microbiota on the Pathological Process. *Cells*. 2024;13(23):1962. doi:10.3390/cells13231962
11. He Z, Sun J. The role of the neurovascular unit in vascular cognitive impairment: Current evidence and future perspectives. *Neurobiol Dis*. 2025;204:106772. doi:10.1016/j.nbd.2024.106772
12. Shimizu F, Nakamori M. Blood-Brain Barrier Disruption in Neuroimmunological Disease. *Int J Mol Sci*. 2024;25(19):10625. doi:10.3390/ijms251910625
13. Wang X, Lou M, Song Y, et al. Effects of puerarin on chronic cerebral hypoperfusion-induced cognitive dysfunction and white matter injury via activating GLP-1R/PI3K/Akt pathway. *Brain Res Bull*. 2024;208:110563. doi:10.1016/j.brainresbull.2024.110563
14. Kuo WT, Odenwald MA, Turner JR, Zuo L. Tight junction proteins occludin and ZO-1 as

- regulators of epithelial proliferation and survival. *Ann N Y Acad Sci.* 2022;1514(1):21-33. doi:10.1111/nyas.14798
15. Mao X, Li H, Min S, et al. Loss of tricellular tight junction tricellulin leads to hyposalivation in Sjögren's syndrome. *Int J Oral Sci.* 2025;17(1):22. doi:10.1038/s41368-025-00349-9
 16. Cho Y, Haraguchi D, Shigetomi K, Matsuzawa K, Uchida S, Ikenouchi J. Tricellulin secures the epithelial barrier at tricellular junctions by interacting with actomyosin. *J Cell Biol.* 2022;221(4):e202009037. doi:10.1083/jcb.202009037
 17. Rubin LH, Shi W, Azola A, et al. Blood-Brain barrier disruption in long COVID and cognitive correlates: A cross-sectional MRI study. *Brain Behav Immun.* 2025. doi:10.1016/j.bbi.2025.07.024
 18. Ta W, Wang J, Zheng A, et al. Three-Dimensional Dynamic Blood-Brain Barrier Model Incorporating Native Cell Culture Systems Based on Shape Memory Biomaterials. *Adv Healthc Mater.* 2025;14(12):e2500066. doi:10.1002/adhm.202500066
 19. Poh L, Sim WL, Jo DG, et al. The role of inflammasomes in vascular cognitive impairment. *Mol Neurodegener.* 2022;17(1):4. doi:10.1186/s13024-021-00506-8
 20. Voirin AC, Perek N, Roche F. Inflammatory stress induced by a combination of cytokines (IL-6, IL-17, TNF- α) leads to a loss of integrity on bEnd.3 endothelial cells in vitro BBB model. *Brain Res.* 2020;1730:146647. doi:10.1016/j.brainres.2020.146647
 21. Saito AC, Higashi T, Fukazawa Y, et al. Occludin and tricellulin facilitate formation of anastomosing tight-junction strand network to improve barrier function. *Mol Biol Cell.* 2021;32(8):722-738. doi:10.1091/mbc.E20-07-0464
 22. van den Goor L, Miller AL. Closing the gap: Tricellulin/ α -catenin interaction maintains epithelial integrity at vertices. *J Cell Biol.* 2022;221(4):e202202009. doi:10.1083/jcb.202202009
 23. Akın-Kınay F, Uzun N, Kılınç İ, Kılıç AO. Tight Junction Proteins and Blood-brain Barrier Integrity in Pediatric Obsessive-compulsive Disorder: A Study on Claudin-5, Claudin-12, Occludin, Tricellulin, and Angulin-1. *Clin Psychopharmacol Neurosci.* 2025;23(3):444-452. doi:10.9758/cpn.25.1293
 24. Yu H, Luo H, Chang L, et al. The NEDD8-activating enzyme inhibitor MLN4924 reduces ischemic brain injury in mice. *Proc Natl Acad Sci U S A.* 2022;119(6):e2111896119.

doi:10.1073/pnas.2111896119

25. Markov AG, Fedorova AA, Kravtsova VV, et al. Circulating Ouabain Modulates Expression of Claudins in Rat Intestine and Cerebral Blood Vessels. *Int J Mol Sci.* 2020;21(14):5067. doi:10.3390/ijms21145067
26. Che G, Xiao X, Li T, Li J, Gao L. UFMylation: A Potential Modification for Neurological Diseases. *Curr Neuropharmacol.* 2025;23(8):907-917. doi:10.2174/011570159X340639240905092813
27. Rossig C, Paluda A, Chen R, Middleton AJ, Day CL. Arkadia and Ark2C Promote Substrate Ubiquitylation with Multiple E2 Enzymes. *J Mol Biol.* 2025;437(17):169259. doi:10.1016/j.jmb.2025.169259
28. Parashar S, Kaushik A, Ambasta RK, Kumar P. E2 conjugating enzymes: A silent but crucial player in ubiquitin biology. *Ageing Res Rev.* 2025;108:102740. doi:10.1016/j.arr.2025.102740
29. Li Y, Wei JY, Liu H, et al. An oxygen-adaptive interaction between SNHG12 and occludin maintains blood-brain barrier integrity. *Cell Rep.* 2022;39(2):110656. doi:10.1016/j.celrep.2022.110656
30. Zhang GS, Tian Y, Huang JY, et al. The γ -secretase blocker DAPT reduces the permeability of the blood-brain barrier by decreasing the ubiquitination and degradation of occludin during permanent brain ischemia. *CNS Neurosci Ther.* 2013;19(1):53-60. doi:10.1111/cns.12032
31. Zhao B, Yin Q, Fei Y, et al. Research progress of mechanisms for tight junction damage on blood-brain barrier inflammation. *Arch Physiol Biochem.* 2022;128(6):1579-1590. doi:10.1080/13813455.2020.1784952
32. Zhai X, Xie W, Yaqoob MD, et al. Evaluation of the Neuroprotective Effect of Total Glycosides of *Cistanche deserticola* and Investigation of Novel Brain-Targeting Natural MAO-B Inhibitors. *ACS Chem Neurosci.* 2024;15(24):4544-4558. doi:10.1021/acscchemneuro.4c00608
33. Li Y, Jia W, Hu J, et al. *Cistanche tubulosa* improves peripheral neuropathy in MPTP-induced PD mice based on regulation of m6A methylation. *Animal Model Exp Med.* 2025. doi:10.1002/ame2.70039

34. Ai Z, Xie Y, Li X, Lei D, Ambrose K, Liu Y. Revealing color change and drying mechanisms of pulsed vacuum steamed *Cistanche deserticola* through bioactive components, microstructural and starch gelatinization properties. Food Res Int. 2022;162(Pt B):112079. doi:10.1016/j.foodres.2022.112079

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