Review

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Cholinergic dysfunction in Alzheimer's disease: Mechanistic perspectives and multi-target effects of traditional Chinese medicine

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SUMMARY: Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by progressive cognitive decline and complex pathological changes. Cholinergic dysfunction is an early, clinically pivotal feature of AD closely associated with multiple pathological processes, including amyloid-β (Aβ) pathology, neuroinflammatory responses, mitochondrial dysfunction, metal-ion dyshomeostasis, and apolipoprotein E (APOE) ε4-related vulnerability. While disrupted cholinergic signaling impairs memory formation and synaptic function, its bidirectional interaction with AD-related pathological cascades further accelerates disease progression. Traditional Chinese medicine (TCM) has been investigated as a potential multitarget approach with which to modulate cholinergic deficits and related pathological processes. Some TCM-derived compounds, standardized extracts, and formulas have been reported to regulate cholinergic enzymes, acetylcholine levels, cholinergic receptors, oxidative stress, mitochondrial function, neuroinflammatory responses, and Aβ- or tau-related pathology. This review summarizes the role of cholinergic failure in AD pathophysiology and evaluates the evidence surrounding TCM-based interventions, with emphasis on direct cholinergic evidence, indirect collateral mechanisms, and current translational limitations. Indeed, the purpose of these insights is to inform future development of multitarget therapeutic strategies for AD.

Keywords: Alzheimer's disease, cholinergic system, traditional Chinese medicine, multi-target therapy

1. Introduction

As a major neurodegenerative disorder, Alzheimer's disease (AD) predominantly affects older adults worldwide and is characterized by progressive cognitive decline and memory impairment. Patients typically present with initial memory deficits, followed sequentially by language attrition, spatial disorientation, and executive dysfunction. Ultimately, a total loss of independence is observed in the advanced stages (*1*). With the global acceleration of population aging, China faces a particularly acute crisis, harboring the world's largest population of dementia cases, accounting for over 16 million as of 2021 (*2*). Thus, AD imposes a massive burden on patients, families, and healthcare systems worldwide.

The neuropathological landscape of AD is characterized by complex and progressive alterations, including extracellular senile plaques, intracellular neurofibrillary tangles (NFTs), region-specific synaptic loss, and persistent neuroinflammatory activation (*3*). A multi-faceted cascade, including amyloid-β (Aβ) toxicity, pathological tau spreading, cholinergic deficits, oxidative stress, and programmed neuronal death pathways, converges to define selective neuronal vulnerability. Cholinergic deficits have long provided the main rationale for symptomatic treatment in AD (*4*). Cholinergic dysfunction in AD includes diminished choline acetyltransferase (ChAT) activity, impaired high-affinity choline transport, and reduced acetylcholine (ACh) synthesis. These alterations compromise synaptic plasticity and disrupt the integrative function of cognitive networks. The cholinergic system influences Aβ metabolism, neuroinflammatory responses, and mitochondrial bioenergetics in addition to its role in neurotransmission. Loss of cholinergic function can initiate a self-amplifying cascade of complex neurodegenerative processes (*5*).

Rather than representing an isolated neurotransmitter deficit, cholinergic dysfunction interacts with multiple core pathological processes, including Aβ deposition, tau pathology, neuroinflammation, mitochondrial dysfunction, and disturbances in cellular homeostasis. Consequently, cholinergic dysfunction can serve as a

biologically significant entry point for understanding how these mechanisms converge within the broader pathological network of AD. Current pharmacological management includes symptomatic therapies, primarily cholinesterase inhibitors and the NMDA receptor antagonist memantine. These drugs offer mainly symptomatic relief and fail to arrest the underlying neurodegenerative process; long-term administration may be limited by adverse effects, and particularly gastrointestinal disturbances (*5*). Recent advances have led to the clinical approval of anti-amyloid monoclonal antibodies, including lecanemab and donanemab, for select patients with early AD. These disease-modifying therapies have demonstrated the ability to reduce amyloid burden and modestly slow cognitive decline. However, their clinical application remains restricted by stringent patient selection, the need for biomarker confirmation, intravenous administration, intensive imaging monitoring, and safety concerns such as amyloid-related imaging abnormalities (ARIA) (*6*). Their therapeutic benefits are also limited, as they have not yet been shown to fully address the multiple interconnected pathological processes underlying AD.

Against this therapeutic background, complementary strategies capable of acting on multiple pathogenic mechanisms remain of interest. Such a multitarget rationale is particularly relevant for evaluating traditional Chinese medicine (TCM), which has multicomponent and multitarget characteristics that may be suited to modulating interconnected pathological processes. Herbal formulations and TCM-derived compounds have been investigated for their potential effects on cognitive function, activities of daily living, neuropsychiatric symptoms, and AD-related pathological processes (*7*). This review systematically examines cholinergic system dysfunction in AD pathogenesis and evaluates current evidence regarding TCM-mediated modulation of cholinergic function in order to clarify its mechanistic basis, strength of evidence, and translational potential.

2. The cholinergic system in AD

Cholinergic neurotransmission depends on ACh synthesis, vesicular storage, and calcium-triggered release. These cholinergic neurons are widely distributed across the cerebrum and critically regulate learning, memory, and cognition. Pathological degeneration of these projections is a characteristic feature of dementia disorders, and particularly AD. Three features make this system central to AD: early and extensive degeneration, multifunctional disruption, and therapeutic tractability (*8*).

ACh biosynthesis begins with choline acetyltransferase (ChAT), which conjugates choline and acetyl-coenzyme A. The vesicular acetylcholine transporter (VAChT) then loads ACh into synaptic vesicles for release upon calcium-dependent depolarization. Release of ACh activates postsynaptic nicotinic (nAChRs) and muscarinic receptors (mAChRs) until acetylcholine esterase (AChE) hydrolyzes the signal into acetate and choline. Choline returns to the presynaptic terminal *via* the high-affinity choline transporter (CHT1), completing the cycle (*9*) (Figure 1).

Aβ also challenges the integrity of this cycle. In the preclinical stage of AD, physiological $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}16}$ transiently increase the synaptic vesicle pool *via* synaptophysin dephosphorylation and calcineurin activation (*10*). Pathological accumulation of Aβ, however, impairs cholinergic transmission, marking an early transition that precedes neuronal loss and that coincides with initial cognitive decline (*5*).

Cholinergic signaling maintains cerebral homeostasis through two additional mechanisms. These functions extend beyond synaptic transmission. First, ACh serves as an effective modulator and essential prerequisite for the complete expression of sensation-induced neurovascular coupling responses (*11*). Research has established that progressive degeneration of basal forebrain cholinergic neurons in AD leads to chronically reduced cortical ACh levels. These deficits mirror experimental results from models of prolonged ACh deprivation; specifically, both conditions demonstrate substantially impaired neurovascular coupling, constrained dynamic range in sensory processing networks, and distorted interpretation of neural activity through hemodynamic responses (*11*). Second, ACh signaling suppresses neuroinflammation and maintains intracellular homeostasis through multi-level bidirectional regulation *via* protein networks and non-coding microRNAs (*12*). These cortical functions depend on ascending projections from the basal forebrain (*13*). The nucleus basalis of Meynert (NBM) constitutes the principal source of these projections.

The NBM is the largest cholinergic component of the extrathalamic ascending reticular activating system and the primary output nucleus of the central cholinergic system. It integrates dopaminergic, serotonergic (5-HT), and noradrenergic signals from multiple brain regions to govern arousal, attention, and memory encoding (*13*). Its cholinergic Ch4 subdivision, the major cholinergic subdivision of NBM, projects *via* sparse collateral axons to cholinergic neuronal networks in the neocortex, hippocampus, and amygdala, providing the predominant ACh input to both the cerebral cortex and amygdala. Since Ch4 neurons regulate extensive cortical regions through sparse branching, even minor damage disrupts cholinergic signaling across wide territories (*13*). This anatomical vulnerability is associated with molecular susceptibility. Neurofibrillary degeneration within NBM cholinergic neurons emerges during the early stages of AD, strongly associated with the severity of cognitive impairment before substantial neuronal loss occurs. The exact mechanisms linking Ch4 lesions to specific symptoms remain unclear. However, this degeneration is an important factor in early cognitive impairment in AD. Loss of neuroprotective signaling also promotes Aβ and

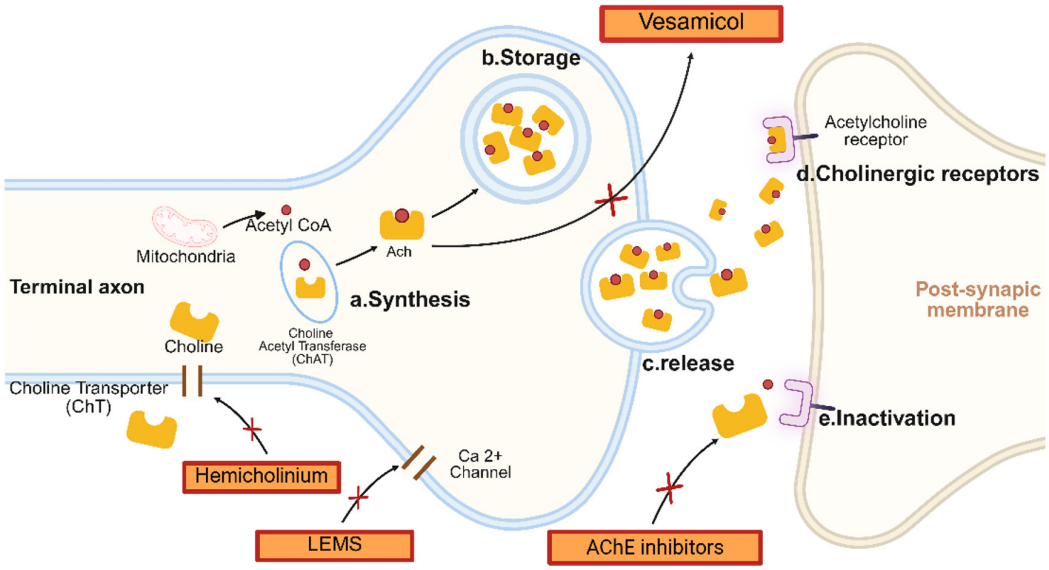


Figure 1. Cholinergic synaptic transmission and pharmacological intervention in Alzheimer's disease (AD). Acetylcholine (ACh) synthesis occurs in the presynaptic terminal, where choline acetyltransferase (ChAT) catalyzes the conjugation of choline and acetyl-CoA. Newly synthesized ACh is sequestered into synaptic vesicles and released into the synaptic cleft following Ca^{2+}-dependent depolarization. Following release, ACh binds to cholinergic receptors on the postsynaptic membrane to initiate signal transmission. Signaling termination proceeds through enzymatic hydrolysis by acetylcholinesterase (AChE), liberating choline for reuptake for subsequent ACh recycling. Pharmacological modulation of cholinergic signaling can target different steps of this process. For example, hemicholinium reduces choline uptake by inhibiting choline transport, vesamicol interferes with vesicular ACh storage through inhibition of the vesicular acetylcholine transporter (VAChT), and AChE inhibitors prolong cholinergic signaling by preventing ACh degradation. Impaired presynaptic release associated with dysfunction of calcium-dependent neurotransmitter release represents another mechanism affecting cholinergic transmission.

tau pathology. These changes further disrupt cholinergic transmission and accelerate cognitive decline (*8*).

The cholinergic crisis in AD encompasses not only presynaptic failure but also postsynaptic deterioration. Concomitant with NBM degeneration, AD reduces the number of nAChRs and mAChRs in basal forebrain cholinergic neurons and their cortical targets.

3. ACh receptors and cholinergic signaling

3.1. Nicotinic ACh receptors

As the first neurotransmitter receptors to be identified, nAChRs serve as prototypical ligand-gated ion channels that are widely distributed in the central and peripheral nervous system. This receptor superfamily also includes γ-aminobutyric acid type A (GABAA) receptors, glycine receptors, and serotonin type 3 (5-HT3) receptors (*14*). The functional diversity of nAChRs arises from various combinations of α and β subunits. At least nine α subunits (α2–α10) and three β subunits (β2–β4) have been identified in the CNS. The prototypical nAChRs typically assemble as a pentamer with an $\alpha_2\beta_3$ stoichiometry, forming a central hydrophilic pore that permits the transmembrane flux of cations such as K^+, Na^+, and Ca^{2+}. Ca^{2+} acts as a critical signaling molecule mediating the sustained regulatory effects of nicotinic receptors. Several neuronal nAChR subtypes, particularly α7 nAChRs, exhibit high Ca^{2+} permeability (*14*). Activation of these receptors increases presynaptic Ca^{2+} levels and promotes the release of multiple neurotransmitters, including glutamate, GABA, dopamine, 5-HT, and ACh.

In recent years, nAChR alterations have been linked to both early diagnosis and disease progression in AD. Positron emission tomography (PET) imaging has consistently demonstrated reduced α4β2 nAChR availability in AD, whereas investigations into α7 nAChRs remain limited but suggest region-specific alterations in receptor binding (*15*). α7 nAChRs are widely distributed and play a critical role in synapse formation, plasticity, learning, memory, and higher cognitive functions. They are reportedly expressed in neurons, microglia, astrocytes, and vascular endothelial cells. In microglia, α7 nAChRs exert neuroprotective effects by mediating anti-inflammatory signaling and modulating oxidative stress; mitochondrial-localized α7β2 nAChRs participate in the regulation of mitochondrial apoptotic pathways (*16*).

Notably, α7 nAChRs exhibit a strong binding affinity for soluble Aβ, forming Aβ-α7 nAChR complexes that undergo clathrin-mediated endocytosis. This process reduces functional α7 nAChRs on neuronal membranes. It also promotes intracellular Aβ accumulation, forming a dual pathological cascade. These observations are consistent with the markedly reduced nAChR expression found in AD brains and suggest a potential link between α7 nAChRs and the progression of AD (*16*). Emerging evidence also suggests that α7nAChRs may modulate

Aβ-induced neurotoxicity and mediate its detrimental effects on synaptic plasticity (*17*). These findings collectively suggest that the interaction between Aβ and α7 nAChRs plays an important role and that its proper balance is required for physiological function.

However, the complexity of α7 nAChR targeting is further compounded by regulatory heterogeneity. At the cellular level, AD patients exhibit upregulated α7 nAChR expression in hippocampal and cortical astrocytes but downregulated neuronal expression (*18*). In AD mouse models, both deletion and stimulation of α7 nAChRs have been reported to improve cognitive function (*19*). Although these findings appear contradictory, they may reflect the context-dependent roles of α7 nAChRs rather than fundamental inconsistencies. Indeed, the functional consequences of α7 nAChR modulation are likely influenced by disease stage, cell type, brain region, and the balance between physiological cholinergic signaling and pathological Aβ–α7 nAChR interactions. Activation of neuronal α7 nAChRs may preserve synaptic plasticity and suppress neuroinflammation during the early stages of disease, whereas under conditions of persistent Aβ accumulation and aberrant receptor signaling, reducing α7 nAChR activity may attenuate downstream neurotoxicity. Moreover, differences in AD models, the timing of the intervention, and experimental paradigms may contribute to the heterogeneity in outcomes across studies. These findings suggest that the function of α7 nAChRs in AD may vary across cell types and brain regions, necessitating precise modulation rather than simple activation or inhibition. Importantly, most evidence supporting α7 nAChR-targeted interventions is derived from preclinical studies, and the lack of consistent efficacy in clinical trials highlights the need for more standardized and disease stage-specific investigations. Although nAChR ligands hold therapeutic promise for some neurodegenerative and psychiatric disorders, no α7 nAChR-targeting ligand has yet succeeded in clinical trials.

3.2. Muscarinic receptors

Current evidence suggests that mAChRs predominantly mediate cholinergic transmission in forebrain regions and their contribution to learning and memory. As G protein-coupled receptors, mAChRs modulate diverse ion channels (*20*). Five subtypes of muscarinic receptors (M1-M5) have been identified and can be functionally grouped into two categories. M1, M3, and M5 couple with Gq/11 proteins. This coupling activates phospholipase C, increases intracellular calcium, and suppresses K^+ channel activity, which enhances neuronal excitability. In contrast, M2 and M4 interact with Gi/o proteins, leading to inhibition of the adenylyl cyclase and modulation of ion channel activity. The M1 subtype significantly influences cognition, attention, and sensory processing. Peripheral M2/M3 activation mediates the main adverse effects of cholinesterase inhibitors and other cholinergic agents, such as bradycardia and gastrointestinal disturbances, reflecting the functional differences between the central and peripheral receptor subtypes (*21*).

Thomson *et al*. examined subtype-specific functions in 2018. Their study showed that M1 and M4 receptors regulate hippocampal circuit formation and influence ACh release, which affects hippocampal function (*22*). Parallel investigations have established that M1 receptor deletion in the mouse prefrontal cortex (PFC) disrupts cholinergic signaling in pyramidal neurons, resulting in behavioral deficits in cue detection tasks (*23*). In contrast, activation of M1 receptors promotes the production of soluble amyloid precursor protein alpha (sAPPα) and reduces Aβ generation (*20*). Collectively, these findings establish M1 mAChR as a promising therapeutic target among the five mAChR subtypes.

Orthosteric agonists activate mAChRs lacking subtype selectivity, often precipitating adverse effects such as salivation, lacrimation, urination, diaphoresis, and gastrointestinal disturbances. To achieve M1 selectivity while avoiding these effects, research efforts have shifted towards allosteric modulation. The positive allosteric modulator VU0467319 (VU319) displays potent M1-mediated efficacy in animal models of AD. It enhances hippocampal synaptic plasticity and increases excitatory activity in the prefrontal cortex. VU319 also improves hippocampus-dependent cognitive function while mitigating the adverse effects of excessive receptor activation, thereby demonstrating an overall favorable safety profile (*24*).

4. Pathological factors associated with the cholinergic system

The hallmark pathological features of AD extend beyond Aβ and NFTs to encompass neuroinflammation, mitochondrial dysfunction, metal ion dyshomeostasis, and genetic abnormalities. These pathological characteristics interact with cholinergic pathology in a complex manner.

4.1. Aβ pathology

Aβ accumulation is a hallmark pathological feature in both familial AD and sporadic AD, and mounting evidence indicates that it is also one of the earliest pathological events contributing to central cholinergic dysfunction. Aβ is generated through sequential cleavage of amyloid precursor protein (APP), a transmembrane glycoprotein, by β-secretase and γ-secretase. This processing occurs predominantly within the endolysosomal system. Under physiological conditions, immune-mediated mechanisms efficiently clear Aβ peptides, whereas pathogenic mutations or impaired clearance promote progressive accumulation of insoluble

Aβ species and ultimately drive senile plaque (SP) formation (*25*) (Figure 2).

Clinical and experimental evidence indicates that abnormal Aβ deposition is closely associated with early impairment of the central cholinergic system. Postmortem evidence indicates that cognitively normal individuals with existing Aβ plaques already exhibit markedly reduced ChAT activity: 70.9% in the superior frontal gyrus and 79.5% in the inferior temporal gyrus. In addition, ChAT activity correlates negatively with Aβ concentration in the inferior temporal gyrus (*26*).

Aβ preferentially damages cholinergic projection regions involved in learning and memory, and particularly the basal forebrain and hippocampus. In Tg2576 transgenic mice, aged animals typically exhibit increased AChE staining around neocortical and hippocampal Aβ plaques. Moreover, cholinergic fibers in these areas exhibit marked structural distortion (*27*). Intracerebral injection of $A\beta_{42}$ in male rats impairs cholinergic function. Observed changes include lower ACh levels, reduced ChAT activity, and increased AChE activity, along with a compromised mitochondrial structure (*28*).

Aβ oligomers (AβOs) have been found to cause multi-target neurotoxicity. Direct interaction with α7 nAChRs represents one of the major mechanisms underlying cholinergic dysfunction. In addition, AβOs disrupt glutamatergic signaling by interfering with synaptic glutamate recycling. As a result, this induces abnormal extracellular glutamate accumulation, excessive NMDAR activation, and subsequent suppression of long-term potentiation (LTP) and exacerbation of long-term depression (LTD), ultimately resulting in a synaptic plasticity imbalance (*29*).

Interestingly, Aβ plaques and phosphorylated tau protein exhibit synergistic effects in the progression of AD. This interaction involves Aβ-facilitated tau aggregation and propagation within neuritic plaques. Tau is a microtubule-associated protein responsible for maintaining cytoskeletal stability (*30*). In AD, aberrant Aβ activates intracellular kinases that phosphorylate tau and promote its detachment from microtubules, which produces neurofibrillary tangles, destabilizes microtubules, impairs normal neuronal function, and may ultimately trigger neurodegeneration or apoptosis (*30*). The Aβ–tau cascade contributes not only to progressive neurodegeneration but also to sustained deterioration of cholinergic function. Postmortem studies have confirmed that Aβ pathology typically precedes and accelerates neocortical tau propagation, synergistically driving cognitive decline, which is closely associated with cholinergic dysfunction (*31*). Cholinergic depletion exacerbates cognitive impairment by promoting tau pathology and Aβ deposition, as shown in animal models. In rodent models of AD, selective basal forebrain cholinergic damage correlates significantly with increased hippocampal and cortical Aβ deposition and tau hyperphosphorylation (*32*). Beyond direct cytotoxicity, aberrant tau oligomers propagate intercellularly (*33*). These species activate neuroinflammatory responses

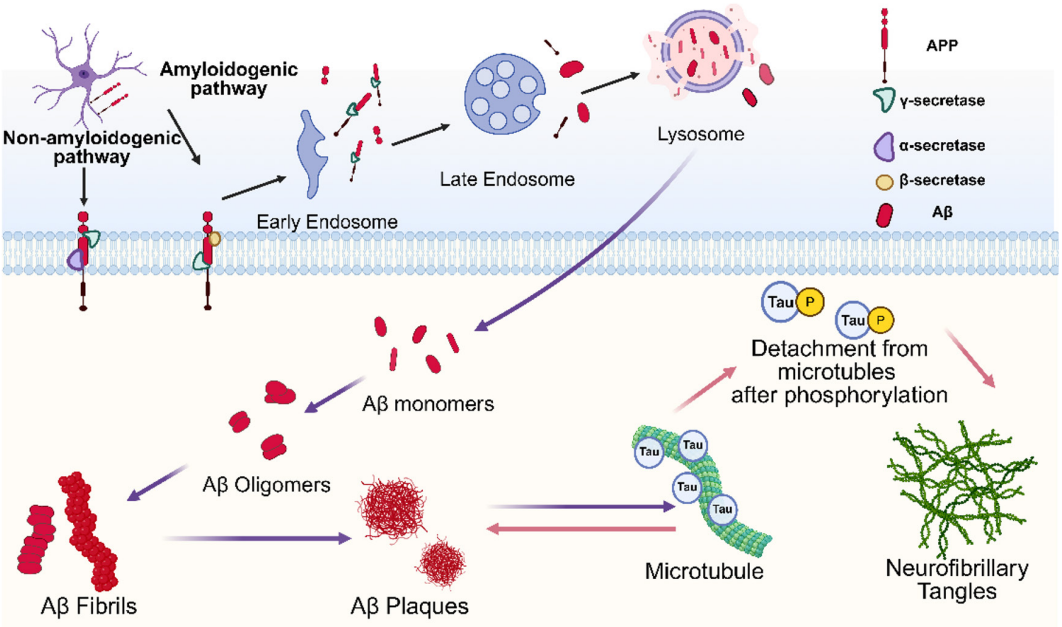


Figure 2. Mechanisms of amyloid-β processing and tau pathology in AD. Aβ accumulation constitutes a hallmark pathological feature of both familial and sporadic AD. Aβ is generated through sequential cleavage of the amyloid precursor protein (APP) by β-secretase and γ-secretase within the endosomal–lysosomal system. Following internalization from the plasma membrane, APP is trafficked through early and late endosomes and lysosomes, where amyloidogenic processing occurs. The resulting Aβ peptides are released as monomers that progressively assemble into oligomers, fibrils, and ultimately extracellular plaques. In parallel, Aβ-associated pathology promotes tau phosphorylation, triggering its detachment from microtubules and subsequent aggregation into neurofibrillary tangles. These interconnected processes drive the progressive neuropathological alterations characteristic of AD.

and, through astrocyte and microglia interactions, induce programmed cell death (*33*). The cholinergic system possesses intrinsic anti-inflammatory properties, and compromised cholinergic function may amplify these pathological processes. These findings collectively suggest that Aβ-mediated cholinergic impairment is not merely a consequence of amyloid pathology. Loss of cholinergic function may further encourage Aβ accumulation and tau-associated neurodegeneration, thereby accelerating disease progression.

4.2. Neuroinflammation

Neuroinflammation is closely linked to cholinergic dysfunction in AD, driven in part by the disruption of the cholinergic anti-inflammatory pathway (CAP). The CAP was first characterized in 2000 by Borovikova and colleagues, who showed that the vagus nerve and its neurotransmitter ACh suppress excessive inflammatory responses by modulating immune cell function. This interaction inhibits key signaling pathways (including NF-κB), downregulates pro-inflammatory cytokine production, and thereby limits excessive inflammation while protecting tissue homeostasis (*34*).

Impaired CAP signaling weakens cholinergic regulation of neuroinflammation and contributes to persistent glial activation during the progression of AD. Microglia and astrocytes are the principal drivers of central nervous system inflammation. In AD, cerebral Aβ clearance depends largely on microglial phagocytosis. Given that cholinergic signaling can restrain glial activation through CAP, impairment of this pathway may render microglia and astrocytes more susceptible to persistent inflammatory activation. However, chronic microglial activation induced by Aβ accumulation triggers sustained release of inflammatory factors, which paradoxically impairs phagocytic and degradative capacity. This inflammatory loop increases Aβ deposition and promotes tau aggregation, culminating in neuronal injury and cell death and accelerating the progression of AD (*35*). *In vitro* studies have demonstrated that Aβ exposure induces microglia and astrocytes to generate neurotoxic reactive oxygen species (ROS) and reactive nitrogen species, activate the NLRP3 inflammasome, and release pro-inflammatory cytokines (*36*). Simultaneously, activated microglia can indirectly induce astrocytes to adopt an A1-like reactive phenotype through the release of IL-1α, TNF, and the complement component C1q. This transformation causes astrocytes to lose their normal neuroprotective functions, including support of neuronal survival, synapse formation, and synaptic pruning, while reducing neurotrophic factor expression, thereby further exacerbating neuronal injury (*37*). Pathological cholinergic neuronal loss, coupled with declining ACh release, attenuates CAP-mediated control, effectively removing a vital regulatory checkpoint that restricts neuroinflammatory cascades. Persistent neuroinflammation, in turn, further compromises cholinergic neuronal survival and neurotransmission, forming a feed-forward cycle that amplifies cholinergic dysfunction throughout the progression of AD (*34*).

4.3. Mitochondrial dysfunction

Cholinergic neurons are particularly vulnerable to mitochondrial dysfunction given their exceptionally high metabolic demands. Mitochondrial dysfunction is an early feature of AD. It involves reduced energy metabolism, disrupted Ca^{2+} homeostasis, and increased oxidative stress. Mitochondria facilitate neuronal survival not only by generating ATP through oxidative phosphorylation but also by regulating neurotransmitter synthesis and Ca^{2+} buffering, both of which are essential to maintaining cholinergic neurotransmission (*38*).

Mitochondrial metabolism is directly coupled to cholinergic function. Under physiological conditions, mitochondria generate ATP and critical metabolites including acetyl-CoA, the acetyl donor required for ACh synthesis, through the tricarboxylic acid (TCA) cycle. Given that ACh synthesis relies on both acetyl-CoA availability and an adequate ATP supply, mitochondrial dysfunction can directly compromise ACh production and cholinergic synaptic activity (*39*) (Figure 3). Reduced ATP production also impairs the maintenance and function of cholinergic neurons, thereby exacerbating cholinergic dysfunction during the progression of AD.

In addition to facilitating ACh synthesis, mitochondrial metabolism influences neuronal survival through multiple protective mechanisms. For example, the TCA cycle intermediate oxaloacetate (OAA) plays several protective roles in neurons. It enhances energy metabolism, promotes mitochondrial biogenesis, reduces neuroinflammation, and facilitates hippocampal neurogenesis. These effects may help preserve cholinergic neuronal function, although they are attenuated under the hypometabolic conditions characteristic of AD (*40*). Enhancing mitophagy facilitates the clearance of Aβ plaques and hyperphosphorylated tau, restores mitochondrial homeostasis, and may interrupt the propagation of pathological cascades and even reverse memory impairment (*38*). These observations highlight the close metabolic dependence of cholinergic neurotransmission on mitochondrial integrity throughout the progression of AD.

4.4. Dysregulation of metal ion homeostasis

Disruption of metal ion homeostasis is closely associated with cholinergic dysfunction in AD. Iron, copper, and calcium affect cholinergic neurons through distinct but convergent mechanisms, including oxidative stress, mitochondrial dysfunction, abnormal cholinesterase regulation, and calcium homeostasis disruption, particularly within the vulnerable basal forebrain

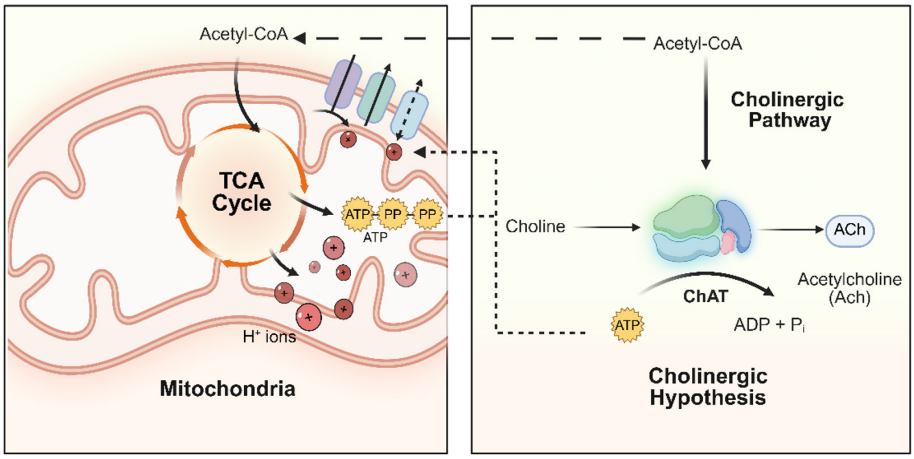


Figure 3. Mitochondrial metabolic regulation of the cholinergic pathway in AD. The tricarboxylic acid (TCA) cycle generates ATP and metabolic intermediates, including acetyl-CoA, which supply essential substrates and energy for ACh synthesis. Acetyl-CoA condenses with choline *via* ChAT to produce ACh. In AD, mitochondrial hypometabolism and impaired mitophagy promote the accumulation of dysfunctional mitochondria, disrupting energy homeostasis and amplifying the mitochondrial cascade. Reduced metabolic output restricts acetyl-CoA availability and ATP production, thereby impairing cholinergic function. Restoration of mitochondrial integrity may attenuate pathological protein accumulation and indirectly facilitate cholinergic neuronal survival and signaling.

cholinergic system (*41*). Iron has been extensively implicated in cholinergic degeneration. Excess iron and copper may also indirectly damage cholinergic neurons by promoting APP expression, accelerating Aβ production, and enhancing tau hyperphosphorylation.

Cholinergic neurons exhibit heightened susceptibility to iron dysregulation (*42*). Abnormal iron accumulation promotes oxidative stress and ferroptosis, thereby increasing the vulnerability of cholinergic neurons. Anatomical evidence indicates that the ventral extension area of the iron-rich globus pallidus overlaps highly with the distribution of cholinergic neurons (AChE-positive neurons). This anatomical overlap may, to some extent, account for the selective susceptibility of cholinergic neurons to iron-mediated oxidative injury (*43*). Iron overload further enhances ROS generation through the Fenton reaction, leading to oxidative damage, lipid peroxidation, and progressive neuronal degeneration (*38*). Iron dysregulation may also impair cholinergic neurotransmission by altering butyrylcholinesterase (BChE) activity. In addition, current evidence suggests that abnormally elevated BChE activity in the hippocampus and cortex accelerates ACh hydrolysis, thereby further exacerbating cholinergic dysfunction (*42*).

Disruption of copper homeostasis represents another mechanism linking a metal ion imbalance to cholinergic dysfunction in AD. Excessive intracellular copper can trigger cuproptosis, a form of regulated cell death associated with mitochondrial metabolism disruption. Several cuproptosis-related genes, including *FDX1*, *DLAT*, *LIAS*, and *PDHA1*, have been reported to be dysregulated in AD and may contribute to mitochondrial dysfunction and oxidative stress. Reduced PDHA1 activity limits acetyl-CoA production, thereby not only impairing ATP generation but also restricting ACh synthesis. Dysregulated copper homeostasis may therefore impair cholinergic neurotransmission by disrupting mitochondrial metabolism and promoting cuproptosis-associated neuronal injury (*44*).

Iron dysregulation and calcium homeostasis disruption are closely interconnected, together intensifying neuronal injury in AD. Iron-mediated calcium signaling activates downstream kinase cascades involved in synaptic plasticity. Under physiological conditions, iron-dependent ROS generation facilitates normal calcium-dependent signaling, whereas excessive ROS disrupts calcium signaling and mitochondrial function, ultimately impairing neurons, including cholinergic neurons (*45*). Given the dependence of cholinergic neurotransmission on tightly regulated intracellular Ca^{2+} dynamics, persistent disruption of calcium homeostasis is likely to further exacerbate cholinergic dysfunction during the progression of AD (*46*). Taken together, these findings suggest that metal ion dyshomeostasis may diminish cholinergic neurotransmission and reduce neuronal survival, thereby contributing to a self-reinforcing degenerative process during the progression of AD.

4.5. Apolipoprotein E

The apolipoprotein E (APOE) ε4 allele, the strongest genetic risk factor for sporadic AD, is closely associated with cholinergic vulnerability in AD. In addition, ApoE is essential for cholesterol transport, synaptic maintenance, and neuronal repair in the central nervous system. (*47*).

Compared to non-carriers, individuals carrying the APOE ε4 allele have been reported to exhibit poorer therapeutic responses to cholinesterase inhibitors such as tacrine, possibly owing to impaired ACh biosynthesis (*48*). ApoE4 transgenic mice were found to display

a reduced capacity to regenerate cholinergic nerve fibers following entorhinal cortex injury (*49*), while postmortem studies have demonstrated significantly lower ChAT activity in APOE ε4 carriers than in non-carriers. Although a direct causal relationship between APOE ε4 and reduced ChAT activity has not been established, available evidence suggests that this genotype may exacerbate cholinergic degeneration by impairing neuronal repair and limiting the functional reserve of the cholinergic system (*50*).

APOE ε4 also contributes to cholinergic dysfunction through its effects on Aβ pathology. In this respect, ApoE4 promotes the early deposition and spread of Aβ while reducing its clearance, thereby increasing pathological stress on vulnerable cholinergic neurons (*51*). In addition, Aβ can interact with BChE and ApoE to form a soluble complex with enhanced ACh-hydrolyzing activity, thereby increasing ACh degradation and further disrupting cholinergic neurotransmission (*52*).

These interrelated pathological events do not occur in isolation but converge on and interact with the cholinergic system, forming a key point of intersection among multiple neurodegenerative cascades. A cholinergic-centered perspective helps integrate these mechanisms and highlights the complex interplay underlying disease progression, as shown in Figure 4.

5. Progress in TCM for the treatment of AD

At present, the main medications for AD in clinical use include centrally acting AChE inhibitors, such as rivastigmine, galantamine, and donepezil, together with the NMDA receptor antagonist memantine. Although these agents can alleviate cognitive symptoms to some extent, they have limited ability to prevent the progressive loss of cholinergic neurons or modify the complex pathological processes underlying AD, and their long-term use may be constrained by adverse effects, including gastrointestinal disturbances and sleep disorders (*5*). Consequently, multitarget therapeutic strategies that simultaneously affect cholinergic dysfunction and its associated pathological networks have attracted increasing attention. In this context, TCM and its bioactive constituents have, given their multicomponent composition and multitarget characteristics, been investigated as potential therapeutic approaches for AD. Current evidence indicates that some TCM interventions directly modulate cholinergic-related markers, including ACh, AChE, BChE, ChAT, and cholinergic receptors. Other studies suggest that their reported effects may involve the regulation of AD-related pathological processes closely associated with cholinergic degeneration, including Aβ deposition, tau pathology, oxidative stress, mitochondrial dysfunction, and neuroinflammation (*7*).

Accordingly, the available evidence was categorized into three groups: direct cholinergic evidence, indirect cholinergic-related evidence, and supportive or weak evidence. Direct cholinergic evidence is defined as studies demonstrating the direct modulation of established cholinergic markers, including ACh, AChE, BChE, ChAT, or cholinergic receptors. Indirect cholinergic-related evidence includes studies in which the main findings involve AD-associated

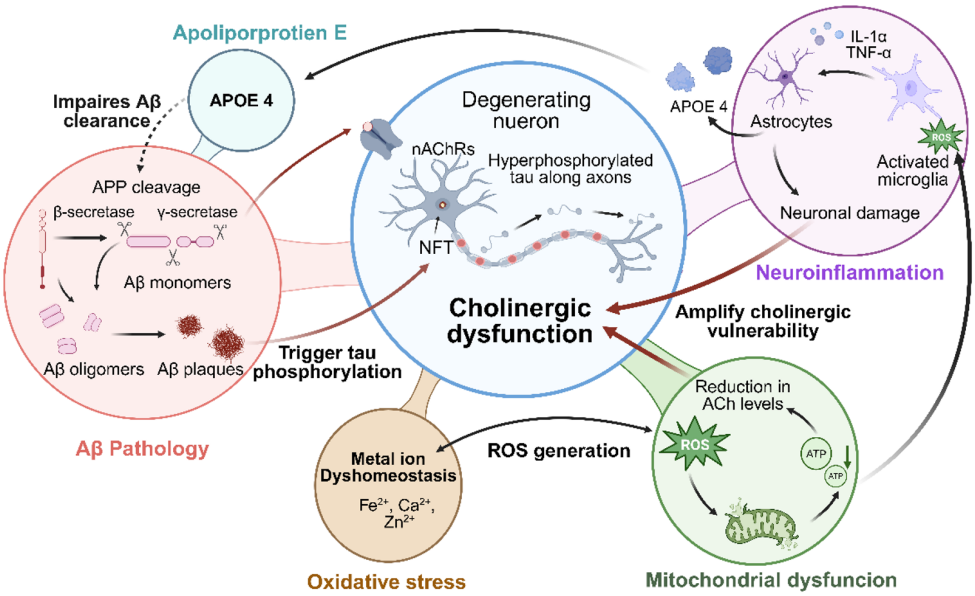


Figure 4. Interconnected mechanisms underlying cholinergic dysfunction in AD. APP cleavage by β- and γ-secretases generates Aβ monomers, oligomers, and plaques, along with impaired Aβ clearance, and particularly in the presence of APOE4. These changes are linked to tau hyperphosphorylation, neurofibrillary tangle (NFT) formation, and neuronal degeneration. Oxidative stress is reflected by reactive oxygen species (ROS) production and metal ion dyshomeostasis (Fe^{2+}, Cu^{2+}, Ca^{2+}). Mitochondrial dysfunction is characterized by reduced ATP production and decreased ACh levels. Astrocytes and activated microglia release pro-inflammatory mediators, contributing to neuronal damage. These alterations converge, resulting in cholinergic dysfunction. Red arrows indicate detrimental effects on the cholinergic system.

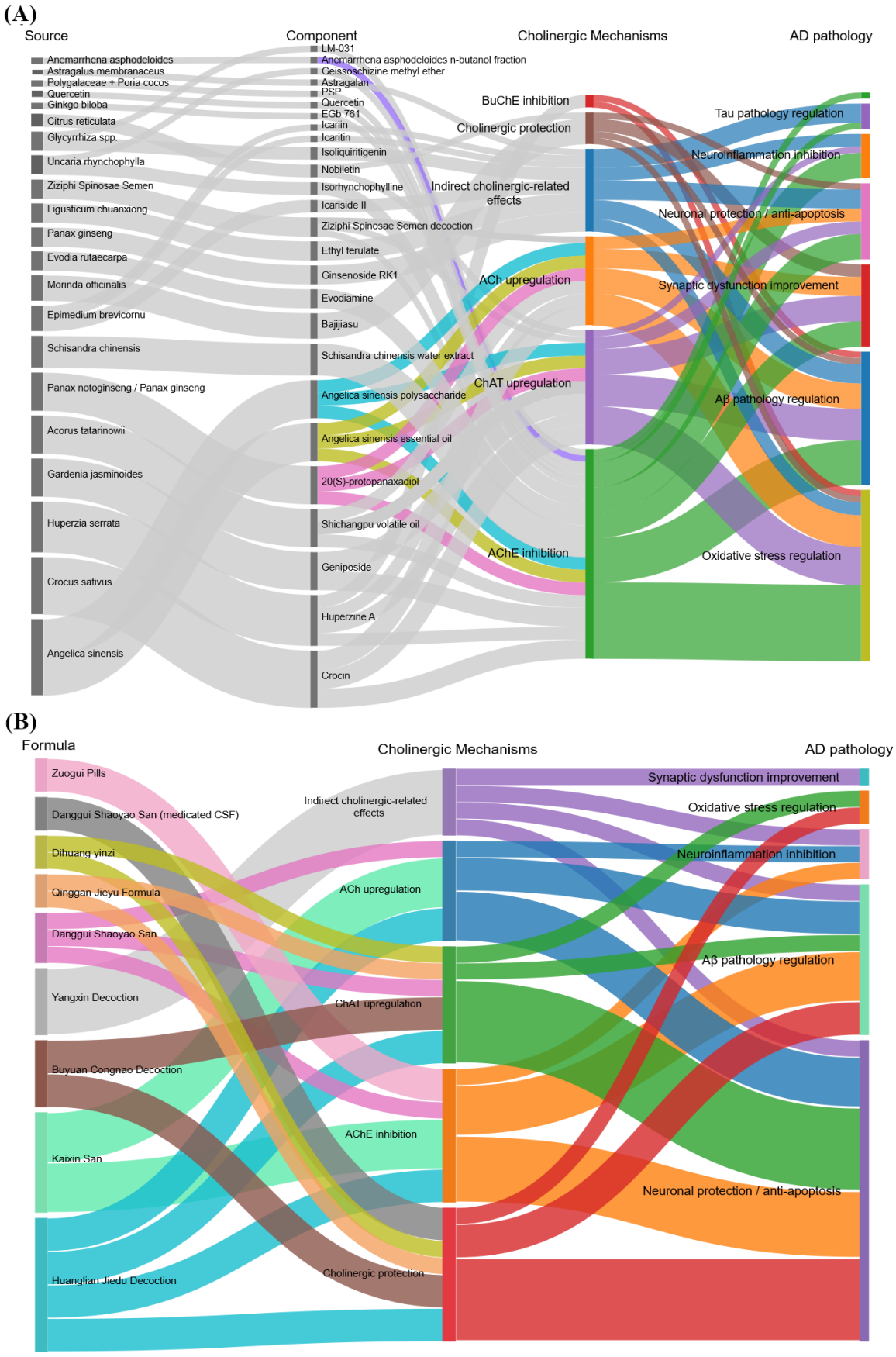


Figure 5. Sankey diagrams of active components from single herbs and herbal formulations in traditional Chinese medicine (TCM). **(A)** Sankey diagram of TCM compounds regulating the cholinergic system in AD. **(B)** Sankey diagram of TCM herbal formulations regulating cholinergic mechanisms in AD. The Sankey diagrams were generated using the online visualization tool RAWGraphs 2.0 (https://app.rawgraphs.io/)

pathological processes, such as Aβ deposition, tau pathology, oxidative stress, mitochondrial dysfunction, neuroinflammation, or synaptic impairment, without direct assessment of cholinergic markers. Supportive or weak evidence refers to studies reporting only general neuroprotective effects, showing limited relevance to disease models, or lacking clear experimental validation. Based on this classification scheme, the experimental evidence evaluating single herbs, standardized extracts, and pure TCM-derived constituents is shown in Table 1, with multi-herb formulas summarized in Table 2. Table 3 presents a comprehensive summary of the available clinical evidence and translationally relevant information.

5.1. Single herbs, standardized extracts, and TCM-derived compounds

Among the currently investigated TCM-based therapeutic strategies, bioactive constituents that directly modulate cholinergic neurotransmission have yielded relatively substantial experimental evidence. Among these, Huperzine A, an active constituent isolated from *Huperzia serrata* (serrate clubmoss), represents the most extensively investigated example. Huperzine A is a potent AChE inhibitor that enhances cholinergic neurotransmission by reducing the degradation of ACh. In APP/PS1 mice, intranasal administration of Huperzine A reduced AChE activity, increased ChAT expression, and alleviated Aβ pathology through the modulation of APP processing and activation of the Wnt/β-catenin signaling pathway (*53*). These findings collectively suggest that the effects of directly enhancing cholinergic function are not limited to the regulation of neurotransmitter signaling. Direct cholinergic modulation may also influence multiple interconnected pathological pathways in AD, thereby producing broader modulation of AD-related pathological processes.

In addition to Huperzine A, several compounds derived from TCM have been reported to have direct cholinergic activity by targeting different components of the cholinergic system. For example, nobiletin, a polymethoxylated flavonoid isolated from *Citrus reticulata* (tangerine peel), restored ChAT expression and cholinergic fiber density in the hippocampus of olfactory bulbectomized mice (*54*). Likewise, crocin, a major bioactive constituent of *Crocus sativus* (saffron), increased ACh and ChAT levels while reducing AChE activity in animal models of AD (*55*). Similar cholinergic regulatory activities have been reported for geniposide, *Schisandra chinensis* (Chinese magnolia vine) extract, isorhynchophylline, *Angelica sinensis* (Chinese Angelica) polysaccharides, 20(S)-protopanaxadiol, β-asarone, and other natural products summarized in Table 1 (*56-61*). Collectively, these findings indicate that TCM-derived compounds may directly regulate cholinergic signaling at multiple levels, including neurotransmitter synthesis, cholinergic neuron integrity, and other key components of the cholinergic system.

Notably, many compounds with direct cholinergic activity modulate pathological processes that are closely associated with cholinergic neurodegeneration. In addition to restoring cholinergic neurotransmission, crocin attenuates oxidative stress, mitochondrial dysfunction, and Aβ deposition. Geniposide not only normalizes abnormalities in ChAT and AChE but also reduces the amyloid burden by suppressing the Aβ-RAGE/MAPK signaling pathway (*56*). Likewise, *Schisandra chinensis* extract decreases AChE activity while ameliorating neuroinflammation and metabolic disturbances (*57*). Taken together, these findings suggest that cholinergic dysfunction in AD is not an isolated neurotransmitter deficiency but an integral component of the broader pathological network underlying disease progression.

Another group of compounds has not been evaluated for their direct effects on cholinergic markers, such as ACh, AChE, ChAT, or cholinergic receptors. At present, these compounds are more appropriately regarded as indirect cholinergic-related evidence. Representative examples include ginsenoside RK1, icariin, icariside II, icaritin, and ethyl ferulate. Their reported effects mainly involve the regulation of Aβ production and clearance, tau phosphorylation, oxidative stress responses, mitochondrial homeostasis, and neuronal survival (*62-65*). In addition, some compounds have been assessed in models with limited relevance to AD-related cholinergic dysfunction. In this regard, quercetin has been reported to improve abnormal AChE activity only in a cadmium-induced neurotoxicity model and is therefore classified as supportive or weak evidence in Table 1. Collectively, evidence from isolated compounds and standardized extracts indicates that TCM-derived interventions may act through both direct cholinergic regulation and indirect modulation of AD-related pathological processes. Nevertheless, the assessment of cholinergic markers remains inconsistent across studies, and the translational value of these findings needs to be validated further.

5.2. TCM formulas

Compared to individual compounds, TCM formulas are characterized by multicomponent composition, multitarget actions, and network-based regulation. These features theoretically align well with the complex pathological network in which cholinergic dysfunction develops during the progression of AD. In contrast to several TCM-derived natural compounds with mechanisms that have been investigated in considerable detail, only a limited number of TCM formulas have been supported by direct evidence of cholinergic regulation. Most studies have instead demonstrated their ability to modulate pathological processes that are closely associated with cholinergic degeneration. Accordingly, the collective evidence for TCM formulations remains

Table 1. Preclinical evidence of TCM-derived compounds and standardized extracts targeting cholinergic dysfunction in AD

Intervention	Main constituent(s)	Model	Dose	Evidence category	Cholinergic evidence	Main findings	Reference
Intranasal administration with Huperzine A	Huperzine A	APP/PS1 mice	167 or 500 μg/kg/day, intranasal, 4 months	Direct	AChE↓, ChAT↑	Improved cognition; reduced Aβ plaques; activated Wnt/β-catenin signaling.	(*53*)
Nobiletin (*Citrus reticulata*)	Nobiletin	OBX mice	50 mg/kg/day, i.p., 11 days	Direct	ChAT↑; restored cholinergic fiber density	Improved memory through ERK/CREB signaling.	(*54*)
Crocin (*Crocus sativus*)	Crocin	HT22 cells; D-galactose/AlCl3 mice	5 or 20 mg/kg/day	Direct	ACh↑, ChAT↑, AChE↓	Reduced Aβ deposition; attenuated oxidative stress and mitochondrial dysfunction.	(*55*)
Geniposide	Geniposide	APP/PS1 mice; primary hippocampal neurons	12.5–50 mg/kg/day for 3 months	Direct	ChAT↑, AChE↓	Reduced amyloid pathology *via* the Aβ–RAGE/MAPK pathway.	(*56*)
Schisandra chinensis extract	Gomisin A, Schisandrin, Longikaurin A	$A\beta_{25–35}$ rats	0.5% SCF for 7 weeks	Direct	AChE↓	Reduced neuroinflammation and Aβ deposition; improved cognition.	(*57*)
Isorhynchophylline	Isorhynchophylline	AlCl3-induced AD in mice	20 or 40 mg/kg/day for 8 weeks	Direct	AChE↓	Improved cognition; suppressed oxidative stress and NF-κB activation.	(*58*)
Angelica sinensis polysaccharide	Angelica sinensis polysaccharide	$A\beta_{25–35}$ rats	2 mL/100 g/day for 28 days	Direct	ACh↑, ChAT↑, AChE↓	Reduced APP/Aβ levels and oxidative stress.	(*59*)
20(S)-Protopanaxadiol	20(S)-PPD	Scopolamine-induced memory impairment in mice	20 or 40 μmol/kg/day for 14 days	Direct	AChE↓, ChAT↑, ACh↑	Improved cognition; reduced oxidative stress.	(*60*)
β-Asarone (*Acorus tatarinowii*)	β-Asarone	$A\beta_{1–42}$ rats	25 mg/kg/day for 28 days	Direct	ACh↑, ChAT↑, AChE↓	Increased NGF and reduced neuronal apoptosis.	(*61*)
Ginsenoside RK1	Ginsenoside RK1	PC12 cells; mice with AD	Not reported	Indirect	Not directly evaluated	Improved cognition; mitochondrial protection; activated AMPK/Nrf2.	(*62*)
Icariin/Icaritin	Icariin; Icaritin	OA-treated SH-SY5Y cells	ICA 2.5 μM; ICT 1 μM	Weak	Not directly evaluated	Reduced tau phosphorylation through GSK-3β inhibition.	(*63*)
Icariside II	Icariside II	APP/PS1 mice	10 or 30 mg/kg/day for 3 months	Indirect	Not directly evaluated	Reduced Aβ production; improved cognition.	(*64*)
Ethyl ferulate	Ethyl ferulate	$A\beta_{1–42}$ rats	100 mg/kg/day for 6 weeks	Indirect	Not directly evaluated	Reduced Aβ, oxidative stress and neuroinflammation.	(*65*)

Table 1. Preclinical evidence of TCM-derived compounds and standardized extracts targeting cholinergic dysfunction in AD (continued)

Intervention	Main constituent(s)	Model	Dose	Evidence category	Cholinergic evidence	Main findings	Reference
Quercetin	Quercetin	Cadmium-exposed rats	5–50 mg/kg/day	Direct	Prevented abnormal AChE↑	Neuroprotection in cadmium toxicity rather than AD.	(*78*)
LM-031 (chalcone–coumarin hybrid)	LM-031	Tet-On Aβ-GFP293/SH-SY5Y cells	Not reported	Indirect	AChE inhibition	Reduced Aβ aggregation and oxidative stress.	(*79*)
Anemarrhena asphodeloides n-butanol fraction	Timosaponins	Scopolamine-induced memory impairment in mice	20 mL/kg/day for 21 days	Direct	AChE↓	Improved cognition and antioxidant capacity.	(*80*)
Geissoschizine methyl ether	Geissoschizine methyl ether	AChE assay	IC50 18.3 μM	Direct	Reversible AChE inhibition	Potential AChE inhibitor.	(*81*)
Ziziphi Spinosae Semen	Jujuboside A/B	PCPA-induced insomnia in mice	Not reported	Direct	AChE mRNA↓, ACh↑, ChAT↑	Improved cholinergic neurotransmission.	(*82*)
Angelica sinensis essential oil	Angelica sinensis Essential oil	$A\beta_{25-35}$ rats	Not reported	Direct	ACh↑, ChAT↑, AChE↓	Improved cognition and reduced APP/Aβ.	(*83*)
PSP (*Polygala tenuifolia* saponins + *Poria cocos* polysaccharides)	PSP	Memory-impaired mice	Not reported	Direct	AChE↓	Improved learning and antioxidant capacity.	(*84*)
EGb 761 + donepezil	Standardized Ginkgo biloba extract	Scopolamine-induced memory impairment in rats	EGb761 8.27 mg/kg + donepezil	Direct	AChE↓	Enhanced cognitive improvement with donepezil.	(*85*)
Bajijiasu	Bajijiasu	$A\beta_{25-35}$ rats	See original study	Direct	Attenuated cholinergic dysfunction	Improved cognition and reduced neuronal apoptosis.	(*80*)
Evodiamine	Evodiamine	STZ-induced AD in mice	50 or 100 mg/kg/day for 21 days	Direct	AChE↓	Improved cognition; suppressed oxidative stress and neuroinflammation.	(*81*)

Table 2. Preclinical evidence of TCM formulas targeting cholinergic dysfunction in AD

Formula	Main herbal components	Model	Dose	Evidence category	Cholinergic evidence	Other major mechanisms	Main findings	Major limitations	Reference
Huanglian Jiedu Decoction	*Coptis chinensis, Scutellaria baicalensis, Phellodendron chinense, Gardenia jasminoides*	$A\beta_{1-42}$-induced AD rats	1.5, 3, or 6 g/kg/day	Direct	ACh↑; ChAT↑; AChE↓; α7nAChR↑	Anti-Aβ activity; neuroprotection	Increased ACh, ChAT and α7nAChR expression while reducing AChE activity, accompanied by improved cognition and alleviated neuronal injury.	Downstream cholinergic signaling was not investigated; receptor antagonist validation lacking.	(*66*)
Danggui Shaoyao San	*Angelica sinensis, Paeonia lactiflora, Atractylodes macrocephala, Alisma orientale, Poria cocos, Ligusticum chuanxiong*	Ibotenic acid (IBO)-induced AD rats	0.139, 0.372, or 1 g/kg/day	Direct	ACh↑; ChAT↑; AChE↓	Neuroprotection; behavioral improvement	Restored cholinergic function by increasing ACh and ChAT and decreasing AChE, while improving neuronal survival and cognitive performance.	Limited mechanistic investigation beyond cholinergic biomarkers.	(*67*)
Kaixin San	*Panax ginseng, Polygala tenuifolia, Acorus tatarinowii, Poria cocos*	SCOP-induced cognitive impairment in C57BL/6 mice	6.5 g/kg/day	Direct	ACh↑; AChE↓	NF-κB/STAT3 inhibition; anti-neuroinflammation; neuroprotection	Increased ACh, reduced AChE, improved cognitive function, and inhibited NF-κB/STAT3 signaling *in vivo*.	Only selected predicted targets were experimentally validated.	(*68*)
Yang Xin Decoction	*Poria cocos, Polygala tenuifolia, Panax ginseng, Astragalus membranaceus, Angelica sinensis, etc.*	Primary mouse cortical neurons; ReNcell VM; SH-SY5Y; PC12 cells	100 μg/mL (extract); Polygala tenuifolia extract 10 μg/mL	Indirect	None (no direct cholinergic evidence)	Anti-Aβ aggregation; anti-inflammatory activity; CREB/ERK activation; neuronal differentiation; neuroprotection	Reduced Aβ aggregation, suppressed inflammation, activated CREB/ERK signaling, and promoted neuronal differentiation.	No *in vivo* validation; cholinergic biomarkers were not assessed; synergistic interactions remain unclear.	(*69*)
Zuogui Pills	*Rehmannia glutinosa, Dioscorea opposita, Cornus officinalis, Lycium barbarum, etc.*	D-galactose + $A\beta_{1-40}$-induced AD in rats	2.7 or 5.4 g/kg/day	Direct	AChE↓	HSP70 upregulation; anti-apoptosis; neuroprotection	Suppressed brain AChE activity, enhanced HSP70 expression, and protected hippocampal neurons.	Active constituents and downstream pathways remain unclear.	(*88*)
Buyuan Congnao Decoction	*Astragalus membranaceus, processed Polygonum multiflorum, etc.*	$A\beta_{1-42}$ + IBO-induced AD in rats	~2.39 g/kg/day	Direct	ChAT↑; AChE modulation	Antioxidant activity; neuroprotection	Improved learning and memory while increasing serum ChAT activity and antioxidant capacity.	Mechanistic pathway remains unclear.	(*89*)
Qinggan Jieyu Formula	*Angelicae Sinensis Radix, Paeoniae Radix Alba, Poria, Atractylodis Macrocephalae Rhizoma, etc.*	Naturally occurring dementia in aged mice	Not reported	Direct	ChAT↑; AChE-positive fiber density↑	Neuroprotection	Enhanced central cholinergic activity and improved learning and memory in aged mice.	Formula composition and dosage were incompletely reported.	(*90*)

Table 2. Preclinical evidence of TCM formulas targeting cholinergic dysfunction in AD (continued)

Formula	Main herbal components	Model	Dose	Evidence category	Cholinergic evidence	Other major mechanisms	Main findings	Major limitations	Reference
Dihuang Yinzi	*Rehmannia glutinosa, Morinda officinalis, Cornus officinalis, Dendrobium officinale, etc.*	2VO-induced chronic cerebral hypoperfusion in rats	10 or 20 g/kg/day	Indirect	ChAT↑; AChE↑	Neuroprotection	Improved cognitive performance and partially restored hippocampal cholinergic activity in chronic cerebral hypoperfusion rats.	Disease model differs from classical AD pathology. The increase in AChE complicates interpretation of its cholinergic effect.	(*91*)

heterogeneous, with the extent of mechanistic validation varying substantially across studies (Table 2).

Huanglian Jiedu Decoction, Danggui Shaoyao San, and Kaixin San are among the few representative TCM formulas for which direct evidence of cholinergic regulation has been reported. In animal models of Aβ-induced synaptic dysfunction, Huanglian Jiedu Decoction increased the levels of ACh and the expression of ChAT and hippocampal α7nAChR while reducing AChE activity. It also alleviated neuronal injury, suggesting that the formula regulates both cholinergic neurotransmission and related pathological processes (*66*). Similarly, Danggui Shaoyao San restored cholinergic function by increasing ChAT and ACh levels while decreasing AChE activity. It also improved neuronal survival and behavioral performance (*67*). Kaixin San was consistently found to increase ACh levels and reduce AChE activity *in vivo*. Further experimental studies showed that its cholinergic effects were accompanied by inhibition of the NF-κB/STAT3 signaling pathway (*68*). These studies collectively indicate that the effects of TCM formulas on cholinergic signaling are unlikely to occur in isolation. Instead, their cholinergic effects usually extend to multiple interconnected pathological pathways.

At present, most TCM formulas have yielded only evidence indirectly related to cholinergic dysfunction. The reported mechanisms primarily involve the regulation of amyloid pathology, neuroinflammation, oxidative stress, mitochondrial homeostasis, synaptic function, or neuronal survival. In many of these studies, however, classical cholinergic biomarkers were not evaluated. For example, Yangxin Decoction has demonstrated broad neuroprotective activities, including inhibition of Aβ aggregation and inflammatory signaling pathways, activation of the CREB/ERK-dependent pathway, and promotion of neuronal differentiation. However, direct regulation of AChE, ChAT, or other cholinergic biomarkers by this formula has not been demonstrated (*69*). For studies of TCM formulas that rely solely on network pharmacology predictions or preliminary computational target analyses without experimental validation, the findings can only suggest potential regulatory mechanisms rather than provide mechanistic confirmation. Accordingly, these studies were classified as supportive or weak evidence in Table 2.

Overall, the available evidence suggests that TCM formulas may influence cholinergic dysfunction in AD through the coordinated regulation of interconnected pathological processes (Table 2). Compared to studies of individual compounds, the principal challenge in investigating TCM formulas is not the identification of a single molecular target. Rather, it lies in the incomplete characterization of their chemical basis, key bioactive constituents, and the synergistic interactions among different components. At present, only a limited number of studies have directly evaluated ACh levels, AChE or

Table 3. Clinical evidence of TCM interventions targeting cholinergic dysfunction in AD

Intervention	Disease type	Treatment duration	Dose	Clinical evidence	Main clinical outcomes	Safety	Reference
Huperzine A	AD	8 weeks–6 months	0.2–0.45 mg/day, oral	Systematic review, meta-analysis, and TSA (25 studies; *n* = 1,813); RCT (*n* = 90)	Improved MMSE, HDS-R, memory performance, ADL, and overall clinical response.	Mild cholinergic adverse events (mainly gastrointestinal discomfort and dizziness); serious adverse events uncommon.	(*70*)
EGb 761 (*Ginkgo biloba* extract)	Mild dementia (including probable AD, VD, and mixed dementia)	22–24 weeks	240 mg/day, oral	Meta-analysis (*n* = 782)	Improved cognition, ADL, global assessment, and quality of life, and alleviated neuropsychiatric symptoms.	Generally well tolerated; no significant increase in serious adverse events.	(*71*)
Buyuan Congnao Decoction + piracetam	AD	12 weeks	Buyuan Congnao Decoction + piracetam (0.8 g, three times daily)	RCT (*n* = 196)	Improved MMSE, ADAS-Cog, overall clinical response; increased IGF-1 and reduced Aβ.	No obvious treatment-related adverse events reported.	(*72*)
Dihuang Yinzi + donepezil	Mild-to-moderate AD	3 months	Dihuang Yinzi (1 dose/day) + donepezil (5 mg/day)	RCT (*n* = 114)	Improved MMSE, ADAS-Cog, ADL; regulated Notch1, ADAM10, BACE1, and acrolein.	Similar incidence of adverse events between groups; no serious adverse events.	(*73*)
Jianpi Yangxin Decoction + donepezil	Mild-to-moderate AD	3 months	Jianpi Yangxin Decoction (1 dose/day) + donepezil (5–10 mg/day)	RCT (*n* = 150)	Improved MMSE, ADL, and TCM syndrome scores.	Lower incidence of adverse events than donepezil alone.	(*74*)
Hengqing II + donepezil	AD	3 months	Hengqing II Formula (1 dose/day) + donepezil (5–10 mg/day)	RCT (*n* = 107)	Improved MMSE, MoCA, ADAS-Cog, ADL, and NPI; reduced APP, Aβ, inflammatory markers, Hcy, and CysC.	Well tolerated; no significant difference in adverse events between groups.	(*75*)
Bushen Capsules	aMCI	24 months	4 capsules, three times daily	Randomized, double-blind, placebo-controlled trial (*n* = 60)	Improved episodic memory and altered brain activation patterns on fMRI.	Similar adverse-event profile to a placebo.	(*76*)
Di-Tan Decoction	AD	24 weeks (+6-week follow-up)	13.5 g twice daily, oral	Randomized, double-blind, placebo-controlled study protocol (planned *n* = 40)	Primary outcomes: ADAS-Cog, C-MMSE, C-DAD (trial ongoing).	Safety monitoring predefined; no efficacy or safety results available.	(*92*)
Buyuan Congnao Decoction + acupuncture	AD	12 weeks	Buyuan Congnao Decoction (1 dose/day) + acupuncture	RCT (*n* = 98)	Improved MMSE, overall clinical response; reduced serum Hcy and Aβ.	No serious safety concerns reported.	(*93*)

ChAT activity, or the expression of cholinergic receptors. Most investigations remain focused on the modulation of pathological pathways or on predictions derived from network pharmacology. Future studies should establish standardized quality control procedures and provide comprehensive chemical characterization of TCM formulas. Further investigations need to be conducted to verify their direct effects on the cholinergic system and to clarify how these effects are related to the regulation of multiple pathological processes.

5.3. Clinical evidence and translational considerations

Clinical studies of TCM and related interventions for AD have primarily focused on a limited number of standardized extracts, isolated compounds, TCM formulas, Chinese patent medicines, and combination therapies. The most assessed outcomes include cognitive function, activities of daily living, overall clinical improvement, and select AD-related pathological biomarkers (Table 3).

Among the currently available interventions, Huperzine A has yielded one of the relatively strongest bodies of clinical evidence supporting a cholinergic therapeutic approach. As a well-characterized AChE inhibitor, its clinical efficacy has been evaluated in multiple randomized controlled trials and is further supported by systematic reviews and meta-analyses. Collectively, these studies suggest that Huperzine A is associated with improvements in cognitive function, as assessed with the Mini-Mental State Examination (MMSE), the Revised Hasegawa Dementia Scale (HDS-R), and memory-related evaluations. Improvements in activities of daily living have also been reported, indicating potential functional benefits. Although mild cholinergic adverse events, such as gastrointestinal discomfort and dizziness, were reported more frequently in the treatment groups than in the control groups, serious adverse events were uncommon (*70*).

The standardized extract EGb 761 from *Ginkgo biloba* L. is another intervention supported by a relatively substantial body of clinical evidence. A recent meta-analysis involving patients with mild dementia reported improvements in cognition, activities of daily living, global clinical assessment, and quality of life and alleviation of neuropsychiatric symptoms after 22–24 weeks of treatment. However, the study populations likely included patients with AD, vascular dementia (VD), and mixed dementia. In addition, treatment efficacy was evaluated solely based on clinical outcome measures, without assessing cholinergic function (*71*).

Current clinical evidence for TCM formulas is derived primarily from studies in which they were used as adjunctive therapies in combination with conventional treatments, such as donepezil, piracetam, or acupuncture. In those randomized controlled trials, formulas such as Buyuan Congnao Decoction, Dihuang Yinzi, Jianpi Yangxin Decoction, and Hengqing II were reported to improve cognitive outcomes, including scores on the MMSE, the Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog), and the Montreal Cognitive Assessment (MoCA), and activities of daily living. Changes in AD-related biomarkers, including Aβ, amyloid precursor protein (APP), inflammatory mediators, insulin-like growth factor-1 (IGF-1), and Notch-associated signaling molecules, were also reported (Table 3) (*72-75*). In addition to studies involving patients with clinically diagnosed AD, the current clinical evidence includes investigations conducted in different stages of cognitive impairment and using diverse study designs. For example, Bushen Capsules have been evaluated in patients with amnestic mild cognitive impairment (aMCI). The study findings highlight the potential value of TCM-based interventions during the prodromal stage of cognitive decline (*76*). Overall, the current clinical evidence suggests that some TCM-derived interventions may provide cognitive or functional benefits, particularly when standardized compounds, extracts, and TCM formulas are used as adjunctive therapies. Nevertheless, considerable heterogeneity remains across studies with respect to patient populations, intervention strategies, outcome measures, and study design. These factors should be taken into account when interpreting the evidence, and particularly in the context of the translational limitations discussed below.

5.4. Translational limitations, standardization, and quality control

The translational reliability of the clinical evidence described thus far remains subject to several important limitations. From the perspective of the cholinergic system, which is the focus of this review, the most important limitation is the lack of mechanistic endpoints in clinical studies. Most studies have relied predominantly on neuropsychological assessments, activities of daily living, or global clinical improvement as their primary endpoints. Mechanistic indicators, including ACh, AChE, ChAT, VAChT, cholinergic receptors, and cholinergic neuroimaging, have rarely been incorporated into clinical evaluations. Taken together, the available clinical studies provide preliminary support for selected TCM-based interventions, and particularly as adjunctive therapies. However, the current evidence remains insufficient to establish their cholinergic-specific clinical effects in AD.

The methodological quality of the available clinical evidence is also inconsistent. Although most studies were described as randomized controlled trials, details regarding allocation concealment, blinding procedures, and follow-up were often incomplete. Moreover, the investigations were predominantly limited by small sample sizes and single-center designs, with diagnostic criteria varying substantially across the literature, including the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), the

National Institute on Aging-Alzheimer's Association (NIA-AA) criteria, the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria, and Chinese diagnostic guidelines. Some studies also enrolled patients with vascular dementia, mixed dementia, or aMCI. This heterogeneity limits the extent to which their findings can be directly interpreted as evidence in patients with clinically diagnosed AD. Evidence regarding long-term safety also remains limited. Most studies reported only mild and transient adverse events, and a consistent increase in serious adverse events was not observed. Nevertheless, treatment durations were typically limited to three to six months, precluding a comprehensive assessment of long-term safety. Combination therapy is another important factor affecting the interpretation of the available clinical evidence. In many studies, TCM formulas were administered as adjuncts to treatments such as donepezil, piracetam, or acupuncture, hampering attribution of the observed cognitive or functional improvements solely to the TCM intervention. Moreover, few studies have systematically evaluated potential pharmacokinetic or pharmacodynamic interactions associated with combination therapy. Whether co-administration with AChE inhibitors alters drug exposure or increases the risk of cholinergic adverse effects remains unclear.

Standardization and quality control remain major challenges for TCM formula-based preparations. Most clinical studies provide limited information on botanical authentication, chemical characterization, batch-to-batch consistency, and quality assurance. This limits the reproducibility and comparability of findings across studies. Future studies should include well-designed multicenter clinical trials using standardized TCM preparations and rigorous methodological standards. Longer follow-up periods and systematic safety evaluations are also needed. In addition, validated cholinergic biomarkers should be incorporated to bolster the translational evidence linking TCM-based interventions to cholinergic dysfunction in AD (*77*).

6. Summary and prospects

AD is a multifactorial disorder with multiple pathological changes. One early feature is the selective vulnerability of the basal forebrain cholinergic system. The cholinergic hypothesis emphasizes the importance of this system in the pathology of AD and links cholinergic deficits to Aβ deposition, neuroinflammation, and mitochondrial bioenergetics. These interactions suggest that the cholinergic system may act as an important contributor to disease progression.

Current cholinesterase inhibitors offer limited symptomatic benefit without demonstrable disease-modifying effects, highlighting the urgent need for novel therapeutic strategies. TCM may, given its multicomponent and multitarget characteristics, provide a complementary perspective. Some TCM-derived compounds, standardized extracts, and formulas have been reported to modulate cholinergic neurotransmission, influence Aβ metabolism, reduce neuroinflammation, and regulate other AD-related pathological processes. Emerging clinical evidence also suggests potential cognitive and functional benefits of selected TCM-derived interventions, and particularly when used as standardized preparations or adjunctive therapies. In addition, combining TCM with conventional drugs may be a promising complementary therapeutic strategy, although this possibility requires further confirmation in well-designed clinical studies.

TCM research faces several challenges, including the complexity of herbal components, unclear mechanisms, lack of standardized methods of preparation, and insufficient clinical evidence. Greater emphasis should also be placed on improving study quality, standardization, quality control, and translational validation, including the incorporation of mechanistic cholinergic biomarkers in future clinical studies. Future efforts should integrate traditional TCM theories with modern biomedical approaches, elucidate mechanisms at the molecular level, and establish standardized evaluation systems.

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