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REVIEW ARTICLE

# A Review on Neuroprotective Activity and Pharmacological Mechanisms of *Bacopa monnieri* in Various Animal Models of Alzheimer's Disease

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**Abstract**: Alzheimer's disease is a progressive neurodegenerative condition characterized by complex multifactorial neuropathology, including amyloid-beta aggregation, tau hyperphosphorylation, oxidative stress, cholinergic deficits, and neuroinflammation. *Bacopa monnieri* and its bioactive dammarane-type triterpenoid saponins display multifaceted neuroprotective actions across transgenic, naturally aged, invertebrate, and neurotoxin-induced experimental models. An evaluation of these experimental models shows that *Bacopa monnieri* exerts polypharmacological effects by suppressing soluble amyloid-beta accumulation, downregulating glycogen synthase kinase-3 beta (GSK-3β) activity to decrease tau hyperphosphorylation, upregulating the Nrf2/HO-1 antioxidant pathway, inhibiting nuclear factor kappa B (NF-κ B)-driven inflammatory cascades, and stabilizing central cholinergic transmission through acetylcholinesterase inhibition. Additional structural and functional benefits include the enhancement of hippocampal neurogenesis and synaptic plasticity. Despite these consistent preclinical findings, barriers remain due to extract composition variability, a predominant focus on prophylactic rather than therapeutic treatment paradigms, male-biased animal cohorts, and limited pharmacokinetic profiling. Extract standardization and evaluating therapeutic intervention windows in clinically representative models are the main requirements for advancing this botanical candidate toward clinical validation.

**Keywords:** *Bacopa monnieri;* Alzheimer's disease; Bacosides; Neuroprotection; Translational pharmacology.

## 1. Introduction

Alzheimer's disease (AD) is a primary neurodegenerative disorder and the predominant driver of dementia worldwide, pathologically marked by a progressive loss of memory, executive dysfunction, and severe behavioral alterations [1]. The historical identification of the condition dates to 1907, when Alois Alzheimer described presenile dementia in a patient named Auguste Deter, noting characteristic cortical amyloid deposits and neurofibrillary tangles (NFTs) upon post-mortem examination [2]. Modern neuropathological definitions confirm that the primary structural hallmarks of AD consist of extracellular senile plaques composed of amyloid-beta (Aβ) peptides and intracellular NFTs formed by hyperphosphorylated microtubule-associated protein tau [3]. These primary proteinaceous lesions initiate downstream neurotoxic cascades, including sustained oxidative stress, severe mitochondrial impairment, disturbed neuronal calcium homeostasis, dysregulation of the gut-brain axis, and persistent neuroinflammation mediated by reactive microglia and astrocytes [4, 5].

Despite major clinical investments targeting these pathology markers, available interventions remain predominantly palliative. Standard clinical options, such as acetylcholinesterase (AChE) inhibitors, the N-methyl-D-aspartate (NMDA) receptor antagonist memantine, and monoclonal antibodies directed against Aβ, offer temporary functional stabilization or modest reductions in plaque burden without arresting neuronal loss [6, 7]. The failure of numerous single-target agents in late-stage clinical trials over recent decades reflects the reality that single-mechanism approaches are generally insufficient against the complex, overlapping pathologies of AD [8].

Consequently, research attention has increasingly shifted toward multi-target therapeutic strategies. Natural products and botanical formulations represent structural diversity and polypharmacological capabilities. Derived from traditional systems such as Ayurveda and Traditional Chinese Medicine, these multi-component agents can modulate multiple pathological pathways simultaneously, suppressing amyloidogenesis, oxidative injury, synaptic loss, and inflammatory cascades [9, 10].

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Validating botanical candidates requires well-designed preclinical *in vivo* models [11]. Although *in vitro* systems allow isolated mechanistic evaluations and high-throughput screening, they cannot replicate the complex systemic, circuit-level, and behavioral interactions of human neurodegenerative disease [12]. Animal models, incorporating transgenic and neurotoxin-induced approaches, remain necessary for evaluating candidate efficacy, quantifying cognitive outcomes, and supporting translation toward clinical testing [13].

*Bacopa monnieri* (BM), recognized as Brahmi in Ayurvedic practice, has long been categorized as a *medhya rasayana*, or cognitive-enhancing botanical agent. Its principal pharmacological constituents are dammarane-type triterpenoid saponins known as bacosides, with Bacoside A serving as a key analytical and bioactive marker [14, 15].

**Figure 1. Leaves and Flowers of *Bacopa monnieri***

Preclinical evaluations of BM across diverse experimental paradigms including heavy metal toxicity, accelerated aging, cholinergic blockade, and transgenic pathology indicate cognitive recovery and structural synaptic preservation, such as increased dendritic arborization and elevated synaptic density. Pharmacologically, BM preparations attenuate AChE activity to sustain hippocampal acetylcholine levels, directly interact with Aβ oligomers to limit lipid peroxidation, and upregulate endogenous antioxidant defenses including superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT). The following sections provide an evaluation of BM's pharmacological profiles and therapeutic potential across established animal models of AD.

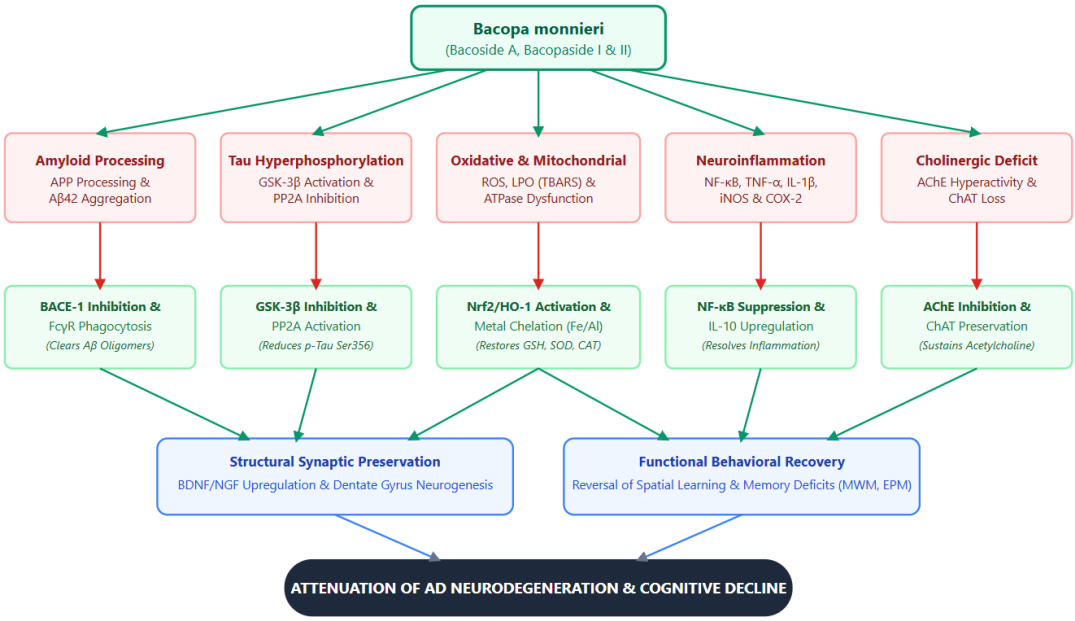


**Figure 2. Multi-Target Polypharmacological Actions of *Bacopa monnieri* across Alzheimer's Pathological Pathways**

## 2. Transgenic Mammalian Models of Amyloidosis and Tauopathy

Transgenic rodent models overexpressing human mutations associated with familial AD provide valuable tools for evaluating specific proteinopathies under controlled genetic conditions.

### 2.1. PSAPP Double Transgenic Mouse Model

The PSAPP model co-expresses the Swedish amyloid precursor protein ($APP_{Swe}$) and M146L presenilin-1 (PS1) mutations, driving rapid Aβ aggregation in the cerebral cortex and hippocampus beginning at approximately three months of age. This system provides a sensitive environment for testing anti-amyloidogenic compounds under conditions of elevated Aβ production [16].

Oral administration of a standardized BM extract (BME) at doses of 40 mg/kg/day or 160 mg/kg/day for two or eight months, initiated at two months of age, decreased cortical $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations by approximately 60 % compared to vehicle-treated controls. This reduction coincided with improved performance in Y-maze spatial exploration and open-field behavioral tests. However, long-term administration did not alter Congo Red-positive fibrillar plaque burden or overall plaque dimensions [17].

The distinction between reduced soluble Aβ species and unchanged dense-core plaque load suggests that BME acts upstream of fibril formation, potentially modulating secretase processing or accelerating oligomer clearance, rather than disaggregating established mature plaques. The specific phytochemicals driving these outcomes remain uncharacterized due to limited extract profiling, highlighting a common methodological constraint in preclinical botanical research.

### 2.2. APP/PS1 Double Transgenic Mouse Model

The APP/PS1 model incorporates mutant human APP and PSEN1, yielding age-dependent Aβ accumulation and gradual plaque formation within cortical and hippocampal regions. This system allows evaluation of long-term interventions targeting plaque clearance and associated cognitive deficits [18].

Oral administration of Bacopaside I (BS-I), a purified triterpenoid saponin derived from BM, at 15 mg/kg/day or 50 mg/kg/day for two months significantly reduced Morris Water Maze (MWM) escape latencies and improved probe trial performance. Interestingly, the lower dose (15 mg/kg/day) yielded greater behavioral recovery than the higher dose (50 mg/kg/day). Thioflavin-S histological staining confirmed reductions in fibrillar plaque burden that correlated with the behavioral observations. Proteomic and transcriptomic evaluations revealed alterations in neuroactive ligand-receptor interactions, calcium homeostasis, mitogen-activated protein kinase (MAPK) signaling, endocytosis, and Fcγ receptor-mediated phagocytosis, indicating that enhanced microglial Aβ clearance contributed to the observed effects [19].

This study indicates plaque reduction following administration of a purified saponin fraction, providing clear mechanistic linkages to phagocytic clearance. However, the non-linear dose-response relationship suggests possible target saturation, altered bioavailability, or off-target effects at higher concentrations. While using an isolated active constituent allows clearer mechanistic attribution, it limits direct extrapolation to complex whole-plant extracts.

**Table 1. Transgenic Mammalian Model Studies**

| Model and Species | AD Pathology Mimicked | Extract / Active Compound | Dosage Regimen | Route and Duration | Molecular and Behavioral Findings |
|---|---|---|---|---|---|
| PSAPP double transgenic mouse ($APP_{Swe}$ / $PS1_{M146L}$) | Accelerated Aβ plaque deposition in cortex and hippocampus | Standardized BME | 40 mg/kg/day or 160 mg/kg/day | Oral; 2 or 8 months (initiated at 2 months) | ~ 60% reduction in cortical $A\beta_{1-40}$ and $A\beta_{1-42}$; improved Y-maze exploration; unchanged Congo Red plaque load [17] |
| APP/PS1 double transgenic mouse (APP / PSEN1) | Age-dependent Aβ accumulation and plaque deposition | Bacopaside I (BS-I) | 15 mg/kg/day or 50 mg/kg/day | Oral; 2 months | Reduced MWM escape latency (15 mg/kg > 50 mg/kg); decreased Thioflavin-S plaque density; activation of FcγR-mediated phagocytosis [19] |

## 3. Natural Aging Models

Evaluating interventions in non-transgenic aged animals allows assessment of neuroprotective potential against age-associated cognitive decline and metabolic breakdown without genetic over-expression.

### 3.1. Chronologically Aged Female Wistar Rats

Middle-aged (17-18 months) and aged (>24 months) female Wistar rats received an oral bacoside mixture containing Bacopaside I, Bacoside A3, Bacopaside II, and isomers of Bacopasaponin C at 200 mg/kg/day for three months following dose-escalation screening. Behavioral assessments revealed cognitive restoration in passive avoidance tasks starting from day 30 in middle-aged cohorts, along with reduced immobility in tail-suspension testing. Cortical acetylcholine concentrations recovered to approximately 85% of youthful control levels, accompanied by adaptive adjustments in AChE activity. Monoamine analysis revealed partial restoration of serotonin (105%) and dopamine (82%), whereas norepinephrine levels remained unchanged. Antioxidant measurements showed marked reductions in lipid peroxidation markers, including thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides, alongside preservation of glutathione peroxidase (GPx) and total glutathione (GSH). Histological analysis verified preserved pyramidal cell morphology within the CA1 and CA3 hippocampal regions [20].

This design provides clinically relevant insights into intervention timing during middle age, supporting a preventive neuroprotective window. However, the exclusive use of female cohort limits generalizability, and the relatively high dosage (200 mg/kg/day) requires careful consideration regarding human dose translation.

### 3.2. Naturally Aged Mice with Bacopa-Phospholipid Complex

Aged male Swiss albino mice (12-15 months) were treated orally with either standard BM extract (BE, 40 mg/kg/day) or a specialized Bacopa-phospholipid complex (BPC, 40 mg/kg/day) for 15 days, using piracetam as a reference comparator. Behavioral testing across the elevated plus-maze, MWM, and passive avoidance tasks indicated that both treatments attenuated age-related memory deficits, with BPC consistently outperforming standard BE. Animals receiving BPC exhibited significantly higher brain AChE inhibition. Pharmacokinetic analysis in rats confirmed that BPC achieved substantially higher peak serum concentrations ($C_{max}$) of Bacopaside I (12.21 μg/mL) and Bacopaside II (12.28 μg/mL) within one hour, driven by enhanced lipid membrane permeability and protection against intestinal hydrolysis [21]. These pharmacokinetic findings indicate that phospholipid formulation strategies can improve saponin bioavailability and functional efficacy. However, the short 15-day treatment duration limits conclusions regarding long-term neuroprotective impacts on progressive structural degeneration.

**Table 2. Natural Aging Model Studies**

| Model and Species | AD Pathology Mimicked | Extract / Active Compound | Dosage Regimen | Route and Duration | Molecular and Behavioral Findings |
|---|---|---|---|---|---|
| Chronologically aged female Wistar rats (17-24+ months) | Age-associated memory loss, monoaminergic decline, and oxidative stress | Bacoside mixture | 200 mg/kg/day | Oral; 3 months | Improved passive avoidance retention; restored cortical ACh, dopamine, and serotonin; suppressed TBARS; preserved CA1/CA3 neurons [20] |
| Aged male Swiss albino mice (12-15 months) | Age-induced amnesia and cholinergic decline | Standard BE vs. Phospholipid Complex (BPC) | 40 mg/kg/day | Oral; 15 days | BPC > BE across MWM and EPM tasks; greater AChE inhibition; elevated serum $C_{max}$ for Bacopaside I and II [21] |

## 4. Invertebrate and Lower Organism Transgenic Models

Invertebrate models offer efficient genetic platforms for identifying targeted pathways and screening bioactive constituents.

### 4.1. Transgenic *Caenorhabditis elegans*

A leaf hexane extract of BM (7-10 μg/mL) was evaluated in transgenic *Caenorhabditis elegans* expressing human Aβ (CL2006) and in insulin/IGF-1 signaling mutants (daf-2 and daf-16). The extract extended median and maximal lifespan in Aβ-expressing strains at

7-8 µg/mL, reduced ultraviolet-A-induced reactive oxygen species (ROS) accumulation, and upregulated longevity-associated genes including daf-16, mpk-1, mek-2, ace-1, and ace-2. The survival extension depended on intact DAF-16 signaling and was completely abolished in daf-16 loss-of-function mutants. Molecular docking analyses suggested potential interactions between active saponins and the transcription factors SKN-1 and DAF-16 [22]. These results establish a clear dependence on the conserved DAF-16/FOXO signaling pathway. However, simple worm longevity assays represent general cell survival under acute Aβ toxicity rather than human neurodegenerative progression, requiring validation in higher order mammalian neural circuits.

### 4.2. Transgenic *Drosophila melanogaster*

Pan-neuronal expression of human $A\beta_{42}$ was driven using the elav-GAL4 × UAS-$A\beta_{42}$ driver system in *Drosophila melanogaster*. Flies received feed containing 0.1 % aqueous BM leaf extract for 25 days. Quantitative proteomic analysis of head tissue using tandem mass tagging (TMT) indicated that BM treatment restored over 90% of the $A\beta_{42}$-altered proteome back toward control levels. Restored proteins included subunit components of electron transport chain (ETC) complexes I and III, endogenous antioxidants (GstO1, Trx-2), stress response proteins (TotA), and autophagy regulators (Atg8a, Atg8b). These molecular changes correlated with restored climbing capacity in negative geotaxis functional assays [23].

These proteomic findings highlight mitochondrial bioenergetic support and autophagic clearance as key mechanisms of BM activity. However, cross-species translation requires empirical verification in mammalian systems with relevant tissue architecture and vascular delivery.

**Table 3. Invertebrate and Lower Organism Transgenic Model Studies**

| Model and Species | AD Pathology Mimicked | Extract / Active Compound | Dosage Regimen | Route and Duration | Molecular and Behavioral Findings |
|---|---|---|---|---|---|
| Transgenic C. elegans (CL2006 Aβ) | Aβ toxicity and accelerated metabolic senescence | Hexane leaf extract | 7-10 µg/mL | Media addition; Lifespan assay | Extended lifespan via DAF-16 activation; attenuated ROS; upregulated mpk-1, mek-2, ace-1/2 [22] |
| Transgenic Drosophila melanogaster (elav-GAL4/$A\beta_{42}$) | Pan-neuronal $A\beta_{42}$ toxicity and locomotor impairment | 0.1% aqueous leaf extract | 0.1% in feed | Dietary; 25 days | Restored >90% of $A\beta_{42}$ altered proteome; elevated ETC complexes I/III, Atg8a, and antioxidant proteins; improved climbing [23] |

## 5. Neurotoxin-Induced Models of Alzheimer's Disease Pathology

Chemically induced models allow targeted disruption of specific biological pathways, such as cholinergic transmission, tau phosphorylation, or redox balance, providing insights into isolated pathological mechanisms.

### 5.1. Okadaic Acid-Induced Tau Hyperphosphorylation

Okadaic acid (OKA) acts as a selective inhibitor of protein phosphatases PP1 and PP2A, driving rapid tau hyperphosphorylation, neuroinflammation, oxidative stress, and cognitive dysfunction. Male Sprague-Dawley rats received a single intracerebroventricular (ICV) infusion of OKA (200 ng), followed by 13 days of oral BM extract (40 mg/kg/day or 80 mg/kg/day). Treatment at 80 mg/kg/day restored MWM performance, activated the Nrf2/HO-1/GCLC antioxidant pathway, restored depleted GSH pools, and reduced ROS and nitrite accumulation. BM restored PP2A enzyme activity, inhibited GSK-3β, and reduced hyperphosphorylated tau levels. Pro-inflammatory signaling was suppressed through down-regulation of nuclear factor kappa B (NF-ϰ B), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β), alongside upregulation of anti-inflammatory IL-10. Apoptotic markers, including the Bax/Bcl-2 ratio and caspase-3 cleavage, were similarly normalized [24].

These findings indicate that BM modulates tau pathology through simultaneous PP2A activation and GSK-3β suppression. However, acute chemical phosphatase inhibition does not fully replicate the slow, progressive development of human tauopathies.

### 5.2. Microtubule Disruption and Cholinergic Impairment via Colchicine

ICV administration of colchicine (15 µg) disrupts neuronal microtubule dynamics, impairs axonal transport, increases oxidative stress, and damages cholinergic pathways. In experiments by Saini et al., oral BM extract (50 mg/kg/day for 15 days) attenuated

spatial memory deficits in male Wistar rats across passive avoidance and MWM tasks. Treated animals showed reduced lipid peroxidation and protein carbonylation, restored GSH levels, and normalized activities of SOD, CAT, GR, and glutathione S-transferase (GST). AChE hyperactivity and $Na^+/K^+$-ATPase inhibition were reversed [25]. Subsequent investigations confirmed down-regulation of beta-site amyloid precursor protein cleaving enzyme 1 (BACE-1), reduced Aβ plaque density, and suppression of inflammatory mediators including IL-6, TNF-α, monocyte chemoattractant protein-1 (MCP-1), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) [26]. These data highlight broad antioxidant, anti-inflammatory, and cholinergic protective properties. However, because colchicine acts primarily as a microtubule depolymerizing agent rather than an amyloidogen, the observed reductions in Aβ deposition likely stem from general neuroprotection rather than primary anti-amyloid mechanisms.

### 5.3. Muscarinic Cholinergic Blockade via Scopolamine

Intraperitoneal administration of scopolamine (1 mg/kg) induces acute central cholinergic blockade by competing with acetylcholine at muscarinic receptor sites. Male Wistar rats pretreated with BM extract (200 mg/kg/day orally for 14 days) showed preserved MWM escape latencies and step-down passive avoidance retention following scopolamine challenge. BM attenuated scopolamine-induced AChE hyperactivity, preserved synaptic acetylcholine availability, and restored tissue GSH, SOD, CAT, and malondialdehyde (MDA) levels back toward baseline values [27]. While the scopolamine paradigm provides an established model for testing pro-cholinergic agents, it produces transient neurotransmitter blockade without structural neurodegeneration or proteinopathy, limiting its predictive value for chronic disease.

### 5.4. Selective Cholinergic Lesioning via Ethylcholine Aziridinium (AF64A)

Bilateral ICV infusion of the selective cholinergic neurotoxin ethylcholine aziridinium (AF64A) induces loss of basal forebrain cholinergic neurons and associated hippocampal deficits. Oral BM extract containing 5% total saponins (20 mg/kg/day, 40 mg/kg/day, or 80 mg/kg/day) was administered to male Wistar rats for three weeks (two weeks pre-lesioning, one week post-lesioning). All doses improved MWM acquisition performance to a degree comparable to donepezil, although probe trial retention improvements were less pronounced. Histological studies showed dose-dependent preservation of pyramidal neurons within the CA2, CA3, and dentate gyrus regions, with 40 mg/kg/day yielding maximal preservation of choline acetyltransferase (ChAT)-positive neurons in CA1 and CA2 regions [28]. These results show structural preservation of cholinergic pathways following chemical insult. However, the requirement for a two-week pretreatment period highlights a reliance on prophylactic administration strategies.

### 5.5. GABAergic and Long-Term Potentiation Disruption via Diazepam

Male Swiss albino mice received daily BM extract (120 mg/kg, containing 55.35% bacosides) for six days, with acute diazepam (1.75 mg/kg intraperitoneally) administered 30 minutes prior to behavioral testing to induce $GABA_A$-mediated amnesia. BM treatment reversed diazepam-induced deficits during MWM acquisition. Molecular analyses showed that while diazepam upregulated MAPK, phosphorylated cAMP response element-binding protein (pCREB), and iNOS, BM administration normalized MAPK, pCREB, and iNOS expressions while partially restoring nitrite levels [29]. This study shows interaction with non-cholinergic memory cascades, specifically $GABA_A$-regulated long-term potentiation. However, acute pharmacological amnesia models lack key pathological features of neurodegenerative conditions.

### 5.6. Cerebral Metabolic Impairment via Intracerebroventricular Streptozotocin

Sub-diabetogenic ICV administration of streptozotocin (STZ, 3.0 mg/kg bilaterally) impairs central insulin signaling, disrupts cerebral glucose metabolism, and induces oxidative stress and cholinergic dysfunction. Male Wistar rats were pretreated with BM extract (30 mg/kg/day orally for 14 days) prior to ICV-STZ infusion. BM treatment reduced MWM swim distances and escape latencies while increasing target quadrant retention. Biochemical analysis revealed reduced TBARS concentrations, preserved $Na^+/K^+$-ATPase activity, and restored GSH, GPx, GST, and CAT activities. Immunofluorescence confirmed preservation of hippocampal Cu/Zn-SOD, and histological staining revealed intact CA1 pyramidal neuron morphology [30]. The ICV-STZ model provides relevant insights into sporadic AD metabolic dysregulation. Nevertheless, the study design relied entirely on prophylactic dosing, and downstream effects on primary proteinopathies were not evaluated.

### 5.7. Neuroinflammation and TLR4 Activation via Lipopolysaccharide

Systemic administration of lipopolysaccharide (LPS, 0.175 mg/kg/day intraperitoneally for 7 days) activates toll-like receptor 4 (TLR4) pathways, driving neuroinflammation and cognitive impairment. Male Sprague-Dawley rats received oral BM extract (70 mg/kg/day, standardized to 21.24% bacoside A) for 14 days prior to and concurrently with LPS challenge. While behavioral improvements in MWM and Y-maze tasks did not achieve statistical significance, BM significantly suppressed cortical expression

of IL-1β, TNF-α, iNOS, and COX-2. Interestingly, hippocampal inflammatory markers were less affected, and local prostaglandin $E_2$ ($PGE_2$) and AChE levels showed modest elevations [31].

This study shows regional variation in anti-inflammatory responsiveness between cortical and hippocampal tissue. The lack of significant behavioral recovery highlights the need to optimize dosing schedules in strong inflammatory environments.

### 5.8. Heavy Metal-Induced Oxidative Stress via Aluminium Chloride

Chronic exposure to aluminium chloride ($AlCl_3$) promotes iron-mediated oxidative stress, protein aggregation, and cholinergic degeneration. In an evaluation by Jyoti et al., concurrent administration of BM extract (40 mg/kg/day) with $AlCl_3$ (50 mg/kg/day orally for 5 weeks) attenuated cortical TBARS, 4-hydroxynonenal (4-HNE), and protein carbonyls, while restoring SOD, GPx, GST, and GSH levels. Transmission electron microscopy revealed decreased lipofuscin accumulation and preserved nuclear architecture in somatosensory neurons [32]. Thippeswamy et al. showed that combining BM granules (100 mg/kg/day) with rivastigmine (5 mg/kg/day) during $AlCl_3$ exposure (100 mg/kg/day for 42 days) produced additive improvements in spatial memory, exceeding responses to either agent alone [33]. Anand et al. evaluated a standardized Brahmi Herbal Drink (BHD, 0.05% BM) against $AlCl_3$ toxicity (100 mg/kg/day for 23 days), observing reduced escape latencies, decreased AChE, iNOS, and HSP-70 expression, restored antioxidant capacity, and elevated brain-derived neurotrophic factor (BDNF) expression [34].

These findings consistently support the antioxidant and metal-chelating properties of BM across independent laboratories. The observed synergy with rivastigmine offers support for multi-agent therapeutic strategies.

### 5.9. Hippocampal Lesioning and Neurogenesis via Trimethyltin

A single systemic injection of trimethyltin (TMT, 2.8 mg/kg intraperitoneally) induces selective loss of pyramidal neurons in the CA1, CA3, and dentate gyrus regions. Standardized BM extract (50 mg/kg/day) containing 21.8% bacoside A and 11.0% Bacopaside I was administered to mice for 15 or 30 days post-injury. Treatment for 30 days significantly improved object location discrimination, Y-maze exploration, and passive avoidance performance. Histological evaluation confirmed preserved CA1 and CA3 pyramidal cell density. Importantly, 5-bromo-2'-deoxyuridine (BrdU) labeling showed that 30-day (but not 15-day) BM administration significantly increased proliferating cell counts in the subgranular zone of the dentate gyrus, confirming stimulation of endogenous neurogenesis [35].

This study provides valuable evidence of active neurorepair and neurogenesis following established damage. The distinct timeframe between early structural protection and late progenitor proliferation provides a clear biological timeline for treatment response.

### 5.10. Accelerated Senescence via D-Galactose and Sodium Nitrite

Co-administration of D-galactose (120 mg/kg/day) and sodium nitrite (90 mg/kg/day) for 60 days in young mice models chronic oxidative stress and metabolic aging, causing widespread inhibition of membrane-bound $Na^+/K^+$-ATPase, $Mg^{2+}$-ATPase, and $Ca^{2+}$-ATPase enzymes across central nervous system tissues. Oral BM extract (100 mg/kg/day) administered from day 10 to day 180 gradually restored MWM performance, with latencies approaching control values by day 165. Membrane ATPase activity across six evaluated brain regions returned to normal levels by day 180 [36]. This long-term evaluation shows extended functional stability under chronic oxidative challenge. However, the model reflects generalized cellular aging rather than AD-specific proteinopathy.

### 5.11. Targeted Amyloid Toxicity via Intrahippocampal Amyloid-Beta 42 Fibril Injection

Stereotaxic intrahippocampal injection of preformed $A\beta_{42}$ aggregates (4 μL at 1.25 μg/μL) creates localized amyloid toxicity, tau hyperphosphorylation, neuroinflammation, cholinergic deficits, and neuronal loss. Male Wistar rats received oral BM extract (40 mg/kg/day or 80 mg/kg/day) for 28 days following surgery. BM treatment improved open-field exploration, novel object recognition, and MWM performance. Molecular analyses confirmed suppression of AChE activity, reduced IL-6 and TNF-α expression, decreased Bax/Bcl-2 ratios, and restored BDNF and nerve growth factor (NGF) levels. Importantly, BM treatment lowered overall tau protein expression and normalized tau hyperphosphorylation at Ser356. Molecular docking and 50-nanosecond molecular dynamics simulations indicated stable interactions between major saponins (Bacopasaponin C, Bacopaside I, Bacoside A) and the catalytic domain (Asp133/Val135) of GSK-3β, leading to reduced GSK-3β expression and upregulation of downstream Wnt-3a and β-catenin signaling [37].

This investigation provides clear mechanistic linkages connecting $A\beta_{42}$ toxicity, GSK-3β inhibition, Wnt/β-catenin activation, and reductions in tau hyperphosphorylation. However, the model reflects secondary tau changes resulting from acute fibril injection rather than progressive genetic tauopathy.

**Table 4. Neurotoxin-Induced Studies**

| Model and Species | AD Pathology Mimicked | Dosage Regimen | Route and Duration | Molecular and Behavioral Findings |
|---|---|---|---|---|
| OKA ICV (Male Sprague-Dawley rats) | PP1/PP2A inhibition, tau hyperphosphorylation, and neuroinflammation | 40 mg/kg/day or 80 mg/kg/day | Oral; 13 days post-OKA | Restored MWM performance; activated Nrf2/HO-1; inhibited GSK-3β; decreased p-tau; lowered NF-ϰ B, TNF-α, and caspase-3 [24] |
| Colchicine ICV (Male Wistar rats) | Microtubule disruption, oxidative damage, and cholinergic deficits | 50 mg/kg/day | Oral; 15 days | Reduced TBARS and protein carbonyls; normalized AChE and $Na^+/K^+$-ATPase; downregulated BACE-1 and Aβ plaque density [25, 26] |
| Scopolamine (Male Wistar rats) | Muscarinic cholinergic blockade | 200 mg/kg/day | Oral; 14 days | Preserved MWM escape latency and passive avoidance retention; suppressed AChE; restored SOD, CAT, and GSH [27] |
| AF64A ICV (Male Wistar rats) | Selective cholinergic lesioning | 20 mg/kg/day, 40 mg/kg/day, or 80 mg/kg/day | Oral; 3 weeks | Improved MWM learning comparable to donepezil; preserved CA2/CA3 neurons and $ChAT^+$ immunoreactivity (40 mg/kg) [28] |
| Diazepam amnesia (Male Swiss albino mice) | $GABA_A$-mediated LTP disruption | 120 mg/kg/day | Oral; 6 days | Reversed MWM acquisition deficits; suppressed elevated MAPK, pCREB, and iNOS expression [29] |
| ICV-Streptozotocin (Male Wistar rats) | Central metabolic impairment and insulin resistance | 30 mg/kg/day | Oral; 2 weeks pre- + 2 weeks post-STZ | Reduced MWM path lengths; restored $Na^+/K^+$-ATPase and antioxidant enzymes; preserved CA1 pyramidal neurons [30] |
| LPS neuroinflammation (Male Sprague-Dawley rats) | TLR4-mediated microglial activation | 70 mg/kg/day | Oral; 14 days pre- + 7 days concurrent | Suppressed cortical IL-1β, TNF-α, iNOS, and COX-2; limited behavioral recovery; elevated hippocampal $PGE_2$ [31] |
| AlCl3 toxicity (Male Wistar rats) | Iron-mediated oxidative damage and lipofuscin accumulation | 40-200 mg/kg/day | Oral; 21 to 42 days | Reduced TBARS and 4-HNE; restored antioxidant enzymes; synergized with rivastigmine; upregulated BDNF [32-34] |
| TMT hippocampal lesion (Male mice) | Pyramidal cell loss and spatial memory deficits | 50 mg/kg/day | Oral; 15 or 30 days post-TMT | Improved spatial exploration; preserved CA1/CA3 neurons; 30-day treatment enhanced dentate gyrus $BrdU^+$ neurogenesis [35] |
| D-Galactose / $NaNO_2$ (Male mice) | Accelerated oxidative aging | 100 mg/kg/day | Oral; 170 days | Restored MWM performance by day 165; normalized membrane $Na^+/K^+$-ATPase across brain regions by day 180 [36] |
| Intrahippocampal Aβ42 (Male Wistar rats) | Direct focal $Aβ_{42}$ toxicity | 40 mg/kg/day or 80 mg/kg/day | Oral; 28 days post-injection | Improved behavioral scores; suppressed AChE and inflammatory cytokines; lowered p-tau; inhibited GSK-3β; upregulated Wnt/β-catenin [37] |

## 6. Polypharmacological Targets

Across diverse experimental paradigms, the neuroprotective activity of *Bacopa monnieri* converges on several primary biological targets.

### 6.1. Anti-Amyloidogenic and Anti-Tau Activity

BM constituents interrupt amyloidogenic processing by inhibiting BACE-1 activity, altering secretase dynamics, and promoting microglial Aβ clearance through Fcγ receptor mechanisms. In soluble form, bacosides bind Aβ oligomers directly, suppressing initial membrane aggregation and fibril formation. Regarding tau pathology, BM restores protein phosphatase PP2A function while inhibiting GSK-3β activity via activation of Wnt/β-catenin signaling cascades. This dual modulation limits tau hyperphosphorylation at critical serine residues (Ser356), supporting microtubule stabilization and structural cell integrity.

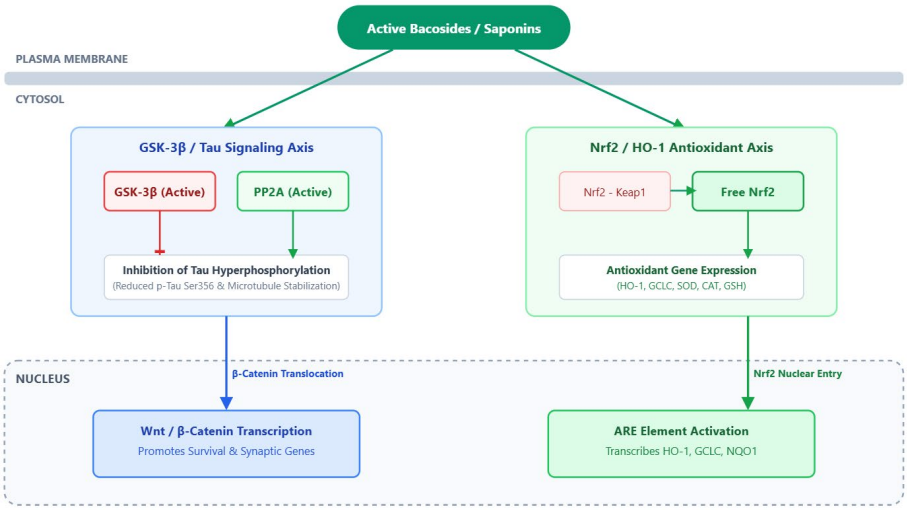


**Figure 3. Dual Modulation of GSK-3β / Wnt / β-Catenin and Nrf2 / HO-1 Signaling Pathways by *Bacopa monnieri***

### 6.2. Redox Homeostasis and Antioxidant Signaling

Oxidative damage represents a major pathological feature across all evaluated animal models. BM administration upregulates the Nrf2/HO-1/GCLC transcriptional pathway, driving the synthesis of endogenous antioxidants including GSH, SOD, CAT, GPx, and GST. Saponin constituents chelate free transition metals ($Fe^{2+}$, $Al^{3+}$), preventing Fenton-type hydroxyl radical generation. These direct and indirect antioxidant actions suppress lipid peroxidation (TBARS, 4-HNE) and protein carbonylation, protecting mitochondrial membrane integrity and preserving cellular ATPase function.

### 6.3. Anti-Inflammatory Modulation

Pro-inflammatory responses driven by microglial and astrocytic activation are attenuated following BM administration. Active saponins suppress NF-ϰ B nuclear translocation, decreasing downstream transcription of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and inflammatory enzymes (iNOS, COX-2). Concurrently, expression of anti-inflammatory cytokines, such as IL-10, is increased, aiding the resolution of neuroinflammatory cascades.

### 6.4. Cholinergic Preservation and Synaptic Plasticity

BM preserves central cholinergic transmission by inhibiting AChE activity and supporting ChAT expression, maintaining synaptic acetylcholine levels within cortical and hippocampal circuits. Beyond neurotransmitter modulation, BM promotes neurotrophic support by upregulating BDNF, NGF, and glial cell line-derived neurotrophic factor (GDNF). These trophic changes drive structural plasticity, reflected by increased dendritic spine density, enhanced arborization, and progenitor proliferation within the dentate gyrus.

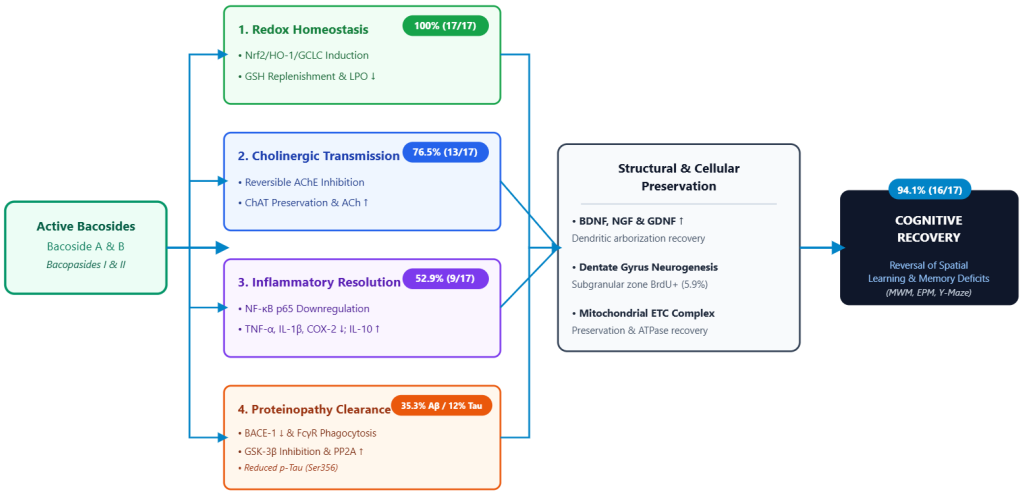


**Figure 4. Multi-target pharmacological cascade flowchart detailing the percentage coverage and functional convergence of *Bacopa monnieri* across 17 reviewed preclinical studies. Active bacosides drive primary molecular cascades across redox rebalancing (100%), cholinergic stabilization (76.5%), inflammatory resolution (52.9%), and proteinopathy clearance (35.3% Aβ, 11.8% tau), converging on structural synaptic preservation and robust cognitive recovery (94.1%).**

## 7. Methodological Limitations and Challenges

While preclinical studies show positive outcomes, several methodological limitations affect translational potential.

### 7.1. Extract Heterogeneity and Standardization

A primary challenge in botanical research involves extract variability. Across the reviewed literature, tested formulations ranged from crude aqueous or alcoholic extracts to specific enriched fractions (BS-I) and lipid complexes (BPC). Many studies lacked detailed chromatographic profiling or quantified bacoside percentages, complicating cross-study comparisons and hindering reproducible dosing standards required for clinical trial designs.

### 7.2. Prophylactic Dosing Bias and Timing of Intervention

The majority of preclinical studies utilized prophylactic dosing designs, initiating BM administration prior to or concurrently with toxin exposure or transgene expression. While prophylactic testing provides insights into protective actions, it does not replicate the clinical presentation of AD, where treatment begins long after neuropathology and cognitive deficits are established. Therapeutic interventional designs in symptomatic animals remain essential.

### 7.3. Demographic and Sex Biases in Preclinical Cohorts

Preclinical evaluation displays a significant sex bias, relying almost exclusively on male animal cohorts. Human epidemiological data show a higher prevalence and altered progression of AD in females. Evaluating candidates solely in male animals omits potential interactions with female sex hormones, cerebrovascular differences, and dimorphic immune responses.

### 7.4. Pharmacokinetic and Bioavailability Constraints

Detailed pharmacokinetic profiling for major triterpenoid saponins remains sparse. Saponins generally exhibit poor gastrointestinal permeability, low oral bioavailability, and extensive metabolic conversion. Although phospholipid formulation strategies (BPC) indicate improved absorption, detailed data detailing blood-brain barrier passage, regional tissue distribution, half-life, and active metabolic constituents require further systematic investigation.

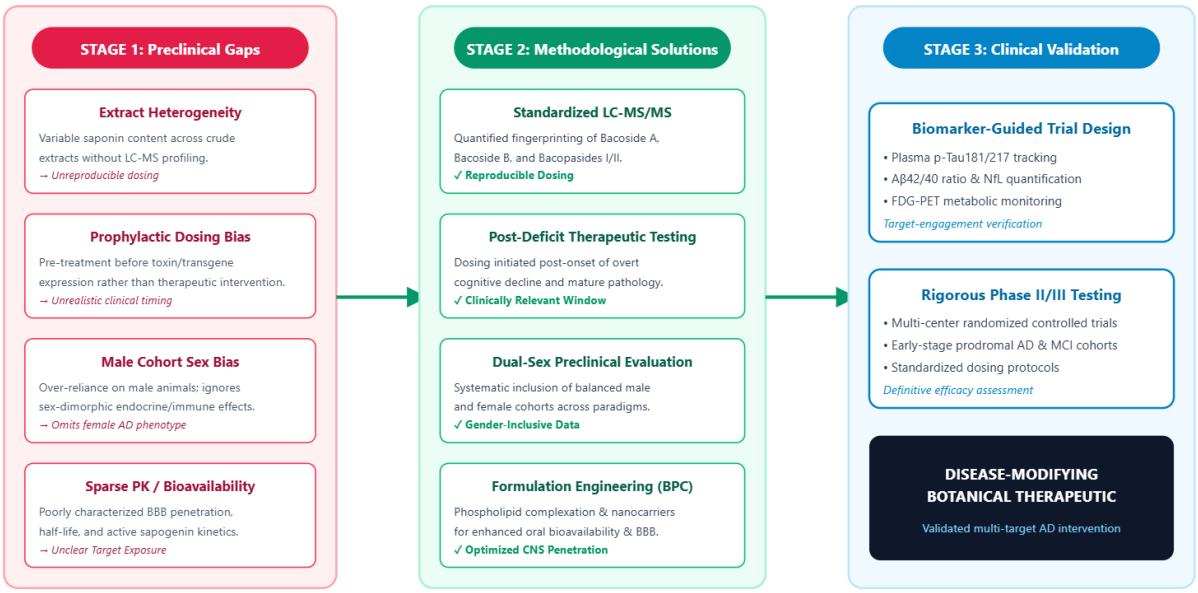


**Figure 5. Overcoming Preclinical Gaps for Human Clinical Validation**

## 8. Conclusion

Preclinical evaluations show that *Bacopa monnieri* exerts multi-target neuroprotective actions across diverse experimental models of Alzheimer's disease pathology. Its combined actions including suppression of amyloid accumulation, reduction of tau hyperphosphorylation via GSK-3β inhibition, upregulation of Nrf2-driven antioxidant defenses, suppression of NF-ϰ B inflammatory signaling, and restoration of cholinergic transmission ameliorate multiple pathways of neurodegenerative disease. Overcoming methodological limitations through standardized phytochemical profiling, therapeutic dosing schedules in symptomatic models, inclusion of both sex cohorts, and detailed pharmacokinetic characterization will be important steps for clinical application on large scale.

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