

Bacopa monnieri, a Neuroprotective Lead in Alzheimer Disease: A Review on Its Properties, Mechanisms of Action, and Preclinical and Clinical Studies

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ABSTRACT: Alzheimer disease is a neurodegenerative disease that is signified by cognitive decline, memory loss, and erratic behavior. Till date, no cure for Alzheimer exists and the current Alzheimer medications have limited effectiveness. However, herbal medicines may slow down the disease's progression, which may hopefully reduce the number of cases in the years to come. Numerous studies have been done on characterizing the neuroprotective properties from plants belonging to Scrophulariaceae family, particularly *Bacopa monnieri* and its polyphenolic compounds known as bacosides. This review presents the findings on bacosides in therapeutic plants and their impact on Alzheimer disease pathology. These reports present data on the clinical, cellular activities, phytochemistry, and biological applications that may be used in new drug treatment for Alzheimer disease.

KEYWORDS: Alzheimer, aging, therapeutic plant, *Bacopa monnieri*, bacosides

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Alzheimer disease (AD) is a devastating, progressive, and irreversible neurodegenerative disorder, which is clinically characterized by the deterioration of memory, disorientation, increased confusion, and other psychological as well as physical manifestations (Figure 1).¹ The appearance of extracellular amyloid-beta ($A\beta$) deposits in senile plaques and the development of intracellular neurofibrillary tangles, reactive microgliosis, and astrogliosis are the primary histopathological characteristics of AD.² Alzheimer disease primarily affects the elderly,³ is the most common and feared type of dementia, represents 70% of all dementia cases, and is a worldwide epidemic. Bertram et al⁴ postulated that in addition to the sporadic form of AD, for which aging is the primary factor, mutations in the amyloid-beta precursor protein ($A\beta$ PP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) cause autosomal dominant early-onset familial AD. Polidori et al⁵ found that genetic and environmental factors, vascular pathology, and other risk factors also play crucial roles in AD pathogenesis.

Due to the lack of effective disease-modifying treatments, findings on pharmacological or nonpharmacological strategies to slow disease progression are of significant importance. In addition, the failure of potential pharmaceuticals in human clinical trials has highlighted the need for research into early AD diagnosis. As synaptic and neuronal loss along with brain shrinkage has already occurred when AD's clinical symptoms appear, current treatments that seek to slow disease progress are more likely to be effective before the onset of AD symptoms,

ideally at the earliest preclinical stage. The lack of effective AD treatments and pharmaceuticals has led to the assessment of alternative therapeutics, such as nutraceuticals. For example, many antioxidants may enhance cognitive ability.^{6–8} Nutraceuticals have an effect on various neurodegenerative diseases as they modulate signaling pathways.⁹ Nutraceuticals are nutrients, herbals, and dietary supplements that can help in maintaining physical wellbeing, work against various diseases, and ensure a better quality of life. Bacosides from *Bacopa monnieri* (*B monnieri*) are examples of valuable therapeutic agent for AD due to their anti-inflammatory, antioxidant, and $A\beta$ aggregation inhibitor properties. This review presents current clinical studies and scientific evidences that document the therapeutic potential of *B monnieri* extracts (BME) such as bacosides in AD.

Traditional Aspects of *B monnieri*

According to World Health Organization, traditional medicine is defined as “the sum total of knowledge, skills and practices based on the theories, beliefs and experiences of different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses.”¹⁰ Many population in the developing countries have reverted to the use of traditional plants in maintaining their health and wellbeing.¹¹ In this age where migration has taken a leap, immigrants tend to bring traditional plants from their country of origin to use as supplements. This has caused the promotion



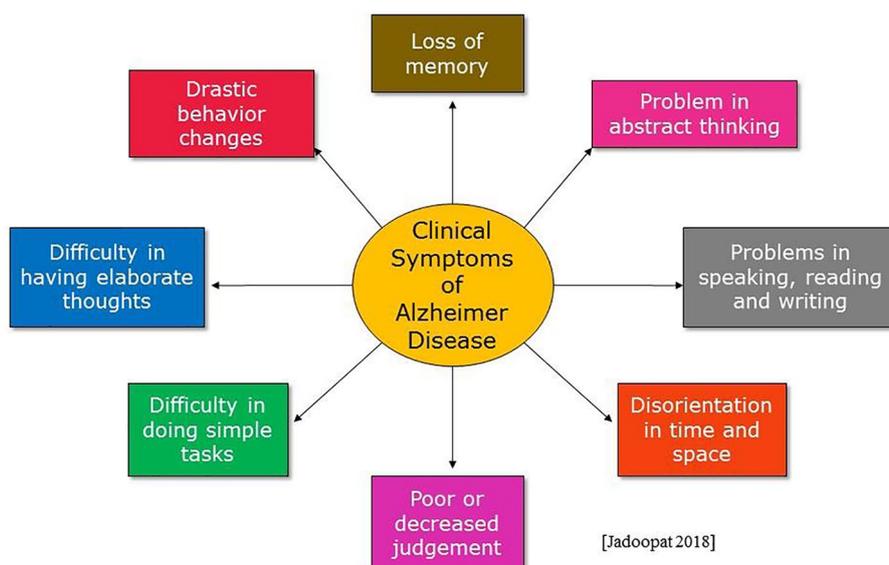


Figure 1. Clinical symptoms of Alzheimer disease.



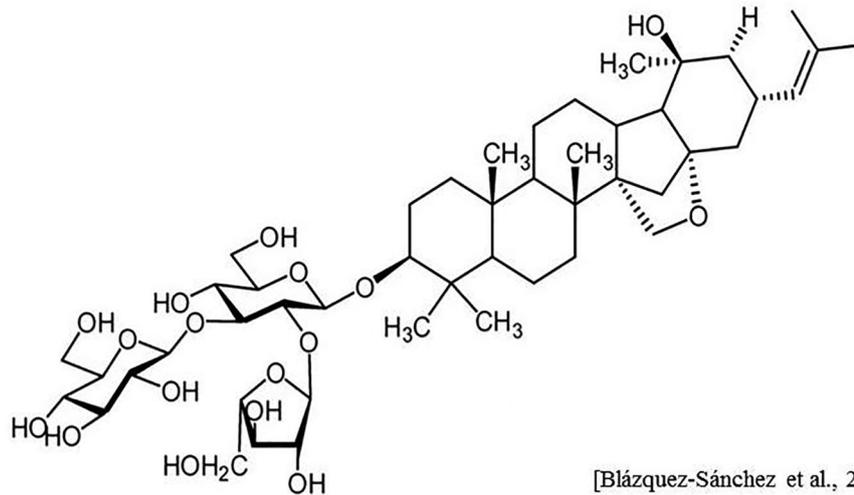
Figure 2. *Bacopa monnieri* plants.

of such non-native plants in a foreign country, particularly the ones used in Ayurvedic and Chinese traditional medicine.¹² These plants or plant compounds are known as complementary or alternative medicines in non-native countries. Notably, *B monnieri*, otherwise known as Brahmi and Aindri (Sanskrit) is classified into the Scrophulariaceae family and found throughout the Indian subcontinent in moist soil, humid, and muddy environments.¹³ The genus *Bacopa* has 146 aquatic herbal species dispersed throughout the subtropical regions of the globe, including Nepal, India, Sri Lanka, Taiwan, China, and Vietnam, as well as Florida and other US southern regions. Although it can be seen in the United States, these plants are perceived as weeds in rice fields and abundantly grown in wetlands and marshes of warmer districts.¹⁴ As shown in Figure 2, Brahmi is a succulent herb commonly grown in subtropical nations up to 1500 m altitude. Brahmi, which is traditionally known as “medhya rasayana,” which means brain tonic or nootropic, or in Sanskrit word, referring to intellectual,

cognition, and rejuvenation because it enhances the brain’s cognitive properties, is popular among Ayurveda practitioners, who use it to treat various ailments, (ie, memory loss, inflammation, epilepsy, fever, and asthma).¹⁵

Structure and Components of Bacosides

The chemical compound that has neuropharmacological properties and pseudo-jujubogenin moieties, known as aglycone units, is Bacoside A (dammarane-type triterpenoid saponin; Figure 3).^{16,17} This compound is composed of bacoside III, bacoside X, bacoside A3, and bacosasaponin C.¹⁵ Through structural similitude analysis, 12 analogues derived from the bacosides have been characterized and various saponin types have been identified as essential ingredients, known as bacosides I–XII.¹⁸ *Bacopa’s* additional components include apigenin, cucurbitacin, alkaloids brahmine, monnierin, hersaponin, monnierasides I–III, plantain side B, D-mannitol, herpestine, and nicotine.^{19,20} Table 1 shows molecular composition of Bacoside A.



[Blázquez-Sánchez et al., 2017]

Figure 3. Chemical structure of Bacoside A.¹

The Neuropharmacological Activity of Bacoside A

Numerous studies suggested that *B monnieri*'s bioactive components (ie, bacosides) protect the brain against oxidative damage and age-related cognitive deterioration with several mechanisms of action.^{22,23} In addition, bacosides prevent A β aggregation and formation of fibrils²⁴ as well as protect neurons against A β -induced toxicity.²⁵ From high-performance liquid chromatography (HPLC) analysis, the bioactive constituent, bacoside A, was present in the *B monnieri* extract (BME)-treated rat serum and could directly or indirectly interact with the neurotransmitter systems to improve memory and learning ability.²⁶ Bacosides present in *B monnieri* are commonly non-polar glycosides,²⁷ which enable it to cross the blood-brain barrier (BBB) via simple lipid-mediated passive diffusion.²⁸ Similarly, the bioavailability in the brain has been affirmed by the radiopharmaceuticals biodistribution.²⁹ De et al, using an animal model, described BME as being capable of altering the uptake of radioactivity of ^{99m}Tc-labeled ethylene dicysteine diethyl ester (^{99m}Tc-ECD) and ^{99m}Tc-labeled cystine dimethyl ester (^{99m}Tc-CDM) in brain and other organs. The results revealed an increased and significant uptake ($P < .05$) of ^{99m}Tc-ECD and ^{99m}Tc-CDM in brain and other organs after treatment with BME. As BME is a good antioxidant and has cognitive function on human memory, these findings have evaluated pharmacokinetic interactions of BME and suggested that BME can act on the biodistribution of ^{99m}Tc-ECD and ^{99m}Tc-CDM in specific organs.²⁹

Likewise, clinical studies also showed that oral treatment with *B monnieri* was able to enhance memory in both adults and children. The effects of *B monnieri* administration on hepatic and intestinal P-glycoprotein (Pgp) as well as Cytochrome 3A (CYP3A) expression levels were examined in Watkins' studies.³⁰ According to him, individually, Pgp-mediated efflux and Cytochrome P450 (CYP450)-mediated metabolism play a vital role in modulating the oral bioavailability of corresponding drug. However, *B monnieri* mediating

effects on CYP3A4 and alterations in Pgp were measured according to the mRNA expression level and functional activity in the intestine and liver of male Sprague Dawley (SD) rats after a week of *B. monnieri* administration. The results showed that *B. monnieri* downregulated both intestinal Pgp and CYP3A expression levels, depending on the testosterone hydroxylase catalytic activity in liver and intestine.³¹ Further studies also showed that in vivo pharmacokinetic interaction between digoxin (Pgp substrate) and carbamazepine (CYP 3A substrate) along with the administration of *B monnieri* extract in male SD rats could alter the pharmacokinetics of both Pgp and CYP3A probe drugs. Probe drug is known to lessen both biological and technical risk factors of tracking a particular target to be selective as well as potent to their target. The results showed that treatment with *B monnieri* and carbamazepine caused a change in the carbamazepine pharmacokinetic profile with a significant increase in C_{max} (maximum serum concentration of the drug achieved in the plasma) and AUC (the area under the plasma drug concentration-time curve) (0- ∞) as well reduction in CL/F (apparent total clearance of the drug from plasma after oral administration) opposing to the vehicle control rats.

The Role of Other Compounds in BME

CDR1-08

Also known as Synapsa, or KeenMind, a nootropic CDR1-08 is a well-characterized ethanolic extract of *B monnieri*. Several lines of evidence demonstrated that CDRI-08 significantly enhances the cognitive performance in the elderly and patients with impaired neurological functions³²⁻³⁴ as well as healthy human participants.^{35,36} Moreover, bacosides present in the CDRI-08 are nonpolar glycosides, and it can enter the brain by crossing the BBB through lipid-mediated passive diffusion.²⁸ The biodistribution in brain also has been affirmed by radiopharmaceuticals.²⁹ Study by Preethi et al investigated whether treatment with the CDRI-08 could change the methylation

Table 1. Molecular composition of Bacoside-A²¹.

COMPOSITION	FUNCTIONAL UNIT
Bacoside	3-O- α -L-arabinofuranosyl-(1 \rightarrow 2)-[6-O-sulfonyl- β -D-glucopyranosyl-(1 \rightarrow 3)]- α -L-arabinopyranoside, 3-O- β -D-glucopyranosyl-(1 \rightarrow 3)- α -L-arabinofuranosyl
Bacoside V	3-O- α -L-arabinofuranosyl-(1 \rightarrow 2)-[6-O-sulfonyl- β -D-glucopyranosyl-(1 \rightarrow 3)]- α -L-arabinopyranoside, 3-O- β -D-glucopyranosyl-(1 \rightarrow 3)- α -L-arabinofuranosyl
Bacoside II	3-O- α -L-arabinofuranosyl-(1 \rightarrow 2)-[β -D-glucopyranosyl-(1 \rightarrow 3)]- β -D-glucopyranoside
Bacoside III	3-O-(6-O-sulfonyl- β -D-glucopyranosyl-[1 \rightarrow 3])- α -L-arabinopyranoside
Bacoside IV	3-O- β -D-glucopyranosyl-(1 \rightarrow 3)- α -L-arabinopyranosyl
	3-O-{ β -D- glucopyranosyl (1 \rightarrow 4)(α -L-arabinofuranosyl-[1 \rightarrow 2])}- β -D-glucopyranosyl}-20-O- α -L-arabinopyranosyl
Bacoside IX	3-O- β -D-glucopyranosyl-(1 \rightarrow 3)- α -L-arabinopyranosyl 3-O-{ β -D- glucopyranosyl (1 \rightarrow 4)(α -L-arabinofuranosyl-[1 \rightarrow 2])}- β -D-glucopyranosyl}-20-O- α -L-arabinopyranosyl
Bacoside XI	3-O-{ β -D- glucopyranosyl (1 \rightarrow 4)(α -L-arabinofuranosyl-[1 \rightarrow 2])}- β -D-glucopyranosyl}-20-O- α -L-arabinopyranosyl
Bacoside XII-12	3-O-{ β -D-glucopyranosyl(1 \rightarrow 3)[β -D-arabinofuranosyl(1 \rightarrow 2)]- β -D-glucopyranosyl}-20-O-
Bacopasaponin A	3,20-di-O- α -L-arabinopyranoside
Bacoside A₁	3-O-(α -L-arabinofuranosyl[1 \rightarrow 3])- α -L-arabinopyranoside
Bacoside A₂	3-O- α -L-arabinopyranosyl-(1 \rightarrow 5)-[α -L-arabinofuranosyl-(1 \rightarrow 6)]- α -D-glucofuranoside
Bacoside A₃	3-O- α -L-arabinofuranosyl-(1 \rightarrow 2)-[β -D-glucopyranosyl-(1 \rightarrow 3)]- β -D-glucopyranoside
Bacopasaponin B	3-O-(α -L-arabinofuranosyl-[1 \rightarrow 2])- α -L-arabinopyranoside
Bacopasaponin C	3-O- α -L-arabinofuranosyl-(1 \rightarrow 2)-[β -D-glucopyranosyl-(1 \rightarrow 3)]- α -L-arabinopyranoside
Bacopasaponin D	3-O-(α -L-arabinofuranosyl-[1 \rightarrow 2])- β -D-glucopyranoside
Bacopasaponin E	3-O- α -L-arabinofuranosyl-(1 \rightarrow 2)-[β -D-glucopyranosyl-(1 \rightarrow 3)]- α -L-arabinopyranoside, 20-O- α -L-arabinopyranoside
Bacopasaponin F	3-O- α -L-arabinofuranosyl-(1 \rightarrow 2)-[β -D-glucopyranosyl-(1 \rightarrow 3)]- β -D-glucopyranoside, 20-O- α -L-arabinopyranoside
Bacopasaponin G	3-O-(α -L-arabinofuranosyl-[1 \rightarrow 2])- α -L-arabinopyranoside
Bacopasaponin H	3-O-[α -L-arabinopyranosyl]

status of reelin and brain-derived neurotrophic factor (BDNF) to enhance the memory through the interaction of *N*-methyl-D-aspartate receptor (NMDAR) with synaptic proteins. Using rat pups as a model in the study, after treatment with CDRI-08/5-azacytidine (80 mg/kg/3.2 mg/kg), their results demonstrate a higher discrimination toward novel objects than with old objects during the testing. They also observed an elevated level of unmethylated DNA in reelin and BDNF-promoted region, which suggested that this mechanism might contribute to the modulation of synaptic plasticity and thus can enhance learning and memory.³⁷ However, study by Rai et al provides the evidence for the mechanism underlying the role of the CDRI-08 in restoring spatial memory in amnesic mice. In their study, upon daily oral administration of CDRI-08 (200 mg/kg body weight [BW]) to scopolamine-treated

amnesic mice for 7 days, the spatial memory was restored, which was found to be related with significant upregulation of the GluN2B (ionotropic glutamate receptors) subunit expression and reduction in the acetylcholinesterase activity in prefrontal cortex as well as hippocampus.³⁸

Bacognize[®]

The standardized extract of *B monnieri* (Bacognize) has been shown to improve some aspects of cognitive functions in a 6-month trial in geriatric Alzheimer patients.³⁹ In this study, all patients who took 300 mg of Bacognize orally twice a day showed a statistically significant improvement in various components of Mini-Mental State Examination Scale (MMSES) including orientation of time, place and person,

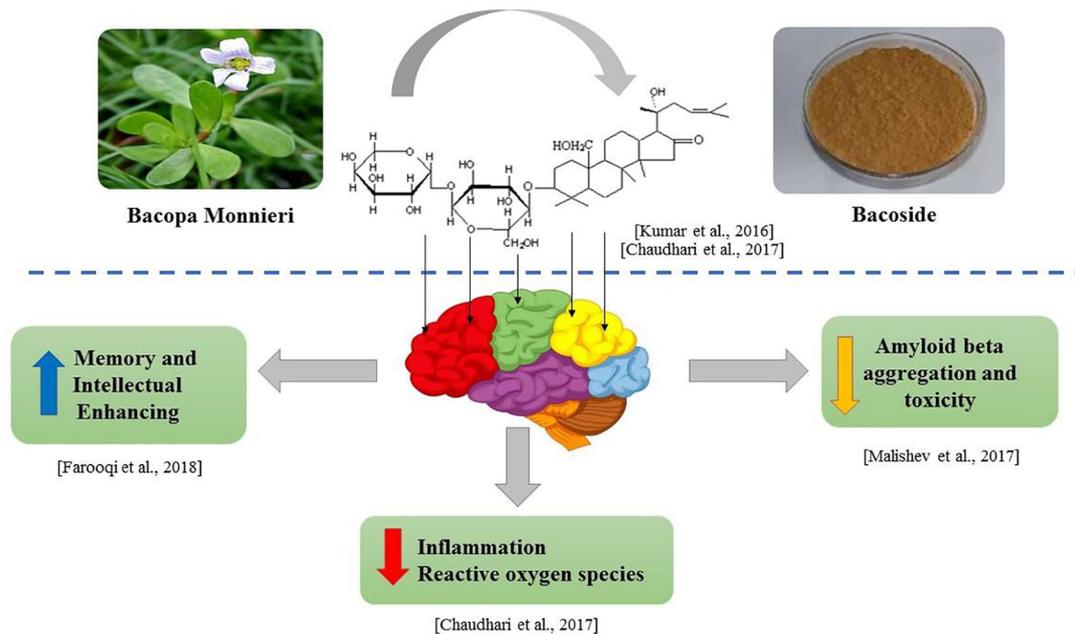


Figure 4. Neuroprotective effects of bacoside from *Bacopa monnieri*.

attention, and their language ability in terms of reading, writing, and comprehension at the end of trial. Another study refers this extract to be safe and have sustained cognitive effects when used for 12 weeks in healthy older adults.⁴⁰ Kumar et al had evaluated the effect of Bacognize on memory of 60 medical students with 42 days of administration. This randomized placebo-controlled trial exhibited a significant improvement in the tests relating to the cognitive functions in the participants who had taken 150 mg of Bacognize.⁴¹ The poor solubility of Bacognize also has been improved by recent study of Thakkar et al using the inclusion complex of Bacognize (contained 16% bacosides) and β -cyclodextrin prepared in different molar ratios of *B monnieri* via co-precipitation method. The results revealed that the inclusion of complex at molar ratio of 1:4 can enhance threefold solubility and stability of *B monnieri* in inclusion complex.⁴²

Mechanisms as a Neuroprotective Agent

The mechanisms that underlie the progression of neuronal degeneration are described in the following sections. Figure 4 illustrates the neuroprotective effects of bacoside from *B monnieri* from various studies.^{43–45}

Bacosides and Reactive Oxygen Species

Wide studies have reported the role of superoxide anion, hydroxyl radical, hydrogen peroxide, and nitric oxide in the oxidative stress-mediated neurodegeneration in AD.^{46,47} Neuronal lesions can activate microglia activation, which further generates excessive superoxide radicals.⁴⁸ Thus, mitochondrial autophagy serves as a vital source of reactive oxygen species (ROS) production.⁴⁹ As mitochondria functions as both the source and target of toxic ROS, mechanisms by which

mitochondrial dysfunction leads to neuron degeneration in AD are believed to be associated with ROS generation, activation of mitochondrial permeability transition, excitotoxicity, impaired production of adenosine triphosphate, and altered calcium homeostasis.⁵⁰ Various studies have shown an increased level of 4-hydroxynonenal, the byproduct of oxidative stress in the brain of AD patients.^{51,52} An increased level of lipid peroxidation (LPO) marker has been reported as well.^{53,54} Besides that, iron-induced oxidative stress, as demonstrated by iron accumulation in the brain of AD, is responsible for neurodegeneration in patients diagnosed with AD.⁵⁵

Extensive studies have been performed on neuroprotection of *B monnieri* against ROS. The administration of *B monnieri* inhibited LPO especially in the hippocampus, prefrontal cortex, and striatum areas of the rat cerebrum.⁵⁶ As for the rat's astrocytes, it significantly reduced the harm done by high concentrations of nitric oxide.⁵⁷ Likewise, different reports recommended that bioactive components from *B monnieri* can protect the brain against oxidative harm and improve cognitive capability via a few mechanisms.^{22,23} The enhanced cognitive capability was attributed to the free radical scavenging properties of the bacosides. Superoxide dismutase (SOD), heat shock protein 70 (Hsp70), and cytochrome P450 (CytP450) in the rat's cerebrum have critical role in both the production of ROS and scavenging activity.⁵⁸ The detoxification and binding of free radical scavenging metal ions or increasing the antioxidant properties are the mechanisms involved in the neuroprotection from bacosides^{25,34,58} (Figure 5). It also reduces the formation of lipid peroxides, divalent metals, scavenging ROS, and restraining lipoxygenase action. As results indicated that ROS level had declined when neurons were treated with BME, we propose that it may control the intracellular oxidative stress.²⁵

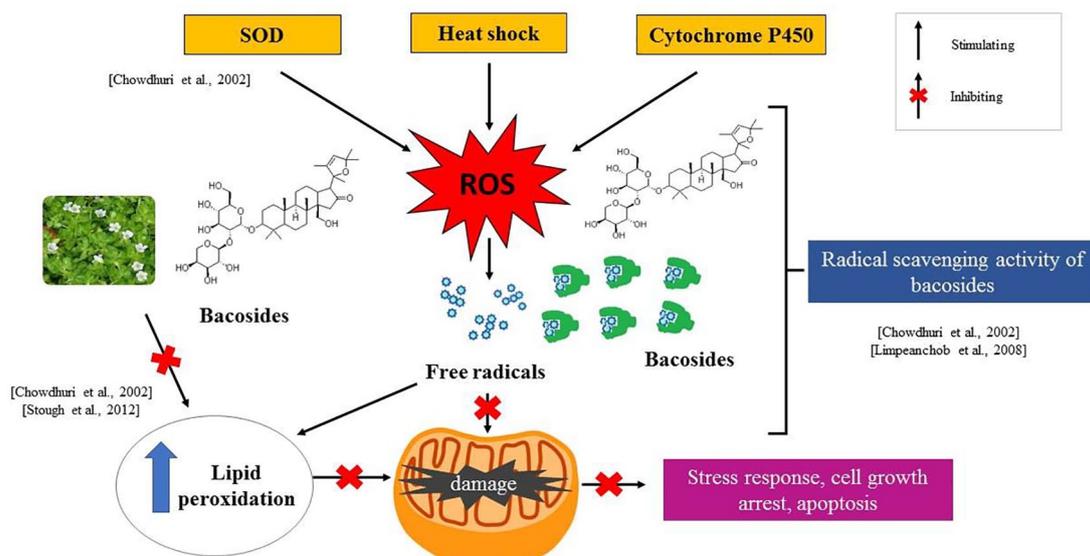


Figure 5. The action mechanism of bacoside against ROS induces mitochondrial damage. ROS indicates reactive oxygen species; SOD, superoxide dismutase.

Likewise, in neonatal hypoglycaemia, *B monnieri* has potent neuroprotective capability in reversing the modified dopamine D1 receptor function, BAX (BCL2 Associated X, Apoptosis Regulator), and gene expressions, respectively. Thus, SOD level is lowered, which in turn causes cortical cell death.⁵⁹

Furthermore, in rat models of neurotoxicity incitation by ibotenic corrosive and colchicine, *B monnieri* indicated a dosage-related intellectual deficiencies.⁶⁰ Acrolein is an exceptionally active compound shaped as a LPO byproduct and acts as an oxidative stress inducer by framing adducts of cell nucleophilic groups. It demonstrates a significant elevation of acrolein levels in the hippocampus; *B monnieri* extract is accounted to have neuroprotection in human neuroblastoma cell line SK-N-SH against hydrogen peroxide and acrolein-induced toxicity.³ It also protects through ROS scavenging, maintains the mitochondrial membrane integrity, modulates the expression of several redox regulatory proteins, that is, Sirt1 (Sirtuin 1), NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), p66Shc (a member of the SHC family of protein adapters), and ERK1/2 (extracellular signal-regulated protein kinases 1 and 2), and protects the cells from oxidative stress. The counter pressure impact of bacosides of *B monnieri* was studied in adult male SD rats. The results portrayed that it is able to de-stress the modulation of SOD, Hsp70, and CytP450 under unfavourable conditions, for example, stress.⁵⁸

Bacoside A and Beta Amyloid Toxicity

A significant inhibitory effect of cytotoxicity, fibrillation, and membrane interactions of beta-amyloid (1-42) were observed by preincubation of Bacoside A with A β 42 in SH-SY5Y cell line model.⁴³ A β is a peptide that plays a prominent role in AD progression and toxicity. In AD, A β assembles into insoluble amyloid fibrils that aggregate in extracellular neuritic or senile

plaques^{61,62} and is accompanied by synaptic dysfunction, neuronal deterioration, dementia, and cognitive declination.⁶³

Therefore, it can suggest that A β may be directly toxic to neuronal cells and synapses. The previous study showed that extracts containing soluble A β aggregates can induce amyloidosis in an animal model that otherwise never develop amyloid plaque.⁶⁴ Inhibition of A β aggregates and assembly is one of the primary therapeutic strategies in AD treatment and prevention. Another study reported a substantial link between the toxic peptide of A β with its membrane interaction.⁴³ In their experiment, A β 42 monomer initially produces oligomeric species that were membrane-active and cytotoxic. It then aggregated into fibrils, which were promoted through interactions with the bilayer interface. However, aggregation of A β 42 was reduced and its membrane interaction was inhibited following incubation with Bacoside A. Figure 6 shows the mechanism of action of bacoside on A β .^{43,61}

Synergistic Action of *B monnieri*

The synergistic effects of *B monnieri* have been investigated, providing information on its possible neuropharmacological effects between this herbal medicine and other plant extracts or synthetic drugs. The synergistic action of *B monnieri* (320 mg), L-theanine (100 mg), *Crocus sativus* (30 mg), copper (2 mg), folate (400 μ g) with vitamin B (450-9 μ g) and vitamin D (25 μ g) in a cohort of elderly subjects (1 capsule per day) for 8 weeks of treatment were investigated.⁶⁶ The results showed a significant improvement of cognitive decline, perceived stress, and depression tested with Mini-Mental State Examination (MMSE), Perceived Stress Questionnaire (PSQ) Index, and Self-Rating Depression Scale (SRDS) scores.

In another study done in an in vivo model, combination of *B monnieri* (100 mg/kg) with rivastigmine (5 mg/kg) showed significant protection against aluminum chloride (AlCl₃)-induced

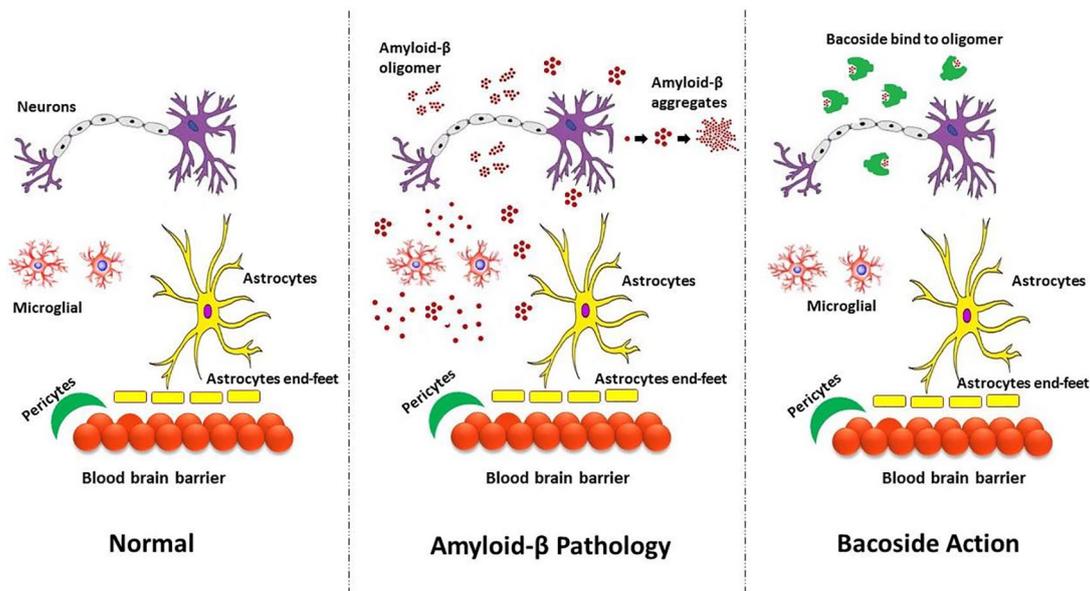


Figure 6. Mechanism of action of bacoside against beta-amyloid (adaptation and modifications from previous works^{2,3}).

However, Bacoside A exhibited anti-amyloid toxicity properties upon membrane interactions and bilayer-induced fibrillation of pathogenic substance prion protein (PrP). The experimental data revealed that preincubation of PrP (106-126) with Bacoside A before addition to vesicle bilayers might possibly enhance fibril formation and in parallel had inhibited membrane interactions of the peptide assemblies. The findings from this study revealed a significant interaction of the compound with the amyloidogenic determinant of PrP and noticeable effects upon the structural and functional properties of the peptide even though Bacoside A has not been explored yet in conjunction with the prion protein. In a more extensive context, the anti-amyloid properties of Bacoside A might be discovered to its impact in ameliorating the amyloid protein toxicity via stimulating and enhancing fibrillation.⁶⁵

memory impairment in rats compared to those treated with $AlCl_3$ per se.⁶⁷ In their study, chronic administration of $AlCl_3$ caused functional deficits in learning and memory skills, which were tested using the Morris water maze and Elevated Plus Maze (EPM) tasks. However, rats treated with combination of rivastigmine and *B monnieri* showed better acquisition and retention latencies compared to groups treated only with $AlCl_3$, indicating significant protection against $AlCl_3$ -induced deterioration in learning and memory skills. They concluded that *B monnieri* and rivastigmine act through synergistic mechanisms to prevent neuronal damage and enhance cholinergic neurotransmission, thus showing better therapeutic effect compared to treatment alone.

Antidementia and anticholinesterase activities in adult male Swiss mice also were studied using combined extracts of *B monnieri* and *Ginkgo biloba* (GB).⁶⁸ In this study, anti-dementia activity was tested against scopolamine (3 mg/kg BW)-induced impairment in passive avoidance (PA) test. Their results indicate a significant increase in transfer latency time (TLT) and no transfer response (NTR) after treatment of combined extracts of *B monnieri* at 30 mg/kg and GB at 15, 30, and 60 mg/kg for 7 days of administration. All the extracts showed potent effects toward attenuating the effects of dementia.

In vivo and in vitro Study on Neuroprotective Effects of BME

Research on neuroprotective effects of BME has been widely studied before by in vivo and in vitro models. Most of the study conducted by in vivo were performed on male Wistar rats and

male Swiss albino mice and rats. However, for in vitro study, different cell line models were chosen to study the effect of BME such as PC12, SH-SY5Y, as well as primary cortical neuron cells. Uabundit et al demonstrated protective effects of BME in male Wistar rat model that had been induced with 2 nmol/2 μ L ethylcholine aziridinium ion (AF64A). Result showed that 20, 40, and 80 mg/kg BW of BME was able to mitigate the memory impairment and neurodegeneration in the rats by enhancing the escape latency time ($P < .01$) in the Morris water maze test. They also observed that both cholinergic neuron and neuron density reduction were lessened.⁶⁹ Other than that, BME administered orally at 40 mg/kg/day for 5 weeks was able to prevent the neurotoxicity in the cerebral cortex of male Wistar rat brain exposed with aluminum chloride ($AlCl_3$).⁷⁰ Research done by Khan et al revealed that BME given orally at 30 mg/kg BW for 2 weeks significantly improved the memory and learning capability in intracerebroventricular-streptozotocin (ICV-STZ)-induced male Wistar rats. Their finding demonstrates the therapeutic efficacy of BME on cognitive impairment and oxidative damage, observed by significant reduction in LPO levels, increased GSH (glutathione) contents, and upregulated antioxidant enzymes activity such as SOD, GST (glutathione S-transferases), CAT (catalase), and GPx (glutathione peroxidase) in the hippocampus infused by ICV-STZ model.⁷¹ On a different study, BME at 100 mg/kg BW for 180 days lessened both the sodium nitrate ($NaNO_2$) and D-galactose (D-Gal) levels, which improved the BW, memory, and learning skills. *B monnieri* extracts also normalized the ATPase system in AD-induced mice.⁷² Dwivedi et al⁷³

also demonstrate attenuation of Okadaic acid (OKA)-induced memory dysfunction in SD rats treated with BME at 40 and 80 mg/kg BW for 13 days.

Moreover, interesting extensive finding by Rastogi et al revealed the protective effect of bacosides, against the age-associated neurodegeneration and promotion of healthy brain aging in female Wistar rats. In this study, bacosides were administered orally at 200 mg/kg BW for 3 months in middle aged and aged rats, and its impact on the prevention of Senile Dementia of Alzheimer Type (SDAT) was evaluated. Their findings demonstrated that bacosides was found to display significant anti-aging property by preventing the lipofuscin aggregation in the brain cortex of middle-aged and aged rat. Other than that, cholinergic neurotransmission was observed in aged-rat brain cortex, and treatment with bacosides was able to mitigate this age-associated cholinergic degeneration. Based on the potential findings on bacosides, they suggested that bacosides exerted multitargeted pharmacological action by preventing the lipofuscin accumulation, enhancing the synthesis of cholinergic neurotransmitter acetylcholine, modulating the metabolism of monoaminergic neurotransmitters, and inhibiting LPO in the aged rats.⁷⁴

A previous study also investigated a new nanotechnology approach for the brain delivery of the Bacoside A for the treatment of neurodegenerative disorders using poly-(D, L)-Lactide-co-Glycolide (PLGA) as surfactant. Bacoside-A-loaded PLGA nanoparticles were prepared via oil-in-water (o/w) emulsion solvent evaporation technique. Surface of nanoparticles were modified by coating with polysorbate 80 to enhance the crossing of BBB. The ability of nanoparticles in targeting the brain was evaluated by in vivo studies using Wistar albino rats. Their results suggested that PLGA nano Bacoside A formulation with a size range of 70–200 nm and a relatively low polydispersity index of 0.391 ± 1.2 showed encapsulation efficiency at $57.11\% \pm 7.11\%$, with a drug loading capacity of $20.5\% \pm 1.98\%$. Scanning electron microscopy (SEM) and X-ray studies also revealed its spherical shape and low crystallinity. This verified that there were no chemical interactions between both polymer and drug molecules. The in vitro study showed a constant pattern with maximum release of $83.04\% \pm 2.55\%$ in 48 hours, while in vivo study showed a higher brain concentration of Bacoside A ($23.94 \pm 1.74 \mu\text{g/g}$ tissues) that implied a significant role of surface-coated nanoparticles on brain targeting. The overall results suggested the efficiency of surface-modified PLGA nanoparticles in delivery of Bacoside A to the brain.⁷⁵

In vitro study demonstrated that scopolamine induced PC12 cell death was significantly ameliorated by BME pretreatment, and the viability was restored at 85.75% of the control with 100 $\mu\text{g/mL}$ of BME. *B monnieri* extracts pretreated cells also showed a decreased release of lactate dehydrogenase (LDH) up to 22.42% of total as compared with 30% of scopolamine-treated group. *B monnieri* extracts also found to ameliorate scopolamine effect by downregulating acetylcholinesterase

(AChE) and upregulating BDNF as well as muscarinic-1 receptor expression.⁷⁶ While pretreatment of BME with different doses (2.5–100 $\mu\text{g/mL}$) for 3 hours in SK-N-SH cells prior to the addition of 200 μM of H_2O_2 or 15 μM of acrolein can significantly protect against acrolein-induced cytotoxicity. *B monnieri* extracts also showed to inhibit the generation of intracellular ROS in addition to preserving the mitochondrial membrane potential. *B monnieri* extracts pretreatment also prevented the modifications caused by the activity of several redox regulated protein.³ Furthermore, Limpeanchob et al revealed the neuroprotective effect of BME against $\text{A}\beta$ -induced cell death in primary cortical cultured neuron cells. They found that the cell viability of cultured cortical cells was increased when treated with 100 $\mu\text{g/mL}$ of BME. From their study, they postulated that Brahmi extract can diminish neuronal death induced by $\text{A}\beta$ peptide through the suppression of AChE activity. Brahmi extract also exhibited antioxidant properties in both in vitro and cell-based assays.²⁵ By using SH-SY5Y cell as a model, BME at 0.1 to 25 μM significantly reduced neurotoxicity of oxidized low-density lipoprotein (LDL) in a dose-dependent manner as well as suppressed the elevation of cellular AChE activity mediated by oxidized LDL.⁷⁷ Using the same SH-SY5Y model, Bacoside A at 50 μM exerted significant inhibitory effects upon cytotoxicity, fibrillation, and particularly membrane interactions of $\text{A}\beta$ (1–42) ($\text{A}\beta 42$).⁴³ Table 2 outlines the specific effects of bacosides on various study designs (in vivo and in vitro) of AD.

Clinical Studies in Humans Using BME

Upon the promising neuroprotective effect of *B monnieri* in in vitro and in vivo studies, numerous clinical studies on human subjects have been performed using *B monnieri* for cognitive improvement. A clinical study of standardized extract of *B monnieri* (150 mg) on 60 medical students from Government Medical College, Nagpur, India over a period of 15 days revealed significant improvement in biochemical analyses, that is, significant elevation in serum calcium levels and enhanced memory test.⁴¹ Another group of researchers reported that individual doses of *B monnieri* and *Sideritis scardica* extracts in 10 mild cognitive impairment subjects from Germany (mean age: 61.88 ± 6.69 years) resulted in improvement in the d2-concentration test.⁷⁹ However, treatment with *B monnieri* (2×150 mg) for 90 days in 107 participants (between ages 18 and 60 years) in Swinburne University, Australia led to an improved performance in a structural working remembrance task in healthy participants with no history of neurological diseases, gastrointestinal disorders, as well as chronic infections. Above it all, none of the healthy participants took any cognitive-enhancing drugs.⁸⁰

Besides cognitive improvement, *B monnieri* can also enhance learning capability. Consumption of *B monnieri* for 3 months in 76 human subjects between 40 and 65 years of age in University of Wollongong, Australia resulted in significant effects on retention of new information.⁸¹ The consistent consumption of

Table 2. Effects of *Bacopa monnieri* extract (BME) on various study designs of AD.

THE MODEL USED AND STUDY DESIGN	DOSE OR FREQUENCY	EFFECT OF BME TREATMENT	REFERENCES
<i>In vivo model</i>			
Ethylcholine aziridinium ion (AF64A) (2nmol/2 µL)—ICV-induced male Wistar rats	20, 40, and 80mg/kg BW	BME enhanced the escape latency time ($P < .01$) in the Morris water maze test. Both cholinergic neuron and neuron densities reduction were lessened.	Uabundit et al ⁶⁹
Oral administration of aluminum chloride (AlCl ₃ ; 50mg/kg, p. o.)—IP-induced male Wistar rats	40 and 50mg/kg BW	BME treated significantly prevented the reduction in SOD activity and decreased the lipid peroxides and protein oxidation. Both electron and fluorescence microscopic studies uncovered substantial inhibition of necrotic change and intra-neuronal lipofuscin in the hippocampus CA1 region.	Jyoti et al ⁷⁰
Streptozotocin (STZ) (3mg/kg, p. o.)—ICV-induced male Wistar rats	30 mg/kg BW	BM treated improved the memory and learning capability in ICV-STZ rats. BME treated significantly reduced in LPO levels, increase GSH contents and increase the activity of antioxidant enzymes such as SOD, GST, CAT and GPx in the hippocampus infused by ICV-STZ model.	Khan et al ⁷¹
D-Galactose (120mg/kg, p. o.) and sodium nitrite (90mg/kg, p. o.)—IP-induced male Albino mice	100 mg/kg BW	BME lessened both the NaNO ₂ and D-Gal levels, which improved the body weight, memory, and learning skills. BME also normalized the ATPase system in AD-induced mice.	Kunte and Kuna ⁷²
Okadaic Acid (300mg/kg, p. o.)—ICV-induced male Sprague Dawley rats	40 and 80 mg/kg BW	BME treated significantly enhanced the memory-enhanced memory dysfunction in AD rats as appeared by a reduction in path length and latency time. BM also restored GCLC, HO1, and Nrf2 as well reduced the neuronal loss, oxidative stress and neuroinflammation	Dwivedi et al ⁷³
Female Wistar rats; young (2-3 months), middle-aged (17-18 months), aged (>24 months old)	200 mg/kg BW	BME (bacosides) significantly ($P < .05$) prevents the lipofuscin aggregation in the middle-aged and aged rat brain cortex. Bacosides also enhances the synthesis of cholinergic neurotransmitter acetylcholine, modulates the metabolism of monoaminergic neurotransmitters and inhibits lipid peroxidation in the aged brain rats.	Rastogi et al ⁷⁴
Adult albino Wistar rats	20mg/kg BW	PLGA nano BME formulation with a size range of 70-200 nm and a relatively low polydispersity index of 0.391 ± 1.2 showed that encapsulation efficiency was $57.11\% \pm 7.11\%$, with a drug loading capacity of $20.5\% \pm 1.98\%$. SEM revealed the PLGA nanoparticles' spherical shape, as well as it appeared to have low crystallinity by X-ray studies. This verified there were no chemical interactions between both polymer and drug molecules. The <i>in vitro</i> study showed a constant pattern with a maximum release of $83.04\% \pm 2.55\%$ in 48 hours and <i>in vivo</i> study showed higher brain concentration of Bacoside A (23.94 ± 1.74 µg/g tissues), which implied a significant role of surface-coated nanoparticles on brain targeting.	Jose et al ⁷⁵
Male Swiss albino mice and Wistar rats were used to evaluate nootropic activity and bioavailability studies, respectively.	40 mg/kg BW	Bacopaephospholipid complex (BPC) portrayed 2 endothermal peaks (80.90°C and 171°C) in DSC studies. BPC treated significantly improved cognitive ability and anti-amnesic activity in aged mice in most memory-related models studied. BPC also retained effective bacosides concentration for a longer period in rat serum.	Habbu et al ⁷⁸
Scopolamine—IP-induced male Swiss albino mice	120mg/kg BW	BME treated reversed both retrograde and anterograde amnesia.	Saraf et al ⁸²
PSAPP mice	40 and 160mg/kg BW	BME reversed both Y-maze and open-field hyperlocomotion behavioral changes in PSAPP mice. Thus, suggested BME reduced Aβ 1-40 and 1-42 levels in the cortex by 60%.	Holcomb et al ⁸⁴
<i>In vitro model</i>			
PC12 cell line	100 µg/mL	BME ameliorated the mitochondrial and plasma membrane damage induced by 3 µg/ml scopolamine to 54.83% and 30.30%.	Pandareesh and Anand ⁷⁶
SK-N-SH cell line	12.5, 25, 50, 75, and 100 µg/mL	BME pretreatment significantly protects against H ₂ O ₂ and acrolein-induced cytotoxicity and inhibited the generation of intracellular reactive oxygen species in addition to preserving the mitochondrial membrane potential.	Singh et al ⁸³
Primary cortical cultured neurons cell line	100g/mL	BME protected neurons from beta-amyloid-induced cell death. BME inhibited the lipid peroxidation reaction of brain homogenate in a dose-dependent manner.	Limpeanchob et al ⁸⁵
SH-SY5Y cell line	0.1 to 25 µM	BME diminished the neurotoxicity of oxidized LDL in a dose-dependent manner potentially by suppression of cellular oxidative stress.	Yamchuen et al ⁷⁷
SH-SY5Y cell line	Bacoside-A (50 µM)	Bacoside-A exerted significant inhibitory effects upon cytotoxicity, fibrillation, and particularly membrane interactions of amyloid-beta (1-42) (Aβ ₄₂).	Malishev et al ⁸³

Abbreviations: AlCl₃, aluminum chloride; AD, Alzheimer disease; BME, *Bacopa monnieri* extract; BPC, Bacopaephospholipid complex; BW, body weight; CAT, catalase; D-Gal, D-galactose; GCLC, glutamate-cysteine ligase catalytic subunit; GSH, glutathione; GST, glutathione S-transferase; GPx, glutathione peroxidase; HO1, heme oxygenase 1; H₂O₂, hydrogen peroxide; ICV-STZ, intracerebroventricular-streptozotocin; IP, intraperitoneal; LDL, low-density lipoprotein; LPO, lipid peroxidation; NaNO₂, sodium nitrate; Nrf2, nuclear factor erythroid 2-related factor 2; OKA, okadaic acid; PLGA, poly-(D, L)-lactide-co-glycolide; PSAPP, presenilin/amyloid precursor protein; SEM, scanning electron microscopy; SOD, superoxide dismutase; DSC, differential scanning calorimetry.

Table 3. Summary of clinical studies of *Bacopa extract* in cognition.

PARTICIPANTS/STUDY DESIGN/GEOGRAPHICAL REGION	INTERVENTION	CLINICAL OUTCOME	REFERENCES
Healthy children, 6-8 years from rural India. Double-blind, randomized placebo-controlled independent-group study was employed	One teaspoonful of <i>Bacopa</i> syrup 3 times daily for 3 months. (Each teaspoonful was equivalent to 350mg of crude <i>Brahmi</i>)	Strengthened exploratory drive (as measured by maze learning), improved perceptual images of patterns, and increased perceptual organization and reasoning ability (as measured by reaction time)	Sharma et al ⁸³
Healthy adults, between 18 and 60 years, in Swinburne University, Australia. A double-blind, placebo-controlled independent group design in which subjects were randomly allocated to 1 of 2 treatment conditions.	<i>Bacopa</i> extract, 300 mg daily, for 12 weeks	Significant improvement in speed of visual information processing measured by the IT task, learning rate, and memory consolidation measured by the AVLT ($P < .05$) and state anxiety ($P < .001$) compared to placebo, with maximal effects evident after 12 weeks.	Stough et al ⁸⁴
Healthy adults, between the ages of 40 and 65 years in University of Wollongong, Australia. Double-blind, randomized placebo-controlled independent group study was employed	<i>Bacopa</i> extract, 300 mg if subject <90 kg and 450 mg if >90 kg, for 12 weeks	Significant effect on a task requiring the retention of new information ($P < .05$) where the group who received the <i>Brahmi</i> retained more word pairs over the delay than the placebo group.	Roodenrys et al ⁸¹
Healthy adults (mean age 73.5 years) in University of Catania, Italy. Double-blind, randomized placebo-controlled clinical trial with a placebo run-in of 6 weeks	<i>Bacopa</i> extract, 300 mg daily, for 12 weeks	Enhanced AVLT delayed word recall memory scores relative to placebo, significant improvement in Stroop results ($P < .05$) and also decreased in CES-D-10 depression scores over time, as well as decreased in combined state plus trait anxiety scores and heart attack.	Calabrese et al ⁸³
Healthy adults, between 18 and 60 years, in Swinburne University, Australia. A double-blind, placebo-controlled independent group design was employed	<i>Bacopa</i> extract, 300 mg daily, for 90 days	Significant improvement in working memory ($P = .035$), spatial working memory ($P = .0510$), and significant reduction ($P = .029$) in the amount of false alarms produced during RVP task.	Stough et al ⁸⁰
Children requiring individual educational support, 10.5 years in Center for Research in Mental Retardation (CREMERE), Mumbai, India. The study was conducted as outpatient procedure in hospital settings with close monitoring.	<i>Bacopa</i> extract, 225 mg daily, for 16 weeks	Significant change in the baseline value of working memory and short-term verbal memory from 5.21 ± 0.32 to 6.38 ± 0.25 ($P \leq .05$) and 5.33 ± 0.44 to 6.54 ± 0.35 ($P \leq .05$). Significant improvement ($P \leq .05$) was also seen in logical memory, memory related to personal life and also in visual as well as auditory memory.	Usha et al ⁸⁵
Ninety eight healthy subjects, age ≥ 55 years in Lismore, New South Wales, Australia. Double-blind, randomized placebo-controlled design was employed	<i>Bacopa</i> extract, 300 mg daily, for 12 weeks	Significantly enhanced the memory acquisition, verbal learning, and delayed recall measure by Rey Auditory Verbal Learning Test (AVLT): Trial a4 ($P = .000$), Trial a5 ($P = .016$); Trial a6 ($P = .000$); Trial a7 (delayed recall, $P = .001$); Total learning ($P = .011$) as well retroactive interference ($P = .048$). Scores including MAC-Q, TMT, and CFT improved the group differences and nevertheless were not significant	⁸²
Sixty healthy adults, mean age: 62.62 ± 6.46 years (37 females and 23 males) in Thailand. Double-blind, randomized placebo-controlled design was employed	<i>Bacopa</i> extract, 300 mg or 600 mg daily, for 12 weeks	Treated extract group displayed an enhanced working memory as well a reduction in both P300 and N100 latencies. The plasma AChE activity suppression was also seen, which suggest that it could enhance the cognitive ability and working memory and improve attention	Peth-Nui et al ⁸⁶
Seventeen healthy volunteers (13 females and 4 males), mean age 25.23 ± 5.97 in Melbourne, Australia. Double-blind, placebo-controlled cross-over study was employed	<i>Bacopa</i> extract, 320 mg or 640 mg daily, 1 hour and 2 hour	<i>Bacopa</i> consumption showed a change from baseline score indicative of positive cognitive effects at first and second hour post consumption on the Stroop tasks as well Letter Search. It produced some nootropic and adaptogenic effects. Positive mod effects and reduction in cortisol levels (physiological stress response) were associated with <i>Bacopa</i> consumption by participants.	Benson et al ⁸⁵
Sixty healthy adults between 19 and 22 years from Government Medical College, Nagpur, India. Double-blind, randomized placebo-controlled no-crossover, parallel trial was employed	<i>Bacopa</i> extract, 150 mg, for 15 days	Significant improvement in memory test, neuropsychological tests (digit span memory task, paired associate task, logical memory test (story recall), memory span for nonsense syllables) and computerized tests (finger tapping test, simple reaction test, choice reaction test, choice discrimination test, and digit picture substitution test (symbol digit modalities test). Blood biochemistry showed significant elevation in serum calcium levels (still within normal range).	Kumar et al ⁸¹
Ten subjects (mean age: 61.88 ± 6.69 years) from Germany with mild cognitive impairment.	Sideritis extract, 500 mg combined with <i>Bacopa</i> extract, 160 and 320 mg	<i>Sideritis</i> extract combined with <i>Bacopa</i> extract indicated better performances in d2-test test only contrasted with memory test and arithmetic calculation test (CPT). Quantitative EEG assessment revealed that <i>Sideritis</i> extract combined with <i>Bacopa</i> extract at lower dose (160 mg) increased the spectral power while combined with <i>Bacopa</i> extract at higher dose (320 mg) formed attenuation of all waves except for Delta in frontal-temporal brain areas, indicating massive differences between both extracts.	Dimpfel et al ⁷⁹
Thirty elderly subjects mean age 66 ± 3 years in Bologna, Italy. Double-blind, cross-over designed trial versus placebo group study was employed	Combined nutraceuticals containing <i>Bacopa</i> dry extract (320 mg), L-theanine (100 mg), <i>Crocus sativus</i> (30 mg), copper (2 mg), folate (400 µg), and vitamins of B (4.50 µg-9 mg) and D (25 µg)	After 2 months of nutraceutical therapy, MMSE and PSQ index significantly improved in the active treatment arm, both versus baseline and versus the parallel arm. Both groups experienced a significant improving in the SRDS scores	Cicero et al ⁸⁶

Abbreviations: AVLT, Rey Auditory Verbal Learning Test; CESD, Center for Epidemiologic Studies Depression scale; CFT, Complex Figure Test; MAC-Q, Memory Complaint Questionnaire; MMSE, Mini-Mental State Examination; PSQ Index, Perceived Stress Questionnaire; RVP, Rapid visual information processing; SRDS, Self-Rating Depression Scale; TMT, Trail Making Test.

BME (300 mg/day) for 84 days in participants without dementia aged 65 years and above in University of Catania, Italy also showed improvement in their performance in a restraint recall and Stroop Task, that is, evaluating the capability to bypass unnecessary input.³³ Moreover, in Lismore, New South Wales, Australia, the administration with *B monnieri* (300 mg/day) in healthy volunteers over 55 years of age showed improvement in their oral learning, memory attainment, and suppressed recall.⁸²

In another research done at Swinburne University of Technology, Melbourne, Australia using higher single dose in a double-blind, placebo-controlled trial among normal healthy subjects between the age of 18 and 44 years demonstrated an improved and preserved cognitive ability.³⁶ Significant enhancement in prompt memory and response performance was also observed when *Bacopa* in the form of syrup (proportionate to 10g dried *Bacopa* daily) was administered in 40 school children aged between 6 and 8 years for 90 days from rural India.⁸³ The overall clinical trials in humans using BME are summarized in Table 3.

Conclusions

Many traditional plants especially *B monnieri* have intricate mixtures of chemical compounds, which exhibit various pharmacological and biological activities. They have been used as traditional medicines and for anti-aging. According to the long-established hypothesis, plant compounds are able to maintain the fundamental vitality in the body and have various neuroprotective mechanisms that empower them to be used as part of our well-being. This review reveals the effective use of *B monnieri* in cognition and neuroprotection and its phytoconstituents that can be used in novel drug discovery.

Author Contributions

ASAM, SV and PM designed the flow and wrote sub-sections of the manuscript. All other authors contributed to manuscript revision, proof reading and approval of the submitted version.

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