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Review

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Neuroprotective and Nootropic Effect of *Bacopa monnieri* (L.) Wettst. (Brahmi) through Its *in vivo* Data Focused on Alzheimer's Disease

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Abstract

Bacopa monnieri (L.) Wettst. (Plantaginaceae) (BM), known as "brahmi", is a reputed medicinal plant, particularly in Ayurvedic medicine. Since BM has been traditionally used for memory dysfunction, experimental studies at the pre-clinical level support the nootropic effect of the plant relevant to Alzheimer's disease (AD) demonstrated through various mechanisms. It has been reported that saponins (*e.g.* bacosides, bacopasides, and bacopasaponins) are largely responsible for the memory-enhancing and other neuropharmacological effects of BM. Bacosides, which are non-polar molecules that can easily cross the blood-brain barrier, are reported to directly lead to anti-inflammatory and antioxidant effects in the brain. BM extracts rich in bacosides are considered advantageous due to their higher nootropic efficacy. The findings suggest that only bacoside enrichment increases antiamnesic activity; other components also contribute to the extract with a synergism. Adverse side effects of BM in humans have rarely been reported. In the current review, we aimed to scrutinize *in vivo* data derived from the studies related to the cognitive-enhancement effect of BM with a special focus on AD.

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1. Introduction

Bacopa monnieri (L.) Wettst. (Plantaginaceae) (BM) is a member of the genus *Bacopa*, which has about 100 taxa worldwide. It is a perennial and creeping plant growing in various parts of the world, such as southern and northern India, Nepal, Sri Lanka, China, Vietnam, Australia, Africa, the Arabian Peninsula, America, and the Caribbean. Known as "brahmi" in India and "water hyssop, thyme-leaved gratiola, herb of grace, and Indian pennywort" in English, the plant has been widely used in Ayurvedic medicine for hundreds of years. Brahmi (BM) is recognized as a "natural nootropic/brain tonic" in Ayurvedic medicine (Suhdakaran et al., 2020).

Since the primary therapeutic activity of BM is to improve cognitive function or cognitive dysfunction, much of the research has focused on the mechanisms associated with this effect. The triterpene saponins (especially bacosides) in the plant are responsible for enhancing the transmission of nerve impulses. Bacosides have been found to help repair damaged neurons by increasing kinase activity, synthesis, and restoration of neuronal synaptic activity, and, ultimately, nerve impulse transmission (Singh & Dhawan, 1997). According to the results of animal studies, bacosides have antioxidant activity against oxidative damage in the hippocampus, frontal cortex, and striatum. *In vivo* studies have also shown that BM extracts modulate the expression of some enzymes involved in the formation and clearance of reactive oxygen species in the brain (Chowdhury et al., 2002). *In vitro* reports imply the protective effect of BM against DNA damage both in human fibroblasts and astrocytes (Russo et al., 2003). Since there are many studies on the nootropic effect of BM, this review aims to summarize *in vivo* data of the plant. For this purpose, Web of Science (WoS), PubMed, Scopus, ResearchGate, and Google Scholar academic databases were used for the literature summary.

2. *In vivo* studies related to the nootropic effect of BM

CDRI 08 is the ethanol extract of BM, a nootropic plant, standardized over 55% bacosides. CDRI 08 reduced oxidative stress and memory dysfunction induced by decabrominatediphenylether-209 (PBDE-209) in mice (Verma et al., 2015). The effect of CDRI 08 on *N*-methyl-D-aspartate receptor (NMDA-R1, NR1 variant) expression and repressor element-1 silencing transcription factor (REST)/neuron-restrictive silencer factor *(*NRSF) binding to the NR1 promoter, male mouse pups were given CDRI 08 orally at doses of 40, 80 or 120 mg/kg in combination with PBDE-209 (20 mg/kg). Results showed that NR1 expression increased and REST/NRSF binding to the NR1 promoter decreased after post-natal exposure to PBDE-209. Furthermore, CDRI 08 supplementation significantly restored NR1 expression and binding of REST/NRSF to the NR1 promoter, close to the control value at a dose of 120 mg/kg. In conclusion, the findings suggest that CDRI 08 acts on the glutamatergic system, possibly through the expression and regulation of NR1. In another study (Rastogi et al., 2012), by selecting the optimum dose of bacoside, dose-dependent activity on the neurotransmitter acetylcholine, a biomarker of aging biomarker lipofuscin and senile dementia of Alzheimer's type (SDAT) biomarker neurotransmitter acetylcholine was screened in the brains of the aged female Wistar rats. The designated dose of bacosides at 200 mg/kg

(b.w.) was administered orally to middle-aged and aged rats for 3 months. Its protective effect against age-related changes in the neurotransmission mechanism, oxidative stress markers, behavioral paradigms as well as hippocampal neuronal loss was further investigated. According to the results, bacosides may act as impending natural agents in preventing the harmful effects of aging and preventing age-related pathologies such as SDAT.

The memory-enhancing (acute and chronic) potentials of commercial extracts of BM (200 mg/kg, *p.o.*), *Ginkgo biloba* L. (150 mg/kg, *p.o.*), and *Lavandula angustifolia* Mill. (200 mg/kg, *p.o.*) and their mixtures (BM and *L. angustifolia* at 100 mg/kg, *G. biloba* at 75 mg/kg, *p.o.)* were compared for their synergistic/additive effects in scopolamine-induced amnesia in mice by Morris water maze (MWM) test and elevated plus maze (EPM) tests. In the MWM test, escape latency and accumulated path length parameters were significantly (n: 8, $p < 0.05$) reduced in animals given BM, *G. biloba, and L. angustifolia* and their mixtures (Rehman et al., 2021). Furthermore, in the experiments (acute on the 7th day and chronic on the 15th day), the crossings at the platform position and the time spent in the platform quadrant were significantly augmented. On the other hand, transfer latency in the EPM test was reduced in treated animals compared to the saline group (n: 8, $p < 0.05$). The mixture indicated a synergistic effect on memory enhancement in mice compared with each extract individually.

Prenatal stress (PNS) affects the neurodevelopment of offspring, causing anxiety-like behaviors and memory deficits. A related study (Sivasangari & Rajan, 2020) investigated whether pretreatment administration of BME (CDRI 08/BME) affects PNS-induced changes in signaling molecules and behavioral changes in Wistar rat offspring. Pregnant rats were randomly divided into control and PNS groups and BME was administered. Animals were first placed in a social defeat cage to induce PNS and exposed to social defeat from the 16-18th gestational day. BME treatment was applied to pregnant rats inserted in the PNS + BME group from the 10th day of gestation until the 23rd postnatal day of their offspring. PNS-induced anxiety-like behaviors and increased levels of poor memory, corticosterone, adrenocorticotropic hormone, glucocorticoid receptor, pro-apoptotic caspase-3, and 5-HT2C receptor. On the other hand, anti-apoptotic B cell leukemia (B cell leukemia, Bcl-2), 5-HT1A receptor, some synaptic proteins (synaptophysin and synaptotagmin-1), calmodulin-dependent protein kinase II/neurogranin phosphorylation, NMDARs (2A and 2B), postsynaptic density protein 95 levels decreased. It was determined that BME inhibited PNS-induced changes in anxiety-like behaviors and memory impairments, also due to its antioxidant properties.

The effects of the alcohol extract of BM on olfaction were studied in olfactory deficits in olfactory bulbectomized (OBX) mice and the molecular mechanisms underlying this effect (Le et al., 2013). OBX mice were given BM (50 mg/kg, *p.o.*) or the reference drug tacrine (2.5 mg/kg, *i.p.*, daily) one week before OBX and continuously 3 days after OBX. The cognitive performance of the animals was analyzed using a modified Y-maze, fear conditioning, and novel object recognition (NOR) tests. Neurochemical and immunohistochemical analyses were carried out in the brain tissues obtained from OBX animals. BM administration ameliorated

memory impairments and reversed the adverse neurochemical and histological changes induced by OBX [except for reduced GluR1 phosphorylation and enhanced cAMP response element binding protein (CREB) phosphorylation]. BM also inhibited the AChE activity in the brains of the mice. These results suggested that BM treatment ameliorated OBX-induced cognitive dysfunction through the improvement of synaptic plasticity-related signaling, protection of brain-derived neurotrophic factor (BDNF) transcription and cholinergic systems from OBX-induced neuronal damage.

Reduced amounts of gamma-aminobutyric acid (GABA)-ergic neurons in the brains of both schizophrenia patients and *in vivo* experimental models suggest that impaired GABAergic function is involved in the pathophysiology of the disease. Decreased GABAergic neurotransmission may also be involved in the cognitive impairment that develops in schizophrenia. The cognitive enhancement and neuroprotective effects of BM on NOR memory and GABAergic neuronal mass, as outlined by the presence of calcium-binding proteins including CBPs, *e.g.* calbindin (CB), parvalbumin (PV), and calretinin (CR), were studied in a sub-chronic (2 mg/kg, *i.p.*) phencyclidine (PCP) mouse model of schizophrenia (Piyabhan et al., 2019). In the novel object recognition task, a discrimination rate (DR) representing cognitive ability was obtained. CB, PV, and CR immune density was measured in the prefrontal cortex, striatum, and cornu ammonis areas. DR decreased in the PCP group, which co-occurred with CB, PV, and CR at decreased levels in the brain except CA1-3 in the CR and cognitive enhancement effect experiment in the striatum. PCP + BM indicated a greater DR score with intensified CB in the prefrontal cortex and striatum, increased PV in the prefrontal cortex and CA1-3, and increased CR levels in the prefrontal cortex. This study demonstrated both partial refurbishment of cognitive deficit and neuroprotective activity of BM. In another similar study (Mishra et al., 2018), brahmi vati (BV) containing BM was prepared in strict adherence to the traditional Ayurvedic formula. A bacoside A-rich fraction of BM (BA) was obtained by extraction and fractionation and administered at a dose of 32.5 mg/kg (b.w.) to different animal groups for 7 days. BV showed significant anticonvulsant, memory enhancing, and anti-schizophrenic activity and caused higher brain glutathione levels and much lower AChE activity compared to control groups and BA.

In a study on whether dietary intake of BM leaf powder tends to modulate endogenous markers of oxidative stress, protein oxidation, redox status [low glutathione (GSH), thiol status], the response of antioxidant defenses (enzymatic), and cholinergic function in various brain regions of prepubertal (PP) mice, it was determined whether a BM-enriched diet (4. 5 and 1%) for 4 weeks revealed a significant reduction in key oxidative markers [malondialdehyde (MDA) levels, reactive species production, hydroperoxide (HP) levels, and protein carbonyls] in both the strophasm and mitochondria of all brain regions of PP mice (Shinomol & Muralidhara, 2011). This was accompanied by increased levels of reduced GSH, thiols, and improved actions of antioxidant enzymes [catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD)]. The significant decrease in the activity of AChE in all brain regions revealed the potential of BM leaf powder to modulate cholinergic function. Based on these results, it was reported that dietary intake of BM leaf powder provides a neuroprotective advantage, and

BM is effective as a prophylactic/therapeutic agent for neurodegenerative disorders involving oxidative stress.

The exact molecular mechanism of the role of CDRI 08, a standardized BM extract, in the improvement of diabetes mellitus (DM)-induced memory impairments is unknown. In a study by Pandey et al. (2015), low doses of CDRI 08 (50- or 100 mg/kg, b.w.) can significantly improve spatial memory in streptozocin (STZ)-DM2 mice. Thus, it was reported to be linked to upregulation of *α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (*AMPA) receptor GluR2 subunit gene expression along with a noteworthy decline in oxidative stress and in the hippocampus. In addition to the effect of reversing DM2-induced high oxidative stress and decreased GluR2 subunit expression and improving spatial memory impairment, treatment of DM2 mice with a higher dose of CDRI 08 (150 mg/kg, b.w. or above) indicated antidiabetic effect. The results provide evidence for the molecular basis of the memory-enhancing and antidiabetic role of BM extract in STZ-induced DM2 mice.

In another study on the ability of BME to improve memory *via* improved cell proliferation and neuroblast differentiation in the dentate gyrus, 7-week-old mice were administered BME once daily for 4 weeks and a NOR memory test was implemented (Kwon et al., 2018). The mice were then euthanized, followed by immunohistochemistry analysis for Ki67, double cortin (DCX) and phosphorylated CREB as well as BDNF western blot analysis. BME-treated mice exhibited moderate increases in the exploration of novel objects compared to familiar objects. Ki67 and DCX immunohistochemistry indicated that BME administration to the dentate gyrus induced cell proliferation and neuroblast differentiation. In addition, administration of BME increased BDNF protein expression and CREB phosphorylation in the hippocampal dentate gyrus. These data suggest that BME enhances NOR by rising cell proliferation and neuroblast differentiation in the dentate gyrus, which may be closely related to high levels of BDNF and CREB phosphorylation in the dentate gyrus.

In a study to determine the effect of alcohol extract of BM (BME) on cognitive function and neurodegeneration in an animal model of ethylcholine aziridinium ion (AF64A)-induced AD, male Wistar rats were given BME orally at doses of 20, 40 and 80 mg/kg (b.w.) (Uabundit et al., 2010). The effect of BME on the spatial memory of rats was tested using the MVM test and the density of neurons. Cholinergic neurons were identified using histological techniques 7 days after AF64A administration. In the MWM test, the extract led to a rise in the escape latency time ($p < 0.01$). In addition, cholinergic neuron densities were also decreased. These findings suggest that BM has a cognitive-enhancing potential and neuroprotective effect against AD.

To further clarify the pharmacological properties and usefulness of BME, which is considered a new herbal anti-dementia agent, whether BME affects neuronal repair was investigated using a trimethyltin (TMT)-induced neuronal loss/self-repair mouse model in the hippocampus (Pham et al., 2019). Mice pretreated with TMT (2.8 mg/kg, *i.p.*) on day 0 were administered BME (50 mg/kg, *p.o.*) once daily for 15–30 days. Cognitive performance of animals, 17-20th days (Phase I) and 32-35th illuminated twice with the object location test and the modified Y maze test on

days (Phase II) or with the passive avoidance test in Phase II. TMT lessened hippocampus-dependent spatial working memory and amygdala-dependent fear-induced memory. BME administration significantly prevented TMT-induced cognitive deficits. The protective effect of BME on spatial memory deficits was confirmed by Nissl staining of hippocampal tissues and organotypic hippocampal slice cultures stained by propidium iodide propidium iodide. Immunohistochemical analyses performed on 17^{th} and 32^{nd} days disclosed that 30 days of BME administration augmented 5-bromo-2'-deoxyuridine (BrdU)-immunopositive cell quantity in the dentate gyrus part of TMT-treated mice. However, 15 days of application with BME did not cause any effect. The obtained outcomes proposed that BME ameliorated TMT-induced cognitive dysfunction mainly by protecting hippocampal neurons from TMT-induced hippocampal lesions and partly by inducing neuroregeneration in dentate gyrus regions.

Saini et al. (2012) evaluated the neuroprotective capacity of BM, which is a medicinal plant in ayurvedic medicine efficient for cognitive impairment in dementia induced with colchicine. Intracerebroventricular administration of colchicine (15 μg/5 μL) caused cognitive impairment in rats as assessed by EPM. This was accompanied by enhanced lipid peroxidation (LPO) and a significant increase in oxidative stress in terms of protein carbonyl levels. However, a diminution in the activity of antioxidant enzymes was detected in animals administered colchicine. BM (50 mg/kg, b.w.) supplementation reversed the memory impairment observed in rats treated with colchicine. BM weakened oxidative damage, as evidenced by reduced LPO and protein carbonyl levels and the restoration of antioxidant enzyme activities. The activity of membrane-bound enzymes (Na^+K^+ ATPase and AChE) was changed in brain regions administered colchicine, and BM administration repaired the activity of the enzymes, which were comparable to the values in control. The results demonstrate the therapeutic potential of BM in treating cognitive decline associated with AD.

3. Conclusion

The studies suggest that BM may enhance cognitive function and protect against neurodegenerative diseases by reducing oxidative stress, inflammation, and Aβ accumulation in the brain. As can be seen from our literature summary, the molecular basis of the neuroprotective effectiveness of bacosides is ascribed to surface expression of neuroreceptors such as AMPA, NMDA, and GABA in various parts of the brain and the regulation of mRNA translation. The general mechanisms of action that provide the neuroprotective activity of the plant are also reported as antioxidant, AChE inhibition, choline acetyltransferase activation, Aβ reduction, cerebral blood flow increase, monoamine potentiation, and modulation. Additionally, it may promote neuronal growth and synaptic plasticity, contributing to improved memory and learning abilities. In order to clinically detect these effects of the plant and to confirm the pre-clinical data, more clinical studies should be conducted by comparing the effects with reference drugs. These studies should be standardized, and the accepted neuropsychological tests should be applied. Besides, more research is needed to fully understand the mechanisms and potential benefits of BM for its neuroprotective nootropic effects on AD.

Ethical Statement

Since this is a review, ethical approval was not obtained.

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This study did not receive any financial support.

Conflicts of Interest

The authors declare that there is no conflict of interest between them.

Author Contributions

Both of the authors contributed to the literature search, data collection, writing and editing the text.

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