Insights into the Molecular Aspects of Neuroprotective Bacoside A and Bacopaside I

Vini C. Sekhar#, Gayathri Viswanathan# and Sabulal Baby#+*

Phytochemistry and Phytopharmacology Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Pacha-Palode 695562, Thiruvananthapuram, Kerala, India

Abstract: Bacopa monnieri, commonly known as Brahmi, has been extensively used as a neuromedicine for various disorders such as anxiety, depression and memory loss. Chemical characterization studies revealed the major active constituents of the herb as the triterpenoid saponins, bacosides. Bacoside A, the vital neuroprotective constituent, is composed of four constituents viz., bacopasides A3, bacopasides II, jujobogenin isomer of bacopasaponin C (bacopaside X) and bacopasaponin C. *B. monnieri* extracts as well as bacosides successfully establish a healthy antioxidant environment in various tissues especially in the liver and brain. Free radical scavenging, suppression of lipid peroxidation and activation of antioxidant enzymes by bacosides help to attain a physiological state of minimized oxidative stress. The molecular basis of neuroprotective activity of bacosides is attributed to the regulation of mRNA translation and surface expression of neuroreceptors such as AMPAR, NMDAR and GABAR in the various parts of the brain. Bioavailability as well as binding of neuroprotective agents (such as bacosides) to these receptors is controlled by the Blood Brain Barrier (BBB). However, nano conversion of these drug candidates easily resolves the BBB restriction and carries a promising role in future therapies. This review summarizes the neuroprotective functions of *B. monnieri* extracts as well as its active compounds (bacopaside A, bacopaside I) and the molecular mechanisms responsible for these pharmacological activities.

Keywords: *Bacopa monnieri*, bacoside A, bacopasides, neuroprotection, antioxidants, nanoparticles.

1. INTRODUCTION

*Bacopa monnieri* is a nootropic herb distributed throughout the warm wetlands of the world. *B. monnieri* has various medicinal properties, and these medicinal aspects are discussed in several reviews. This article is envisaged in the context of neuroprotection by this herb and its major active constituents. Ancient Vedic scholars frequently used *B. monnieri* to memorize lengthy sacred hymns and scriptures. In India, in Ayurvedic prescriptions, *B. monnieri* has been consumed as ‘medhyarasaya’ (in Sanskrit, ‘medhya’ - intellect or cognition, ‘rasayana’ - rejuvenation). Many Ayurvedic preparations prescribed for cognitive dysfunction contain *B. monnieri* as a prime constituent. In Charaka Samhita (6th century AD), *B. monnieri* is mentioned as a medicine for the management of mental dysfunctions such as anxiety, poor cognition and lack of concentration [1]. Due to its ability to nourish neurons, *B. monnieri* is traditionally used as a neural tonic and memory enhancer. *B. monnieri* is also known to help attenuating dementia or decline in mental ability [2].

2. CHEMISTRY

*B. monnieri* produces various metabolites such as saponins, alkaloids and sterols [3]. The main active constituents of *B. monnieri* are dammarane-type triterpenoid saponins known as bacosides with jujobogenin or pseudojujobogenin as their aglycone units. Bacosides are known for their nootropic and various other biological activities [4-9]. Another recently identified group of saponins from *B. monnieri* are bacopasides [10-14]. Bacopaside A is the most studied triterpenoid saponin from *B. monnieri*. Deepak and co-workers reported it as a mixture of four saponins: bacopasides A3, bacopasides I, jujobogenin isomer of bacopasaponin C (bacopaside X) and bacopasaponin C [15] (Table 1, Fig. 1). Rastogi et al. reported the major bacosides in *B. monnieri* as bacopasides I, bacopasides I, bacopasides A3, bacopasides II, bacopasaponin C isomer and bacopasaponin C, of which the last four saponins constituted bacopaside A [16]. *B. monnieri* accessions, with elite contents of bacoside A and bacopaside I, were recently reported from the southern Western Ghats in India [17].

* Address correspondence to this author at the Phytochemistry and Phytopharmacology Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Pacha-Palode 695562, Thiruvananthapuram, Kerala, India; Tel: +91 472 2869626, 2869628; Mob: +91 9495832391; Fax: +91 472 2869646; E-mails: sabulal@jnbgri.res.in; sabulal@gmail.com

* All three authors contributed equally to this work.
3. NEUROPROTECTION

In vitro and animal model studies revealed the promising role of B. monnieri in the treatment of epilepsy, anxiety and other neurodegenerative disorders. Oxidative stress is the state where free radicals cause an imbalance in the homeostatic defense mechanisms of the cell [23]. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and catalase (CAT) are the free radical-quenching enzymes present in our body. Antioxidant compounds including vitamins A, C, E and phenols also play crucial protective roles [24, 25]. Oxidative stress leads to many diseases, even aging, by degrading lipids, peroxidizing lipids, blocking metabolic pathways, destabilizing DNA strands and denaturing proteins [26, 27]. Metabolically active brain which possesses high levels of pro-oxidant iron and unsaturated lipids is more prone to oxidative stress and lipid peroxidation [28]. Furthermore, due to the BBB, many exogenous antioxidants are not capable of quenching reactive oxygen species in the brain [29].

Saini et al. assessed the activity of B. monnieri against colchicine-induced oxidative stress and found that B. monnieri treatment diminished lipid peroxidation and protein carbonyl levels. Colchicine-induced changes in the activities of acetylcholine esterase (AChE), NaK*ATPase, SOD, CAT, GPx, GR and GST were all restored to significant levels compared to controls [30]. The administration of B. monnieri ethanolic extract on 6-OHDA-induced lesions in rats showed that both neurobehavioral deficits and enzyme levels were dose-dependently restored by B. monnieri [31]. Singh et al. examined the effect of B. monnieri on paraquat and 1-methyl-4-phenyl-pyridinium iodide-induced toxicities in a dopaminergic SK-N-SH cell line. Normal levels of glutathione, mitochondrial membrane potential and mitochondrial complex I activity were maintained upon B. monnieri treatment. The administration of B. monnieri activated the nuclear factor (erythroid-derived) 2 (Nrf2) pathway by regulating the expression of Keap1 and enhancing glutathione synthesis [32].

Shinomol and Bharath investigated the neuroprotective role of the alcohol extract of B. monnieri against 3-nitropropionic acid (3-NPA)-induced oxidative stress in N27 cells and pre-pubertal male mice. B. monnieri diminished 3-NPA-induced oxidative stress in isolated striatal mitochondria by depleting the levels of malondialdehyde, hydroperoxide, protein carbonyls and reactive oxygen species (ROS). These results recommend B. monnieri as an adjuvant in oxidation-mediated neurodegenerative ailments [33]. Sumathi et al. demonstrated methyl mercury (MeHg) induced activation of GR and inhibition of the activities of SOD, CAT and GPx in the cerebellum of rat brain. These alterations were prevented by the administration of B. monnieri extract [34]. Pretreatment with B. monnieri methanolic extract (mBME) reduced the increased serum level of ALT, AST and creatinine in rats, and provided protection against opioid-induced hepatotoxicity and nephrotoxicity [5]. In another recent study, the neuroprotective role of B. monnieri against decabrominated diphenyl ether (PBDE-209)-induced antioxidant alterations in neonate and young female mice were revealed. B. monnieri significantly preserved the levels of antioxidants and enhanced the activities of antioxidant enzymes in frontal cortex and hippocampus of the brain [35].

Creatine kinase (CK) has three isoforms (CK-MM, MB, BB) which are considered to be sensitive markers in the diagnosis of cardiac and cerebral damage. Anbarasi et al. (2005a) studied the effects of cigarette smoke exposure in rats. The results showed reduction in the CK activity in the heart and brain tissues but marked increase in serum CK activity. This is attributed to free radical-mediated lipid peroxidation, neuronal and cardiac cell membrane damage and leakage of CK into the blood. Bacoside A is known to prevent lipid peroxidation and maintain the structural integrity of cell membranes. It blocked the leakage of CK into the blood and reversed the enzyme level variations in the tissues and blood [36]. The same research group also found that bacoside A treatment inhibited the mitochondrial dysfunction in rat brain induced by cigarette smoke exposure. The mitochondria membrane-stabilizing properties of bacoside A protect the brain, which is evident from the depleted levels of lipid peroxides, cholesterol, and cholesterol/phospholipid (C/P) ratio, and increased levels of mitochondrial enzymes [37]. Further, experimental data
revealed the presence of higher levels of serum lactate dehydrogenase (LDH) with a concomitant decrease in the tissue enzyme level in the respective organs upon cigarette smoke exposure. The administration of bacoside A stabilized the cell membranes through its anti-lipid peroxidative effect and these alterations were prevented [38]. In another study, Anbarasi et al. (2005b) evaluated the neuroprotective role of bacoside A against oxidative stress in the brains of rats exposed to cigarette smoke, and found that bacoside A significantly enhanced brain levels of vitamins A, C, E and glutathione. Bacoside A administration inhibited lipid peroxidation and increased the activities of antioxidant enzymes. It also improved the activity of thiol-dependent enzymes like adenosine triphosphatases (ATPases), by protecting the -SH groups in their active sites from free radical-mediated inactivation. Thus bacoside A maintained ionic equilibrium in the brains of cigarette smoke-exposed rats [39].

Bacoside-rich extract from *B. monnieri* showed antifatigue effect in rats having impairments associated with physical fatigue. Marked reduction in the malondialdehyde (MDA) levels, a significant increase of SOD, CAT and HSP-70 expression in brain, liver and muscle tissues compared with the control group confirmed the antifatigue property of *B. monnieri* [8]. Rastogi et al. revealed that purified...
Bacopside I exhibited neuroprotective role by reducing neurological deficits, cerebral infarct volume and edema against injury caused by cerebral ischemia in rats. Oral treatment with bacopside I markedly increased brain ATP content, antioxidant enzymes, Na\(^+\)K\(^+\)ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)ATPase activities. At the same time, bacopside I decreased MDA content of the brain [41].

4. MOLECULAR EXPRESSION OF RECEPTORS AND REGULATORS

Neurotransmitters are the chemical messengers that transmit signals from one neuron to another and regulate brain functions through intracellular signaling pathways. Some of the important neurotransmitters are dopamine, noradrenaline, serotonin, neuropeptides, adrenaline, glucocorticoids and acetylcholine [42]. During synaptic signal transmission, neurotransmitters released from the presynaptic neuronal end of one neuron bind to receptors at the postsynaptic membranes of another neuron and produce variations in membrane potential or initiate signaling cascades. Synapses in the brain are of two types: excitatory and inhibitory synapses. In excitatory synapses, neurotransmitters cause depolarization of postsynaptic membranes and glutamate functions as a major neurotransmitter. In contrast, GABA (gamma-aminobutyric acid) and glycine act as major inhibitory neurotransmitters in inhibitory synapses. Glutamate receptors are classified into two - ionotropic or metabotropic glutamate receptors. This classification is based on whether the neurotransmitter binding site and the ion channel are components of the same protein or components of different proteins. Based on the sensitivity to pharmacological agents, ionotropic glutamate receptors are further classified as AMPA-, NMDA-, and kainate-sensitive glutamate receptors. NMDA- and kainate-type receptors are involved in the synaptic plasticity or slower transmission to cause nerve membrane depolarization after glutamate binding. The fast synaptic excitation occurring in the entire brain parts associated with neuronal disorders is controlled primarily by the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazololepropionic acid receptors (AMPA\(^\text{Rs}\)). Therefore, this activity is determined by the number of AMPARs at synapses. Interestingly, it is proven that AMPAR antagonists can block epileptiform activity by inhibiting glutamate-mediated excitation in animal models and in vitro preparations. AMPARs are heterotetrameric protein receptors having four subunits designated either as GluA1–GluA4 or GluR1–GluR4 or GluRA-GluRD. Among them, the GluR2 subunit plays a vital role in synaptic plasticity [43, 44]. The reduction in the AMPAR expression could lead to receptor dysfunction and motor learning deficit in epileptic rats [45]. Since AMPARs mediate the majority of excitatory synaptic neurotransmissions, inhibitors of this class of ionotropic glutamate receptors help to prevent seizures in animal epilepsy models [46, 47].

A fraction of \(B.\) monnieri extract (CDRI-08) significantly improved spatial memory with a significant decline in oxidative stress and up-regulation of the AMPAR GluR2 subunit gene expression in the hippocampus of streptozotocin-induced diabetes mellitus type 2 mice [48]. Fmr-1 gene encoded protein, (Fragile X mental retardation protein, FMRP), is a neuronal translational repressor and has been associated with learning, memory and cognition. CDRI-08 administration reversed the memory loss, and this was correlated with significant downregulation of Hif-1α and upregulation of Fmr-1 expression [49]. In another study, the neuroprotective role of \(B.\) monnieri methanol extract was investigated in the hippocampus of the temporal lobe of epileptic rats. This study revealed that \(B.\) monnieri methanol extract treatment potentiates the therapeutic effect by reversing the alterations in glutamate receptor binding and N-methyl-D-aspartate receptor1 (NMDA R1) gene expression which occurs during epilepsy, thus resulting in reduced glutamate-mediated excitotoxicity in the over-stimulated hippocampal neurons [50]. 5-HT2C receptors have been involved in stress, whose alterations were reversed by treatment with \(B.\) monnieri in pilocarpine-induced temporal lobe epileptic rats. Similarly, NMDAR functions and IP3 (inositol 1,4,5-trisphosphate) content were modulated with \(B.\) monnieri treatment, thereby efficiently balancing the neurotransmitter level in the cerebral cortex [51].

The neuroprotective role of \(B.\) monnieri in the prevention of cognitive deficit in schizophrenia in sub-chronic phencyclidine (PCP) rat model has also been studied. PCP administration resulted in the cognitive deficit in rats due to up-regulation of NMDAR1 in CA2/3 and DG. Interestingly, on treatment with \(B.\) monnieri prior to PCP administration, NMDAR1 expression was reduced in these brain areas [52]. Reduced expression of vesicular glutamate transporter 1 (VGLUT1), 2 (VGLUT2) and 3 (VGLUT3) is an indication of glutamatergic hypofunction leading to cognitive impairment in schizophrenia. The administration of PCP resulted in low levels of cerebral VGLUT3 accompanied by the cognitive deficit in rats. A standardized extract of \(B.\) monnieri containing 20% of bacosides A+B prevented cognitive impairment by increasing VGLUT3 in the prefrontal cortex, striatum and CA1-3 [53]. BME (\(B.\) monnieri extract) exhibited neuroprotective properties by mediating hippocampal neurogenesis in rats with an increase in the brain-derived neurotrophic factor (BDNF) level and antioxidant parameters against oxidative stress [54]. Daily administration of BME significantly controls the mechanisms underlying antidepressant-like action by maintaining the normal levels of BDNF, total and phospho-Akt, total and phospho-CREB in the hippocampus and reverses the behavioural changes [55].
The treatment with *B. monnieri* extract prevented neurochemical alterations related to thioacetamide (TAA) induced hepatic encephalopathy in rats by modulating the expression of two NMDAR subunits - NR2A and NR2B, and their downstream mediators and also normalized the expression of nNOS-apoptotic factors in the cerebellum of rats [56]. The exposure of a flame retardant, PBDE-209, enhanced the expression of NR1 and diminished the binding of REST/NRSF to NR1 promoter in mice. Interestingly, *B. monnieri* extract modified the glutamatergic system through regulating the expression of NR1 and binding of REST/NRSF to NR1 promoter. Hence it restored the impaired memory caused by PBDE-209 in mice [57].

Scopolamine exposure resulted in a remarkable downregulation of the NMDAR GluN2B subunit expression in prefrontal cortex and hippocampus in mice. The oral administration of *B. monnieri* extract to scopolamine-treated amnesic mice restored the spatial memory, by the upregulation of GluN2B subunit expression and reduction in the acetylcholinesterase activity in prefrontal cortex as well as hippocampus [58].

Olfactory bulbectomy (OBX) altered the non-spatial short-term memory, spatial working memory and long-term fair memory in mice. OBX decreased the phosphorylation of synaptic plasticity-related signaling proteins: glutamate receptor 1 (GluR1), NR1 subunit of NMDAR, calmodulin-dependent kinase II and reduced the BDNF mRNA in the hippocampus. *B. monnieri* treatment eliminated OBX-induced cognition dysfunction through upregulation of signaling associated with synaptic plasticity and BDNF transcription thereby preventing neuronal damage in cholinergic systems [59]. Okadaic acid (OKA) resulted in severe memory deficit, oxidative stress, neuroinflammation and neuronal damage accompanied by reduced expression of Nrf2, HO1 and GCLC in rats. Treatment with *B. monnieri* and melatonin significantly improved memory dysfunction in OKA rats. The supplementation also restored the expression levels of Nrf2, HO1 and GCLC, which led to reduction in oxidative stress, neuroinflammation and neuronal loss. This protective effect of *B. monnieri* and melatonin in OKA induced memory impairment is mediated through Nrf2 pathway modulation [60].

P-glycoprotein (Pgp) is a major drug efflux transporter which limits drug permeability across the BBB into the central nervous system. Pregnan X Receptor (PXR) is a transcriptional regulator of Pgp and helps to restore Pgp at the BBB. The drug-mediated PXR activation induces upregulation of Pgp and potentially increases drug penetration through BBB [61]. *In silico* docking studies showed considerable interaction between pregnane X receptor (PXR) and bacopaside I [62]. Bacopaside II blocked the rhodamine 123 (Rh123) passage across a LLC-GA5-COL150 cell monolayer thereby reduced P-gp efflux ratio of Rh123 compared to control. This study helps to understand herb-drug interactions of bacopaside II upon P-gp substrate drug treatment [63].

Gamma amino butyric acid (GABA) is the primary inhibitory neurotransmitter in the cerebral cortex, which balances neuronal excitation by maintaining the inhibitory excitaton. When this balance is disturbed, seizures may ensue. *B. monnieri* and bacoside A treatments reverse epilepsy associated changes to near control, suggesting that reduced GABA receptors in the cerebral cortex contribute to the epileptic occurrence [64]. *B. monnieri* extract and bacoside A administration rectified the dopaminergic and cAMP imbalance in hypoglycaemic neonatal rats. *B. monnieri* extract treatment re-established the altered gene expression parameters of Bax and SOD. Dopamine D1 and D2 receptor subtype expressions were improved whereas cAMP signaling and cell death resulting from oxidative stress were attenuated [65].

Bacopaside I showed antidepressant-like activity. Exposure to chronic unpredictable mild stress (CUMS) increased the level of plasma corticosterone and glucocorticoid receptor expression in mice. Bacopaside I treatment reversed the condition by modifying the hypothalamic-pituitary-adrenal (HPA) axis hyperactivity of CUMS-exposed mice [66]. Aquaporin (AQP) family of water channels which facilitate water flux and CSF movement across the plasma membrane could function as an important target in the treatment of neuropathologies like cerebral edema. Based on the screening conducted in the Xenopus oocyte expression system, it was proved that bacopaside I and bacopaside II can act as selective modulators of Aquaporin-1 (AQP1) channels. Bacopaside I blocked both the water and ion permeability, but bacopaside II selectively inhibited the AQP1 water permeability without impairing the ionic conductance [67]. Bacopaside I attenuated oxygen- and glucose-deprivation (OGD) induced necrosis, apoptosis and neuronal cell damage in an *in vitro* model of ischemia. The neuroprotective activity of bacopaside I was blocked by the inhibitors of PKC and PI3K, but not by the ERK inhibitor. Bacopaside I restored the level of phospho-Akt (p-Akt), an anti-apoptotic factor. Thus, bacopaside I, via PKC and PI3K/Akt pathways, played a neuroprotective role [68].

Monoamine oxidase inhibitors are potent anti-depressant drugs which prevent oxidative deamination of monoamine type neurotransmitters. Singh *et al.* evaluated the effect of major constituents (bacaposide I, bacopaside II, bacosate A3, bacopasaponin C, bacosine and bacosate A mixture) of *B. monnieri* on recombinant human monoamine oxidase (MAO) enzymes. Bacoside A and bacopaside I mixture showed inhibition towards MAO-A and MAO-B enzymes. Bacopaside I selectively inhibited the MAO-A enzyme [69].

5. NANOPHYTOMEDICINE

Despite promising medical properties, biomolecules suffered from low water solubility, which in turn resulted in their poor bioavailability and less clinical efficacy. Hence, researchers attempted to enhance water solubility and bioavailability of bioactive molecules by loading them in biodegradable polymeric nanoparticles. Other benefits of nanoparticle-drug formulations include minimum patient expenses and low risk of toxicity. Nanocapsulation improves specificity, efficacy, tolerability and therapeutic potential of drugs [70, 71]. Bioavailability and controlled delivery of drugs related to various diseases were improved successfully by nanocapsulation [72]. Some of the
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CONCLUSION

Extensive scientific research on the neuropharmacological potential of B. monnieri extracts as well as its major triterpenoid saponin constituents is discussed in this mini review. Bacoside A and bacopaside I exhibited neuroprotective role by reducing oxidative stress, inducing expression of antioxidant enzymes and regulating surface expression of various neuroreceptors. This review also provides valuable information on the scope and safe use of B. monnieri secondary metabolite nanoparticles as excellent drugs against neurodegenerative disorders.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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