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Neuropharmacological and cognitive effects of *Bacopa monnieri* (L.) Wettst – A review on its mechanistic aspects

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ABSTRACT

Bacopa monnieri (L.) – (BM) is a perennial, creeping herb which is widely used in traditional ayurvedic medicine as a neural tonic to improve intelligence and memory. Research into the biological effects of this plant has burgeoned in recent years, promising its neuroprotective and memory boosting ability among others. In this context, an extensive literature survey allows an insight into the participation of numerous signaling pathways and oxidative mechanism involved in the mitigation of oxidative stress, along with other indirect mechanisms modulated by bioactive molecules of BM to improve the cognitive action by their synergistic potential and cellular multiplicity mechanism. This multi-faceted review describes the novel mechanisms that underlie the unfounded but long flaunted promises of BM and thereby direct a way to harness this acquired knowledge to develop innovative approaches to manipulate its intracellular pathways.

1. Introduction

Bacopa monnieri (L.) – (BM) (Common names: Brahmi, Bacopa, Waterhyssop), renowned as a nootropic plant; belonging to the family Scrophulariaceae, is a well-known ayurvedic medicinal plant used in India, as a neural tonic to improve intelligence and memory, increase brain function and promote longevity.[1–3] Being mainly of complex formulations rather than a single bioactive compound, ayurvedic system of medicine contains several individual components that have a synergic interaction among phytochemicals, that mutually enhance their efficacy and allows them to act on multiple targets to control complex molecular recognition sites.[4] Encouragingly, recently there is a growing support for the 'black-box' approach – a whole-system approach considering the entire compounds rather than individual active compounds, for the reason that they can contribute to the synergistic action of various molecules to achieve a physiological or pharmacological effect.[5]

Of the most noticeable therapeutic influences of BM, researchers generally pointed at the neuroprotection against dementia,[6] amnesia,[7] memory dysfunction,[8,9] Parkinson's disease (PD),[10] Alzheimer's disease (AD),[11] epileptic seizures[12] and schizophrenia.[13] Nevertheless, BM is reported to possess sedative,[14] antimicrobial,[15] anti-inflammatory,[16] calcium antagonistic,[17] anticonvulsant,[18] anti-aging,[19] cognitive enhancive,[20–22] antinociceptive,[23] broncho-vasodilatory,[24] anticancer,[25] hepatoprotective,[26] anti-allergic,[27] antidepressant,[28] anti-hypercholesterolemic effect,[29] anti-emetic[30] and antiulcer[31] activities. Preliminary animal studies of BM whole plant or alcohol extracts have reported cognition-enhancing effects, including a decrease in walking errors,[32] enhanced learning ability, increased memory retrieval and prevented dendritic atrophy following hypoxic exposure in rats.[33]

Beneficial effects of the plant may be attributed to its vast range of closely related saponins, which differs slightly in structure. Some of the earlier work in this regard, reported the effects of BM on cognitive function.[34,35] Hence, its effects on cognitive performance, holds a great promise for the amelioration of age-related cognitive decline as well as cognitive enhancement in the young.[33] A controlled study has been done by Roodenrys et al.[36] with various memory functions and the levels of anxiety measured, demonstrated the significance of BM on the retention of new information. Later, Peth-Nui et al.[37] verified the effect of BM on boosted attention and cognitive processing skill together with improved working memory and cholinergic function.

Several *in vitro* and *in vivo* studies investigating the pharmacological properties of BM have been published and have especially highlighted its neuroprotective ability which is attributed to its antioxidative, anti-apoptotic and anti-inflammatory properties. Herein, not just blue-sky thinking, but to decipher the molecular pathways that are already being used to guide systematic procedures and algorithms for neurochemical/biological properties of BM, suggesting its protection against neurotoxic and memory enhancing ability are revised. Moreover, in this review, we evaluate the ways in which BM contributes to cognitive health beyond

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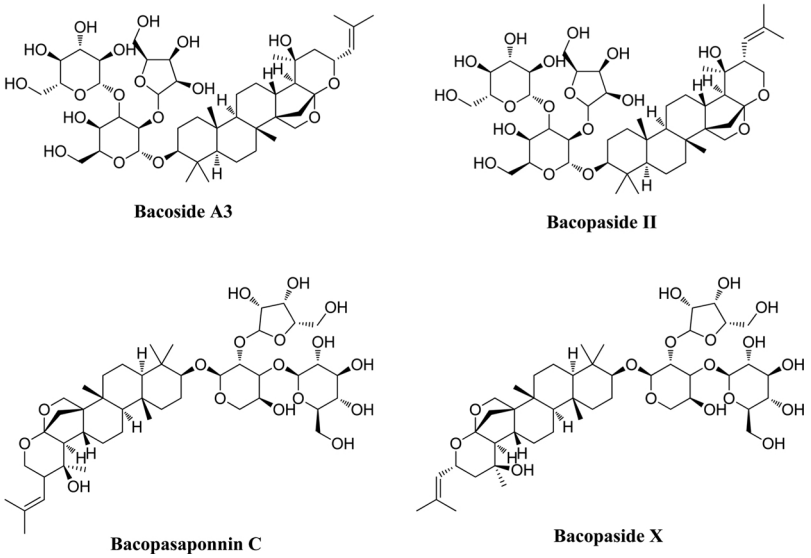

Fig. 1. Chemical structure of Bacoside A.

the known mechanisms of its action and to explore the molecular crosstalk between these mechanisms and other related pathways in cell survival, neuroprotection, and cognitive enhancing capabilities that are critical to its underlying molecular mechanism to formulate therapeutic interventions.

2. Active constituents of BM

The characteristic bioactive constituents of BM responsible for its cognitive effects are saponins called bacosides.[38] The active ingredients specifically bacosides A (Fig. 1) and B (Fig. 2) are believed to underlie the memory-enhancing effects of BM.[33] Bacosides, comprise a family of 12 known analogs, is a dammarane type of triterpenoid saponin with jujubogenin, or pseudo-jujubogenin moieties (glycone units). The most studied constituent bacoside A (3-(a-L-arabinopyranosyl)-O-b-D-glucopyranoside-10,20-dihydroxy-16-keto-dammar-24-ene) is a mixture of four triglycosidic saponins, namely bacoside A3, bacopaside II, bacopasaponin C and the jujubogenin isomer of bacosaponin C (bacopaside X),[39] whereas bacoside B consists of bacopaside N1, bacopaside N2, bacopaside IV, and bacopaside V.[40]

Levorotatory bacoside A, a mixture of compounds co-occurs usually with dextrorotatory bacoside B.[41] Rastogi et al.[42] demonstrated triterpenoid saponin, bacoside A3 (3-b-[O-b-Dglucopyranosyl (1–3)-O-[a-L-arabinofuranosy(1→2)]-Ob-D-glucopyranosyl)oxy] jujubogenin by chemical and spectral analyses. Along with another bacogenin A4, which was identified as ebelin lactone pseudojujubogenin and a minor saponin bacoside A1 was isolated and characterized as 3-O-[a-L-arabinofuranoyl(1→3)-β-L-arabinopyranosyl] jujubogenin.[42] Garay et al.[43] isolated three dammarane-type triterpenoid saponins: bacopasaponins A, B and C, which was identified as 3-O-a-L-arabinopyranosyl-20-O-a-L-arabinopyranosyl-jujubogenin, 3-O-[a-L-arabinofuranosyl (I→2) a-L-arabinopyranosyl]pseudojujubogenin and 3-O-[β-D-glucopyranosyl(1→3){a-L-arabinofuranosyl(1→2)}a-Larabinopyranosyl]. Along with a dammarane-type pseudojujubogenin glycoside, bacopasaponin D was also isolated and defined as 3-O-[α-Larabinofuranosyl (I→2) β-D-glucopiranosyl] pseudojujubogenin. Four cucurbitacins, bacobitacin A, B, C and D and a known cytotoxic, cucurbitacin E, together with three known phenylethanoid glycosides *viz.* monnieraside I, III and plantioside B were isolated from aerial parts of BM.[44] A triterpene bacosine (3alpha)-3-Hydroxylup-20(29)-en-27-oic acid), was also isolated and identified from aerial parts of BM.[45,46]

Recently, novel saponins called bacopasides I–XII have been identified by Le et al.,[39] in a study to clarify the anti-dementia effects of BM. Other medicinally important chemical entities include alkaloids (brahmine and herpestine), saponins (D-mannitol and hersaponin), monnierin, betulic acid, stigmastarol, beta-sitosterol, wogonin,

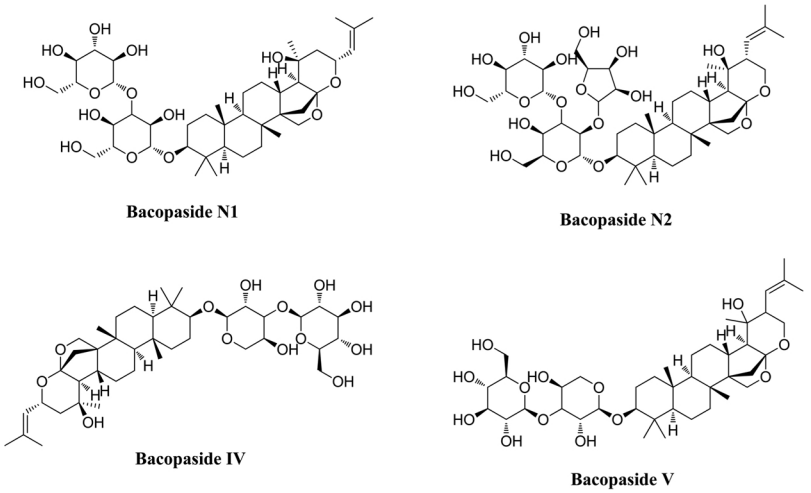

Fig. 2. Chemical structure of Bacoside B.

Table 1
Major active constituents of BM.

Compound	IUPAC name	Biological activity	Molecular formula	References
Bacoside A3	3-β-[(O-β-D-glucopyranosyl[(1→3)-O-(α-L-arabinofuranosyl[(1→2)]-O-β-D-glucopyranosyl) oxy]jujubogenin	Anti-depression activity, Hepatoprotective	$C_{47}H_{76}O_{18}$	26,28,47
Bacopaside II	3-O-[α-L-arabinofuranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl] pseudojujubogenin	Anti-depression activity, Hepatoprotective	$C_{47}H_{76}O_{18}$	26,28,47
Bascopasaponin C	3-O-[β-D-glucopyranosyl-(1→3)-[α-L-arabinofuranosyl-(1→2)]-α-L-arabinopyranosyl] pseudojujubogenin	Anti-depression activity, Hepatoprotective	$C_{46}H_{74}O_{17}$	26,28,47
Bacopaside X	3-O-[β-D-glucopyranosyl-(1→3)-[α-L-arabinofuranosyl-(1→2)]-α-L-arabinopyranosyl] jujubogenin	Hepatoprotective	$C_{46}H_{74}O_{17}$	26,47
Bacopaside N2	3-O-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl] pseudojujubogenin	Cognitive enhancive, antinociceptive	$C_{42}H_{68}O_{14}$	22,23,47
Bacopaside N1	3-O-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl] jujubogenin	Cognitive enhancive, anticonvulsant	$C_{42}H_{68}O_{14}$	18,20,47
Bacopaside IV	3-O-[α-L-arabinofuranosyl-(1→2)-[6-O-sulfo-β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl] pseudojujubogenin	Cognitive enhancive, anti-aging	$C_{46}H_{74}O_{20}S$	19,22,47
Bacopaside V	3-O-b-D-glucopyranosyl-(1→3)-a-Larabinofuranosyl pseudojujubogenin	Cognitive enhancive, anticancer	$C_{41}H_{66}O_{13}$	25,48

oroxindin as well as numerous bacosides and bacopasaponins.[15,49] Among these bioactive compounds, bacoside A and bacopaside I are used as chemical markers for the quality control of BM products.[41] Major bioactive compounds and their chemistry are summarized in Table 1.

3. Neuroprotection and its mechanism of action

Neuroprotection refers to an effect that may result in salvage, recovery or preservation of neuronal structure and/or function.[50] The preservation of neuronal integrity against a neurodegenerative insult often target oxidative stress and excitotoxicity, which could further lead to neuronal death and aggravate neuronal loss over time (neurodegeneration).[51] Neuroprotective properties of BM on ruining neuronal status in the brain and the subsequent rescue by BM are well documented.[52] The cholinergic neurotoxin AF64A (ethylcholine aziridinium) induced animal model of AD, studied by Uabundita et al.[11] demonstrated that, BM extract could mitigate the memory impairment and the degeneration of neurons in hippocampus. Nevertheless, they reported lack of dose dependent response due to masking effect of the active ingredient with increasing concentration. Further, Shinomol et al.[53] validated the *in vitro* neuroprotection of an alcoholic leaf extract of BM against 3-NP challenge, by ameliorating 3-NP induced elevation of oxidative markers and cytotoxicity. Not to mention, it was also reported that studies on polyherbal formulations like Saraswatarishta, Saraswat Choorna, Brahmi Ghrita *etc.* indicated effective in promoting restorative and neuroprotective action in convulsions. The anti-convulsion activity study of BM thus revealed an effective potential in protecting the epileptic seizure intensity and fostering recovery.[12]

Another study was carried out on anti-ischemia effects of BM with isolated bacosides (bacopaside I, bacopaside N2, and a mixture of bacopaside II and bacosaponin D) exhibited the suppressive effect on ischemia, using induced neuronal cell damage (oxygen and glucose-deprivation), on organotypic hippocampal slice cultures.[39] Again, the neurotoxicant MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced motor skill abnormalities on mouse model studied was studied by Babita et al.[32] And the results reported an improvement in grip strength when treated with BM. Furthermore, their study also revealed a significant decrease in nitrite levels in the nigrostriatal region upon BM treatment. To boot, the newer studies that fit with a bigger heap of evidence showing the neuroprotective and anti-apoptotic activities of BM have reported (Table 2). Post treatment with a methanolic extract of BM on oxidatively stressed Neuro-2a cell lines showed increased cell viability of neuronal cells, through a reduction in fluorescent intensities after microscopic examinations of Neuro-2a cells with fluorescent dyes, which indicated the cytoprotective effect of BM against oxidative stress.[57]

An insight into the mechanisms of action paves the way for a biological readout of specific molecular targets to which the active compounds bind such as an enzyme or receptor. Suggested mechanisms of action include a synergic combination of antioxidant, calcium channel blocking activity,[24] pro-cholinergic effects,[58] GABA-ergic modulation,[38] reduction of β-amyloid,[52] brain protein synthesis,[59] suppresses neuronal oxidative stress,[60] inhibition of acetylcholinesterase,[52] modulation of brain stress hormones,[59] and/or anti-dopaminergic and anti-serotonergic properties.[23] An overview of mechanisms of action of BM in neuroprotection is depicted in Fig. 3.

3.1. Antioxidant neuroprotection

The brain, being a highly metabolic active organ with high metabolic rate is susceptible to oxidative stress.[61] This oxidative stress leads to an imbalance between oxidation/reduction reactions (redox state) within the cells, which causes a progressive loss in cellular functions. Under oxidative stress, as a first line of defense antioxidants come for scavenging the free radicals.[62,63] The antioxidant defense systems

Table 2
Neuroprotective effects of BM through protein interaction.

Neurodegenerative diseases	Major protein(s) involved	Cell line/animal model studied	Mechanism of action	References
Dementia	AChE	Parkin mutant drosophila	Restored membrane bound enzymes	6
Parkinson's disease	α-synuclein	Two *Caenorhabditis elegans* of transgenic model	Through overexpression of Hsp-70	9
Schizophrenia	VGluT1, VGluT2, VGluT3	Rat model of schizophrenia	Transports glutamate into synaptic vesicles	13
Alzheimer's disease	β-amyloid	SH-SY5Y cells	Prevent self-assembly of oligomers	54
Amnesia	AChE	Swiss mice	Decreased AChE activity	55
Epileptic seizures	Glutamate-8 receptor (mGluR8)	Neonatal rats	Impaired declarative memory was restored	56

include both enzymatic (superoxide dismutase, glutathione peroxidase, and catalase) and non-enzymatic defense systems [ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), β-Carotene, and vitamin A]. Various animal studies on the effect of BM as antioxidant inducer of the endogeneous antioxidant system are reported.[19,49,54]

3.1.1. Intracellular protein oxidation

Intracellular protein oxidation has been usually considered as hallmarks of cellular damage due to oxidative stress which further leads to become key players in a broad spectrum of diseases, from neurodegenerative disorders to various kinds of cancer.[65] Covalent modification of a protein induced by reactive oxygen species (ROS) may be caused by oxidation in both amino acid side chains and protein backbones, resulting in protein fragmentation by cytosolic proteases or protein-protein cross-linkages. Among the amino acids, cysteine and methionine are prone to oxidative changes, due to high nucleophilic property of the sulfur group in those amino acids.[66] Since amino acids from cellular proteins are used to make the neurotransmitters, the oxidized amino acids can cause alterations in neurotransmitter. Furthermore, oxidative modifications of proteins can change their chemical and physical properties, including structure, conformation, solubility, enzyme activities, and susceptibility to proteolysis.[65] The BM has been reported to have protection against from oxidative damage through decreased protein carbonyl levels in the cytosol and mitochondrial fractions in all brain regions.[53]

3.1.2. Lipid peroxidation

Lipid peroxidation is a mechanism of cell injury by free radicals or chemicals, that affects cellular membranes, lipoproteins, and other molecules that contain lipids, which often precedes with irreversible cell damage thus being an early cause of cell death.[67,68] Substantial evidence on inhibitory activity of BM on lipid peroxidation in the prefrontal cortex, striatum, and hippocampus were reported in rats.[69] Since, lipid peroxidation has the significant potential to contribute in the pathogenesis of numerous diseases, through free radical initiation, thereby resulting in altered receptor functions, membrane fluidity loss and permeability changes.[3]

Malondialdehyde (MDA), a terminal compound of lipid peroxidation serves as an index of oxidative injury due to oxygen free radicals.[70] In this direction, peroxidative damage caused by oxygen free radicals leads to increase in levels of MDA formation.[71] A significant reduction of MDA levels was observed in the BM treated diabetic rats when compared with untreated diabetic rats, which reveals that the BM has the considerable neuroprotecting ability.[47] In like manner, Muralidhara[72] studied mitigation of rotenone induced oxidative stress and neurotoxicity in Drosophila system, through BM exposure. The endogenous levels of MDA in whole body homogenates of flies, on exposure to BM, are kindred to above reduced MDA levels. In another experiment, the role of Bacopaside I on enhanced MDA levels in animal models studied by Liu et al.[73] concluded a similar effect in MDA levels. In agreement with these reports, a study using dichlorvos-poisoned mice to study the antioxidative protective effect of Bacoside A, which exhibited similar results.[74] Correspondingly, another study on MPTP treated mouse, revealed a significant increase in MDA levels when treated with BM extract.[32]

3.1.3. Superoxide dismutase (SOD) activity

In the presence of free radicals, as a first line of antioxidant defense strategy and as a fast neutralizing antioxidant, SOD activity increases in the biological system. A decreased SOD activity to normal levels was found on BM treated diabetic rats, thus indicating a homeostasis between the oxidant and antioxidant species.[47] Investigations into age-associated decline in sympathetic noradrenergic nerve fibers in the lymphoid organs done by Priyanka et al.[75] reported a significant decline in the SOD activity in old rats after treatment with BM, in comparison to young and early middle-aged rats. More studies supporting this

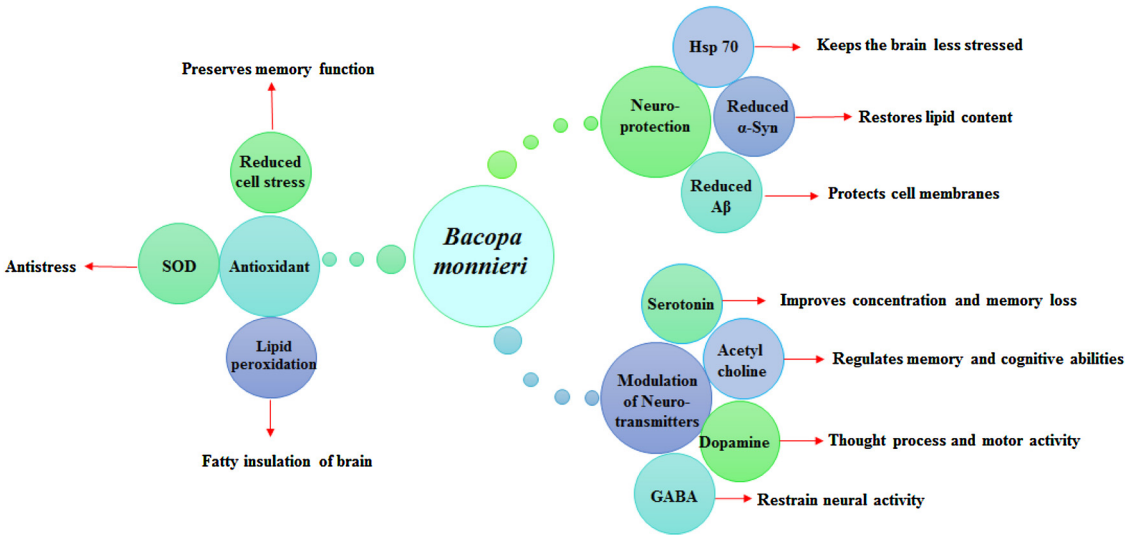

Fig. 3. An overview on the neuroprotective effects on BM.

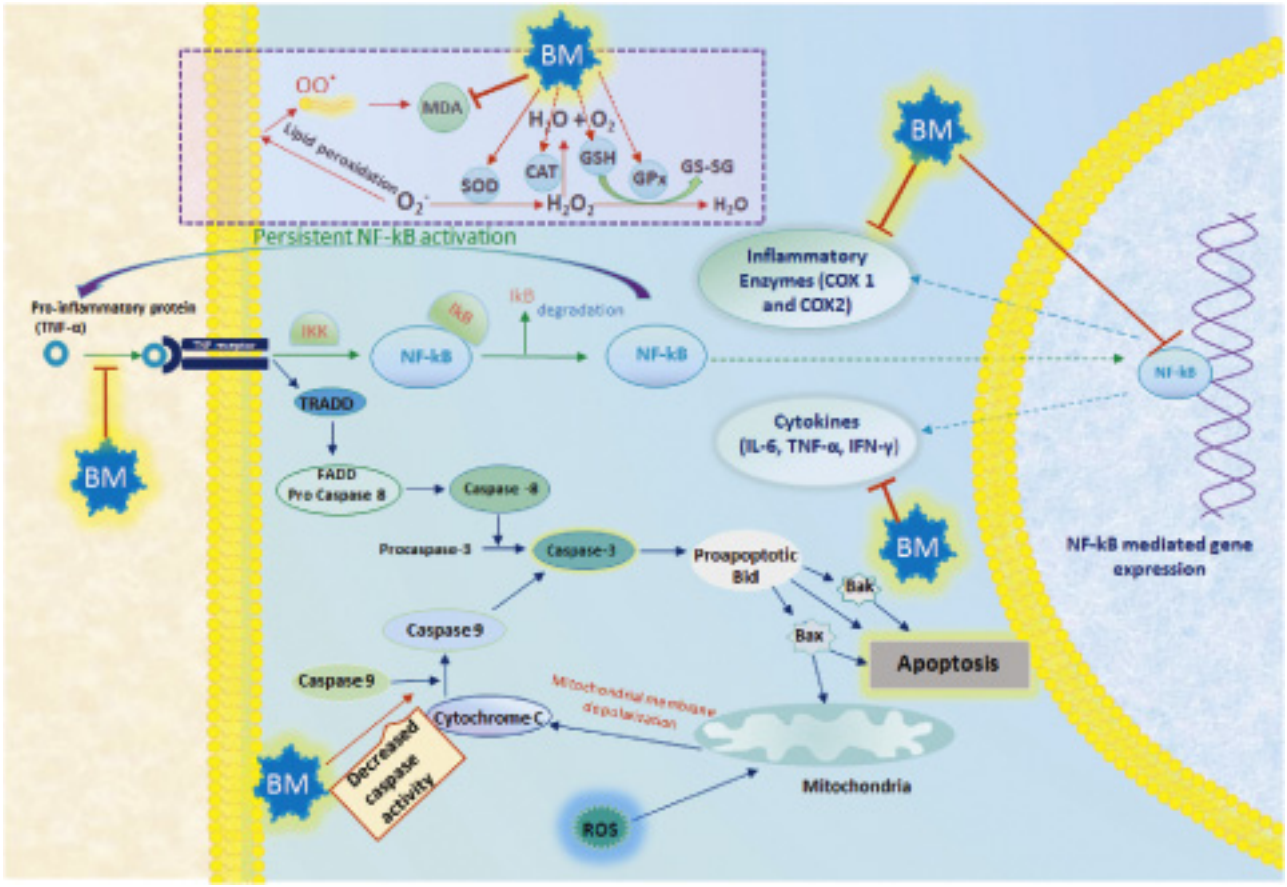

Fig. 4. Illustration of role of BM in antioxidant, anti-inflammatory and anti-apoptotic activity.

restored SOD activities on treatment with the BM were described.[3,32,64] Likewise, a study done by Agarwal et al.[74] to investigate the effects of BM on the oxidative stress *in vitro*, BM exhibited a similar protection from dichlorvos poison in animals through restoring SOD activity. Supportively, Liu et al.[73] explored the ability of bacopaside I on regulating antioxidants in the 'Forced swim test' in mice and the results indicated a decreased SOD activity. The antioxidant activity of BM could be mainly attributed to the presence of bacopaside I.

3.1.4. Reduced glutathione (GSH)

Significant depletion of glutathione (GSH-reduced form) was found normally in diabetic rats as compared to non-diabetic rats due to oxidative stress.[76] Formerly, the ability of BM to increase the level of reduced glutathione was reported.[49] Accordingly, in a study the dietary ingestion of BM leaf powder on modulation of endogenous markers of oxidative stress, redox status, response of antioxidant defenses, protein oxidation and cholinergic function in several brain regions of mice were assessed.[53] Interestingly, their study unveiled a substantial rise in the levels of GSH, total thiols and non-protein thiols in both cytosol and mitochondria of diverse brain regions of mice, that are fed with BM. The significant increases of oxidative biomarkers such as thiobarbituric acid reactive substances and protein carbonyl content due to the production of free radicals in serum of mice by the injection of dichlorvos were significantly restored in proximity of normal level after administration of bacoside A.[74]

3.1.5. Catalase activity

Catalase, a marker enzyme for peroxisomes, is a common antioxidant enzyme found in nearly all living organisms that are exposed to oxygen.[77] In the mammalian brain, catalase is the key enzyme that catalyzes the peroxidatic oxidation of ethanol to acetaldehyde.[62,63] Reduced catalase activity was found in induced diabetic rats, differing to maximum enhancement in BM treated rats which was comparable to glibenclamide treated diabetic rats.[49] This was supported by a similar study[32] involving PD mouse with elevated levels of catalase upon treatment with BM, which was well in correlation with previous studies.[3,64] Notably, apparent age-associated decline in the activity of catalase in the lymphocytes of early middle-aged and older male rats was significantly increased over BM treatment.[75] In a related study, the ameliorative efficacy of bacoside A and bromelain on oxidative stress biomarkers in the serum of dichlorvos intoxicated mice was evaluated and the results showed low catalase activity in BM treated mice.[74] An *in vitro* study on bacopaside I by Liu et al., reported its ability to increase catalase activity, which gives an insight into the ability behind its antioxidant activity.[73]

3.1.6. Glutathione peroxidase

Glutathione Peroxidase (GPx) is a cytosolic selenium-containing antioxidant enzyme that catalyzes the reduction of hydrogen peroxide (H_2O_2) and lipid peroxides to water and lipid alcohols, respectively, as well as catalyzing the oxidization of glutathione to glutathione disulfide.[78] In the absence of adequate GPx activity detoxication process is withdrawn, which leads to the formation of OH-radicals and lipid peroxyl radicals, thus GPx system is considered to be a significant defense in low-level oxidative stress.[79] The decreased rate of GPx activity found in diabetic rats were found normal when treated with BM.[49] Likewise, a study was conducted by Priyanka et al. on the effect of BM on age dependent GPx activity decline, demonstrated a significant increase in the lymphocytes of early middle-aged and old rats after the treatment.[75] Similarly, on dichlorvos poisoned animals when treated with BM, was reported to have a reduced level of GPx[74] which is in line with a previous study on the effect of BM on aluminum induced

oxidative stress and hippocampus damage in rats.[3]

3.2. Anti-neuroinflammatory activity

More exciting still, in recent studies, several interesting scientific investigations have uncovered a much more complicated participation of regulatory impacts of BM on a wide range of molecular targets in the neuronal cells, than has previously been accepted and has exposed novel ways in which inhibition of proinflammatory cytokine, that could eventually lead to inflammation and thereby activating apoptotic pathway.[80] Proinflammatory cytokines, the positive mediators of inflammation, are a type of signaling molecule (a cytokine) that is excreted from cell types that promote inflammation.[81] They include proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-6, and chemokines (*e.g.*, monocyte chemoattractant protein-1 [MCP-1]).[82] Along these lines, neuroinflammation is a complex response to brain injury involving the activation of glia, release of proinflammatory cytokines, and generation of reactive oxygen and nitrogen species, thus the response of BM to them is of paramount importance.[83] An illustrative representation of anti-inflammatory activity of BM along with antioxidant and apoptotic activity is given in Fig. 4.

3.2.1. Nuclear factor kappa-B (NF-κB)

NF-κB is a protein complex, initially described as a B-cell–specific factor for the immunoglobulin-κ light chain gene. It is a pro-inflammatory transcription factor that controls the transcription of different proinflammatory mediators such as cytokines, chemokines, and adhesion molecules.[84,85] Viji et al.[86] have studied the anti-inflammatory function of BM through suppression of lipopolysaccharide (LPS) stimulated IL-6 production in peripheral blood mononuclear cells. In their study, betulinic acid isolated from BM could mitigate inflammation through inhibition of IL-6 production *via* inhibition of NF-κB or p38/ERK MAPK (Mitogen activated protein kinases) pathways. Paradoxically, NF-κB signaling have important implications for neural development and for learning and memory in part by modulating synaptic plasticity, synapse function, as well as by regulating the growth of dendrites.[87,88] Besides, higher concentration of IL-6 was reported in another study on lymphocytes stimulated with concanavalin A and LPS when treated with BM.[89]

3.2.2. Tumor necrosis factor-α (TNF-α)

TNF-α is a cell signaling protein (cytokine) involved in systemic inflammation that is produced by macrophages, monocytes, NK cells (Natural killer cells), endothelial cells, neutrophils, smooth muscle cells, activated lymphocytes, mast cells, astrocytes, neurons and adipocytes.[90] Most significantly, LPS induced TNF-α production in mononuclear cells are inhibited by the triterpenoid and the bacoside enriched fraction of BM. This inhibitory effect was predominantly due to their activity on the NF-κB pathway.[91]

3.2.3. Interleukin 1 beta (IL-1β)

IL-1β, an early response proinflammatory cytokine, is a protein that is encoded by the *IL1B* gene, which can up-regulate a broad range of proinflammatory activity in cells.[91] Captivatingly, studies have shown that bacoside treatment has reduced IL-1β, thereby having a modulatory effect over inflammatory signaling cascades, that would otherwise lead to the neuronal degeneration in the aged brain.[19] Further, the reduction in the IL-1β was well correlated with the decreased lipofuscin accumulation in rat brain. The anti-aging property of BM could be attributed to its effect on lipofuscins, as this pigment is produced mainly by peroxidation of unsaturated fatty acids in complex with proteins and are deposited as yellow brown pigments.[92] Moreover, its accumulation is closely associated with oxidative stress and mitochondrial dysfunction.[19]

3.3. Anti-apoptotic and pro-apoptotic protein activity

Demise or existence of a cell is determined by the ratio between anti-apoptotic and pro-apoptotic protein in each cell. The prosurvival proteins (Bcl-2) are termed so, due to their ability to bind and sequester their pro-apoptotic relatives. Pore forming proteins (Bax) involve in pore formation (apoptotic pore) in the mitochondria through homo-oligomerization.[93] Caspase-3, a pro-apoptotic protein, is one of the most important caspases in the cytochrome C-dependent apoptosis pathways and their activation appears to be a key event in apoptosis. Furthermore, mitochondrial dysfunction triggered by neurotoxin induces release of cytochrome C, and the activation of caspase-3[9] Of importance to the present review, the protective effect of BM could perhaps be associated with numerous pathways; activation of Bcl-2, thus maintaining the stability of MMP, and decreasing the activation of caspase-3 and Bax protein, through the mitochondria-dependent pathway.

Recently, dopaminergic neuron survival study done by Babitha et al.[32] investigated the effect of an ethanolic BM extract on MPTP induced PD model animals. Their results showed a significant increase in expression of Bcl-2 protein, whereas the expression of both caspase-3 and Bax decreased, compared to the substantia nigra region of MPTP induced PD model animals. Interestingly, they could conclude the ability of BM to reinstate the cell loss (degeneration of dopaminergic neurons) in the substantia nigra of mice brain. An earlier study also supported the associated antioxidant and antiapoptotic activities in the neuroprotective role of BM when 6-OHDA (catecholaminergic neurotoxin 6-hydroxy dopamine) induced dopaminergic neurodegeneration model was used.[9]

Consistent with these studies, Benzo-a-pyrene (B-a-P) induced apoptosis was reported to be mended by BM, thus providing cytoprotection through Beclin-1-dependent autophagy activation in human keratinocytes (HaCaT) cells. This effect of BM on cytoprotection, deciphers its role in the clearance of mitochondria-generated ROS in mitochondria through mitophagy, a process in which damaged mitochondria and cellular contents, including cytoplasmic material, protein aggregates are degraded through the formation of double-layered autophagosome. Thus, providing a nutrient pool to cope with different types of stress, while maintaining cellular homeostasis.[94] Reciprocally, their study also showed a decreased caspase activity in Caspase-Glo assay, in the presence of BM, together with decreased the expression of cytochrome C, thus giving a compelling reason to confirm the role of BM in protection of mitochondria from induced toxicity. Conversely, Yang et al.[95] suggested over expression of Bcl-2 could attenuate MPTP-induced neuronal cell death. This is true in considering, as the pro-apoptotic protein Caspase-3 and Bax is highly expressed in the substantial nigra and by its ablation neuronal apoptosis is attenuated.[32,96] To boot, a recent study done by Manu et al. have reported down-regulated expression of Bax pro-apoptotic protein and up-regulated expression of Bcl2 anti-apoptotic protein, while restoring Bcl2/Bax ratio when compared to tBHP induced apoptosis in H9C2 cardiomyocyte.[97]

3.4. Mitochondrial dysfunction

Cerebral mitochondrial oxidative energy metabolism and membrane function is a crucial factor of both excitability and the viability of neurons.[98] Mitochondrial travel (mobility) is essential to satisfy the energy needs for neuronal function.[99] The quality of mitochondrial function is assured by the fitness of the systems involved in metabolism (glycolysis, TCA cycle, oxidative phosphorylation).[100] Nevertheless, its dysfunction compromises the mitochondrial bioenergetics and dynamics. Administration of BM was found to decrease oxidative stress, plasma corticosterone levels and neuronal degeneration, which increases cytochrome C oxidase activity and ATP levels, recommending a positive effect on mitochondrial function and thereby brain energy

metabolism.[33] A study was recently conducted by Manu et al. to assess the effect of hydroalcoholic extract of BM and Bacoside A for cardio-protective property in H9C2 cells with tBHP induced oxidative stress.[97] In their study, a decreased ROS level with restored mitochondrial membrane potential upon pre-treatment with BM and Bacoside A was reported, which was consistent with their earlier findings of ROS generation inhibition by BM pretreatment in H_2O_2 induced ROS generation in L-132 cells.[101]

Being a key determinant of both excitability and the viability of neurons, mitochondrial respiratory chain (MRC) function is vital. Its dysfunction leads to decreased levels of brain mitochondrial enzyme status in rats.[102] Mitochondrial MDH, an enzyme catalyst in the citric acid cycle, which converts malate into oxaloacetate using NAD^+ and *vice versa*, serves as an indicator of anaerobic metabolism. It can bind to purified complex I of the electron transport system. A study on the protective role of BM exhibited increased MDH activity in the muscle of the epileptic rats, indicating an increased metabolic rate in the muscle of the epileptic rats.[103]

3.5. Interactions with receptors

3.5.1. Nicotinic acetylcholine receptors

Nicotinic acetylcholine receptors (nAChRs) are receptors for the neurotransmitter acetylcholine (hence, cholinergic receptors) that referee fast cholinergic transmission at the peripheral and central nervous system. nAChRs are receptor proteins that are members of the 'Cys-loop' family of pentameric ligand-gated ion channels (LGICs).[103,104] Their fast-cholinergic transmission has an important role in cognitive and addictive processes.[103] Also, Nicotine, from cigarette smoke can upregulates nAChR (pre- and postsynaptic), which sequentially could interact with the noradrenergic, cannabinoid, dopaminergic, cholinergic, and serotonergic systems thereby increasing the levels of norepinephrine, dopamine, acetylcholine, and serotonin.[105] An investigation into neuroactive agents in BM by Vani et al.[106] demonstrated that the Bacoside A in BM could alter changes in rat brain histologically and at the neurotransmitter level after cigarette smoke exposure, which include lipid peroxidation states, mitochondrial functions, membrane alterations, and apoptotic damage in rat brain, mainly through down-regulating nAChRs.

3.5.2. N-methyl-D-aspartate (NMDA) receptor

The *N*-methyl-D-aspartate receptor (also known as the NMDA receptor), is a glutamate receptor and ion channel protein found in neuronal cells, which is considered as the foremost molecular device for regulating memory function and synaptic plasticity.[107] Khan et al.[108] demonstrated the therapeutic benefit of BM on glutamate receptor gene expression and binding. Boosted extracellular hippocampal glutamate levels during induced epileptic model, leads to declining receptor binding in the hippocampus. Increased glutamate dehydrogenase (GDH) and decreased glutamate decarboxylase (GAD) in the hippocampus indicates accumulation of glutamate. In their study, Increased GDH activity was brought down to near-control levels after BM treatment. Additionally, the NMDA receptor and nitric oxide syntheses are the emerging target sites for development of novel drug molecules because their modulation affects the long-term potentiation process.[109]

3.5.3. GABA receptors

Ionotropic receptors for gamma-amino butyric acid (GABA) – a major inhibitory neurotransmitter in the mammalian brain, are essential for learning and memory.[110,111] They are heteropentameric complexes of α, β and γ integral membrane-protein subunits creating Cl^--channels functioned by GABA.[111] Treatment of BM and Bacoside A on epileptical rats, demonstrated by Mathew et al.[103] conversed its effect on GABA receptors. It has been found that a significant decrease in the B_{max} of epileptic rats compared to controls in the scatchard analysis of [^{3}H] GABA against GABA in the striatum. Moreover, expression of $GABA_{A\alpha1}$, $GABA_{A\gamma3}$, and $GABA_{A\delta}$ gene showed significant down-regulation in the striatum of the epileptic rats compared to the control. Also, a significant upregulation of $GABA_{A\alpha5}$ receptor subunit was reported.[103]

3.5.4. Serotonin receptors (5-HTRs)

Neurotransmitter serotonin exerts a modulatory action on neurological function through serotonin receptors (5-HTRs), which are most abundant in cerebral cortex, predominantly in the hippocampal region and the ventromedial prefrontal cortex.[112] They mediate both central and peripheral control on numerous physiological functions consequently contributes in multifarious ways to the regulation of brain function, mood and mental health.[113,114] There are seven general serotonin receptor classes with a total of 14 different receptor subtypes of which, cataloged into either an excitatory (increase in activity) or inhibitory (decrease in activity). Thus, diverse receptors modulate diverse neuronal excitability through the cell-type-specific expression patterns.[115] Krishnakumar et al.[116] demonstrated the neuroprotective role of BM through the upregulation of 5-HT_{2C} receptor in epileptic rats, together with a reversed down-regulation of the 5-HT content, 5-HT_{2C} gene expression and 5-HT_{2C} receptor binding, to normal level in BM treated epileptic rats. A summary of biological action of BM on neuroreceptor signaling is depicted in Fig. 5.

3.6. Interactions with proteins

3.6.1. β-Amyloid reduction

The highly membrane-active neurotoxic oligomers formed due to the adverse biological effects of amyloid β-protein (Aβ) fibril aggregates have the ability to damage the cell membrane. Indeed, Aβ play a major role in AD progression and pathogenicity.[117] Aβ is derived by proteolytic cleaving of the extracellular and transmembrane regions of the amyloid precursor protein (APP). A study on neuronal protection activity of BM was evaluated, *via* assessing the viability of cultured cortical cells that are injured with Aβ and glutamate, have confirmed that certain compounds in BM extract could protect cultured neurons from beta-amyloid protein mediated neuronal damage.[60]

Exceptionally, Malishev and coworkers[118] evidenced the anti-amyloid activity of bacoside-A through its effects on fibrillation and membrane interactions of the amyloidogenic fragment of prion protein, with suggestive induction of fibril formation and corresponding inhibition of membrane interactions. Furthermore, in their another study, the inhibitory effects of bacoside A upon cytotoxicity, fibrillation, and particularly membrane interactions of Aβ were determined and their results exhibited the ability of BM to prevent oligomers from self-assembly and membrane perturbation which could possibly be behind the mechanism of its ameliorating amyloid toxicity by bacoside-A and its supposed physiological benefits.[118]

3.6.2. Alpha synuclein

The progression of memory loss and cognitive decline resulting from selective degeneration of specific neuronal cells and the accretion of aggregated proteins are associated with pathogenicity of neurodegenerative diseases.[9] Alpha-synuclein (α-Syn) aggregation in the neuronal cells is an important pathophysiological characteristic of synucleinopathies.[119] It is a small (14 kDa) protein, abundant in human neural tissues, plays an important role in maintaining a supply of synaptic vesicles in presynaptic terminals.[120]

Jadiya et al.[9] quantified α-Syn aggregation pattern by its fluorescence intensity by using α-Syn expressing model of *Caenorhabditis elegans*. Significant reduction in fluorescence intensity of aggregation was reported in BM treated worms as compared to BM untreated *C. elegans*. This preventive effect on α-Syn aggregation was mainly due to the expression of stress proteins within the system which is BM mediated. A previous study on the ability of BM to induce chaperoning protein called HSP-70, backs this overexpression of stress proteins.[121] Also, BM

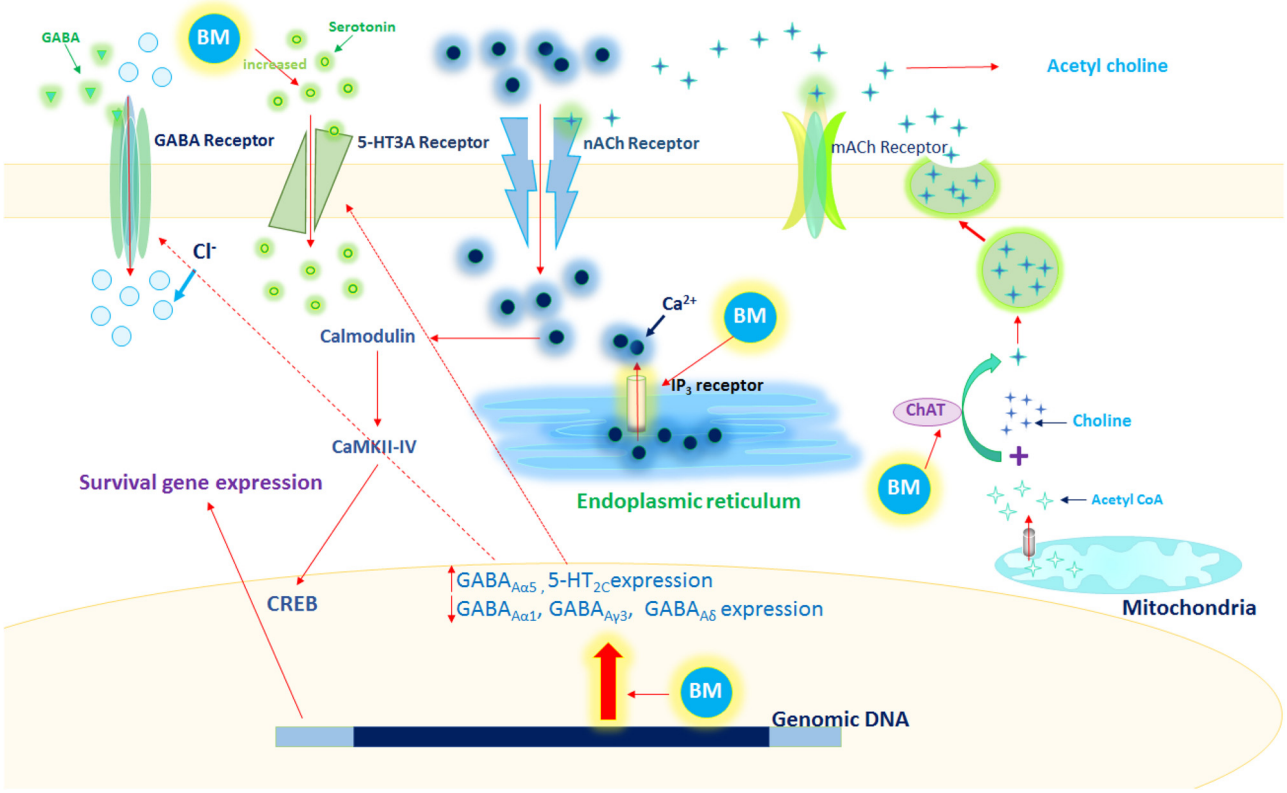

Fig. 5. Putative action of BM on neuroreceptor signaling.

could restore the lipid content in an α-Syn expressing model of *C. elegans*, with reduced lipid content due to disturbed lipid composition, caused as a result of α-Syn toxicity within the worms. Conceptually, central nervous system (CNS) being abundant in lipid molecules with neurotransmitting function, this protective effect of BM could be based on its anti-oxidant properties thus resulting in a reduced lipid peroxidation.[9]

3.6.3. Chaperone proteins

The heat shock protein Hsp70, with its molecular chaperone functioning in cells plays an important role in protecting cells – *via* a mechanism involving binding of the chaperone to various species on the α-Syn self-assembly pathway. Thus, inhibiting α-Syn fibril formation.[122,123] Hsp70 protein profile studied in different parts of the rat brain in BM pretreated rats were found to have decreased Hsp70 expression. The study was conducted on adult male Sprague Dawley rats by oral administration of distilled water dissolved BM.[121] On this ground, anti-stress activity of BM could be attributed to its modulatory effect on Hsp70 expression.

3.7. Interactions with enzymes

3.7.1. Acetylcholinesterase (AChE) inhibition

The enzyme AChE, abundant in the synaptic cleft, has its role in clearing free acetylcholine from the synapse by converting acetylcholine into inactive metabolites; choline and acetate. Hence, AChE activity is considered as a marker for cholinergic activity.[103] The role of AChE in ACh-cycle includes extending ACh at the synaptic cleft along with the release of Ach.[124] A study was assessed the memory enhancing potential of BM containing product using amnesia induced mice model, was reported to possess decreased AChE activity.[55] In support of this, epileptic rats with increased AChE activity was also reversed to normal when treated with BM.[105] Interestingly, in a human trial, BM consumed subjects demonstrated enhanced attention and cognitive processing capability, together with enhanced working memory and cholinergic function. The likely mechanism is that BM could suppress the function of AChE in the cerebral cortex, particularly in the parietal cortex and hippocampus. Accordingly, the levels of available ACh increases, thus giving rise to enhanced attention and memory operation capability.[37]

3.7.2. Proteases Inhibition

Proteases (peptidase or proteinase) constitute a very large and complex group of enzymes that catalyze the process of protein breakdown.[125] Midchannel proteolysis is a novel form of homeostatic negative-feedback processing that could overpoweringly affect neuroprotection, neuronal excitability, neurotransmission, and calcium signaling in physiological and disease states.[126] With this in mind, it is worth noting that, in a study to access the wound healing property, Sharath et al.[127] pioneered the analysis of BM activity on protease inhibition. They could demonstrate the ability of bacoside-A to cleave metalloproteases (MMPs), that would eventually lead to complete inactivation of enzymes.

3.7.3. Inducible nitric oxide synthase (iNOS)

Inducible nitric oxide synthase (iNOS) is one among the nitric oxide synthases (NOSs) family of enzymes, that catalyze the production of nitric oxide (NO) from L-arginine.[128] Since NO is an important cellular signaling molecule, iNOS stands apart among other mammalian NOSs, as it generates more NO after cytokine exposure.[129] A report on the neuroprotective effect of bacosides against age associated neurodegeneration depicted that it acts as an efficient neuroprotector, when tested against Wister rats brain.[19] Researchers from aforesaid study, could demonstrate the elevated pro-inflammatory cytokines, that escorted with the up-regulation of iNOS protein expression in the aged rat brain cortex, which on administration of bacosides was down-regulated, thus reducing the age-related nitrite generation. Supportively, a recent study on BM pre-treated H9C2 cells with tBHP induced cytotoxicity has significantly attenuated the toxicity, mainly through the

down regulation of iNOS gene expression in comparison to tBHP treated H9C2 cells.[100]

3.7.4. Brain neuronal cytochromes P450

Brain neuronal cytochromes P450 (CYPs) enzymes are a type of hemoproteins with important functions in the metabolism of endogenous signaling molecules, xenobiotics, and drugs by specific involvement in brain development, function, and plasticity.[130,131] Chowdhuri et al.[121] reported the modulatory effect of BM on CYPs enzyme activity with male Sprague-Dawley rats pretreated with BM (dissolved in distilled water) and the results revealed that, lesser CYPs activity was found on stress administration, compared to higher CYPs activity in control rats.

3.8. Cerebral blood flow and vasodilation

Typically, the brain depends on energy generated from a continuous supply of glucose and oxygen from the blood.[132] Hence, the brain is highly perfused and is extremely sensitive to any alteration in its blood supply.[133] Again, cerebral blood flow (CBF) is a main mediator for cellular mechanisms to alter brain function related to cognitive impairment and stroke.[134] A study reported by Kamkaew et al.[135] found that BM acted as a vasorelaxant in animal arteries, with potency mostly on basilar artery. Their subsequent follow-up studies[136] reported an increased CBF with improved cognitive function in rats. Classically, cognitive decline is associated with the vascular dysfunction in cerebral cortex and the hippocampus. Thus, studies regarding increased CBF are of supreme importance. As well, hippocampus, being critical in learning and memory, is reported to have increased neocortical blood flow after 8 weeks of BM treatment, which could improve cognition.[136,137]

4. Learning and memory and their mechanisms

Memories are accounted as deeply personal, ephemeral possessions – snippets of emotions, words, colors and smells that are stitched into our unique neural tapestries as life goes on.[138] Technically, it is the ability to encode, store, retain and subsequently recall information and past experiences in the human brain,[139] whereas learning, is the process by which acquiring the knowledge of the world.[140] But still, the distinction between learning and memory is hazy, so researchers often treat the two as similar actions. Recent years have seen the field of neuroscience has transformed with the development of new technologies, that can manipulate neuronal cells in ways that can harness their interactions in a different but more tangible perspective. The long-overlooked memory transfer – substantiated with extracted RNA from shocked worms and injected into unconditioned ones – even though bends the rules of nature, can provide the prospect of new functional technology.[141] Being recorded as a memory enhancer in Materia Medica of Ayurveda, BM through recent studies, offers mind-bending results that have gained unexpected support.

The mechanism behind ‘neuroprotection’ of BM, in particular, is very clear, but the mechanism behind ‘memory and learning’ potential of BM has to be unpacked with respect to the distinct ways in which it can be entangled. A schematic representation of molecular interactions and cross-talks behind the enhanced ‘learning and memory’ of BM are portrayed in Fig. 6.

4.1. Synaptic plasticity

Synaptic plasticity referring to any change in the efficacy of a given synapse, is believed to be the basis of learning and memory. Even though much is known about the cellular and molecular basis of synaptic plasticity, these processes appear to be very complex.[142,143] Plastic changes at the synapse due to complicated interplay of neurotransmitter release, the number and variety of postsynaptic receptors and synchronous activation of neighboring structures, can extent to overall strengthening or weakening of synaptic connections. Suchlike functional consequences of strengthening of a synapse (LTP; long-term potentiation) or weakening of a synapse (LTD; long-term depression) in the hippocampus hallmarks the structure for studying synaptic plasticity, that have a direct link to learning and memory. The key molecular players identified having an important role in LTP and LTD includes different types of glutamate receptors, calcium-signaling molecules (calcium-calmodulin kinase II, calcium-response element binding protein) and the neurotrophin brain-derived neurotrophic factor (BDNF).[144]

In particular, as reviewed previously by Shouval et al.[142] hippocampal NMDA receptor-dependent plasticity is sufficient for episodic memory that involve a hypothetical ligand-activated Ca^{2+} channel exogenously. Such Ca^{2+} influx is adequate to induce early-LTP that would result in synapse-specific potentiation.[145] In a report, phosphorylation of synaptic plasticity-related signaling proteins (NR1 subunit of NMDA receptor and calmodulin-dependent kinase II) down-regulated by OBX (Olfactory bulbectomy) was reversed by administration of BM along with enhanced CREB (cyclic AMP-responsive element binding protein) and BDNF (brain-derived neurotrophic factor) mRNA in the hippocampus.[2]

4.2. Electrical stimulation

Electrical stimulation through voltage-gated ion channels, is considered an important event in neuronal activation which further persuades membrane potential depolarization.[146] ‘Depolarization’ which is essential for the communication between cells, is termed to be a change within a cell, during which the cell undergoes a shift in electric charge distribution, resulting in less negative charge inside the cell. This change in charge from the resting potential to a positive charge occurs due to an influx of sodium ions into a cell. Thus Na^+ and Ca^{2+} are called “depolarizers” of the membrane potential.[147] The depolarization causes the voltage-gated Ca^{2+} channels to open, allowing the influx of Ca^{2+} that signals the release of neurotransmitter into the synaptic cleft. Remarkably, Saini et al.[6] evaluated the neuroprotective effects of BM on colchicine induced cognitive impaired rat models, where the altered enzyme activities of Na^+-K^+ ATPase was reported to be restored by BM. The Na^+-K^+ ATPase is the solute pump that maintain the right concentration of ions. The restored activity along with Ca^{2+}-ATPase, and Mg^{2+}-ATPase was in sync with prior studies.[53,148] Since, the transmembrane electrochemical gradient of Ca^{2+} into cells is large, in order to maintain low concentrations of Ca^{2+} for proper cell signaling, plasma membrane Ca^{2+}-ATPase (PMCA) is necessary to remove Ca^{2+}.[149] More interestingly, an increase in intracellular inositol phosphates (IP3) content in hippocampus of epileptic rats after BM treatment was explored. Since, the IP3 are known to regulate membrane trafficking and intracellular Ca^{2+} homeostasis, predominantly the release of stored Ca^{2+} *via* IP3 receptors, the ability of BM thus to maintain intracellular Ca^{2+} can be through IP3 receptors.[116]

Besides, another study on the administration of active constituent (Bacoside I) from BM administered has reduced neurological deficits together with increased Na^+ K^+-ATPase and $Ca^{2+}Mg^{2+}$-ATPase activity. In this study, the release of intracellular Ca^{2+} was glutamate and neurotrophin dependent, in the post-synaptic cell through a number of downstream protein kinase that are crucial for synaptogenesis.[150] Subsequently, these results reveal the specific components of BM can encourage in neuroprotective processes. Equally, the modulation of calcium entry in the cells in *C. elegans* by BM was reported by Pandey et al.[151] while studying the effect of Bacoside A on the seizure activity in T-type Ca^{2+} channel CCA-1 mutant worms. Taken together, these findings not only indicate the neuroprotective properties of BM, but also the post-synaptic kinase and neurotrophin activity which is an indicative of synaptic formation and enhancement that improves with BM consumption.

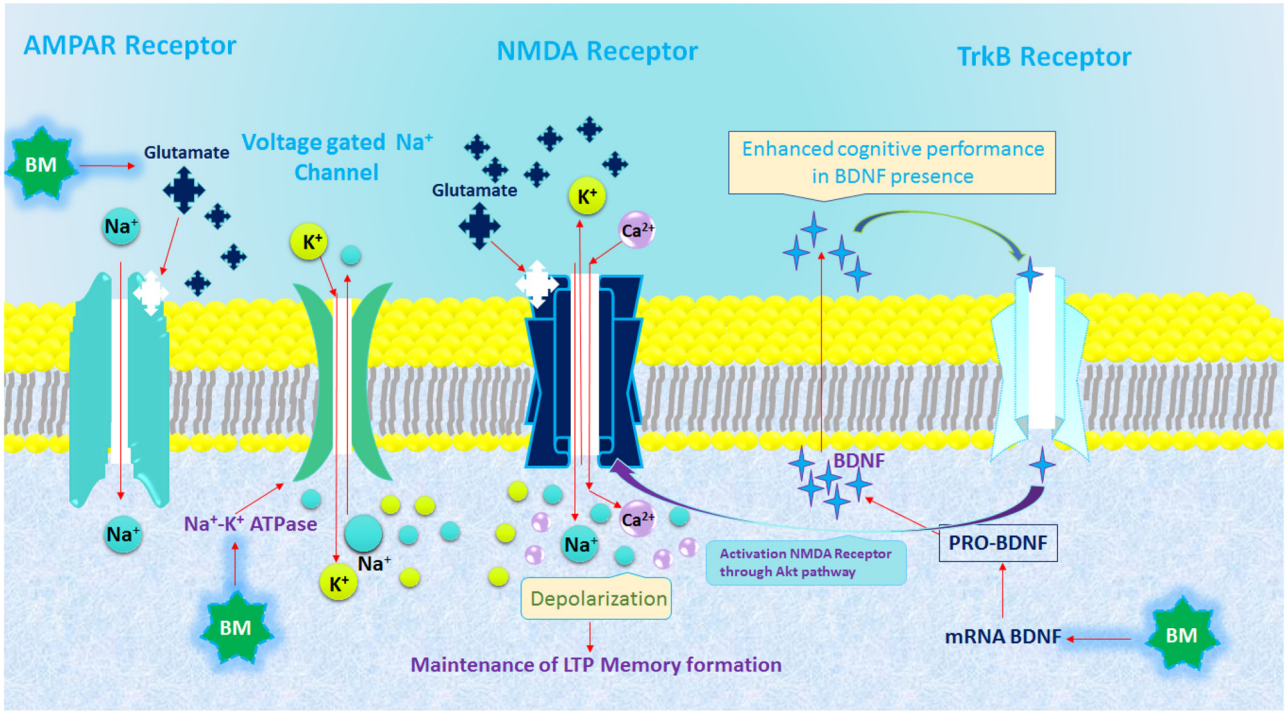

Fig. 6. Schematic representation of molecular interactions and cross-talks behind enhanced 'learning and memory' of BM.

4.3. Cerebral blood flow

The human brain being one of the most complex biological systems in the world, with its very dynamic nature, the analysis of microstates for better understanding of underlying mechanisms can be challenging. However, brain functional assessment techniques have contributed to information about the localization and organization of cerebral areas involved in higher brain functions including the organization of neural networks supporting verbal memory.[152] Cerebral ischemia can lead to the decline in learning and memory function. In a clever set of experiments, levels of AChE, GABA, SOD, and MDA in the frontal lobe and hippocampus area as well as the change of the content of some amino acids, are associated with cerebral ischemia and the decline of the learning memory function. In support of this, an investigation to study the effect of regional cerebral blood flow (rCBF) in special brain areas, on adult male healthy Sprague-Dawley rats by Kong et al.[153] which demonstrated that, reduced rCBF impaired the learning and memory function as well as its molecular mechanism. As mentioned earlier, the ability of BM to have an effect on AChE, GABA, SOD, and MDA levels, may be the reason behind increased cerebral blood flow.

4.4. Stress resilience and proteostasis

On the grounds that, hippocampus stage a significant role in processing and remembering spatial and contextual information, by converting 'short term memories' to 'long term memories' while briefly storing them prior to eternal storage in the cortex and retrieving contextual fear memory for up to 2–3 weeks after learning.[154] Pleasingly, in stress induced hippocampal animal model, significant regression in the expression of Hsp70 on BM treatment was detailed, which points toward the enhanced performance of BM in learning and memory through stress re-silence. Moreover, the study also promised that BM supports in surviving with the combined hypoxic, hypothermic and immobilization stress by modulating the activity of P450s and SOD, in addition to the expression of Hsp70.[121]

Extensively, heat shock proteins have a role in preserving protein integrity through counteracting the misfolding of cellular proteins that are under stress, thus they can be considered as a biomarker of proteostasis (the maintenance of proteins in their conformation, concentration, and location for their correct function) in stressed environments.[155] Consequently, this mechanism will result in homeostatic/proteostatic plasticity – a form of neuroprotection, that enables neurons and their target cells to maintain their activity levels.[156]

4.5. Cholinergic markers

The neurotransmitter acetylcholine (ACh), being stored in vesicles in the terminal portions of the nerves is released in response to an action potential, which has an important role in attention, recall, and memory formation.[157,158] Particularly, ACh release in the hippocampus aids in the construction or retrieval of memories depending on the concentration of extracellular ACh.[157] In presynaptic terminals the cholinergic marker choline acetyltransferase enzyme (ChAT) catalysts the biosynthesis of ACh from Acetyl-CoA and choline.[159] A neuropharmacological review on BM had described the hypothesis of the restored ACh through the primary ACh-boosting mechanism of BM *via* choline acetyltransferase activation (synthesis of ACh), but not due to the AChE inhibition.[160]

Another cholinergic marker, AChE is an ACh hydrolyzing enzyme, involved in the termination of ACh neurotransmission in numerous cholinergic pathways. Hence, the decreased AChE activity would eventually lead to increased ACh levels in the brain, thus improving learning and memory.[159,160] Promisingly, the neuroprotective effects of BM studied by Saini et al.[6] in an experimental model of dementia have reported the modulatory effect on the enzymes which is in line with previous studies.[95,161] In the study, the activity of membrane bound enzymes (Na^+, K^+, ATPase) were investigated and had restored their activity, whereas AChE had decreased activity after BM supplementation which could be attributed to its cognitive enhancing effect.

4.6. Glutamatergic markers

Glutamate, an excitatory neurotransmitter has a role in cognitive

functions such as learning and memory in the brain.[162] Hippocampal glutamatergic neurotransmission is a molecular basis underlying learning and memory. In support of this possibility, a study on subchronic phencyclidine administrated rat with deficits in novel object recognition could recover cognitive deficits after BM treatment. Most importantly, the immunohistochemistry study in this rat model of schizophrenia-like psychosis suggested the increased expression of VGluT3 (vesicular glutamate transporter 3), VGluT1 (Vesicular glutamate transporter 1) and VGluT2 (vesicular glutamate transporter 2), density in the prefrontal cortex, striatum and CA1-3 of the hippocampus. This transporter protein can transport the neurotransmitter glutamate into synaptic vesicles before it is released into the synaptic cleft.[13] Worth mentioning, an interesting study has done previously on pilocarpine induced temporal lobe epilepsy in rats and reported the regulatory effect of BM on metabotropic glutamate-8 receptor (mGluR8) and NMDA receptor 1 (NMDAR1) gene expression. Since, hippocampal formation is critical for procedural memories, the restoration of these receptors by BM forms the motif behind the refurbishment of pilocarpine impaired declarative memory.[56]

4.7. Serotonergic markers

Serotonin (5-hydroxytryptamine, 5-HT) a small molecule that functions as a neurotransmitter in the CNS, and its receptors are involved in normal, pathophysiological and therapeutic aspects of learning and memory.[163,164] More specifically, an increased turnover of 5-HT in the hippocampus may play a role in long-term potentiation and improvement in memory. An investigation led to study the role of BM in relation to depression in pilocarpine-induced temporal lobe epileptic rats, showed upregulation of 5-HT_{2C} receptors, together with increase in 5-HT_{2C} gene expression and IP3 content in the epileptic hippocampus. As mentioned earlier, this leads to excess Ca^{2+} release from IP3-sensitive stores in retort to 5-HT_{2C} receptor hyperfunction.[116] Correspondingly, a former study by Charles et al.[165] on postnatal rats have also improved learning and retention of memory after oral administration of BM. The study reported the increased 5-HT after treatment with BM, along with associated up-regulation of serotonin synthesizing enzyme tryptophan hydroxylase-2 (TPH2) mRNA expression.[165] These effects highpoint a novel facet of BM in learning and memory.

5. Blood brain barrier (BBB) permeability and excipient compatibility

Blood-brain barrier (BBB) being the high resistant tight junctions within the endothelium of capillaries perfusing the vertebrate brain, the BBB penetration of bacosides is of great interest. Possible enough, the bacosides being nonpolar glycosides, could cross the BBB by simple lipid-mediated passive diffusion.[166] Supportively, an *in-silico* study done on the constituents of BM for its cognitive effects, showed that the parent bacosides were not able to dock into the chosen CNS targets and had poor molecular properties as a CNS drug. But their study revealed that the aglycones and their derivatives showed a better binding affinity and good CNS drug-like properties. Moreover, they were well absorbed through the intestines and had good blood brain barrier (BBB) penetration. Suggestively, BM constituents transform *in vivo* to its active form before exerting their pharmacological activity.[167]

Stability testing, now being the key procedural component in the pharmaceutical development program ensures the scientific and commercial success of a pharmaceutical product.[168] In this regard, physical and chemical stability of bacosides is of paramount importance. Nevertheless, the reports regarding the stability studies of bacosides are less. Assuringly, a study done by Bajaj et al.[168] investigated the stabilities of crude and diluted BM extracts at various temperatures. The study involved the usage of saponin glycosides, bacopaside I and bacoside A3 as markers for quantitative analysis, where the temperature stability was found to be between 60 °C and 5 °C. Furthermore, the amount of both compounds in the standard solution were stable between the pH 6.8 and 9.0.[169] Thus, further stability studies of BM will be beneficial for pharmaceutical pre-formulation to ensure the quality of the final products.

Oral bioavailability, being the fundamental concept for pharmacology which is defined as the rate and degree of chemical absorption into the systemic circulation.[170] In a study to validate the effect of BM on the behavioral improvements, Charles et al. evidenced that orally treated BM was uptaken into the system where bacoside A was present in the serum of animals.[165] Another study was attempted to evaluate the drug (natural plant extract or synthetic) interactions with radiopharmaceuticals, where the influence of ethanolic BM extract on the biodistribution of the radiopharmaceuticals (99mTc-ECD and 99mTc-CDM) in female Sprague Dawley rats was evaluated for its bioavailability. Amazingly, the bioavailability has been confirmed by the biodistribution of radiopharmaceuticals effectively activating the cascade which participates in the memory enhancing mechanism.[38,171] Captivatingly, a BM extract formulation based on phospholipid using solvent evaporation method was prepared with an attempt to improve the solubility and permeability of bioactive compounds. The formulation was reported to have higher aqueous solubility and *in vitro* dissolution efficiency. Moreover, *ex vivo* permeation studies showed improved permeation of BM molecules.[172]

6. Toxicity studies

Safety profiling through 'preclinical toxicity testing' of new pharma candidate is essential for drug development process. Relevantly, the BM is reported to have no incidence of genotoxicity, while analyzing the genotoxic activities of its compounds Bacoside A and B with three cytogenetic end points *i.e.* chromosomal aberration, sister chromatid exchange and micronuclei formation *in vivo*.[173] Similarly, a bacopa enriched phytochemical composition when evaluated for short-term safety and tolerability in healthy adult volunteers, and the results was testified to be safe.[174] Further, a subchronic oral toxicity study for 90 days in rats at different dose levels (85, 210 and 500 mg/kg) did not expose any indication of toxicity in the matter of clinical signs, neurological examination, food consumption, body weight gain, hematological and blood biochemistry parameters.[175] Additionally, Dipanwita et al. demonstrated a dose-dependent protection of BM against the clastogens together with no mutagenic effect on the tested strains in the Ames test.[176] Then again, another pharmacokinetic study was done by *in silico* on BM molecules revealed desirable quantum chemical and ADMET (absorption, distribution, metabolism, and excretion-toxicity) properties.[177]

7. Perspectives

Within this review, we have concisely accounted the neurochemical processes involved in signaling events, and the roles of BM played as a neuroprotective agent associated in enhanced learning and memory. The available literature point toward a significant progress, that has been made in determining the role of such molecular events in cognition and neuroprotection. But in some aspects, an integrative approach that can utilize the advances in molecular biology and analysis of cellular signaling networks using an interactive array of bioinformatic resources, should be used to explore the possible impact on understanding the biological basis of molecular pathway crosstalks. Consequently, it will be interesting to determine exactly how intracellular signaling cascades involve in pharmacological interventions and the specific molecules intricate in mediating beneficial aspects of BM, pertaining to its influence over cognition and memory. Accordingly, a facility to untangle and hopefully creating *in silico* models of molecular cross talks, may be one of the greatest advances that will bring us closer to understand the mechanism of action without

missing any molecular links.

8. Conclusion

Over the years, the researchers had tweaked hacking cellular communication, giving up its secrets to plenty of unanswered questions thus predicting potential signal transduction cascade activity which forms the cornerstone for future functional food. Current trend, especially among the neuropharmacological research, is to define brain systems and its neural networks. For this, research strategies that cover alternative promising approach to provide fundamental insights in neuroscience must be oriented. Promisingly, breathtaking advances have occurred in molecular and cellular neurosystems. A bottom-up bridge like this, advance our understanding in neuronal cell biology driven by biochemistry, illuminate the molecular paths to researches in neuropharmacology.

In this review, we attempted to decipher a wider array of mechanism in which BM promotes cognitive health and neuroprotection. Although, it stands to reason that different molecules of BM, have very different biological effects like antioxidative, anti-inflammatory, anti-apoptotic *etc*. Noteworthy, this myriad of phytochemical components in the BM itself is the reason that would work together to flow information into a cascade of cell signaling that leads to its physiological and pharmacological actions.

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