ROS

REVIEW ARTICLES

A Review on the Antioxidant and Therapeutic Potential of *Bacopa monnieri*

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ABSTRACT | Molecular oxygen, a basic component of aerobic metabolism, is an inevitable component of all living organisms. However, a portion of the oxygen used by the cells is converted to several harmful reactive oxygen species (ROS) and free radicals. In low/moderate concentrations, free radicals serve as beneficial compounds and are involved in normal physiological functions. Excessive free radical generation results in oxidative stress, with the prime targets being the fundamental building blocks of cell, such as lipids, protein, and DNA. Oxidative damage to these molecules leads to growth arrest, senescence, or apoptosis which play crucial role in the development of several pathological conditions of humans. A growing body of recent literature suggests that scavenging these ROS alleviates the diseases completely. The documented literature suggests that many herbal extracts possess antioxidant effects. Among them, brahmi (*Bacopa monneiri*) has received increasing attention as a traditional medicine for treatment of several disorders. Previous research has shown that *Bacopa monneiri* is used to cure various ailments such as cardiovascular diseases, cancer, neurodegenerative diseases, and diabetes, among others. The present review focuses on the therapeutic potential of *Bacopa monneiri* in the management of diseases that are caused by oxidative stress and ROS by acting as a free radical scavenger.

KEYWORDS | Antioxidants; *Bacopa monnieri*; Bacoside A; Oxidative stress; Reactive oxygen species

ABBREVIATIONS | AD, Alzheimer's disease; CytP450, cytochrome P450; GPx, glutathione peroxidase; GSR, glutathione reductase; NO, nitric oxide; OS, oxidative stress; PD, Parkinson's disease; RNS, reactive nitrogen species; ROS, reactive oxygen species; SNP, sodium nitroprusside; SOD, superoxide dismutase

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1. INTRODUCTION

Even though oxygen is an inevitable element for all living cells, its surplus level would come up with certain nuisances also. For a living system, one important type of free radical is reactive oxygen species (ROS), which is a partially reduced form of oxygen and is responsible for its unusual toxicity [1]. When ROS levels exceed physiological levels by its continuous generation and accumulation, cellular damage occurs [2]. ROS can be classified into two categories: (i) free oxygen radicals and (ii) non-radical ROS. Examples of free oxygen radicals are superoxide, hydroxyl, peroxyl, and hydroperoxyl radicals which are ascertained to have an impact on various pathological conditions including cancer. Non-radical ROS include certain non-radical oxidizing agents like hydrogen peroxide, hypochlorous acid, and ozone which can be converted into free radicals. ROS are produced during normal metabolism and are involved in enzymatic reactions, mitochondrial electron transport, signal transduction, activation of nuclear transcription factors, gene expression, and the antimicrobial action of neutrophils and macrophages. About 95–98% of ROS such as hydrogen peroxide, hydroxyl free radical, superoxide anion, and peroxynitrite are formed in the mitochondria as a result of cellular respiration.

The major harmful effects of free radicals include oxidative stress and nitrosative death. Oxidative death is caused by ROS and nitrosative death by reactive nitrogen species (RNS), which leads to oxidative stress with subsequent generation of ROS [3] and resultant oxidative injury. Potential biological damage occurs as a result of an overproduction of ROS and RNS and a down regulation of enzymatic and non-enzymatic antioxidants. The ROS generated by the powerful oxidizing agents interfere with the expression of a number of genes involved in the activation of signal transduction cascades [4–7] leading to important cellular events such as apoptosis. The reducing environment inside the cells aids prevention of free radical-mediated damage. This reducing environment is maintained by the action of enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione, ascorbate (vitamin C), alpha-tocopherol, and thioredoxin. An imbalance between the cellular oxidant and antioxidant status results in disease condition.

Oxidative stress has been implicated in various pathophysiological conditions either directly or indirectly (**Figure 1**). They are cardiovascular disease, cancer [8], Parkinson's disease (PD), Alzheimer's diseases (AD) [9, 10], amyotrophic lateral sclerosis (ALS), Huntington's disease, multiple sclerosis, diabetes mellitus [11], ischemia/reperfusion, fibrosis, kidney disease, ototoxicity of drugs such as cisplatin, and congenital deafness in both animals and humans, obstructive sleep apnea, obesity, hypertension, and other diseases related to aging [12]. These diseases fall into two groups. The first group involves the diseases characterized by prooxidants shifting the thiol/disulfide redox state and impairing glucose tolerance—the so-called the mitochondrial stress conditions (cancer and diabetes mellitus).The second group involves diseases characterized by "inflammatory and oxidative conditions" and enhanced activity of either NADPH oxidase leading to atherosclerosis and chronic inflammation or xanthine oxidase-induced formation of ROS (implicated in ischemia/reperfusion injury).

The most promising line of attack against the oxidative damage caused by these reactive species is the use of antioxidant molecules. These compounds can act as direct antioxidants through free radical scavenging mechanisms as well as indirect antioxidants by boosting the antioxidant status (enzymatic and non-enzymatic). The bioactive molecules isolated from the herbs contain potent antioxidant property and protect the cells from various injuries caused by reactive oxygen and nitrogen species. The present work reviews the biological and pharmacological activities of bioactive components isolated from *Bacopa monnieri.*

2. BASICS OF *Bacopa monnieri*

Bacopa monnieri, also known as aindri and brahmi in Sanskrit belongs to the family *Scrophulariaceae*. It is a creeper, glabrous, and somewhat succulent

herb which grows in wet places of India and neighboring tropical countries up to 1500-meter altitude. *Bacopa* is known as medhya rasayana, due to its ability to improve the cognitive properties of brain and is a very commonly used herb among all Ayurvedic practitioners to treat conditions such as fever, inflammation, pain, asthma, epilepsy, insanity, and memory loss. The major chemical compound responsible for these neuropharmacological effects is a dammarane-type triterpenoid saponin called bacoside A [13] with jujubogenin or pseudo-jujubogenin moieties as their aglycone units. Bacoside A is composed of bacoside A3, bacopaside II, jujubogenin isomer of bacopasaponin C, and bacopaside X [14–16]. Based on the structural similarity, 12 analogs from the family of bacosides have been elucidated. In the recent past, different classes of saponins have been identified as important constituents of the herbal extract and are named as bacopasides I–XII [17]. The plant has also been reported to contain hersaponin, apigenin, D-mannitol, cucurbitacin, monnierasides I–III, and plantainoside B, as well as the alkaloids brahmine, herpestine, nicotine, and monnierin [18, 19]. The strong antioxidant property is imparted by the significant amounts of flavanoids and phenolic compounds contained in the herb.

Several research studies reported the therapeutic potential of *B. monnieri* in various disease conditions [20–26] (**Figure 2**). In experimental models of diabetes, ischemia, and aluminum- and cigarette smoking-induced toxicity, pretreatment with extracts of *B. monnieri* and bacoside A prevented lipid peroxidation and enhanced antioxidant enzyme activities.

The hydroxyl radical scavenging activities of *B. monnieri* as assessed by DPPH assay revealed that the methanol extract is more effective in preventing DNA damage than chloroform, hexane, acetone, ethyl acetate, and methanol/aqueous extracts. A dose-dependent free radical scavenging capacity and a protective effect against DNA damage by methanolic extract of *B. monnieri* was also reported by Russo et al. [26] and Anand et al. [20]. Lipid peroxidation brought about by $FeSO_4$ and cumene hydroperoxide was significantly reduced by bacopa treatment in animal models by chelating Fe^{2+} [27]. Radical quenching activity of ethanol and aqueous extracts of *B. monnieri* was also widely reported [28]. Effects of *B. monnieri* extract on ROS generation and lipid peroxidation are evaluated by exposing the lung epithelial cell line, L132 cells to nitric oxide (NO) donor, sodium nitroprusside (SNP), a generator of RNS. The results of the study showed that pretreatment with *B. monnieri* extract significantly reduced the nitrosative stress by inhibiting the generation of RNS in SNP-treated cells. *B. monnieri* extract pre-treatment modulated the SNP induced up-regulation of Bax, cytochrome c, and caspase 3 expression along with a down-regulation of Bcl2 expression by maintaining mitochondrial integrity indicating the cytoprotective role of *B. monnieri* extract against SNP-induced damage [29]. Methanolic extract of *B. monnieri* and its isolated constituent bacoside A exhibited profound wound healing activity [30]. These results suggest that *B. monnieri* might have good impact in the treatment of human ailments in which free radical production plays a key role.

Extracts of *B. monnieri* exhibit strong antioxidant properties such as adsorption and neutralization of free radicals by quenching singlet oxygen or decomposing peroxides. It has been reported that glial cells produce NO upon superoxide radical stimulation by an enzyme-independent mechanism. Russo et al. [26] examined the effect of a methanolic extract of *B. monnieri* against S-nitroso-*N*-acetyl-penicillamine (SNAP) (a well-known nitric oxide donor)-induced toxicity in rat astrocytes. Their results pointed out that SNAP-induced increase in reactive species and DNA fragmentation was inhibited by the treatment of *B. monnieri* extract in a dose- dependent manner.

Furthermore, oxidative stress generated by lead exposure is ameliorated by *B. monnieri* in various areas of rat brain [31]. Lead exposure raised the levels of ROS, lipid peroxidation, the carbonyl content in total protein, and metal content in rat brain tissues. *B. monnieri* pretreatment prevented these oxidative changes and protected against lead toxicity.

3. *Bacopa monnieri* IN DIABETES

Oxidative stress is known to cause complications such as neuropathy, nephropathy, and cardiomyopathy in the case of diabetics patients. The protective role of *B. monnieri* on tissue antioxidant defense system and lipid peroxidative status in streptozotocin-induced diabetic rats was investigated by Bhattacharya *et al.* [32]. The effects of the oral administration of *B. monnieri* extracts on the enzymatic and non-enzymatic antioxidant levels and lipid peroxidation were observed in the kidney, cerebellum,

cerebrum, and midbrain of diabetic rats and the results were compared to a reference drug, glibenclamide. The results revealed a significant increase in the antioxidant status and a concomitant decrease in the peroxidative damage. The diabetic rats showed significant improvement of the glycemic index and body weight upon treatment with ethanolic extract of *B. monnieri* compared to untreated rats. The glycemic status of *B. monnieri* extract-treated diabetic rats is comparable to that of the reference drug. Further improvement was observed in rats receiving administration of both the extract and glibenclamide with respect to oxidative stress and hyperglycemic status in the liver of diabetic rats [32]. These results suggest that *B. monnieri* has antidiabetic activity comparable to the existing oral antidiabetic drug and the combinatorial regimen helps in reducing oral antidiabetic drug-induced toxicity.

4. *Bacopa monnieri* IN CANCER

The anticancer potential of *B. monnieri* has been reported earlier by several investigators. Prakash *et al*., have reported the anticancer activity of bacoside A from whole plant of *B. monnieri* in MCF-7 (human breast cancer), HT-29 (human colon adenocarcinoma), and A-498 (human breast cancer) cell lines [33]. Janani et al. showed that bacoside A exhibits its chemopreventive effects against DEN (diethyl nitrosamine)-induced carcinoma by decreasing the level of lipid peroxidation and enhancing the antioxidant status probably through its free radical scavenging activity [31]. The antioxidant and tumor inhibiting property of *B. monnieri* were also reported in 3-methylcholanthrene-induced fibrosarcoma in rats [34]. *B. monnieri* supplementation enhanced the antioxidant enzyme status, reduced the rate of lipid peroxidation, and downregulated tumor development markers. Among the five crude samples such as the whole plant of *B. monnieri* and four different fractions (petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH fractions) of the methanol extract, *n*-BuOH fraction was noted to have the highest anticancer activity as per bioassay-guided methods conducted by Peng et al. The studies showed that dammarane triterpene saponins isolated from *n*-BuOH fraction, bacopaside É and bacopaside VII, have potential anticancer effect as revealed from the cytotoxicity studies in various human cancer cell lines MDA-MB-231, SHG-44, HCT-8, A-549, and PC-3M in MTT assay in vitro, and showed 90.52 % and 84.13 % inhibition in mouse implanted with sarcoma S180 in vivo at the concentration of 50 μmol/kg, respectively. Further studies revealed significant inhibition of human breast cancer cell line MDA-MB-231 adhesion, migration, and matrigel invasion in vitro at the concentration of 50 μM [35].

Manoharan et al. studied the effect of bacoside fraction from *B. monnieri* against oxidative stress-induced apoptosis in untransformed (buccal) and transformed (kb oral carcinoma) cells. Interestingly, the results have shown anti-apoptosis in bacoside-treated buccal cells, whereas a steep increase in apoptotic cells was observed in bacoside-treated kb oral carcinoma cells. These observations clearly indicate that bacoside-induced toxicity is specific to cancer cells while sparing normal cells. Their studies suggest combinatorial regimen of bacoside along with established anticancer drugs during chemotherapy for the reduction of undesirable side effects [36].

5. NEUROPROTECTIVE EFFECT OF *Bacopa monnieri*

Chronic administration of *B. monnieri* inhibited the lipid peroxidation particularly in the prefrontal cortex, striatum, and hippocampus regions of the rodent brain, via a mechanism of action similar to vitamin E [26]. In the astrocytes of rodents, bacopa treatment resulted in significant reduction in the damage produced by high concentrations of nitric oxide [21]. Furthermore, various studies suggested that bioactive components from *B. monnieri*, protect the brain against oxidative damage and age-related cognitive decline with several modes of action and also enhance memory [37, 38]. Improved cognitive function is noted with standardized CDR108 extract in both in vivo and in vitro studies. The improved cognitive action of extract of *B. monnieri* was attributed to the increase in the free radical scavenging activity of bacosides. A similar increase was also noted in the case of diabetic rats showing a significant reversal of redox imbalance and peroxidative damage to enhance a defense system against ROS [39]. Heat shock protein 70 (Hsp70), cytochrome P450 (CytP450), and SOD in the rat brain play an important role in the production and scavenging of ROS, and determine stress responses in rats [40]. The binding and detoxification

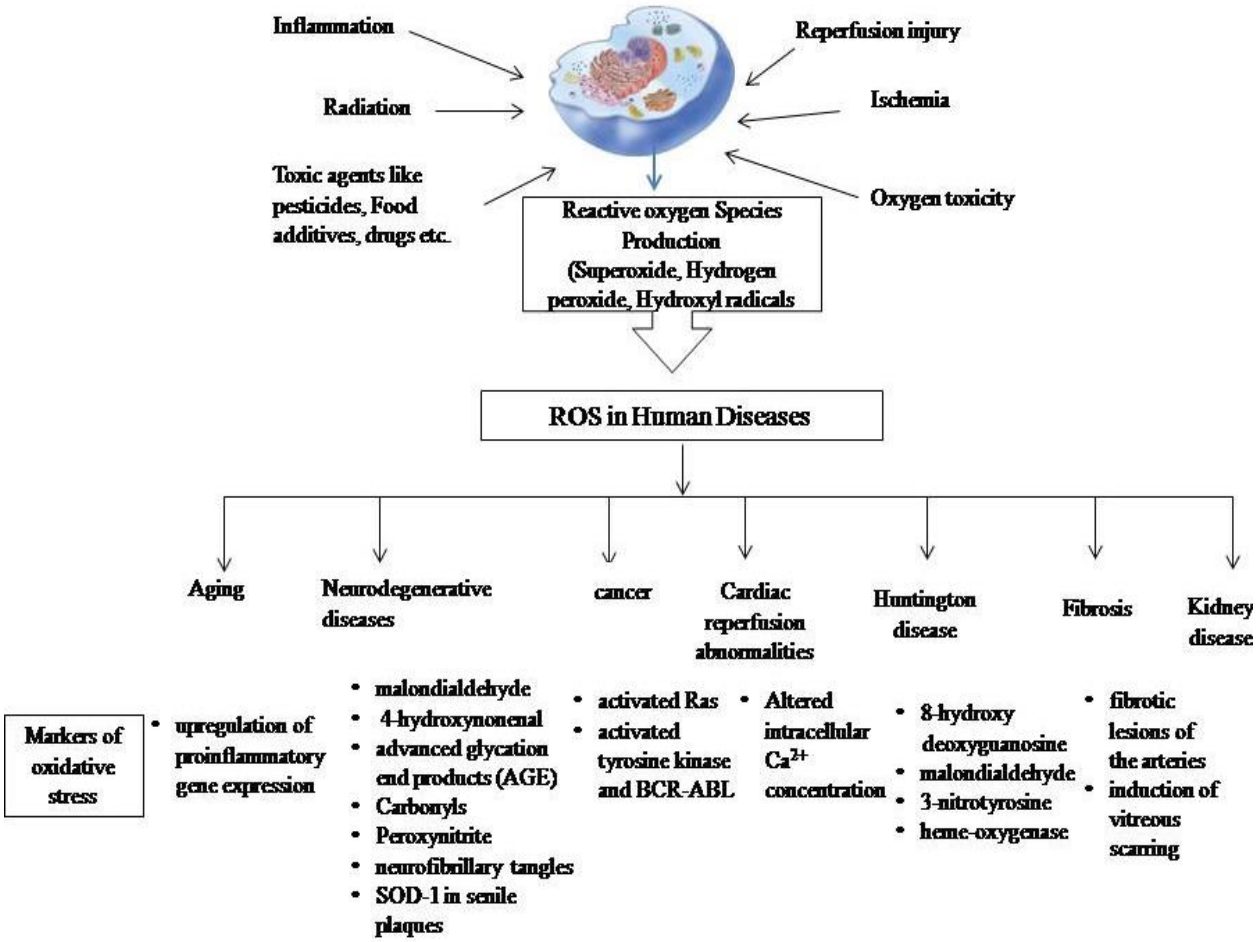

FIGURE 1. Generation of reactive oxygen species (ROS) and its implications in human diseases. Inflammation, ischemia, radiation, reperfusion injuries, and toxic agents (e.g., food additives, pesticides, and drugs) induce a cell to generate ROS including superoxide, hydrogen peroxide, and hydroxyl radicals, among others. ROS are implicated in a variety of human diseases, and each disease is characterized by a set of oxidative stress markers.

of metal ions, free radical scavenging, or increasing antioxidant activity are some of the mechanisms involved in the neuroprotective and memory enhancing effects of extract of *Bacopa monnieri*. *B. monnieri* was reported to act by reducing divalent metals, scavenging ROS, decreasing lipid peroxides formation, and inhibiting lipoxygenase activity. The results showed *B. monnieri* extract-treated neurons expressed a lower level of ROS, suggesting that brahmi could control intracellular oxidative stress [41].

Roshni et al. clearly reported that bacoside A and *B. monnieri* act as potent neuroprotective agents in reversing the altered dopamine D1 receptor function, gene expression, and altered Bax expression due to neonatal hypoglycemia. Free radical accumulation in neonatal hypoglycemia results in decreased level of SOD which in turn causes cortical cell death. Bacoside A and *B. monnieri* supplementation helps to maintain the level of SOD by free radical scavenging and thus overcome the stress by prevention of free radical accumulation [42].

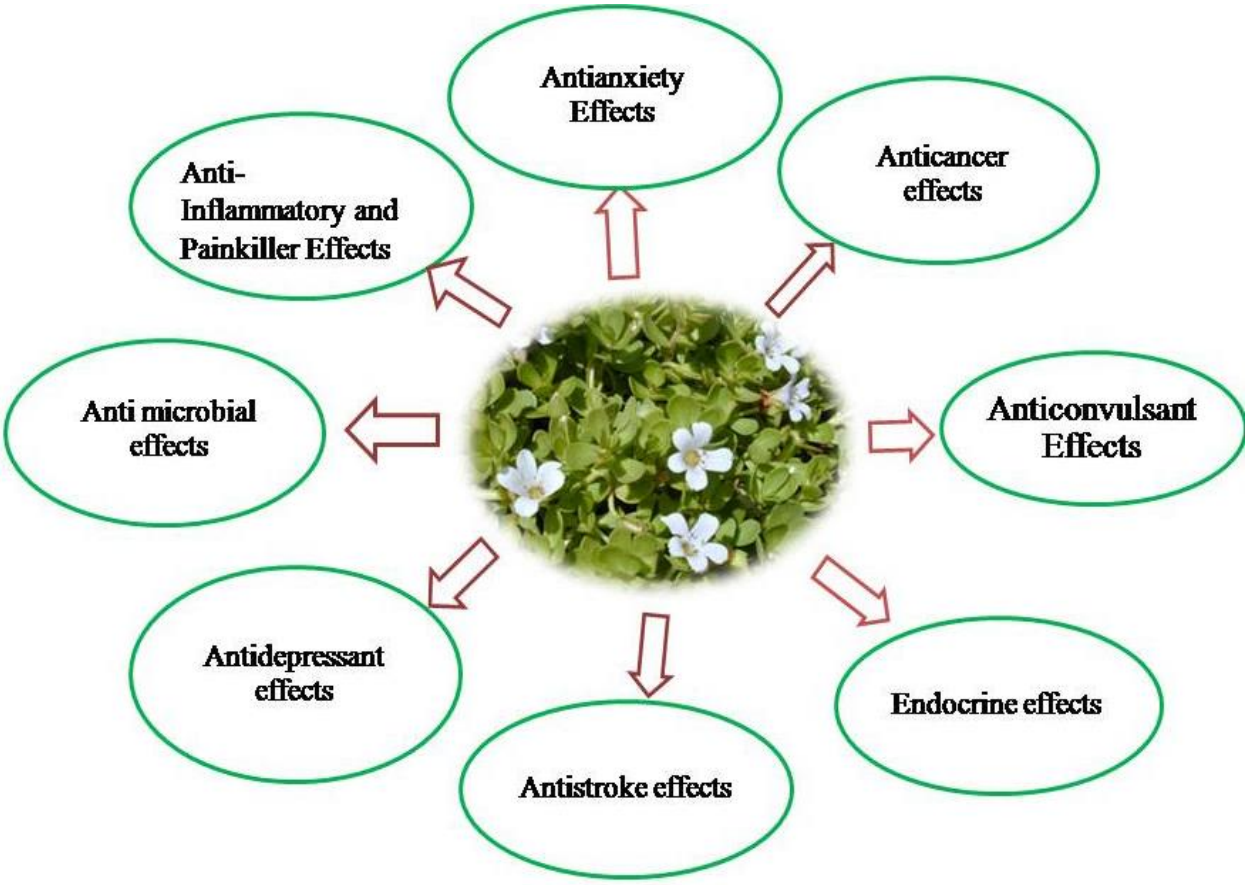

FIGURE 2. Biological effects of *Bacopa monneiri.* Besides neuroprotective function, Ayurveda texts quoted the use of *Bacopa monnieri* in other pathophysiological conditions. Researchers evaluated its antidepressant activity [23], anti- potential [43], anticancer effect [44], anti-inflammatory property [45], anti-stress action [42, 46], bronchodilatory effect [47], anti-convulsive activity [48], endocrine effect [49], and antianxiety effect [50].

6. *Bacopa monnieri* IN PARKINSON'S DISEASE

B. monnieri might be useful in age-related neurodegeneration including PD by maintaining redox homeostasis and mitochondrial activities. Anbarasi et al. [35] assessed the neuroprotective role of bacoside A against oxidative stress in the brains of rats. The researchers administered 10 mg/ml bacoside A through oral gavage daily and found the *B. monnieri* significantly increased brain levels of glutathione, vitamin C, vitamin E, and vitamin A in addition to the increased activities of antioxidant enzymes such as SOD, catalase, glutathione peroxidase (GPx) and glutathione reductase (GSR) in rats exposed to cigarette smoke.

7. *Bacopa monnieri* IN ALZHEIMER'S DISEASE

A dose-related reversal of cognitive deficits was observed by treatment with *B. monnieri* in animal models of neurotoxicity induced by colchicine and ibotenic acid [16]. The neuroprotective effect of standardized extracts of *B. monnieri* against acrolein and H_2O_2 was investigated by Singh et al. Acrolein is a highly reactive compound formed as a byproduct

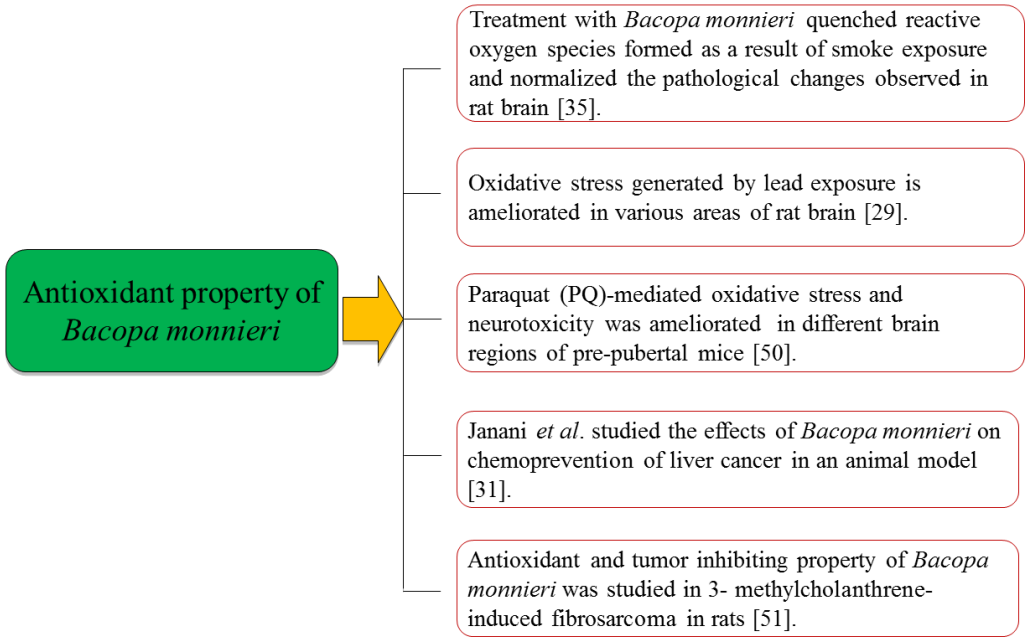

FIGURE 3. Antioxidant property of *Bacopa monnieri* as reported in the literature. The figure depicts the free radical scavenging potential of *Bacopa monnieri* in various disease models such as liver cancer, fibrosarcoma, paraquat-induced oxidative stress, and smoke exposed rats.

of lipid peroxidation. It is not only a marker of lipid peroxidation but also serves as an initiator of oxidative stress by forming adducts of cellular nucleophilic groups. The brains of AD patients showed significantly higher levels of acrolein in vulnerable brain region like hippocampus. Furthermore, it is reported to be more toxic in primary hippocampal culture than 4-hydroxyl-2-nonenal. Actually, hydrogen peroxide (H_2O_2) is the compound which contributes toxicity to the amyloid-β peptide. Neuroprotective effect of *B. monnieri* extract was reported in human neuroblastoma cell line SK-N-SH against H_2O_2- and acrolein-induced toxicity. *B. monnieri* offered cytoprotection through ROS scavenging and maintaining the mitochondrial membrane integrity. *B. monnieri* also modulated expression of several regulatory proteins, such as NF-κB, Sirt1, ERK1/2, and $p66^{Shc}$, so as to favor cell survival in response to oxidative stress.

Stress also promotes free radical generation. The anti-stress effect of bacosides of *B. monnieri*, dissolved in distilled water, was studied in adult male Sprague Dawley rats. The data indicate that *B. monnieri*'s ability to destress involves the modulation of the expression/activities of Hsp70, CytP450, and SOD under adverse conditions such as stress [40].

8. CONCLUDING REMARKS

From the current understanding of *B. monnieri*, it can be concluded that some of the constituents of bacopa act as an efficient free radical scavenger and have the potential to serve as a phytoremedy in the disease conditions involving ROS and oxidative stress (**Figures 2** and **3**). The antioxidant and neuroprotective roles of the individual components of *B. monnieri* need to be explored further which might help in evolution of new drugs.

REFERENCES

1. Gerschman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. Oxygen poisoning and x-irradiation: a mechanism in common. *Science* 1954;

119(3097):623–626.
2. Anand T, Pandareesh MD, Bhat PV, Venkataramana M. Anti-apoptotic mechanism of Bacoside rich extract against reactive nitrogen species induced activation of iNOS/Bax/caspase 3 mediated apoptosis in L132 cell line. *Cytotechnology* 2014; 66(5):823–838. doi: 10.1007/s10616-013-9634-7.
3. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; 82(1):47–95. doi: 10.1152/physrev.00018.2001.
4. Flescher E, Tripoli H, Salnikow K, Burns FJ. Oxidative stress suppresses transcription factor activities in stimulated lymphocytes. *Clin Exp Immunol* 1998; 112(2):242–247.
5. Pahlavani MA, Van Remmen H. New directions for studying the role of free radicals in aging. *Age (Omaha)* 1997; 20(3):151–163. doi: 10.1007/s11357-997-0014-0.
6. Li PF, Dietz R, von Harsdorf R. Reactive oxygen species induce apoptosis of vascular smooth muscle cell. *FEBS Lett* 1997; 404(2–3):249–252.
7. Li PF, Dietz R, von Harsdorf R. Superoxide induces apoptosis in cardiomyocytes, but proliferation and expression of transforming growth factor-beta1 in cardiac fibroblasts. *FEBS Lett* 1999; 448(2–3):206–210.
8. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov* 2009; 8(7):579–591. doi: 10.1038/nrd2803.
9. Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? *Nat Med* 2004; 10 Suppl:S18–25. doi: 10.1038/nrn1434.
10. Shukla V, Mishra SK, Pant HC. Oxidative stress in neurodegeneration. *Adv Pharmacol Sci* 2011; 2011:572634. doi: 10.1155/2011/572634.
11. Paravicini TM, Touyz RM. Redox signaling in hypertension. *Cardiovasc Res* 2006; 71(2):247–258. doi: 10.1016/j.cardiores.2006.05.001.
12. Haigis MC, Yankner BA. The aging stress response. *Mol Cell* 2010; 40(2):333–344. doi: 10.1016/j.molcel.2010.10.002.
13. Anand T, Prakash KB, Pandareesh MD, Khanum F. Development of bacoside enriched date syrup juice and its evaluation for physical endurance. *J Food Sci Technol* 2014; 51(12):4026–4032. doi: 10.1007/s13197-013-0955-5.
14. Srivastava P, Raut HN, Puntambekar HM, Desai AC. Stability studies of crude plant material of Bacopa monnieri and quantitative determination of bacopaside I and bacoside A by HPLC. *Phytochem Anal* 2012; 23(5):502–507. doi: 10.1002/pca.2347.
15. Deepak M, Amit A. Bacoside B: the need remains for establishing identity. *Fitoterapia* 2013; 87:7–10. doi: 10.1016/j.fitote.2013.03.011.
16. Singh R, Ramakrishna R, Bhateria M, Bhatta RS. In vitro evaluation of *Bacopa monniera* extract and individual constituents on human recombinant monoamine oxidase enzymes. *Phytother Res* 2014; 28(9):1419–1422. doi: 10.1002/ptr.5116.
17. Rauf K, Subhan F, Al-Othman AM, Khan I, Zarrelli A, Shah MR. Preclinical profile of bacopasides from *Bacopa monnieri* (BM) as an emerging class of therapeutics for management of chronic pains. *Curr Med Chem* 2013; 20(8):1028–1037.
18. Kapoor R, Srivastava S, Kakkar P. Bacopa monnieri modulates antioxidant responses in brain and kidney of diabetic rats. *Environ Toxicol Pharmacol* 2009; 27(1):62–69. doi: 10.1016/j.etap.2008.08.007.
19. Deepak M, Amit A. The need for establishing identities of bacoside A and B, the putative major bioactive saponins of Indian medicinal plant *Bacopa monnieri*. *Phytomedicine* 2004; 11(2–3):264–268. doi: 10.1078/0944-7113-00351.
20. Anand T, Naika M, Swamy MSL, Khanum F. Antioxidant and DNA damage preventive properties of *Bacopa monniera* (L) Wettst. *Free Radic Antioxid* 2011; 1:84–90. doi: 10.5530/ax.2011.1.13.
21. Tripathi YB, Chaurasia S, Tripathi E, Upadhyay A, Dubey GP. *Bacopa monniera* Linn. as an antioxidant: mechanism of action. *Indian J Exp Biol* 1996; 34(6):523–526.
22. Bhattacharya SK, Bhattacharya A, Kumar A, Ghosal S. Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum and hippocampus. *Phytother Res* 2000; 14(3):174–179.
23. Sairam K, Dorababu M, Goel RK, Bhattacharya SK. Antidepressant activity of standardized

extract of *Bacopa monniera* in experimental models of depression in rats. *Phytomedicine* 2002; 9(3):207–211. doi: 10.1078/0944-7113-00116.
24. Sairam K, Rao CV, Babu MD, Goel RK. Prophylactic and curative effects of *Bacopa monniera* in gastric ulcer models. *Phytomedicine* 2001; 8(6):423–430. doi: 10.1078/S0944-7113(04)70060-4.
25. Sumathy T, Govindasamy S, Balakrishna K, Veluchamy G. Protective role of *Bacopa monniera* on morphine-induced brain mitochondrial enzyme activity in rats. *Fitoterapia* 2002; 73(5):381–385.
26. Russo A, Borrelli F, Campisi A, Acquaviva R, Raciti G, Vanella A. Nitric oxide-related toxicity in cultured astrocytes: effect of *Bacopa monniera*. *Life Sci* 2003; 73(12):1517–1526.
27. Peth-Nui T, Wattanathorn J, Muchimapura S, Tong-Un T, Piyavhatkul N, Rangseekajee P, et al. Effects of 12-week *Bacopa monnieri* consumption on attention, cognitive processing, working memory, and functions of both cholinergic and monoaminergic systems in healthy elderly volunteers. *Evid Based Complement Alternat Med* 2012; 2012:606424. doi: 10.1155/2012/606424.
28. Radha P. *Antioxidant Responses Evoked in Vitro and in Vivo by Bacopa monnieri Leaf Extracts*. Dessertation. Avinashilingam University, India. 2010.
29. Velaga MK, Basuri CK, Robinson Taylor KS, Yallapragada PR, Rajanna S, Rajanna B. Ameliorative effects of Bacopa monniera on lead-induced oxidative stress in different regions of rat brain. *Drug Chem Toxicol* 2014; 37(3):357–364. doi: 10.3109/01480545.2013.866137.
30. Sharath R, Harish BG, Krishna V, Sathyanarayana BN, Swamy HM. Wound healing and protease inhibition activity of Bacoside-A, isolated from *Bacopa monnieri* wettest. *Phytother Res* 2010; 24(8):1217–1222. doi: 10.1002/ptr.3115.
31. Janani P, Sivakumari K, Geetha A, Ravisankar B, Parthasarathy C. Chemopreventive effect of bacoside A on N-nitrosodiethylamine-induced hepatocarcinogenesis in rats. *J Cancer Res Clin Oncol* 2010; 136(5):759–770. doi: 10.1007/s00432-009-0715-0.
32. Bhattacharya SK, Kumar A, Ghosal S. Effect of *Bacopa monniera* on animal models of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. *Res Commun Pharmacol Toxicol* 1999; 4(3&4):1–12.
33. Prakash NS, Sundaram R, Mitra SK. In vitro and in vivo anticancer activity of bacoside a from whole plant of *Bacopa Monnieiri* (Linn). *Am J Pharmacol Toxicol* 2011; 6(1):11–19. doi: 10.3844/ajptsp.2011.11.19.
34. Peng L, Zhou Y, Kong de Y, Zhang WD. Antitumor activities of dammarane triterpene saponins from *Bacopa monniera*. *Phytother Res* 2010; 24(6):864–868. doi: 10.1002/ptr.3034.
35. Anbarasi K, Sabitha KE, Devi CS. Lactate dehydrogenase isoenzyme patterns upon chronic exposure to cigarette smoke: protective effect of bacoside A. *Environ Toxicol Pharmacol* 2005; 20(2):345–350. doi: 10.1016/j.etap.2005.03.006.
36. Manoharan M, Padma PR. Effect of the bacoside fraction from *bacopa monnieri* against oxidative stress-induced apoptosis in untransformed (buccal) and transformed (kb oral carcinoma) cells. *IJPRBS* 2016; 5(2):75–88.
37. Saraf MK, Prabhakar S, Khanduja KL, Anand A. *Bacopa monniera* attenuates scopolamine-induced impairment of spatial memory in mice. *Evid Based Complement Alternat Med* 2011; 2011:236186. doi: 10.1093/ecam/neq038.
38. Mukherjee S, Dugad S, Bhandare R, Pawar N, Jagtap S, Pawar PK, et al. Evaluation of comparative free-radical quenching potential of Brahmi (*Bacopa monnieri*) and Mandookparni (*Centella asiatica*). *Ayu* 2011; 32(2):258–264. doi: 10.4103/0974-8520.92549.
39. Stough CK, Pase MP, Cropley V, Myers S, Nolidin K, King R, et al. A randomized controlled trial investigating the effect of Pycnogenol and Bacopa CDRI08 herbal medicines on cognitive, cardiovascular, and biochemical functioning in cognitively healthy elderly people: the Australian Research Council Longevity Intervention (ARCLI) study protocol (ANZCTR12611000487910). *Nutr J* 2012; 11:11. doi: 10.1186/1475-2891-11-11.
40. Chowdhuri DK, Parmar D, Kakkar P, Shukla R, Seth PK, Srimal RC. Antistress effects of bacosides of *Bacopa monnieri*: modulation of Hsp70 expression, superoxide dismutase and

cytochrome P450 activity in rat brain. *Phytother Res* 2002; 16(7):639–645. doi: 10.1002/ptr.1023.

41. Limpeanchob N, Jaipan S, Rattanakaruna S, Phrompittayarat W, Ingkaninan K. Neuroprotective effect of *Bacopa monnieri* on beta-amyloid-induced cell death in primary cortical culture. *J Ethnopharmacol* 2008; 120(1):112–117. doi: 10.1016/j.jep.2008.07.039.
42. Thomas RB, Joy S, Ajayan MS, Paulose CS. Neuroprotective potential of *Bacopa monnieri* and Bacoside A against dopamine receptor dysfunction in the cerebral cortex of neonatal hypoglycaemic rats. *Cell Mol Neurobiol* 2013; 33(8):1065–1074. doi: 10.1007/s10571-013-9973-0.
43. Rao CV, Sairam K, Goel RK. Experimental evaluation of *Bocopa monniera* on rat gastric ulceration and secretion. *Indian J Physiol Pharmacol* 2000; 44(4):435–441.
44. Elangovan V, Govindasamy S, Ramamoorthy N, Balasubramanian K. In vitro studies on the anticanceractivity of *Bacopa monnieri*. *Fitoterapia* 1995; 66:211–215.
45. Jain P, Khanna NK, Trehan N, Pendse VK, Godhwani JL. Antiinflammatory effects of an Ayurvedic preparation, Brahmi Rasayan, in rodents. *Indian J Exp Biol* 1994; 32(9):633–636.
46. Rai D, Bhatia G, Palit G, Pal R, Singh S, Singh HK. Adaptogenic effect of *Bacopa monniera* (Brahmi). *Pharmacol Biochem Behav* 2003; 75(4):823–830.
47. Dar A, Channa S. Bronchodilatory and cardiovascular effects of an ethanol extract of *Bacopa monniera* in anaesthetized rats. *Phytomedicine* 1997; 4(4):319–323. doi: 10.1016/S0944-7113(97)80040-2.
48. Shanmugasundaram ER, Akbar GK, Shanmugasundaram KR. Brahmighritham, an Ayurvedic herbal formula for the control of epilepsy. *J Ethnopharmacol* 1991; 33(3):269–276.
49. Kar A, Panda S, Bharti S. Relative efficacy of three medicinal plant extracts in the alteration of thyroid hormone concentrations in male mice. *J Ethnopharmacol* 2002; 81(2):281–285.
50. Singh RH, Singh L. Studies on the anti-anxiety effect of the Medyha Rasayana drug, Brahmi (*Bacopa Monniera* (L.) Pennell Wettst.) – Part 1. *J Res Ayur Siddha* 1980; 1:133–148.
51. Hosamani R, Krishna G, Muralidhara. Standardized *Bacopa monnieri* extract ameliorates acute paraquat-induced oxidative stress, and neurotoxicity in prepubertal mice brain. *Nutr Neurosci* 2016; 19(10):434–446. doi: 10.1179/1476830514Y.0000000149.