ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 6, Issue 3, 2013

ISSN - 0974-2441

Research Article

PROPHYLACTIC EFFICACY OF *BACOPA MONNIERI* ON DECABROMODIPHENYL ETHER (PBDE-209)-INDUCED ALTERATIONS IN OXIDATIVE STATUS AND SPATIAL MEMORY IN MICE

PRIYA VERMA, POONAM SINGH, BEHROSE S. GANDHI*

Department of Zoology, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi-221005, India. Email: bsgandhi.bhu@gmail.com

Received: 15 April 2013, Revised and Accepted: 18 may 2013

ABSTRACT

Bacopa monnieri (BM) is extensively used as a nerve tonic in traditional medicine systems, originated from India and neighbouring countries. BM possesses antioxidative and memory enhancing properties. The present study has been designed to investigate the neuroprotective role of BM against PBDE-209-induced alterations in spatial memory and oxidative status in the frontal cortex (FC) and hippocampus (Hc) of mice. The mice were orally administered with PBDE-209 at the dose of 20 mg/kg body weight (bw) from postnatal day (PND) 3-10. BM at the doses of 40, 80 or 120 mg/kg bw were co-administered with 20 mg/kg of PBDE-209 from PND 3-10. The spatial memory of young mice was evaluated by Morris water maze (MWM) and radial arm maze (RAM). We also tested the levels of cellular oxidants e.g. malondialdehyde (MDA), protein carbonyl (PC) and and the activities of antioxidant enzymes e.g. superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) in the FC and Hc of mice at neonate (PND 11) and young age (PND 60). Supplementation with BM significantly restored the levels of MDA and protein carbonyls in FC and Hc, which were elevated significantly after PBDE-209 exposure. PBDE-209-induced decrease in the activities of antioxidant enzymes recovered significantly following supplementation with BM. Further, BM-supplementation resulted in significant improvement in the working and reference memory of PBDE-209-exposed mice. The findings, therefore, suggest the neuroprotective efficacy of BM against PBDE-209-induced impairments in spatial memory and oxidative status.

Keywords: *Bacopa monnieri*; neuroprotection; oxidative stress; PBDE-209; spatial memory.

INTRODUCTION

Bacopa monnieri (Brahmi), a nootropic plant, belonging to family Scrophulariaceae, is a creeping annual herb found in wet, damp and marshy areas of tropical regions. BM has been an important constituent of the ¨ayurvedic materia medica¨ and is mentioned in Charaka Samhita (compilation of Charaka around 6th century ad), Bhavprakasa (16th century ad). It is used for the treatment of oxidative stress, epilepsy and memory impairment for centuries[1,2]. The presence of the active saponins like bacosides A and B in BM are highly responsible for its pharmacological properties [3,4]. The bacoside repairs the damaged neurons by enhancing the kinase activity and neuronal synthesis coupled with restoration of synaptic activity thereby enhancing the nerve impulse transmission[2]. These mechanisms of action could be partly responsible for its memory facilitation activity. Recently, BM has been reported to exert protective effect on scopalamine-induced amnesia[5]. Further, BM extract has shown to inhibit multiple components of the β-amyloid-induced oxidative stress pathway that can contribute to Alzheimer's pathology [6]. BM also plays antioxidant and antistress activities in rat by modulating the activities of Hsp 70, P450 and SOD[1,7,8]. It has been involved in antioxidant defence through scavenging of free radicals, improving activities of antioxidative enzymes, chelating of metal ions and breaking oxidative chain reaction [9,10]. A recent study has reported that BM extract plays a neuroprotective role against aluminium-induced oxidative stress in the hippocampus of rat brain[8]. Moreover, protective role of bacoside A against chronic cigarette-induced oxidative damage is reported in rat brain [7]. Despite the extensive uses of BM plant, its extracts and isolated bacosides, studies related to its prophylactic efficacy against PBDE-induced dysfunctions are rare.

Polybromininated diphenyl ethers (PBDEs) are the group of additive flame retardants that have caused great concern in recent years due to their increasingly detectable levels in the environment and in humans[11]. 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (PBDE-209), one of the major congener of PBDEs, is a persistent organic pollutant , which can bioaccumulate in the environment and biomagnify up the food chain [12]. PBDE-209 can be extensively metabolized and debrominated down to lower brominated congeners in animal bodies, so the potential toxicity of PBDE-209 may be higher than that of the lower PBDEs [13, 14]. Furthermore, recent reports indicate the presence of higher level of PBDE-209 in infants and toddlers than that of adults [15]. Therefore, PBDE-209-induced effects must be properly evaluated for their potential toxicity during neonatal period especially in the brain. Brain is one of the most affected organs by bioaccumulant toxins due to its high lipid content and high energy requirements, especially during neonatal period in mice and third trimester in humans, called brain growth spurt period [16, 17]. During this period, rapid growth and various remarkable changes occur in the brain. Neuronal oxidative damage might be one of the primary mechanisms of neurotoxicity caused by PBDEs [18]. Recently, N-acetylcysteine (anti-oxidant) has been reported to attenuate the PBDE-209-induced apoptosis, alterations in the expression of p38 MAPK, in the calcium ion concentration and in the ROS level in hippocampal neurons *in vitro*[19]. Therefore, it is worth to analyze the role of BM against PBDE-209-induced alterations in the brain. The aim of the present study is to evaluate the neuroprotective effects of BM against PBDE-209-induced impairments in spatial memory. Neuroprotective efficacy of BM against acute and long-term effects of PBDE-209 on oxidative stress markers in FC and Hc of neonate and young mice have also been evaluated.

MATERIALS AND METHODS

Chemicals

The standardized ethanolic extract of BM (BESEB CDRI-08), containing 58.18% Bacosides, is generously gifted by Dr. H. K. Singh, Ex-director, Central Drug Research Institute, Lucknow, India [20]. The chemical profiling of the plant is done by Lumen Marketing Company and Research Foundation, Chennai, India. PBDE-209 (98%, CAS no. 1163-19-5) was obtained from Aldrich-Chemie. Corn oil was purchased from Sigma (St. Louis, MO, USA) while rest of the chemicals was purchased from Aldrich-Chemie, Sigma, Merck and

Sisco Research Laboratory (India). PBDE-209 was dissolved in corn oil whereas ethanolic extract of BM was suspended in 5% tween 80.

Animals

Male and female adult Swiss albino mice weighing 25–30 g were maintained in an animal house as per the recommendations from central animal ethical committee of the university (CAECU) for the care and use of laboratory animals. The animals were maintained at ambient temperature at 12L/12D cycle. They were fed with standardized pelleted food and tap water *ad libitum*. Two females were housed with one male for breeding. Females were examined every morning to observe the formation of a vaginal plug. The vaginal plug-positive females were caged individually. The day of litter born was designated PND 0. The size of the litter was adjusted as much as possible in order to obtain litters of the same size (6-8 pups).

Experimental Design

At PND 0, male pups within the same litter were randomly assigned to five treatment groups of twenty eight each:

Group I: Control
Group II: 20 mg/kg bw of PBDE-209
Group III: 20 mg/kg bw of PBDE-209+40 mg/kg bw of BM
Group IV: 20 mg/kg bw of PBDE-209+80 mg/kg bw of BM
Group V: 20 mg/kg bw of PBDE-209+120 mg/kg bw of BM

All the treatments were given orally via a micropipette with 100 µl microtip at a volume of 5.0 µl/gm bw of pups from PND 3-10. The pups of each group were divided into two subgroups I and II, comprising of 7 and 21 pups respectively. Seven pups from both subgroups were sacrificed on PND 11 and 60 for biochemical analyses. Seven pups of subgroup II were used for Morris water maze study from PND 60-66 while in rest of the 7 pups, radial arm maze study was conducted from PND 60-74. FC and Hc were collected and stored at -80°C for biochemical analyses as these two brain regions are involved in spatial memory function[21].

Preparation of tissue homogenate

Homogenates of FC and Hc were prepared in 50 mM phosphate buffer (pH 7.0) containing 1 mM phenylmethanesulfonylfluoride and centrifuged at 10,000 g for 10 min at 4°C. The supernatants were aliquoted and stored at −80°C to measure the levels of MDA and PC and the activities of SOD and GSH-Px. Protein content was determined by the method of Bradford with minor modifications using bovine serum albumin as standard (E. Merck, Darmstadt, Germany) [22,23].

Biochemical Analyses

Lipid peroxidation assay

Lipid peroxidation, an indicator of oxidative stress, was determined by measuring the MDA level, adopting the method of Ohkawa et al.[24] with small modifications. Absorbance was determined at 532 nm. MDA was measured by using a molar absorption coefficient (ε) of 1.56×10^5 $M^{-1} \cdot cm^{-1}$. All the results were expressed as nmoles of MDA (malondialdehyde)/ mg protein.

Protein carbonylation assay

Carbonylated protein concentration was determined according to the method of Levine et al.[25]. The absorbance was measured at 366 nm against the appropriate blank sample. Carbonylated protein content was determined by using a molar absorption coefficient (ε) of 22000 $M^{-1} \cdot cm^{-1}$. The amount of protein carbonyl groups was expressed as nmoles of CO/mg of protein.

In-gel activity assay of antioxidant enzymes

Superoxide dismutase

In-gel activity assay of SOD was performed by non-denaturing polyacrylamide gel electrophoresis (PAGE), followed by the method of Beauchamp et al.[26] with minor modifications. Equal amounts of protein from each sample were separated by PAGE using a 10% non-denaturing gel at 4°C. After electrophoresis, the gel was submerged in 2.5 mM Nitro Blue Tetrazolium, 28 µM riboflavin, and 28 mM N, N, N, N-tetramethylethylenediamine. After 20 min incubation in the dark, gels were exposed to a fluorescent light to develop achromatic bands against dark blue background corresponding to SOD protein in the gel. The intensity of bands was analyzed by densitometric scanning using an Alpha Image Analyser System (Alpha Innotech).

Glutathione peroxidase

In-gel activity assay of GSH-Px was performed by non-denaturing PAGE, followed by the method of Lin et al. [27] with minor modifications. Equal amounts of protein from each sample were separated by PAGE using a 10% non-denaturing gel at 4°C. After electrophoresis the gel was submerged in 50 mM Tris/HCl buffer (pH 7.9) containing 13 mM GSH and 0.01% H_2O_2 with gentle shaking for 20 min at room temperature (25°C). The gel was stained with solution containing 1.2 mM NBT and 1.6 mM phenazine methosulfate (PMS) for 20 min at room temperature in the dark and then exposed to bright light until the appearance of clear zone of GSH-Px bands with purple background. The intensity of bands was analyzed by densitometric scanning using an Alpha Image Analyser System (Alpha Innotech).

Spatial memory behavioral tests

Morris water maze

Modified Morris water maze [28] has been used to evaluate the memory deficit caused by PBDE-209. In brief, a black-painted circular water tank (diameter: 122 cm, height: 51 cm) was divided into four equal quadrants. The tank was filled with water upto the height of 31 cm. A square platform (area: 10 cm^2, height: 30 cm), placed in the center of one of these four quadrants was typically submerged 1.0 cm below the water surface filled. The position of the platform was kept fixed throughout the training session. In the present study, the quadrant Q2 was taken as a target. The four consecutive trials were given to each animal on each day during which they were allowed to board the hidden platform and allowed to remain there for 10 sec. When the animal was unable to locate the hidden platform in the 120 sec period, it was gently guided to the platform and to remain there for 10 sec. Escape latency time (ELT), as a quantitative measure to locate the hidden platform in water maze, was recorded as an index of acquisition. Each animal was subjected to four acquisition trials per day for 6 consecutive days. On the seventh day, the platform was removed, and the time spent by the animal in each of the four quadrants (Q1, Q2, Q3, and Q4) was noted. The time spent by the animal in the target quadrant (Q2) while searching for the hidden platform was recorded as an index of probe trial.

Radial arm maze

The Radial arm maze [29] consisted of a round central platform (40 cm) elevated 50 cm above the floor, with eight radiating arms 32 cm long and 5.0 cm wide attached to it at equal distances from each other. Each arm forms a corridor leading to an 8.0 cm square platform. A small cup, 1.0 cm in diameter, embedded in each platform, contained a hidden reward. The surrounding wall of the room had several extra maze cues. During pre-training, each mouse was placed on the platform and allowed to explore the paths and consume food scattered in the whole maze for 10 minutes. This pre-training procedure was repeated for 3 consecutive days. The actual training procedure started on the fourth day. Before each trial, four arms were baited with a gram placed in each food cup. The arms chosen to be baited must be the same for a mouse. The mice ran a trial per day during 12 consecutive days and the two consecutive days considered as a session block (session block 1st: days 1-2, 2nd: days 3-4, 3rd: days 5-6, 4th: days 7-8, 5th: 9-10 and 6th: days 11-12). On each trial, the mice were placed on the central platform and allowed to make choices. The trial ended when the animal visited all baited arms or made 8 visits or the trial lasted for more than 10 min. The following data were recorded (1) the number of correct entries into baited arms (2) the number of entries into unbaited arms (reference memory error) and (3) the number of re-entries into baited arms (working memory error).

Statistical Analysis

Statistical evaluations were done with one-way analysis of variance (ANOVA), using SPSS (16.0) software. All the analyses were followed by post hoc Tukey HSD (honestly significant difference) test [30]. A difference of $p < 0.05$ was considered statistically significant.

RESULTS

Lipid peroxidation and protein carbonylation

The levels of MDA (Fig. 1A and 1B) and PC (Fig. 2A and 2B) were significantly elevated in FC and Hc of PBDE-exposed neonate and young mice as compared with their respective controls ($p < 0.05$). However, administration of various doses of BM (40, 80 and 120 mg/kg bw) along with PBDE-209 showed that only maximum dose of BM (120 mg/kg bw) was effective in reverting back the levels of MDA (Fig. 1A and 1B) and PC (Fig. 2A and 2B) in both regions of the brain to the control values.

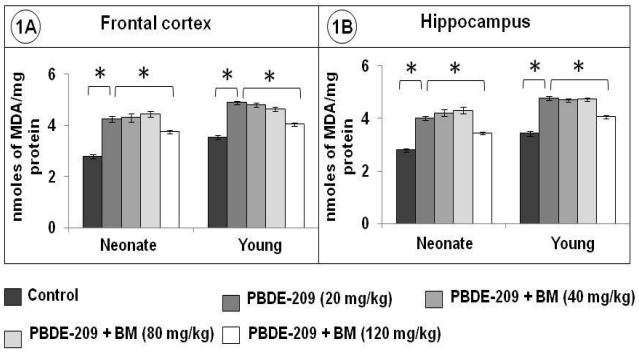

Figure 1: It shows the effect of *Bacopa monnieri* (40, 80 and 120 mg/kg bw) against PBDE-209 (20 mg/kg bw) on the levels of MDA in frontal cortex (1A) and hippocampus (1B). The units of lipid peroxidation (mean ±SEM) are expressed as nmoles malondialdehyde produced per mg protein *p < 0.05, control vs experimental groups.

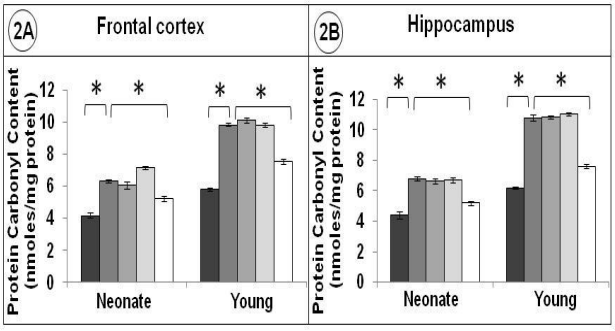

Figure 2: It shows the effect of *Bacopa monnieri* (40, 80 and 120 mg/kg bw) against PBDE-209 (20 mg/kg bw) on the levels of protein carbonyls in frontal cortex (2A) and hippocampus (2B). The units of protein carbonylation (mean ± SEM) are expressed as nmoles protein carbonyl produced per mg protein. *p < 0.05, control vs experimental groups.

SOD and GSH-Px activities

The activities of SOD (Fig. 3A and 3B) and GSH-Px (Fig. 4A and 4B) were significantly reduced in FC and Hc of PBDE-exposed neonate and young mice as compared with their r espective controls ($p < 0.05$). However, administration of BM, only at the maximum dose (120mg/kg bw) in PBDE-209-exposed mice, caused significant increase ($p < 0.05$) in the activities of SOD (Fig. 3A and 3B) and GSH-Px (Fig. 4A and 4B) in both regions of the brain, attaining the values of control.

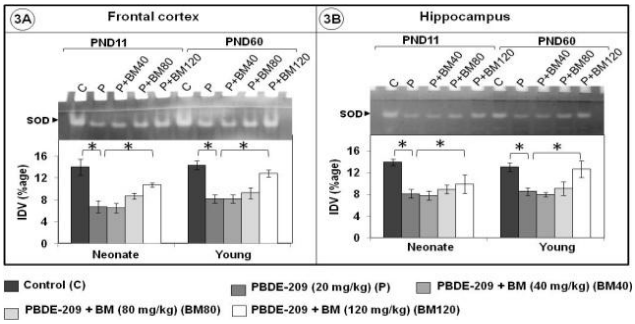

Figure 3: It shows the effect of *Bacopa monnieri* (40, 80 and 120 mg/kg bw) against PBDE-209 (20 mg/kg bw) on the activity of SOD in frontal cortex (3A) and hippocampus (3B). The gel photographs are representative of three independent SOD in-gel activity assays. The histograms are representative of integrated densitometric values (IDV) of SOD bands. Results presented as mean ± SEM from the 3 independent sets of experiments. *p < 0.05, control vs experimental groups.

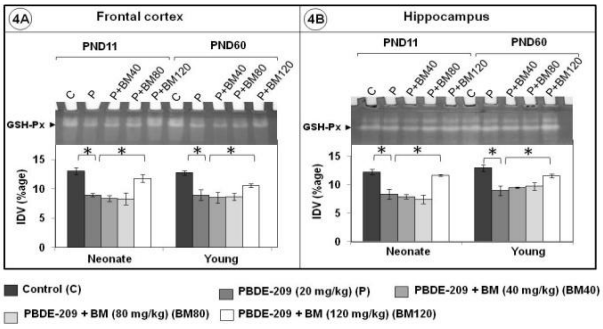

Figure 4: It shows the effect of *Bacopa monnieri* (40, 80 and 120 mg/kg bw) against PBDE-209 (20 mg/kg bw) on the activity of GSH-Px in frontal cortex (4A) and hippocampus (4B). The gel photographs are representative of three independent GSH-Px in-gel activity assays. The histograms are representative of integrated densitometric values (IDV) of GSH-Px bands. Results presented as mean ± SEM from the 3 independent sets of experiments. *p < 0.05, control vs experimental groups.

Morris water maze test

Mice of control group showed a significant decrease in the ELT (ELT on day 1 vs ELT on day 2-6: $p < 0.05$; Fig. 5A) while increase in the time spent in the target quadrant (time spent in Q2 vs time spent in other three quadrants: $p < 0.05$; Fig. 5B) during the probe trial at day 7. PBDE-209 exposure also produced significant decrease in the ELT during successive acquisition trials (ELT on day 1 vs ELT on day 2-6: $p < 0.05$; Fig. 5A) and in the time spent in the target quadrant (Q2) during the probe trial similar with the control group (time spent in Q2 vs time spent in other three quadrants: $p < 0.05$; Fig. 5B). ELT of mice exposed with PBDE-209 showed a significant decrease in successive acquisition trials during day 5 and 6, as compared with the control (Fig. 5A). A significant decrease was noticed in the time spent in the target quadrant after exposure with both the doses of PBDE-209 (time spent in Q2 vs time spent in other three quadrants: $p < 0.05$; Fig. 5B). BM at the dose of 120 mg/kg bw reverted back the PBDE-209-induced impairment of ELT with acquisition trials as compared with PBDE-209-exposed group (Fig. 5A). These mice also spent more time in target quadrant (Q2) as compared with PBDE-209-exposed mice during retrieval trial (Fig. 5B).

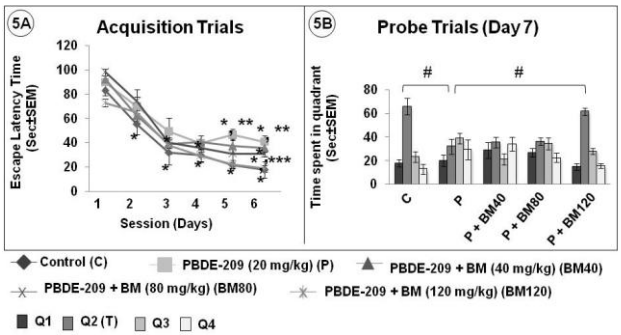

Figure 5: It shows the effect of *Bacopa monnieri* (40, 80 and 120 mg/kg bw) against PBDE-209 (20 mg/kg bw) on the acquisition (5A) and probe trial (5B) in mice at PND 60 by Morris water maze. In acquisition trials, each value represents mean±SEM. (*) indicates significance at p < 0.05 of the particular day's Escape Latency Time (ELT) i.e., ELT of days 2 to 6 vs ELT on day 1. (**) indicates p < 0.05 of PBDE-209 vs ELT of control group for the same day. (***) indicates p < 0.05 of BM dose vs ELT of PBDE-209 group for the same day. During probe trials, the histogram shows effect of PBDE-209 on retrieval of memory as compared to control male mice and each value represents mean±SEM. (#) indicates p < 0.05 vs the control group's time spent in the target quadrant (Q2).

Radial arm maze test

Control mice showed a significant increase in the percentage of correct choices (trial on session block 1st vs trial on session block 2nd-6th: p < 0.05; Fig. 6A) while a significant decrease in reference and working memory (trial on session block 1st vs trial on session block 2nd-6th: p < 0.05; Fig. 6B and 6C). In PBDE-209-treated group, significant changes were observed in the percentage of correct choices on session block 6th (p < 0.05; Fig. 6A), in reference memory error on session block 5th (p < 0.05; Fig. 6B) and in working memory error on session block 4th (p < 0.05; Fig. 6C), as compared with same session blocks of the controls. However, BM at the dose of 120 mg/kg bw significantly reverted back PBDE-209-induced depression in the percentage of correct choices (Fig. 6A) and elevation in the reference (Fig. 6B) and working memory (Fig. 6C) in comparison to PBDE-209-exposed mice.

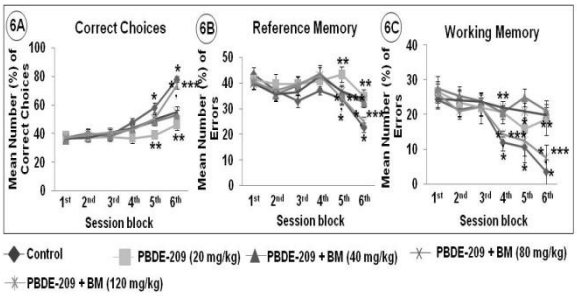

Figure 6: It shows the effect of *Bacopa monnieri* (40, 80 and 120 mg/kg bw) against PBDE-209 (20 mg/kg bw) on the percentage of correct choices (6A), reference memory error (6B) and working memory error (6C) in mice at PND 60 by radial arm maze. The average of two consecutive days considered as a session block. Data shows as mean±SEM. (*) indicates significance at p < 0.05 (trial on session block 1st vs trial on session blocks 2nd-6th), (**) indicates significance at p < 0.05 of PBDE-209 vs control group for the same session block and (***) indicates significance at p < 0.05 of BM dose vs PBDE-209 group for the same session block.

DISCUSSION

Brain is prone to free-radical damage due to its high level of oxygen metabolism and the presence of large amount of oxidant-sensitive polyunsaturated fatty acids in the membrane. Brain is also relatively deficient in both free radical scavenging enzymes and endogenous antioxidants as compared to other organs [16]. The present study was designed to investigate the role of *Bacopa monnieri* against PBDE-209-induced impairments in spatial memory and alterations in the levels of cellular oxidants e.g. MDA, PC and the activities of antioxidant enzymes e.g. SOD and GSH-Px in the FC and Hc.

Our results showed that postnatal exposure of PBDE-209 disrupted spatial memory of young mice. PBDE-209-exposed mice demonstrated spatial memory impairment compared to the control group as seen in their increased latencies to find the hidden platform during acquisition trial in Morris water maze test. During retrieval trial, PBDE-209-exposed mice spent less time in Q2 in comparison to control. Similarly, the percentage of correct choices decreased significantly while percentage of reference and working memory error were increased in radial arm maze test. Overall, working and reference memory were impaired in young mice following postnatal exposure of PBDE-209. The alterations in the spontaneous behaviour in young mice are reported following neonatal exposure of PBDE-209. According to this author other congeners of PBDE (PBDE-99 and 153) and PCBs can also affect learning and memory behavior and cholinergic system of adult mice and rats[31]. Further, Liu and others [32] have suggested that decline in spatial memory is associated with a significant increase in the levels of lipid peroxidation and protein oxidation suggesting the oxidative stress in the brain. We have found the increased levels of MDA and PC in PBDE-209-exposed mice at neonate and young age. These are secondary products of lipid peroxidation and protein carbonylation. Both are the important markers of oxidative stress and have been shown to catalyze the process of oxidative insult to membranes causing diminished specific lipid and protein functions [33]. Oxidative modification of proteins in vivo may affect a variety of cellular functional proteins like receptors, signal transduction mechanisms, transport systems, and enzymes [34]. The increased accumulation of these by-products (MDA and PC) as noticed in our study strongly reflects PBDE-209-inflicted oxidative damage.

These results support the hypothesis that oxidative stress contributes to PBDE-209-induced impairment in the spatial learning and memory. As oxidative stress is mediated by free radicals, it becomes therefore, necessary to investigate the status of endogenous antioxidant enzymes. SOD and GSH-Px present the first line of defense against free radical damage under oxidative stress conditions [35]. We have observed the significantly reduced activity of antioxidant enzymes like SOD and GSH-Px in PBDE-209 exposed mice as compared with the control. The decreased activity might have resulted from oxidative modification of proteins. The lower activity of natural antioxidants resulted in a decrease of antioxidant versus oxidant ratio. Usually, the decreased antioxidant versus oxidant ratio plays a crucial role in generating a condition of oxidative stress[36]. PBDE-209 increases reactive oxygen species (ROS) levels in hippocampal neurons [19]. ROS are ions and very small molecules having high reactivity because of the presence of unpaired valence shell electrons[37]. ROS are cellular messengers having both positive and negative impact to spatial memory. Several authors have reported that ROS act both as signaling molecules required for normal synaptic function and as oxidative stressors causing harmful effects on synaptic plasticity[38,39]. Therefore, alterations in the cellular oxidants and antioxidant enzymes as noticed in the FC and Hc of PBDE-209-exposed mice in our study may be one of the mechanisms involved in consolidation of spatial memory.

In the present study, co-administration of ethanolic extract of higher dose of BM (120 mg/kg bw) along with PBDE-209 significantly recovered the working and memory towards control while the lower doses of BM (40 and 80 mg/kg bw) could not attenuate the working and reference memory error induced by PBDE-209. We have also found that the ethanolic extract of BM at the dose of 120 mg/kg bw inhibited the accumulation of lipid and protein damage in PBDE-209-exposed mice as evidenced by restoration of the levels of MDA and PC. BM also prevented the decrease in the activities of SOD and GSH-Px in PBDE-209-exposed mice. These findings therefore, suggest that ethanolic extract of BM has potential to attenuate the oxidative damage inflicted by PBDE-209 exposure. The results of our findings also suggest that PBDE-209 neurotoxicity is mediated

through oxidative damage and BM extract has potential to encounter it. These antioxidants play a pivotal role in preventing both free radical damage and generating oxidative stress like conditions. A number of reports are available confirming neuroprotective action of BM extracts and isolated bacosides [2, 7]. The mode of action of neuroprotective effects of BM appears to be the results of its antioxidant property which suppresses neuronal oxidative stress and the acetylcholinesterase activities. Further study showed that BM treated neurons expressed lower level of reactive oxygen species suggesting that BM restrained intracellular oxidative stress which in turn prolonged the lifespan of the culture neurons [40]. It has reported that BM is able to prevent lipid peroxidation in vitro and in vivo and quench the superoxide and hydroxide radicals effectively in vitro[10].The earlier finding indicates that BM has potential to modulate the activities of Hsp 70, P450 and SOD thereby possibly allowing the brain to be prepared to act under adverse conditions such as stress[1]. Hence, we can propose that probably elevation of antioxidant enzymes could be one of the mechanism by which BM extract encounters PBDE-209-induced oxidative stress and impairment in spatial memory.

CONCLUSION

We conclude from present studies that Bacoside-A, an active component of *Bacopa monnieri* improves the working and reference memory by restoring the alterations in cellular oxidants and antioxidant enzymes in the frontal cortex and hippocampus of postnatally PBDE-209-exposed mice.

ACKNOWLEDGEMENT

First author is thankful to Banaras Hindu University for providing Junior Research Fellowship and to Indian Council of Medical Research (ICMR), Government of India for Senior Research Fellowship (ICMR Award No. 45/56/2011/TRM-BMS). We thank to Late Padma Shri M.S. Kanungo, Professor, Department of Zoology, Banaras Hindu University, Varanasi, India for extending laboratory facilities and invaluable suggestions, and Kanungo's PhD student Mr. Rajaneesh K. Gupta for fruitful discussion on the work. We also express our sincere thanks to Dr. H.K. Singh, Ex-director, Central Drug Research Institute, Lucknow, India, for gift of ethanolic extract of *Bacopa monnieri*.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Chowdhuri DK, Parmar D, Kakkar P. Antistress effects of bacosides of Bacopa monnieri: modulation of Hsp70 expression, superoxide dismutase and cytochrome P450 activity in rat brain. Phyto Res 2002; 16:639–45.
2. Kishore K, Singh M. Effect of bacosides, alcoholic extract of Bacopa monnieri Linn. (brahmi), on experimental amnesia in mice. Indian J Exp Biol 2005; 43:640–45.
3. Nathan PJ, Clarke J, Lloyd J, Hutchison CW, Downey L, Stough C. The acute effects of an extract of Bacopa monnieri (Brahmi) on cognitive function in healthy normal subjects. Human Psychopharmacol 2001; 16:345–51.
4. Stough C, Lloyd J, Clarke J, Downey LA, Hutchison CW, Rodgers T, et al. The chronic effects of an extract of Bacopa monnieri (Brahmi) on cognitive function in healthy human subjects. Psychopharmacology 2001; 156:481–84.
5. Saraf MK, Anand A, Prabhakar S. Scopolamine induced amnesia is reversed by Bacopa monnieri through participation of kinase-CREB pathway. Neurochem Res 2010; 35:279–87.
6. Dhanasekaran M, Tharakan B, Holcomb LA, Hitt AR, Young KA, Manyam BV. Neuroprotective mechanisms of ayurvedic antidementia botanical Bacopa monnieri. Phyto Res 2007; 21:965–69.
7. Anbarasi K, Vani G, Balakrishna K, Devi CS. Effect of bacoside A on brain antioxidant status in cigarette smoke exposed rats. Life Sciences 2006; 78:1378–84.
8. Jyoti A, Sharma D. Neuroprotective role of *B. monnieri* extract against aluminium induced oxidative stress in the hippocampus of rat brain. Neurotoxicology 2006; 27:451-57.
9. Bhattacharya SK, Bhattacharya A, Kumar A, Ghosal S. Antioxidant activity of *B. monnieri* in rat frontal cortex, striatum and hippocampus. Phyto Res 2000; 14:174–79.
10. Russo A, Izzo AA, Borrelli F, Renis M, Vanella A. Free radical scavenging capacity and protective effect of Bacopa monnieri L. on DNA damage. Phyto Res 2003; 17:870–75.
11. Hites RA. Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations. Environ Sci Technol 2004; 38:945–56.
12. Law RJ, Allchin CR, deBoer J, Covaci A, Herzke D, Lepom P. Levels and trends of brominated flame retardants in the European environment. Chemosphere 2006; 64:187-08.
13. Mörck A, Hakk H, Örn U, Klasson-Wehler E. Decabromodiphenyl ether in the rat: absroption, distribution, metabolism, and excretion. Drug Metab Dispos 2003; 31:900–07.
14. Van den Steen E, Covaci A, Jaspers VL, Dauwe T, Voorspoels S, Eens M, et al. Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (Sturnus vulgaris). Environ Pollut 2007; 148:648–53.
15. Toms L, Sjödin A, Harden F, Hobson P, Jones R, Edenfield E, et al. Serum polybrominated diphenyl ether (PBDE) levels are higher in children (2-5 years of age) than in infants and adults. Environ Health Perspect 2009; 117:1461-65.
16. Giordano G, Kavanagh TJ, Costa LG. Neurotoxicity of a polybrominated diphenyl ether mixture (DE-71) in mouse neurons and astrocytes is modulated by intracellular gluthatione levels. Toxicol Appl Pharmacol 2008; 232:161–68.
17. Davison AN, Dobbing. Journal of Applied neurochemistry. Oxford: Blackwell, 1968.p.178–21.
18. Daubié S, Bisson JF, Lalonde R, Schroeder H, Rychen G. Neurobehavioral and physiological effects of low doses of polybrominated diphenyl ether (PBDE)-99 in male adult rats. Toxicology Letters 2011; 204:57–63.
19. Zhang C, Liu F, Liu X, Chen D. Protective effect of N-acetylcysteine against BDE-209-induced neurotoxicity in primary cultured neonatal rat hippocampal neurons in vitro. Int. J. Dev.l Neuroscience 2010; 28:521–28.
20. Singh HK The Memory-Enhancing and Associated Effects of a Bacosides-Enriched Standardized Extract of Bacopa monniera (BESEB—CDRI-08). In: Stough CKK, Scholey A, editors. Advances in Natural Medicines, Nutraceuticals and Neurocognition. CRC Press; 2013. p. 251-88
21. Davachi L, Mitchell JP, Wagner AD. Multiple routes to memory: distinct medial temporal lobe processes build item and source memories. Proc Natl Acad Sci USA 2003; 100:2157-62.
22. Bradford MM. "Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding". Anal. Biochem. 1976; 72:248–54.
23. Gupta RK, Kanungo M. Glial molecular alterations with mouse brain development and aging: up-regulation of the Kir4.1 and aquaporin-4. Age (Dordr) 2013; 35:59-67.
24. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351-58.
25. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990; 186:464-78.
26. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971; 44:276-87.
27. Lin CL, Chen HJ, Hou WC. Activity staining of glutathione peroxidase after electrophoresis on native and sodium dodecylsulfate polyacrylamide gels. Electrophoresis 2002; 23:513-16.
28. Vorhees CV, Williams MT. Morris water maze: procedure for assessing spatial and related forms of learning and memory. Nature protocols 2006; 1:848-58.
29. Roullet P. Inter-session delay and its effects on performance and retension of spatial learning on a radial maze with mice. Neurobiol Learn Mem 1995; 64:4-9.
30. Kirk RE. Procedures for the behavioral science. Belmont, CA: Brooks/Cole; 1968.

31. Viberg H, Johansson N, Fredriksson A, Eriksson J, Marsh G, Eriksson P. Neonatal exposure to higher brominated diphenyl ethers, hepta-, octa-, or nonabromodiphenyl ether, impairs spontaneous behavior and learning and memory functions of adult mice. Toxicol Sci 2006; 92:211-18.
32. Liu J, Head E, Gharib AM, Yuan W, Ingersoll RT, Hagen TM, et al. Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-l-carnitine and/or R-alphalipoic acid. Proc Natl Acad Sci USA 2002; 99:2356–61.
33. Butterfield DA, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. Free Radic Biol Med 2002; 32:1050–60.
34. Dröge W. Free Radicals in the Physiological Control of Cell Function. Physiol Rev 2002; 82:47-95.
35. Linares V, Alonso V, Albina ML, Bellés M, Sirvent JJ, Domingo JL, et al. Lipid peroxidation and antioxidant status in kidney and liver of rats treated with sulfasalazine. Toxicology 2009; 256:152-56.
36. Helliwell B, Gutteridge JMC. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem Sci 1990; 15:129–35.
37. Siddique HR. Adverse effect of tannery waste leachates in transgenic Drosophila melanogaster: role of ROS in modulation of Hsp70, oxidative stress and apoptosis. J. Applied Toxicol 2008; 28:734–748.
38. Murphy MP. How mitochondria produce reactive oxygen species. Biochem. J. 2009; 417:1–13.
39. Massad CA, Klann E. Reactive Oxygen Species in the Regulation of Synaptic Plasticity and Memory. Antioxid Redox Signal 2011; 10:2014-54.
40. 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; 30:112–17.