Neuromodulatory role of *Bacopa monnieri* on oxidative stress induced by postnatal exposure to decabromodiphenyl ether (PBDE -209) in neonate and young female mice

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**Abstract**

**Objective(s):** *Bacopa monnieri* (BM), a traditional ayurvedic medicine, is a well-known memory enhancer. We have explored the role of BM against decabrominated diphenyl ether (PBDE-209)-induced alterations in oxidative status.

**Materials and Methods:** Mice were orally administered with PBDE-209 (20 mg/kg body weight) postnatal day (PND) 3-10. Levels of malondialdehyde, protein carbonyl and activities of superoxide dismutase and glutathione peroxidase were measured at both ages. The correct choices and reference/working memory errors of young mice were evaluated by Morris water radial arm maze.

**Results:** The results showed that BM at the dose of 120 mg/kg significantly (*P*<0.05) restored the levels of oxidants and the activities of antioxidant enzymes in frontal cortex and hippocampus of neonates against PBDE-209-induced toxicity.

**Conclusion:** BM plays a neuroprotective role against PBDE-209-induced alterations in oxidative status.

**Keywords:** Bacopa monnieri, Oxidative stress, PBDE-209, Reference memory, Working memory

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**Introduction**

*Bacopa monnieri* (BM), a nootropic plant belongs to Scrophulariaceae family, is found in wet, damp and marshy areas of tropical regions. *B. monnieri* is an important constituent of “Ayurveda” and has been mentioned in Charaka Samhita, Susruta-Samhita and other treatise (1). The presence of active saponins like bacosides A and B in BM act as antioxidant and memory enhancer (2). The antioxidant property of BM has been studied against various toxicants including morphine and aluminium-induced oxidative damage in the rat brain (3, 4). The memory enhancing effects of BM has been reported against scopolamine-induced impairment of spatial memory performance in Morris water maze test in mice (5).

Being a highly brominated congener of polybrominated diphenyl ether, 2,2’,3,3’,4,4’,5,5’,6,6’-decabrominated diphenyl ether (PBDE-209) is used as flame retardant in wide variety of everyday products, from polyurethane foam in furniture to high-impact plastics used in computer casings (6). Due to its lipophilicity, PBDE-209 bioaccumulates easily in body organs and breast milk, affecting human health including developmental and neurological functions (7). Hence, concerns have been raised about the potential adverse health effects of PBDE-209, especially in the area of developmental neurotoxicity. However, attenuation of PBDE-209-induced alterations has not been established so far. Therefore, in the present study, we are interested to explore the neuroprotective role of BM on (a) alterations in oxidative status caused by postnatal exposure to PBDE-209 in frontal cortex (FC) and hippocampus (HC) of neonate and young female mice, and on (b) the correct choices, working and reference memory in young female mice.

**Materials and Methods**

**Chemicals**

The standardized ethanolic extract of BM (containing 58.18% Bacosides), was a gift from Dr HK Singh, Central Drug Research Institute, India. PBDE-209 (98%, CAS no. 1163-19-5) was obtained from Aldrich-Chemie while rest of the chemicals were purchased from Sigma, Merck and Sisco Research Laboratory (India). PBDE-209 was dissolved in corn oil whereas ethanolic extract of BM...
was suspended in tween 80 (5% v/v).

**Animals and treatment**

Male and female Swiss albino mice were kept in an animal house as per the guidelines of animal ethical committee, Banaras Hindu University (BHU), India. The day of litter born was designated as postnatal day 0 (PND 0). At PND 3, female pups within the same litter were randomly assigned into five groups of twenty eight in each: (a) Group I: control; (b) Group II: 20 mg/kg body weight of PBDE-209 and (c) Groups III, IV and V: 40, 80 and 120 mg/kg body weight of ethanolic extract of BM, respectively, 60 min after administration of 20 mg/kg of PBDE-209 (8). All the treatments were given orally via a micropipette with 100 μl micropipet at a volume of 5 μl/g of pups from PND 3 to 10. The pups of each group were further divided into two subgroups I and II, comprising of 7 and 21 pups, respectively. Seven pups from both subgroups were sacrificed on PND 11 (neonate) and 60 (young), the FC and Hc were collected and stored at -80°C for biochemical analyses. Seven pups of subgroup II were used for Morris water maze (MWM) performance, while radial arm maze (RAM) performance was conducted with the rest of the seven pups.

**Biochemical estimations**

Homogenates of FC and Hc were prepared in 50 mM phosphate buffer (pH 7.0) containing 1 mM of phenylmethanesulfonyl fluoride and centrifuged at 10,000×g for 10 min at 4°C. Protein contents of supernatants were measured by modified Bradford method (9, 10). The levels of malondialdehyde (MDA) and protein carbonyl (PC) were measured by biochemical assay, whereas the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were detected by in-gel activity assay as described in detail previously (8).

**Spatial memory tests**

Modified Morris water maze (MWM) and radial arm maze (RAM) were used to evaluate the memory deficit caused by PBDE-209. In MWM, a black-painted circular water tank (diameter: 122 cm, height: 51 cm), was divided into four equal quadrants – Q1, Q2, Q3 and Q4. The tank was filled with water up to the height of 31 cm. A square platform (area: 10 cm², height: 30 cm) placed in the center of one of these four quadrants was typically submerged 1.0 cm below the water surface filled. Platform was kept in the target quadrant Q2 (South-West) throughout the training session. Acquisition trials (working memory) and probe trials (reference memory) were performed by MWM as described in our previous report (8).

The RAM was consisted of a round central platform (40 cm) elevated 50 cm above the floor with eight radiating 32 cm long and 5 cm wide arms. Each arm formed a corridor leading to a square platform (8 cm²) having a small cup of 1 cm in diameter containing a hidden reward. The correct choices, working and reference memory errors were tested by RAM as described previously (8).

**Statistical analysis**

Data are presented as means ± standard error of mean (SEM). The biochemical estimations and in-gel activity assay were evaluated with one-way analysis of variance ANOVA followed by Tukey HSD (honestly significant difference) post hoc test. In Morris water maze, ELT was analyzed by two-way ANOVA between subject factors treatment and session, whereas probe trial was analyzed by one-way ANOVA followed by least significant difference (LSD) post hoc test. The radial arm maze data were also analyzed by using two-way ANOVA between subject factors treatment and session block followed by LSD post hoc test. All statistical analyses were conducted using SPSS (16.0) software. A difference of P<0.05 was considered statistically significant for main effects, however, for interactions at P< 0.1.

**Results**

**Lipid peroxidation and protein carbonylation in the brain of neonate and young females**

Supplementation with graded doses of BM in PBDE-209-exposed mice showed that only the maximum dose (120 mg/kg) was significantly effective in restoring the increased levels of MDA (Figure 1A and 1B) and PC (Figure 1C and 2D) in the FC and Hc of neonate mice (P<0.05). However, no significant alterations were observed in young females at any doses of B. monnieri compared with PBDE-209-exposed as well as control groups, as the levels of these remained unchanged in PBDE-209-exposed group.

**SOD and GSH-Px activities in the brain of neonate and young female**

BM, only at the dose of 120 mg/kg in PBDE-209-exposed mice, caused significant restoration (P<0.05) of SOD (Figure 1E and 1F) and GSH-Px (Figure 1G and 1H) activities in both regions of the brain. However, no significant changes were observed in BM-supplemented young females at any doses compared with PBDE-209-exposed and control groups, as these are remained unchanged in PBDE-209-exposed group.

**Morris water maze test in young female mice**

By comparing PBDE-209-exposed group with control, we found significant effect of session (F5, 72 5.115, P=.000), but not for treatment (F1, 72 0.215, P=0.644) and treatment × session (F5, 72 0.067, P=0.997) interaction. Similarly, we observed a significant effect of session (F5, 144 26.315, P=.000),
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Figure 1. Effect of Bacopa monnieri (40, 80 and 120 mg/kg) against decabrominated diphenyl ether (20 mg/kg) in frontal cortex and hippocampus on the levels of malondialdehyde (A and B), protein carbonyls (C and D) and the activities of superoxide dismutase (E and F) and glutathione peroxidase (G and H). The units of lipid peroxidation and protein carbonylation are expressed as nmoles malondialdehyde and nmoles protein carbonyl produced per mg protein, respectively. The gel photographs are representative of three independent SOD and GSH-Px in gel activity assays. The histograms are representative of integrated densitometric values (IDV) of bands. Results are presented as mean ± SEM. *P<0.05, control vs experimental groups

whereas no significant changes were observed for treatment (F_{3, 144} 1.138, P=0.336) and treatment × session (F_{15, 144} 0.610, P=0.863) interaction comparing BM-supplemented groups with PBDE-209-exposed group (Figure 2A).

During the probe trial at day 7, significant increase was noticed in the target quadrant Q2 (time spent in Q2 vs. time spent in other three quadrants: P<0.05; Figure 2B) in all groups. The time spent in the target quadrant (Q2) during the probe trials of PBDE-209-treated group with the control showed no significant changes. Similarly, BM-supplemented groups, at any doses, did not produce significant change in the time spent in the Q2 during the probe trials compared to PBDE-209-exposed and control groups (Figure 2B).

Radial arm maze test in young female mice

With respect to % correct choices, two-way ANOVA indicated significant effect of session block (F_{5, 72} 19.127, P<0.001) but no significant changes for treatment (F_{1, 72} 0.446, P=0.920) and treatment × session (F_{15, 72} 0.067, P=0.997) interaction in PBDE-209-treated group compared to control. Similarly, we observed a significant effect of session block (F_{5, 144} 17.526, P=.000) only, whereas no significant changes were observed for treatment (F_{33, 144} 0.568, P=0.637) and treatment × session (F_{15, 144} 0.363, P=0.986).
interaction when the BM-supplemented groups were compared with PBDE-209-exposed group (Figure 2C).

Regarding reference memory error, two-way ANOVA indicated main significant effects of session block (F_{5, 72} = 8.073, P = 0.000), whereas no significant changes were observed for treatment (F_{1, 72} = 3.937, P = 0.057) and treatment × session block (F_{15, 72} = 1.338, P = 0.258) interaction when compared the PBDE-209-treated group with control group. Similarly, we observed a significant effect of session block (F_{5, 144} = 3.524, P = 0.005), while no significant changes were observed for treatment (F_{3, 144} = 0.532, P = 0.661) and treatment × session block (F_{15, 144} = 0.593, P = 0.877) interaction when all BM-supplemented groups compared with PBDE-209-exposed group (Figure 2D).

With respect to working memory error, two-way ANOVA indicated main significant effects of session block (F_{5, 72} = 5.414, P = 0.000), whereas no significant changes were observed for treatment (F_{1, 72} = 0.653, P = 0.422) and treatment × session block (F_{5, 72} = 0.122, P = 0.987) interaction comparing PBDE-209-treated group with control. Similarly, we observed a significant effect of session block (F_{5, 144} = 4.648, P = 0.001) only, whereas no significant changes were observed for treatment (F_{3, 144} = 0.051, P = 0.985) and treatment × session block (F_{15, 144} = 0.523, P = 0.925) interaction when all BM-supplemented groups compared with PBDE-209-treated group (Figure 2E).

Discussion

Our results showed that BM (120 mg/kg) has potential to assuage the PBDE-209-induced oxidative damage possibly by inhibiting the accumulation of lipid and protein damage, and restoring the levels of antioxidant enzymes in neonate female mice. These findings are supported by our previous study therein BM reversed the alterations in the oxidative status caused by postnatal exposure to PBDE-209 in male mice (8).

Several recent findings have reported the potential role of BM in preventing the morphine and aluminium-induced changes in oxidative status in the brain of rodents (3, 4, 11). Reversal effects of BM extract on cognitive deficits in neurodegenerative disorders such as in Alzheimer disease and epilepsy have been well documented (12). Similar to our finding, BM has also been reported to reverse scopolamine-induced acquisition and retrieval of memory in Morris water maze task (5, 13). It also repairs the damaged neurons by enhancing kinase activity and neuronal synthesis coupled with restoration of synaptic activity, thereby improving the nerve impulse transmission (12). In the present study, postnatal exposure to PBDE-209 has neither altered the levels of cellular oxidants and the activities of antioxidant enzymes nor the correct choices, working and reference memory in young female mice. Furthermore, BM supplementation in PBDE-209-exposed mice did not induce any
alterations in the same parameters. Postnatal exposure to parathion has also been reported to cause unaltered cognitive behaviour in young female rats (14). By contrast, postnatal exposure to PBDE-209 in male mice causes significant alterations in the oxidative status and correct choices, working and reference memory in young mice, which is attenuated by BM-supplementation (8). Moreover, endogenous estrogen has been implicated in neuroprotection; therefore, we hypothesize its prominent role in better protection from PBDE-209-induced alterations in young female mice. It has been also shown that neuroprotective capability of estrogen is due to its antioxidant effects, interaction with membrane binding sites and modulation of neurotransmitter systems (15). Therefore, we suggest presence of optimal level of estrogen in young females can be responsible for maintaining the unaltered levels of oxidants/antioxidants even after PBDE-209 exposure.

**Conclusion**

In conclusion, *Bacopa monnieri* reverses the PBDE-209-induced alterations in cellular oxidants/antioxidants in the frontal cortex and hippocampus of neonate female mice while young female mice are protected from PBDE-209-induced alterations.

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**Conflict of Interests**

All authors declare that they have no conflicts of interest.

**References**

1. Sharma P. *Charak Samhita: Agnivesa's Treatise Refined and Annotated by Charak and Redacted by Dridhala, Text with English Translation*. Varanasi, India: Chaukhamba Publications; 2009.p. 1–2.
2. Singh HK, Rastogi RP, Srimal RC, Dhawan BN. Effects of bacosides A and B on avoidance response in rats. Phytotherap Res 1988; 2:70–75.
3. Sumathi T, Nathiya VC, Sakthikumar M. Protective effect of bacoside-A against morphine-induced oxidative stress in rats. Indian J Pharm Sci 2011; 73:409–415.
4. Jyoti A, Sethi P, Sharma D. *Bacopa monniera* prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. J Ethnopharmacol 2007; 115:56–62.
5. Saraf MK, Prabhakar S, Khanduja KL, Anand A. *Bacopa monniera* attenuates scopalamine-induced impairment of spatial memory in mice. Evid Based Complement Alternate Med 2011; 1:1–10.
6. Bromine Science and Environment Forum (BSEF). An introduction to brominated flame retardants. BSEF, Belgium; 2000.
7. Mazdai A, Dodder NG, Abernathy MP, Hites RA, Bigsby RM. Polybrominated diphenyl ethers in maternal and fetal blood samples. Environ Health Perspect 2003; 111:1249–1252.
8. Verma P, Singh P, Gandhi BS. Propylactic efficacy of *Bacopa monnieri* on decabromodiphenyl ether (PBDE-209)-induced alterations in oxidative status and spatial memory in mice. Asian J Pharm Clin Res 2013; 6:242–247.
9. 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.
10. Gupta RK, Prasad S. Early down regulation of the glial Kir4.1 and GLT-1 expression in pericontusional cortex of the old male mice subjected to traumatic brain injury. Biogerontology 2013; 14:531–541.
11. Sadeghnia HR, Kamkar M, Assadpour E, Boroushaki MT, Ghorbani A. Protective effect of safranal, a constituent of crocus sativus, on quinolinic acid-induced oxidative damage in rat hippocampus. Iran J Basic Med Sci 2013; 16:73–82.
12. Dhanasekaran M, Tharakan B, Holcomb LA, Hitt AR, Young KA, Manyam BV. Neuroprotective mechanisms of ayurvedic antioxidant botanical *Bacopa monnieri*. Phytother Res 2007; 21:965–969.
13. Tamaddonfar E, Farshid AA, Asri-Rezaee S, Javadi Sh, Khosravi V, Rahman B, et al. Crocin improved learning and memory impairments in streptozotocin-induced diabetic rats. Iran J Basic Med Sci 2013; 16:91–100.
14. Levin ED, Timofeeva OA, Yang L, Petro A, Ryde IT, Wrench N. Early postnatal parathion exposure in rats causes sex-selective cognitive impairment and neurotransmitter defects which emerge in aging. Behav Brain Res 2010; 208:319–327.
15. Amantea D, Russo R, Bagetta G, Corasaniti MT. From clincial evidence to molecular mechanisms underlying neuroprotection afforded by estrogens. Pharmacol Res 2005; 52:119–132.