

***In vitro* Safety Evaluation and Anticlastogenic Effect of BacoMind™ on Human Lymphocytes**

DIPANWITA DUTTA DEB*, PREETI KAPOOR, R. P. DIGHE, R. PADMAJA, M. S. ANAND, P. D'SOUZA,
M. DEEPAK, B. MURALI, AND AMIT AGARWAL

*Natural Remedies Pvt. Ltd, 19 K. M. Stone, Hosur Road, Plot No. 5B Veerasandra Indl. Area
Bangalore 560100, India*

Objective BacoMind™ (BM) is a standardized extract of *Bacopa monnieri*, which belongs to the family Scrophulariaceae and is a creeping annual plant found throughout the Indian subcontinent. It has been used by Ayurvedic medicinal practitioners in India for almost 3000 years and is classified as a *medharasayana*, a substance which improves memory and intellect. With the widespread traditional use as well as scientific validation of *Bacopa monnieri* for nootropic activity, a bioactive-rich unique phytochemical composition-BacoMind™ was developed from *B. monnieri* for use as a cognition and memory enhancing agent. The present study aimed to investigate the *in vitro* toxicity of this formulation of BacoMind™ on human lymphocytes and to rule out its possible contribution to mutagenicity. **Methods** In the present investigation the active ingredients present in BM were identified and quantified by high performance liquid chromatography (HPLC) and high performance thin-layer chromatography (HPTLC). Antioxidant and anticlastogenic properties of BM were studied *in vitro* with and without metabolic activation. Doses of BM were chosen on the basis of mitotic index (MI) and cytokinesis-block proliferation index (CBPI). Clastogenicity assays were performed at 31.2 µg/mL, 62.5 µg/mL, and 125 µg/mL, while the *Salmonella* reverse mutation assay (Ames test) was performed at doses of 61.72, 185.18, 555.55, 1666.67, and 5000.00 µg/plate. **Results** HPLC and HPTLC analysis of BM revealed the presence of bacoside A₃, bacopaside I, bacopaside II, jujubogenin isomer of bacopasaponin C, bacosine, luteolin, apigenin, bacosine, and β-sitosterol D glucoside. BM demonstrated significant antioxidant activity. The number of chromosomal aberrations and the frequency of micronuclei induced by BM were not statistically significant up to a dose of 62.5 µg/mL. A subsequent dose of 125 µg/mL prior to metabolic activation induced mild clastogenicity, but it was found to be biologically insignificant as this effect was not seen post metabolic activation. BM also demonstrated a dose-dependent protection against the clastogens used in this study using the above tests for clastogenicity. Maximum protection was observed in presence of metabolic activation. Moreover, BM demonstrated no mutagenic effect on the tested strains, as observed in the Ames test. **Conclusion** BM protected human lymphocytes against various clastogens. BM also exhibited high antioxidant activity which might be responsible for the observed protective effects against the clastogens since the used clastogens are known to induce their clastogenic effects *via* production of oxidative radicals.

Key words: BacoMind™, Cytotoxicity; Chromosomal aberration; Ames test; Micronucleus; Clastogens; Antioxidant; High performance liquid chromatography; High performance thin-layer chromatography (HPTLC)

REFERENCES

1. Russo A, Borrelli F (2005). *Bacopa monniera*, a reputed nootropic plant: an overview. *Phytomedicine* **12**, 305-317.
2. Murthy P B, Raju V R, Ramakrishna T, *et al.* (2006). Estimation of Twelve Bacopa Saponins in *Bacopa monnieri* Extracts and Formulations by High-Performance Liquid Chromatography. *Chem Pharm Bull* (Tokyo) **54**(6), 907-911.
3. Ernst E (2006). Herbal remedies for anxiety—a systematic review of controlled clinical trials. *Phytomedicine* **13**(3), 205-208.
4. Sairam K, Dorababu M, Goel R K, *et al.* (2002). Antidepressant activity of standardized extract of *Bacopa monniera* in experimental models of depression in rats. *Phytomedicine* **9**(3), 207-211.
5. Bhattacharya S K, Bhattacharya A, Kumar A, *et al.* (2000). Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum and hippocampus. *Phytother Res* **14**, 174-179.
6. Rao Ch V, Sairam K, Goel R K (2000). Experimental evaluation of *Bacopa monniera* on rat gastric ulceration and secretion. *Indian J Physiol Pharmacol* **44**, 35-41.
7. Goel R K, Sairam K, Dora Babu M, *et al.* (2003). *In vitro* evaluation of *Bacopa monniera* on anti-*Helicobacter pylori* activity and accumulation of prostaglandins. *Phytomedicine* **5**, 523-527.
8. Samiulla D S, Prashanth D, Amit A (2001). Mast cell stabilizing activity of *Bacopa monnieri*. *Fitoterapia* **72**, 284-285.
9. Stough C, Lloyd J, Clarke J, *et al.* (2001). The chronic effects of an extract of *Bacopa monniera* (Brahmi) on cognitive

*Correspondence should be addressed to Dipanwita Dutta DEB, Tel: 91-80-40209999. Fax: 91-80-40209817. E-mail: dipanwita@naturalremedy.com, dipanwita.dd@gmail.com

Biographical note of the first author: Dipanwita Dutta DEB, female, born in 1977, majoring in genetic toxicology, environmental toxicology and preventive and protective properties of natural products against environmental carcinogens.

- function in healthy human subjects. *Psychopharmacology* **156**(4), 481-484.
10. Singh H K, Dhawan B N (1982). Effect of *Bacopa monniera* Linn. (brahmi) extract on avoidance responses in rat. *J Ethnopharmacol.* **5**(2), 205-214.
 11. Singh R H, Singh L (1980). Studies on the anti-anxiety effect of the medhya rasayana drug, Brahmi (*Bacopa monniera* Wettst). *J Res Ayur Siddha* **1**, 133-148.
 12. Singh H K, Dhawan B N (1992). Drugs affecting learning and memory. In: Tandon, P. N., Bijiani, V., Wadhwa, S. (Eds.), *Lectures in Neurobiology*, vol. 1. Wiley Eastern, New Delhi, pp. 189-207.
 13. Halliwell B, Gutteridge J M, Aruoma O I (1987). The deoxyribose method: A simple "test tube" assay determination of rate constants for reactions for hydroxyl radicals. *Anal. Biochem* **165**, 215.
 14. Yen G C, Chen H Y (1995). Antioxidant activity of various tea extracts in relation top their antimutagenicity. *J Agric Food Chem* **43**, 27.
 15. Vani T, Rajani M, Sarkar S, *et al.* (1997). Antioxidant properties of the Ayurvedic formulations triphala and its constituents. *Int J Pharmacog* **35**, 313.
 16. Auddy B, Ferreira M, Blasina F (2003). Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. *J Ethnopharmacol* **4**, 131.
 17. Sreejayan, Rao M N (1997). Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol* **49**, 105.
 18. OECD-Guideline for Testing of Chemicals, 21 July (1997). *In vitro* Mammalian Chromosomal Aberration Test, No. **473**.
 19. OECD-Guideline for Testing of Chemicals, draft proposal for a new guideline, June 14 (2004). (1st version). *In vitro* Micronucleus Test, No. **487**.
 20. Garner R C, Miller E C, Miller J A *et al.* (1972). Liver microsomal metabolism of aflatoxin B1 to a reactive derivative toxic to *Salmonella typhimurium* TA 1530. *Cancer Res* **32**, 2058-2066.
 21. Ames B N, Durston W E, Yamasaki E, *et al.* (1973). Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. *Proceedings of the National Academy of Science USA* **70**, 2081-2285.
 22. Api A M, San R H C (1999). Genotoxicity tests with 6-acetyl-1,1,2,4,4,7-hexamethylcyclopenta-y-2-benzopyran. *Mutat Res* **446**, 67-81.
 23. Ozkul Y, Silici S, Eroğlu E (2005). The anticarcinogenic effect of propolis in human lymphocytes culture. *Phytomedicine* **12**, 742-747.
 24. Surralles J, Xamena N, Creus A, *et al.* (1995). Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures. *Mutat Res* **341**(3), 169-184.
 25. Bhattacharya S K, Kumar A, Ghosal S (1999). Effect of *Bacopa monniera* on animal models of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. In: Siva Sankar, D.V. (Ed.), *Molecular Aspects of Asian Medicines*. PJD Publications, New York.
 26. Shanker G, Singh H K (2000). Anxiolytic profile of standardized Brahmi extract. *Indian J Pharmacol* **32**, 152.
 27. Deepak M, Amit A (2004). The need for establishing identities of 'bacoside A and B', the putative major bioactive saponins of Indian medicinal plant *Bacopa monnieri*. *Phytomedicine* **11**, 264-268.
 28. Deepak M, Sangli G K, Arun P C, Amit A (2005). Quantative determination of the major saponin mixture Bacoside A and *Bacopa monnieri* by HPLC. *Phytochem Anal* **16**, 24-29.
 29. Roneada T, Vicentini V E P, Mantovani M S (2004). Possible modulating actions of plant extracts on the chromosome breaking activity of MMC and Area-C in human lymphocytes *in vitro*. *Toxicology* **5**, 617-622.
 30. Stella M A, Montaldi R, Rossi G, *et al.* (1982). Clastogenic effects of chromium on human lymphocytes *in vitro* and *in vivo*. *Mutat Res* **101**, 151-164.
 31. Gregory L Erexson, Periago M V, Carol S Spicer (2001). Differential sensitivity of Chinese hamster V79 and Chinese hamster ovary (CHO) cells in the *in vitro* micronucleus screening assay. *Mutat Res* **495**, 75-80.
 32. Wei Q, Gu J, Cheng L, *et al.* (1996). Benzo[a]pyrene diol epoxide-induced chromosomal aberrations and risk of lung cancer. *Cancer Res* **56**, 3975-3979.
 33. Mauthe R J, Cook V M, Coffing S L, *et al.* (1995). Exposure of mammalian cell culture to benzo[a]pyrene and light results in oxidative DNA damage as measured by 8-hydroxydeoxyguanosine formation. *Carcinogenesis* **16**, 133-137.
 34. Wei Q, Spitz M R, Gu J, *et al.* (1996). DNA repair capacity correlates with mutagen sensitivity in lymphoblastoid cell lines. *Biomarkers Prev* **5**, 199-204.

(Received January 21, 2007 Accepted October 8, 2007)