Crude Extracts of Bacopa Monnieri Induces Dendrite Formation in Rodent Neural Stem Cell Cultures—A Possible Use in Neuronal Injury

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Abstract

Background  Repair of nervous tissue injury impairs positive functional outcome. Major challenges involved are formation of new neuronal cells at the site of injury, growth and development of existing or stem cell-derived neuronal cells, and proper anatomical alignment of the cells required for the functional organization of the nervous system. Stem cells and various agents have been tried to overcome the above challenges yielding only limited positive results. Bacopa has been in frequent usage for cognitive impairment in Ayurvedic medicine. The assumption that Bacopa monnieri (BM) extracts may lead to certain specific changes at the cellular structural level benefitting the central nervous system repair, prompted us for the present study.

Objective  This is an in vitro study evaluating the effect of BM extracts (bacopasides and analogues) on the neuronal stem cells (NSC) culture in various concentrations. The study investigates the possibility of BM as an agent for the regeneration and differentiation of nervous tissue injury. This may have clinical and therapeutic implications.

Material and Method  NSC were harvested from the newborn albino rats, Rattus norvegicus, and the BM extracts were obtained from product “brahmi” manufactured by Himalaya Drug Company. Aqueous suspension of 2 μL of alcoholic extract of BM was locally added to the culture plates of NSC in concentrations of 5, 10, and 20 μg/mL after development of NSC in the media. The control NSC (without BM) and BM-rich NSC were simultaneously observed at regular unit intervals after inoculation. The morphological change in the NSC were observed and recorded.

Result  NSC could be successfully cultured from the newborn rat’s brain harvested at 3 and 6 hours of birth. NSCs derived at 3 hours of birth were more primitive (predominantly neurospheres) than derived those at 6 hours of birth. BM had significant positive effect on the neurospheres, that is, dendritic formation was seen in the NSC.
Introduction

Recovery from injury of the central nervous system (CNS) is grossly limited because of inadequate capacity to regenerate. Functional deficits following CNS injury results from mechanical insult leading to demyelination, axonal damage, and loss of glia and neurons. The damaging effects are further pronounced due to further secondary processes like ischemia, anoxia, free radical formation, and excitotoxicity. The solution seems to be possibly in the regenerative capacity of the neuronal or/other stem cells at the site of injury. Neural progenitor cells (NPCs) isolated from the adult CNS differentiate into neurons and glia after transplantation into the brain. They also differentiate into oligodendrocytes and astrocytes after transplantation into the spinal cord. Even rat and human mesenchymal stem cells retain the capacity to differentiate into nonmesenchymal derivatives, specifically into neurons. Embryonic stem cells derived oligodendrocytes have been shown to produce myelin in vitro and after transplantation are capable of myelinating axons in vitro in injured CNS. Human and rat CNS stem cells grow as neurospheres (CNS-stem cells) and are capable of surviving and differentiating without contributing to the glial scars. An agent that can facilitate the differentiation of these stem cells into the mature nerve cells promoting anatomical and functional organization at the injured site shall be highly beneficial for the CNS recovery. Bacopa Monnieri (BM), a herb belonging to the family Scrophulariaceae and commonly known as “Brahmi” in India, has been implicated to have beneficiary role in improving learning and memory in animals and humans. BM extracts help to repair damaged neurons and exhibit neuroprotective effect against oxidative stress in the hippocampus of the rat brain. The neuroprotective effects of BM may be attributed to its ability to suppress neuronal oxidative stress and also to its acetylcholinesterase inhibitory activities. We hypothesized that since BM has neuroprotective effect it may have a significant role in the differentiation of the neuronal stem cells (NSCs) leading to significant morphological changes which may contribute to recovery from CNS injury. Hence, we designed a preliminary in vitro experiment to study the effect of BM extracts on indigenously cultured NSC. In this study, NSCs were cultured from the brain of newborn rat and once the neurospheres were evident they were divided into control group (NSC-CON) and BM incorporated (NSC-BM) group. The NSC-BM group was subjected to the different concentrations of BM extracts. The results were analyzed based on the morphological changes in the NSCs. It was found that the BM extract had a positive effect on the dendritic formation of the NSC which was biologically significant. This study provides for an alternative approach to repair of traumatized neural tissue. It may have future implication in the central and peripheral nervous tissue treatment.

Materials and Method

The study is a preliminary in vitro experimental study evaluating the role of BM on the dendritic formation of NSC. The study involved isolation of NSCs, expansion, culture, and differentiation of isolated stem cells, preparation of BM in various concentrations, inoculation of the prepared BM in the stem cell culture, and comparative assessment of the various groups. The study was approved and cleared by the Institutional Ethical Committee and Animal Ethical Committee (vide letter no. MC/SMIMS/IAEC/01/2015).

Isolation of Neonatal Neuronal Stem Cells

NSCs were isolated from the brain of newly born albino laboratory rodents (Rattus norvegicus) at 3rd and 6th hours after birth. The rodents were procured from the animal house of Sikkim Manipal Institute of Medical Sciences. Neonatal rodents less than 6 hours old were brought to the research laboratory and sterilized by wiping the whole body twice with 70% alcohol and then by keeping under ultraviolet rays for 20 minutes in laminar flow. The rodents were euthanized by decapitation and skin with scalp was removed by surgical blades (No. 15). Skull bone was removed and dura with brain was exposed. Brain was chipped out with blades in small pieces and kept in a Petri dish with phosphate-buffered saline (PBS) solution. Brain was then macerated with blade to fine homogenous mixture and then washed by transferring the material to 2 mL conical centrifuge tubes (Eppendorf) and centrifuged for 2 minutes at 300 × g (1,380 revolutions per minute). Supernatant was decanted carefully and then fresh PBS solution was poured and mixed thoroughly. The fresh solution was again spun for 2 minutes at...
300 × g and supernatant was decanted carefully. This freshly prepared media solution was poured into the Eppendorf and mixed thoroughly. Viable cell count was done with using Trypan blue solution in hemocytometer.

**Media Preparation for Expanding Rat Neonatal Neural Stem Cells**

The media for expanding the stem cells was prepared as per instructions in the kit. The StemProNSF SFM 100 mL solution was prepared with 1 × 97 mL of KnockOut D-MEM/F12 (Invitrogen, United States), 2 mL of 2% StemPro neural supplement (Invitrogen), 1 mL of 2 mM GlutaMAX-1 Supplement (Invitrogen), 20 µL of 20 µg/mL basic fibroblast growth factor (bFGF) (Invitrogen), 20 µL of 20 µg/mL epidermal growth factor (EGF) (Invitrogen), and 1 mL antibiotics to make total solution volume of 100 mL.

**Differentiating Media**

Differentiating media was used for allowing BM to help neurospheres to differentiate into nerve cells. This media did not have growth factor in them and the rest of the media was same as complete StemProNSF SFM without bFGF and EGF.

**Preparation of Bacopa Monnieri (Brahmi) for Inoculation**

Dry powder form of BM “brahmi” 250 mg/capsule (Himalaya Drug Company). Stock solution of BM was made by mixing contents of one capsule of BM (250 mg) thoroughly with 1,000 mL of distilled water. Sonication was done for 2 minutes to mix the product in distilled water by ultrasonic homogenizer (Hieshler, model UP 100 H). The solution was filtered with microsyringe filter and sterilized with microspore filter size 45 µm. The required concentration of 5, 10, and 20 µg/mL was prepared from stock solution. Standardizing of Bacopa was done using Agilent QTOF liquid chromatography system.

**Culture and Inoculation of BM**

The culture of rat NSCs was done from the cell solution prepared from the isolated cells harvested from brain of newborn rat. About 200 µL of cell suspension was plated on uncoated culture flask (200 mL) containing 20 mL of complete StemProNSF SFM media and antibiotic solution. The culture bottle was incubated in CO2 incubator (New Brunswick, model: Galaxy –40R) at 37°C with 5% CO2 and 95 to 98% humidity for 24 hours. The culture which showed neurospheres at 96 hours were further centrifuged and cells were taken out in StemProNSF SFM media without growth factors, that is, bFGF and EGF. The fresh culture was suspended in the flask and then taken in cell viewer culture plates in duplicates. The culture plates were marked as BM 5 µg/mL, BM 10 µg/mL, and BM 20 µg/mL. Each plate was inoculated with 200 µL of media and 200 µL of cell suspension. Thus, NSC-CON (without BM) and NSC-BM (with BM) at 5, 10, and 20 µg/mL groups were created. Note that 2 µL of BM solution at various concentrations were inoculated in the respective culture plates and all groups were incubated. Phase microscopy was done at 24, 48, 72, and 96 hours using fluorescence Leica Microscope DMIL – LED FLUO. Photography was done at each time by the inbuilt digital camera DFC 295 and the results were assessed.

**Result**

**Neuronal Stem Cell can be Harvested from Newborn Rodent Whole Brain Irrespective of Area Specificity**

NSC was isolated from the rodent brains at 3 and 6 hours of birth. The newborn rodent brain could yield a sufficient number of viable NSC which could be successfully cultured (Fig. 1). NSC harvested from the rat brain was first cultured with growth factors (bFGF and EGF) but without BM. The NSC on culturing with the growth factors formed dendrites after 48 hours in both the groups. It was found that at 72 hours, NSC obtained at 6 hours of birth showed more evident dendritic formation as compared with NSC harvested at 3 hours of birth (Fig. 2). Both the groups developed dendrite by 96 to 120 hours. It was inferred that the NSCs can be harvested and cultured from the newly born rat’s brain and the NSCs obtained at 6 hours of birth were more mature considering the extent of dendritic formation. This finding indicated the regenerating capacity of newly born rat’s brain due to presence of stem cells. This regenerating capacity shall presumably be higher at earlier age in lieu of presence of more primitive stem cells.

**Bacopa Monnieri has a Positive Effect on Neuronal Stem Cell Growth and Dendrite Formation**

The NSC formed neurospheres after 24 hours of culture (Fig. 2 and 3). The formation of neurospheres was better appreciated in BM-instilled specimens in comparison to the control group. The BM-instilled cultures (NSC-BM) formed dendrite in 24 hours, irrespective of the concentration; but the control (NSC-CON) formed dendrite only after 48 hours. The extent and size of dendrite formation in NSC-CON was less than the NSC-BM group even at 72 to 120 hours. The difference in these characteristics of the dendrites was biologically significant in NSC-BM in comparison to NSC-CON. The concentration variation of the BM also played a significant role on the dendrite formation. The concentration of 5 and 10 µg/mL had relatively better positive effect than 20
proposed as rapidly proliferating cells which generate neu-
axons after spinal cord injury. Ependymal cells have been
believed to end after few days of life but Reynolds and
Weiss demonstrated that adult mouse striatum cells
have the capacity to divide and differentiate into neurons and astrocytes. The present in vitro study explored the
feasibility of harvesting NSC from the newborn rat’s brain
within few hours of birth. This study successfully
establishes the possibility of generating NSC from the newborn
rat’s whole brain irrespective of area specificity in the
laboratory set up. However, the multipotentiality needs to
be studied in detail. The study helps in establishing the
hypothesis that NSC thus derived can be differentiated into
neurons with dendrites. In vitro generated NSC can possibly
play a significant role in managing neurological disorders. In
vitro expanded NSCs have been transplanted in spinal cord
injury and shown to repair the CNS with neurogenesis and
provide functional recovery.

In vitro culturing of NSCs is feasible in the presence of
growth factors like FGF, EGF, and others. An agent that can
replace or supplement the existing factors shall be highly
beneficial for the neuronal regenerative treatment. The
present study evaluated the efficacy of the traditional Ayur-
vedic Indian medicine namely BM. It is a traditional medi-
cinal herb found in India, China, Nepal, Sri Lanka, and Taiwan
and has been recommended for treatment of various mental
disorders. Studies show BM extracts to be nontoxic, non-
teratogenic, and nonmutagenic in rats and monkeys. A
longer period BM extract treatment in rats with higher dose
brought about induced structural changes in basolateral
amygdaloid neurons which went on to improve learning
and memory. Brahmi (local Indian name of BM) antagonize
the effect of scopolamine in mice thus improving cognitive
functions of brain. It has also been found to enhance wound
healing and have a broad spectrum of antibacterial activi-
ty. Dried whole plant of BM with 50% ethanol extract
decrease free radical levels, promoting antioxidant status,
and has anxiolytic effect, antidepressant activity, and anti-
convulsive action. BM extracts help to repair damaged
neurons and show neuroprotective effect against oxidative
stress in the hippocampus of the rat brain. BM extracts have
also been used for treatment of neonatal hypoglycemia.
Treatment with BM extracts significantly reduced lipid per-
oxidation, lipofuscin deposition, and attenuated structural
derangements in the hippocampus. Considering extensive
positive effect of the BM extracts on neuronal cells, the
present study was planned with the novel idea of checking
its ability in vitro setup. In the present study, higher doses
of BM (20, 40, and 80 mg/kg) over long duration of 4 to
6 weeks have shown dendritic formation in Wister rats’
basolateral amygdala neurons. The present study demon-
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µg/mL concentration. A significant difference due to the
concentration was appreciated at 120 hours of culture (Fig. 3).
However, no toxic effect was noticed at the used
concentrations of 5, 10, or 20 µg/mL. BM thus acted like a
catalytic agent in promoting neuronal growth and formation
of dendrite. The arborization pattern was also evident at
120 hours of the culture.

**Discussion**

The existence of adult CNS multipotent neural stem cells has
opened a new avenue for the growing field of cell therapy as a
means to repair CNS injuries and provides a platform to
explore stem cell-based therapy in CNS injury repair. Several
studies have been performed to evaluate the possi-
bility of presence of multipotent NSC in adult CNS and their
capability in treating neurological diseases. Fetal CNS stem
cells transplant have shown clinical improvement in Parkin-
son’s patients. Transplant of fetal CNS tissue has been per-
formed in human patients with Parkinson’s disease, resulting
in some clinical improvement. Transplantation of brain-
derived adult NPC is an effective strategy to replace oligo-
dendrocytes and promote the remyelination of surviving
axons after spinal cord injury. Ependymal cells have been
proposed as rapidly proliferating cells which generate neu-
rons which can differentiate astrocyte and participate in scar
formation. The spinal cord epidermal region-derived
stem/progenitor cells have the ability to self-renew and of
multipotentiality for neurons and glia. In most of the
researches a lot of focus has been on generation of NSCs
from embryonic tissue and placental cord tissue. Subven-
tricular tissue in adult mammalian brain has been implicated
to be the source of neural stem cell. Neurogenesis has been
believed to end after few days of life but Reynolds and
Weiss demonstrated that adult mouse striatum cells
have the capacity to divide and differentiate into neurons and astrocytes. The present in vitro study explored the
feasibility of harvesting NSC from the newborn rat’s brain
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![Fig. 2](image_url) **Formation of dendritic formation (black arrows) of the**
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**growth factors.**
the culture media, with the best response at dose between 5 and 10 µg/mL. Further studies need to be done in animals to see if the same effects are present at the plasma levels of BM extract at concentration of 5 and 10 µg/mL. It remains to be ascertained which oral dosing would achieve such plasma levels. The dendritic formation was evident after 24 hours of culture and significant changes were found after 120 hours of culture. BM has been earlier implicated for exhibiting neuroprotective effects on oxidative stress, but the present study hints toward its role in neuron growth and differentiation. The structural changes due to BM extracts in the neurons have also been earlier delineated with electron microscopic evaluation. The present study also demonstrated structural changes that were beneficial in the in vitro neuronal growth and differentiation. It is noteworthy that even in the absence of growth factors, BM had positive effect on neuronal growth and differentiation. Detail evaluation of BM and its extracts is needed to ascertain the exact mechanism resulting to the beneficiary structural changes.

**Conclusion**

The brain of newborn rats contains NSCs that can be harvested and cultured. The stem cells are more primitive at earlier age.
As seen in tissue culture, BM, used in proper concentration (5–10 µg/mL), has a positive effect on NSC derived from the newborn rat's brain in terms of dendritic formation. The feasibility of BM as an agent for neuronal growth and differentiation can be further explored. The study opens up a new area of pharmacologic usage in nervous tissue injury repair and neuronal regeneration. It may have future clinical implication in central and peripheral nervous tissue disorder treatment especially using stem cells.

However, study is limited by the following issues. It is a preliminary study and the result can only be validated after adequate number of further experimental runs required for confirmation of the hypothesis. Further, the positive effect of BM needs to be ascertained on the NSCs derived from other sources. The result may have to be evaluated in the in-vivo set up or actual clinical conditions of nervous tissue repair.

Authors’ Contributions
R.S., M.S., and A.G. designed this study and edited the figures. R.K.S., R.S., M.S., and A.G. conducted the study and performed the analysis.

Note
The present study is a part of ongoing research on nervous tissue injury repair at Sikkim Manipal Institute of Medical Sciences, Gangtok, India. The ongoing research has been approved for funding by the Indian Council of Medical Research (ICMR), Ministry of Health, and Government of India. The Bacopa Monnieri extract has been obtained from the traditional medicine sold by generic name “brahmi” manufactured by Himalayan Drug Company. No financial support has been taken from the pharmaceutical company for the study; however, formal clearance for the product use has been obtained from the company. The study has been approved by the institute’s ethical committee and animal ethics committee.

Conflict of Interest
None declared.

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References
1 Gage FH, Coates PW, Palmer TD, et al. Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc Natl Acad Sci U S A 1993;92(25):11870–11883
2 Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000;61(04):364–370
3 Liu S, Qu Y, Stewart TJ, et al. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci U S A 2000;97(11):6126–6131
4 Cummings BJ, Uchida N, Tamaki SJ, et al. Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc Natl Acad Sci U S A 2005;102(39):14069–14074
5 Singh H, Dhawan BN. Drugs affecting learning and memory. Flj 189;1992
6 Singh HK, Dhawan BN. Effect of Bacopa monniera Linn. (brahmi) extract on avoidance responses in rat. J Ethnopharmacol 1982;5(02):205–214
7 Singh HK, Dhawan BN. Neuropsychopharmacological effects of the Ayurvedic nootropic Bacopa monniera Linn.(Brahmi). Indian J Pharmacol 1997;29(05):359
8 Jyoti A, Sharma D. Neuroprotective role of Bacopa monniera extract against aluminium-induced oxidative stress in the hippocampus of rat brain. Neurotoxicology 2006;27(04):451–457
9 Limpeanchob N, Jaapan S, Rattanakaruna S, Phrompitayarat W, Ingkaninan K. Neuroprotective effect of Bacopa monnieri on beta-amyloid-induced cell death in primary cortical culture. J Ethnopharmacol 2008;120(01):112–117
10 Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 1992;255(5052):1707–1710
11 Freed CR, Breeze RE, Rosenberg NL, et al. Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson’s disease. N Engl J Med 1992;327(22):1549–1555
12 Freeman TB, Olanon CW, Hauser RA, et al. Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson’s disease. Ann Neurol 1995;38(03):379–388
13 Lindvall O, Brundin P, Widner H, et al. Grafts of fetal dopamine neurons survive and improve motor function in Parkinson’s disease. Science 1990;247(4942):574–577
14 Karimi-Abdolrezae S, Efekharpour E, Wang J, Morshead CM, Fehlings MG. Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J Neurosci 2006;26(13):3377–3389
15 Johansson CB, Momma S, Clarke DL, Rising M, Lendahl U, Frisen J. Identification of a neural stem cell in the adult mammalian central nervous system. Cell 1999;96(01):25–34
16 Parr AM, Kulbatski I, Tator CH. Transplantation of adult rat spinal cord stem/progenitor cells for spinal cord injury. J Neurotrauma 2007;24(05):835–845
17 Duetsch F, Caillé I, Lim DA, García-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 1999;97(06):703–716
18 Ogawa Y, Sawamoto K, Miyata T, et al. Transplantation of in vitro-expanded fetal neural progenitor cells results in neurogenesis and functional recovery after spinal cord contusion injury in adult rats. J Neurosci Res 2002;69(06):925–933
19 Dhawan BN, Singh HK. Nootropic activity of Bacopa monniera Linn. Int J Neuropharmacol 2000;3:53
20 Russo A, Borrelli F. Bacopa monniera, a reputed nootropic plant: an overview. Phytomedicine 2005;12(04):305–317
21 Vollala VR, Upadhya S, Nayak S. Enhancement of basolateral amygdaloid neuronal dendritic arborization following Bacopa monniera Linn. extract. Int J Neuropharmacol 2000;3:S3
22 Yadav KD, Reddy KR, Kumar V. Study of Brahmi Ghrīta and piracetam in amnesia. Anc Sci Life 2012;32(01):11–15
23 Mihkal’chik EV, Anurov MV, Titkova SM, et al. Activity of antioxidant enzymes in the skin during surgical wounds. Bull Exp Biol Med 2006;142(06):667–669English, Russian.
24 Sampathkumar P, Dheeba B, Vidhyasagar ZV, Arulprakash T, Vinothkannan R. Potential antimicrobial activity of various extracts of Bacopa monnieri (Linn.). Int J Pharmacol 2008;4(03):230–232
25 Shanker G, Singh HK. Anxiolytic profile of standardized Brahmi extract. Indian J Pharmacol 2000;32:152
26 Sairam K, Dorabu M, Goel RK, Bhattacharya SK. Antidepressant activity of standardized extract of Bacopa monniera in experimental models of depression in rats. Phytomedicine 2002;9(03):207–211
27 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(08):1065–1074
28 Nannepaga JS, Korivi M, Tirumanyam M, Bommavaram M, Kuo CH. Neuroprotective effects of Bacopa monniera whole-plant extract against aluminum-induced hippocampus damage in rats: evidence from electron microscopic images. Chin J Physiol 2014;57(05):279–285