Mitogenic properties of *Bacopa monnieri* extract play a significant role in human endothelial cell expansion

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**Abstract**

**Introduction**

*Bacopa monnieri* is widely used as an Ayurvedic medicinal herb for rejuvenating neuronal cells and facilitates in curing many neurological disorders. In this study, we investigated the anti-oxidant nature of *B. monnieri* extract, which plays a significant role in vitro vascular endothelial cell expansion.

**Materials and methods**

The model of our study involves using the human umbilical vein endothelial cell culture approach. The experiments were set up for control (without any treatment) and human umbilical vein endothelial cells treated with H2O2 (100 µM) for 1 h, *B. monnieri* extract (100 µg) for 2 h, pre-*B. monnieri* extract (100 µg) for 2 h plus post-H2O2 (100 µM) for 1 h and pre-H2O2 (100 µM) for 1 h plus post-*B. monnieri* extract (100 µg) for 2 h.

**Results**

HUVEC cell count for day 0 was 1 × 10^4 cells, and it showed 70% confluence with an expansion rate of 1.60312 × 10^5 on the twelfth day for the control group of cells.

**Conclusion**

These experiments support the findings that *B. monnieri* extract has a potential role in proliferation of human umbilical vein endothelial cells and can be used to improve the mitogenic properties and functions of vascular endothelial cells in culture.

**Introduction**

Oxidant and free radical attacks damage the cardiovascular cells and system, which causes inappropriate cellular proliferation and apoptosis. To get rid of the oxidant and free radical damage, cells have to develop self-defence antioxidant mechanisms, but due to continuous free radical attacks, this mechanism gets impaired and the cells require antioxidants to rejuvenate themselves. Nature has provided many ways to acquire antioxidants from various sources such as plants and herbs. One such important herb is *Bacopa monnieri*. *B. monnieri* is known as Brahmi or Jalnimba in India. *B. monnieri* is a potent, established Ayurvedic (antioxidant) medicine valued for its diverse roles in the treatment of various challenging diseases of the modern world and its anti-aging property. Plant antioxidants are a complex group of enzymes essential for repair of damaged DNA, damaged cell membrane, damaged protein and oxidized lipids and peroxides; they also stop chain propagation of peroxyl lipid radicals. These enzymes repair the damage to biomolecules and reconstitute the damaged cell membrane, e.g. lipase, protease, DNA repair enzymes, transferase and methionine sulfoxide reductase. *B. monnieri* extract (BME) and its isolates have been extensively investigated for their neuropharmacological effects.

However, research related to the role of BME in cultured vascular endothelial cell proliferation has not yet been investigated. In this study, we developed an endothelial cell culture model to investigate the mitogenic properties of BME and the significance of their role in human umbilical vein endothelial cell (HUVEC) proliferation.

**Materials and methods**

The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed.

**BME**

Crude methanolic extract was prepared from fresh, clean, washed and dried *B. monnieri* herb as per the protocol of Phrompittayarata et al.2.

**HUVEC expansion study for control (without any treatment)**

Cell count was taken using a haemocytometer. To study the HUVEC proliferation rate, HUVECs were diluted to a cell density of 1 × 10^4 cells/mL and seeded. One millilitre of 20% endothelial cell growth medium was added to all the wells, and the plate was incubated in 5% CO₂ incubator. Cell count was taken on the second and twelfth day. Graph was plotted as follows: number of days (x-axis) vs. HUVEC proliferation (y-axis) along with dosage of treatments.

**HUVEC expansion study for treatments**

HUVECs were treated with H₂O₂ (100 µM) for 1 h, BME (100 µg) for 2 h, pre-BME (100 µg) for 2 h plus post-H₂O₂ (100 µM) for 1 h and pre-H₂O₂ (100 µM) for 1 h plus post-BME (100 µg) for 2 h. HUVECs were diluted to a cell density of 1 × 10^4 cells/mL and seeded. Cell count was taken on the second and twelfth day. Cell count was taken using a haemocytometer. Graph was plotted as follows:

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Research study

**Results**

**BME**

Twelve grams of sticky crude BME was obtained and stored in the refrigerator and used for experiments.

**HUVEC expansion and cell counts**

HUVEC cell count for day 0 was $1 \times 10^4$ cells, and it showed 70% confluence with an expansion rate of $1.60312 \times 10^5$ on the twelfth day for the control group of cells (Figure 1). H$_2$O$_2$ (100 µM; oxidative stress) treatment for 1 h resulted in a cell count of $1.51972 \times 10^5$ cell/cm$^2$ on the twelfth day (Figure 2). BME (100 µg) treatment for 2 h resulted in a cell count of $1.982 \times 10^5$ cell/cm$^2$ on the twelfth day (Figure 3). Pre-BME (100 µg) treatment for 2 h plus post-H$_2$O$_2$ (100 µM) treatment for 1 h resulted in a cell count of $1.96121 \times 10^5$ cell/cm$^2$ on the twelfth day (Figure 4). Pre-H$_2$O$_2$ (100 µM) treatment for 1 h plus post-BME (100 µg) treatment for 2 h resulted in a cell count of $1.50772 \times 10^5$ on the twelfth day (Figures 5 and 6).

**Discussion**

BME contains a mixture of active constituents such as bacosides and saponins, sterols, betulic acid, stigmasterol, β-sitosterol and...
some alkaloids including brahmine, herpestine and acid A4.

A lag phase after seeding is followed by a period of exponential growth called the log phase. The attachment of cells to each other and to culture substrate is mediated by cell surface glycoproteins and Ca2+. Routine passage leads to standard growth cycle. It is essential to become familiar with this cycle for each endothelial cell that is cultured as it controls the seeding concentration, the duration of growth before subculture, the duration of experiments and the appropriate times for sampling to achieve highest consistency3. For control culture, i.e. without any treatment, HUVEC count was maximum on the twelfth day. In comparison with the control group, a 1-h treatment of HUVECs with H2O2 (100 µM) resulted in a decreased cell count on the twelfth day. HUVEC cell count dramatically increased after a 2-h treatment with BME compared with that for cells in the control group. Based on these observations, it was proved that BME (100 µg) dose exposure enhances cell growth or provides a mitogenic factor that play a significant role in HUVEC proliferation and rejuvenation of cells.

Conclusion
This finally concludes that BME has some beneficial antioxidant and mitogenic properties that play a significant role in HUVEC proliferation and rejuvenation of cells.

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Abbreviations list
BME, B. monnieri extract; HUVEC, human umbilical vein endothelial cell.

References