Original Article

"Preclinical Investigation On The Protective Effect Of Bacopa Monnieri On Human Neuroblastoma Cell Line SH-SY5Y On Its Progressive Development And Finding On Autism Spectrum Disorder"

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Abstract

Autism is a common neurodevelopmental disorder characterised by differences in societal behaviour and interactive communication, as well as stereotyped, monotonous behaviours, unjustifiably partial to males and females by a ratio of 4:1, and a lack of understanding of the role of sex hormones in autism susceptibility. MTT & SRB Cell Viability Assay Proliferation of Cells Assay The entire study was carried out on differentiated SH-SY5Y cells with varying passage numbers. SH-SY5Y human neuroblastoma cell lines were used in this investigation to expose the circumstances. SH-SY5Y cells seldom produce and distribute neuron-specific proteins at levels equivalent to mature neurons. As a result, differentiated SH-SY5Y cells were used in the majority of neuro research. SH-SY5Y cells were grown and allowed to differentiate for a period of time. Autism spectrum disorder (ASD) is a group of neurodevelopmental diseases marked by a lack of social skills and nonverbal interactions in early childhood, such as decreased eye contact, facial expression, and body motions. SH-SY5Y cell lines were chosen to explore the pattern of ASD. MTT & SRB Cell Viability Assay.

KEYWORDS: Cell Proliferation, SH-SY5Y, Neuroblastoma, Bacopa monnieri

INTRODUCTION:

Autism is a spectrum disorder, this means that a child's symptoms might appear in a variety of ways, ranging from minor to severe. A youngster with autism may find it challenging to speak and engage with others, Neurodegenerative disorders are a serious hazard to people's health. The central nervous system is affected by neurodegenerative illnesses, resulting in increasing nervous system dysfunction. [1]. For centuries, Bacopa monnieri, often known as Brahmi, has been utilised as a brain tonic. It has been shown to improve memory, concentration, encourage healthy blood cells, and reduce weariness. B. monnieri has been found to be a possible cognitive enhancer and neuroprotection. [2].

Aside from that, it has anti-inflammatory, antitumor, cytotoxic, anti - oxidant, antiulcer, tissue repair, and other properties. SH-SY5Y is a research cell line for human neuroblastoma. In vitro models of brain function and differentiation, SH-SY5Y cells are generally implemented. The original cell line, SK-N-SH, was sub cultured and isolated from a bone marrow biopsy of a four-year-old female with neuroblastoma [3].

Bacopa monnieri is an essential medicinal herb that has been used as a memory enhancer, anti-inflammatory, painkiller, antipyretic, sedative, and anti-epileptic drug for thousands of years. Many commercial and therapeutic researches have focused their resources on this plant for several years. Anti-bacterial, anti-fungal, anti-cancer, anti-oxidant, anti-inflammatory, anti-hyperglycaemic, anti-depressant, anti-epileptic, memory enhancer, anti-ulcer, Hepatoprotective, Analgesic, anti-diarrheal, anti-hypertensive, anti-toxicity, anti-ulcer, Hepatoprotective, analgesic, anti-diarrheal, As a result, steps should be taken to ensure its long-term use and conservation [4]. The phytoconstituents, traditional applications, and pharmacological actions of Bacopa monnieri are discussed in this paper. In summary, biotechnology approaches such as tissue culture, elicitation, and genetic transformation have been used to develop this plant and its active component production. My research utilizes SH-SY5Y cell lines to assess the efficacy of Bacopa monnieri. [5].

METHODS

I. PLANT COLLECTION AND IDENTIFICATION

Professor P. Jayaraman, PhD Director Plant Anatomy Research Centre, Chennai, authenticated Bacopa monnieri L., which was gathered in November from SSP Herbs Marthandam, Tamil Nadu. A voucher specimen of the plant (PARC/2019/4048) has been identified. The medication was studied macroscopically with the naked eye. On the basis of literature descriptions, the size, shape, colour, and organoleptic properties of the plant were observed, and the plant was confirmed.

II. EXTRACTION OF PLANT MATERIAL

The plant material was cleansed in water and dried in the shade before being ground into powder with an electric mixer. The powder (75g) was placed into a Soxhlet apparatus and extracted for 24 hours at 650C to 750C with 500ml of hydro alcoholic solvent (350ml alcohol-95 percent and 150ml water). The extract was vacuum-dried, and dry mass weights were calculated and recorded [6].

III. PRELIMINARY PHYTOCHEMICAL SCREENING

The control node for detecting alkaloids, tannin, glycosides, terpenoids, flavanoids, steroids, and other phytochemicals was followed. [7].

IV SH-SY-5Y (Human neuroblastoma) cell line study

NCCS provided the SH-SY-5Y (Human Neuroblastoma) cell line. Stock cells were grown until confluent in media supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), and streptomycin (100 g/ml) in a humidified environment of 5% CO2.

TPVG solution was used to separate the cell (0.2 percent trypsin, 0.02 percent EDTA, 0.05 percent glucose in PBS). The cells were tested for viability and centrifuged. A total of 50,000 cells were planted per well in a 96-well plate and cultured for 24 hours at 37°C in a 5% CO2 incubator. [8].

V. MTT ASSAY

Using appropriate medium containing 10% FBS, the monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml. 100l of diluted cell suspension (50,000 cells/well) was put to each well of the 96 well microtiter plate.(Yang, Z., Tang, et al(2015). The supernatant was flicked off after 24 hours, the monolayer was washed once with media, and 100l of varied doses of test medicines (3.125–100g) were introduced to the partial monolayer in microtiter plates. The plate was then incubated for 24 hours at 37°C in a 5% CO2 environment. The test solutions in the wells were removed after incubation, and 100l MTT (1 mg/1 ml MTT in PBS) was added to each well. The plate was incubated for 4 hours at 37 degrees Celsius in a 5% CO2 environment. [9]

The supernatant was removed, and 100 l of DMSO was added to the plate, which was gently agitated to dissolve the formazan that had formed. A microplate reader was used to measure the absorbance at a wavelength of 570 nm.

The percentage of viability was calculated using the following formula: % of viability = sample abs/control abs x 100 [10] [11].

VI. SRB ASSAY

Using appropriate medium containing 10% FBS, the monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml. 100l of diluted cell suspension (50,000 cells/well) was put to each well of the 96 well microtiter plate. [12] [13]The supernatant was flicked off after 24 hours, the monolayer was washed once with media, and 100l of varied doses of test medicines (3.125–100g) were introduced to the partial monolayer in microtiter plates. After 48 hours, the cells were fixed for 1 hour at 4°C using ice-cold tri-chloro acetic acid (100µl/well, 10% w/v). 100l SRB (0.057% w/v in 1% aqueous acetic acid) solution was added to the washed and dried plates and maintained at room temperature for 30 minutes. Washing the plates five times with 1 percent v/v acetic acid removed the unbound SRB solution. The plates were then dried after being washed. To solubilize the bound SRB, 200l of 10 mM Tris Base (pH 10.5) was added to each well. After that, it was shaken for 5–10 minutes. A microplate reader was used to measure the absorbance at a wavelength of 570 nm.The percentage of viability was calculated using the following formula: % of viability = sample abs/control abs x 100. [14] [15]

RESULTS

MTT assay was identified as the IC50 value of the given test samples Bacopa monnieri and the standard Cisplatin was determined to be 85.71 g and 3.44 g, respectively, according to the study report. According to my research, as the concentration of MTT and SRB rises, cell viability and proliferation decrease correspondingly in both experiments. When compared to conventional cisplatin, Bacopa monnieri demonstrates significant viability in the MTT graph. When compared to conventional Cisplatin, Bacopa monnieri demonstrates substantial proliferation in the SRB assay.

Table 1. Preliminary phytochemical screening of Hydro alcoholic extract of Bacopa monnieri

S. No	Phytochemical	Test	Hydro alcoholic extract of Bacopa monnieri
1.	Alkaloids	Wagner's Reagent	+ve
2.	Glycosides	Kellerkellani's Test	-ve
3.	Flavanoids	Alkaline Reagent	+ve
4.	Phenols	Ferric Chloride Test	+ve
5.	Saponins	Foam Test	+ve
6.	Tanins	Braymer's Test	+ve
7.	Terpenoids	Salkowki's Test	-ve
8.	Quinones	Acid Test	-ve

Table 2: MTT ASSAY ON SH-SY 5Y CELL LINE

S. No	Concentration (ug)	MTT	CISPLATIN
		% Viability	
1.	3.125	90.4	56.75
2.	6.25	87.92	38.49
3.	12.5	82.97	28.99

4.	25	76.78	11.04
5.	50	62.53	4.12
6.	100	45.61	3.19

Table 3: SRB ASSAY ON SH-SY 5Y CELL LINE

S. No	**Concentration (ug)**	**SRB**	**CISPLATIN**
		% Viability	
1.	3.125	90.6	63.04
2.	6.25	85.91	43.03
3.	12.5	74.98	26.09
4.	25	65.05	14.54
5.	50	58.27	4.84
6.	100	49.03	1.61

Figure 1. **EFFECT OF HAEBM ON MTT ASSAY-SH-SY5Y CELL LINE**

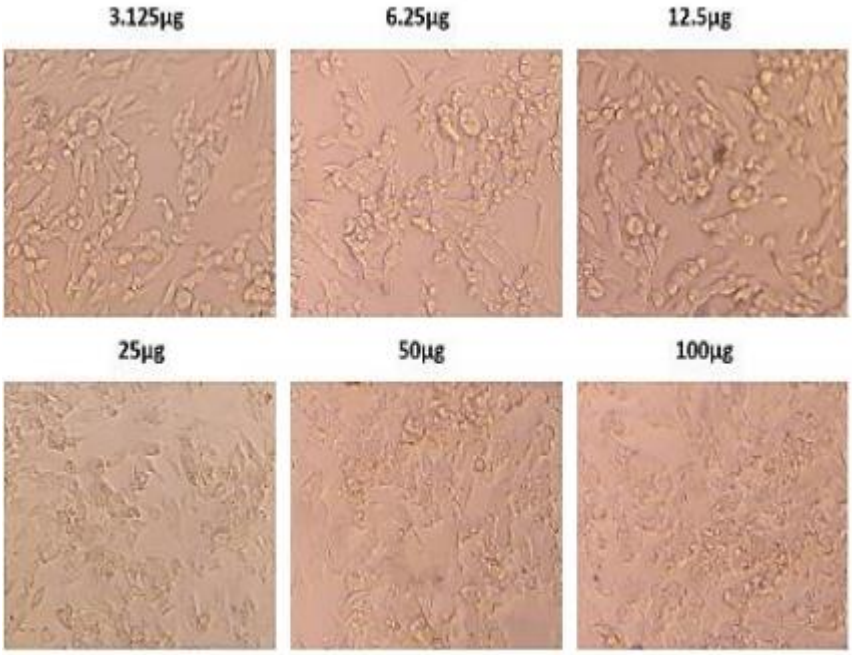


Figure 2. EFFECT OF HAEBM ON SRB ASSAY-SH-SY5Y CELL LINE

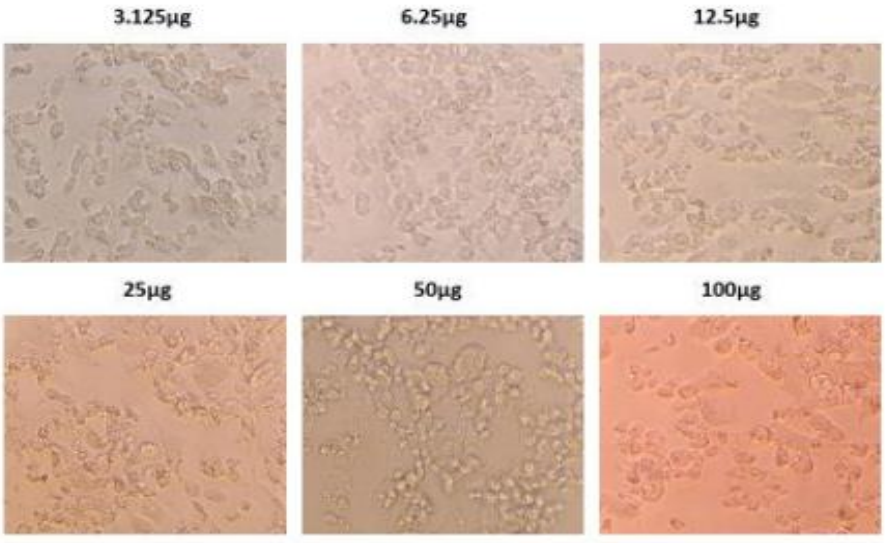


Figure 3. STANDARD CISPLASTIN

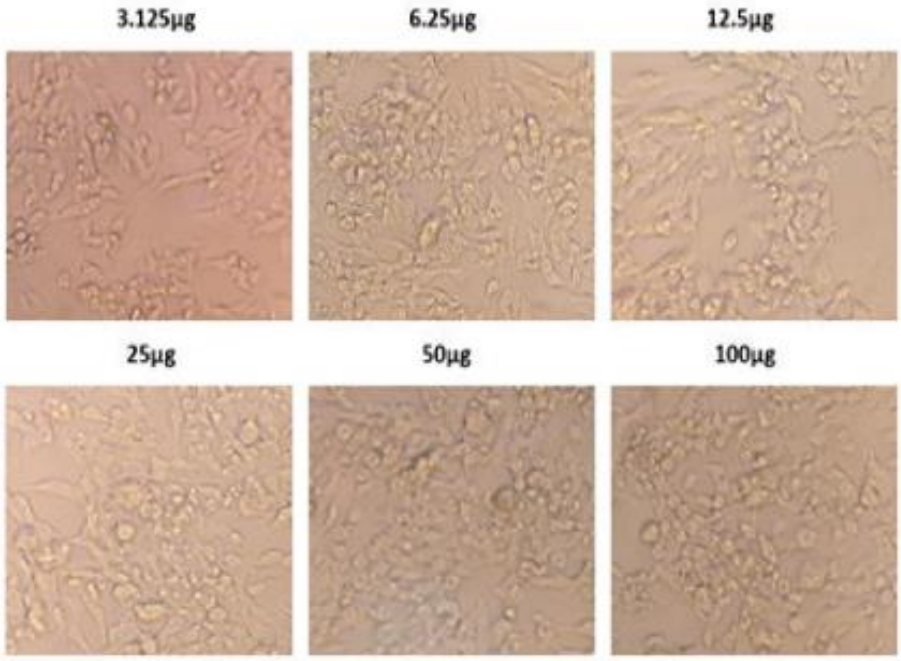


Figure 4. PERCENTAGE VIABILITY GRAPH OF HAEBM ON MTT ASSAY -SH-SY5Y CELL LINE

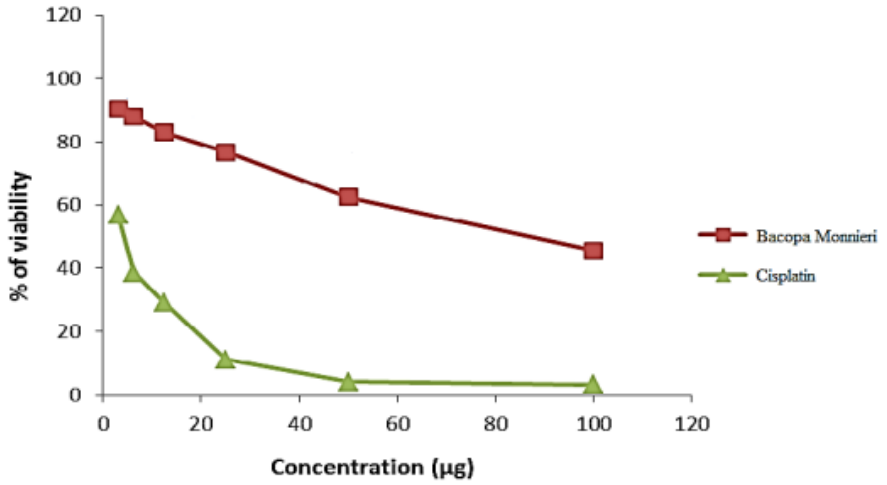
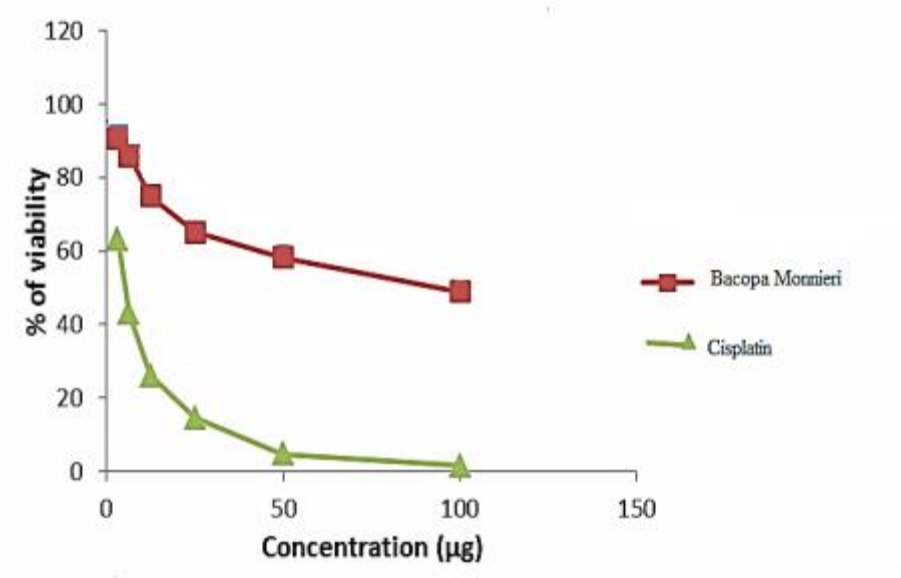


Figure 5. PERCENTAGE VIABILITY GRAPH OF HAEBM ON SRB ASSAY -SH-SY5Y CELL LINE



DISCUSSION

The IC50 values of the test samples Bacopa monnieri and Cisplatin, respectively, were found to be 93.61 g and 4.49 g. On the cell line employed in the study, the data clearly showed significant neuroprotection (P>0.001).

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REFERENCES:

1. Lonappan D, Dineshkumar B, Krishnakumar K, Nair SK, Rajeshkumar R. Formulation of para-coumaric acid loaded chitosan nanoparticles: In Vitro neuroprotective effect against neuroblastoma cell line. Research Journal of Pharmacy and Technology. 2021 Apr 1;14 (4):2286-90.
2. George A, Jacob KM, Priya CL, Rao KV. Phytochemical Composition and Antioxidant Activities of Various Stem Extracts of Bacopa monnieri Linn. Research Journal of Pharmacy and Technology. 2013 Sep 1;6(9):1074.
3. Frith, U., & Mira, M. (1992). Autism and Asperger syndrome. Focus on Autistic Behavior, 7(3), 13-15.
4. Amudhan, S. M., & Begum, H. V. (2008). Protective effect of areca catechu extract on ethanol induced gastric mucosal lesions in rats. Pharmacology online, 1, 97-106.
5. Banerjee, S., Anand, U., Ghosh, S., Ray, D., Ray, P., Nandy, S& Dey, A. (2021). Bacosides from Bacopa monnieri extract: An overview of the effects on neurological disorders. Phytotherapy Research, 35(10), 5668-5679.
6. Krishna, A., Biryukov, M., Trefois, C., Antony, P. M., Hussong, R., Lin, J., & May, P. (2014). Systems genomics evaluation of the SH-SY5Y neuroblastoma cell line as a model for Parkinson's disease. BMC genomics, 15(1), 1-21.
7. Sinclair, J., & Hechtman, L. (2013). Herbal medicine. Clinical naturopathic medicine-eBook, 103.
8. Sanyal R, Nandi S, Pandey S, Chatterjee U, Mishra T, Datta S, Prasanth DA, Anand U, Mane AB, Kant N, Jha NK. Biotechnology for propagation and secondary metabolite production in Bacopa monnieri. Applied Microbiology and Biotechnology. 2022 Feb 26:1-8.
9. Gopalasatheeskumar, K., Senthilnathan, B., Vijayalakshmi, A., Bhavya, E., Jeyamani, V., Masilamani, K., & Swarnapriya, B. (2018). Design and development of dexibuprofen loaded chitosan nanoparticles. Drug Invent. Today, 10, 248-252.
10. Gopalasatheeskumar, K., Kumar, G. A., Sengottuvel, T., Devan, V. S., & Srividhya, V. (2019). Quantification of Total Phenolic and Flavonoid content in leaves of Cucumis melo var agrestis using UV-spectrophotometer. Asian Journal of Research in Chemistry, 12(6), 335-337.
11. Evans, W. C. (2009). Trease and Evans'pharmacognosy. Elsevier Health Sciences.
12. Jiang, L., Zeng, X., Wang, Z., & Chen, Q. (2009). Cell line cross-contamination: KB is not an oral squamous cell carcinoma cell line. European journal of oral sciences, 117(1), 90-91.

13. Yang, Z., Tang, W., Luo, X., Zhang, X., Zhang, C., Li, H., & Liu, J. (2015). Dual-ligand modified polymer-lipid hybrid nanoparticles for docetaxel targeting delivery to Her2/neu overexpressed human breast cancer cells. Journal of Biomedical Nanotechnology, 11(8), 1401-1417.
14. Romero, C., Benedí, J., Villar, A., & Martín-Aragón, S. (2010). Involvement of Hsp70, a stress protein, in the resistance of long-term culture of PC12 cells against sodium nitroprusside (SNP)-induced cell death. Archives of toxicology, 84(9), 699-708.
15. Xie HR, Hu LS, Li GY: SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. Chin Med J (Engl). 2010, 123 (8): 1086-1092.

PAYMENT TRANSACTION RECEIPT