

Protective role of *Bacopa monnieri* against Rotenone induced parkinson's disease in PC 12 cell lines

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Abstract

Parkinson disease (PD) is a chronic neurodegenerative disorder characterized by the loss of dopaminergic (DA) neurons in the substantia nigra, decreased striatal dopamine (DA) levels, and consequent extrapyramidal motor dysfunction. PC 12 cells originate from pheochromocytoma cells of rat adrenal medulla and share many common characteristics with substantia nigra cells. They produce DA, several growth factors, such as nerve growth factor, fibroblast growth factor and transforming growth factor, and express DA receptors. Hence, these cells are being used to study the alterations in dopaminergic neurons, in vitro, that occur during Parkinson's disease. Since long term usage of antiparkinsonian drugs cause high incidence of pharmacoresistance and untoward side effects, attention has been paid in recent years to screen bioactive compounds from natural medicinal plants for treatment of several neurological disorders including Parkinson's disease. Keeping in view of relative importance of natural medicinal plants, the present study is mainly focused to characterize the anti-parkinsonian effect of *Bacopa monnieri* (BM), an Indian herb which is being extensively used in ayurveda treatments related to neurological complications. The present study was designed to assess the neurotoxicity of rotenone on DA-producing PC12 cells and explore the possible antiparkinsonian effect of BM in comparing with Levodopa (LD) (Reference control). The survivability studies of PC 12 cell-lines were analysed using MTT assay. Pre-treatment with BM extract significantly ameliorated morphological damage, cell viability, and apoptosis of PC12 cells exposed to RT. Hence BM extract can be effectively used in the treatment of PD and other related neurological disorders.

Keywords: Parkinson's disease (PD), *Bacopa monnieri* (BM), PC 12 Cell lines

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by the loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). The major symptoms of PD include muscle rigidity, tremors and bradykinesia [1] and also causes depression, memory loss, sleep disturbance, speech impairments and dysphagia [2]. Worldwide, the prevalence of PD is 1% of the population over the age of 60 years [3] and the relative risk of mortality ranges from 1.6 to 3.0 compared with age matched control populations [4]. A small group of patients with PD also show secondary symptoms, which include depression, pain, abnormal facial expression due to lack of fitness in the facial muscle and constipation. In the present study Parkinson disease was induced by treating the PC-12 cell lines with rotenone [5]. Since PC-12 cell lines may be used as an *in vitro*

model simulating DA producing neuronal cells, *in vivo*. PC-12 cell lines originate from pheochromocytoma cells of rat adrenal medulla [6] and share many characteristics with substantia nigra cells. They produce DA, several growth factors, such as nerve growth factor, fibroblast growth factor, transforming growth factor and express DA receptors [7].

Although there are many medications for PD, prolonged use of them caused untoward side effects. One of the most used medications is Levodopa and chronic usage of Levodopa is associated with the development of disabling motor complications in the majority of PD patients [8].

During the past few years, there is a growing interest to screen the bioactive factors isolated from the medicinally important plants for treating several neurodegenerative disorders including Parkinson, schizophrenia etc. Keeping in view the relative importance of medicinal plants, the primary focus of the present investigation is to investigate the protective effect of *Bacopa monnieri* (BM), an

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endemic medicinal plant, against Rotenone-induced Parkinson's disease using PC-12 cell lines.

Materials and Methods

Collection of plant material

Bacopa monnieri plant used in this work was collected in bulk from Tirumala Hills, Andhra Pradesh in India and authenticated by qualified botanist at Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh in India.

Extract Preparation

The whole plant (including roots) of *Bacopa monnieri* was dried in shade, and then powdered plant material was macerated with ethanol for 7 days. The plant material was percolated with circulating 95% ethanol (200 ml) for three rounds. The residue was extracted twice using the same procedure. The extract was filtrated and concentrated under reduced pressure in the Buchi rota vapour yielding a greenish-black sticky residue. Finally the extract was freeze-dried and was used for further studies.

Experimental design

PC12 cells were cultured in Dulbecco's-Modified Eagle's Medium (DMEM), which was supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum, 100 kU/L penicillin, and 100 mg/L streptomycin and incubated at 37 C in 5% CO₂. Cells in the exponential phase of growth were used in the experiment and were divided into four groups.

GROUP I: Control (no treatment, culture for 24 hours)

GROUP II: Rotenone [9] (1.6 µg/ml rotenone exposure for 24 hours)

GROUP III: BM -rotenone (10 µg/ml pre-treatment with BM extract for 2 hours prior to the addition of 1.6 µg/ml rotenone for 24 hours)

GROUP IV: LD -rotenone (10 µg/ml treatment with LD for 2 hours after the addition of 1.6 µg/ml rotenone for 24 hours)

10 mg of drug is dissolved in 10 mL of serum free MEM giving a concentration of 1mg / 1 mL. The stock is prepared fresh and filtered through 0.45 µ filters before each assay. Working concentrations of drug ranging from 1mg/ml to 7.8125 mg/ml were prepared for further analysis.

Cell viability assay

A fixed number (5 × 10⁶) of exponentially growing cells were seeded into 96 well micro titre plates and allowed to grow. Twenty four hours later, several of such individual cell cultures were used for the experiments. The cell viability is measured using the MTT assay [10] and the treated cells were incubated with 10µL of MTT for 3h followed by 10µL solubilisation solution and mixed thoroughly using a pipette. MTT medium was carefully aspirated from the wells and formazan dye was eluted using DMSO. Absorbance was measured using spectrophotometer (Micro plate reader) at wave length of 570 nm.

Statistical Analyses

Results are presented as mean ± SEM. One-way analysis of variance (ANOVA) followed by Student–Newman–Keuls (SNK) test was used to compare the differences between means in more than two groups. A probability value of < 0.05 was considered to be statistically significant. All statistical analysis was performed using the SPSS software.

Result

Following 24-hour treatment with RT, the survival rate of PC 12 cells was significantly decreased during rotenone-induced PD compared to the control (Table 1; Figure 1). The PC 12 cell lines were pre treated with BM extract for 2 hrs followed by RT treatment for 24 hrs and the survival rate was studied. Pre treatment with BM to the RT treatment caused significant protection as evidenced by 27% increase in cell viability as compared to RT-treatment (Table 1; Figure 1). Similarly the PC 12 cell lines were treated with RT for 24 hrs and the reference drug, LD was added to the RT-treated cell line after 2hrs of treatment. The LD treated cell lines showed 19% increase in survival rate when compared to RT-treatment (Table 1; Figure 1).

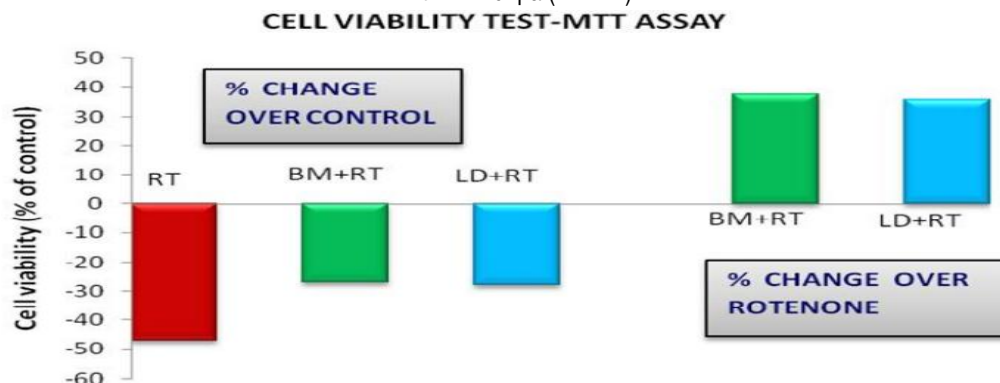
Table 1: Assessment of percent change in PC-12 cells following the exposure of RT and on pre-treatment with BM and treatment with LD.

S.No	Brain regions	Cell viability
1	SC	100±0
2	RT	53±18.25
3	BM+RT	73±10.25
4	LD+RT	72±10.09

The values are expressed in Mean ± SEM (n=3).



Figure.1: Percent changes in the cell viability during Rotenone (RT) induced PD, pre-treatment with *Bacopa monnieri* (BM+RT) and treatment with Levodopa (LD+RT).



Discussion

In the present study, the protective role of BM on RT induced PD was investigated in PC12 cell lines. PC12 cells, derived from a pheochromocytoma of a rat adrenal medulla, are similar to neurons in morphology, structure, and function. PC12 cells release dopamine, nor epinephrine, acetylcholine, and various membrane receptors under certain conditions [11]. Systemic administration of RT, a specific inhibitor of mitochondrial complex, can reproduce the neurochemical, behavioural, and pathological features similar to PD in rats. Therefore, PC12 cells provide a robust model for carrying out neurotoxicological studies [12]. Mitochondrial complex I inhibitors, such as Rotenone, cause degeneration of dopaminergic neurons, motor dysfunction [13], which may play a central role in the cascade of events that terminates in nigral neuronal loss of PD [14]. Hence, chronic *in vivo* exposure of RT can be used as a better model to simulate the pathological features of PD [15]. In this study, PC12 cells treated with RT showed significant morphological damage and apoptosis when compared to Control showing progression of PD. RT was responsible for induction of apoptosis in PC12 cells, which may be due to the accumulation of reactive oxygen species (ROS) caused by mitochondrial dysfunction. This supports the hypothesis that mitochondrial dysfunction might have been involved in the pathogenesis of PD. It is well documented that free radicals, produced as a result of mitochondrial dysfunction might have been implicated in different varieties of disorders such as cancer, atherosclerosis, ageings, ischemia, diabetes and neurodegenerative disorders [16].

Several lines of evidence indicate that natural antioxidants present in medicinal plants play a pivotal role in scavenging free radicals produced excessively during different neurological disorders. In support of this, it has been demonstrated that Chinese herbal medicinal plants, *Cistanche salsa* [17] and *Radix paeoniae alba* [18] play a significant neuroprotective role using PC 12 neural cell lines [9]. It has been reported that bacoside A isolated from BM inhibited lipid per oxidation [19] and modulate the expression of certain enzymes involved in the generation and scavenging the

reactive oxygen species in the brain [20]. *In vivo* studies of [21] suggest that bacosides present in BM exhibit antioxidant activity in different regions of brain. Earlier studies from our laboratory showed that pre-treatment with BM extract exhibited antiparkinsonian effect during RT-induced Parkinson disease in rats [22]. On par with LD

treatment pre-treatment with BM extract significantly ameliorated morphological damage, cell viability, and decreased apoptosis of PC12 cells exposed to rotenone suggesting that BM has the ability to provide neuroprotection against rotenone toxicity in an *in vitro* model of PD. Many studies have revealed the pharmacological role of BM as cognition-enhancer [23], antidepressant [24], antioxidant [25], antiulcerogenic agent [26], and calcium antagonist [23] and hence it has been used as a brain tonic from ancient times for promoting mental health and improving memory [27]. The present findings coupled with earlier reports suggest that the bioactive factors present in the BM extract have the ability to scavenge the reactive oxygen species (ROS) which ameliorate the progressive accumulation of oxidised protein aggregates and of dysfunctional mitochondria.

Conclusion

In spite of the advent of modern high technology on drug discovery and screening techniques, traditional knowledge on medicinal plants has given clues to the discovery of valuable drugs. Traditional medicinal plants are often cheaper, locally available and easily consumable as raw or as simple medicinal preparations with less or no side effects. The present finding using *in vitro* models using PC 12 cell lines coupled with the earlier reports clearly suggests that the bioactive factors present in the BM offers protection against rotenone- induced Parkinson's disease.

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