Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 3| Issue 2 |APR-JUN |2013|286-292

Research Article
Biological Sciences

ALTERATIONS OF CHOLINERGIC NEUROTRANSMISSION IN ROTENONE INDUCED PARKINSON'S DISEASE: PROTECTIVE ROLE OF *BACOPA MONNIERI*

Gunduluru Swathi, Cherukupalle Bhuvaneswar & Wudayagiri Rajendra*

Department of Zoology, Division of Molecular Biology, Sri Venkateswara University, Tirupati-517502. A.P. INDIA.

***Corresponding Author Email:** rajendraw2k@yahoo.co.in*

ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Dopaminergic anti-parkinsonian medications, cause drug-induced development of disabling motor complications in majority of patients with PD. Bacopa monnieri (BM), an Indian herb extensively used in Ayurveda, was tested on cholinergic system in Rotenone (RT) induced rat model of PD. Cholinergic system is affected due to imbalance between Acetylcholine (ACh) and Dopamine (DA) neurotransmitters. In the experiment conducted rats were divided into four groups of six in each group, group 1 received Saline water (1 ml/kg), group 2 received RT (2.5 mg/kg) through i.p. route administration for 60 days to induce PD. The third group received BM extract (180 mg/kg/day) for 20 days orally before induction of PD and group 4 received Levodopa (LD) (10 mg/kg/day) orally which is referred as reference control. The Acetylcholine (ACh) and Acetylcholinesterase (AchE) had been elevated and depleted respectively when PD was induced. In LD and BM treated rats the Ach was decreased and AchE was increased when compared to the PD induced rats. Our results suggest the ability of BM extract to modulate cholinergic system in different brain regions of PD induced rats.

KEY WORDS

Parkinson's disease (PD), Bacopa monnieri (BM), Rotenone (RT), Levodopa (LD), Acetylcholinesterase (AChE), Acetylcholine (ACh).

INTRODUCTION

PD is a chronically progressive, age-related neurodegenerative disease characterized by progressive resting tremor, rigidity, bradykinesia, gait disturbance, postural instability and dementia. A major neuro-pathological feature is PD is neurodegeneration in substantia nigra pars compacta (SNpc), by loss of DA neurons. In PD, cognitive dysfunction may be related to impairment of the ascending cholinergic system [1]. The key feature of cognitive profiles in PD is to executive dysfunction that has difficulty in tasks that require generation of cognitive sequencing. Cholinergic fibres originate from the brainstem and the basal forebrain is impaired in dementia associated with Lewy bodies which can be a consequence of Parkinson's disease [2]. Although a neural basis for cognitive dysfunctions in PD remains unknown, pathological and functional neuroimaging studies suggest that the cholinergic system arising from the basal forebrain has an important role in cognitive functions of PD patients [3]. Moreover, the loss of functioning in the dopaminergic system of the substantia nigra and the loss of cells in the cholinergic pathways in the nucleus basalis are responsible for the most significant cognitive deficits in PD [4]. Chronic use of current anti-parkinsonian medications including Levodopa therapy causes disabling abnormal involuntary movements known as drug-induced dyskinesias in the majority of PD patients [5,6].

There are significant number of evidences indicating the oxidative stress involvment in the

pathophysiology of these diseases which can induce neuronal damages, modulate intracellular signaling and ultimately leading to neuronal death by apoptosis or necrosis[7]. Thus, antioxidants have been studied for their effectiveness in reducing these deleterious effects and neuronal death in many in vitro and in vivo studies 8. Hence there is a need to find out newer pharmacologically active agents obtained from natural sources as plant extracts, without side effects and can act as natural antioxidants. *Bacopa monnieri* (BM), a medicinal plant commonly known as Brahmi in Sanskrit, has been used in the indigenous systems of medicine reported for its pharmacological roles as memory enhancer [9], cognition-enhancer, [10-12], antidepressant and also antioxidant properties [13]. In this present investigation BM was used in treatment of induced PD.

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 material was collected and shade-dried to powder. 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 filtered and concentrated under reduced pressure in the Buchi rotavapour yielding a greenish-black sticky residue. Finally the extract was freeze- dried and was used for further studies.

Experimental design:

The present work was conducted on male Wistar rats weighing 150±25g, they were maintained at a temperature of 25±5^{o}C and relative humidity of 45-55% with 12:12 h dark: light cycle. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing no.04a/a/CPCSEA/IAEC/08-09/SVU/Zool/WR-GS/dt.1.9.2009.

GROUP I: Served as normal control group, received vehicle (1.0 ml/kg/day) i.p. for 60 days.

GROUP II: PD was induced by RT (emulsified in natural oil to a concentration of 2.5 mg/ml), given i.p. route administration (2.5 mg/kg/day) for 60 days [14].

GROUP III: RT -induced PD rats were treatedwith BM extract (180 mg/kg/day) orally for 80 days, started before 20 days from induction of PD.

GROUP IV: RT -induced PD rats were treated with LD (10 mg/kg/day) orally started after 20 days from induction of PD [15].

The development of PD was detected after 20 days from induction with rotenone, by occurrence of tremors and exhibiting specific symptoms such as bradykinesia and rigidity in rats. The treatment with BM extract was started 20 days before induction of PD and LD was started after 20 days from induction of PD and continued for 60 days. After stipulated duration, the animals were sacrificed by cervical dislocation and the brain regions [Cerebral cortex (CC), Cerebellum (CB), Mid brain(MB) and Pons-Medulla (PM)] were immediately isolated, frozen in liquid nitrogen and were stored at -40^{o}C until further analysis.

Biochemical Analysis:

The level of ACh content and activity of AChE were estimated by the method of Hestrin (1949) as given by Augustinson (1957) [16] and with slight modification of the Ellman et al., 1961 [17] respectively in different brain regions of control and experimental animals.

Statistical Analyses:

Values of the measured parameters were expressed as mean ± SEM. One way- ANOVA (F value) was used to test the significance of difference among more than two arithmetic means, followed by Post-hoc test (Scheffe multiple comparison) to test the difference between each two means. The significance was considered at p values < 0.05. All the statistical analyses were processed using Statistical Program of Social Sciences (SPSS) for Windows, version 11.5.

RESULTS

The levels of Acetylcholine (ACh) content and the activity of Acetylcholinesterase (AChE) in different brain regions Cerbral cortex (CC), Cerebellum (CB), Mid brain (MB) and Pons medulla (PM) of control and experimental rats were represented in (**Table 1 and 2; Fig 1 and 2**).

Table 1: Changes in the Acetylcholine (Ach) content in different brain regions of rats during RT-induced PD and pretreatment with ethanolic extract of BM.

(Values are expressed in µ moles of Acetylcholine/g wet wt of tissue)

Ach content	SC	RT	BM±RT	LD±RT
CC	4.38±0.12	7.01±0.25*	5.29±0.15#	5.32±0.19#
CB	3.44±0.11	7.35±0.20*	5.60±0.12#	5.52±0.13#
MB	3.72±0.17	7.07±0.24*	5.54±0.12#	5.48±0.17#
PM	3.46±0.15	7.57±0.16*	5.21±0.09#	5.59±0.14#

Values are expressed in Mean ± SEM,
* p < 0.05 as compared with Control.
\# p < 0.05 as compared with PD rats.

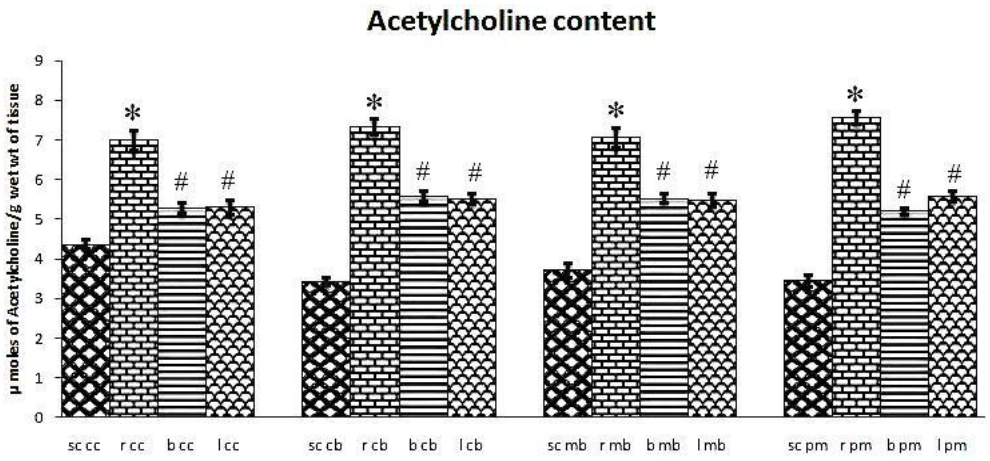
Table 2: Changes in the Acetylcholinesterase (AChE) activity in different brain regions of rats during RT-induced PD and pretreatment with ethanolic extract of BM.

(Values are expressed in µ moles of Acetylthiocholine iodide hydrolyzed/mg protein/hr)

AChE activity	SC	RT	BM±RT	LD±RT
CC	4.93±0.08	2.41±0.13*	4.18±0.14#	3.63±0.07#
CB	4.65±0.10	2.60±0.17*	3.91±0.31#	3.59±0.20#
MB	4.63±0.16	2.37±0.16*	3.75±0.11#	3.70±0.07#
PM	4.54±0.08	2.46±0.13*	3.67±0.16#	3.61±0.13#

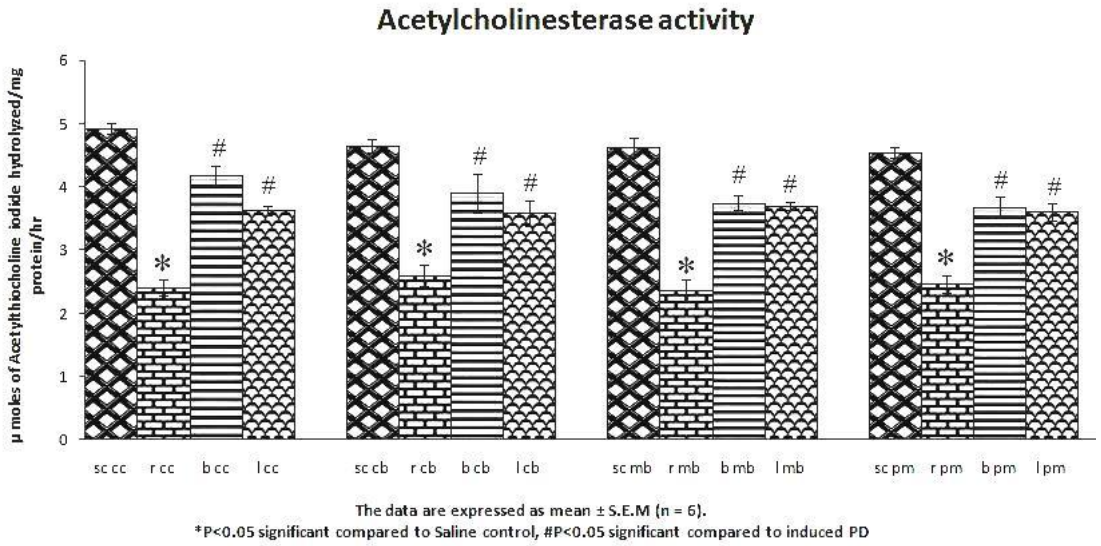
Values are expressed in Mean ± SEM,
* p < 0.05 as compared with Control.
\# p < 0.05 as compared with PD rats.

Fig 1: Changes in the Acetylcholine (ACh) content in different brain regions of rats during RT-induced PD and pretreatment with ethanolic extract of BM.



The data are expressed as mean ± S.E.M (n = 6).
*P<0.05 are significant compared to Saline control, #P<0.05 are significant compared to induced PD

Fig 2: Changes in the Acetylcholinesterase (AChE) activity in different brain regions of rats during RT-induced PD and pretreatment with ethanolic extract of BM.



In RT-induced rats, AChE activity ($P < 0.05$) was significantly depleted, whereas ACh content ($P < 0.05$) was elevated in all the brain regions, when compared with control rats. Pretreatment with BM extract and treatment with LD caused significant elevation of AChE activity ($P < 0.05$) and depletion of ACh content ($P < 0.05$) were observed in different brain regions, when compared to RT induced PD rats.

DISCUSSION

The central cholinergic system is considered to be the most important neurotransmitter involved in the regulation of cognitive functions [18]. The main neurotransmitter in cholinergic system is ACh, important in motor control, the autonomic, the enteric, and in the central nervous system (CNS). In cholinergic neurotransmission, the transmitted signal is terminated by cleavage of the transmitter, ACh, yielding acetate and choline. This cleavage is mediated by acetylcholinesterase (AChE), an enzyme of the α/β-fold family of proteins. The present study was designed to explore the protective effect BM on Cholinergic system (ACh content and AChE activity) in the brain of induced PD rats. The main feature of PD is relatively selective nigrostriatal dopaminergic degeneration. The interaction between molecules of DA and AChE resulted not only in modification of catecholamine oxidation, but caused the inactivation of AChE catalytic activity as well [19] which occurred mainly due to the direct interaction of a quinone or semiquinone oxidation products with the enzyme when induced by RT. Reactive oxygen species (ROS) are generated during dopamine metabolism and by mitochondrial respiration, which are shown to cause protein damage [20]. In this study, decline in AChE activity in PD induced rats as compared to the control rats was observed in different regions of brain. The AChE activity is an important marker of cholinergic neurotransmission dysfunction. In our study all the brain regions of RT induced PD rats showed the decreased activity of AChE compared to controls, by which we can conclude the progression of PD by cholinergic neurotransmission dysfunction.

Numerous reports in literature show decrease in AChE activity is reflected by acetylcholine content in the brain [21]. Imaging studies [22] agree with post-mortem evidence suggesting that basal forebrain cholinergic system degeneration appears early in PD and worsens coincident with the appearance of dementia. Early cholinergic denervation in PD without dementia appears to be heterogeneous and may make specific contributions to the PD. Apart from well-known cognitive and behavioral deficits; central in particular limbic, cholinergic denervation may be

associated with progressive deficits of odor identification in PD.

The main motor symptoms of PD as rigidity/tremors and muscle fatigness may be due to the abnormal increase of ACh content in induced PD rats (Table 1) when compared to control rats. The loss of dopaminergic inhibition for increased cholinergic activity in the striatum causes an imbalance between dopaminergic and cholinergic modulation of the striatal output to the motor program may be due to increased level of ACh (**Table 1; Fig 1**) which causes overactivity and due to continuous stimulation without inhibition leading to the characteristic symptoms of tremor, rigidity and muscle fatigness leading to postural instability.

According to Henk Konings (1995) [23] observations cholinergic dysfunction exists in PD patients with cognitive impairment. The increment of ACh (Table 1) correlates with decrement of AChE (Table 2) expressed as alterations in both motor and cognitive behaviors respectively. Although the major factors involved in PD declines remain to be specified, oxidative stress has been mainly implicated in cognitive impairment and neuropathologic disorders [24]. The RT induces complex I inhibition which causes the symptoms of PD, and increases the oxidative stress.The central nervous system is particularly more vulnerable to oxidative damage because of its high oxygen consumption, high tissue concentration of iron and relatively low levels of some antioxidants system [25]. This implication has led to the notion that antioxidant defense mechanisms in the brain are not sufficient to prevent PD which increases in oxidative damage. The dietary intake of a variety of antioxidants might be beneficial for preserving brain functions and maintaining protective role to prevent progression of PD.

The ethanolic extract of BM is reported to be rich in saponins, the saponins in general possess antioxidant activity. BM pretreatment in the present study resulted in declined the content of ACh (**Table 1; Fig 1**) in PD induced rats (Group III) as compared to the PD induced rats (Group II).which clearly indicates reduction of motor complications. Pretreatment with BM significantly increased the AChE activity in the brain of induced PD rat (**Table 2; Fig 2**).The BM extract group showed that it had significance difference compared to RT induced PD which indicates its protective effect on PD induced rats. Which clearly indicates that progression of PD had been slowed down in case of BM pretreated group compared to untreated PD group. It also showed that there was no significance differences compared to controls, showing recovery of this group of rats from PD. The results of BM were on par with LD treated group which specify the protective role of BM. This shows the improvement of motor and cognitive functions in pretreated BM and LD treated rats. The BM treated group results are similar to the results of LD treated group, showing that it can act as antiparkinsonian agent with antioxidant properties and/or its effect on the cholinergic system. Chowdhuri *et al*., (2002) [26] have demonstrated that Brahmi extract modulated the expression of important enzymes involved in generation and scavenging of reactive oxygen in the brain. Bacopa's antioxidant action and free radical scavenging activity,especially in memory related structures in the brain including the hippocampus [9] Oral administration of BM extract markedly reduced the memory deficits as well as acetylcholine concentrations, choline acetylase activity, and muscuranic receptor binding in the hippocampus and frontal cortex [9]. From the present results coupled with earlier reports it is obvious that the saponins which are present in BM extract might have offered a protective role from the oxidative stress and toxicity caused during RT-induced PD.

The study concludes that BM extract administration is effective in enhancing AChE and also demonstrates inhibition of ACh in PD induced rats. Hence, BM administration might serve useful in reversing PD symptoms. The cholinergic/cognitive enhancing properties of BM ethanolic extract warrants further studies with larger group of animals, on its individual active constituents and their mechanism of action. The rapid development in the field of animal models hopefully lead to an improved understanding of the pathophysiology of PD, and finally permit a rational designing of novel therapeutic strategies for PD.

ACKNOWLEDGEMENTS

One of the authors (G. Swathi) is grateful to CSIR-UGC (NET) (India) for providing Junior Research Fellowship.

REFERENCES

1. Lange KW, Wells FR, Jenner P, Marsden CD. Altered muscarinic and nicotinic receptor densities in cortical and subcortical brain regions in Parkinson's disease. J Neurochem, 1993; 60:197–203.
2. Perry EK, Irving D, Kerwin JM, McKeith I, Thompson P, Collerton D, Fairbairn A, Ince PG, Morris CM, Cheng AV et al., Cholinergic transmitter and neurotrophic activities in Lewy body dementia: similarity to Parkinson's and distinction from Alzheimer disease. Alzheimer Dis. Assoc. Disord, 1993; 7, 69-79.
3. Caviness JN, Driver-Dunckley E, Connor DJ, Sabbagh MN, Hentz JG, Noble B, Evidente VG, Shill HA, Adler CH. Defining mild cognitive impairment in Parkinson's disease. Mov. Disord. 2007; 22, 1272–1277.
4. Ferreri F, Agbokou C, and Gauthier S. Recognition and management of neuropsychiatric complications in Parkinson's disease. CMAJ 2006; 175(12): 1545–1552.
5. Deogaonkar M, Subramanian T. Pathophysiological basis of drug-induced dyskinesias in Parkinson's disease. Brain Res Rev 2005; 50:156-68.
6. Obeso JA, Olanow CW, Nutt JG. Levodopa motor complications in Parkinson's disease. Trends Neurosci 23(10 Suppl): 2000; S2-7.
7. Palla `SM, & Camins A. Molecular and biochemical features inAlzheimer's disease. Current Pharmaceutical Design, 2006; 12: 4389–4408.
8. Ramassamy C. Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: A review of their intracellular targets. European Journal of Pharmacology, 2006; 545, 51–64.
9. Bhattacharya SK, Bhattacharya A, Kumar A, & Ghosal S. Antioxidant activity of Bacopa monniera in rat frontal cortex, striatum and hippocampus. Phytotherapy Research, 2000; 14(3), 174-179.
10. Das A, Shanker G, Nath C, Pal R, Singh S, Singh HK. A comparative study in rodents of standardized extracts of Bacopa monniera and Ginkgo biloba anticholinesterase and cognitive enhancing activities. Pharmacol Biochem Behav 2002; 73:893-900.
11. Singh HK, Dhawan BN. Neuropsychopharmacoloical effects of the Ayurvedic nootropic Bacopa monnieri Linn (Brahmi). Indian J Pharmacol 1997; 29:S359-S365.
12. Sumathi T, Nayeem M, Balakrisshna K, Veluchamy G, Devarraj NS. Alcoholic extract of Bacopa monniera reduces the in vitro effects of morphine withdrawal in guinea-pig ileum. J Ethnopharmacol 2002; 82:75-81.
13. Sairam K, Rao CV, Babu MD, Goel RK. Prophylactic and curative effects of Bacopa monniera in gastric ulcer models. Phytomedicine 2001; 8:423-430.
14. Alam M, Schmidt WJ. Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. Behav Brain Res 2002; 136(1):317-324.
15. Alam M, Schmidt WJ. L-DOPA reverses the hypokinetic behaviour and rigidity in rotenone-treated rats. Behav Brain Res 2004; 153(2):439-446.
16. Augustinson KB. Assay methods for cholinesterases. In: *Methods of Biochemical Analysis* (D. Glick, Ed.), Vol. 5. Interscience Publishers Inc., New York, pp. 1957; 1-63.
17. Ellman GL, Courtney KL, Andres VJr Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol*. 1961; 7: 88-95.
18. Kim HK, Kim M, Kim S, Kim M, & Chung JH. Effects of green tea polyphenol on cognitive and acetylcholinesterase activities.Bioscience, Biotechnology, and Biochemistry, 2004; 68:1977–79.
19. Klegeris A, Korkina LG, Greenfield SA. A possible interaction between acetylcholinesterase and dopamine molecules during autoxidation of the amine. Free Radic. Biol. Med. 1995; 18: 223–230.
20. Simpson JA, Narita S, Gieseg S, Gebicki S, Gebicki JM, Dean RT. Long-lived reactive species on free-radical- damaged proteins. Biochem. J., 1992; 282:621-624;
21. Das A, Dikshit M, Nath C. Profile of acetylcholinesterase in brain regions of male and female rats of adult and old age. Life Sci. 2001; 68 (13): 1545–1555.
22. Oikawa H, Sasaki M, Ehara S, Abe T. Substantia innominata: MR findings in Parkinson's disease. Neuroradiology 2004; 46: 817–821.
23. Henk Konings C, Kuiper MA, Mulder C, Calliauw J, Wolters ECh. CSF acetylcholinesterase in Parkinson disease: decreased enzyme activity and immunoreactivity in demented patients. Short communication. Clinica Chimica Acta 1995; 235: 101-105.
24. Murali G, Panneerselvam C. Age-associated oxidative macromolecular damages in rat brain regions: role of glutathione monoester. J. Gerontol. A Biol. Sci Med. Sci, 2007; 62:824–830.
25. Droge W, Schipper HM. Oxidative stress and aberrant signaling in aging and cognitive decline. Aging Cell. 2007; 6: 361–370.

26. Chowdhuri DK, Parmar D, Kakkar P, Shukla R, Seth PK, & Srimal RC. Antistress effects of bacosides of Bacopa monnierei: Modulation of Hsp70 expression, superoxide dismutase and cytochrome P450 activity in rat brain. Phytotherapy Research, 2002; 16: 639-645.

***Corresponding Author:**
Wudayagiri Rajendra*
Professor, Department of Zoology,
Division of Molecular Biology,
Sri Venkateswara University,
Tirupati-517502. INDIA.
E-mail: rajendraw2k@yahoo.co.in
Mobile: +91 9849667236