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Research article

EVALUATION OF ROTENONE INDUCED PARKINSON'S DISEASE ON GLUTAMATE METABOLISM AND PROTECTIVE STRATEGIES OF *BACOPA MONNIERI*

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ABSTRACT: *Bacopa monnieri* (BM; Family: Scrophulariaceae), also referred as Brahmi has been used for centuries in Ayurvedic system of medicine as a brain tonic, memory enhancer, revitaliser of sensory organs, anti-anxiety, cardio-tonic, diuretic, antidepressant and anticonvulsant agent, and the pharmacological actions are mainly attributed to the saponin compounds present in the alcoholic extract of the plant. The present study was carried out with a specific aim to examine the neuroprotective effect of Rotenone (RT) induced Parkinson's disease (PD) with particular reference to glutamate metabolism in different regions of rat brain. The rats were divided into four groups of six in each, group 1 received Saline water, group 2 received RT through i.p. route for 60 days to induce PD. The BM extract was given orally 20 days before induction of the PD to group 3 and group 4 received Levodopa (LD) orally, referred as drug control. The levels of Glutamine content, Glutamate dehydrogenase (GDH), Glutamine synthetase (GS) and Glutaminase were measured. Glutamine content and activity levels of GDH, GS were significantly depleted and elevated Glutaminase activity was found in different brain regions of rat during RT induced PD when compared to control rats. Treatment with BM and LD caused significant elevation in Glutamine content and the activity levels of GDH, GS and depletion in glutaminase activity in different brain regions of rats when compared to induced PD rats. Our results suggest the ability of BM extract to modulate glutamate metabolism in different brain regions of induced rodent model of PD.

Key words: Parkinson's disease (PD), *Bacopa monnieri* (BM), Rotenone (Rt), Levodopa (LD), Glutamate metabolism.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). It shows symptoms such as muscle rigidity, tremor and bradykinesia [1] and also causes depression, memory loss, sleep disturbance, speech impairments and dysphagia [2]. It has increased relative risk of mortality ranges from 1.6 to 3.0 compared with matched control populations [3]. In recent years there has been an enormous demand for further scientific development of animal models that can mimic the progressive motor impairments as in PD. The novel model of PD is based on chronic systemic exposure of rotenone [4], which is used widely as a pesticide and an insecticide. It is highly lipophilic and readily gains access to all organs [5] and Complex I mitochondrial inhibitor. 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 [6, 7]. Hence there is a need of newer pharmacologically active agents obtained from natural sources as plant extracts. *Bacopa monnieri* (BM), family scrophulariaceae is a medicinal plant commonly known as Brahmi in Sanskrit. It has been used in the indigenous systems of medicine for the treatment of various nervous systems ailments such as insomnia, anxiety, epilepsy, hysteria, etc. [8]. Although the mechanisms by which the degeneration of neurons in PD are poorly understood, indirect evidence suggests involvement of glutamatergic mechanisms in the pathogenesis of this disorder. Glutamate, the major excitatory transmitter in the mammalian central nervous system, is known to be neurotoxic when present in excess at the synapses. These observations may support the hypothesis that many of the signs and symptoms of advanced stages of PD may be driven by an increase in glutamate in the striatum [9]. The present study is mainly focused to investigate the antiparkinsonian effect of *Bacopa monnieri* (BM) with particular reference of glutamate metabolism in different brain regions of rat during 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 collected was shade-dried and powdered. The plant material was percolated with circulating 95% ethanol (200 ml) for 3-4 rounds. The residue was extracted twice using the same procedure. The extract was filtrated 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 ± 25 g, they were maintained at a temperature of $25 \pm 1^\circ\text{C}$ 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 of 1.0 ml/kg/day i.p. for 60 days.

GROUP II: Parkinson's disease was induced by rotenone (emulsified in natural oil to a concentration of 2.5 mg/ml), given i.p. route administration at a dose of 2.5 mg/kg once daily for 60 days [10].

GROUP III: Rotenone-induced Parkinson's diseased rats were treated with BM extract with a dose of 180 mg/kg/day orally for 80 days, started before 20 days from induction of PD.

GROUP IV: Rotenone-induced Parkinson's diseased rats were treated with Levodopa (reference control) with a dose of 10 mg/kg/day orally started after 20 days from induction of PD [11].

The development of Parkinson's disease 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°C until further analysis.

Biochemical Analysis

The level of Glutamine content and activities of Glutamate dehydrogenase, Glutamine synthetase, Glutaminase were estimated by the method of Colowick and Kaplan (1967) [12], Lee and Lardy (1965) [13], Wu (1963) [14] and Meister (1955) [15] respectively in different brain regions of control and experimental animals.

Statistical Analyses

Values of the measured parameters were expressed as mean \pm 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 SPSS.

RESULTS

The levels of glutamine content and the activities of enzymes viz. GDH, GS, Gln.ase in different brain regions (CC, CB, MB, PM) of control and experimental rats were represented in Tables 1, 2, 3 and 4.

In PD induced rats, Glutamine content and the activity levels of enzymes viz., GDH, GS were significantly decreased, whereas Glutaminase activity was increased in all the brain regions, when compared with control rats. Pretreatment with BM extract and Levodopa caused significant depletion in glutaminase activity in different regions of brain when compared to Rotenone induced Parkinson's diseased rats.

Table 1: Changes in the Glutamine (Gln) content in different brain regions of rats during RT-induced PD and pretreatment with ethanolic extract of BM.(Values are expressed in μg of glutamine/g wet wt of tissue)

Gln content	SC	RT	BM+RT	LD+RT
CC	4.24±0.09	2.49±0.12*	3.29±0.06 [#]	3.261±0.12 [#]
CB	3.98±0.04	2.29±0.11*	3.28±0.09 [#]	3.01±0.05 [#]
MB	4.35±0.11	2.38±0.14*	3.30±0.09 [#]	3.35±0.12 [#]
PM	4.26±0.08	2.37±0.08*	3.35±0.12 [#]	3.20±0.08 [#]

Values are expressed in Mean \pm SEM, * $p < 0.05$ as compared with Control.# $p < 0.05$ as compared with PD rats.**Table 2: Changes in the Glutamate Dehydrogenase (GDH) activity in different brain regions of rats during RT-induced PD and pretreatment with ethanolic extract of BM.**(Values are expressed in μ moles formazan formed/mg protein/hr)

GDH activity	SC	RT	BM+RT	LD+RT
CC	4.36±0.06	2.42±0.13*	3.41±0.12 [#]	3.383±0.09 [#]
CB	4.73±0.04	2.29±0.12*	3.68±0.13 [#]	3.48±0.12 [#]
MB	3.77±0.08	2.36±0.16*	3.41±0.11 [#]	3.41±0.26 [#]
PM	3.99±0.031	2.33±0.13*	3.12±0.24 [#]	3.30±0.12 [#]

Values are expressed in Mean \pm SEM, * $p < 0.05$ as compared with Control.# $p < 0.05$ as compared with PD rats.**Table 3: Changes in the Glutamine synthetase (GS) 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 γ -glutamyl hydroxymate formed/mg protein/hr)

GS activity	SC	RT	BM+RT	LD+RT
CC	4.43±0.07	2.14±0.07*	3.41±0.11 [#]	3.25±0.09 [#]
CB	4.36±0.094	2.37±0.12*	3.30±0.09 [#]	3.60±0.079 [#]
MB	4.27±0.08	2.16±0.14*	3.02±0.22 [#]	3.26±0.08 [#]
PM	4.15±0.07	2.07±0.13*	3.16±0.05 [#]	3.20±0.04 [#]

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

Table 4: Changes in the Glutaminase 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 ammonia formed/mg protein/hr)

Glutaminase activity	SC	RT	BM+RT	LD+RT
CC	3.13 \pm 0.06	4.46 \pm 0.13*	3.24 \pm 0.09 [#]	3.46 \pm 0.09 [#]
CB	2.93 \pm 0.05	4.38 \pm 0.09*	3.34 \pm 0.11 [#]	3.41 \pm 0.11 [#]
MB	3.27 \pm 0.09	4.37 \pm 0.14*	3.40 \pm 0.11 [#]	3.24 \pm 0.06 [#]
PM	2.41 \pm 0.10	4.37 \pm 0.15*	3.40 \pm 0.11 [#]	3.54 \pm 0.12 [#]

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

DISCUSSION

Parkinson's disease (PD) is associated with degeneration of the pigmented dopaminergic neurons. Although the mechanisms by which these neurons degenerate in PD are poorly understood, indirect evidence suggests involvement of glutamatergic mechanisms in the pathogenesis of this disorder. Glutamate, the major excitatory transmitter in the mammalian central nervous system, is known to be neurotoxic when present in excess at the synapses. There is evidence that the severe loss of dopaminergic innervations of the striatum in PD is associated with over-activity of glutamate [16, 17]. There is evidence that the decreased dopaminergic innervations of the striatum in PD are associated with over-activity of glutamate [16]. The enzymes participated in Glutamate metabolism are GDH which catalyzes the glutamate to 2-oxoglutarate [18], GS plays a vital role in the ω -amidation of glutamate to form glutamine and Glutaminase strongly favors glutamate formation rather than glutamine synthesis. The purpose of this investigation was to verify the role of BM ethanolic extract in treatment of PD with particular reference to glutamate metabolism, with the aim to elucidate the metabolic changes in parameter related to the release of the glutamate under BM administration. Glutamine content and activities of GDH, GS and Glutaminase, were estimated that showed differential changes in different brain regions of induced PD, BM and LD treated rats.

The chronic exposure to rotenone causes highly selective nigrostriatal dopaminergic degeneration that is features of PD (19, 20). It develops slow onset of PD symptoms that makes suitable to study neuroprotective strategies (20, 21). During rotenone induced PD, the activities of GDH, GS (Table 2, 3) were decreased and Glutaminase activity was increased in all brain regions. These clearly indicate formation of Glutamate and also justify the lowered level of glutamine content (Table 1). This clearly indicating the progression of PD compared with control rats. Glutamate in excess causes neuronal degeneration correlating to neurotoxicity and described this interaction known as 'neuroexcitotoxicity' [22]. Glutamate is a major cause of neuronal cell death in a number of different neurodegenerative diseases [23]. Two pathways for glutamate toxicity have been described: excitotoxicity, which occurs through the activation of glutamatergic receptors [24, 25], and oxidative glutamate toxicity, which is mediated via a series of disturbances to the redox homeostasis of the cell [26]. These pathways are incompletely characterized, but both result in the production of free radicals [27]. Glutamatergic neuronal stimulation may be a common final pathway in several brain conditions in which oxidative stress and ensuing excitotoxicity plays a role. A contributing factor in many such conditions is excessive glutamate release, and subsequent glutamatergic neuronal stimulation, that causes increased production of reactive oxygen species (ROS), oxidative stress, excitotoxicity and neuronal damage (28). Loss of dopamine and stimulation of dopamine receptors on the glutamatergic terminals result in an increased level of glutamate in substantia nigra (SN) (29) may cause excitotoxicity in PD. Glutamate excitotoxicity causes ROS production which inducing oxidative stress which may be an important factor in several pathological brain conditions of PD.

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years [30]. *Bacopa monnieri*, used in this study is one of the ancient ayurvedic plant used for a long time in as brain tonic for promoting mental health and improving memory [31]. Preclinical and clinical studies have shown that BM improves memory and mental function [32]. The chronic effects of an extract of BM on cognitive function in human subject have been reported [33] and also its pharmacological roles as memory enhancer [31], antidepressant and also antioxidant properties [34]. Human consumption of BM is on the increase owing to its multiple beneficial effects [35]. In the present study, pretreatment with BM significantly increased the Glutamine content, GDH, GS activities (Table 1, 2 and 3), whereas Glutaminase activity (Table 4) was found to be increased in the different brain regions of induced PD rat. 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 demonstrates the 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 difference compared to controls, which clearly indicates recovering of this group of rats from PD. The BM treated group results are similar to the results of LD treated group which shows that it can act as antiparkinsonian agent. These results may show the overall improvement of Glutamate metabolism, increased the brain glutamine level (36) and proves the decrease of glutamate level. These data together indicate the neuroprotective role of BM extract which were almost comparable to LD treated rats.

In conclusion, our findings demonstrated the ability of BM extract to modulate glutamate metabolism in different brain regions of Rotenone induced rodent model of Parkinson's disease. Further, the *Bacopa monnieri* extract, effectively regulates glutamatergic hyperexcitation and thus can be used as "antiparkinsonian agent".

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