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Original Article

PROTECTIVE ROLE OF *BACOPA MONNIERI* ON INDUCED PARKINSON'S DISEASE WITH PARTICULAR REFERENCE TO CATECHOLAMINE SYSTEM

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ABSTRACT

Objective: Parkinson's disease (PD) is the second most neurodegenerative disorder. *Bacopa monnieri* (BM), an Indian herb extensively used in Ayurveda, used in treatments related to neurological complications was used now in our study.

Methods: In the present experiment, rats were divided into four groups of six in each group: group 1 received saline water, group 2 received rotenone (RT) through i.p. for 60 days to induce PD, group 3 received for 20 days orally before induction of PD and group 4 received Levodopa (Reference drug; LD) orally.

Results: The levels Dopamine (DA), Serotonin (5-HT), Epineprine (EP), Nor-epineprine (NEP) were decreased and Monoamine oxidase (MAO) activity was increased in different brain regions such as Cerebral cortex (CC), Cerebellum (CB), Mid brain (MB) and Pons medulla (PM) during induced PD compared with controls. These results were reversed after treatment with ethanolic extract of BM on par with reference drug (LD).

Conclusion: Our results suggest the ability of BM extract to modulate catecholamine system in different brain regions of RT induced rodent model of PD and thus offers protection. When compared overall the BM is better than the LD drug. The BM may provide a platform for future drug discoveries and novel treatment strategies in PD and can act as antiparkinsonian agent.

Keywords: Parkinson's disease (PD), *Bacopa monnieri* (BM), Rotenone (RT), Levodopa (LD), Dopamine (DA), Serotonin (5-HT), Epineprine (EP), Nor-epineprine (NEP) and Monoamine oxidase (MAO).

INTRODUCTION

PD is the second most common progressive neurodegenerative disease after Alzheimer's disease. PD is characterized by difficulties in movement control. Its classic symptoms include resting tremor, inability to start movements, rigidity, and difficulties in maintaining gait. PD results from the slow degeneration of dopaminergic neurons in the pars compacta of the substantia nigra and subsequent reduction of the modulatory actions of dopamine in the striatum. The etiology of PD remains unknown and no disease-modifying drugs are available. Thus, the need for more information on the pathogenesis of PD is obvious. There is considerable evidence suggesting that mitochondrial dysfunction and oxidative damages may play a role in the pathogenesis of PD. Among the toxic animal models of PD, chronic systemic exposure of rotenone represents one of the most recently used approaches. (Betarbet *et al.,* 2000). It is highly lipophilic and readily gains access to all organs (Talpade *et al.,* 2000) and functions as Complex I mitochondrial inhibitor. The chronic exposure to rotenone causes highly selective nigrostriatal dopaminergic degeneration, the characteristic feature of PD (Betarbet *et al.,* 2000; Alam and Schmidt, 2002; Sherer *et al.,* 2002). It develops slow onset of PD symptoms that makes suitable to study neuroprotective strategies (Sherer *et al.,* 2003; Schmidt and Alam, 2006).

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 (Deogaonkar and Subramanian, 2005). Hence there is a need to discover newer pharmacologically active agents obtained from natural sources as medicinal plant extracts. Several agents were suggested to modulate the cellular energy metabolism and could exert antioxidative effects. Agents that have shown to be beneficial in animal models of PD include creatine, Ginkgo biloba leaves extract (Bastianetto *et al.,* 2002), Vicia faba beans (Shetty *et al.,* 2002) coenzyme Q_{10}, nicotinamide, and acetyl-L-carnitine. *Bacopa monnieri* (called Brahmi in Sanskrit); an ayurvedic medicinal plant used as a brain tonic, can be used in this PD treatment. Its ethanolic extract contains a mixture of triterpenoid saponins designated as bacosides A and B (Chatterjee *et al.,* 1963, 1965). Several studies have reported its pharmacological roles as memory enhancer (Bhattacharya *et al.,* 2000), cognition-enhancer, (Das *et al.,* 2002), antidepressant and also antioxidant properties (Sairam *et al.,* 2001). In the present study *Bacopa monnieri* (BM) was used to study neuroprotection of 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 (including roots) of B. monnieri was dried in shade, and then powdered plant material was macerate 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 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±2^{0}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. The rats were divided into 4 groups each consisted of 6 rats.

Group I: Served as normal control group, Oil (sunflower oil) was injected as vehicle to the control rats (1 ml/kg) of 1.0 ml/kg/day i.p. for 60 days.

Group II: Rotenone (RT) emulsified in sunflower oil at 2.5 mg/ml was given intraperitoneally once a day at 1 ml/kg for 60 days (Alam and Schmidt, 2002), which induces PD.

Group III: RT-induced PD 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: RT -induced PD rats were treated with Levodopa (reference control) with a dose of 10 mg/kg/day orally started after 20 days from induction of PD (Alam and Schmidt, 2004).

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^{0}C until further analysis.

Biochemical Analysis

Epinephrine (EP), Nor-epinephrine (NE) and dopamine (DA) and 5-HT were estimated in all the brain regions were estimated by the method of Kari *et al.* (1978) and Monoamine oxidase (MAO) activity was estimated by the method of Green and Haughton (1961).

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 differences between means in more than two groups. A probability value of < 0.05 was considered to be statistically significant. All statistical analysis were performed using the SPSS statistical software package version 11.5.

RESULTS

The levels of biogenic amines, viz. dopamine (DA), norepinephrine (NE), epinephrine (EP), 5-hydroxytryptamine (5-HT), and the content of the enzyme monomaine oxidase (MAO) were estimated 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, 2, 3 and 4).

Table 1: Changes in the Nor epinephrine (NE) content in different brain regions of rats during RT-induced PD and on pretreatment with ethanolic extract of BM and treatment with LD. (Values are expressed in µg of Nor epinephrine/g wet wt of tissue)

S. No.	Brain regions	SC	RT	BM+ RT	LD+ RT
1	CC	3.12±0.05	1.13±0.03* (-63.78 [123.00]	2.52±0.12# (-19.23) [102.65]	2.29±0.10# (-26.60) [102.65]
2	CB	2.93±0.05	1.35±0.11* (-53.92 [92.59]	2.60±0.07# (-11.26) [79.25]	2.42±0.03# (-17.406) [79.25]
3	MB	3.10±0.06	1.06±0.01* (-65.80 [154.71]	2.70±0.03# (-12.90) [162.26]	2.78±0.14# (-10.32) [162.26]
4	PM	3.17±0.09	1.40±0.15* (-55.83) [85.71]	2.60±0.14# (-17.98) [81.42]	2.54±0.02# (-19.87) [81.42]

Table 2: Changes in the Epinephrine (EP) content in different brain regions of rats during RT-induced PD and on pretreatment with ethanolic extract of BM and treatment with LD

S. No.	Brain regions	SC	RT	BM+ RT	LD+ RT
1	CC	3.91± 0.02	1.18±0.25* (-69.82) [113.55]	2.50±0.10# (-55.15) [94.06]	2.31±0.12# (-41.43) [94.06]
2	CB	3.57±0.17	1.38±0.10* (-61.34) [88.40]	2.62±0.02# (-27.17) [75.36]	2.22±0.03# (-32.21) [75.36]
3	MB	3.92±0.23	1.06±0.07* (-72.95) [154.71]	2.69±0.01# (-82.14) [162.26]	2.77±0.24# (-29.08) [162.26]
4	PM	3.89±0.13	1.53±0.14* (-60.66) [69.93]	2.71±0.11# (-33.16) [66.01]	2.34±0.09# (-34.70) [66.01]

(Values are expressed in µg of Epinephrine/g wet wt of tissue)

DISCUSSION

Dopamine, along with the neurotransmitters norepinephrine and epinephrine, forms part of a system called the catecholamine system. Since these three monoamines play a pivatol role in motor control, so enhancing the catecholaminergic can be considered as one of the preferred method to reduce the Parkinson's symptoms and to improve overall health. All of these neurotransmitter pathways play a crucial role in the emotional well-being of an individual given their linkages to neurobehavioral processes as emotion, mood and cognation (Aarsland, 2006). Chronic use of current anti-parkinsonian medications causes disabling abnormal involuntary movements known as drug-induced dyskinesias (DID) in majority of patients with advanced Parkinson's disease (PD) that are expensive and difficult to treat.

However, anticonvulsant treatment with herbal medicines has not received much attention. The present study was aimed to know the effect of ethanolic extract of BM on induced PD, with the particular reference to catecholaminergic system.

Table 3: Changes in the Dopamine (DA) content in different brain regions of rats during RT-induced PD and on pretreatment with ethanolic extract of BM and treatment with LD.(Values are expressed in μg of Dopamine/g wet wt of tissue)

S. No.	Brain regions	SC	RT	BM+ RT	LD+ RT
1	CC	6.47±0.07	2.20±0.10* (-65.99)	5.42±0.12# (-16.22) [146.36]	5.27±0.09# (-18.54) [139.54]
2	CB	6.40±0.08	2.40±0.13* (-62.5)	5.40±0.10# (-15.62) [125]	5.61±0.08# (-12.34) [133.75]
3	MB	6.31±0.08	2.55±0.14* (-59.58)	4.39±0.63# (-30.42) [72.15]	5.31±0.07# (-15.84) [108.23]
4	PM	6.21±0.10	2.79±0.07* (-55.07)	4.88±0.38# (-21.41) [74.91]	5.29±0.17# (-14.81) [89.60]

Table 4: Changes in the 5-Hydroxytryptamine (5-HT) content in different brain regions of rats during RT-induced PD and on pretreatment with ethanolic extract of BM and treatment with LD

S. No.	Brain regions	SC	RT	BM+ RT	LD+ RT
1	CC	5.40±0.05	2.18±0.08* (-59.62)	4.38±0.11# (-18.88) 100.91	4.25±0.07# (-21.29) 94.95
2	CB	5.40±0.05	2.18±0.08* (-59.62)	4.38±0.11# (-18.88) 100.91	4.25±0.07# (-21.29) 94.95
3	MB	5.27±0.06	2.53±0.14* (-51.99)	3.68±0.41# (-30.17 45.45	4.28±0.05# (-18.78) 69.16
4	PM	5.19±0.09	2.75±0.09* (-47.01)	4.03±0.21# (-22.35) 46.54	4.27±0.05# (-17.72) 55.27

(Values are expressed in μg of 5-Hydroxytryptamine /g wet wt of tissue)

Table 5: Changes in the Monoamine oxidase (MAO) activity in different brain regions of rats during RT-induced PD and on pretreatment with ethanolic extract of BM and treatment with LD

S. No.	Brain regions	SC	RT	BM+ RT	LD+ RT
1	CC	3.38±0.12	7.01±0.25* (107.39)	4.28±0.15# (26.62) [-38.94]	4.32±0.19# (27.81) [-38.37]
2	CB	2.44±0.11	7.35±0.20* (201.22)	4.59±0.12# (88.11) [-37.55]	4.52±0.13# (85.24) [-38.50]
3	MB	2.73±0.17	7.07±0.24* (158.97)	4.50±0.12# (64.83) [-36.35]	4.45±0.17# (63.00) [-37.05]
4	PM	2.45±0.15	7.57±0.16* (208.97)	4.21±0.09# (71.83) [-44.38]	4.56±0.15# (86.12) [-39.76]

(Values are expressed in μ moles of p-hydroxyphenyl acetaldehyde formed/mg protein/hr). All the values are expressed in Mean ± SEM of six individual observations, * Values with the same superscript is significant at $p<0.05$ compared with control, # Values with the same superscript is significant at $p<0.05$ compared with PD rats, Values in '( )' paranthesis are % change over saline control, Values in '[ ]' paranthesis are % change over RT induced PD. In RT-induced rats, DA, NE, EP, 5-HT levels ($P < 0.05$) were signi ficantly depleted,whereas MAO activity ($P < 0.05$) was elevated in all the brain regions, when compared with control rats. Pretreatment with BM extract and treatment with LD caused significant elevation of DA, NE, EP,5-HT levels ($P < 0.05$) and depletion of MAO activity ($P < 0.05$) was observed in different brain regions, when compared to RT induced PD rats.

In the present study, NE levels were decreased in RT-induced rats may be due to the loss of 50% of neurons in PD (Kellstein *et al.,* 1988). Similarly EP levels were also decreased (Ahlskog *et al.,* 1996) during RT-induced PD (Ahlskog *et al.,* 1996). DA levels and levels of 5-HT (Rao *et al.,* 2007) were decreased in RT-induced rats. In the present study NE, EP, DA and 5-HT levels increased significantly in pretreated BM and LD treated RT-induced rats compared to the RT-induced rats. Sheikh *et al*. (2007) also have reported significant elevation in NE, DA and 5-HT levels in cortex and levels of NE and 5-HT in hippocampus when treated with BM in acute and chronic unpredictable stress. On par with the decreased monoamines levels one of the main metabolizing enzymes monoamine oxidase (MAO) was found to be increased in all areas of rat brain during RT-induced Parkinson's disease. Which suggest that the MAO is partially responsible for the metabolism of catecholamines and hence the rate of catecholamine depletion after synthesis/inhibition can be used as an index of catecholamine turnover (Brodie *et al.,* 1966). The major problem encountered with Parkinson's is the degeneration of the dopaminergic system, leading to lowered dopamine levels. Thus dopamine levels, when abnormally low, due to degeneration of the dopamine system in the brain, leads to the loss of motor control and other conditions associated with Parkinson's disease. The most prominent symptoms of PD reflect deficits in motor function, such as tremors, postural disturbance, and difficulty in initiating voluntary movements, it is clear that catecholamine tract alterations had played a crucial role in the control of movement during induced PD. As in case of RT induced PD rats, overall catecholamine system is reduced when compared to control rats due to progression of disease. The main motor symptoms of PD as rigidity/tremors and muscle fatigness may be due to the abnormal increase of Acetylcholine (Ach) content in PD. 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 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. As in our previous study (Swathi *et al.,* 2013) the ACh levels were decreased significantly in pretreated BM and LD treated RT-induced rats compared to the RT-induced rats showing the rescuing effect of BM. Decrease in Ach levels shows the balance with DA was maintained, indicating increase in DA content when treated with BM extract to PD induced rats.

It was recently demonstrated that a moderate level of complex I inhibition characteristic for PD leads to significant ROS formation (Sipos *et al.,* 2003). Dopaminergic neurons are also likely to face a higher baseline level of oxidative stress due to ROS formed from the degradation of dopamine. On par with the Levodopa, pretreatment with the extract of BM caused conspicuous augmentation in the levels of monoamines and decline in the levels of monoamine oxidase. Thus the BM extract caused due to the PD and thus offers antiparkin sonian effect by modulating the monoamine neurotransmitters in different regions of rat brain. It has been reported that BM extract the dopamine content was found to be increased significantly compared with RT, in order for normal dopaminergic neurons to survive, they may require more intrinsically increased antioxidant capacity than other neurons. Among all of this experimental therapeutic refinement as LD, the use of BM extract has been most successful in that it has been shown that BM extract may have naturally occurring antioxidant capacity for rescuing striatal neurons and prolonging the survival of PD induced rats. In our present investigation, midbrain region in RT induced rat found DA content to be decreased by 80.98%, which shows the progression of the disease, were as in BM pretreated rats showed decrease of 30.42% compared to controls which showed its recovering against PD.

CONCLUSION

The present findings coupled with the earlier reports clearly suggests that abatement of all the biogenic amines play a pivotal role in the neuronal damage during PD and the bioactive factors present in the BM offers protection against PD induced catecholaminergic abnormalities.

CONFLICT OF INTERESTS

Declared None

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