



## NEUROCHEMICAL MODULATION BY *BACOPA MONNIERI* IN ROTENONE INDUCED HEMI-PARKINSON'S DISEASE

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### ABSTRACT

Parkinson's disease is the second most common neuro-degenerative disorder. Treatment with L-DOPA improves the motor symptoms, but lead to dyskinesia. The herbal extract of *Bacopa monnieri* is used as a cognitive enhancer in ayurvedic system of medicine. The efficacy of saponin rich extract of *Bacopa monnieri* in the treatment of Parkinson's disease is evaluated in the present study. The changes in the brain levels of monoamine neurotransmitters and their metabolites were analyzed by HPLC technique. The levels of dopamine, levodopa, 3,4-dihydroxyphenylacetic acid, homovallic acid and serotonin were decreased and norepinephrine was increased incredibly in the midbrain and striatum of PD induced animals. On treatment with *Bacopa monnieri* whole plant extract the animals exhibited a marked increase in the level of dopamine and its metabolites and a decrease in norepinephrine. A trivial increase in the level of epinephrine was observed on PD induction which improved on *Bacopa* treatment. This clearly indicates the neuroprotection imparted by *Bacopa monnieri* in rotenone induced Parkinson's disease.

**Keywords:** Parkinson's disease, Neurotransmitter, Dopamine, *Bacopa monnieri*

### 1. INTRODUCTION

First described by James Parkinson in 1817, Parkinson's disease is the second most-prevalent neurodegenerative disorder, next to Alzheimer's disease. It is a progressive disorder affecting several regions of the brain, especially the substantia nigra that controls balance and movement. Intra-neuronal proteinaceous inclusions known as Lewy bodies, characterized by depigmentation of the substantia nigra is one of the pathological hallmark of Parkinson's disease [1]. A majority of cases of the disorder are of unknown origin and sporadic, but several genes have been identified that, when mutated, give rise to familial forms of the disease. Even though it was categorized under disorder of senility, neuronal damage in normal ageing is not adequate to cause Parkinson's disease. Stress response, with reduced mitochondrial function resulting from variable expression of genes involved in mitochondrial energy metabolism, is one of the contributors of the pathogenesis of this condition. Malfunctioning of the stress attenuating system of the body could lead to an accelerated neurodegeneration in a positive feedback-type scenario [2]. There is a general agreement that mitochondrial dysfunction,  $\alpha$ -synuclein aggregation, oxidative stress, neuro-inflammation, and

impaired protein degradation are involved in the neurodegeneration of dopaminergic neurons. The depletion of striatal dopamine causes deregulation of the motor circuits that project throughout the basal ganglia, resulting in the clinical manifestations of the disease [3]. Parkinson's disease exhibit both motor and non-motor symptoms. The cardinal motor symptoms of Parkinson's disease are bradykinesia resulting from failure of basal ganglia to reinforce cortical mechanisms to execute motor commands [4] tremor due to the intrinsic biophysical property of thalamic neurons [5] and rigidity due to the exaggerated beta band activity from the subthalamic nuclei [6]. The non-motor manifestations include olfactory, visual, sleep disturbances, neuropsychiatric, autonomic, pain and other sensory symptoms which are attributed to the widespread distribution of  $\alpha$ -synuclein [7].

In the neuropathology of Parkinson's disease a complex, interconnected neuronal circuit systems are involved. This pathology is regulated by a number of different neurotransmitters in addition to dopamine. The degeneration of dopaminergic neurons in nigro-striatal pathway has led to a neurotransmitter imbalance in the extrapyramidal system with a dopamine and GABA

deficiency and an acetylcholine and glutamate surplus [8]. The non-dopaminergic neurotransmission systems modulate and interact with dopamine in causing non-motor symptoms of the disease. PD treatments like levodopa administration that focus primarily on the dopaminergic system alone are unable to alleviate the non-motor symptoms [9]. Therefore assessment of the neurotransmitters is crucial in determining the severity of the disease condition and the progression of the disorder. *Bacopa monnieri*, commonly known as 'water hyssop', is an herb often used in Ayurvedic medicine since ages. Supplementing it with food has been shown to improve cognition, thereby reducing anxiety, improve memory formation and consolidation. Even though the effects are usually studied in the elderly population, it appears to affect young people as well, making it a useful nootropic. There are documented evidences suggesting many herbal extracts possessing antioxidant effects and *Bacopa monnieri* has gained an increasing attention as traditional medicine for treatment of several disorders [10]. It was revealed that the antioxidant property of *Bacopa monnieri* is due to its free radical scavenging activity and its methanolic extracts have greater antioxidant properties as observed by the low  $IC_{50}$  value and high phenolic content [11]. *Bacopa monnieri* also possess an effective DPPH scavenging activity indicating its antioxidant potential [12]. In the present study the effect of methanolic extracts of *Bacopa monnieri* in alleviating the neurotransmitter imbalance due to unilateral infusion of neurotoxicant rotenone in medial forebrain bundle of male Wistar albino rats were compared.

## 2. MATERIAL AND METHODS

### 2.1. Plant extract preparation

Pre-identified *Bacopa monnieri* plants specimen was procured from a nursery in Chennai. The dried plants were coarsely powdered and macerated with methanol and extracted in Soxhlet apparatus. The plant extract obtained was freeze dried and stored in 4°C temperature. The extract was dissolved in 0.9% saline solution (10 mg/ml). The animals were treated with a dosage of 50 mg/kg body weight of this methanolic extract.

### 2.2. Chemicals

Analytical grade of Rotenone (R8875), Dimethyl sulfoxide (D8418) and neurotransmitter standards were purchased from Sigma Aldrich (USA). All other chemicals of analytical grade were procured from Sisco Research Laboratories (India).

### 2.3. Animals

All the experimental procedures were carried under ethical guidelines and the permission was obtained from the institutional animal ethical committee (IAEC number- 01/18/2015). Five cohorts of young adult male Wistar albino rats, each containing six animals weighing 230-250 gram were used in the present study. The animals were housed in a temperature controlled room on a 12 h light-dark cycle with access to both food and water *ad-libitum*. The first group of animals was used as control which was not lesioned and untreated. The second group was sham operated animals which were infused with 4µl of dimethyl sulfoxide (DMSO) into the right medial forebrain bundle stereotactically. The third group of animals were not operated but received *Bacopa monnieri* (BM) extract at a dosage of 50 mg/kg body weight [13] for 60 days and the fourth group included Parkinson's disease induced animals which were infused with 3µg of rotenone dissolved 4µl of DMSO into the right medial forebrain bundle (MFB) and the fifth group comprised of animals that were treated with BM for 30 days before and after surgery.

### 2.4. Rotenone-induced Hemi-Parkinson's disease

The induction of Hemi-Parkinson's disease condition was done according to the standard protocol adopted by Sindhu et al., [14]. Rotenone was dissolved in DMSO at a concentration of 0.75µg /µl. The reference for the location of medial forebrain bundle (MFB) is AP: 4.0 mm; L: 1.8 mm; DV: 7.6 mm from the bregma [15]. The animals were anaesthetised with ketamine (87 mg/kg of body weight) and xylazine (13 mg/kg of body weight) by a single dosage of intra-peritoneal injection [16] fastened on the stereotaxic frame and the head was fixed in flat skull position. A midline incision is made in the skin over the skull and a bore is made using a hand held drill for access of stereotaxic cannula (RM SBL - Braintree Scientific, U.S.A.). The cannula is inserted into the right MFB region and rotenone is infused for twenty minutes at a rate of 0.2 µl/min, using syringe pump (BS-300 - Braintree Scientific, USA.) The cannula was left in place for ten minutes and after complete diffusion of the drug it was retracted subsequently. The skin was sutured and proper post-operative care was given until the animal recovered completely. The animals exhibiting spontaneous rotational behavioural towards the side of lesion in the first three days of recovery were included in the study.

## 2.5. Preparation of Brain tissue Sample

The animals were stunned by cervical dislocation followed by decapitation. The brain of the animal was immediately removed and the discrete regions were separated. The midbrain region containing substantia nigra and the striatum were used for the estimation of neurotransmitters. These regions were isolated weighed in a physical balance. 60 mg of the tissue was taken in a tube containing 1 ml of 0.1M perchloric acid solution and homogenised. To this 10µl of DHBA solution as internal standard was added. This homogenate was centrifuged at 2000 rpm in a cooling centrifuge maintained at 4°C. After centrifugation the supernatant was taken and centrifuged at 12000 rpm at 4°C for 20 minutes. The supernatant was filtered using a 0.2 µm syringe filter and collected in a tube.

## 2.6. High Performance Liquid Chromatography

The mobile phase was prepared by adding 3.674 gram of Citric acid, 0.8872 gram of Disodium hydrogen orthophosphate, 0.054 gram of 1-Octanesulfonic acid, 0.0092 gram of Ethylenediaminetetraacetic acid and 90 ml of methanol to 410 ml of double distilled water [17]. This mobile phase is allowed to flow through the detector using a HPLC pump that pumps at a rate of 0.9 ml/min. The detector was set on and the current range of 50nA and voltage of +0.10V was set in the detector. The data acquisition system started and the baseline was obtained. 20 µl of the sample solution was taken in a

Hamilton glass syringe and injected. The peak of each analyte was observed in the data acquisition system.

## 2.7. Estimation of neurotransmitters

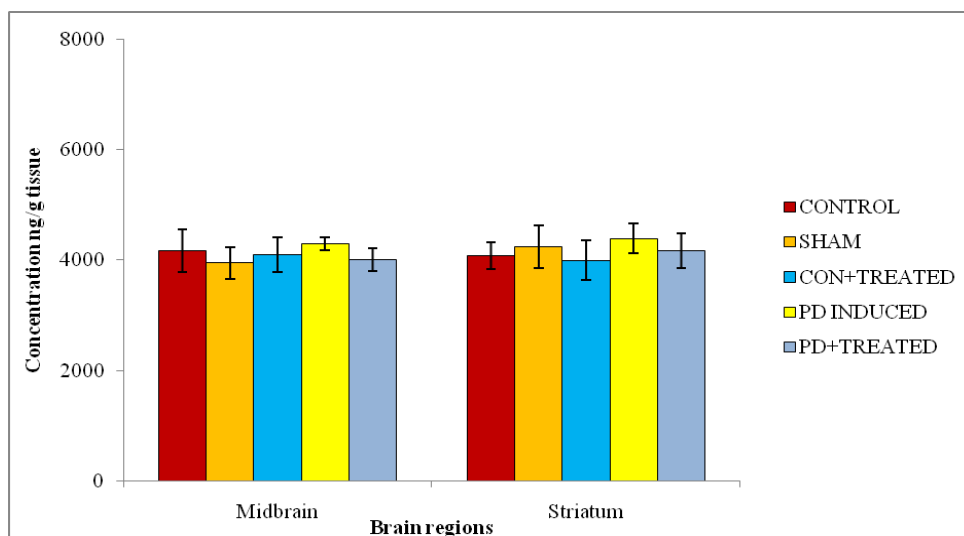
The area of the peak of each analyte was determined by extrapolation. This area was compared to that of the internal standard and the concentration of the neurotransmitters in the brain discrete region was estimated.

## 2.8. Statistical Analysis

All the data of the tests were expressed as Mean ± Standard Deviation (SD). The results observed were analyzed by SPSS (version 25.0, SPSS Institute Inc., Cary, USA). The statistical significance between different groups of the study was determined by one-way analysis of Variance (ANOVA) and the Tukey's honest significant difference (HSD) test for post hoc analysis by setting the P value less than 0.05.

## 3. RESULTS AND DISCUSSION

The neurotransmitters are endogenous chemical messengers transmitting signals across synapses in nervous tissue. The estimation of neurotransmitters in the brain discrete regions is most vital in the present study as rotenone cause dopaminergic degeneration leading to neurotransmitter imbalance in the basal ganglia.

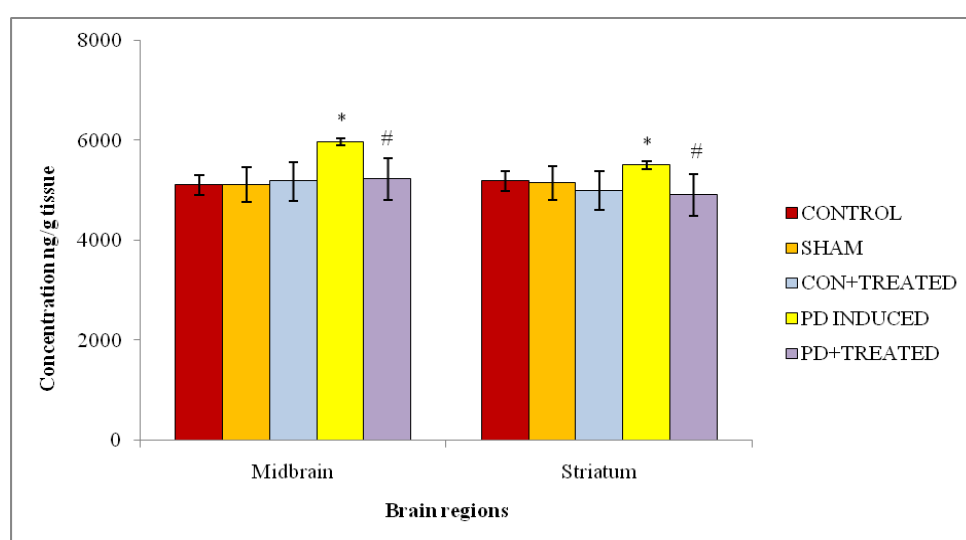


**Fig. 1: Showing the level of Epinephrine in midbrain and striatum among the experimental groups. The level of epinephrine was increased in PD induced group which was insignificant as compared to that of the control animals and on treatment with Bacopa extract the animals showed a decrease in the level of epinephrine**

The monoamine neurotransmitters epinephrine, norepinephrine and dopamine were studied in the midbrain and striatum of right side. Since dopamine is the key neurotransmitter in PD, its precursor levodopa and its metabolites DOPAC and homovallic acid (HVA) in the striatum and substantia nigra regions were also studied. The neurotransmitter imbalance in Parkinson's disease induction was studied in the midbrain and striatal region of the experimental animals. The estimated values of neurotransmitters were compared between the groups and the efficacy of *Bacopa monnieri* in imparting neuroprotection was analyzed.

Rotenone, on infusion in MFB altered the dopaminergic system and led to neurotransmitter imbalance. Therefore

the disease is a multisystem disorder affecting other neurotransmitters in the associated brain regions. In the present study, as shown in figure 1, it was observed that the brain level of epinephrine was decreased in PD induced animals but there was no statistical significance among the groups. The probable reason for this could be that, in the present study since the rotenone infusion is targeted only in MFB affecting SNpc region. The adrenergic innervation in midbrain was in the lateral ventral tegmental area which receives a strong innervation from C1 A2 area of medulla [18]. These adrenergic neurons may also have a vital impact in regulation of DA neurons during stress.



**Fig. 2: Showing the level of norepinephrine in midbrain and striatum among the experimental groups. The level of norepinephrine was remarkably increased in PD induced group as compared to that of the control animals. On treatment with *Bacopa* extract the animals showed a near normal level of NE as compared to that of control.**

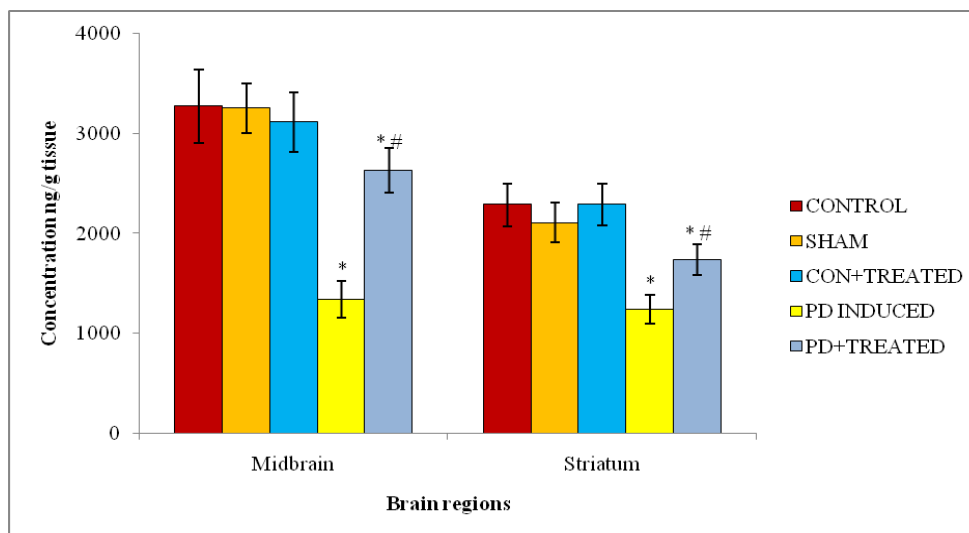
The level of norepinephrine in midbrain and striatum was shown in figure 2. It was observed that the level was significantly increased in the PD induced animals when compared to that of the control animals in the midbrain and striatum regions. This effect may be due to the effect of rotenone on dopaminergic neurons in the SNpc thereby, affecting the neurochemical balance in brain. The motor symptoms attributed to Parkinson's disease are not solely attributed due to dopaminergic loss but also other neurotransmitter imbalance mainly norepinephrine. Even, depletion of dopamine alone in animal models has failed to simultaneously elicit both the motor and non-motor deficits of Parkinson's disease [19]. Since, stress is a common activator of adrenergic neurons, traumatic stress could also be the probable

reason for increasing the sympathetic activity on locus coeruleus which is the principal source of norepinephrine in brain. Administration of *Bacopa monnieri* had a potent effect on the norepinephrine levels in brain bringing the levels of NE back to normal. The inherent antioxidant and neuroprotective property of *Bacopa* might provide this protective effect. It was also reported that, in 6-OHDA lesion of dopaminergic neurons, over activity of locus coeruleus noradrenergic neurons with an irregular pattern of discharge was observed, suggesting their magnified influence in DA-lesioned rats [20].

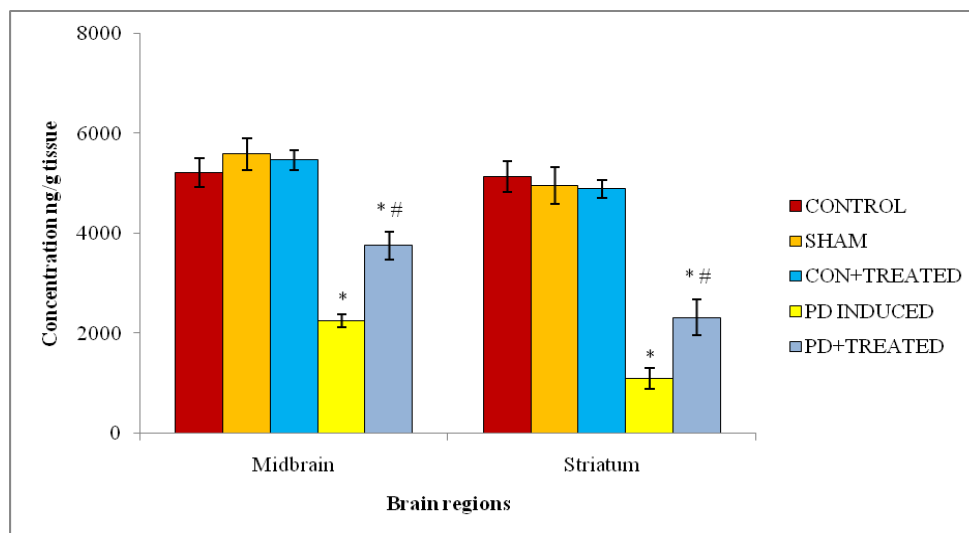
Since, levodopa (LD) is a precursor for dopamine synthesis its level in brain regions provides a vital indication of neural damage. The most effective therapeutic strategy for PD is oral administration of

levodopa. It crosses the blood brain barrier and gets converted to dopamine in so reversing the symptoms mainly, hypokinetic state and rigidity [21]. It is well documented that LD has the ability to overwhelm the cardinal symptoms mainly akinesia and rigidity.

Therefore the estimation of the brain levels of LD plays a significant role and is one of the useful biomarker to delineate the extent of neural damage in PD. The levels of LD in brain discrete regions were depicted in figure 3.



**Fig. 3: Showing the level of levodopa in midbrain and striatum among the experimental groups.** The level was markedly decreased in PD induced animals when compared to that of the control animals and on treatment with Bacopa extract the animals showed a notable increase in the brain level of levodopa but normalcy was not restored.



**Fig. 4: Showing the level of dopamine in midbrain and striatum among the experimental groups.** The levels of dopamine was markedly decreased in PD induced animals as compared to that of the control animals and on treatment with Bacopa the animals showed a notable increase in the brain level of dopamine as compared to that PD induced animals.

It was inexplicably observed that the level was also altered significantly in the PD induced groups as compared to that of the control group of animals. This may be due to the fact that the conversion of

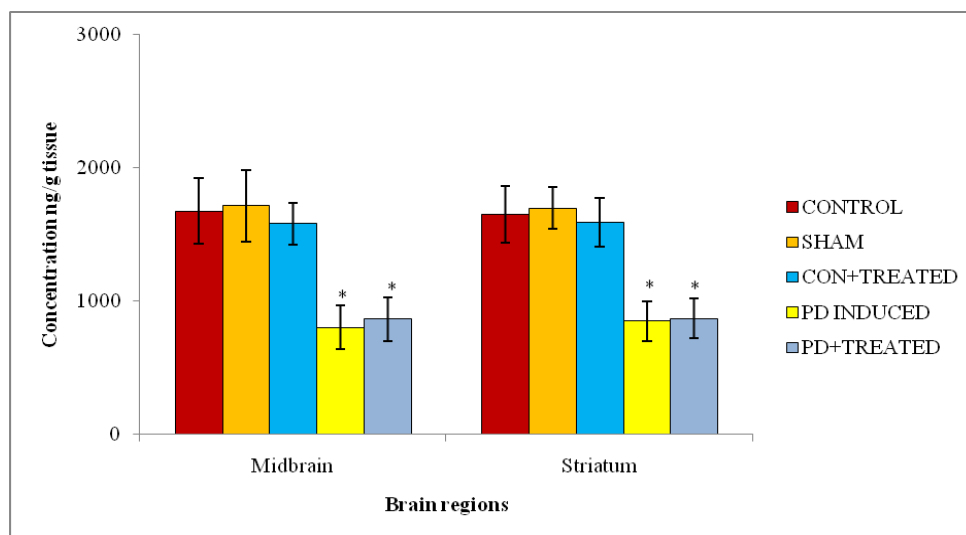
phenylalanine to tyrosine is the rate limiting step in the dopamine synthesis [22] when blocked the further steps in dopamine synthesis were blocked. This prominently emphasizes the role of enzyme tyrosine hydroxylase in

dopamine synthesis. Treatment with *Bacopa* in the experimental group with induced PD showed a significant increase in the levels of LD. This highlights the inherent action of *Bacopa* on dopaminergic neurons increasing the synthesis of dopamine. It also signifies the effectiveness of tyrosine hydroxylase enzyme activity in the dopamine synthesis. The level of levodopa in substantia nigra of control, sham operated and *Bacopa* treated animal groups did not varied perceptibly.

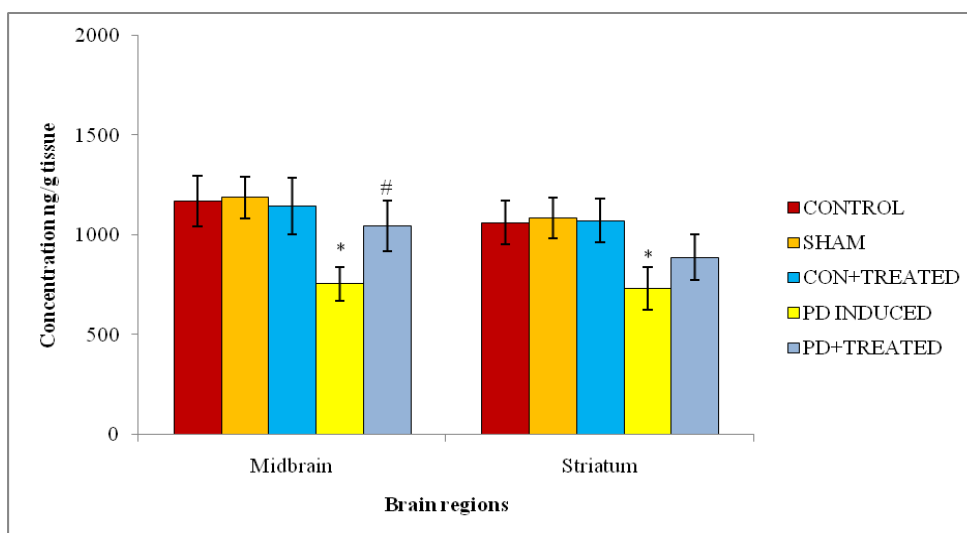
As shown in figure 4, the levels of dopamine in the brain discrete regions were significantly altered among the experimental groups in midbrain and striatum. On induction, PD caused a significant decrease in the dopamine levels in these regions. This evidences that, infusion of rotenone in dopaminergic neurons at the level of MFB cause an anterograde and retrograde degeneration of the nerve terminal and cell body respectively. This degeneration affects the rate of synthesis and dopamine turnover. This degeneration of the nervous tissue may be due to the generation of  $H_2O_2$  produced due to the oxidation of dopamine by MAO in the presence of  $Fe^{2+}$  through the Fenton reaction [23]. Even though alpha synuclein formation is a protective mechanism by which the degenerating neurons accumulate the oligomers in lewy bodies, the release of dopamine from the neurons were affected [24]. This has led to a lack of dopamine at the nerve terminal, which was the prime reason for the symptoms of PD observed in the study. The interaction between dopamine from SNpc with neurons of basal ganglia at the biochemical

level is responsible for fine tuning of voluntary movements. Treatment with *Bacopa monnieri* act as an antioxidant having a check over the ROS produced due to complex-I inhibition in mitochondria [25]. This prevents the degenerating neurons from further damage and has a protective effect over them. In this study it was observed that treatment with *Bacopa monnieri* had a protective effect over damage caused by rotenone, evidenced as an escalation of dopamine level in the brain regions. It was found that standardized *BM* powder protected against rotenone-induced oxidative damage and dopamine depletion [26]. Even though *Bacopa monnieri* exhibit shielding effects, the damage caused by rotenone was undying. Therefore in the experimental group treated with *Bacopa monnieri* and induced PD the level of dopamine was increased significantly but normalcy was not brought back.

The availability of dopamine in the nigral nerve terminals is a key factor in determining the severity of PD. In the present study, rotenone infusion produced an apoptotic degeneration of the dopaminergic neurons of basal ganglia and thereby decreasing its availability in striatum. The degradation of dopamine is catalyzed by MAO and COMT enzymes [27]. The expression of these enzymes in the nigro-striatal neurons is also a key factor in determining the severity as their over expression cause rapid uptake and oxidation of dopamine with DOPAC and homovallic acid (HVA) as the non-toxic by-products.

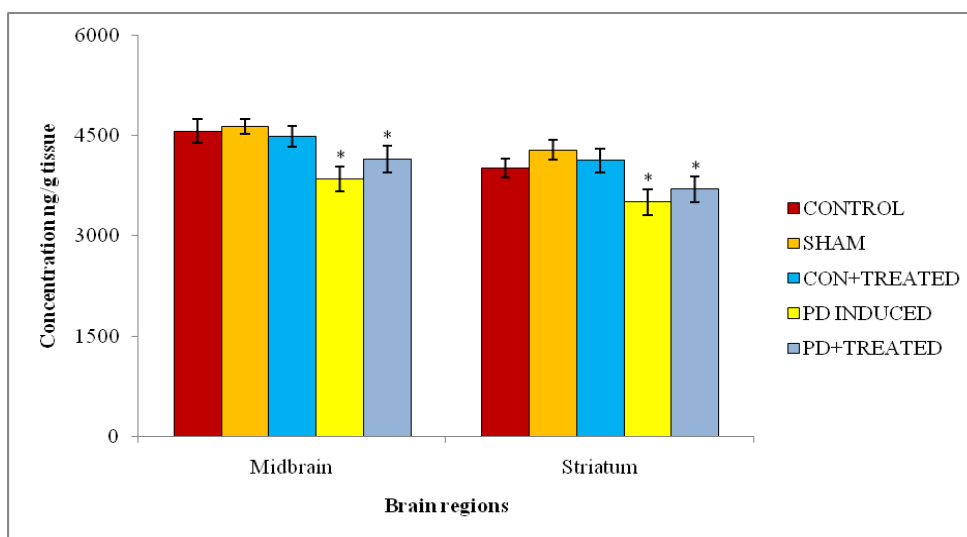


**Fig. 5: Showing the level of DOPAC in midbrain and striatum among the experimental groups. The levels of DOPAC in these regions were markedly decreased in PD induced animals when compared to that of the control animals and on treatment with *Bacopa* extract the animals showed a trivial increase in the brain level of DOPAC which was insignificant as compared to that of the PD induced animal group.**



**Fig. 6: Showing the level of HVA in midbrain and striatum among the experimental groups.**

The levels of HVA in the brain discrete regions were markedly decreased in PD induced animals when compared to that of the control animals and on treatment with *Bacopa extract* the animals showed immense increase in the brain level of HVA which was statistically significant when compared to that of the PD induced animal group and near normalcy in the levels of HVA were restored.



**Fig. 7: Showing the level of serotonin in midbrain and striatum among the experimental groups.**

The levels were decreased in PD induced animals when compared to that of the control animals and on treatment with *Bacopa* the animals showed immense increase in the brain level of serotonin when compared to that of the PD induced animal group

The levels of DOPAC and HVA were depicted in figure 5 and 6 respectively. It was observed that these metabolites were decreased in the Parkinson's disease induced animals when compared to control animals. This has specified that rotenone reformed dopamine synthesis and also had a trivial effect on its metabolism. On treatment with *Bacopa* extracts in PD induction the level of the metabolites were increased and normalcy was restored. The metabolism of dopamine is dependent on the

monoamine oxidase enzymes. Previous reports suggest that *Bacopaside I*, one of the major constituent of *Bacopa monnieri* inhibits the MAO-A enzyme selectively and also Catechol-O-methyl transferase (COMT) [28, 29]. Therefore further investigations of the level of MAO and COMT enzymes in the nigro-striatal region will provide information about the role of rotenone in dopamine degradation. It was also observed that the level



of metabolites was not altered in the control, sham operated and the *Bacopa* treated group of animals.

In brain, the neurotransmitter serotonin has a multifaceted function in modulation of information processing. The largest populations of these neurons are located in the midbrain region, mainly the raphe nuclei. The serotonergic innervations of basal ganglia come from the dorsal raphe nuclei. It was reported that the electrical stimulation of this neurons inhibit the spontaneous activity of SNpc neurons although its chemical lesion of raphe nuclei did not produce significant alteration in the dopamine release from SNpc. Therefore the estimation of the brain levels of serotonin will also serve as a tool to recognize the neurochemical

imbalance in PD. In the present study as shown in figure 7, it was also observed that the level of serotonin was affected significantly in the midbrain and striatum of PD induced animals when compared to control animals. Even though selective degeneration of dopaminergic neurons was achieved by infusion of rotenone in MFB, the level of serotonin was also altered. The probable reason could be that the neurochemical imbalance imparted by dopamine depletion in the nigral region has led to the depletion of the serotonin in the striatal region of the affected side [30]. Treatment with *Bacopa monnieri* improved the serotonin level but did not bring them back to normal.

**Table 1: Showing the level of various neurotransmitters in the midbrain region of the experimental groups**

|               | Norepinephrine | Epinephrine    | Levodopa       | Dopamine       | DOPAC          | HVA            | Serotonin      |
|---------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Control       | 5112.07±556.05 | 4171.58±384.38 | 3274.00±364.75 | 5223.11±292.74 | 1676.20±247.49 | 1171.92±126.94 | 4572.05±173.61 |
| Sham          | 5123.33±465.98 | 3949.27±283.86 | 3252.78±250.11 | 5587.77±317.09 | 1716.87±268.32 | 1189.59±105.47 | 4636.76±107.83 |
| Con + Treated | 5184.20±176.64 | 4104.61±318.88 | 3116.52±300.84 | 5465.42±196.62 | 1581.83±155.07 | 1145.38±142.30 | 4491.71±157.54 |
| PD Induced    | 5975.56±168.56 | 4296.97±111.65 | 1341.61±187.69 | 2253.47±134.97 | 802.29±163.84  | 756.78±84.73   | 3856.77±185.35 |
| PD + Treated  | 5231.77±269.55 | 4009.71±214.82 | 2633.26±223.75 | 3756.49±286.19 | 865.93±162.33  | 1046.0±128.23  | 4152.26±203.85 |

All the values are expressed as Mean ± SD. The values are expressed as ng of neurotransmitter per gram weight of wet brain tissue sample

**Table 2: Showing the level of various neurotransmitters in the striatum region of the experimental groups.**

|               | Norepinephrine | Epinephrine    | Levodopa       | Dopamine       | DOPAC          | HVA            | Serotonin      |
|---------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Control       | 5188.57±198.25 | 4087.50±240.85 | 2288.48±212.91 | 5139.56±314.02 | 1651.65±212.56 | 1061.84±109.44 | 4012.67±141.27 |
| Sham          | 5156.19±339.17 | 4247.03±389.40 | 2108.29±197.67 | 4963.12±365.50 | 1699.99±156.26 | 1086.47±103.53 | 4291.09±142.11 |
| Con + Treated | 5004.07±392.51 | 3999.10±353.36 | 2292.84±207.69 | 4886.27±176.40 | 1591.47±184.89 | 1073.07±109.72 | 4129.90±175.93 |
| PD Induced    | 5512.00±74.09  | 4394.93±268.10 | 1244.71±143.52 | 1102.24±213.77 | 850.05±147.80  | 733.25±108.60  | 3507.67±196.13 |
| PD + Treated  | 4911.12±413.18 | 4164.96±316.39 | 1736.97±152.68 | 2318.14±355.70 | 871.94±149.21  | 889.36±113.00  | 3699.02±188.90 |

All the values are expressed as Mean ± SD. The values are expressed as ng of neurotransmitter per gram weight of wet brain tissue sample

The key problem encountered with PD is the degeneration of the dopaminergic system, leading to dopamine depletion. It was observed that the dopamine levels were abnormally low due to degeneration of the dopamine system in the SN region, leading to the loss of motor control and other conditions associated with Parkinson's disease. Treatment with *Bacopa monnieri* produced a neuroprotective effect in rotenone induced degeneration. The potent antioxidant properties inherent to *Bacopa monnieri* may be the reason for the changes observed in the study. Apart from combating the

neurotransmitter imbalance *Bacopa monnieri* also decreased the alpha synuclein protein expression in PD due to the induction of stress buffer protein Hsp-70. Similar results were observed by Swathi and co-workers [31] that upon intra-peritoneal injection of rotenone, the brain levels of neurotransmitters were decrease and *Bacopa* treatment increased the neurotransmitter synthesis. Similarly it was reported that 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 [32].



#### 4. CONCLUSION

Parkinson's disease is a neuro-degenerative disorder due to the selective death of dopaminergic neurons in the substantia nigra region of the midbrain. This has led to a neurochemical imbalance in the basal ganglia leading to perceptible symptoms. The brain levels of neurotransmitters were altered among the study groups. It was observed that the level of dopamine, its precursor levodopa and its metabolites DOPAC and HVA were decreased in the SNpc and striatum of the PD induced animals. There was a substantial increase on *Bacopa* treatment indicating the effect of *Bacopa monnieri* in dopamine metabolism and neuro-protection. The levels of norepinephrine in PD induced group were increased in the PD induced group which returned to near-normal on *Bacopa* treatment and the levels of epinephrine were unaltered in the brain regions in the study. This signifies the therapeutic efficacy of methanolic extracts of *Bacopa monnieri* in alleviating the neurochemical imbalance imparted by rotenone infusion in midbrain.

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