

## Comparative evaluation of extract of *Bacopa monnieri* and *Mucuna pruriens* as neuroprotectant in MPTP model of Parkinson's disease

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Parkinson's disease (PD) results primarily from the death of dopaminergic neurons in substantia nigra. Treatment of PD has been shifted recently towards herbal medicines. *Bacopa monnieri* (L.) Wettst. (BM) and *Mucuna pruriens* (L.) DC (MP) are traditional herbal plants known to have neuroprotective effects due to the presence of bacosides in whole plant extract of *Bacopa monnieri* (BME) and L-DOPA in MP seed extract (MPE). In this study, the comparative effect of BME and MPE in Parkinsonian mice induced by chronic exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was evaluated. Twenty four Swiss albino mice (35-45 g) were grouped into Control, MPTP, MPTP+BME and MPTP+MPE (6 mice in each). Experimental mice were given 40 mg/kg body wt. BME, 48 mg/kg body wt. MPE treatment was given orally for one month with prior use of 15 mg/kg body wt. of MPTP for 2 wk. After the treatment period, behavioral study was performed and assessment of neuroprotective effect was done via neurochemical analysis, Immunohistochemical parameters studied included functional viability of dopaminergic neurons in substantia nigra by Tyrosine hydroxylase (TH) using monoclonal antibody against TH and apoptotic study through caspase-3 and m-RNA expression of neurogenic gene in substantia nigra region of brain. Treatment with BME or MPE for one month significantly decreased the elevated levels of oxidative stress found in Parkinsonian mice. In behavioral tests, comparative analysis of BME and MPE showed a significant increase in spontaneous locomotor activity and grip strength test. Moreover, it was found that the use of BME considerably improved the tyrosine hydroxylase activity, caspase-3 and expression of neurogenic gene in the substantia nigra region of the brain. The results suggest that BME may provide a better platform for future drug discoveries and novel treatment strategies for PD as compared to MPE.

**Keywords:** Ayurvedic, Brahmi, Velvet bean, Water hyssop, Neurodegeneration, Neurogenesis.

Parkinson's disease (PD) is the second most frequent neurological disorder, characterized by a selective loss of dopaminergic neurons in the substantia nigra (SN) region of ventral midbrain, causing a consequent reduction of dopamine (DA) levels in the striatum. Loss of dopamine supply to striatum causes imbalance with neurotransmitters like acetylcholine and DA, resulting in PD symptoms. Four typical characteristic symptoms observed in PD patients are akinesia, dyskinesia, rigidity and tremor<sup>1,2</sup>. The three main strategic developments in drug discovery for therapeutic management of PD patients have focused on the alleviation of motor symptoms using dopaminergic mimetics; the development of

novel non-dopaminergic drugs for symptomatic improvement; and the discovery of neuroprotective compounds that have disease modifying effects in PD<sup>3,4</sup>. The pathogenesis and etiology of PD are not completely understood. Accumulating evidence suggests that glial activation-derived oxidative stress increases the risk of developing PD<sup>5</sup>. *In vivo* and *in vitro* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD have shown that key enzymes involved in the production of reactive oxygen species (ROS) are upregulated in damaged areas, and thereby contribute to the death of dopaminergic neurons<sup>6-8</sup>. Extensive study of various models mimicking key features of PD has outlined the important cellular factors of dopaminergic cell death, including neuroinflammation, oxidative stress, mitochondrial dysfunction and excitotoxicity<sup>9,10</sup>. Although, no model has thus far been able to reiterate all the pathological features of PD<sup>11</sup>, the neurotoxic models have proved themselves to be an admirable tool for developing novel therapeutic strategies and assessing the efficacy and adverse effects of symptomatic treatments of

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Abbreviations: BME, *Bacopa monnieri* extract; DA, Dopamine; DOPAC, Dopamine, 3-4 dihydroxy phenyl acetic acid; HVA, Homo-vanillic acid; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPE, *Mucuna Pruriens* seed extract; PND, Post Natal days; ROS, Reactive Oxygen Species; SNpc, Substantia nigra pars compacta; TH, Tyrosine hydroxylase.

PD<sup>12</sup>. All current therapies are aimed at symptomatic management.

Currently, there is no therapy clinically available that delays the neurodegenerative process itself, and therefore modification of the disease course via neuroprotective therapy is an important unmet clinical need. Thus, understanding the pathophysiology and etiology of the disease at cellular and molecular levels and finding molecular targets for neuroprotective/disease-modifying therapy is the crucial issue in the field of basic PD research. "Neuroprotection" aims to slowdown the disease progression and secondary injuries by halting the loss of neurons<sup>13</sup> or may be by promoting generation of new neurons (neurogenesis) which has not been explored in PD. An initial good response to symptomatic pharmacological treatment declines with time, and severe side effects develop.

*Bacopa monnieri* (L.) Wettst., commonly known as Brahmi since prehistoric time and generally as Water hyssop, have bacosides as major component which are known to improve memory by modulating acetylcholinesterase activity<sup>14</sup>. Locals from Assam, India used Brahmi to treat memory loss since ages<sup>15</sup>. *B. monnieri* is also known to possess antioxidant properties due to sulfhydryl and polyphenol components that scavenge ROS. BM extract, bacosides compounds in particular, have been investigated extensively for neuropharmacological activities<sup>14,16</sup>.

*Mucuna pruriens* (L.) DC, commonly called Velvet bean or cowhage, is popular in India and China for treatment of Parkinson's disease<sup>17,18</sup>. Apart from its use as aphrodisiac<sup>19</sup> and in snakebite<sup>17,19</sup>, it is also known for treating ulcer as well as psychological disorder<sup>19</sup>. While tribals from Malda, West Bengal use it as a sex stimulant<sup>20</sup>, locals in Rajasthan feed female camels along with jaggery or wheat bread to bring them into heat<sup>21</sup>. *M. pruriens* seeds contain significant amounts of levodopa. The endocarp of *M. pruriens* is non-toxic and is 2-3 times more potent than synthetic levodopa. *M. pruriens* has also shown to exhibit neuroprotective effect by increasing brain mitochondrial complex-I activity and significantly restoring dopamine and nor-epinephrine levels in Parkinsonism animal model<sup>18</sup>.

In view of the above mentioned beneficial qualities of *B. monnieri* and *M. pruriens*, here, we tried to elucidate the comparative neuroprotective effects of *Bacopa monnieri* extract (BME) and *Mucuna*

*pruriens* seed extract (MPE) in 1-methyl-4-phenyl-1,2,3,6 tetra-hydro-pyridine (MPTP) treated animal model of Parkinson's disease. In the present study, the efficacy of BME and MPE in providing protection as neurogenesis to dopaminergic neurons against and neurodegeneration caused by oxidative stress was examined.

## Materials and Methods

### Animals

Swiss albino male mice (8-10 wk old, 30-45 g) were used for the study. Animals were obtained from the breeding colony of CSIR-Indian Institute of Toxicological Research (IITR) Lucknow, India and were used throughout the study with the approval of the Institutional Animal Ethics Committee in accordance with the CPCSEA guideline (646/02/a/CPCSEA dated on 19/07/02, Ethical clearance letter No.39/IAH/Pharma/14)..All the mice were maintained on Hindustan Lever LTD (Mumbai; India) Pellets diet and water *ad libitum*. The cages were kept in temperature and humidity controlled room with 12 h light:dark cycle.

### Plant material

Prepared ethanolic whole plant extract of *B. monnieri* and seed extract of *M. pruriens* were purchased from Natural remedies (tested for pesticides, heavy metals, mycotoxins & microbial contaminants, to comply with USP limits, in ISO 17025 certified) Bangalore, India for treatment of animals.

### Experimental design

For experimental study, healthy male Swiss albino mice (30-45 g) were divided into four groups of 6 mice in each group. Group I (Control), normal control, received normal saline i.p. from Post Natal days (PND) 45-90 and scarified at PND 90; Group II (MPTP), MPTP treated, received neurotoxin MPTP 15 mg/kg<sup>22</sup> i.p. from PND 45-60 and scarified at PND 90; Group III (MPTP+BME), *Bacopa monnieri* extract (40 mg/kg body wt.)<sup>23</sup> treatment orally from PND 60-90 with prior use of MPTP from PND 45-60 and scarified at PND 90; Group IV (MPTP+MPE), *Mucuna pruriens* extract (48 mg/kg body wt.)<sup>24</sup> treatment orally from PND 60-90 with prior use of MPTP from PND 45-60 and scarified at PND 90.

At the end of the entire treatment, behavioral studies were performed to understand motor skill abnormalities by spontaneous loco-motor activity

(SLA) in each group. Five mice from each group were sacrificed by decapitation, brains were removed quickly and the substantia nigra and striatum region of the brain were dissected in cold condition on ice pack. Assessment of neuroprotective efficacy of BME and MPE was done using neurobehavioral, biochemical, immune-histochemical parameters and gene expression analysis. Flow chart of parameters measured is given as Scheme 1.

### Neurobehavioral analysis

#### *Spontaneous locomotor activity (SLA)*

Spontaneous locomotor activity in mice was carried out using computerized Actimot (TSE, Germany) following the method as described by Ali *et al.*<sup>25</sup>. Effect on different parameters including total distance travelled, resting time, stereotypic time, time moving and rearing was studied in the control and treated groups.

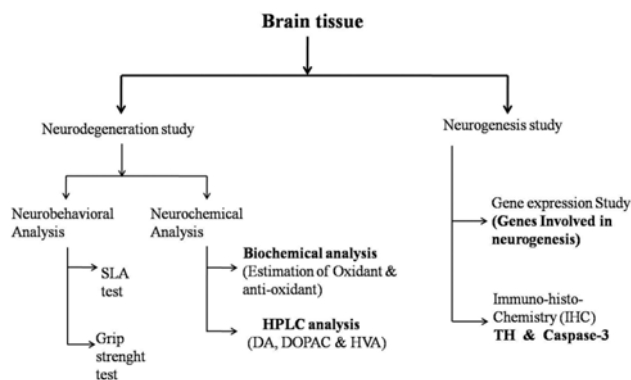
#### *Grip strength*

A computerized grip strength meter (TSE, Germany) was used to measure the forelimb grip strength in the control and treated animals following the standard procedure as described by Terry *et al.*<sup>26</sup>.

### Neurochemical analysis

#### *Assessment of oxidative stress*

To assess the free radical mediated effects of MPTP neurotoxicity and scavenging potential of *Bacopa monnieri* whole plant extract and *Mucuna pruriens* seed extract, estimation of lipid per-oxidation (LPO), conjugated dienes (CD), superoxide dismutase (SOD) and catalase was carried out in tissue homogenate [ten percent (w/v)] of striatum region of mice brain. LPO was measured by estimating malonaldehyde (MDA) levels following the method of Ohkawa *et al.*<sup>27</sup> and



Scheme 1—Flow chart presentation of parameters measured.

conjugated dienes by Racknell *et al.*<sup>28</sup> method. Catalase activity was determined spectrophotometrically by the method of Aebi<sup>29</sup>. SOD activity was determined spectrophotometrically according to the method of McCord and Fridovich<sup>30</sup>.

### Estimation of dopamine and its metabolites

Determination of dopamine and its metabolites were performed by HPLC, an Agilent 1260 Infinity Quaternary LC Series system consisting of 1260 infinity Diode Array Detector (DAD), quaternary solvent delivery system with thermostatted auto sampler, thermostatted columns compartment and equipped with an Agilent Zorbax C-18 (4.6 mm×250 mm, 5 μm) column. Five mice from each group were sacrificed by decapitation after the completion of treatment. The brains were removed quickly and the striatum was dissected in cold condition on ice pack and was homogenized in PBS. The resultant supernatant was collected after centrifugation at 15000 g for 10 min at 4°C. Buffer acetate (pH 4.66) and methanol with a flow rate of 0.8 mL/min were used as the components of the mobile phase<sup>31</sup>. The measurements were performed at temperatures of 4 and 40°C. One mg/mL sample of each compound was introduced into the chromatographic column. Detection was conducted with different wavelength settings 220, 260 and 280 nm for the estimation of Dopamine, DOPAC and HVA, respectively. Flow rate and injection volume were set at 1.0 mL/min and 5 μL, respectively. The concentrations are presented as ng/mg tissue<sup>32</sup>.

### Immunohistochemical analysis

#### *Tyrosine hydroxylase (TH) immunoreactivity and Caspase-3 activity*

Analysis was carried out in the substantia nigra region of the brain by immunohistochemical method<sup>33</sup>. In brief, mice from each group were deeply anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and perfused transcardially by 0.1 M phosphate-buffered saline (PBS, pH 7.2), followed with 4% paraformaldehyde in PBS for fixation of tissue. Brains were removed immediately and post-fixed in the same fixative for 24 h followed by transfer to 10, 20, and 30% sucrose (w/v) in PBS. The fixed tissues were embedded in OCT compound (polyvinyl glycol, polyvinyl alcohol, and water) and frozen at -20°C. About 20 μM thick coronal sections were cut in freezing microtome (Slee Mainz Co., Germany), and collected in PBS and stored at 4°C. Then sections

were transferred to gelatin-coated slides and immersed in wash buffer (sodium phosphate 100 mM, sodium chloride 0.5 M, Triton X-100, sodium azide) pH 7.4 for 20 min. For non-specific binding, sections were blocked in PBS containing 1.5% NGS, 0.5% BSA and 0.1% Triton X-100 and were incubated in primary antibody (anti-TH antibody, 1:500) and activated caspase-3 (indicator of apoptosis) primary monoclonal antibody (1:300) for 48 h. Sections were washed thrice and incubated in Alexa Flour labeled secondary antibody (1: 400) at room temperature (25°C) for 2 h followed by three washes with PBS. Sections were transferred on to gelatinized glass slides, mounted in DAPI, cover slipped and then visualized in microscope.

The total numbers of TH-immunoreactive (TH-ir) neurons in the substantia nigra were determined by a computerized image analysis system (Nikon Eclipsa Ti BR Imaging system, Japan) as described in earlier method<sup>33</sup>.

**Gene expression analysis by quantitative real time q-PCR**

Total RNA was isolated from substantia nigra region of all group mice using the TRIzol Reagent. Genomic DNA was removed using RNase-free DNase (Ambion). RNA pellets were resuspended in DEPC treated water (Ambion). Equal amounts of RNA were reverse transcribed using the Superscript first-strand cDNA synthesis kit with Oligo-dT (Invitrogen, USA) and diluted in nuclease-free water (Ambion) to a final concentration of 10 ng/μL. Real time PCR was carried out to detect changes in messenger RNA (mRNA) expression. Expression of the cellular housekeeping gene b-actin or GAPDH served as a control to normalize values. All quantitative real time-PCR plating was performed on ice. Targets were detected and quantified in real-time using the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems; Foster City, CA) and SYBR Green (Applied Biosystems). Relative expression was calculated using the delta Ct method<sup>34</sup>. The sequences for primers were neurogenin: F-ctcggggagcacccttac R-caggtatcctcccctcttta, Double-cortin (DCX): F- gctctgaggttccacaaa R- ggcttgattgtactctgga.

**Statistical analysis**

All data were expressed as means ± SE. The test of one-way variance (ANOVA) followed by Student Newman Keuls test. Compare experimental vs. Control in InStat 3 package program was used to

detect the significant difference between the treated groups and the control. The *P*-value <0.05 were considered statistically significant.

**Results**

**Effect of BME or MPE on Neuro-behavioral analysis in PD mice**

Spontaneous locomotor activity (SLA) and grip strength test measures motor function in animal model of PD. SLA includes time moving behavior. A significant decrease was found in the locomotor activity in MPTP treated mice as compared to control group which was restored by MPTP+BME treated group as well as in MPTP+MPE treated group as compared to MPTP treated group. This improvement was found to be significantly better in MPTP+BME as compared to treatment of MPTP+MPE (Fig. 1A).

The grip strength was found to be significantly decreased in mice treated with MPTP as compared to control group. Significant improvement in grip strength was observed in mice simultaneously treated with MPTP+BME compared to MPTP+MPE treated group. The motor coordination in Parkinsonian mice was greatly compromised, but it was protected significantly by the treatment with MPTP+BME which was better than other groups (Fig. 1B).

**Neurochemical analysis results**

*Effect of BME or MPE on Oxidative stress associated study*

MPTP treatment produced significant changes in oxidant parameter (LPO) and antioxidant parameters (SOD and catalase) as compared to control. MPTP+BME treated group exhibited attenuation of oxidative stress by increasing the SOD and catalase activity, and thereby decreasing the LPO and CD levels. MPTP+MPE treated group exhibited no significant change in the LPO and CD levels when

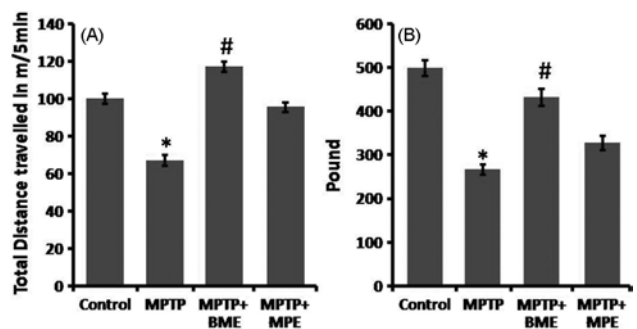


Fig. 1—Effect of BME and MPE on Spontaneous locomotor activity (SLA) in terms of distance travelled meter/5 min. [\**P* <0.05 compared to control group, #*P* <0.05 compared to MPTP treated group]

Table 1—The effect of BME and MPE on oxidative stress associated study in straitum region of brain tissue.

[Values are expressed mean  $\pm$  SE (n = 6 mice per group). \* $P$  < 0.05 compared to control group, # $P$  < 0.05 compared to MPTP treated group]

Groups	MDA (nmole MDA/gram tissue)	Conjugated dienes (nmole CD/gram tissue)	SOD activity (unit/gram tissue)	Catalase activity (Unit/gram tissue)
Control	46.44 $\pm$ 1.10	115.11 $\pm$ 8.36	49.88 $\pm$ 5.26	22.75 $\pm$ 2.58
MPTP	61.60 $\pm$ 1.98*	254.96 $\pm$ 9.50*	32.78 $\pm$ 2.25*	11.96 $\pm$ 1.28*
MPTP+BME	46.48 $\pm$ 0.60#	115.8 $\pm$ 8.68#	47.23 $\pm$ 3.37#	24.26 $\pm$ 1.99#
MPTP+MPE	42.48 $\pm$ 1.93	108.35 $\pm$ 8.59	42.88 $\pm$ 2.56	19.68 $\pm$ 2.64

compared to control group. In comparative study of BME and MPE with MPTP, BME group significantly reduced the elevated levels of MDA which was found to be better than MPE group (Table. 1).

#### Effect of BME or MPE on Dopamine, DOPAC and HVA levels

A significant decrease in DA, DOPAC and HVA levels was observed in MPTP treated mice ( $P$  < 0.001) as compared to the control indicating significant loss of DA neurons in MPTP treated animals. DA, DOPAC and HVA levels in BME and MPE treated group were restored significantly ( $P$  < 0.05) when compared to the MPTP treated group. However, in comparative analysis more significant change was observed in the BME treated group as compared to MPE treated group (Fig. 2).

#### Immunohistochemical studies

##### Effect of BME or MPE on tyrosine hydroxylase immunoreactivity

IHC analysis of TH-positive dopaminergic neurons in frozen brain sections was conducted to evaluate the effect of BME and MPE on MPTP treated mice. MPTP treatment led to a significant decline in the TH positive neurons, whereas MPTP+BME treatment group led to a significant increase in TH-positive dopaminergic neurons in the SN region, which was comparable to controls. The improvement in the BME + MPE treated group was expressed in terms of number of TH positive cells in the SN region. The higher number of TH-ir immuno-reactive neurons in SN of MPTP + BME treated group suggests the recovery and more neuroprotective action of BME on dopaminergic neurons as compare to MPTP+MPE group (Fig. 3).

##### Apoptosis analysis by caspase -3 activity

In order to assess the comparative effect of BME and MPE treatment on apoptotic cell death, we carried out labeling of activated caspase-3 (indicator of apoptosis). Quantitative analysis showed significant increase in activated caspase-3 positive cells in MPTP

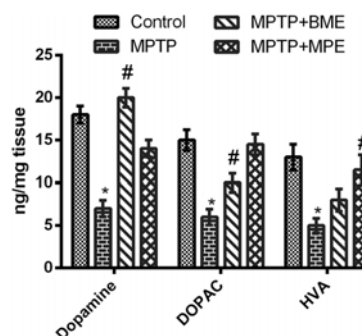


Fig. 2—Effect of BME and MPE on Dopamine and its metabolites DOPAC and HVA. [\* $P$  < 0.05 compared to control group, # $P$  < 0.05 compared to MPTP treated group.]

treated group when compared to control mice. MPTP+BME treated mice exhibited significant decrease in caspase-3 positive cells when compared with MPTP treated mice. We found no significant change in MPTP+MPE treated mice compared to the control (Fig. 4).

#### Gene-expression study

We studied the comparative effect of BME or MPE treatment on the mRNA expression of neurogenic genes/neuronal transcription factors in the substantia nigra region of brain. A significant change was found in neurogenic genes/transcription factors including neurogenin and DCX in MPTP+BME treated group compared to control (Fig.5). MPE also altered the expression of neurogenic genes in substantia nigra region of brain. Neurogenin is basic helix-loop-helix (bHLH) family proneural transcription factor, which acts as master regulators of neuronal differentiation. It is involved in the differentiation of nervous system. The DCX is a marker of newborn neurons. MPTP+BME treated groups significantly upregulated the expression of neurogenic genes as compared to control group. However, in comparative study of MPTP+BME and MPTP+MPE, BME showed significant change.

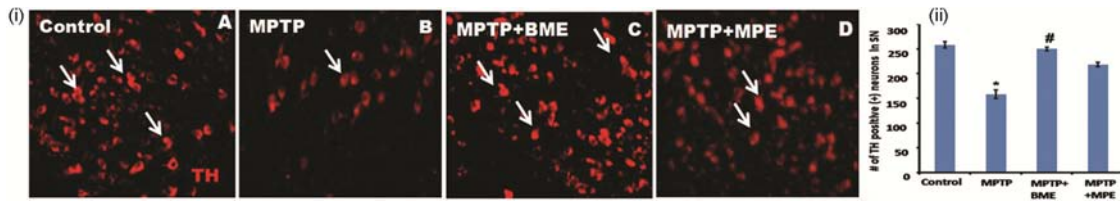


Fig. 3—TH-immunoreactive (TH<sup>+</sup>) counts in the Substantia nigra pars compacta (SNpc) of Control, MPTP, MPTP+BME and MPTP+MPE treated mice. (i) Representative photomicrographs of TH-immunoreactive positive counts neurons in the SNpc of (A) control, (B) MPTP, (C) MPTP+BME and (D) MPTP+MPE treated mice. Arrows indicate TH + neurons. (ii) Quantification analysis suggested significantly decreased number of TH + neurons in the SNpc of MPTP-treated mice (B). [Values are expressed mean ± SEM (n = 5 mice per group)]

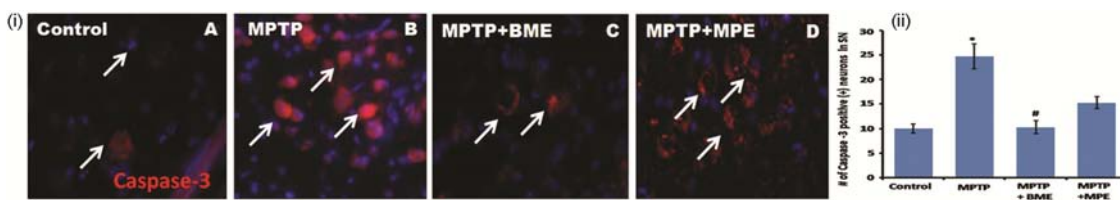


Fig. 4—MPTP exposure induces apoptosis in the Substantia nigra pars compacta (SNpc). (i) Representative photomicrographs showing neurons with activated caspase-3 (marker of cell apoptosis) in the SNpc of (A) Control, (B) MPTP, (C) MPTP+BME and (D) MPTP+MPE- treated mice. Arrows indicate apoptotic neurons. (ii) Quantification analysis suggested significantly increased number caspase-3 positive neurons in the SNpc of MPTP-treated mice (B) and decreased number of neurons in MPTP+BME-(C). [Values are expressed mean ± SEM (n = 5 mice per group)]

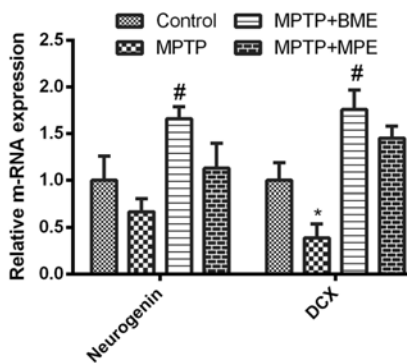


Fig. 5—Altered mRNA expression of neurogenic genes/neuronal transcription factors in the substantia nigra of control and all treated mice. [Total RNA was isolated from the Substantia nigra of Control and all treated group mice. Quantitative real-time PCR analysis was performed for relative mRNA expression of Neurogenin and DCX and normalized to beta-actin. \*P < 0.05]

**Discussion**

Several mechanisms for neuronal injury or neurodegeneration have been proposed including increased excitotoxicity, neuroinflammation, formation of free radicals, mitochondrial dysfunction and inhibition of protein synthesis<sup>35</sup>. These factors may not be sequential, but certainly are interlinked. There are several therapeutic options for the early treatment of PD. The present study was planned to evaluate the comparative efficacy of BME and MPE in MPTP mice model of PD. Our findings shows that

BME can be a better neuroprotectant as it has significant effect on retention of locomotor activity, preventing neurodegeneration and promoting neurogenesis as compared to MPE. It is also pertinent to note that biotechnologists have already developed improvised methods including bioreactor systems for improved biosynthesis of bacoside<sup>36</sup>.

The present study clearly demonstrated that MPTP results in oxidative damage to mice brain, as evidenced by significant increase in brain malondialdehyde (MDA, an end product of lipid peroxidation) and conjugated dienes, whereas decrease in antioxidant status. There is growing evidence that generation of ROS in the SNpc neurons are implicated in the neuronal death in PD<sup>35</sup>. MPTP is oxidized to a toxic molecule, MPP+(1-methyl-4-phenylpyridinium) by monoamine oxidase and inhibits the mitochondrial complex I in the electron transport chain and thereby disrupts the flow of electrons resulting in decreased ATP production and increased generation of ROS<sup>37</sup>. There are similar reports claiming oxidative damage in nervous tissue in PD disease<sup>38</sup>. Dopaminergic neurons provide fertile environment for the generation of ROS, as the metabolism of DA produces hydrogen peroxide and superoxide radicals, and auto-oxidation of DA produces DA-quinone<sup>39</sup>, a molecule that damages proteins by reacting with cysteine residues, so

effective antioxidant agents capable of augmenting the intracellular concentrations of these species should be present.

Studies have shown that treatment with BM extract increased the antioxidant enzyme activities such as of superoxide dismutase, catalase, glutathione peroxidase and levels of GSH and also inhibited the content of lipid per-oxidation in the frontal cortex, striatum and hippocampus<sup>40</sup>. Various other studies reported neuroprotective effects of BM aqueous extract against oxidative stress induced damage in the hippocampus region of rat brain<sup>41,42</sup>.

Seeds of *M. pruriens* possess antioxidant, hypoglycemic, lipid lowering and neuroprotective activities<sup>43</sup> because it contains the alkaloids, mucunine, mucunadine and a number of other bioactive substances<sup>44</sup>. Our results are in concurrence with the earlier reports that *M. pruriens* is a known adaptogen and its alcoholic extract reduces lipid per-oxidation and maintains the levels of glutathione and SOD activity<sup>45</sup>. BME and MPE both showed neuroprotective effect on dopaminergic neurons, which could be a result of its promising antioxidant capacity, but BME showed a better effect as compared to MPE, the reason for the same can be explained as *M. pruriens* seeds are rich source of L-DOPA and its metabolites. Therefore, increase in dopamine level in the brain with *M. pruriens* seed extract treatment may cause increase in the oxidative stress, and increased activity of antioxidants may not suffice to combat increased free radical damage.

Treatment with MPTP causes reduction of TH rate limiting enzyme in dopamine synthesis pathways<sup>46</sup>. The comparative effect of BME or MPE on restoring the functional viability of dopaminergic neurons in substantia nigra was studied by TH using monoclonal antibody against TH. The present study showed that in MPTP treated mice, number of surviving TH positive neurons were significantly less and BME treated group exhibited a significant increase in TH-ir neurons as compared to the MPE treated group. The increase in number of striatal DA neurons following BME treatment can also be associated with the ability of BME to prevent DA degradation or possibly decreased DA reuptake. The result of this study supports previous studies<sup>46,47</sup>. The enhanced numbers of dopaminergic neurons in MPTP+BME showed its better protective effect on restoring cell loss.

In most mammalian species including the human, new neurons are generated throughout the life from NSC through a process known as neurogenesis. For many investigators as of now, center of attention is neurogenesis in PD. Several neurogenic genes/transcription factors such as neurogenin, neuroigin, nestin, DCX regulate the neurogenesis<sup>34,48,49</sup>. We, therefore, studied the comparative effects of BME and MPE on expression of these neurogenic genes and neuronal transcription factors in the substantia nigra. A significant effect of BME treatment on newborn neuron populations was observed in the present study.

Further, our results showed that chronic MPTP exposure in mice causes decreased neurogenesis and increased cell death in the hippocampus region. These finding offers a realistic support that decrease in the neurogenesis due to MPTP treatment and may be due to reduction in proliferation rate or increase in cell death (apoptosis).

Neurogenin, in addition to promoting neurogenesis, maintains progenitor cells in an undifferentiated state, allowing them to proliferate prior to maturation<sup>34</sup> and also inhibits astrocyte differentiation by inhibiting the Stat pathway<sup>50</sup>. DCX is a marker of immature neurons and neuroblasts and is expressed specifically in migrating neuronal precursors/neuroblasts in the brain. Results showed that MPTP exposure in mice model caused decreased neurogenesis, altered neuronal differentiation, increased cell death. Overall, alterations in the expression of neurogenic genes/transcription factors result in increased neurogenesis, but the exact mechanism by which BME regulates the expression of these genes is still unknown and remains to be explored.

## Conclusion

From the results of the present study, it can be suggested that BME treatment promotes significant neurogenesis and declines apoptosis promotes antioxidant capacity, restores dopamine concentration and its metabolites as compared to MPE in MPTP-induced Parkinson disease in mice. The whole plant extract of BM contain multiple classes of chemical entities with synergic properties, which might be holding better promise, therapeutic benefits and applicability in neuroprotection as compared to single chemical entity L-DOPA in MPE.

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