Summary

1. 6-OHDA infused unilateral Parkinson’s disease rats were used as models to study the alterations in brain glutamatergic and NMDA receptors; second messengers - IP3, cAMP and cGMP; apoptotic factors - Bax, TNF-α; intercellular protein - α-synuclein; transcription factor - CREB and their regulation by 5-HT, GABA and BMC individually and in combinations.

2. The body weight was analyzed to study the changes in body weight in 6-OHDA infused rats compared to control. Parkinson’s disease induction in rats caused a reduction in the body weight and treatment combinations with 5-HT, GABA and BMC regained the body weight near to control and 5-HT, GABA and BMC supplemented alone showed no significant reversal in the body weight.

3. Behavioural studies: apomorphine induced rotational analysis, limb use asymmetry test, rotarod test, swim test, Y maze and radial arm test were conducted to assess the motor learning and memory in control and experimental rats. 6-OHDA infused rats showed a significant deficit in cognition, memory and motor learning. Rats treated with 5-HT, GABA and BMC in combinations reversed the behavioural response to near control. BMC treated alone showed no significant reversal in the behavioural deficits towards the control.

4. Glutamate content increased in the corpus striatum, cerebral cortex, hippocampus, cerebellum and brain stem of 6-OHDA infused rats compared to control. Individual treatment with 5-HT, GABA and 5-HT, GABA and BMC in combinations functionally reversed the alteration to near control. BMC alone treated group did not show any significant reversal to control.
5. Glutamatergic receptor functional status was analysed by Scatchard analysis using $[^3]H$ glutamate. The total glutamate receptors in corpus striatum, cerebral cortex, hippocampus, cerebellum and brain stem of 6-OHDA infused rats were increased compared to control with no significant change in the $K_d$ representing the affinity. Treatment with 5-HT, GABA individually and 5-HT, GABA and BMC in combinations restored the total glutamatergic receptors in brain regions near to control. There was no significant reversal in BMC alone treated rats.

6. NMDA receptor functional status was analysed by Scatchard analysis using $[^3]H$ MK801. The NMDA receptors in corpus striatum, cerebral cortex, hippocampus, cerebellum and brain stem of 6-OHDA infused rats were increased compared to control with no significant change in the $K_d$. Individual treatment with 5-HT, GABA and 5-HT, GABA and BMC in combinations functionally reversed the NMDA receptors to near control. BMC alone treated rats did not show any significant reversal to control.

7. Glutamate mediates its action through its receptor subunits – NMDAR1, NMDA2B, mGluR5. NMDA receptor binding parameters were confirmed by studying the mRNA status of the corresponding receptor using Real-Time PCR. NMDAR1, NMDA2B, mGluR5 receptors showed an increased expression in corpus striatum, cerebral cortex, hippocampus, cerebellum, brain stem and SNpc of 6-OHDA infused rats compared to control. The results showed a co-actvation of NMDA receptors subunits that affect glutamate mediated functions. This enhanced activity of NMDA receptors produce intracellular signals through activation of signaling pathways. Treatment with 5-HT, GABA individually and 5-HT, GABA and BMC in combinations reversed the receptor gene expression status to control. There was no significant reversal in BMC alone treated rats.
8. To prevent glutamate mediated excitotoxic effects it should be cleared from the extracellular space by the glutamate transporters. The gene expression of GLAST glutamate transporter was studied in control and experimental rats. GLAST glutamate transporter showed decreased expression in corpus striatum, cerebral cortex, hippocampus, cerebellum, brain stem and SNpc of 6-OHDA infused rats compared to control. The results showed less reuptake of extracellular glutamate formed in the diseased condition. Individual treatment with 5-HT, GABA and 5-HT, GABA and BMC in combinations functionally reversed the alteration in GLAST glutamate transporter gene expression to near control. BMC alone treated rats did not show any significant reversal to control.

9. Second messengers IP3, cAMP contents were increased and cGMP content was decreased significantly in corpus striatum, cerebral cortex, hippocampus, cerebellum, and brain stem of 6-OHDA infused rats compared to control. Individual treatment with 5-HT, GABA and 5-HT, GABA and BMC in combinations functionally reversed the alteration in Second messengers to near control. There was no significant reversal in BMC alone treated rats.

10. A significant up regulation of pro-apoptotic factors Bax and TNF-α was observed in the corpus striatum, cerebral cortex, hippocampus, cerebellum, brain stem and SNpc which indicated the mitochondrial dysfunction and apoptosis in 6-OHDA infused rats. Individual treatment with 5-HT, GABA and 5-HT, GABA and BMC in combinations reversed the gene expression to near control. BMC alone treated group did not show any significant reversal to control.

11. Increased gene expression of the α-Synuclein in the corpus striatum, cerebral cortex, hippocampus, cerebellum, brain stem and SNpc of 6-OHDA infused rats
advanced the neurodegeneration. Treatment with 5-HT, GABA individually and 5-HT, GABA and BMC in combination reversed α-Synuclein gene expression near to control. There was no significant reversal in BMC alone treated rats.

12. Transcription factor, CREB expression in the brain regions - corpus striatum, cerebral cortex, hippocampus, cerebellum, brain stem and SNpc showed a significant decrease in expression in 6-OHDA infused rats. Treatment with 5-HT, GABA individually and 5-HT, GABA and BMC in combinations reversed CREB gene expression status towards control values. There was no significant reversal in BMC alone treated rats.

13. The increased expression of NMDAR1, NMDA2B and mGluR5 receptors in 6-OHDA infused rats observed from the receptors analysis and Real-time PCR was confirmed by confocal studies using receptor specific antibodies in the brain slices. Treatment with 5-HT, GABA individually and 5-HT, GABA and BMC in combination reversed the mean pixel value towards the control. BMC alone treated group did not show any significant reversal to control.

14. We demonstrated using specific fluorescent cell tracking dye - PKH2GL to bone marrow cells and Nestin to premature neurons – the autologous differentiation of bone marrow cells to neurons. PKH2GL tagged BMC when injected into the SNpc started expressing nestin later differentiating to neurons in vivo. The BMC division and differentiation was increased when it was infused along with 5-HT and GABA. The prominent expression was seen in rats treated with 5-HT, GABA and BMC in combination. The Confocal studies were confirmed with the gene expression analysis of nestin in the SNpc in which the maximum expression was observed in the rats treated with 5-HT, GABA and BMC in combination.
15. We observed a marked activation of astrocytes in the SNpc. In the Confocal analysis the mean pixel value of GFAP in the SN showed an increase in 6-OHDA infused rats and the rats individually treated with 5-HT and GABA compared to control. Maximum expression of GFAP was observed in the rats treated with individual BMC treated group and combinational 5-HT, GABA and BMC treated groups. Activated astrocytes made connections with the transplanted BMC and helped its differentiation. Confocal analysis confirmed astrocytes migration into the SNpc region after BMC infusion. This was confirmed with the Real-time PCR analysis of GFAP which also showed the similar pattern of gene expression.

16. DA quantification, tyrosine hydroxylase analysis using confocal and Real-time PCR was done in the SNpc to confirm the DA production after BMC differentiation. 6-OHDA infusion reduced DA production and Tyrosine hydroxylase expression in SNpc. Treatment with 5-HT, GABA individually and 5-HT, GABA and BMC in combinations reversed DA content and Tyrosine hydroxylase expression near to control. BMC alone treated group did not show any significant reversal to control.

Our results showed that glutamate and NMDA receptor functional balance plays a major role in Parkinson’s disease management. Gene expression studies of NMDAR1, NMDA2B, mGluR5 receptor subunits and GLAST glutamate transporter showed a prominent glutamatergic functional disturbance in brain regions of 6-OHDA infused rats. These findings have important implications for understanding the molecular mechanisms underlying motor, memory and cognitive impairment by second messengers, pro-apoptotic factors, intercellular proteins and transcription factors due to 6-OHDA infusion. The enhanced receptor activity and the second messenger cascades will lead to \( \text{Ca}^{2+} \) overload and thereby excitotoxic
neurodegeneration which affect the cognitive, memory and motor ability of the PD rats. Our results proved that the autologous BMC differentiate to neurons and glial cells when they are infused with 5-HT and GABA. The BMC transformed to neurons and glial cells with 5-HT and GABA, was confirmed with PKH2GL, nestin and GFAP. These newly formed neurons have functional significance in the therapeutic recovery of Parkinson’s disease.