Summary

1. Spinal Cord Injured rats were used as models to study the alterations in spinal cord and brain cholinergic receptors and cholinergic enzymes; second messengers - IP$_3$, cAMP and cGMP; transcription factor CREB; second messenger enzyme PLC; apoptotic factors - Bax, caspase-8, TNF$\alpha$ and NF-$\kappa$B; antioxidant enzymes- GPx and SOD; neuronal survival factors - BDNF, GDNF, IGF-1,Akt-1, and cyclin D2 and their regulation by 5-HT, GABA and BMC individually and in combinations.

2. The examination of body weight showed a significant decrease in SCI rats compared to control. The treatment combinations with 5-HT, GABA and BMC regained the body weight when compared to SCI rats whereas 5-HT, GABA and BMC supplemented individually showed no significant reversal in the body weight.

3. Behavioural studies: rotarod, grid walk and narrow beam tests were conducted to assess the motor control and co-ordination in control and experimental rats. SCI rats showed a significant deficit in motor control and co-ordination. Rats treated with 5-HT, GABA and BMC in combinations reversed the behavioural response when compared to SCI rats. 5-HT, GABA and BMC supplemented individually showed no significant improvement in locomotor functions when compared to SCI rats.

4. Acetylcholine esterase expression was analyzed in the spinal cord and brain regions. In SCI rats, the expression was up regulated in the spinal cord and brain regions- cerebral cortex, cerebellum, brain stem and corpus striatum region. Treatment with 5-HT and GABA individually and SCI +
5-HT+ BMC and SCI + GABA + BMC showed a reversal in the acetylcholine esterase expression in the spinal cord region, whereas SCI+5-HT+GABA+BMC reversed the altered expression in spinal cord and different brain regions. SCI+BMC group did not show any significant reversal in the spinal cord and brain regions.

5. Choline acetyl transferase expression level is used as a marker for acetylcholine synthesis. In SCI rats, ChAT expression was down regulated in the spinal cord and cerebellum whereas it was up regulated in cerebral cortex, brain stem and corpus striatum when compared to control. In the spinal cord region, all the treatment groups except SCI + BMC reversed the changes with BMC combination groups showing a reversal with up regulation. In the brain regions, SCI+5-HT+GABA+BMC group reversed the ChAT expression when compared to SCI rats.

6. Total muscarinic receptor binding was analysed in the spinal cord and brain regions of control and experimental rats. Total muscarinic receptor binding parameter $B_{\text{max}}$ was decreased in spinal cord region and brain regions and $K_d$ showed a decrease in spinal cord, cerebral cortex, cerebellum and brain stem. All treatment groups except SCI + BMC reversed the altered parameters in the spinal cord region. $B_{\text{max}}$ showed a reversal in the cerebral cortex, cerebellum, brain stem and corpus striatum of SCI+5-HT+GABA+BMC with a significant reversal in $K_d$ in the cerebral cortex and brain stem.

7. The Scatchard analysis and gene expression studies of muscarinic M1 receptor was analysed in the spinal cord and brain regions. The Scatchard analysis revealed a decrease in $B_{\text{max}}$ in the spinal cord and brain regions in SCI rats with a decreased $K_d$ in the spinal cord, cerebellum and brain stem. In the cortex and striatum of SCI rats, $K_d$ was significantly
increased. $B_{\text{max}}$ showed a significant reversal in all the treatment groups in spinal cord region whereas in the brain regions only SCI+5-HT+GABA+BMC group showed reversal. In the spinal cord region, $K_d$ showed a significant reversal in all treatment groups except SCI+BMC. In the brain regions of SCI+5-HT+GABA+BMC group, $K_d$ showed a significant reversal in cerebral cortex and brain stem. The gene expression of muscarinic M1 receptor mRNA was down regulated in SCI rats in the spinal cord and brain regions. In the spinal cord region, the gene expression was reversed in all treatment groups. The muscarinic M1 receptor mRNA expression was reversed in cerebellum and reversed with up regulation in cerebral cortex, brain stem and corpus striatum of SCI+5-HT+GABA+BMC group. Immunohistochemistry studies using specific antibodies confirmed the scatchard analysis and Real Time PCR analysis of muscarinic M1 receptor expression at protein level in control and experimental rats in the spinal cord and brain regions.

8. Muscarinic M3 receptor binding and gene expression were studied in spinal cord and brain regions. In SCI rats, $B_{\text{max}}$ was decreased significantly in the spinal cord region and brain stem whereas it showed an increase in cortex, cerebellum and corpus striatum. The $K_d$ in SCI rats showed no significant change in the spinal cord region whereas it was significantly increased in cortical and cerebellar regions and significantly decreased in brain stem and corpus striatum. In the spinal cord region, all treatment groups showed a reversal in $B_{\text{max}}$ with an increased $K_d$ in SCI + GABA group. $K_d$ showed no significant change in any other group. In the brain regions of SCI+5-HT+GABA+BMC group, both $B_{\text{max}}$ and $K_d$ were reversed significantly compared to SCI group. Immunohistochemistry studies using specific antibodies confirmed the scatchard analysis and
Real Time PCR analysis of muscarinic M3 receptor expression at protein level in control and experimental rats in the spinal cord and brain regions.

9. α7 nicotinic acetylcholine receptor gene expression was studied in spinal cord and brain regions of control and experimental rats. In SCI rats, α7 nicotinic acetylcholine receptor was down regulated in spinal cord region, cerebellum, brain stem and corpus striatum when compared to control. In the spinal cord region, the expression showed a significant reversal in SCI+5-HT and SCI+GABA groups when compared to SCI rats whereas SCI+5-HT+BMC, SCI+GABA+BMC and SCI+5-HT+GABA+BMC showed a reversal with up regulation. SCI+BMC rats showed no significant reversal when compared to SCI rats. The gene expression was significantly reversed in the cerebellum, brain stem and corpus striatum of SCI+5-HT+GABA+BMC treated groups when compared to SCI rats. Immunohistochemistry studies using specific antibodies confirmed the gene expression of α7 nicotinic acetylcholine receptor expression at protein level in control and experimental rats in the spinal cord and brain regions.

10. Second messenger enzyme - PLC mRNA expression showed down regulation in SCI rats in the spinal cord region, cerebellum and brain stem when compared to control. In the cortex and corpus striatum of SCI rats the gene expression was significantly up regulated compared to control. In the spinal cord region, all the treatment groups significantly reversed and up regulated the gene expression when compared to SCI rats. In the cerebellum and brain stem of SCI+5-HT+GABA+BMC treated groups the altered PLC expression was significantly reversed and up regulated, but in the cortex and corpus striatum the expression was further up regulated.
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11. Transcription factor, CREB was analyzed in the spinal cord and brain regions. CREB gene expression was down regulated in SCI rats when compared to control in spinal cord and brain regions. In the spinal cord region of SCI+5-HT, SCI+GABA, SCI+5-HT+BMC and SCI+GABA+BMC groups the gene expression was significantly reversed and SCI + BMC showed no significant change when compared to SCI rats. In SCI+5-HT+GABA+BMC rats, the gene expression was reversed in the cerebellum and reversed with an up regulation in the spinal cord, cerebral cortex, brain stem and corpus striatum when compared to SCI rats.

12. Apoptotic factor Bax was up regulated in SCI rats when compared to control in spinal cord and brain regions. In the spinal cord region, 5-HT and GABA treatment alone and SCI+5-HT+BMC and SCI+GABA+BMC groups showed significant reversal in the Bax gene expression whereas BMC alone showed no significant reversal when compared to SCI rats. In SCI+5-HT+GABA+BMC group, the expression was significantly reversed in corpus striatum and significantly reversed with a down regulation in spinal cord, cerebral cortex, cerebellum and brain stem when compared to SCI rats.

13. Caspase-8 mRNA gene expression was studied in the spinal cord and brain regions to study apoptosis and its gene expression was up regulated in SCI rats when compared to control in spinal cord and brain regions. All groups except SCI+BMC showed significant reversal in the gene expression of caspase-8 when compared to SCI rats in the spinal cord region. It was significantly reversed in corpus striatum and reversed with down regulation in cerebral cortex, cerebellum and brain stem of SCI+5-HT+GABA+BMC treated groups.
14. Antioxidant enzyme, SOD gene expression was studied in spinal cord and brain regions of control and experimental rats. In SCI rats, the gene expression of SOD was down regulated in spinal cord, cerebellum and brain stem whereas it was up regulated in the cerebral cortex and corpus striatum when compared to control. In the spinal cord region, SCI+5-HT, SCI+GABA, SCI+5-HT+BMC and SCI+GABA+BMC treated groups significantly reversed the gene expression when compared to SCI rats. In SCI+5-HT+GABA+BMC treated groups, the SOD expression was reversed with an up regulation in the spinal cord, cerebellum and brain stem when compared to SCI rats. In the cerebral cortex and corpus striatum of SCI+5-HT+GABA+BMC rats the expression was further up regulated when compared to SCI rats.

15. Gene expression of GPx was analysed to study the oxidative stress in spinal cord and brain regions. GPx expression was down regulated in SCI rats when compared to control in spinal cord and cerebellum and up regulated in cerebral cortex, brain stem and corpus striatum of SCI rats when compared to control. In the spinal cord region, SCI+5-HT, SCI+GABA, SCI+5-HT+BMC and SCI+GABA+BMC groups significantly reversed the gene expression where as SCI+BMC showed no significant reversal when compared to SCI rats. In SCI+5-HT+GABA+BMC treated group, the GPx gene expression was reversed with an up regulation in the spinal cord and cerebellum when compared to SCI rats. In SCI+5-HT+GABA+BMC rats, a further significant up regulation was observed in cerebral cortex, brain stem and corpus striatum when compared to SCI rats.
16. Pro-apoptotic factor TNFα showed up regulation in its gene expression in spinal cord of SCI rats when compared to control. SCI+5-HT, SCI+GABA, SCI+BMC, SCI+5-HT+BMC and SCI+GABA+BMC treated groups reversed these changes when compared to SCI rats in the spinal cord region.

17. Gene expression of NF-κB mRNA showed up regulation in the spinal cord of SCI rats when compared to control. SCI+5-HT, SCI+GABA and SCI+BMC rats reversed these changes when compared to SCI rats. SCI+5-HT+BMC, SCI+GABA+BMC and SCI+5-HT+GABA+BMC rats showed significant reversal with down regulation when compared to SCI rats.

18. Cell survival factor BDNF was up regulated in spinal cord of SCI rats when compared to control. SCI+5-HT, SCI+GABA, SCI+5-HT+BMC, SCI+GABA+BMC and SCI+5-HT+GABA+BMC rats showed significant up regulation when compared to SCI rats. SCI+BMC rats showed no significant change when compared to SCI rats.

19. Gene expression of GDNF mRNA showed down regulation in the spinal cord of SCI and rats compared to control. SCI+5-HT, SCI+GABA, SCI+5-HT+BMC, SCI+GABA+BMC and SCI+5-HT+GABA+BMC treated groups showed significant reversal and up regulation when compared to SCI rats. SCI+BMC rats showed no significant change when compared to SCI rats.

20. IGF-1 gene expression was also studied in spinal cord region. Gene expression of IGF mRNA showed up regulation in the spinal cord of SCI rats compared to control. SCI+5-HT, SCI+GABA, SCI+BMC, SCI+5-HT+BMC, SCI+GABA+BMC and SCI+5-HT+GABA+BMC treated
groups showed further significant up regulation when compared to SCI rats.

21. Akt-1, involved in cell survival was analyzed in the spinal cord of control and experimental rats. Akt-1 gene expression showed up regulation in the spinal cord of SCI rats compared to control. SCI+5-HT, SCI+GABA, SCI+BMC SCI+5-HT+BMC, SCI+GABA+BMC and SCI+5-HT+GABA+BMC treated groups showed further significant up regulation when compared to SCI rats.

22. Gene expression of cyclin D2 mRNA showed up regulation in the spinal cord of SCI rats compared to control. SCI+5-HT, SCI+GABA and SCI+BMC treated groups showed a significant reversal when compared to SCI rats. The combination treatment group of rats, SCI+5-HT+BMC, SCI+GABA+BMC and SCI+5-HT+GABA+BMC showed significant reversal and down regulation in the gene expression of cyclin D2 mRNA when compared to SCI rats.

23. IP3 content was measured in spinal cord and brain regions of control and experimental rats. IP3 content was decreased in spinal cord, cerebral cortex, cerebellum, brain stem and corpus striatum of SCI rats when compared to control. In the spinal cord region, SCI+5-HT, SCI+GABA, SCI+5-HT+BMC, SCI+GABA+BMC and SCI+5-HT+GABA+BMC groups showed significant reversal in the IP3 content whereas SCI+BMC showed no significant change when compared to SCI rats. It was significantly reversed in cerebral cortex, brain stem and corpus striatum of SCI+5-HT+GABA+BMC when compared to SCI rats. It was significantly reversed and increased in cerebellum of SCI+5-HT+GABA+BMC when compared to SCI rats.
24. cAMP content was analyzed in spinal cord and brain regions of control and experimental rats. cAMP content was decreased in spinal cord, cerebral cortex, cerebellum, brain stem and corpus striatum of SCI rats when compared to control. In the spinal cord region, all groups except SCI+BMC showed a significant reversal in the cAMP content when compared to SCI rats. In SCI+5-HT+GABA+BMC treated group, the cAMP content was significantly reversed and increased in cerebellum and corpus striatum when compared to SCI rats.

25. cGMP content in the spinal cord, cerebral cortex, cerebellum, brain stem and corpus striatum was significantly decreased in SCI rats when compared to control. In the spinal cord region, the treatment groups with 5-HT, GABA and BMC alone showed no significant change whereas the combination treatment group of rats, SCI+5-HT+BMC, SCI+GABA+BMC and SCI+5-HT+GABA+BMC showed a significant reversal in the cGMP when compared to SCI rats. In SCI+5-HT+GABA+BMC treated group, the cGMP content was significantly reversed in cerebral cortex and reversed and increased in cerebellum, brain stem and corpus striatum when compared to SCI rats.

26. We demonstrated the autologous differentiation of BMC to neurons using BrdU-NeuN co-labelling studies. BMC injected into the spinal cord were tagged by proliferative marker BrdU and was seen to express NeuN which indicated neuronal cells. The BMC division and differentiation was increased when it was infused along with 5-HT and GABA. The most prominent expression was seen in rats treated with 5-HT, GABA and BMC in combination. However, BMC injected alone did not express any NeuN.
In the present study, we summarize that the cholinergic transmission was decreased in the spinal cord and brain regions of SCI rats and it has a significant role in locomotor function. The alterations in cholinergic enzymes in spinal cord and brain regions of SCI rats suggested a dysfunction of cholinergic neurotransmission in these regions. PLC, CREB, second messengers, antioxidant enzymes, apoptotic factors and cell survival factors were also altered in SCI rats. Treatment with 5-HT and GABA along with BMC effectively restored the altered cholinergic signaling. Treatment with 5-HT and GABA along with BMC also ameliorate the oxidative stress and apoptosis and induced neuronal cell differentiation and survival. Our results showed the autologous BMC differentiation to neurons in spinal cord when administered along with 5-HT and GABA. The findings from this study give an insight on the molecular mechanisms underlying SCI and the therapeutic role of 5-HT and GABA along with BMC in the functional recovery of SCI.
Spinal cord injury causes permanent and irrevocable motor deficits and neurodegeneration. Disruption of the spinal cord leads to diminished transmission of descending control from the brain to motor neurons and ascending sensory information. Behavioural studies showed deficits in motor control and coordination in SCI rats. Cholinergic system plays an important role in SCI, the evaluation of which provides valuable insight on the underlying mechanisms of motor deficit that occur during SCI. The cholinergic transmission was studied by assessing the muscarinic and nicotinic receptors; cholinergic enzymes- ChAT and AChE; second messenger enzyme PLC; transcription factor CREB and second messengers - IP3, cAMP and cGMP. We observed a decrease in the cholinergic transmission in the brain and spinal cord of SCI rats. The disrupted cholinergic system is the indicative of motor deficit and neuronal degeneration in the spinal cord and brain regions. SCI mediated oxidative stress and apoptosis leads to neuronal degeneration in SCI rats. The decreased expression of anti oxidant enzymes – SOD, GPx and neuronal cell survival factors - BDNF, GDNF, IGF-1, Akt and cyclin D2 along with increased expression of apoptotic factors - Bax, caspase-8, TNFα and NF-κB augmented the neuronal degeneration in SCI condition. BMC administration in combination with 5-HT and GABA in SCI rats showed a reversal in the impaired cholinergic neurotransmission and reduced the oxidative stress and apoptosis. It also enhanced the expression of cell survival factors in the spinal cord region. In SCI rats treated with 5-HT and GABA, the transplanted BMC expressed NeuN confirming that 5-HT and GABA induced the differentiation and proliferation of BMC to neurons in the spinal cord. Neurotrophic factors and anti-apoptotic elements in SCI rats treated with 5-HT and GABA along with BMC rendered neuroprotective effects accompanied by
improvement in behavioural deficits. This resulted in a significant reversal of altered cholinergic neurotransmission in SCI. The restorative and neuro protective effects of BMC in combination with 5-HT and GABA are of immense therapeutic significance in the clinical management of SCI.