Summary

1. GABA and 5-HT, both individually and in combination, were encapsulated in chitosan nanoparticles by ionic gelation method. Spherical particles with 80 nm size were obtained. The interaction between the amino group of chitosan with the carboxyl group of GABA and sulphate group of 5-HT were studied by FT-IR spectroscopy.

2. Encapsulation efficiency in coupling GABA and 5-HT with chitosan nanoparticles were examined to obtain maximum entrapment of neurotransmitters with the nanoparticles.

3. Uptake of fluorescently labelled GABA and 5-HT chitosan nanoparticles by hepatocytes were confirmed by confocal microscope.

4. Partially hepatectomised rats were used as a model to study the liver regeneration by GABA and 5-HT chitosan nanoparticles.

5. Liver cell proliferation in partially hepatectomised rats treated with and without nanoparticles was assessed by quantifying DNA and protein syntheses using \(^{3}\text{H}\) thymidine and \(^{3}\text{H}\) leucine uptake studies \textit{in vitro}. Thymidine kinase activity in the cells of regenerating liver was also studied. DNA and protein syntheses were increased by individual treatment with GABA or 5-HT chitosan nanoparticles and a prominent increase was observed with a combination of GABA and 5-HT chitosan nanoparticles.

6. The incorporation of thymidine analogue BrdU was increased in the partially hepatectomised rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to the rats with single treatment of GABA or 5-HT chitosan nanoparticle and the one with no treatment. This confirmed that combination of GABA and 5-HT chitosan nanoparticles increased DNA synthesis, which is a part of rapid cell proliferation in the regenerating liver.
7. GABA$_B$ receptor functional status was analysed by Scatchard analysis using $[^3H]$ baclofen. GABA$_B$ receptor number was decreased in regenerating liver of partially hepatectomised rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to the rats with single treatment of GABA or 5-HT chitosan nanoparticle and the one with no treatment. This favoured an increased cellular signalling for active liver cell proliferation.

8. GABA$_B$ receptor binding parameters were confirmed by studying the mRNA expression of the corresponding receptor subtype using Real Time PCR. The receptor expression was decreased in partially hepatectomised rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to the rats with single treatment of GABA or 5-HT chitosan nanoparticle and the one with no treatment.

9. Confocal microscopic imaging of GABA$_B$ receptors in the liver sections showed decreased mean pixel intensity for those obtained from the groups treated with both GABA and 5-HT chitosan nanoparticles compared to GABA or 5-HT chitosan nanoparticles treatment individually and the one with no treatment.

10. 5-HT$_2A$ receptor functional status was analysed by Scatchard analysis using $[^3H]$ ketanserin. 5-HT$_2A$ receptor number was increased in rats treated with GABA and 5-HT chitosan nanoparticles individually compared to that with no treatment. A prominent increase was observed in the rats treated with a combination of GABA and 5-HT chitosan nanoparticles.

11. 5-HT$_2A$ receptor binding parameters were confirmed by studying the mRNA expression of the corresponding receptor subtype using Real Time PCR. The receptor expression was increased by individual treatment of GABA or 5-HT chitosan nanoparticles and a prominent increase was observed in the groups with both GABA and 5-HT chitosan nanoparticles treatment.
12. Confocal microscopic imaging of 5-HT$_{2A}$ receptors in the liver sections showed increased mean pixel intensity for partially hepatectomised rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to the rats with single treatment of GABA or 5-HT chitosan nanoparticle and the one with no treatment.

13. cAMP, IP$_3$ contents, CREB and phospholipase C expressions were decreased in the actively regenerating liver of partially hepatectomised rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to the rats with single treatment of GABA or 5-HT chitosan nanoparticle and the one with no treatment. This confirmed a delayed signalling through protein kinase A and C mediated signalling cascade in S phase of cell cycle.

14. NF-κB, TNF-α, and Akt-1 were involved in the subsequent activation of downstream transcription cascades, which effect the transition of the quiescent hepatocytes to the active cell multiplication phase. The gene expressions were decreased in the rats with individual treatment of GABA and 5-HT chitosan nanoparticles and a prominent decrease was observed with a combination of GABA and 5-HT chitosan nanoparticles.

15. SOD activity was assessed by gene expression and enzyme activity studies. The SOD expression was decreased in the regenerating liver of partially hepatectomised rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to the rats with single treatment of GABA or 5-HT chitosan nanoparticle and the one with no treatment. The active cell proliferation was assisted with increased ROS content and decreased SOD.

16. Apoptotic factors like Bax and caspase-8 were decreased prominently in partially hepatectomised rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to the rats with single treatment of GABA or 5-HT chitosan nanoparticle and the one with no treatment. This decrease was due to trigger in cell proliferation rather than death.
17. Rapid DNA synthesis prior to mitosis resulted in decreased DNA methylation. \[^{3}\text{H}\] Methyl group incorporation, in vitro was increased in the liver DNA of rats treated with GABA and 5-HT, individually and in combination compared to that with no treatment. MAT2A, the enzyme required for the synthesis of methyl donor, was also decreased and supported active regeneration by GABA and 5-HT chitosan nanoparticles.

18. Hepatocyte growth factor (HGF) is the most potent stimulator of hepatocyte growth and DNA synthesis identified. The HGF gene expression was increased 24 hours post hepatectomy in GABA and 5-HT chitosan nanoparticles treatment compared to the other groups. The gene expression was decreased seventh day post hepatectomy in the rats treated with nanoparticles compared to the one with no treatment. When the liver gains complete mass after regeneration, hepatocyte growth factor expression was decreased considerably.

19. Liver cell proliferation is initiated and progressed by the combined effect of growth factors. Real Time PCR amplification of Insulin like growth factor-1 mRNA in the liver of experimental rats were increased in the rats treated with a combination of GABA and 5-HT compared to the individual treatment of GABA or 5-HT chitosan nanoparticles.

20. GABA\(_B\) and 5-HT\(_2A\) receptors binding in the cerebral cortex, corpus striatum and brain stem were decreased by combination of GABA and 5-HT chitosan nanoparticles compared to individual GABA or 5-HT chitosan nanoparticles. The active liver cell proliferation by nanoparticles restored GABAergic and serotonergic neurotransmission compared to the regeneration without treatment.

21. GABA\(_B\) and 5-HT\(_2A\) receptors binding parameters in the cerebral cortex, corpus striatum and brain stem were confirmed by studying the mRNA expression of the corresponding receptor subtype using Real Time PCR. Both receptors in the brain regions of rats treated with a combination of GABA and 5-HT chitosan nanoparticles showed a decreased expression
compared to individual treatment with GABA or 5-HT chitosan nanoparticles. The active liver cell proliferation by nanoparticles restored GABAergic and serotonergic neurotransmission in brain regions compared to the regeneration without treatment.

22. The confocal microscopic imaging of GABA$_B$ and 5-HT$_{2A}$ receptors in the brain sections showed decrease in mean pixel intensity for partially hepatectomised rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to the rats with single treatment of GABA or 5-HT chitosan nanoparticle and the one with no treatment.

23. cAMP, IP$_3$ contents, CREB and phospholipase C gene expressions were decreased in the brain regions like cerebral cortex, brain stem and corpus striatum of partially hepatectomised rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to the rats with single treatment of GABA or 5-HT chitosan nanoparticle and the one with no treatment. Disturbed serotonergic and GABAergic neurotransmissions resulted from the over influx of improper metabolism of compounds during liver injury altered these parameters. G-protein mediated signalling was decreased in the GABA and 5-HT chitosan nanoparticles treated rats compared to the rats with no treatment, which restored the normal neuronal activity in liver injured rats.

24. NF-$\kappa$B, TNF-$\alpha$ and Akt-1 expressions showed a significant increase in the corpus striatum, cerebral cortex and brain stem of all partially hepatectomised rats. Partial hepatectomy induces activations of factors responsible for neuronal survival. GABA and 5-HT chitosan nanoparticles, individually and in combination, activated these genes prominently compared to PHNT.

25. Decreased gene expression of apoptotic factors like Caspase-8 and Bax in cerebral cortex, corpus striatum and brain stem was observed in all partially hepatectomised rat groups due to reduction in neurodegeneration by apoptosis. Treatment with 5-HT and GABA chitosan nanoparticles in
combination increased gene expressions of caspase-8 and Bax compared with either GABA chitosan or 5-HT chitosan nanoparticle treatment. This confirmed a decreased apoptosis of neurons in the brain regions due to improper influx of metabolites and altered neurotransmission.

26. Neurotrophic factors BDNF and GDNF showed a significant up regulation in the corpus striatum, cerebral cortex and brain stem of all partially hepatectomised rats. Combined treatment with 5-HT and GABA encapsulated chitosan nanoparticles increased the expression of these neurotrophic factors considerably compared to individual treatment with GABA or 5-HT chitosan nanoparticles. This suggested an increase in neuronal survival achieved through GABA and 5-HT chitosan nanoparticles.

27. cGMP content and IGF-1 expression were related to neuronal survival. cGMP content was decreased and IGF-1 expression was up regulated in corpus striatum, cerebral cortex and brain stem of all partially hepatectomised rats. The neuronal survival conditions favoured by cGMP and IGF-1 was high in the rats treated with a combination of GABA and 5-HT chitosan nanoparticles compared to that with individual treatment of GABA or 5-HT chitosan nanoparticles treatment.

28. Behavioural studies: Partial hepatectomy induced behavioural deficits in rats due to disturbed neurotransmission and neuronal death in brain. The rats treated with a combination of GABA and 5-HT chitosan nanoparticles showed a better capability to retain on the rotating rod, walk along the grid and cross narrow beam compared to the rats with individual treatment of GABA or 5-HT chitosan nanoparticle.

Our results thus showed that GABA_B and 5-HT_{2A} receptors functional regulation and modulation of apoptosis and growth factors plays a critical role for enhancing liver cell proliferation that achieved through GABA and 5-HT chitosan nanoparticles. Gene expression studies of 5-HT_{2A} and GABA_B receptors subunits
showed a prominent GABAergic and serotonergic dysregulation in neurotransmission in brain regions of partially hepatectomised rats. Partially hepatectomised rats slowly tried to restore the neuronal activity without any treatment, whereas GABA and 5-HT chitosan nanoparticles triggered the neuronal survival mechanisms and restored motor co-ordination rapidly. The findings from this study gives insight on understanding the molecular mechanisms underlying liver cell proliferation and maintaining routine neuronal functions in rats with actively regenerating liver. A combination of GABA and 5-HT encapsulated in chitosan nanoparticles showed functional recovery from liver injury mediated tissue loss and neuronal death that is of immense therapeutic significance in the management of liver diseases.