Summary

1. Insulin induced hypoglycemia and streptozotocin induced diabetes in Wistar rats were used as models to study the alterations in cholinergic, GABAergic receptors, GLUT3, insulin receptors, SOD, Bax protein, second messenger enzyme - phospholipase C and transcription factor - CREB in the brain and pancreas during hypoglycemia and diabetes.

2. The body weight was analyzed to study the changes in body weight in hypoglycemic and diabetic rats compared to control. Diabetes caused a reduction in the body weight while hypoglycemic rats showed increased body weight compared to diabetic rats.

3. Blood glucose level in the serum was measured to analyze the circulating glucose level changes due to hypoglycemia and diabetes in rats compared to control. Diabetic rats showed increased blood glucose level. The D + IIH and C + IIH rats showed significant reduction in blood glucose level. The blood glucose analysis also revealed that the D + IIH rats became hypoglycemic at the 3rd hour and the C+IIH rats became hypoglycemic at the 1st hour.

4. The circulating insulin level was analysed to study the changes in insulin concentration in hypoglycemic and diabetic rats compared to control. Diabetic rats showed a significant decrease in insulin level. The D + IIH and C + IIH rats showed significant increase in the insulin concentration.

5. Behavioural studies: Y maze and grid walk test were conducted to assess the exploratory, memory deficit, motor function and learning in control and experimental rats. The experiment demonstrated the impairment in the motor
function, learning and memory in the diabetic, D + IIH and C + IIH rats compared to control. Behavioural deficit was more prominent in hypoglycemic rats.

6. AChE expression level has been used as a marker for cholinergic activity. AChE expression was analysed in the brain regions and pancreas. During diabetes, the expression was increased in the cerebral cortex, cerebellum, brainstem, hippocampus and pancreas while hypoglycemia caused further increase in expression. In corpus striatum, AChE expression decreased significantly in hypoglycemic and diabetic rats.

7. ChAT expression level has been used as a marker for acetylcholine synthesis. ChAT showed decreased expression in the cerebral cortex, cerebellum, corpus striatum, hippocampus, pancreas while in brainstem it was increased in diabetic rats. D + IIH and C + IIH group showed significant decreased expression in all the brain regions and the pancreas.

8. Total muscarinic receptor was analysed in the brain regions and pancreas of control and experimental rats. The receptor binding was decreased in cerebral cortex, corpus striatum and hippocampus while cerebellum and brainstem showed increased expression in diabetic, D + IIH and C + IIH rats. The Scatchard analysis and gene expression studies of muscarinic M1 receptor revealed a down regulation in cerebral cortex, brainstem and hippocampus whereas in cerebellum and corpus striatum it was up regulated. Muscarinic M3 receptor binding and expression in cerebral cortex, cerebellum, brain stem and hippocampus were increased and in corpus striatum there was a decrease in diabetic, D + IIH and C + IIH rats compared to control. In pancreas, total muscarinic, muscarinic M1 and muscarinic M3 receptors were down regulated in hypoglycemia and diabetic condition. Immunohistochemical studies using
specific antibodies confirmed the Scatchard analysis and Real Time PCR analysis of muscarinic receptor expression at protein level in control and experimental rats.

9. α7 nicotinic acetylcholine receptor gene expression was studied in brain regions of experimental rats. In hypoglycemic and diabetic condition, the receptor was increased in cerebral cortex, cerebellum, brain stem, corpus striatum and decreased in hippocampus when compared to control. Immunohistochemical studies using specific antibody confirmed the gene expression of α7 nicotinic acetylcholine receptor expression at protein level in control and experimental rats.

10. Total GABA receptor binding and GAD expression was analysed in the brain regions and pancreas of control and experimental rats. The receptor binding decreased in cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus of hypoglycemic and diabetic rats. The gene expression studies of GABA_A1 and GABA_B receptor showed down regulation in cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus. GAD mRNA expression significantly decreased in all the brain regions studied. In pancreas, GABA receptor binding along with GABA_A1, GABA_B and GAD significantly decreased during hypoglycemia and diabetes. Immunohistochemical studies using specific antibody confirmed the binding analysis and Real Time PCR analysis of GABA_A1 receptor expression in control and experimental rats.

11. Decreased GLUT3 in brain regions- cerebral cortex, cerebellum, brainstem, corpus striatum and hippocampus of diabetic rats were decreased further in hypoglycemic group compared to diabetic and control.
12. Insulin receptor mRNA level was studied in the brain regions and pancreas of experimental rats. A decreased expression of insulin receptor was observed in cerebral cortex whereas in cerebellum, brain stem, corpus striatum and hippocampus, there was an increased expression in hypoglycemic and diabetic rats. Pancreas of both hypoglycemic and diabetic rats showed decreased insulin receptor expression.

13. Antioxidant enzyme, SOD expression was studied in experimental rats. Results showed that in diabetic rats, its mRNA level was down regulated in cerebral cortex, cerebellum and hippocampus whereas in brain stem and corpus striatum, it was up regulated when compared to control. In D + IIH and C + IIH rats, SOD expression decreased in cerebral cortex, cerebellum, corpus striatum and hippocampus whereas brain stem showed increased expression. Pancreatic expression of SOD mRNA in hypoglycemic rats decreased significantly compared to diabetic and control. Oxidative stress seen in diabetic brain and pancreas were found to exacerbate by hypoglycemia.

14. Pro-apoptotic protein- Bax mRNA expression significantly up regulated in hypoglycemic brain regions - cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and pancreas compared to diabetic and control.

15. Second messenger enzyme - phospholipase C showed a decreased expression in hypoglycemic and diabetic brain regions - cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus. In pancreas, there was a significant decrease in mRNA expression of phospholipase C in diabetic, D + IIH and C + IIH rats compared to control.
16. Transcription factor, CREB expression in the brain regions - cerebral cortex, cerebellum, brain stem and hippocampus showed decreased expression in hypoglycemic and diabetic rats. In corpus striatum, there was an increased CREB expression in diabetic rats compared to control whereas D + IIH and C + IIH rats showed decreased expression compared to diabetic.

It is evident from our results that cholinergic, GABAergic receptor functional balance plays a major role in hypoglycemia and diabetes associated disorders. Gene expression studies along with immunohistochemistry showed a prominent functional disturbance in brain regions and pancreas of hypoglycemic and diabetic rats. These findings have important implications for understanding the molecular mechanisms underlying memory and cognitive impairment at second messenger and transcription level during recurrent hypoglycemia. Our studies showed hypoglycemic and hyperglycemic effect on brain functions by acetylcholine and GABA mediated through their receptor subtypes, second messenger enzymes and transcription factors. It is suggested that the corrective measures for the brain functional damage caused during diabetes and its treatment, through cholinergic and GABAergic receptors have therapeutic role in the management of diabetes and hypoglycemia - induced by anti-hyperglycemic treatment in diabetes.