Summary

1. Streptozotocin induced diabetic rats were used as model to study the alterations of cholinergic, dopaminergic, insulin, Vitamin D receptors, GLUT3, second messenger enzyme phospholipase C, CREB and antioxidant enzyme super oxide dismutase and their regulation by curcumin and Vitamin D₃ in insulin secretion.

2. Antihyperglycemic activity of curcumin and Vitamin D₃ were evaluated by the blood glucose and circulating insulin level measurement of experimental rats. Diabetic rats showed increased blood glucose and decreased insulin level. Curcumin and Vitamin D₃ supplementation to diabetic rats reversed the blood glucose and circulating insulin level to control.

3. Serum T3 concentration was decreased in diabetic rats. Insulin, curcumin and Vitamin D₃ treatment reversed the T3 concentration to near control.

4. Behavioural studies: Y maze, rotarod, beam walk and grid walk test were conducted to assess the motor learning and memory in control and experimental rats. Diabetic rats showed a significant deficit in cognition, memory and motor learning. Insulin, curcumin and Vitamin D₃ treated diabetic rats reversed the behavioral response to near control when compared to diabetic rats.

5. Acetylcholine esterase expression level has been used as a marker for cholinergic activity. Acetylcholine esterase expression was analysed in the brain regions and pancreas. During diabetic stage the expression was increased in the cerebral cortex, cerebellum, brainstem, hippocampus and
hypothalamus while in corpus striatum it was decreased. Pancreas showed an up regulation in diabetic rats compared to control. In insulin treated, curcumin and Vitamin D$_3$ treated diabetic rats, the expression of the enzyme reversed to near control. Immunocytochemical studies using specific antibodies of acetylcholine esterase confirmed the mRNA expression at protein level in pancreas of control and experimental rats by reversing the changes in diabetic rats.

6. Choline acetyltransferase expression level has been used as a marker for acetylcholine synthesis. Choline acetyltransferase expression was analysed in the brain regions and pancreas. During diabetes, the expression was decreased in the cerebral cortex, cerebellum, corpus striatum, hippocampus and hypothalamus while in brain stem it was increased. Pancreas showed a down regulation in diabetic rats compared to control. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats, the expression of the enzyme reversed to near control.

7. Total muscarinic receptor was analysed in the brain regions and pancreas of control and experimental rats. Total muscarinic receptor binding was decreased in cerebral cortex, corpus striatum and hippocampus while cerebellum and brainstem showed increased expression in diabetic rats. The Scatchard analysis and gene expression studies of muscarinic M1 receptor revealed a down regulation in cerebral cortex, brainstem, hippocampus and hypothalamus whereas in cerebellum and corpus striatum it was up regulated. Muscarinic M3 receptor binding and expression in cerebral cortex, cerebellum, brain stem, hippocampus and hypothalamus were increased and in corpus striatum there was a decrease in diabetic rats compared to control. In pancreas total muscarinic, muscarinic M1 and muscarinic M3 receptors were down regulated in
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diabetic condition. Insulin, curcumin and Vitamin D₃ supplementation restored the binding and expression of total muscarinic, muscarinic M₁ and muscarinic M₃ receptors in brain regions and pancreas to near control. Immunohistochemistry 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.

8. α7 nicotinic acetylcholine receptor gene expression was studied in brain regions of experimental rats. In diabetic condition α7 nicotinic acetylcholine receptor was increased in cerebral cortex, cerebellum, brain stem and corpus striatum and decreased in hippocampus when compared to control. Treatment using curcumin and Vitamin D₃ in diabetic rats reversed the altered expression in the brain regions to near control whereas insulin treatment to diabetic rats did not significantly restore the altered α7 nicotinic acetylcholine receptor gene expression to control. Immunohistochemistry studies using specific antibodies confirmed the gene expression of α7 nicotinic acetylcholine receptor expression at protein level in control and experimental rats.

9. Total dopamine receptor binding was analysed in the brain regions of control and experimental rats. Total dopamine receptor binding was increased in cerebral cortex, brain stem and hippocampus while cerebellum and corpus striatum showed increased expression in diabetic rats. The gene expression studies of dopamine D₁ receptor revealed an up regulation in cerebral cortex, brain stem and hippocampus whereas in cerebellum, corpus striatum and hypothalamus it was down regulated. Dopamine D₂ receptor expression in cerebral cortex, cerebellum, corpus striatum and hippocampus was increased and in brain stem there was a
decrease in diabetic rats compared to control. In pancreas dopamine D1 and D2 receptor expression decreased in diabetic condition. Insulin, curcumin and Vitamin D₃ supplementation brought back the altered expression of total dopamine, DA D1 and DA D2 receptors to near control.

10. Vitamin D receptor status in the brain regions and pancreas of experimental rats were analysed using Real Time PCR. Cerebral cortex, corpus striatum, and hippocampus showed a decreased Vitamin D receptor mRNA level while an increased mRNA expression level in cerebellum, brain stem and hypothalamus of diabetic rats. There was decreased expression of Vitamin D receptor in pancreas of diabetic rats when compared to control. Restoration of disrupted Vitamin D receptor expression was seen with insulin, curcumin and Vitamin D₃ treatment to diabetic rats.

11. 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, hippocampus and hypothalamus, there was an increased expression in diabetic rats. Pancreas of diabetic rats showed decreased insulin receptor expression. Insulin, curcumin and Vitamin D₃ treatment to diabetic rats considerably ameliorated the altered insulin receptor expression to near control.

12. Gene expression studies showed insulin, curcumin and Vitamin D₃ treatment substantially reversed the increased expression of GLUT3 in brain regions- cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and hypothalamus of diabetic rats to near control. GLUT2
expression was studied in the pancreas and showed down regulation in diabetic rats when compared to control. The treatment groups reversed the decreased expression of GLUT2 to near control.

13. Second messenger enzyme - phospholipase C showed a decreased expression in diabetic brain regions - cerebral cortex, cerebellum, brain stem, hippocampus, hypothalamus and increased expression in corpus striatum. Diabetic pancreas also showed a decreased phospholipase C expression when compared to control. Insulin, curcumin and Vitamin D₃ administration to diabetic rats reversed the altered phospholipase C expression to near control.

14. Transcription factor, CREB expression in the brain regions - cerebral cortex, cerebellum, brain stem, hippocampus and hypothalamus showed decreased expression in diabetic rats. In corpus striatum, there was an increased CREB expression in diabetic rats compared to control. Diabetes induced altered CREB expression in brain regions was reversed with insulin, curcumin and Vitamin D₃ treatment to near control.

15. Antioxidant enzyme, superoxide dismutase expression was studied in experimental rats. Results showed that in diabetic rats, its mRNA level was down regulated in cerebral cortex, cerebellum, hippocampus and hypothalamus whereas in brain stem and corpus striatum, it was up regulated when compared to control. Pancreatic expression of superoxide dismutase in diabetic rats was decreased compared to control. Oxidative stress seen in diabetic brain regions and pancreas was considerable lowered by reversing the expression of superoxide dismutase to near control by treatment with insulin, curcumin and Vitamin D₃.
In summary, we conclude that brain and pancreatic cholinergic, dopaminergic, Vitamin D, insulin receptor, GLUT3/2, phospholipase C, CREB and superoxide dismutase functional balance has a major role in regulating the insulin secretion and modulating behavioural and cognitive process. The present study demonstrates the therapeutic role of nutritional agents, curcumin and Vitamin D3 in ameliorating CNS dysfunctions and insulin synthesis and secretion from pancreas. Thus our results confirmed neuroprotective role of curcumin and Vitamin D3 through cholinergic and dopaminergic functional regulation and glucose homeostasis which in turn lead to a novel therapeutic management of diabetes.