

Summary

1. Streptozotocin induced diabetic rat model was used to study the alterations of NMDA and AMPA receptor and their functional regulation by curcumin and vitamin D₃.
2. Body weights were determined and diabetic rats showed a significant reduction in the body weight when compared to control. Curcumin and vitamin D₃ treatment showed a significant reversal in body weight when compared with diabetic group.
3. Antihyperglycemic activity of curcumin and vitamin D₃ were evaluated by measuring the blood glucose and circulating insulin level in experimental rats. Diabetic rats showed increased blood glucose and decreased insulin level. Curcumin and vitamin D₃ supplementation in diabetic rats reversed the blood glucose and circulating insulin, when compared with diabetic group.
4. Oxidative stress and free radical scavenging capability of diabetic and other experimental conditions were evaluated by studying the SOD activity and GPx gene expression. In diabetic condition, SOD activity was decreased and GPx gene expression was down regulated in the cerebral cortex, cerebellum, hippocampus, brain stem and pancreas. Oxidative stress in diabetic brain regions and pancreas was significantly reduced in insulin, curcumin and vitamin D₃ treated diabetic rats by reversing the altered antioxidant enzyme activity and expression. Curcumin treatment showed prominent reversal in the antioxidant enzyme activity when compared with other treatments.

5. Glutamate content increased in the cerebral cortex, cerebellum, hippocampus, brain stem and pancreas of diabetic rats. Treatment with insulin, curcumin and vitamin D₃ reversed these changes when compared to diabetic in brain regions. Insulin treatment did not show any significant reversal in pancreas.
6. NMDA receptor functional status was analysed by Scatchard analysis using [³H] MK801. NMDA receptor number was increased in cerebral cortex, cerebellum, hippocampus and brain stem of diabetic rats compared to control with no significant change in K_d. NMDA receptor number did not show any significant change in the pancreas of control and experimental rats. Treatment with insulin reversed the alteration in NMDA receptors of cerebral cortex, hippocampus and brain stem but Insulin treatment did not show any significant reversal in cerebellum when compared with diabetic group. The treatment with curcumin and vitamin D₃ significantly reversed the alteration in brain NMDA receptors when compared with diabetic group.
7. AMPA receptor functional status was analysed by Scatchard analysis using [³H] AMPA. AMPA receptor number was increased in cerebral cortex, cerebellum, hippocampus, brain stem and pancreas of diabetic rats compared to control with no significant change in K_d. The treatment with insulin, curcumin and vitamin D₃ reversed the alteration in AMPA receptor when compared with diabetic group.
8. Glutamate mediates its action through its receptor subunits – NMDA R1, NMDA 2B, AMPA GluR2, AMPA GluR4. Real Time PCR analysis of NMDA R1, NMDA 2B, AMPA GluR2, AMPA GluR4 receptors subunit gene expression confirmed the receptor binding data. There was a significant up regulation in NMDA R1, NMDA 2B and AMPA GluR4 and down regulation in AMPA GluR2 gene expression in the cerebral

cortex, cerebellum, hippocampus and brain stem of diabetic rats compared to control, indicating receptor function alterations in the brain regions of diabetic rats. The treatment with insulin, curcumin and vitamin D₃ reversed these changes when compared with diabetic rats.

9. Real Time PCR studies in the pancreas showed that AMPA GluR4 receptor subunit expression was down regulated and AMPA GluR2 receptor subunit expression was up regulated leading to altered pancreatic function. The treatment with insulin, curcumin and vitamin D₃ reversed the gene expression to near control.

10. Differential expression of NMDA R1, NMDA 2B, AMPA GluR4 and AMPA GluR2 receptor subunits in the cerebral cortex, cerebellum, hippocampus and brain stem of diabetic was confirmed by immunohistochemical studies using confocal microscope with specific antibodies in the brain slices. The treatment with insulin, curcumin and vitamin D₃ reversed the mean pixel value to near control.

11. Immunohistochemical studies using confocal microscope with specific antibodies of AMPA GluR4, AMPA GluR2 receptor subunits co-labelled with insulin antibody was carried out to detect the expression of AMPA receptor subunit in the pancreatic islets. AMPA GluR4 subunits expression was significantly decreased and AMPA GluR2 receptor subunits expression was significantly increased in the insulin positive cells in pancreatic islets of diabetic rats. The treatment with insulin, curcumin and vitamin D₃ reversed the mean pixel value when compared with diabetic group.

12. To prevent glutamate mediated excitotoxic effects, glutamate should be cleared from the extracellular space by glutamate transporters. The gene expression of Glutamate aspartate transporter (GLAST) was studied in

control and experimental rats. GLAST showed decreased expression in cerebral cortex, cerebellum, hippocampus, brain stem and pancreas of diabetic rats leading to impaired reuptake of extracellular glutamate formed in the diabetic condition. Insulin treatment showed significant reversal only in brain regions. Curcumin and vitamin D₃ showed prominent reversal of GLAST gene expression in the brain regions and pancreas when compared with diabetic rats.

13. Real time PCR gene expression analysis of Glutamate decarboxylase (GAD) was done in cerebral cortex, cerebellum, hippocampus and brain stem of control and experimental rats. A significant down regulation of GAD mRNA was observed in diabetic rat brain. Decreased GAD gene expression in the diabetic rat leads to increased accumulation of glutamate. Treatment using insulin, curcumin and vitamin D₃ reversed these changes when compared with diabetic rats.
14. Second messenger IP3 content was increased significantly in cerebral cortex, cerebellum, hippocampus and brain stem of diabetic rats. The increased levels of IP3 causes enhanced Ca²⁺ levels leading to neurotoxicity in brain regions. The treatment using Insulin, curcumin and vitamin D₃ reversed these changes when compared with diabetic rats.
15. In pancreas, second messenger IP3 content was significantly reduced and immunohistochemical studies showed that inositol trisphosphate receptor (IP3R3) expression was significantly decreased in the pancreatic islet of diabetic rats when compared with control. Treatment using curcumin and vitamin D₃ reversed these changes when compared with diabetic rats. Insulin treatment did not show any significant change when compared with diabetic group.

16. Calcium imaging results showed decreased calcium release from the pancreatic islets in diabetic rats. Treatment using vitamin D₃ showed prominent increase in Ca²⁺ release when compared with diabetic group.
17. Increased expression of pro-apoptotic factors, caspase-8 and Bax was observed in cerebral cortex, cerebellum, hippocampus, brain stem and pancreas of diabetic rats. Treatment using insulin, curcumin and vitamin D₃ reversed these changes when compared with diabetic rats.
18. A significant down regulation of anti-apoptotic factor Akt-1 was observed in cerebral cortex, cerebellum, hippocampus and brain stem of diabetic rats. Curcumin treatment showed significant reversal in the Akt-1 gene expression when compared with diabetic rats. The treatment with Vitamin D₃ resulted in a prominent reversal and up regulation of Akt-1 gene expression when compared with diabetic and insulin treated rats.
19. Pdx1 and NeuroD1 expression was significantly up regulated in the curcumin and vitamin D₃ treated rats indicating pancreatic β cell survival and reduced pancreatic β cell death in the treatment group. Insulin treatment did not show any significant change.

The alterations in the GAD and GLAST mRNA in diabetic rats can lead to increased production of glutamate content. Increased AMPA and NMDA receptors in the presence of increased glutamate resulted in elevated IP3 levels and Ca²⁺ levels leading to a state of oxidative stress. The activated apoptotic factors Bax and caspase 8 lead to neuronal death. The altered AMPA receptor subunits expression along with increased oxidative stress and activation of apoptotic factors in the pancreas results in decreased secretion of insulin. Thus, from our study it is suggested that curcumin and vitamin D₃ has therapeutic role in diabetes management mediated through glutamatergic function.