Results

BODY WEIGHT

The body weight was significantly decreased (p<0.001) in the diabetic rats when compared to control. Body weight of diabetic rats was decreased significantly in 7th day. After insulin treatment, curcumin and vitamin D₃ supplementation for 14 days, the body weight was significantly reversed (p<0.001) when compared with diabetic rats. (Figure-1, Table-1).

BLOOD GLUCOSE LEVEL

Blood glucose level of all rats before streptozotocin administration was within the normal range. Streptozotocin administration in rats led to a significant increase (p<0.001) in blood glucose level when compared to control group. Insulin curcumin and vitamin D₃ treatments were significantly reversed (p<0.001) the increased blood glucose level when compared to diabetic group (Figure-2, Table-2).

CIRCULATING INSULIN LEVEL

There was a significant decrease in the serum insulin level of the diabetic group when compared to control (p<0.001). Insulin, curcumin and vitamin D₃ treatment for 14 days significantly increased (p<0.001) the serum insulin when compared to diabetic group (Figure-3, Table-3).
**CEREBRAL CORTEX**

Glutamate content in the cerebral cortex of control and experimental rats

Glutamate content was significantly \((p<0.001)\) increased in cerebral cortex of the diabetic rats compared to the control. Treatment using insulin \((p<0.05)\), curcumin \((p<0.001)\) and vitamin D\(_3\) \((p<0.001)\) significantly reversed the glutamate content when compared to diabetic group. Curcumin and vitamin D\(_3\) treatment showed prominent reversal \((p<0.001)\) in glutamate content when compared with insulin treatment (Figure-4, Table-4).

Scatchard analysis of NMDA receptor using \[^{3}H\] MK-801 binding against MK-801 in the cerebral cortex of control and experimental rats

Scatchard analysis of NMDA receptor using \[^{3}H\] MK-801 binding against MK-801 in the cerebral cortex of diabetic rats showed a significant \((p<0.001)\) increase in \(B_{\text{max}}\) compared to control rats. This shows increased NMDA receptor density in the cerebral cortex of diabetic rats. Significant reversal in the \(B_{\text{max}}\) was observed in treatment groups: insulin \((p<0.01)\), curcumin \((p<0.001)\) and vitamin D\(_3\) \((p<0.001)\). There was no significant change in \(K_{d}\) in all experimental groups of rats. Curcumin and vitamin D\(_3\) treatment showed prominent reversal \((p<0.001)\) in \(B_{\text{max}}\) when compared with insulin treatment (Figure- 5, 6 & Table- 5, 6).

Scatchard analysis of AMPA receptor using \[^{3}H\] AMPA binding against AMPA in the cerebral cortex of control and experimental rats

Scatchard analysis of AMPA receptor using \[^{3}H\] AMPA binding against AMPA in the cerebral cortex of diabetic rats showed a significant increase in \(B_{\text{max}}\) \((p< 0.001)\) compared to control rats. This result showed increased AMPA receptor density in the cerebral cortex of diabetic rats compared to control. Treatment using insulin \((p<0.01)\), curcumin \((p<0.01)\) and vitamin D\(_3\) \((p<0.05)\) significantly reversed the changes in receptor binding when compared with diabetic group. There was no significant change in \(K_{d}\) in all experimental groups of rats (Figure- 7, 8 & Table- 7, 8).
Real time PCR amplification of NMDA R1 receptor subunit mRNA from the cerebral cortex of control and experimental rats

Gene expression of NMDA R1 receptor subunit mRNA showed significant up regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and vitamin D3 significantly (p<0.001) reversed the altered gene expression when compared with diabetic group (Figure-9, Table-9).

Real time PCR amplification of NMDA 2B receptor subunit mRNA from the cerebral cortex of control and experimental rats

Real-time PCR gene expression of NMDA 2B receptor subunit showed significant up regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control. There was a significant reversal (p<0.001) in NMDA 2B receptor subunit gene expression in diabetic rats treated with insulin, curcumin and vitamin D3 (Figure-10, Table-10).

Real time PCR amplification of GluR4 subunit of AMPA receptor mRNA from the cerebral cortex of control and experimental rats

Real-time PCR gene expression of GluR4 subunit of AMPA receptor subunit showed significant up regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control rats. There was a significant reversal (p<0.001) in AMPA GluR4 subunit gene expression in diabetic rats treated with insulin, curcumin and vitamin D3 (Figure-11, Table-11).

Real time PCR amplification of GluR2 subunit of AMPA receptor mRNA from the cerebral cortex of control and experimental rats

Gene expression of AMPA GluR2 receptor subunit mRNA showed significant down regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and vitamin D3
significantly (p<0.001) reversed these changes when compared with diabetic group (Figure -12, Table -12).

Real time PCR amplification of GLAST mRNA from cerebral cortex of control and experimental rats

Real-time PCR gene expression of GLAST showed significant down regulation (p<0.001) in the cerebral cortex of diabetic rats. There was significant reversal (p<0.001) in GLAST gene expression in diabetic rats treated with insulin, curcumin and vitamin D3. Curcumin and vitamin D3 treatment showed prominent reversal (p<0.001) in GLAST gene expression when compared with insulin treatment (Figure -13, Table -13).

Real time PCR amplification of GAD mRNA from the cerebral cortex of control and experimental rats

Real-time PCR gene expression of GAD showed significant down regulation (p<0.001) in the cerebral cortex of diabetic rats. Insulin (p<0.01) and curcumin (p<0.001) treated diabetic rats showed a significant reversal when compared to diabetic rats. In vitamin D3 treated rats GAD gene expression was significantly reversed and up regulated (p<0.001) when compared with diabetic rats (Figure-14; Table-14). Curcumin and vitamin D3 treatment showed prominent reversal (p<0.001) in GAD gene expression when compared with insulin treatment.

IP3 content in cerebral cortex of control and experimental rats

IP3 content was significantly increased (p<0.001) in the cerebral cortex of diabetic rats when compared to control rats. Insulin (p<0.001), curcumin (p<0.001) and vitamin D3 (p<0.001) treatment in diabetic rats significantly reversed the IP3 content when compared to diabetic group. Vitamin D3 treatment showed prominent reversal (p<0.05) in IP3 content when compared with insulin treatment (Figure-15; Table-15).
Superoxide dismutase assay in the cerebral cortex of control and experimental rats

There was a significant decrease in SOD activity (p<0.001) in cerebral cortex of diabetic rats. Treatment using insulin (p<0.05), Curcumin (p<0.001) and Vitamin D3 (p<0.001) reversed the activity of SOD enzyme when compared with diabetic rats. Curcumin treatment showed prominent reversal (p<0.05) in SOD activity when compared with insulin treatment (Figure-16; Table-16).

Real time PCR amplification of GPx mRNA from the cerebral cortex of control and experimental rats

Real time PCR gene expression of GPx showed significant down regulation (p<0.001) in the cerebral cortex of the diabetic rats compared to the control. Treatment using insulin, curcumin and vitamin D3 significantly reversed (p<0.001) the changes when compared with diabetic rats. Curcumin treatment showed prominent reversal (p<0.001) in GPx gene expression when compared with insulin treatment (Figure-17; Table-17).

Real time PCR amplification of Akt-1 mRNA from the cerebral cortex of control and experimental rats

Real-time PCR gene expression of Akt-1 showed significant down regulation (p<0.001) in the cerebral cortex of diabetic rats. Treatment using insulin and curcumin significantly reversed (p<0.001) the altered gene expression when compared with diabetic rats. In vitamin D3 treated diabetic rats, there was significant (p<0.001) reversal and up regulation of Akt-1 gene expression when compared to diabetic and control rats respectively. Vitamin D3 treatment showed prominent reversal (p<0.001) in Akt-1 gene expression when compared with insulin treatment (Figure-18; Table-18).
Results

Real time PCR amplification of Bax mRNA from the cerebral cortex of control and experimental rats

Real-time PCR gene expression of Bax showed significant up-regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control rats. The Bax gene expression was reversed significantly in insulin (p<0.01) and curcumin (p<0.001) and vitamin D3 (p<0.001) treated rats when compared with diabetic rats. Curcumin and vitamin D3 treatment showed prominent reversal (p<0.001) in Bax gene expression when compared with insulin treatment (Figure-19; Table-19).

Real time PCR amplification of caspase 8 mRNA from the cerebral cortex of control and experimental rats

Real-time PCR gene expression of caspase 8 showed significant up-regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control rats. There was a significant reversal (p<0.001) in Caspase 8 gene expression in diabetic rats treated with insulin, curcumin and vitamin D3 (Figure-20; Table-20).

NMDA R1 receptor subunit antibody staining in control and experimental groups of rats using confocal microscope

NMDA R1 subunit specific antibody staining in the cerebral cortex showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin (p<0.05), curcumin (p<0.001) and vitamin D3 (p<0.01) treatment in diabetic rats significantly reversed mean pixel value when compared with diabetic rats (Figure-21; Table-21). Curcumin treatment showed prominent reversal when compared with insulin treated rats.

NMDA 2B receptor subunit antibody staining in control and experimental groups of rats using confocal microscope

NMDA 2B subunit specific antibody staining in the cerebral cortex showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin (p<0.01), curcumin (p<0.001) and vitamin D3
treatment in diabetic rats significantly mean pixel value when compared with diabetic rats (Figure-22; Table-22).

**AMPA (GluR4) receptor subunit antibody staining in control and experimental groups of rats using confocal microscope**

AMPA (GluR4) receptor subunit specific antibody staining in the cerebral cortex showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin, curcumin and vitamin D₃ treatment in diabetic rats significantly (p<0.001) reversed the mean pixel value to near control (Figure-23; Table-23).

**AMPA (GluR2) receptor subunit antibody staining in control and experimental groups of rats using confocal microscope**

AMPA (GluR2) receptor subunit antibody staining in the cerebral cortex showed a significant decrease (p<0.001) in the mean pixel value in diabetic rats compared to control. Insulin (p<0.05), curcumin (p<0.001) and vitamin D₃ (p<0.01) treatment to diabetic rats significantly reversed AMPA (GluR2) receptor subunit expression in the cerebral cortex to near control (Table-24, Figure-24).
HIPPOCAMPUS

Glutamate content in the hippocampus of control and experimental rats

Glutamate content was significantly (p<0.001) increased in hippocampus of the diabetic rats compared to the control. Treatment using insulin, curcumin and vitamin D3 reversed (p<0.001) the glutamate content when compared to diabetic group. Curcumin and vitamin D3 treatment showed prominent reversal (p<0.01) in glutamate content when compared with insulin treatment (Figure -25, Table- 25).

Scatchard analysis of NMDA receptor using [3H] MK-801 binding against MK-801 in the hippocampus of control and experimental rats

Scatchard analysis of NMDA receptor using [3H] MK-801 binding against MK-801 in the hippocampus of diabetic rats showed a significant (p<0.001) increase in B_max compared to control rats. Significant reversal (p<0.001) in the B_max was observed in treatment groups: Insulin, curcumin and vitamin D3. There was no significant change in K_d in all experimental groups of rats. Vitamin D3 treatment showed prominent reversal (p<0.001) in B_max when compared with insulin treatment (Figure- 26, 27 & Table- 26, 27).

Scatchard analysis of AMPA receptor using [3H] AMPA binding against AMPA in the hippocampus of control and experimental rats

Scatchard analysis of AMPA receptor using [3H] AMPA binding against AMPA in the hippocampus of diabetic rats showed a significant increase in B_max (p< 0.001) compared to control rats. Treatment using insulin (p<0.05), curcumin (p<0.001) and vitamin D3 (p<0.001) significantly reversed the changes in receptor binding when compared with diabetic group. There was no significant change in K_d in all experimental groups of rats. Vitamin D3 (p<0.001) treatment showed prominent reversal in B_max when compared with insulin treatment (Figure- 28, 29 & Table- 28, 29).
Real time PCR amplification of NMDA R1 receptor subunit mRNA from the hippocampus of control and experimental rats

Gene expression of NMDA R1 receptor subunit mRNA showed significant up regulation (p<0.001) in the hippocampus of diabetic rats compared to control. Treatment using insulin, curcumin and vitamin D3 significantly reversed (p<0.001) the altered gene expression when compared with diabetic group. Curcumin treatment showed prominent reversal (p<0.001) in NMDA R1 receptor subunit gene expression when compared with insulin treatment (Figure-30, Table-30).

Real time PCR amplification of NMDA 2B receptor subunit mRNA from the hippocampus of control and experimental rats

Gene expression of NMDA 2B receptor subunit mRNA showed significant up regulation (p<0.001) in the hippocampus of diabetic rats compared to control. Treatment using insulin, curcumin and vitamin D3 significantly (p<0.001) reversed these changes when compared with diabetic group. Curcumin treatment showed prominent reversal (p<0.001) in NMDA 2B receptor subunit gene expression when compared with insulin treatment (Table-31, Figure-31).

Real time PCR amplification of GluR4 subunit of AMPA receptor mRNA from the hippocampus of control and experimental rats

Real-time PCR gene expression of GluR4 subunit of AMPA receptor subunit showed significant up regulation (p<0.001) in the hippocampus of diabetic rats compared to control rats. AMPA GluR4 subunit gene expression was significantly reversed in diabetic rats treated with insulin (p<0.01), curcumin and vitamin D3 (p<0.001) when compared with diabetic. Curcumin and vitamin D3 treatment showed prominent reversal (p<0.001) in GluR4 subunit of AMPA receptor subunit gene expression when compared with insulin treatment (Figure-32, Table-32).
**Results**

**Real time PCR amplification of GluR2 subunit of AMPA receptor mRNA from the hippocampus of control and experimental rats**

Real-time PCR gene expression of GluR2 subunit of AMPA receptor subunit showed significant down regulation (p<0.001) in the hippocampus of diabetic rats compared to control rats. There was a significant reversal (p<0.001) in AMPA GluR2 subunit gene expression in diabetic rats treated with insulin, curcumin and vitamin D₃ (Figure-33, Table-33).

**Real time PCR amplification of GLAST mRNA from hippocampus of control and experimental rats**

Gene expression of GLAST mRNA showed significant down regulation (p<0.001) in the hippocampus of diabetic rats compared to control. Treatment using insulin, curcumin and vitamin D₃ significantly reversed (p<0.001) the changes to near control. Curcumin and vitamin D₃ treatment showed prominent reversal (p<0.001) in GLAST gene expression when compared with insulin treatment (Figure-34, Table-34).

**Real time PCR amplification of GAD mRNA from the hippocampus of control and experimental rats**

Real-time PCR gene expression of GAD showed significant down regulation (p<0.001) in the hippocampus of diabetic rats. Insulin, curcumin and vitamin D₃ treatment showed a significant reversal (p<0.001) when compared with diabetic rats. Vitamin D₃ treatment showed prominent reversal (p<0.001) in GAD gene expression when compared with insulin treatment (Figure-35; Table-35).

**IP3 content in hippocampus of control and experimental rats**

IP3 content was significantly increased (p<0.001) in the hippocampus of diabetic rats when compared to control rats. Insulin, curcumin and vitamin D₃ treatment in diabetic rats significantly reversed (p<0.001) the IP3 content to near control (Figure-36; Table-36).
Superoxide dismutase assay in the hippocampus of control and experimental rats

There was a significant decrease in SOD activity (p<0.001) in hippocampus of diabetic rats. Treatment using curcumin (p<0.01) and vitamin D3 (p<0.05) reversed the activity of SOD enzyme when compared with diabetes. Treatment using insulin did not show any significant reversal. Curcumin treatment showed prominent reversal (p<0.01) in SOD activity when compared with insulin treatment (Figure-37; Table-37).

Real time PCR amplification of GPx mRNA from the hippocampus of control and experimental rats

Real time PCR gene expression of GPx showed significant down regulation (p<0.001) in the hippocampus of the diabetic rats compared to the control. Treatment using insulin and vitamin D3 significantly reversed (p<0.001) the changes when compared with diabetic rats. Curcumin treatment significantly reversed and up regulate the GPx gene expression when compared with both control (p<0.01) and diabetic (p<0.001). Curcumin (p<0.001) treatment showed prominent reversal in GPx gene expression when compared with insulin treatment (Figure-38; Table-38).

Real time PCR amplification of Akt-1 mRNA from the hippocampus of control and experimental rats

Real-time PCR gene expression of Akt-1 showed significant down regulation (p<0.001) in the hippocampus of diabetic rats. Curcumin and insulin treated diabetic rats showed a significant (p<0.001) reversal of Akt-1 gene expression when compared to diabetic rats. Vitamin D3 treatment showed reversal (p<0.001) and up regulation in Akt-1 gene expression when compared with both control and diabetic group. (Figure-39; Table-39).
Results

Real time PCR amplification of Bax mRNA from the hippocampus of control and experimental rats

Real-time PCR gene expression of Bax showed significant up regulation (p<0.001) in the hippocampus of diabetic rats compared to control rats. The Bax gene expression was reversed significantly (p<0.001) in insulin, curcumin and vitamin D₃ treated rats when compared with diabetic rats. Curcumin (p<0.001) treatment showed prominent reversal in Bax gene expression when compared with insulin treatment (Figure-40; Table-40).

Real time PCR amplification of caspase 8 mRNA from the hippocampus of control and experimental rats

Real-time PCR gene expression of caspase 8 showed significant up regulation (p<0.001) in the hippocampus of diabetic rats compared to control rats. There was a significant reversal (p<0.001) in Caspase 8 gene expression in diabetic rats treated with insulin, curcumin and vitamin D₃. Curcumin (p<0.001) treatment showed prominent reversal in caspase 8 gene expression when compared with insulin treatment (Figure-41; Table-41).

NMDA R1 receptor subunit antibody staining in control and experimental groups of rats using confocal microscope

NMDA R1 subunit specific antibody staining in the hippocampus showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin (p<0.01), curcumin (p<0.001) and vitamin D₃ (p<0.001) treatment in diabetic rats significantly reversed the mean pixel value when compared with diabetic group (Figure-42; Table-42).

NMDA 2B receptor subunit antibody staining in control and experimental groups of rats using confocal microscope

NMDA 2B subunit specific antibody staining in the hippocampus showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin, curcumin and vitamin D₃ treatment in diabetic rats
significantly (p<0.001) reversed the mean pixel value when compared with diabetic group (Figure-43; Table-43).

**AMPA (GluR4) receptor subunit antibody staining in control and experimental groups of rats using confocal microscope**

AMPA (GluR4) receptor subunit specific antibody staining in the hippocampus showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin (p<0.01), curcumin (p<0.001) and vitamin D₃ (p<0.001) treatment in diabetic rats significantly reversed the mean pixel value when compared with diabetic group (Figure-44; Table-44).

**AMPA (GluR2) receptor subunit antibody staining in control and experimental groups of rats using confocal microscope**

AMPA (GluR2) receptor subunit antibody staining in the hippocampus showed a significant decrease (p<0.01) in the mean pixel value in diabetic rats compared to control. Insulin (p<0.05), curcumin (p<0.05) and vitamin D₃ (p<0.05) treatment to diabetic rats significantly reversed AMPA (GluR2) receptor subunit expression in the hippocampus when compared with diabetic rats (Table-45, Figure-45).
Results

BRAIN STEM

Glutamate content in the brain stem of control and experimental rats

Glutamate content was significantly (p<0.001) increased in brain stem of the Diabetic rats compared to the control. The glutamate content was reversed significantly (p<0.001) in insulin, curcumin, and vitamin D₃ treated rats when compared with diabetic rats. (Figure -46, Table- 46). Vitamin D₃ treatment showed prominent reversal (p<0.05) in glutamate content when compared with insulin treatment.

Scatchard analysis of NMDA receptor using [³H] MK-801 binding against MK-801 in the brain stem of control and experimental rats

Scatchard analysis of NMDA receptor using [³H] MK-801 binding against MK-801 in the brain stem of diabetic rats showed a significant (p<0.001) increase in Bmax compared to control rats. Significant reversal (p<0.001) in the Bmax was observed in treatment groups: Insulin, curcumin and vitamin D₃. There was no significant change in Kd in all experimental groups of rats. Vitamin D₃ treatment showed prominent reversal (p<0.001) in Bmax when compared with insulin treatment (Figure- 47, 48 & Table- 47, 48).

Scatchard analysis of AMPA receptor using [³H] AMPA binding against AMPA in the brain stem of control and experimental rats

Scatchard analysis of AMPA receptor using [³H] AMPA binding against AMPA in the brain stem of diabetic rats showed a significant increase in Bmax (p<0.001) compared to control rats. Treatment using curcumin (p<0.01) and vitamin D₃ (p<0.001) significantly reversed the changes in receptor binding when compared with diabetic group. There was no significant change in Kd in all experimental groups of rats. Vitamin D₃ (p<0.001) treatment showed prominent reversal in Bmax when compared with insulin treatment (Figure- 49, 50 & Table- 49, 50).
Real time PCR amplification of NMDA R1 receptor subunit mRNA from the brain stem of control and experimental rats

Gene expression of NMDA R1 receptor subunit mRNA showed significant up regulation (p<0.001) in the brain stem of diabetic rats compared to control. There was a significant reversal (p<0.001) in NMDA R1 receptor subunit gene expression in diabetic rats treated with insulin, curcumin, and vitamin D3 (Figure-51, Table-51).

Real time PCR amplification of NMDA 2B receptor subunit mRNA from the brain stem of control and experimental rats

Real-time PCR gene expression of NMDA 2B receptor subunit showed significant up regulation (p<0.001) in the brain stem of diabetic rats compared to control. Treatment using insulin, curcumin and vitamin D3 significantly reversed (p<0.001) the altered expression when compared with diabetic group. Curcumin and vitamin D3 treatment showed prominent reversal (p<0.001) in NMDA 2B gene expression when compared with insulin treatment (Figure-52, Table-52).

Real time PCR amplification of GluR4 subunit of AMPA receptor mRNA from the brain stem of control and experimental rats

Real-time PCR gene expression of GluR4 subunit of AMPA receptor subunit showed significant up regulation (p<0.001) in the brain stem of diabetic rats compared to control rats. There was a significant reversal (p<0.001) in AMPA GluR4 subunit gene expression in diabetic rats treated with curcumin and vitamin D3. Insulin treatment did not show any significant reversal when compared with diabetes. Curcumin and vitamin D3 treatment showed prominent reversal (p<0.001) in gene expression of GluR4 subunit of AMPA when compared with insulin treatment (Figure-53, Table-53).
Results

Real time PCR amplification of GluR2 subunit of AMPA receptor mRNA from the brain stem of control and experimental rats

Gene expression of AMPA GluR2 subunit receptor mRNA showed significant down regulation (p<0.001) in the brain stem of diabetic rats compared to control. Treatment using insulin, curcumin and vitamin D₃ significantly (p<0.001) reversed these changes when compared with diabetic group. Curcumin treatment showed prominent reversal (p<0.001) in gene expression of AMPA GluR2 subunit when compared with insulin treatment (Table-54, Figure-54).

Real time PCR amplification of GLAST mRNA from brain stem of control and experimental rats

Real-time PCR Gene expression of GLAST showed significant down regulation (p<0.001) in the brain stem of diabetic rats. Treatment using insulin, curcumin and vitamin D₃ significantly reversed (p<0.001) the altered expression when compared with diabetic group. Curcumin and vitamin D₃ treatment showed prominent reversal (p<0.001) in gene expression of GLAST when compared with insulin treatment (Figure-55, Table-55).

Real time PCR amplification of GAD mRNA from the brain stem of control and experimental rats

Real-time PCR Gene expression of GAD showed significant down regulation (p<0.001) in the brain stem of diabetic rats. There was a significant reversal (p<0.001) in GAD gene expression in diabetic rats treated with insulin, curcumin and vitamin D₃. Curcumin (p<0.001) treatment showed prominent reversal in GAD gene expression when compared with insulin treatment (Figure-56; Table-56).

IP3 content in brain stem of control and experimental rats

IP3 content was significantly increased (p<0.001) in the brain stem of diabetic rats when compared to control rats. Curcumin (p<0.001) and vitamin D₃ (p<0.001) treatment in diabetic rats significantly reversed the IP3 content when
compared with diabetic group. Insulin treated rats did not show any significant reversal when compared to diabetic rats. Curcumin and vitamin D₃ treatment showed significant reversal (p<0.05) in IP3 content when compared with insulin treatment (Figure-57; Table-57).

**Superoxide dismutase assay in the brain stem of control and experimental rats**

There was a significant decrease in SOD activity (p<0.001) in brain stem of diabetic rats. Treatment using insulin, curcumin and vitamin D₃ reversed (p<0.001) the activity of SOD enzyme when compared with diabetes. Curcumin (p<0.05) treatment showed prominent reversal in SOD activity when compared with insulin treatment (Figure-58; Table-58).

**Real time PCR amplification of GPx mRNA from the brain stem of control and experimental rats**

Real time PCR gene expression of GPx showed significant down regulation (p<0.001) in the brain stem of the diabetic rats compared to the control. Insulin, curcumin and vitamin D₃ treatment significantly reversed (p<0.001) the changes when compared with diabetes. Curcumin and vitamin D₃ treatment showed prominent reversal (p<0.001) in GPx gene expression when compared with insulin treatment (Figure-59; Table-59).

**Real time PCR amplification of Akt-1 mRNA from the brain stem of control and experimental rats**

Real-time PCR Gene expression of Akt-1 showed significant down regulation (p<0.001) in the brain stem of diabetic rats. Curcumin treatment showed significant down regulation when compared with diabetic group. In vitamin D₃ treated diabetic rats, there was prominent (p<0.001) reversal and up regulation of Akt-1 gene expression when compared to diabetic, control and insulin treated rats. Insulin treated rats did not show any significant reversal when compared to diabetic rats (Figure-60; Table-60).
**Results**

Real time PCR amplification of Bax mRNA from the brain stem of control and experimental rats

Real-time PCR Gene expression of Bax showed significant up regulation (p<0.001) in the brain stem of diabetic rats compared to control rats. There was a significant reversal (p<0.001) in Bax gene expression in diabetic rats treated with insulin, curcumin and vitamin D3. Curcumin and vitamin D3 treatment showed prominent reversal (p<0.001) in Bax gene expression when compared with insulin treatment (Figure-61; Table-61).

Real time PCR amplification of caspase 8 mRNA from the brain stem of control and experimental rats

Real-time PCR Gene expression of caspase 8 showed significant up regulation (p<0.001) in the brain stem of diabetic rats compared to control rats. Insulin, curcumin and vitamin D3 treatment significantly reversed (p<0.001) the changes when compared with diabetic. Curcumin and vitamin D3 treatment showed prominent reversal (p<0.001) in caspase 8 gene expression when compared with insulin treatment (Figure-62; Table-62).

NMAD R1 receptor subunit antibody staining in control and experimental groups of rats using confocal microscope

NMAD R1 subunit specific antibody staining in the brain stem showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin, curcumin and vitamin D3 treatment in diabetic rats significantly (p<0.001) reversed the mean pixel value to near control (Figure-63; Table-63).
NMDA 2B receptor subunit antibody staining in control and experimental groups of rats using confocal microscope

NMDA 2B subunit specific antibody staining in the brain stem showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin, curcumin and vitamin D₃ treatment in diabetic rats significantly reversed (p<0.001) the mean pixel value to near control (Figure-64; Table-64).

AMPA (GluR4) receptor subunit antibody staining in control and experimental groups of rats using confocal microscope

AMPA (GluR4) receptor subunit specific antibody staining in the brain stem showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin, curcumin and vitamin D₃ treatment in diabetic rats significantly (p<0.05) reversed the mean pixel value when compared with diabetic group (Figure-65; Table-65).

AMPA (GluR2) receptor subunit antibody staining in control and experimental groups of rats using confocal microscope

AMPA (GluR2) receptor subunit antibody staining in the brain stem showed a significant decrease (p<0.001) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin (p<0.05) and vitamin D₃ (p<0.01) treatment to diabetic rats significantly reversed AMPA (GluR4) receptor subunit expression in the brain stem to near control. Vitamin D₃ treatment showed prominent reversal when compared with insulin (Table-66, Figure-66).
**CEREBELLUM**

**Glutamate content in the cerebellum of control and experimental rats**

Glutamate content was significantly ($p<0.001$) increased in cerebellum of the diabetic rats compared to the control. There was significant reversal in glutamate content in Insulin ($p<0.01$) curcumin ($p<0.001$) and vitamin D$_3$ ($p<0.001$) treated rats. Curcumin and vitamin D$_3$ treatment showed prominent reversal ($p<0.001$) in glutamate content when compared with insulin treatment. (Figure -67, Table- 67).

**Scatchard analysis of NMDA receptor using $[^3]$H] MK-801 binding against MK-801 in the cerebellum of control and experimental rats**

Scatchard analysis of NMDA receptor using $[^3]$H] MK-801 binding against MK-801 in the cerebellum of diabetic rats showed a significant ($p<0.001$) increase in $B_{max}$ compared to control rats. This shows increased NMDA receptor density in the cerebellum of diabetic rats. Significant reversal in the $B_{max}$ was observed in treatment groups: curcumin ($p<0.001$) and vitamin D$_3$ ($p<0.001$). There was no significant change in $K_d$ in all experimental groups of rats. Curcumin and vitamin D$_3$ treatment showed prominent reversal ($p<0.01$) in $B_{max}$ when compared with insulin treatment (Figure- 68, 69 & Table- 68, 69).

**Scatchard analysis of AMPA receptor using $[^3]$H] AMPA binding against AMPA in the cerebellum of control and experimental rats**

Scatchard analysis of AMPA receptor using $[^3]$H] AMPA binding against AMPA in the cerebellum of diabetic rats showed a significant increase in $B_{max}$ ($p<0.001$) compared to control rats. This result showed increased AMPA receptor density in the cerebellum of diabetic rats compared to control. Treatment using curcumin and vitamin D$_3$ significantly reversed ($p<0.01$) the changes in receptor binding when compared with diabetic group. There was no significant change in $K_d$ in all experimental groups of rats. Treatment using vitamin D$_3$
showed prominent reversal (p<0.05) in $B_{max}$ when compared with insulin treatment (Figure- 70, 71 & Table- 70, 71).

**Real time PCR amplification of NMDA R1 receptor subunit mRNA from the cerebellum of control and experimental rats**

Gene expression of NMDA R1 receptor subunit mRNA showed significant up regulation (p<0.001) in the cerebellum of diabetic rats compared to control. Treatment using insulin, curcumin and vitamin D$_3$ significantly (p<0.001) reversed the altered expression when compared with diabetic group (Figure-72, Table-72). Curcumin and vitamin D$_3$ treatment showed prominent reversal (p<0.001) in NMDA R1 receptor subunit gene expression when compared with insulin treatment.

**Real time PCR amplification of NMDA 2B receptor subunit mRNA from the cerebellum of control and experimental rats**

Real-time PCR Gene expression of NMDA 2B receptor subunit showed significant up regulation (p<0.001) in the cerebellum of diabetic rats compared to control. Treatment using insulin and curcumin reversed (p<0.001) the altered expression when compared with diabetic group. Whereas vitamin D$_3$ treatment significantly (p<0.001) reversed the altered NMDA 2B receptor subunit expression to near control. Vitamin D$_3$ treatment showed prominent reversal (p<0.001) in NMDA 2B receptor subunit gene expression when compared with insulin treatment (Figure-73, Table-73).

**Real time PCR amplification of GluR4 subunit of AMPA receptor mRNA from the cerebellum of control and experimental rats**

Gene expression of AMPA GluR4 subunit receptor mRNA showed significant up regulation (p<0.001) in the cerebellum of diabetic rats compared to control. Treatment using curcumin and vitamin D$_3$ significantly (p<0.001) reversed these changes when compared with diabetic group. Insulin treated rats did not show any significant reversal when compared to diabetic rats. Curcumin
and vitamin D$_3$ treatment showed prominent reversal (p<0.001) in AMPA GluR4 receptor subunit gene expression when compared with insulin treatment (Figure-74, Table-74).

**Real time PCR amplification of GluR2 subunit of AMPA receptor mRNA from the cerebellum of control and experimental rats**

Gene expression of AMPA GluR2 subunit receptor mRNA showed significant down regulation (p<0.001) in the cerebellum of diabetic rats compared to control. Treatment using insulin (p<0.05) curcumin (p<0.001) and vitamin D$_3$ significantly (p<0.001) reversed these changes when compared with diabetic group. Treatment using curcumin and vitamin D$_3$ showed prominent reversal (p<0.001) in AMPA GluR2 receptor subunit gene expression when compared with insulin treatment (Figure-75; Table-75).

**Real time PCR amplification of GLAST mRNA from cerebellum of control and experimental rats**

Real-time PCR Gene expression of GLAST showed significant down regulation (p<0.001) in the cerebellum of diabetic rats. Treatment using insulin (p<0.001) reversed the altered expression when compared with diabetic group. Whereas vitamin D$_3$ and curcumin treatment significantly reversed (p<0.001) the altered GLAST gene expression to near control. Treatment using curcumin and vitamin D$_3$ showed prominent reversal (p<0.001) in GLAST gene expression when compared with insulin treatment (Figure-76; Table-76).

**Real time PCR amplification of GAD mRNA from the cerebellum of control and experimental rats**

Real-time PCR gene expression of GAD showed significant down regulation (p<0.001) in the cerebellum of diabetic rats. Insulin (p<0.01) and curcumin (p<0.001) treated diabetic rats showed a significant reversal when compared with diabetic rats. In vitamin D$_3$ treated rats GAD gene expression was significantly reversed (p<0.001) to near control. Curcumin and vitamin D$_3$
treatment showed prominent reversal (p<0.001) in GAD gene expression when compared with insulin treatment (Figure-77; Table-77).

**IP3 content in cerebellum of control and experimental rats**

IP3 content was significantly increased (p<0.001) in the cerebellum of diabetic rats when compared to control rats. Insulin treatment in diabetic rats showed a significant reversal (p<0.05) when compared with diabetic rats. In curcumin and vitamin D₃ treated rats IP3 content was significantly reversed (p<0.001) to near control. Treatment using curcumin and vitamin D₃ showed prominent reversal (p<0.001) in IP3 content when compared with insulin treatment (Figure-78; Table-78).

**Superoxide dismutase assay in the cerebellum of control and experimental rats**

There was a significant decrease (p<0.001) in SOD activity in cerebellum of diabetic rats. Treatment using curcumin (p<0.001) reversed the activity of SOD enzyme to near control. Insulin and vitamin D₃ did not show any significant reversal when compared to diabetic rats. (Figure-79; Table-79).

**Real time PCR amplification of GPx mRNA from the cerebellum of control and experimental rats**

Real time PCR gene expression of GPx showed significant down regulation (p<0.001) in the cerebellum of the diabetic rats compared to the control. Treatment using insulin and vitamin D₃ significantly reversed (p<0.001) the changes when compared with diabetes. In curcumin treated diabetic rats, there was significant (p<0.001) reversal and up regulation of GPx gene expression when compared to diabetic and control rats respectively. Treatment using curcumin and vitamin D₃ showed prominent reversal (p<0.001) in GPx gene expression when compared with insulin treatment (Figure-80; Table-80).
Results

Real time PCR amplification of Akt-1 mRNA from the cerebellum of control and experimental rats

Real-time PCR gene expression of Akt-1 showed significant down regulation (p<0.001) in the cerebellum of diabetic rats. Treatment using curcumin showed significant reversal when compared with control. Treatment using vitamin D₃ significantly reversed (p<0.001) and up regulated the altered gene expression when compared with both diabetic and control. Treatment using vitamin D₃ showed prominent reversal (p<0.001) in Akt-1 gene expression when compared with insulin treatment (Figure-81; Table-81).

Real time PCR amplification of Bax mRNA from the cerebellum of control and experimental rats

Real-time PCR Gene expression of Bax showed significant up regulation (p<0.001) in the cerebellum of diabetic rats compared to control rats. The Bax gene expression was reversed significantly in insulin (p<0.001) treated rats when compared with diabetic rats. In curcumin and vitamin D₃ treated rats Bax Gene expression was significantly reversed (p<0.001) to near control. Vitamin D₃ treatment showed prominent reversal (p<0.01) in caspase 8 gene expression when compared with insulin treatment (Figure-82; Table-82).

Real time PCR amplification of caspase 8 mRNA from the cerebellum of control and experimental rats

Real-time PCR gene expression of caspase 8 showed significant up regulation (p<0.001) in the cerebellum of diabetic rats compared to control rats. There was a significant reversal (p<0.001) in Caspase 8 gene expression in diabetic rats treated with insulin, curcumin and vitamin D₃. Treatment using curcumin and vitamin D₃ showed prominent reversal (p<0.001) in caspase 8 gene expression when compared with insulin treatment (Figure-83; Table-83).
**NMDA R1 receptor subunit antibody staining in control and experimental groups of rats using confocal microscope**

NMDA R1 subunit specific antibody staining in the cerebellum showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin, curcumin and vitamin D_3_ treatment in diabetic rats significantly reversed (p<0.001) the mean pixel value to near control (Figure-84; Table-84).

**NMDA 2B receptor subunit antibody staining in control and experimental groups of rats using confocal microscope**

NMDA 2B subunit specific antibody staining in the cerebellum showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin, curcumin and vitamin D_3_ treatment in diabetic rats significantly (p<0.001) reversed the mean pixel value to near control (Figure-85; Table-85).

**AMPA (GluR4) receptor subunit antibody staining in control and experimental groups of rats using confocal microscope**

AMPA (GluR4) receptor subunit specific antibody staining in the cerebellum showed a significant increase (p<0.001) in mean pixel value in the diabetic rats when compared to control. Insulin, curcumin and vitamin D_3_ treatment in diabetic rats significantly (p<0.001) reversed the mean pixel value when compared with diabetic (Figure-86; Table-86).

**AMPA (GluR2) receptor subunit antibody staining in control and experimental groups of rats using confocal microscope**

AMPA (GluR2) receptor subunit antibody staining in the cerebellum showed a significant decrease (p<0.001) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and vitamin D_3_ treatment to diabetic rats significantly reversed (p<0.01) AMPA (GluR2) receptor subunit expression in the cerebellum to near control (Table-87, Figure-87).
Results

PANCREAS

Glutamate content in the pancreas of control and experimental rats

Glutamate content was significantly (p<0.001) increased in pancreas of the Diabetic rats compared to the control. There was significant reversal (p<0.001) in glutamate content in curcumin and vitamin D₃ Treated rats. Insulin treated rats did not show any significant reversal when compared to diabetic rats (Figure-88, Table-88)

Scatchard analysis of NMDA receptor using [³H] MK-801 binding against MK-801 in the pancreas of control and experimental rats

Scatchard analysis of NMDA receptor using [³H] MK-801 binding against MK-801 in the pancreas of diabetic and treatment groups did not show any significant change when compared with control. There was no significant change in K_d in all experimental groups of rats (Figure-89, 90 & Table-89, 90).

Scatchard analysis of AMPA receptor using [³H] AMPA binding against AMPA in the pancreas of control and experimental rats

Scatchard analysis of AMPA receptor using [³H] AMPA binding against AMPA in the pancreas of diabetic rats showed a significant increase in B_max (p<0.01) compared to control rats. This result showed increased AMPA receptor density in the pancreas of diabetic rats compared to control. Treatment using insulin (p<0.05), curcumin (p<0.05) and vitamin D₃ (p<0.01) significantly reversed the changes in receptor binding when compared with diabetic group. There was no significant change in K_d in all experimental groups of rats (Figure-91, 92 & Table-91, 92).

Real time PCR amplification of GluR4 subunit of AMPA receptor mRNA from the pancreas of control and experimental rats

Gene expression of AMPA GluR4 subunit receptor mRNA showed significant down regulation (p<0.001) in the pancreas of diabetic rats compared to
control. Treatment using insulin (p<0.01) curcumin and vitamin D3 significantly (p<0.001) reversed these changes when compared with diabetic group. Curcumin and vitamin D3 treatment showed prominent reversal (p<0.001) in AMPA GluR4 subunit receptor gene expression when compared with insulin treatment (Figure-93, Table-93).

Real time PCR amplification of GluR2 subunit of AMPA receptor mRNA from the pancreas of control and experimental rats
Gene expression of AMPA GluR2 subunit receptor mRNA showed significant up regulation (p<0.001) in the pancreas of diabetic rats compared to control. Treatment using insulin, curcumin and vitamin D3 significantly reversed (p<0.001) these changes when compared with diabetic group. Treatment using vitamin D3 showed prominent reversal (p<0.001) in AMPA GluR2 subunit receptor gene expression when compared with insulin treatment (Figure-94; Table-94).

Real time PCR amplification of GLAST mRNA from Pancreas of control and experimental rats
Real-time PCR Gene expression of GLAST showed significant down regulation (p<0.001) in the Pancreas of diabetic rats. Whereas vitamin D3 and curcumin (p<0.001) treatment significantly (p<0.001) reversed the altered GLAST gene expression when compared to diabetic rats. Insulin treatment did not show any significant reversal when compared to diabetic rats (Figure-95; Table-95).

IP3 content in the pancreas of control and experimental rats
IP3 content was significantly decreased (p<0.01) in the Pancreas of diabetic rats when compared to control rats. In curcumin (p<0.01) and vitamin D3 (p<0.01) treated diabetic rats IP3 content was significantly reversed when compared with diabetic group. Insulin treatment did not show any significant reversal when compared to diabetic rats (Figure-96; Table-96).
Superoxide dismutase assay in the Pancreas of control and experimental rats

There was a significant decrease in SOD activity (p<0.001) in pancreas of diabetic rats. Vitamin D₃ treatment showed a significant reversal (p<0.001) when compared with diabetic group. Treatment using curcumin (p<0.001) reversed the activity of SOD enzyme to near control. Insulin treatment did not show any significant reversal when compared to diabetic rats (Figure-97; Table-97).

Real time PCR amplification of GPx mRNA from the Pancreas of control and experimental rats

Real time PCR gene expression of GPx showed significant down regulation (p<0.001) in the Pancreas of the diabetic rats compared to the control. Treatment using vitamin D₃ (p<0.001) significantly reversed the changes when compared with diabetes. In curcumin treated diabetic rats, there was significant reversal (p<0.001) and up regulation of GPx gene expression when compared to diabetic and control rats respectively. Insulin treatment did not show any significant reversal when compared to diabetic rats. (Figure-98; Table-98).

Real time PCR amplification of Bax mRNA from the Pancreas of control and experimental rats

Real-time PCR Gene expression of Bax showed significant up regulation (p<0.001) in the Pancreas of diabetic rats compared to control rats. The Bax gene expression was reversed significantly (p<0.001) in insulin, curcumin and vitamin D₃ treated rats when compared with diabetic rats. Curcumin and vitamin D₃ treatment showed prominent reversal (p<0.001) in Bax gene expression when compared with insulin treatment (Figure-99; Table-99).

Real time PCR amplification of caspase 8 mRNA from the Pancreas of control and experimental rats

Real-time PCR Gene expression of caspase 8 showed significant up regulation (p<0.001) in the Pancreas of diabetic rats compared to control rats. There was a significant reversal (p<0.001) in Caspase 8 gene expression in
diabetic rats treated with insulin, curcumin and vitamin D₃. Treatment using curcumin and vitamin D₃ showed prominent reversal (p<0.001) in caspase 8 gene expression when compared with insulin treatment (Figure-100; Table-100).

**Real time PCR analysis of NeuroD-1 gene expression in Pancreas of control and experimental rats**

Real time PCR gene expression of NeuroD-1 showed significant down regulation (p<0.001) in the Pancreas of the diabetic rats compared to the control. In curcumin and vitamin D₃ treated diabetic rats, there was significant reversal (p<0.001) and up regulation of NeuroD-1 gene expression when compared to diabetic and control rats respectively. Insulin treated diabetic rats did not show any significant change when compared to diabetic rats. Curcumin and vitamin D₃ showed prominent reversal (p<0.001) in NeuroD-1 gene expression when compared with insulin treatment (Figure-101; Table-101).

**Real time PCR analysis of Pdx1 gene expression in Pancreas of control and experimental rats**

Real time PCR gene expression of Pdx1 showed significant down regulation (p<0.001) in the Pancreas of the diabetic rats compared to the control. Treatment using insulin (p<0.001) significantly reversed the changes to near control. In curcumin and vitamin D₃ (p<0.001) treated diabetic rats, there was significant reversal and up regulation of Pdx1 gene expression when compared to diabetic and control rats respectively. Curcumin and vitamin D₃ showed prominent reversal (p<0.001) in Pdx1 gene expression when compared with insulin treatment (Figure-102; Table-102).

**Co-labeling studies using Insulin and AMPA (GluR2) receptor subunit specific antibody in the pancreatic islets of control and experimental groups of rats using confocal microscope**

Insulin positive cells of pancreatic islets showed a significant increase (p<0.001) in the mean pixel value of AMPA (GluR2) receptor subunit antibody
staining in diabetic rats when compared to control. Insulin (p<0.05), curcumin (p<0.01) and vitamin D₃ (p<0.001) treatment to diabetic rats significantly reversed AMPA (GluR2) receptor subunit expression in the insulin positive pancreatic islets to near control (Figure-103, Table-103).

Co-labeling studies using Insulin and AMPA (GluR4) receptor subunit specific antibody in the pancreatic islets of control and experimental groups of rats using confocal microscope

Insulin positive cells of pancreatic islets in diabetic rats showed a significant decrease (p<0.001) in the mean pixel value of AMPA (GluR4) receptor subunit antibody staining when compared to control. Insulin (p<0.01), curcumin and vitamin D₃ treatment to diabetic rats significantly reversed (p<0.001) AMPA (GluR4) receptor subunit expression in the insulin positive pancreatic islets to near control (Table-104, Figure-104).

IP3R3 expression in the pancreatic islets of control and experimental groups of rats using confocal microscope

IP3R3 receptor antibody staining in the pancreatic islets showed a significant increase (p<0.001) in the mean pixel value in diabetic rats compared to control. Curcumin and vitamin D₃ treatment to diabetic rats significantly reversed (p<0.001) IP3R3 expression in the pancreatic islets to near control. Insulin treatment did not show any significant change when compared with diabetic group (Table-105, Figure-105).

Vitamin D receptor expression in the pancreatic islets of control and experimental groups of rats using confocal microscope

Vitamin D receptor antibody staining in the pancreatic islets showed a significant decrease (p<0.001) in the mean pixel value in diabetic rats compared to control. Vitamin D₃ (p<0.001) treatment in diabetic rats significantly reversed the mean pixel value when compared with diabetic rat. Insulin and curcumin
treatment did not show any significant change when compared with diabetic rat (Table-106, Figure-106).

**Calcium release from pancreatic islets using Fluo-4 AM**

The Fluo-4 AM staining showed a significant ($p<0.001$) decrease in mean pixel value indicating reduced calcium release from the pancreatic islets in diabetic rats compared to control. Insulin ($p<0.05$), curcumin ($p<0.05$) and vitamin D$_3$ ($p<0.001$) treatment in diabetic rats significantly reversed the mean pixel value when compared with diabetic Vitamin D$_3$ treatment showed more prominent reversal in calcium release when compared to other treatment group (Figure-107; Table-107).