Results

BODY WEIGHT
The body weight was significantly decreased (p<0.001) in the diabetic rats when compared to control group. After insulin treatment, curcumin and Vitamin D₃ supplementation for 14 days, the body weight reversed to near the initial body weight (Table-1, Figure-1).

BLOOD GLUCOSE LEVEL
Blood glucose level of all rats before streptozotocin administration was within the normal range. Streptozotocin administration led to a significant increase (p<0.001) in blood glucose level of diabetic group when compared to control group. Insulin curcumin and Vitamin D₃ treatments were able to significantly reverse (p<0.001) the increased blood glucose level to near the control level when compared to diabetic group (Table-2, Figure-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 level to near control level when compared to diabetic group (Table-3, Figure-3).

CIRCULATING TRIIODOTHYRONINE (T3) CONTENT LEVEL
There was a significant decrease in the serum T3 level of the diabetic group when compared to control group (p<0.001). Insulin curcumin and Vitamin D₃...
treatment for 14 days significantly increased (p<0.001) the serum T3 level to near control level when compared to diabetic group (Table-4, Figure-4).

BEHAVIOURAL STUDIES

Behavioural response of control and experimental rats on Y-Maze performance

Number of visits and time spent in the novel arm decreased significantly (p<0.001) in the diabetic group compared to control. Lower percentage of arm visits between the novel arm and the start arm and decreased time spent in the novel arm compared to the other two arms within the diabetic rats showed their decreased exploratory behaviour. Time spent in the novel arm and number of visit to the novel arm reversed to near control in the diabetic rats treated with insulin, curcumin and Vitamin D$_3$ (Table-5, Figure-5).

Rotarod performance of control and experimental groups of rats

Rotarod experiment showed a significant decrease in the retention time on the rotating rod in the diabetic rats at 10 (p<0.01), 15 (p<0.001) and 25 (p<0.001) revolutions per minute (rpm) when compared to control. Insulin, curcumin and Vitamin D$_3$ treatment to diabetic rats significantly reversed the retention time near to control at 10 (p<0.001), 15 (p<0.001) and 25 (p<0.001) rpm (Table -6, Figure-6).

Behavioural response of control and experimental rats on grid walk test

There was significant increase (p<0.001) in the foot falls in diabetic rats compared to control. Foot falls significantly reversed to near control in diabetic rats administered with insulin (p<0.001), curcumin (p<0.001) and Vitamin D$_3$ (p<0.001) (Table-7, Figure-7).
Results

Behavioural response of control and experimental rats on narrow beam test

There was significant decrease in the retention of balance on the narrow beam (p<0.001) in diabetic rats compared to control. Balance on the narrow beam significantly reversed to near control in diabetic rats treated with insulin (p<0.001), curcumin (p<0.001) and Vitamin D₃ (p<0.001) (Table-8, Figure-8).

NEUROTRANSMITTERS, VITAMIN D, INSULIN RECEPTORS, GLUT3, PHOSPHOLIPASE C, CREB AND SUPEROXIDE DISMUTASE EXPRESSION IN THE BRAIN REGIONS AND PANCREAS OF EXPERIMENTAL RATS

CEREBRAL CORTEX

Total muscarinic receptor analysis

Scatchard analysis of [³H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebral cortex of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, [³H] QNB and muscarinic general antagonist atropine. The Scatchard analysis showed that the $B_{\text{max}}$ (p<0.001) and $K_{\text{d}}$ (p<0.05) decreased significantly in diabetic rats compared to control group. In insulin, curcumin and Vitamin D₃ treated diabetic rats, $B_{\text{max}}$ (p<0.001) and $K_{\text{d}}$ (p<0.01) significantly reversed to near control value when compared to diabetic group (Table-9, 10 & Fig-9, 10).
**Muscarinic M1 receptor analysis**


Binding analysis of muscarinic M1 receptor was done using $[^3]$H QNB and M1 subtype specific antagonist pirenzepine. The $B_{\text{max}}$ and $K_d$ decreased significantly ($p<0.001$) in diabetic group when compared to control group. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats, $B_{\text{max}}$ ($p<0.001$) and $K_d$ ($p<0.001$) significantly reversed to near control value when compared to diabetic group (Table-11, 12 & Fig-11, 12).

**Muscarinic M3 receptor analysis**


Binding analysis of muscarinic M3 receptors was done using $[^3]$H DAMP and M3 subtype specific antagonist 4-DAMP mustard. The $B_{\text{max}}$ and $K_d$ was increased significantly ($p<0.001$) in diabetic group when compared to control. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats, $B_{\text{max}}$ ($p<0.001$) and $K_d$ ($p<0.001$) significantly reversed to near control value when compared to diabetic group (Table-13, 14 & Fig-13, 14).
Results

Dopamine receptor analysis


Binding analysis of dopamine receptors was done using $[^3]H$ dopamine and unlabelled dopamine. The $B_{max}$ and $K_d$ was increased significantly ($p<0.001$) in diabetic group when compared to control. The $K_d$ also increased significantly when compared to control group ($p<0.001$). In insulin, curcumin and Vitamin D$_3$ treated diabetic rats, $B_{max}$ ($p<0.001$) and $K_d$ ($p<0.001$) significantly reversed to near control value when compared to diabetic group (Table-15, 16 & Fig-15, 16).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation ($p<0.001$) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D$_3$ significantly ($p<0.001$) reversed the altered expression to near control (Table-17, Figure-17).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D$_3$ significantly ($p<0.001$) reversed these changes to near control (Table-18, Figure-18).
Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression was decreased significantly (p<0.001) in diabetic rats and it was reversed significantly (p<0.001) to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-19, Figure-19).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression was increased significantly (p<0.001) in diabetic rats and it was reversed significantly (p<0.001) to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-20, Figure-20).

Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression was increased significantly (p<0.001) in diabetic rats and it was reversed significantly to near control in curcumin and Vitamin D₃ treated diabetic rats. Insulin treatment did not show any significant change in α7 nicotinic acetylcholine receptor gene expression when compared to diabetes (Table-21, Figure-21).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression was increased significantly (p<0.001) in diabetic rats and it was reversed
significantly (p<0.001) to near control in insulin, curcumin and Vitamin D$_3$ treated diabetic rats (Table-22, Figure-22).

**Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats**

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression was increased significantly (p<0.001) in diabetic rats and it was reversed significantly (p<0.001) to near control in insulin, curcumin and Vitamin D$_3$ treated diabetic rats (Table-23, Figure-23).

**Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats**

Real Time-PCR analysis showed that the Vitamin D receptor gene expression was decreased significantly (p<0.001) in diabetic rats and it was reversed significantly (p<0.001) to near control in insulin and Vitamin D$_3$ treated diabetic rats. Curcumin treatment did not show any significant change in Vitamin D receptor gene expression when compared to diabetes (Table-24, Figure-24).

**Real Time-PCR analysis of insulin receptor in the control and experimental rats**

Real Time-PCR analysis showed that the insulin receptor gene expression was decreased significantly (p<0.001) in diabetic rats and it was reversed (p<0.001) significantly to near control in insulin, curcumin and Vitamin D$_3$ treated diabetic rats (Table-25, Figure-25).
Real Time-PCR analysis of GLUT3 in the control and experimental rats

Gene expression of GLUT3 mRNA showed significant down regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed these changes to near control (Table-26, Figure-26).

Real Time-PCR analysis of phospholipase C in the control and experimental rats

Gene expression of phospholipase C mRNA showed significant down regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed these changes to near control (Table-27, Figure-27).

Real Time-PCR analysis of CREB in the control and experimental rats

Gene expression of CREB mRNA showed significant down regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control. Treatment using curcumin (p<0.001) and Vitamin D$_3$ (p<0.001) significantly reversed these changes to near control. Insulin treatment did not show any significant change in CREB mRNA expression when compared to diabetes (Table-28, Figure-28).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant down regulation (p<0.001) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed these changes to near control (Table-29, Figure-29).
CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the cerebral cortex of control and experimental rats

Muscarinic M1 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, curcumin and Vitamin D₃ treatment to diabetic rats significantly reversed (p<0.001) the muscarinic M1 receptor expression in the cerebral cortex to near control (Table-30, Figure-30).

Muscarinic M3 receptor antibody staining in the cerebral cortex of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the cerebral cortex showed a significant increase (p<0.001) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D₃ treatment to diabetic rats significantly reversed (p<0.001) the muscarinic M3 receptor expression in the cerebral cortex to near control (Table-31, Figure-31).

α7 nicotinic acetylcholine receptor antibody staining in the cerebral cortex of control and experimental rats

α7 nicotinic acetylcholine receptor subunit antibody staining in the cerebral cortex showed a significant increase (p<0.001) in the mean pixel value in diabetic rats compared to control. Curcumin and Vitamin D₃ treated diabetic rats significantly reversed (p<0.001) the muscarinic M3 receptor expression in the cerebral cortex to near control. Insulin treatment did not show any significant reversal when compared to diabetic rats (Table-32, Figure-32).
CEREBELLUM

Total muscarinic receptor analysis

Scatchard analysis of $[^3]$H QNB binding against total muscarinic receptor antagonist, atropine in the cerebellum of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, $[^3]$H QNB and muscarinic general antagonist atropine. The Scatchard analysis showed that the $B_{\text{max}}$ ($p<0.001$) and $K_d$ ($p<0.01$) increased significantly in diabetic rats compared to control group. In insulin treated diabetic rats $B_{\text{max}}$ and $K_d$ significantly ($p<0.001$) reversed to near control when compared to diabetic group. Curcumin and Vitamin D$_3$ treatment significantly reversed the $B_{\text{max}}$ ($p<0.01$) to near control value without any change in $K_d$ when compared to diabetic group (Table-33, 34 & Fig-33, 34).

Muscarinic M1 receptor analysis

Scatchard analysis of $[^3]$H QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebellum of control and experimental rats

Binding analysis of muscarinic M1 receptors was done using $[^3]$H QNB and M1 subtype specific antagonist pirenzepine. The $B_{\text{max}}$ and $K_d$ increased significantly ($p<0.001$) in diabetic group when compared to control group. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats $B_{\text{max}}$ ($p<0.001$) and $K_d$ ($p<0.01$) significantly reversed to near control value when compared to diabetic group (Table-35, 36 & Fig-35, 36).
Results

Muscarinic M3 receptor analysis

**Scatchard analysis of [³H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebellum of control and experimental rats.**

Binding analysis of muscarinic M3 receptors was done using [³H] DAMP and M3 subtype specific antagonist 4-DAMP mustard. The $B_{\text{max}}$ and $K_d$ was increased significantly ($p<0.001$) in diabetic group when compared to control group. In insulin and curcumin treated diabetic rats, $B_{\text{max}}$ ($p<0.001$) and $K_d$ ($p<0.01$) significantly reversed to near control when compared to diabetic group. Vitamin D$_3$ treatment significantly reverse the $B_{\text{max}}$ ($p<0.001$) and $K_d$ ($p<0.001$) to near control value when compared to diabetic group (Table-38 & Fig-38).

Dopamine receptor analysis

**Scatchard analysis of [³H] dopamine binding against dopamine in the cerebellum of control and experimental rats.**

Binding analysis of total dopamine receptors was done using [³H] dopamine and unlabelled dopamine. The $B_{\text{max}}$ ($p<0.001$) and $K_d$ ($p<0.01$) decreased significantly in diabetic group when compared to control. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats $B_{\text{max}}$ ($p<0.001$) and $K_d$ ($p<0.01$) significantly reversed to near control value when compared to diabetic group (Table-39, 40 & Fig-39, 40).
REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation (p<0.001) in the cerebellum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed the altered expression to near control (Table-41, Figure-41).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation (p<0.001) in the cerebellum of diabetic rats compared to control. Curcumin and Vitamin D₃ (p<0.001), insulin (p<0.01) treatment significantly reversed the changes to near control (Table-42, Figure-42).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression increased significantly (p<0.001) in diabetic rats and it reversed significantly (p<0.001) to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-43, Figure-43).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression was increased significantly (p<0.001) in diabetic condition and it reversed
significantly (p<0.001) to near control in insulin, curcumin and Vitamin D$_3$ treated diabetic rats (Table-44, Figure-44).

**Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats**

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression increased significantly (p<0.001) in diabetic rats and it reversed to near control value in curcumin and Vitamin D$_3$ (p<0.001) treated diabetic rats. Insulin treatment did not show any significant change in α7 nicotinic acetylcholine receptor gene expression when compared to diabetes (Table-45, Figure-45).

**Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats**

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression increased significantly (p<0.001) in diabetic rats and it reversed significantly (p<0.001) to near control in insulin, curcumin and Vitamin D$_3$ treated diabetic rats (Table-46, Figure-46).

**Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats**

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression increased significantly (p<0.001) in diabetic rats and it reversed significantly (p<0.001) to near control in insulin, curcumin and Vitamin D$_3$ treated diabetic rats (Table-47, Figure-47).
**Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats**

Real Time-PCR analysis showed that the Vitamin D receptor gene expression significantly (p<0.001) increased in diabetic condition and insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed to near control (Table-48, Figure-48).

**Real Time-PCR analysis of insulin receptor in the control and experimental rats**

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly (p<0.001) in diabetic rats and treatment using insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed to near control. (Table-49, Figure-49).

**Real Time-PCR analysis of GLUT3 in the control and experimental rats**

Gene expression of GLUT3 mRNA showed significant down 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 changes to near control (Table-50, Figure-50).

**Real Time-PCR analysis of phospholipase C in the control and experimental rats**

Gene expression of phospholipase C mRNA showed significant down 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 the changes to near control. Insulin treatment did not show any significant change when compared to diabetic (Table-51, Figure-51).
Results

Real Time-PCR analysis of CREB in the control and experimental rats

Gene expression of CREB mRNA showed significant down regulation (p<0.001) in the cerebellum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.01) reversed the changes to near control (Table-52, Figure-52).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant down regulation (p<0.001) in the cerebellum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed these changes to near control (Table-53, Figure-53).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the cerebellum of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the cerebellum showed a significant increase (p<0.001) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D₃ treated diabetic rats significantly (p<0.001) reversed the muscarinic M1 receptor expression in the cerebellum to near control level (Table-54, Figure-54).
Muscarinic M3 receptor antibody staining in the cerebellum of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the cerebellum showed a significant increase (p<0.001) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D\textsubscript{3} treated diabetic rats significantly (p<0.001) reversed the muscarinic M3 receptor expression in the cerebellum to near control level (Table-55, Figure-55).

\textit{α7} nicotinic acetylcholine receptor antibody staining in the cerebellum of control and experimental rats

\textit{α7} nicotinic acetylcholine receptor subunit antibody staining in the cerebellum showed a significant increase (p<0.001) in the mean pixel value of diabetic rats compared to control. Curcumin and Vitamin D\textsubscript{3} treated diabetic rats significantly reversed (p<0.001) the muscarinic M3 receptor expression in the cerebellum to near control level. Insulin treatment did not show any significant reversal when compared to diabetic (Table-56, Figure-56).

BRAIN STEM

Total muscarinic receptor analysis

Scatchard analysis of [\textsuperscript{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the brain stem of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, [\textsuperscript{3}H] QNB and muscarinic general antagonist, atropine. The Scatchard analysis showed that the B\textsubscript{max} increased significantly (p<0.001) in diabetic rats with out any significant change in the K\textsubscript{d} when compared to control. Treatment with insulin,
Results

curcumin and Vitamin D$_3$ significantly (p<0.001) reversed the $B_{\text{max}}$ to near control when compared to diabetic group. $K_d$ did not show any significant change when compared to diabetic (Table-57, 58 & Figure- 57, 58).

Muscarinic M1 receptor analysis

Scatchard analysis of [$^3$H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the brain stem of control and experimental rats.

Binding analysis of muscarinic M1 receptors was done using [$^3$H] QNB and M1 subtype specific antagonist pirenzepine. The $B_{\text{max}}$ and $K_d$ decreased significantly (p<0.001) in diabetic rats when compared to control. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats $B_{\text{max}}$ (p<0.001) and $K_d$ (p<0.01) significantly reversed to near control value when compared to diabetic (Table-59, 60 & Fig-59, 60).

Muscarinic M3 receptor analysis

Scatchard analysis of [$^3$H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the brain stem of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using [$^3$H] DAMP and M3 subtype specific antagonist 4-DAMP mustard. The $B_{\text{max}}$ (p<0.001) and $K_d$ (p<0.01) increased significantly in diabetic group when compared to control group. $B_{\text{max}}$ of insulin, Vitamin D$_3$ (p<0.01) and curcumin (p<0.001) treated diabetic rats significantly reversed to near control when compared to diabetic. In insulin, curcumin (p<0.05) and Vitamin D$_3$ (p<0.001), $K_d$ significantly reversed to near control when compared to diabetic (Table-61, 62 & Fig-61, 62).
Dopamine receptor analysis

Scatchard analysis of \( ^{3} \text{H} \) dopamine binding against dopamine in the brain stem of control and experimental rats.

Binding analysis of total dopamine receptors was done using \( ^{3} \text{H} \) dopamine and unlabelled dopamine. The \( B_{\text{max}} \) and \( K_{d} \) increased significantly (p<0.001) in diabetic group when compared to control group. In insulin, curcumin and Vitamin D\(_{3}\) treated diabetic rats, \( B_{\text{max}} \) and \( K_{d} \) significantly (p<0.001) reversed to near control when compared to diabetic group (Table-63, 64 & Fig-63, 64).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation (p<0.001) in the brain stem of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D\(_{3}\) significantly (p<0.001) reversed the expression to near control (Table-65, Figure-65).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant up regulation (p<0.001) in the brain stem of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D\(_{3}\) significantly (p<0.001) reversed the expression to near control (Table-66, Figure-66).
Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression was decreased significantly (p<0.001) in diabetic rats and it was significantly (p<0.001) reversed to near control in insulin, curcumin and Vitamin D$_3$ (p<0.001) treated diabetic rats (Table-67, Figure-67).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression increased significantly (p<0.001) in diabetic rats and it was significantly (p<0.001) reversed to near control in insulin, curcumin and Vitamin D$_3$ (p<0.001) treated diabetic rats (Table-68, Figure-68).

Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression increased significantly (p<0.001) in diabetic rats and it was significantly reversed (p<0.001) to near control in curcumin and Vitamin D$_3$ treated diabetic rats. Insulin treatment did not show any significant change in α7 nicotinic acetylcholine receptor gene expression when compared to diabetes (Table-69, Figure-69).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression increased significantly (p<0.001) in diabetic rats and it was significantly
(p<0.001) reversed to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-70, Figure-70).

**Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats**

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression decreased significantly (p<0.001) in diabetic rats and it was significantly (p<0.001) reversed to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-71, Figure-72).

**Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats**

Real Time-PCR analysis showed that the Vitamin D receptor gene expression increased significantly (p<0.001) in diabetic rats and insulin (p<0.01), curcumin and Vitamin D₃ (p<0.001) treated diabetic rats, it was reversed significantly to near control (Table-72, Figure-72).

**Real Time-PCR analysis of insulin receptor in the control and experimental rats**

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly (p<0.001) in diabetic condition and it was significantly (p<0.001) reversed to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-73, Figure-73).

**Real Time-PCR analysis of GLUT3 in the control and experimental rats**

Gene expression of GLUT3 mRNA showed significant up regulation (p<0.001) in the brain stem of diabetic rats compared to control. Treatment using
Results

insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed these changes to near control (Table-74, Figure-74).

**Real Time-PCR analysis of phospholipase C in the control and experimental rats**

Gene expression of phospholipase C mRNA showed significant down regulation (p<0.001) in the brain stem of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed these changes to near control (Table-75, Figure-75).

**Real Time-PCR analysis of CREB in the control and experimental rats**

Gene expression of CREB mRNA showed significant down regulation (p<0.001) in the brain stem of diabetic rats compared to control. Treatment using curcumin and Vitamin D$_3$ significantly (p<0.001) reversed these changes to near control. Insulin treatment did not show any significant change in CREB mRNA expression when compared to diabetes (Table-76, Figure-76).

**Real Time-PCR analysis of superoxide dismutase in the control and experimental rats**

Gene expression of superoxide dismutase mRNA showed significant up regulation (p<0.001) in the brain stem of diabetic rats compared to control. Treatment using insulin (p<0.01), curcumin and Vitamin D$_3$ (p<0.001) significantly reversed these changes to near control when compared to diabetic. (Table-77, Figure-77).
CONFOCAL STUDIES

**Muscarinic M1 receptor antibody staining in the brain stem of control and experimental rats**

Muscarinic M1 receptor subunit antibody staining in the brainstem showed a significant decrease (p<0.001) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D₃ treatment to diabetic rats significantly reversed (p<0.001) the muscarinic M1 receptor expression in the brain stem to near control (Table-78, Figure-78).

**Muscarinic M3 receptor antibody staining in the brain stem of control and experimental rats**

Muscarinic M3 receptor subunit antibody staining in the brainstem showed a significant increase (p<0.001) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D₃ treatment to diabetic rats significantly reversed (p<0.001) the muscarinic M3 receptor expression in the brainstem to near control (Table-79, Figure-79).

**α7 nicotinic acetylcholine receptor antibody staining in the brain stem of control and experimental rats**

α7 nicotinic acetylcholine receptor antibody staining in the brainstem showed a significant increase (p<0.001) in the mean pixel value in diabetic rats compared to control. Curcumin and Vitamin D₃ treated diabetic rats significantly (p<0.001) reversed the muscarinic M3 receptor expression in the brain stem to near control level. Insulin treatment did not show any significant reversal when compared to diabetic (Table-80, Figure-80).
CORPUS STRIATUM

Total muscarinic receptor analysis

Scatchard analysis of \(^3\text{H}\) QNB binding against total muscarinic receptor antagonist, atropine in the corpus striatum of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, \(^3\text{H}\) QNB and muscarinic general antagonist, atropine. The Scatchard analysis showed that the \(B_{\text{max}}\) decreased significantly \((p<0.001)\) in diabetic rats with out any significant change in the \(K_d\) when compared to control group. Treatment with insulin, curcumin and Vitamin D\(_3\) significantly \((p<0.001)\) reversed the \(B_{\text{max}}\) to near control when compared to diabetic group. \(K_d\) did not show any significant change when compared to diabetic (Table-81, 82 & Figure- 81, 82).

Muscarinic M1 receptor analysis

Scatchard analysis of \(^3\text{H}\) QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the corpus striatum of control and experimental rats

Binding analysis of muscarinic M1 receptors was done using \(^3\text{H}\) QNB and M1 subtype specific antagonist pirenzepine. The \(B_{\text{max}}\) increased and \(K_d\) decreased significantly \((p<0.001)\) in diabetic group when compared to control group. In insulin \((p<0.001)\), curcumin and Vitamin D\(_3\) \((p<0.01)\) treated diabetic rats \(B_{\text{max}}\) significantly reversed to near control when compared to diabetic group. \(K_d\) in insulin, curcumin \((p<0.001)\) and Vitamin D\(_3\) \((p<0.01)\) treated diabetic rats significantly reversed to near control when compared to diabetic group (Table-83, 84 & Fig-83, 84).
Muscarinic M3 receptor analysis

Scatchard analysis of \[^{3}H\] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the corpus striatum of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using \[^{3}H\] DAMP and M3 subtype specific antagonist, 4-DAMP mustard. The B\(_{\text{max}}\) decreased significantly (p<0.001) in diabetic group without any change in K\(_{d}\) when compared to control group. In insulin, curcumin and Vitamin D\(_{3}\) treated diabetic rats, B\(_{\text{max}}\) was significantly (p<0.001) reversed back to near control when compared to diabetic group. K\(_{d}\) did not show any significant change when compared to diabetic (Table-85, 86 & Fig-85, 86).

Dopamine receptor analysis

Scatchard analysis of \[^{3}H\] dopamine binding against dopamine in the corpus striatum of control and experimental rats.

Binding analysis of total dopamine receptors was done using \[^{3}H\] dopamine and unlabelled dopamine. The B\(_{\text{max}}\) and K\(_{d}\) decreased significantly (p<0.001) in diabetic group when compared to control group. In insulin, curcumin and Vitamin D\(_{3}\) treated diabetic rats, B\(_{\text{max}}\) significantly (p<0.001) reversed back to near control. K\(_{d}\) of curcumin and Vitamin D\(_{3}\) treated diabetic rats significantly reversed the changes when compared to diabetic group whereas K\(_{d}\) of insulin treated diabetic rats did not show any significant change when compared to diabetic group (Table-87, 88 & Fig-87, 88).
REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant down regulation (p<0.001) in the corpus striatum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed these changes to near control when compared to diabetic (Table-89, Figure-89).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation (p<0.001) in the corpus striatum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed these changes to near control when compared to diabetic (Table-90, Figure-90).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression was increased significantly (p<0.001) in diabetic rats and it was reversed significantly (p<0.001) to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-91, Figure-91).
**Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats**

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression decreased significantly (p<0.001) in diabetic rats. Treatment using insulin, curcumin (p<0.01) and Vitamin D$_3$ (p<0.001) significantly reversed these changes to near control when compared to diabetic (Table-92, Figure-92).

**Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats**

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression was increased significantly (p<0.001) in diabetic rats. Treatment using insulin, curcumin (p<0.01) and Vitamin D$_3$ (p<0.001) significantly reversed these changes to near control when compared to diabetic (Table-93, Figure-93).

**Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats**

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression was decreased significantly (p<0.001) in diabetic rats and it was reversed significantly (p<0.001) to near control in insulin, curcumin and Vitamin D$_3$ treated diabetic rats (Table-94, Figure-94).

**Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats**

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression increased significantly (p<0.001) in diabetic rats. Treatment using insulin,
curcumin (p<0.001) and Vitamin D₃ (p<0.01) significantly reversed these changes to near control when compared to diabetic (Table-95, Figure-95).

**Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats**

Real Time-PCR analysis showed that the Vitamin D receptor gene expression decreased significantly (p<0.001) in diabetic rats. Treatment using insulin, curcumin (p<0.01) and Vitamin D₃ (p<0.001) significantly reversed these changes to near control when compared to diabetic (Table-96, Figure-96).

**Real Time-PCR analysis of insulin receptor in the control and experimental rats**

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly (p<0.001) in diabetic rats and it was reversed significantly (p<0.001) to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-97, Figure-97).

**Real Time-PCR analysis of GLUT3 in the control and experimental rats**

Gene expression of GLUT3 mRNA showed significant up regulation (p<0.001) in the corpus striatum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed the changes to near control (Table-98, Figure-98).

**Real Time-PCR analysis of phospholipase C in the control and experimental rats**

Gene expression of phospholipase C mRNA showed significant up regulation (p<0.001) in the corpus striatum of diabetic rats compared to control. Treatment using
insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed the changes to near control (Table-99, Figure-99).

**Real Time-PCR analysis of CREB in the control and experimental rats**

Gene expression of CREB mRNA showed significant up regulation (p<0.001) in the corpus striatum of diabetic rats compared to control. Treatment using curcumin and Vitamin D$_3$ significantly (p<0.001) reversed these changes to near control. Insulin treatment did not show any significant change in CREB mRNA expression when compared to diabetic rats (Table-100, Figure-100).

**Real Time-PCR analysis of superoxide dismutase in the control and experimental rats**

Gene expression of superoxide dismutase mRNA showed significant down regulation (p<0.001) in the corpus striatum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed these changes to near control (Table-101, Figure-101).

**CONFOCAL STUDIES**

**Muscarinic M1 receptor antibody staining in the corpus striatum of control and experimental rats**

Muscarinic M1 receptor subunit antibody staining in the corpus striatum showed a significant increase (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<0.001) the muscarinic M1 receptor expression in the corpus striatum to near control (Table-102, Figure-102).

**Muscarinic M3 receptor antibody staining in the corpus striatum of control and experimental rats**

Muscarinic M3 receptor subunit antibody staining in the corpus striatum showed a significant decrease (p<0.001) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D₃ treatment to diabetic rats significantly reversed (p<0.001) the muscarinic M3 receptor expression in the corpus striatum to near control (Table-103, Figure-103).

**α7 nicotinic acetylcholine receptor antibody staining in the corpus striatum of control and experimental rats**

α7 nicotinic acetylcholine receptor subunit antibody staining in the corpus striatum showed a significant increase (p<0.001) in the mean pixel value of diabetic rats compared to control. Curcumin and Vitamin D₃ treated diabetic rats significantly reversed (p<0.001) the muscarinic M3 receptor expression in the corpus striatum to near control level. Insulin treatment did not show any significant reversal when compared to diabetic (Table-104, Figure-104).
HIPPOCAMPUS

Total muscarinic receptor analysis

Scatchard analysis of $[^3]$H QNB binding against total muscarinic receptor antagonist, atropine in the hippocampus of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, $[^3]$H QNB and muscarinic general antagonist, atropine. The Scatchard analysis showed that the $B_{\text{max}}$ and $K_d$ decreased significantly (p<0.001) in diabetic rats. Treatment with insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed the $B_{\text{max}}$ and $K_d$ to near control (Table-105, 106 & Figure-105, 106).

Muscarinic M1 receptor analysis

Scatchard analysis of $[^3]$H QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the hippocampus of control and experimental rats

Binding analysis of muscarinic M1 receptors was done using $[^3]$H QNB and M1 subtype specific antagonist pirenzepine. The $B_{\text{max}}$ decreased significantly (p<0.001) in diabetic group with out any change in $K_d$ when compared to control group. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats, $B_{\text{max}}$ significantly (p<0.001) reversed to near control when compared to diabetic. $K_d$ did not show any significant change when compared to diabetic (Table-107, 108 & Figure- 107, 108).
Results

Muscarinic M3 receptor analysis

Scatchard analysis of $[^3]$H DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the hippocampus of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using $[^3]$H DAMP and M3 subtype specific antagonist, 4-DAMP mustard. The $B_{\text{max}}$ and $K_{d}$ increased significantly ($p<0.001$) in diabetic group when compared to control group. $B_{\text{max}}$ in insulin ($p<0.001$), curcumin and Vitamin D$_3$ ($p<0.01$) treated diabetic rats significantly reversed back to near control value when compared to diabetic group. $K_{d}$ did not show any significance change when compared to diabetic group (Table-109, 110 & Figure-109, 110).

Dopamine receptor analysis


Binding analysis of total dopamine receptors was done using $[^3]$H dopamine and unlabelled dopamine. The $B_{\text{max}}$ increased significantly ($p<0.001$) in diabetic group without any change in $K_{d}$ when compared to control group. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats, $B_{\text{max}}$ significantly ($p<0.001$) reversed to near control when compared to diabetic group. $K_{d}$ did not show any significance change in treatment groups when compared to diabetic (Table-111, 112 & Figure-111, 112).
REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase 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 the changes to near control when compared to diabetic (Table-113 & Figure-113).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase 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 (p<0.001) reversed the changes to near control when compared to diabetic (Table-114, Figure-114).

Real Time-PCR analysis of muscarinic M₁ receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M₁ receptor gene expression decreased significantly (p<0.001) in diabetic condition and it was significantly reversed to near control in insulin and curcumin (p<0.001), Vitamin D₃ (p<0.01), when compared to diabetic rats (Table-115, Figure-115).
Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression increased significantly (p<0.001) in diabetic condition and it was reversed to near control in insulin, curcumin (p<0.01) and Vitamin D$_3$ (p<0.001) treated diabetic rats (Table-116, Figure-116).

Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression decreased significantly (p<0.001) in diabetic condition and it was significantly reversed to near control value in curcumin (p<0.01) and Vitamin D$_3$ (p<0.001) treated diabetic rats. Insulin treatment did not show any significant change in α7 nicotinic acetylcholine receptor gene expression when compared to diabetic rats (Table-117, Figure-117).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression increased significantly (p<0.001) in diabetic rats and it was reversed significantly (p<0.001) to near control in insulin and curcumin treated diabetic rats. Vitamin D$_3$ did not show any significantly change in dopamine D1 receptor gene expression when compared to diabetic rats (Table-118, Figure-118).
Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression increased significantly (p<0.001) in diabetic condition and it was reversed significantly (p<0.001) to near control in insulin and curcumin treated diabetic rats. Vitamin D3 did not show any significant change in dopamine D2 receptor gene expression when compared to diabetic rats (Table-119, Figure-119).

Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats

Real Time-PCR analysis showed that the Vitamin D receptor gene expression decreased significantly (p<0.001) in diabetic condition and it was significantly (p<0.01) reversed to near control in insulin, curcumin and Vitamin D3 treatment (Table-120, Figure-120).

Real Time-PCR analysis of insulin receptor in the control and experimental rats

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly (p<0.001) in diabetic condition and it was significantly (p<0.001) reversed to near control in insulin, curcumin and Vitamin D3 treated diabetic rats (Table-121, Figure-121).

Real Time-PCR analysis of GLUT3 in the control and experimental rats

Gene expression of GLUT3 mRNA showed significant up regulation (p<0.001) in the hippocampus of diabetic rats compared to control. Treatment using insulin (p<0.001), curcumin and Vitamin D3 (p<0.01), significantly reversed these changes to near control (Table-122, Figure-122).
Real Time-PCR analysis of phospholipase C in the control and experimental rats

Gene expression of phospholipase C 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 (p<0.001) reversed the changes to near control when compared to diabetic rats (Table-123, Figure-123).

Real Time-PCR analysis of CREB in the control and experimental rats

Gene expression of CREB mRNA showed significant down regulation (p<0.001) in the hippocampus of diabetic rats compared to control. Treatment using curcumin and Vitamin D₃ significantly (p<0.01) reversed the changes to near control. Insulin treatment did not show any significant change in CREB mRNA expression when compared to diabetes (Table-124, Figure-124).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase 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 (p<0.001) reversed the changes to near control when compared to diabetic (Table-125, Figure-125).
CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the hippocampus of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the hippocampus 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<0.001) the muscarinic M1 receptor expression in the hippocampus to near control (Table-126, Figure-126).

Muscarinic M3 receptor antibody staining in the hippocampus of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the hippocampus showed a significant increase (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<0.001) the muscarinic M3 receptor expression in the hippocampus to near control (Table-127, Figure-127).

α7 nicotinic acetylcholine receptor antibody staining in the hippocampus of control and experimental rats

α7 nicotinic acetylcholine receptor subunit antibody staining in the hippocampus showed a significant decrease (p<0.001) in the mean pixel value of diabetic rats compared to control. Curcumin and Vitamin D$_3$ treated diabetic rats significantly reversed (p<0.001) the α7 nicotinic acetylcholine receptor expression in the hippocampus to near control level. Insulin treatment did not show any significant reversal when compared to diabetic (Table-128, Figure-128).
HYPOTHALAMUS

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation (p<0.001) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed the changes to near control when compared to diabetic (Table-129 & Figure-129).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation (p<0.001) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed the changes to near control when compared to diabetic (Table-130, Figure-130).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression decreased significantly (p<0.001) in diabetic condition and it significantly reversed to near control in insulin, curcumin (p<0.001) and Vitamin D₃ (p<0.01) treated diabetic rats (Table-131, Figure-131).
Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression increased significantly (p<0.001) in diabetic rats and it was significantly (p<0.001) reversed to near control in insulin, curcumin and Vitamin D3 treated diabetic rats (Table-132, Figure-132).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression decreased significantly (p<0.001) in diabetic rats. Treatment using insulin, curcumin and Vitamin D3 significantly (p<0.001) reversed the changes to near control when compared to diabetic rats (Table-133, Figure-133).

Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression decreased significantly (p<0.001) in diabetic rats. Treatment using insulin, curcumin and Vitamin D3 significantly (p<0.001) reversed the changes to near control when compared to diabetic rats (Table-134, Figure-134).

Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats

Real Time-PCR analysis showed that the Vitamin D receptor gene expression increased significantly (p<0.001) in diabetic condition and it was significantly
reversed to near control value in insulin, Vitamin D$_3$ (p<0.001) and curcumin (p<0.01) treated diabetic rats (Table-135, Figure-135).

**Real Time-PCR analysis of insulin receptor in the control and experimental rats**

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly (p<0.001) in diabetic condition and it reversed to near control value in insulin (p<0.001), curcumin (p<0.001) and Vitamin D$_3$ (p<0.001) treated diabetic rats (Table-136, Figure-136).

**Real Time-PCR analysis of GLUT3 in the control and experimental rats**

Gene expression of GLUT3 mRNA showed significant up regulation (p<0.001) in the hypothalamus of diabetic rats compared to control. Treatment using insulin (p<0.01), curcumin and Vitamin D$_3$ (p<0.001) treatment significantly reversed the changes to near control. (Table-137 & Figure-137).

**Real Time-PCR analysis of phospholipase C in the control and experimental rats**

Gene expression of phospholipase C mRNA showed significant down regulation (p<0.001) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed the changes to near control (Table-138, Figure-138).

**Real Time-PCR analysis of CREB in the control and experimental rats**

Gene expression of CREB mRNA showed significant down regulation (p<0.001) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin (p<0.01) Vitamin D$_3$ (p<0.001) treatment significantly reversed the changes to near control (Table-139, Figure-139).
Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant up regulation (p<0.001) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed the changes to near control (Table-140, Figure-140).

PANCREAS

Total muscarinic receptor analysis

Scatchard analysis of [$^3$H] QNB binding against total muscarinic receptor antagonist, atropine in the pancreas of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, [$^3$H] QNB and muscarinic general antagonist, atropine. The Scatchard analysis showed that the $B_{\text{max}}$ and $K_d$ decreased significantly (p<0.001) in diabetic rats when compared to control group. In insulin, curcumin and Vitamin D₃ treated diabetic rats $B_{\text{max}}$ (p<0.001) and $K_d$ (p<0.01) significantly reversed to near control value when compared to diabetic group (Table-141, 142 & Fig-141, 142).

Muscarinic M1 receptor analysis

Scatchard analysis of [$^3$H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the pancreas of control and experimental rats

Binding analysis of Muscarinic M1 receptors was done using [$^3$H] QNB and M1 subtype specific antagonist, pirenzepine. The $B_{\text{max}}$ decreased significantly (p<0.001) in diabetic rat when compared to control. The $K_d$ did not show any
Results

significant change when compared to control. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats, $B_{\text{max}}$ significantly (p<0.001) reversed back to near control when compared to diabetic group. $K_d$ did not show any significant change in the treatment group. (Table-143, 144 & Fig-143, 144).

Muscarinic M3 receptor analysis

Scatchard analysis of [$^3$H] DAMP binding against muscarinic M3 receptor antagonist 4-DAMP mustard in the pancreas of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using [$^3$H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard. The $B_{\text{max}}$ decreased significantly (p<0.001) in diabetic group when compared to control group. The $K_d$ did not show any significant change when compared to control group. In insulin, curcumin and Vitamin D$_3$ treated diabetic rats, $B_{\text{max}}$ was significantly (p<0.001) reversed back to near control when compared to diabetic group. $K_d$ did not show any significant change when compared to diabetic group. (Table-145, 146 & Fig-145, 146).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation (p<0.001) in the pancreas of diabetic rats compared to control. Treatment using insulin, Vitamin D$_3$ (p<0.001) and curcumin (p<0.01) significantly reversed the altered expression to near control (Table-147, Figure-147).
Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation (p<0.001) in the pancreas of diabetic rats compared to control. Treatment using curcumin, Vitamin D$_3$ (p<0.001) and insulin (p<0.01) significantly reversed the altered expression to near control (Table-148, Figure-148).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression decreased significantly (p<0.001) in diabetic rats. Treatment using insulin, curcumin and Vitamin D$_3$ significantly (p<0.001) reversed the altered expression to near control (Table-149, Figure-149).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression decreased significantly (p<0.001) in diabetic rats and it was significantly (p<0.001) reversed to near control in insulin, curcumin and Vitamin D$_3$ treated diabetic rats (Table-150, Figure-150).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression increased significantly (p<0.001) in diabetic rats and it was significantly
(p<0.001) reversed to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-151, Figure-151).

**Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats**

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression increased significantly (p<0.001) in diabetic rats and it was significantly (p<0.001) reversed to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-152, Figure-152).

**Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats**

Real Time-PCR analysis showed that the Vitamin D receptor gene expression decreased significantly (p<0.001) in diabetic rats and it was significantly reversed to near control in insulin, Vitamin D₃ (p<0.001) and curcumin (p<0.01) treated diabetic rats (Table-153, Figure-153).

**Real Time-PCR analysis of insulin receptor in the control and experimental rats**

Real Time-PCR analysis showed that the insulin receptor gene expression decreased significantly (p<0.001) in diabetic rats and it was reversed significantly (p<0.01) to near control in insulin, curcumin and Vitamin D₃ treated diabetic rats (Table-154, Figure-154).

**Real Time-PCR analysis of GLUT2 in the control and experimental rats**

Gene expression of GLUT2 mRNA showed significant down regulation (p<0.001) in the pancreas of diabetic rats compared to control. Treatment using
insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed the changes to near control (Table-155, Figure-155).

**Real Time-PCR analysis of phospholipase C in the control and experimental rats**

Gene expression of phospholipase C mRNA showed significant down regulation (p<0.001) in the pancreas of diabetic rats compared to control. Treatment using insulin (p<0.001), curcumin (p<0.01) and Vitamin D₃ (p<0.01) significantly reversed the changes to near control (Table-156, Figure-156).

**Real Time-PCR analysis of superoxide dismutase in the control and experimental rats**

Gene expression of superoxide dismutase mRNA showed significant down regulation (p<0.001) in the pancreas of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D₃ significantly (p<0.001) reversed the changes to near control (Table-157, Figure-157).

**CONFOCAL STUDIES**

**Acetylcholine esterase antibody staining in the pancreas of control and experimental rats**

Acetylcholine esterase antibody staining in the pancreas showed a significant decrease (p<0.001) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D₃ treated diabetic rats significantly (p<0.001) reversed the acetylcholine esterase expression in the pancreas to near control (Table-158, Figure-158).
Results

Muscarinic M1 receptor antibody staining in the pancreas of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the pancreas showed a significant decrease (p<0.001) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D3 treated diabetic rats showed a significant reversal (p<0.001) of muscarinic M1 receptor expression in the pancreas to near control level (Table-159, Figure-159).

Muscarinic M3 receptor antibody staining in the pancreas of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the pancreas showed a significant decrease (p<0.001) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D3 treated diabetic rats showed a significant reversal (p<0.001) of muscarinic M3 receptor expression in the pancreas to near control level (Table-160, Figure-160).

Vesicular acetylcholine transporter antibody staining in the pancreas of control and experimental rats

Vesicular acetylcholine transporter antibody staining in the pancreas showed a significant decrease (p<0.001) in the mean pixel value of diabetic rats compared to control. Insulin, Curcumin and Vitamin D3 treated diabetic rats showed a significant reversal (p<0.001) of vesicular acetylcholine transporter expression in the pancreas to near control level (Table-161, Figure-161).