Results

BODY WEIGHT OF CONTROL AND EXPERIMENTAL RATS

The body weight was significantly decreased (p<0.001) in the diabetic rats when compared to control group. After treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine supplementation for 14 days, the body weight was reversed significantly (p<0.001) to near the initial level (Table-1; Figure-1).

BLOOD GLUCOSE LEVEL OF CONTROL AND EXPERIMENTAL RATS

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. Treatment using insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine was able to significantly reduced (p<0.001) the increased blood glucose level to near control when compared to diabetic rats (Table-2; Figure-2).

CIRCULATING INSULIN LEVEL IN THE PLASMA OF CONTROL AND EXPERIMENTAL RATS

Diabetic rats showed a significant decrease (p<0.001) in circulating insulin level compared to control. Treatment using insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine significantly increase (p<0.001) the plasma insulin level to near control when compared to diabetic rats (Table-3; Figure-3).
TRIIODOTHYRONINE (T3) CONCENTRATION IN SERUM OF CONTROL AND EXPERIMENTAL RAT

Diabetic rats showed a significant decrease ($p<0.001$) in T3 concentration compared to their respective controls. Diabetes rats treated with pyridoxine alone significantly increased ($p<0.05$) the plasma insulin level to near control when compared to diabetic rats. Treatment using insulin and *Aegle marmelose* leaf extract alone and in combination with insulin and *Aegle marmelose* leaf extract significantly increased ($p<0.001$) the plasma insulin level to near control when compared to diabetic rats (Table-4; Figure-4).

SEROTONIN AND ITS METABOLITES CONTENT IN DIFFERENT BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS

*Cerebral Cortex*

There was a significant decrease ($p<0.01$) in 5-HT content in the cerebral cortex of diabetic compared to control. The decreased 5-HT content was significantly reversed ($p<0.01$) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract. The 5-HIAA in the cerebral cortex was significantly decreased ($p<0.05$) in diabetic rats compared to control. The decreased 5-HIAA content was significantly reversed ($p<0.05$) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract (Table-5).
Results

**Brain stem**

There was a significant decrease (p<0.05) in 5-HT content in brain stem of diabetic rats compared to control rats. The decreased 5-HT content was significantly reversed (p<0.05) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract. The 5-HIAA in the brain stem was significantly decreased (p<0.05) in diabetic rats compared to control. The decreased 5-HIAA content was significantly reversed (p<0.05) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract (Table-6).

**Cerebellum**

There was a significant decrease (p<0.05) in 5-HT content in the cerebellum of diabetic rats compared to control rats. The decreased 5-HT content was (p<0.05) reversed to near control in diabetic rats treated pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract. The 5-HIAA in the cerebellum was significantly decreased (p<0.05) in diabetic rats compared to control. The decreased 5-HIAA content was significantly reversed (p<0.05) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract (Table-7).

**Hippocampus**

There was a significant decrease (p<0.05) in 5-HT content in hippocampus of diabetic rats compared to control rats. The decreased 5-HT content was significantly reversed (p<0.05) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract. The 5-HIAA in the hippocampus was significantly decreased (p<0.05) in diabetic rats compared to
control. The decreased 5-HIAA content was significantly reversed (p<0.05) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelos* leaf extract (Table-8).

**GLUTAMATE CONTENT IN DIFFERENT BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS**

**Cerebral Cortex**

Our results showed a significant increase (p<0.001) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelos* leaf extract alone and in combination with pyridoxine in diabetic rats significantly reversed (p<0.001) the glutamate content to near control compared to diabetic group (Table-9).

**Brain stem**

Our results showed a significant increase (p<0.001) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelos* leaf extract alone and in combination with pyridoxine in diabetic rats significantly reversed (p<0.001) the glutamate content to near control compared to diabetic group (Table-10).

**Cerebellum**

Our results showed a significant increase (p<0.05) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelos* leaf extract alone and in combination with pyridoxine in diabetic rats significantly
Results

reversed (p<0.001) the glutamate content to near control compared to diabetic group (Table-11).

Hippocampus

Our results showed a significant increase (p<0.001) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats significantly reversed (p<0.001) the glutamate content to near control compared to diabetic group (Table-12).

Pancreas

Our results showed a significant increase (p<0.001) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats significantly reversed (p<0.001) the glutamate content to near control compared to diabetic group (Table-13).

SEROTONIN, 5-HT\textsubscript{2A}, GLUTAMATE RECEPTORS, 5-HT\textsubscript{2A}, 5-HTT, mGluR5 AND GLAST GENE EXPRESSION CHANGES IN THE BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS

Cerebral Cortex

Scatchard analysis of Serotonin receptors using [\textsuperscript{3}H] 5-HT against 5-HT

Scatchard analysis of serotonin receptors showed that the B\textsubscript{max} decreased significantly (p<0.001) in the cerebral cortex of diabetic rats with a significant
increase (p<0.001) in the affinity when compared to control. Treatment groups D+I (p<0.01), D+P (p<0.01), DIP (p<0.001), D+A (p<0.01) and DAP (p<0.001) significantly reversed the $B_{\text{max}}$ and $K_d$ (p<0.01) to near control compared to diabetic group (Table-14, 15; Figure-5, 6).

Scatchard analysis of 5-HT$_{2A}$ receptors using $[^3H]$ Ketanserin against ketanserin

Scatchard analysis showed no significant change in $B_{\text{max}}$ when compared to control. The $K_d$ was significantly decreased (p<0.001) in diabetic group. Treatment groups D+I (p<0.001), D+P (p<0.05), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) significantly reversed the $K_d$ to near control compared to diabetic group (Table-16, 17; Figure-7, 8).

Scatchard analysis of Glutamate receptors using $[^3H]$ Glutamate against glutamate

Scatchard analysis showed that the $B_{\text{max}}$ increased significantly (p<0.001) in the cerebral cortex of diabetic rats with a significant increase (p<0.001) in the affinity. Treatment groups D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) significantly reversed the $B_{\text{max}}$ and $K_d$ to near control compared to diabetic group (Table-18, 19; Figure-9, 10).

Real-Time PCR analysis of 5-HT$_{2A}$ receptors

Real Time-PCR analysis showed that the 5-HT$_{2A}$ receptor mRNA was significantly down regulated (p<0.001) in diabetic rats and it was significantly reversed (p<0.001) to near control in diabetic rats treated with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine. Diabetic rats treated with pyridoxine alone was also significantly reversed (p<0.05) to near control (Table-20; Figure-11).
Results

**Real-Time PCR analysis of 5-HTT transporter**

Real Time-PCR analysis showed that the 5-HTT receptor mRNA was significantly down regulated (p<0.001) in diabetic rats and it was significantly reversed (p<0.001) to near control in diabetic rats treated with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine. Diabetic rats treated with pyridoxine treated alone was also (p<0.001) reversed to near control (Table-21; Figure-12).

**Real-Time PCR analysis of mGluR5 receptors**

Real Time-PCR analysis of mGluR5 receptor mRNA was significantly up regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control in diabetic rats treated with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine (Table-22; Figure-13).

**Real-Time PCR analysis of GLAST glutamate transporter**

Real Time-PCR analysis of GLAST receptor mRNA showed a significant down regulation (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control in diabetic rats treated with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine (Table-23; Figure-14).
**Brain stem**

**Scatchard analysis of Serotonin receptors using [³H] 5-HT against 5-HT**

Scatchard analysis showed that the $B_{\text{max}}$ increased significantly ($p<0.001$) in the brain stem of diabetic rats with a significant decrease ($p<0.001$) in the affinity. Treatment groups D+I ($p<0.001$), D+P ($p<0.01$), D+I ($p<0.001$), D+P ($p<0.001$) and DAP ($p<0.001$) reversed the $B_{\text{max}}$ and D+I ($p<0.001$), D+I ($p<0.001$), D+P ($p<0.001$), D+I ($p<0.001$) and DAP ($p<0.001$) reversed the $K_d$ to near control compared to diabetic group (Table-27,28; Figure-18,19).

**Scatchard analysis of 5-HT$_{2A}$ receptors using [³H] Ketanserin against ketanserin**

Scatchard analysis showed that the $B_{\text{max}}$ decreased significantly ($p<0.001$) in the brain stem of diabetic rats with no change in the affinity. Treatment groups D+I ($p<0.001$), D+I ($p<0.001$), D+I ($p<0.001$) and DAP ($p<0.001$) significantly reversed the $B_{\text{max}}$ to near control compared to diabetic group (Table-29,30; Figure-20,21).

**Scatchard analysis of Glutamate receptors using [³H]Glutamate against glutamate**

Scatchard analysis showed that the $B_{\text{max}}$ increased significantly ($p<0.001$) in the brain stem of diabetic rats with no change in the affinity. Treatment groups D+I ($p<0.001$), D+I ($p<0.001$), D+P ($p<0.01$), D+I ($p<0.001$) and DAP ($p<0.001$) significantly reversed the $B_{\text{max}}$ to near control compared to diabetic group (Table-31, 32; Figure-22,23).
Results

Real-Time PCR analysis of 5-HT$_{2A}$ receptors

Real Time-PCR analysis showed that the 5-HT$_{2A}$ mRNA was significantly upregulated (p<0.001) in diabetic rats compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelos leaf extract alone and in combination with pyridoxine treated diabetic rats (Table-33; Figure-24).

Real-Time PCR analysis of 5-HTT transporter

Real Time-PCR analysis showed that the 5-HTT mRNA was significantly upregulated (p<0.001) in diabetic rats compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelos leaf extract alone and in combination with pyridoxine treated diabetic rats. Diabetic rats treated with pyridoxine alone treated alone was reversed (p<0.001) to near control (Table-34; Figure-25).

Real-Time PCR analysis of mGluR5 receptors

Real Time-PCR analysis showed that the mGluR5 receptor mRNA was significantly upregulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelos leaf extract alone and in combination with pyridoxine in diabetic rats (Table-35; Figure-26).

Real-Time PCR analysis of GLAST glutamate transporter

Real Time-PCR analysis showed that the GLAST receptor mRNA was significantly downregulated (p<0.001) in diabetic rats when compared to control and
it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-36; Figure-27).

**Cerebellum**

**Scatchard analysis of Serotonin receptors using \[^3\text{H}\] 5-HT against 5-HT**

Scatchard analysis showed that the $B_{\text{max}}$ decreased significantly (p<0.001) in the cerebellum of diabetic rats with a significant increase (p<0.001) in the affinity. Treatment groups D+I (p<0.001), D+P (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) significantly reversed the $B_{\text{max}}$ and $K_d$ (p<0.001) to near control compared to diabetic group (Table-40, 41; Figure-31, 32).

**Scatchard analysis of 5-HT$_{2A}$ receptors using \[^3\text{H}\] Ketanserin against ketanserin**

Scatchard analysis showed that the $B_{\text{max}}$ decreased significantly (p<0.001) in the cerebellum of diabetic rats with no change in affinity. Treatment groups D+I (p<0.001), D+P (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) significantly reversed the $B_{\text{max}}$ to near control compared to diabetic group (Table-42, 43; Figure-33, 34).

**Scatchard analysis of Glutamate receptors using \[^3\text{H}\]Glutamate against glutamate**

Scatchard analysis showed that the $B_{\text{max}}$ increased significantly (p<0.001) in the cerebellum of diabetic rats with no change in the affinity. Treatment groups D+I (p<0.001), D+P (p<0.01), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) significantly reversed the $B_{\text{max}}$ to near control compared to diabetic group (Table-44, 45; Figure-35, 36).
Results

Real-Time PCR analysis of 5-HT₉₂₄ receptors

Real Time-PCR analysis showed that the 5-HT₂₆ receptor mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control level on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed (p<0.001) to near control (Table-46; Figure-37).

Real-Time PCR analysis of 5-HTT transporter

Real Time-PCR analysis showed that the 5-HTT mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone was also (p<0.001) reversed to near control (Table-47; Figure-38).

Real-Time PCR analysis of mGluR5 receptors

Real Time-PCR analysis showed that the mGluR5 receptor mRNA was significantly up regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-48; Figure-39).
Real-Time PCR analysis of GLAST glutamate transporter

Real Time-PCR analysis showed that the GLAST receptor mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.01) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed (p<0.05) to near control (Table-49; Figure-40).

Hippocampus

Scatchard analysis of Serotonin receptors using [3H] 5-HT against 5-HT

Scatchard analysis showed that the $B_{\text{max}}$ decreased significantly (p<0.001) in the hippocampus of diabetic rats with a significant increase (p<0.001) in the affinity. Treatment groups D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) significantly reversed the $B_{\text{max}}$ and DIP (p<0.001) and DAP (p<0.001) reversed the $K_d$ to near control compared to diabetic group (Table-53, 54; Figure-44, 45).

Scatchard analysis of 5-HT$_2A$ receptors using [3H] Ketanserin against ketanserin

Scatchard analysis showed that the $B_{\text{max}}$ decreased significantly (p<0.001) in the hippocampus of diabetic rats with a significant decrease (p<0.05) in the affinity. Treatment groups D+I (p<0.001), D+P (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) significantly reversed the $B_{\text{max}}$ and (p<0.05) reversed the $K_d$ to near control compared to diabetic group (Table-55, 56; Figure-46, 47).
Scatchard analysis of Glutamate receptors using $[^3]$H Glutamate against glutamate

Scatchard analysis showed that the $B_{\text{max}}$ increased significantly ($p<0.001$) in the hippocampus of diabetic rats with no change in the affinity. Treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine reversed ($p<0.001$) the $B_{\text{max}}$ to near control compared to diabetic group (Table-57, 58; Figure-48, 49).

Real-Time PCR analysis of 5-HT$_{2A}$ receptors

Real Time-PCR analysis showed that the 5-HT$_{2A}$ receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-59; Figure-50).

Real-Time PCR analysis of 5-HTT transporter

Real Time-PCR analysis showed that the 5-HTT mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.001$) to near control (Table-60; Figure-51).
Real-Time PCR analysis of mGluR5 receptors

Real Time-PCR analysis showed that the mGluR5 mRNA was significantly up regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats (Table-61; Figure-52).

Real-Time PCR analysis of GLAST glutamate transporter

Real Time-PCR analysis showed that the GLAST mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.01) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed (p<0.05) to near control level (Table-62; Figure-53).

Pancreas

Scatchard analysis of Serotonin receptors using [3H] 5-HT against 5-HT

Scatchard analysis showed that the $B_{\text{max}}$ decreased significantly (p<0.001) in the pancreas of diabetic rats with a significant increase (p<0.001) in the affinity. Treatment groups D+I (p<0.001), D+P (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) significantly reversed the $B_{\text{max}}$ and $K_d$ to near control compared to diabetic group (Table-66, 67; Figure-57, 58).
Scatchard analysis of 5-HT_{2A} receptors using [{\textsuperscript{3}H}] Ketanserin against ketanserin

Scatchard analysis showed that the $B_{\text{max}}$ decreased significantly ($p<0.001$) in the pancreas of diabetic rats with no change in the affinity. Treatment groups D+I ($p<0.001$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the $B_{\text{max}}$ to near control compared to diabetic group (Table-68, 69; Figure-59, 60).

Scatchard analysis of Glutamate receptors using [{\textsuperscript{3}H}]Glutamate against glutamate

Scatchard analysis showed that the $B_{\text{max}}$ increased significantly ($p<0.001$) in the pancreas of diabetic rats with a significant increase ($p<0.001$) in the affinity. Treatment groups D+I ($p<0.01$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the $B_{\text{max}}$ and D+I ($p<0.01$), DIP ($p<0.001$), D+A ($p<0.01$) and DAP ($p<0.001$) reversed the $K_d$ to near control compared to diabetic group (Table-70, 71; Figure-61, 62).

Real-Time PCR analysis of 5-HT_{2A} receptors

Real Time-PCR analysis showed that the 5-HT_{2A} receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic
rats. Diabetic rats treated with pyridoxine treated alone also reversed (p<0.001) to near control level (Table-72; Figure-63).

Real-Time PCR analysis of 5-HTT transporter

Real Time-PCR analysis showed that the 5-HTT mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone reversed also (p<0.01) to near control (Table-73; Figure-64).

Real-Time PCR analysis of mGluR5

Real Time-PCR analysis showed that the mGluR5 mRNA was significantly up regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-74; Figure-65).

Real-Time PCR analysis of GLAST glutamate transporter

Real Time-PCR analysis showed that the GLAST mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.01) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats.
Results

Diabetic rats treated with pyridoxine treated alone also reversed (p<0.05) to near control (Table-75; Figure-66).

GENE EXPRESSION STUDIES OF INSULIN RECEPTORS AND STATUS OF ANTIOXIDANTS- SOD AND GPx IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS

Cerebral Cortex

Real-Time PCR analysis of Insulin receptors

Real Time-PCR analysis showed that the insulin mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats (Table-24; Figure-15).

Real-Time PCR analysis of SOD

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats (Table-25; Figure-16).
**Real-Time PCR analysis of GPx**

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-26; Figure-17).

**Brain stem**

**Real-Time PCR analysis of Insulin receptors**

Real Time-PCR analysis showed that the insulin receptor mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-37; Figure-28).

**Real-Time PCR analysis of SOD**

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-38; Figure-29).
Results

Real-Time PCR analysis of GPx

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control level on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats (Table-39; Figure-30).

Cerebellum

Real-Time PCR analysis of Insulin receptors

Real Time-PCR analysis showed that the insulin receptor mRNA was significantly up regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed (p<0.05) to near control (Table-50; Figure-41).

Real-Time PCR analysis of SOD

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed (p<0.01) to near control level (Table-51; Figure-42).

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Real-Time PCR analysis of GPx

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed (p<0.001) to near control (Table-52; Figure-43).

**Hippocampus**

Real-Time PCR analysis of Insulin receptors

Real Time-PCR analysis showed that the insulin mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-63; Figure-54).

Real-Time PCR analysis of SOD

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-64; Figure-55).
Results

Real-Time PCR analysis of GPx

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats (Table-65; Figure-56).

Pancreas

Real-Time PCR analysis of Insulin receptors

Real Time-PCR analysis showed that the insulin receptor mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed (p<0.05) to near control (Table-76; Figure-67).

Real-Time PCR analysis of SOD

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats (Table-77; Figure-68).
Real-Time PCR analysis of GPx

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated (p<0.001) in diabetic rats when compared to control and it was significantly reversed (p<0.001) to near control on treatment with insulin and Aegle marmelose leaf extract alone and in combination with pyridoxine in diabetic rats (Table-78; Figure-69).

BEHAVIOURAL STUDIES IN CONTROL AND EXPERIMENTAL RATS

Rotarod Performance of control and experimental groups of rats

Rotarod experiment at 10 (p<0.001), 25 (p<0.001) and 50 (p<0.001) revolutions per minute (rpm) showed a significant decrease in the retention time on the rotating rod in diabetic group compared to control. Diabetic rats treated with insulin and Aegle marmelose alone and in combination therapy significantly reversed D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) the retention time to near control (Table-79).

Beam walk test

Beam-walk test was used to assess sensorimotor ability. For a slip to be counted, the foot had to lose contact with the balance beam with the leg extended. The diabetic rats displayed a significantly higher (p<0.001) number of footslips. Treatment with insulin and Aegle marmelose alone and in combination therapy in diabetic rats significantly reversed D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) the footslips to near control (Figure-72).
Elevated plus maze experiment in the control and experimental Rats

(i) Behavioral response in streptozotocin induced diabetic rats: Open and closed arm entry in elevated plus-maze test in control and experimental rats

The experimental groups showed a significant increase (p<0.001) in the attempt taken for open arm entry in diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* alone and in combination therapy in diabetic rats significantly reversed D+I (p<0.001), D+P (p<0.01), DIP (p<0.001) and DAP (p<0.001) treated groups showed the open arm entry to near control (Figure-70).

There was a significant increase (p<0.001) in the number of entries made into closed arm by diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* alone and in combination therapy in diabetic rats significantly reversed D+I (p<0.001), D+P (p<0.01), DIP (p<0.001) and DAP (p<0.001) treated groups showed the closed arm entry to near control (Figure-70, Figure-71).

(ii) Behavioral response in streptozotocin induced diabetic Rats: Time spent in open and closed arms in Elevated plus-maze test in control and experimental rats

There was a significant decrease in time spent in open arm by diabetic rats (p<0.001) compared to control (Figure-6). Time spent in closed arm showed a significant increase in diabetic rats (p<0.001) when compared to control. Treatment with insulin and *Aegle marmelose* alone and in combination therapy in diabetic rats significantly reversed D+I (p<0.001), D+P (p<0.01), DIP (p<0.001) DAP (p<0.001) treated groups showed the time spent in open and closed arms near to control (Figure-71).
CONFOCAL STUDIES

Cerebral Cortex

**5-HT\textsubscript{2A} receptor antibody staining in control and experimental groups of rats**

The 5-HT\textsubscript{2A} receptor antibody staining in the cerebral cortex showed significant decrease (p<0.001) in the 5-HT\textsubscript{2A} receptor in diabetic rats compared to control. There was a significant reversal of 5-HT\textsubscript{2A} receptor to near control in D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of with pyridoxine alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-80; Figure-73).

**5-HT transporter antibody staining in control and experimental groups of rats**

The 5-HTT antibody staining in the cerebral cortex showed significant increase (p<0.001) in the 5-HTT in diabetic rats compared to control. There was a significant reversal to near control in expression of D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of 5-HTT on treatment with insulin and *Aegle marmelose* alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-81; Figure-74).

**mGluR5 antibody staining in control and experimental groups of rats**

The mGluR5 receptor antibody staining in the cerebral cortex showed significant increase (p<0.001) in diabetic rats compared to control. There was a significant reversal to near control in expression of D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of mGluR5 on treatment with insulin and *Aegle*
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*marmelose* alone and in combination therapy with pyridoxine compared to diabetic rats (Table-82; Figure-75).

Brain stem

**5-HT\textsubscript{2A} receptor antibody staining in control and experimental groups of rats**

The 5-HT\textsubscript{2A} receptor antibody staining in the brain stem showed a significant increase (p<0.001) in the 5-HT\textsubscript{2A} receptor in diabetic rats compared to control. There was a significant reversal of 5-HT\textsubscript{2A} receptor to near control in D+I (p<0.001), D+P (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of 5-HT\textsubscript{2A} receptors on treatment with pyridoxine alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-83; Figure-76).

**5-HT transporter antibody staining in control and experimental groups of rats**

The 5-HTT antibody staining in the brain stem showed a significant decrease (p<0.001) in the 5-HTT in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of 5-HTT on treatment with insulin and *Aegle marmelose* alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats. (Table-84; Figure-77).

**mGluR5 antibody staining in control and experimental groups of rats**

The mGluR5 receptor antibody staining in the brain stem showed a significant increase (p<0.001) in diabetic rats compared to control. There was a significant reversal to near control in expression of D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of mGluR5 on treatment with insulin and *Aegle*
marmelose alone and in combination therapy with pyridoxine compared to diabetic rats (Table-85; Figure-78).

Cerebellum

5-HT$_{2A}$ receptor antibody staining in control and experimental groups of rats
The 5-HT$_{2A}$ receptor antibody staining in the cerebellum showed a significant decrease (p<0.001) in the 5-HT$_{2A}$ receptor in diabetic rats compared to control. There was a significant reversal of 5-HT$_{2A}$ receptor to near control in D+I (p<0.001), D+P (p<0.05), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) on treatment with pyridoxine alone and in combination therapy with insulin and Aegle marmelose compared to diabetic rats (Table-86; Figure-79).

5-HT transporter antibody staining in control and experimental groups of rats
The 5-HTT antibody staining in the cerebellum showed a significant decrease (p<0.001) in the 5-HTT in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of 5-HTT on treatment with insulin and Aegle marmelose alone and in combination therapy with insulin and Aegle marmelose compared to diabetic rats. (Table-87; Figure-80).

mGluR5 receptor antibody staining in control and experimental groups of rats
The mGluR5 receptor antibody staining in the cerebellum showed a significant increase (p<0.001) in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of mGluR5 receptors on treatment with insulin and
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*Aegle marmelose* alone and in combination therapy with pyridoxine compared to diabetic rats (Table-88; Figure-81).

**Hippocampus**

5-HT$_{2A}$ receptor antibody staining in control and experimental groups of rats

The 5-HT$_{2A}$ receptor antibody staining in the hippocampus showed a significant decrease (p<0.001) in the 5-HT$_{2A}$ receptor in diabetic rats compared to control. There was significant reversal of 5-HT$_{2A}$ receptor to near control level in D+I (p<0.001), D+P (p<0.05), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of 5-HT$_{2A}$ receptors on treatment with pyridoxine alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-89; Figure-82).

5-HT transporter antibody staining in control and experimental groups of rats

The 5-HTT antibody staining in the hippocampus showed a significant decrease (p<0.001) in the 5-HTT in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of 5-HTT on treatment with insulin and *Aegle marmelose* alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats. (Table-90; Figure-83).

mGluR5 receptor antibody staining in control and experimental groups of rats

The mGluR5 receptor antibody staining in the hippocampus showed a significant up regulation (p<0.001) in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I (p<0.001), DIP (p<0.001), D+A (p<0.001) and DAP (p<0.001) of mGluR5 receptors on treatment
with insulin and *Aegle marmelose* alone and in combination therapy with pyridoxine compared to diabetic rats (Table-91; Figure-84).

**Pancreas**

*5-HT$_{2A}$ receptor antibody staining in control and experimental groups of rats*

The 5-HT$_{2A}$ receptor antibody staining in the pancreas showed a significant decrease (p<0.001) in the 5-HT$_{2A}$ receptor in diabetic rats compared to control. There was a significant reversal of 5-HT$_{2A}$ receptor to near control level in D+I (p<0.01), D+P (p<0.05), D+P (p<0.001), D+A (p<0.01) and DAP (p<0.001) of 5-HT$_{2A}$ receptors on treatment with pyridoxine alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-92; Figure-85).

*mGluR5 receptor antibody staining in control and experimental groups of rats*

The mGluR5 receptor antibody staining in the pancreas showed a significant increase (p<0.001) in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I (p<0.01), D+I (p<0.001), D+A (p<0.01) and DAP (p<0.001) of mGluR5 receptors on treatment with insulin and *Aegle marmelose* alone and in combination therapy with pyridoxine compared to diabetic rats (Table-93; Figure-86).

**CALCIUM IMAGING**

*Calcium release from pancreatic islets using Fluo-4*

The Fluo-4 staining showed a significant (p<0.001) increased calcium release from the pancreatic islets in diabetic rats compared to control. Treatment was significantly reversed to near control level in D+I (p<0.05), D+P (p<0.001), D+A (p<0.05) and DAP (p<0.001) with insulin and *Aegle marmelose* alone and in combination therapy with Pyridoxine compared to diabetic rats (Table-94; Figure-87).