Results

Body Weight and Blood Glucose Level

There was no significant change in the body weight and blood glucose levels in the control and experimental groups of neonatal rats (Table 1).

Dopamine and Homovanillic Acid Content (nmoles/g wet wt.) in the Brainstem of Control and Experimental Groups of Neonatal Rats

DA content in the brainstem showed a significant decrease (p<0.001) in Hx; Hx+G; Hx+O; Hx+G+O and Hx+G+E+O compared to C. Glucose treatment to Hx-Hx+G+O significantly (p<0.01) reversed the DA contents near to C. HVA content significantly decreased in Hx (p<0.01); Hx+G (p<0.001); Hx+O (p<0.01), Hx+G+O (p<0.001) and Hx+G+E+O (p<0.001) compared to C. DA/HVA ratio decreased significantly in Hx (p<0.001); Hx+O (p<0.001) and Hx+G+E+O (p<0.01). Glucose treatment to Hx- Hx+G (p<0.001) and Hx+G+O (p<0.01) significantly reversed the DA/HVA ratio towards control (Table 2).

Dopamine and Homovanillic Acid Content (nmoles/g wet wt.) in the Cerebellum of Control and Experimental Groups of Neonatal Rats

DA content in the cerebellum showed a significant decrease (p<0.001) in Hx; Hx+O; and Hx+G+E+O compared to C. Glucose treatment to Hx - Hx+O and Hx+G+O significantly reversed (p<0.001) the DA contents near to C. There was no significant change in HVA content in Hx; Hx+G; Hx+O and Hx+G+O groups. The HVA content significantly decreased in Hx+G+E+O (p<0.001, p<0.01) compared to C and Hx respectively. DA/HVA ratio decreased significantly in Hx (p<0.01); Hx+O (p<0.01) and Hx+G+E+O (p<0.05). Glucose treatment to Hx- Hx+G and Hx+G+O significantly reversed (p<0.01) the DA/HVA ratio towards control (Table 3).
Dopamine and Homovanillic Acid Content (nmoles/g wet wt.) in the Plasma of Control and Experimental Groups of Neonatal Rats

NE content in plasma showed no significant change in all the experimental groups compared to C. There was a significant increase in the EPI content in Hx (p<0.01); Hx+G (p<0.05); Hx+O (p<0.05); Hx+G+O (p<0.05) and Hx+G+E+O (p<0.001) groups. The EPI content remained significantly higher in Hx+G+E+O (p<0.01) compared to Hx. There was no significant change in plasma DA levels in all the experimental groups of neonatal rats. The HVA content increased in Hx+O (p<0.05); Hx+G+O (p<0.05) and decreased in Hx+G+E+O (p<0.01) compared to both C and Hx. There was no significant change in the DA/HVA ratio. (Table 4)

BRAIN DOPAMINE RECEPTOR ALTERATIONS IN THE CONTROL AND EXPERIMENTAL GROUPS OF NEONATAL RATS

Cerebral Cortex
Scatchard analysis using [3H]DA against DA

Scatchard analysis of [3H]DA against DA in cerebral cortex showed a significant increase (p<0.001) in Bmax in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to Hx- Hx+G and Hx+G+O reversed the DA binding parameter near to C. Kd showed no significant change in all the experimental groups compared to C (Fig 1-5; Table 5-9).

Scatchard analysis using [3H]SCH 23390 against SCH 23390

Binding studies of [3H]SCH 23390 against SCH 23390 for DA D1 receptors showed a significant decrease in Bmax (p<0.05) in the Hx groups. Glucose treated groups - Hx+G (p<0.001), Hx+G+O (p<0.01) and HX+G+E+O (p<0.001) groups showed a significant increase in the Bmax. There was a significant increase (p<0.05) of Kd in Hx, Hx+O and Hx+G+E+O compared to C (Fig 6-10; Table 9-14).
Real-Time PCR analysis of DA D<sub>1</sub> Receptors

The gene expression studies by real-time PCR analysis showed that DA D<sub>1</sub> receptor mRNA was significantly up regulated in Hx (p<0.001), Hx+O (p<0.01) and Hx+G+E+O (p<0.001) groups compared to C. Glucose treatment to Hx - Hx+G and Hx+G+O significantly (p<0.001) reversed the up regulation compared to hypoxic groups (Fig 11; Table 15).

Scatchard analysis using [³H]YM-09151-2 against sulpiride

Binding studies of [³H]YM-09151-2 against sulpiride for DA D<sub>2</sub> receptors in cerebral cortex showed that the B<sub>max</sub> decreased significantly (p<0.001) in Hx and Hx+O groups while in Hx+G and Hx+G+O groups the receptor numbers reversed to near control values. The K<sub>d</sub> increased significantly in Hx (p<0.05) and Hx+G+E+O (p<0.001) when compared to control (Fig 12-16; Table 16-20).

Real-Time PCR analysis of DA D<sub>2</sub> Receptors

The gene expression studies by real-time PCR analysis showed that DA D<sub>2</sub> receptor mRNA was significantly (p<0.001) down regulated in Hx, Hx+O and Hx+G+E+O groups. Glucose treatment to hypoxic rats - Hx+G and Hx+G+O significantly (p<0.001) reversed the down regulation compared to Hx (Fig 17; Table 21).

Brainstem

Scatchard analysis using [³H]DA against DA

Scatchard analysis of [³H]DA against DA in brainstem showed a significant increase in B<sub>max</sub> of Hx (p<0.001), Hx+O (p<0.05) and Hx+G+E+O (p<0.001) groups compared to C. Glucose treatment to Hx- Hx+G and Hx+G+O reversed the DA binding parameter near to C. Hx+G+E+O group showed a significant decrease
(p<0.001) in K_d compared to C. There was no change in K_d in other experimental groups compared to C (Fig 18-22; Table 22-26).

**Scatchard analysis using [³H]SCH 23390 against SCH 23390**

Binding studies of [³H]SCH 23390 against SCH 23390 for DA D_1 receptors showed a significant increase in B_max in the Hx (p<0.01) and Hx+G+E+O (p<0.001) groups compared to C. B_max of Glucose treated groups - Hx+G, Hx+G+O reversed towards control level. There was a significant increase in K_d for Hx (p<0.01), Hx+O (p<0.01), Hx+G+O (p<0.01) and Hx+G+E+O (p<0.001) compared to C (Fig 23-27; Table 27-31).

**Real-Time PCR analysis of DA D_1 Receptors**

The gene expression studies by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly up regulated in Hx (p<0.001) and Hx+O (p<0.01) and Hx+G+E+O (p<0.001) groups. Glucose treatment to hypoxic (Hx+G, Hx+G+O) significantly (p<0.001) reversed the up regulation compared to Hx groups (Fig 28; Table 32).

**Scatchard analysis using [³H]YM-09151-2 against sulpiride**

Binding studies of [³H]YM-09151-2 against sulpiride for DA D_2 receptors in brainstem showed that the B_max decreased significantly in Hx (p<0.01) and Hx+O (p<0.05) groups while in Hx+G and Hx+G+O groups the receptor numbers reversed to near control values. The K_d increased significantly in Hx (p<0.001). All other groups did not show any significant change in K_d compared to C. (Fig 29-34; Table 32-37).
Results

Real-Time PCR analysis of DA D2 Receptors

The gene expression studies by real-time PCR analysis showed that DA D2 receptor mRNA was significantly (p<0.001) up regulated in Hx, Hx+O and Hx+G+E+O groups. Glucose treatment to hypoxic rats - Hx+G and Hx+G+O significantly (p<0.001) reversed the up regulation compared to Hx (Fig 34; Table 38).

Cerebellum

Scatchard analysis using [3H]DA against DA

Scatchard analysis of [3H]DA against DA in brainstem showed a significant decrease in B$_{\text{max}}$ of Hx (p<0.01) and Hx+O (p<0.05) groups compared to C. Glucose treatment to Hx- Hx+G and Hx+G+O reversed the DA binding parameters near to C. Hx and Hx+G+E+O groups showed significantly (p<0.01) decreased K$_d$ compared to C. There was no change of K$_d$ in other experimental groups compared to C (Fig 35-39; Table 39-43).

Scatchard analysis using [3H]SCH 23390 against SCH 23390

Binding studies of [3H]SCH 23390 against SCH 23390 for DA D1 receptors showed a significant decrease in B$_{\text{max}}$ in the Hx (p<0.001), Hx+O (p<0.05) and Hx+G+E+O (p<0.001) groups compared to C. Glucose treated groups - Hx+G, Hx+G+O groups reversed towards C in the B$_{\text{max}}$. There was a significant increase in K$_d$ of Hx+G (p<0.01) and Hx+O (p<0.001) compared to C (Fig 40-44; Table 44-48).

Real-Time PCR analysis of DA D1 Receptors

The gene expression studies by real-time PCR analysis showed that DA D1 receptor mRNA was significantly down regulated in Hx (p<0.001) and Hx+O (p<0.01) and Hx+G+E+O (p<0.001) groups compared to C. Glucose treatment to hypoxia - Hx+G, Hx+G+O significantly (p<0.001) reversed the up regulated mRNA expression compared to Hx (Fig 45; Table 49).

Binding studies of $[^3]$H|YM-09151-2 against sulphirde for DA D$_2$ receptors in cerebellum showed that the B$_{\text{max}}$ decreased significantly in Hx (p<0.01) and Hx+O (p<0.05) groups while in Hx+G and Hx+G+O groups the receptor numbers reversed to near control values. There was no significant change in K$_d$ compared to C in all the experimental groups (Fig 46-50; Table 50-54).

Real-Time PCR analysis of DA D$_2$ Receptors

The gene expression studies by real-time PCR analysis showed that DA D$_2$ receptor mRNA was significantly (p<0.001) down regulated in Hx, Hx+O and Hx+G+E+O groups. Glucose treatment to hypoxic rats - Hx+G and Hx+G+O significantly (p<0.001) reversed the down regulation compared to Hx (Fig 51; Table 55).

BRAIN NMDA RECEPTOR ALTERATIONS IN THE CONTROL AND EXPERIMENTAL GROUPS OF NEONATAL RATS

Cerebral Cortex

Scatchard analysis of $[^3]$H|MK-801 against MK-801 in cerebral cortex showed a significant decrease (p<0.01) in B$_{\text{max}}$ in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to Hx - Hx+G and Hx+G+O reversed the DA binding parameter near to C. K$_d$ showed significant decrease (p<0.05) in Hx while in Hx+O and Hx+G+E+O groups the K$_d$ significantly increased (p<0.05) compared to C. In glucose treated groups- Hx+G and Hx+G+O, K$_d$ values reversed to C. (Fig 52-56; Table 56-60).
Results

**Real-Time PCR analysis of mGLU5 Receptors**

The gene expression studies by real-time PCR analysis showed that mGLU5 receptor mRNA was significantly up regulated (p<0.001) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly (p<0.001) reversed the up regulation compared to hypoxic groups (Fig 57; Table 61).

**Real-Time PCR analysis of NMDA 2b Receptors**

The gene expression studies by real-time PCR analysis showed that NMDA 2b receptor mRNA was significantly down regulated (p<0.001) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly (p<0.001) reversed the down regulation compared to hypoxic groups (Fig 58; Table 62).

**Brainstem**

**Scatchard analysis using [³H]MK-801 against MK-801**

Scatchard analysis of [³H]MK-801 against MK-801 in brainstem showed a significant increase in B_{max} in Hx (p<0.001), Hx+O (p<0.001) and Hx+G+E+O (p<0.01) groups compared to C. Glucose treatment to Hx - Hx+G and Hx+G+O significantly reversed (p<0.001) the DA binding parameters near to C. K_{d} showed significant decrease (p<0.05) in Hx, Hx+O and Hx+G+E+O groups compared to C. In glucose treated groups- Hx+G and Hx+G+O, K_{d} values reversed to C. (Fig 59-63; Table 63-67).

**Real-Time PCR analysis of mGLU5 Receptors**

The gene expression studies by real-time PCR analysis showed that mGLU5 receptor mRNA was significantly up regulated (p<0.001) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G,
Hx+G+O significantly (p<0.001) reversed the up regulation compared to hypoxic groups (Fig 64; Table 68).

**Real-Time PCR analysis of NMDA 2b Receptors**

The gene expression studies by real-time PCR analysis showed that NMDA 2b receptor mRNA was significantly up regulated (p<0.001) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly (p<0.001) reversed the down regulation compared to hypoxic groups (Fig 65; Table 69).

**Cerebellum**

**Scatchard analysis using [3H]MK-801 against MK-801**

Scatchard analysis of [3H]MK-801 against MK-801 in cerebellum showed a significant increase (p<0.001) in B<sub>max</sub> in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to Hx - Hx+G and Hx+G+O significantly reversed (p<0.001) the DA binding parameters near to C. K<sub>d</sub> showed significant decrease in Hx+O (p<0.05) and Hx+G+E+O (p<0.01) groups compared to C. In glucose treated groups- Hx+G and Hx+G+O, K<sub>d</sub> values reversed to C (Fig 66-70; Table 70-74).

**Real-Time PCR analysis of mGLU5 Receptors**

The gene expression studies by real-time PCR analysis showed that mGLU5 receptor mRNA was significantly up regulated (p<0.001) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly (p<0.001) reversed the up regulation compared to hypoxic groups (Fig 71; Table 75).
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Real-Time PCR analysis of NMDA 2b Receptors

The gene expression studies by real-time PCR analysis showed that NMDA 2b receptor mRNA was significantly upregulated (p<0.001) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly (p<0.001) reversed the down regulation compared to hypoxic groups (Fig 72; Table 76).

cGMP content in the cerebral cortex of experimental groups of neonatal rats

The cGMP content in the cerebral cortex decreased significantly (p<0.001) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G (p<0.001), Hx+G+O (p<0.01) significantly reversed the cGMP levels to C (Fig 73; Table 77).

cGMP content in the brainstem of experimental groups of neonatal rats

The cGMP content in the brainstem increased significantly (p<0.05) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed (p<0.05) the cGMP levels to C (Fig 74; Table 78).

cGMP content in the cerebellum of experimental groups of neonatal rats

The cGMP content in the cerebellum increased significantly (p<0.001) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed (p<0.01) the cGMP levels to C (Fig 75; Table 79).

cAMP content in the brainstem of experimental groups of neonatal rats

The cAMP content in the brainstem increased significantly (p<0.05) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed (p<0.001) the cAMP levels to C (Fig 76; Table 80).
cAMP content in the cerebellum of experimental groups of neonatal rats

The cAMP content in the cerebellum increased significantly in Hx (p<0.01), Hx+O (p<0.01) and Hx+G+E+O (p<0.001) compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed (p<0.05) the cAMP levels to C (Fig 77; Table 81).

IP3 content in the cerebral cortex of experimental groups of neonatal rats

The IP3 content in the cerebral cortex decreased significantly (p<0.001) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed (p<0.001) the IP3 levels to C (Fig 78; Table 82).

IP3 content in the brainstem of experimental groups of neonatal rats

The IP3 content in the brainstem decreased significantly (p<0.001) in Hx (p<0.001), Hx+O (p<0.001) and Hx+G+E+O (p<0.01) compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed (p<0.001) the IP3 levels to C (Fig 79; Table 83).

IP3 content in the cerebellum of experimental groups of neonatal rats

The IP3 content in the cerebellum increased significantly in Hx (p<0.01), Hx+O (p<0.05) and Hx+G+E+O (p<0.05) compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed (p<0.01) the IP3 levels to C (Fig 80; Table 84).

Body weight of experimental animals used for behavioural studies

Experimental rats on postnatal day 30 were used for behavioural study. Body weight of experimental animals used for behavioural studies showed a significant decrease in Hx (p<0.01) and Hx+G+E+O (p<0.001) when compared to C. There was
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no significant change in Hx+G, Hx+O and Hx+G+O groups compared to C (Table 85).

Rotarod Performance of experimental animals

Rotarod test at 10 (p<0.01), 15 (p<0.001) and 25 (p<0.001) revolutions per minute (rpm) showed a significant decrease in the retention time on the rotating rod in Hx, Hx+O and Hx+G+E+O compared to respective C. Glucose treatment – Hx+G and Hx+G+O significantly reversed the retention time near to control at 10 (p<0.01), 15 (p<0.001) and 25 (p<0.001) rpm compared to Hx group (Fig 81; Table 86).

Calcium Imaging

Calcium imaging studies in neonatal cortical cells showed a significant decrease in extracellular Ca\(^{2+}\) during hypoxia compared to control levels. Administration of 4mM glucose reversed the Ca\(^{2+}\) levels towards control (Fig 82; Table 87).

Studies on the combination effect of 10\(^{-8}\)M DA and 10\(^{-6}\)M Glutamate on Ca\(^{2+}\) release in control and hypoxic cortical cells showed an increase in Ca\(^{2+}\) release during addition of DA and a decrease with glutamate administration (Fig 83,84; Table 88,89).

TO-PRO-3 Staining

TO-PRO-3 staining of neonatal rat brain showed a decrease in the cell numbers in hypoxia compared to control. This is due to the apoptotic processes during hypoxia (Fig 85).