Dopamine receptor subtypes functional regulation in insulin induced hypoglycemic neonatal rats: Effect of Glucose, Bacopa monnieri and Bacoside A resuscitation

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ROSHNI BABY THOMAS

DEPARTMENT OF BIOTECHNOLOGY
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 022, KERALA, INDIA

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DEPARTMENT OF BIOTECHNOLOGY

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

COCHIN- 682 022, INDIA Phone : 0484-2576267 (O), 0485-2812428 (R) Mob: 94470 12428
Email: cspaulose@cusat.ac.in, paulosecs@yahoo.co.in Fax: 91-0484-2576267, 2577595

Dr. C. S. Paulose
Professor - Emeritus
UGC - BSR Faculty Fellow

CERTIFICATE

This is to certify that the thesis entitled **"Dopamine receptor subtypes functional regulation in insulin induced hypoglycemic neonatal rats: Effect of Glucose, *Bacopa monnieri* and Bacoside A resuscitation"** is a bonafide record of the research work carried out by **Ms. Roshni Baby Thomas,** under my guidance and supervision in the Department of Biotechnology, Cochin University of Science and Technology and that no part thereof has been presented for the award of any other degree. All the relavent corrections and modifications suggested by the audience during the pre-synopsis seminar and recommentaions by the Doctoral Committee of the candidate has been incorporated in the thesis.

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DECLARATION

I hereby declare that the thesis entitled **"Dopamine receptor subtypes functional regulation in insulin induced hypoglycemic neonatal rats: Effect of Glucose, *Bacopa monnieri* and Bacoside A resuscitation."** is based on the original research carried out by me at the Department of Biotechnology, Cochin University of Science and Technology, under the guidance of Dr. C. S. Paulose, Professor-Emeritus, UGC-BSR Faculty Fellow, Department of Biotechnology and no part thereof has been presented for the award of any other degree, diploma, associateship or other title or recognition from any University / Institution.

Cochin - 682 022
20-03-2014

Roshni Baby Thomas
Reg. No. 3903
Department of Biotechnology
Cochin University of Science and Technology

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The woods are lovely, dark and deep.

But I have promises to keep, and miles to go before I sleep

Robert Frost

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Dedicated to my beloved family. . .

ABBREVIATIONS

AC	Adenylyl Cyclases
AGE	Advanced glycation end products
ANS	Autonomic Nervous System
ATP	Adenosine triphosphate
Bax	Bcl-2–associated X protein
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
Ca^{2+}	Calcium ions
cAMP	Cyclic adenosine monophosphate
CAD	Caspase activated DNase
CARD	Caspase recruitment domain
CAT	Catalase
Caspases	Cysteine-dependent aspartate-specific proteases
cGMP	Cyclic guanosine monophosphate
ChAT	Choline acetyl transferase
CNS	Central nervous system
CSF	Cerebrospinal fluid
CRE	cAMP response element
CREB	Cyclic AMP response element binding protein
DAG	Diacylglycerol
DA	Dopamine
DAD1	Dopamine D1

DAD2	Dopamine D2
DARPP	DA receptor phosphor protein
DED	Death effector domain
ECD	Electrochemical detector
EDTA	Ethylene diamine tetra acetic acid
ER	Endoplasmic Reticulum
GABA	Gamma amino butyric acid
GAD	Glutamic acid decarboxylase
GDM	Gestational Diabetes Mellitus
GDNF	Glial cell line-derived neurotrophic factor
GLUT	Glucose Transporter
GPCR	G protein coupled receptors
GPx	Glutathione peroxidase
H_2O_2	Hydrogen peroxide
HCN	Hyperpolarization - activated cyclic nucleotide-gated channels
HGF	Hepatocyte growth factor
HI	Hyperinsulinemia
HPLC	High performance liquid chromatography
IP_3	Inositol trisphosphate
IGF-I	Insulin like groth factor-1
IL	Interleukin
IP3R	Inositol trisphosphate receptor
IR	Insulin receptor
IRS	Insulin receptor substrate

JNK	NH_2-terminal Jun kinases
MAPK	Mitogen-activated protein kinase
NF-κB	Nuclear factor-kappa B
NMDA	Presynaptic *N*-methyl-d-aspartate
NGF	Nerve growth factor
NO	Nitric Oxide
PBS	Phosphate buffered saline
PDK 1	Phosphoinositide dependent kinase-1
PFA	Paraformaldehyde
PKA	Protein kinase A
PI3	Phosphatidyl inositol-3
PI3-K	Phosphatidylinositol 3-kinase
PLC	Phospholipase C
PKA	Protein kinase A
PKC	Protein kinase C
RPHN	Repetitive and profound hypoglycemia
ROS	Reactive oxygen species
RNS	Reactive Nitrogen Species
SN	Substantia nigra
SOD	Superoxide dismutase
TK	Tyrosine Kinase
TNF-α	Tumor necrosis factor- α
TCA	Tricarboxylic Acid
TM	Transmembrane domain

TPP	Trisodium penta polyphosphate
WHO	World Health Organization

CONTENTS

Introduction

The term hypoglycemia refers to a reduction in the glucose concentration of the circulating blood. It is almost 100 years since hypoglycemia was first described in children and over 50 years since it was recognized in newborns and infants (Dhananjayaa & Kiran, 2011). Variable incidence has been reported by various authors in different weight and gestational age groups (Mishra *et al*, 1977). The overall incidence of hypoglycemia in neonates varies from 0.2 to 11.4%. However in the presence of certain risk factors like small for date, large for date, infants of diabetic mothers, prematurity etc., the probability of hypoglycemia increases many folds (Dutta *et al*., 2000).

Glucose is the main energy source in the brain, whenever blood glucose concentration decreases below 20 mg/dl, brain activity ceases and the hypoglycemic coma, also known as the isoelectric period, takes place. This induces selective brain damage in vulnerable brain regions, including the cerebral cortex, the hippocampus and the striatum (Auer *et al*., 1984; Kalimo *et al*., 1985). The initial phase of neuronal damage occurs within minutes of hypoglycemic shock. This is because, more than 99% of cerebral energy production results from the oxidation of glucose and the energy failure give rise to selective neuronal necrosis (Auer & Siesjo., 1988). Glucose levels in the newborn decrease in the initial two hours, but steadily rise afterwards and thereafter remain constant. Hypoglycemia occurs when this equilibrium fails (Fernández & Pérez., 2011). When glucose levels fall below threshold glycemic levels, neuroendocrine, autonomic nervous system (ANS) and metabolic glucose counter regulatory mechanisms are activated. These hypoglycemic counter regulatory mechanisms can be blunted irreversibly by disease duration or by acute episodes of prior stress (Ertl & Oavis, 2004). Although hypoglycemia is associated with a number of physiological changes, the most profound effects are seen in the brain, where glucose is the major substrate for energy metabolism. Lack of glucose produces brain damage or even death if the deficit is prolonged.

Hypoglycemia frequently reflects difficulties in adapting to extra uterine life (Fernández & Pérez., 2011). When severe it leads to permanent neurological dysfunction including seizures, microcephaly, motor and/or developmental abnormalities (Blattner., 1968; Hawdon., 1999; Karp., 1989; Ryan *et al*., 1985; Vannucci & Vannucci., 2001). In the presence of persistent hypoglycemia, three main possible scenarios must be considered: depletion of energetic storage (prematurity and intra-uterine growth restriction), increase tissue energetic consumption and foetal hyperinsulinism. (Mitanchez., 2008; Wight., 2006; Platt & Desphande 2005). The mechanisms underlying hypoglycemic neuronal damage are not completely understood but early studies suggested the participation of an excitotoxic mechanism triggered by the release of glutamate, and particularly aspartate, soon after the onset of the isoelectric period (Sandberg *et al*., 1986; Wieloch, 1984). Neuronal death induced in this condition involves energy depletion, activation of glutamate receptors, elevation of the intracellular calcium concentration and ROS production (Hernández-Fonseca *et al*., 2008). In addition, recent investigations have suggested that oxidative stress is associated with hypoglycemic neuronal damage (Suh *et al*., 2007, 2008; Haces *et al*., 2008, 2010). The hypoglycemic condition favours the production of reactive oxygen and nitrogen (ROS/ RNS) species, which might participate in the induction of the subsequent neuronal death.

In the central nervous system (CNS), the dopaminergic system is important in regulating neuronal growth and development. Insulin-induced hypoglycemia causes the death of neurons in particular brain regions including the cerebral cortex, striatum and hippocampus, while the cerebellum and the brain stem are more resistant. The mechanisms underlying this selective vulnerability to hypoglycemic damage are unknown (Haces *et al*., 2010). The dopamine receptors (DA) are widely expressed in the central nervous system because they are involved in the control of locomotion, cognition, emotion and affect neuro-endocrine secretion (Missale *et al*., 1998). Dopamine peripherally modulates insulin secretion in the pancreatic islets (Nogueira *et al.,* 1994). Any disturbance in the central dopaminergic function will affect the normal memory processing

and cognition. Dopaminergic innervations appear to be sensitive to stress and relatively low intensity levels of stress are capable of disrupting functions like 'working memory' and attention (Goldberg *et al*., 1991; Schneider & Roeltgen., 1993).

DA is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised. DA interacts with specific membrane receptors to produce its effects. These effects are terminated by reuptake of dopamine into the presynaptic neuron by a dopamine transporter. DA plays an important role both central1y and peripherally. The five dopamine receptor subtypes provides a basis for understanding dopamine's central and peripheral actions. DA receptors are classified into two major groups: DA D1 like and DA D2 like. DA D1 like receptors consists of DA D1 and DA D5 receptors.

DA D2 like receptors consists of DA D2, DA D3 and DA D4 receptors. Stimulation of the DA D1, receptor gives rise to increased production of cAMP. DA D2 receptors inhibit cAMP production, but activate inositol phosphate second messenger system (Seeman, 1980). An imbalance between dopaminergic neurotransmission and DA receptors is known to be associated with the symptomatology of numerous neuropsychiatric disorders, like schizophrenia, psychosis, mania and depression as well as neuropathological disorders, like Parkinson's disease and Huntington's disease (Carlsson, 1988, 1993; Bermanzohn & Siris, 1992; Brown & Gershon, 1993; Lakel & Maragos, 2000; Kostrzewa & Segura-Aguilar, 2003). The dopaminergic cells in particular are highly sensitive to excitotoxicity and oxidative stress when the energy metabolism is impaired (Callahan *et al.,* 1998). At the cellular level, dopamine D1/D5 receptor agonists regulate neuronal excitability by altering ion channel activity. In addition, there is evidence that DA D1-like receptors modulate various forms of synaptic plasticity, including long-term potentiation and long-term depression in neocortex (Gurden *et al*., 2000; Otani *et al*., 1998).

There are many treatments for neonatal hypoglycemia (Cornblath & Schwartz., 1976; Jones & Roberton., 1984): glucose infusion is one but it induces complications such as hyperglycaemia, rebound hypoglycemia after interruption

of the infusion and hypersecretion of insulin which can induce recurrence of hypoglycemia; it also inhibits the compensative gluconeogenesis (Kalhan *et al*., 1986) and ketogenesis. Studies show that glucose reintroduction after the isoelectric period correlates with the presence of superoxide and nitrotyrosine immunoreactivity, and suggest that glucose reintroduction stimulates oxidative stress through the activity of NADPH oxidase (Suh *et al*., 2007, 2008). Corticoids are sometimes useful to prevent hypoglycemia but it limit the peripheral uptake of glucose in some tissues (Sann *et al*., 1983), an effect which results in an increased incidence of neurological and electro-encephalographic abnormalities (Jones & Roberton., 1984).

Treatment with herbal drugs has been in use since ancient times and herbs have been an effective source of treatment regimens for different diseases. In modern medicine, medicinal herbs are an integral part of alternative therapy. *Bacopa monnieri* L. (Fam. Scrophulariaceae) is a creeping, glabrous, succulent herb, rooting at nodes, distributed throughout India in all plain districts, ascending to an altitude of 1,320 m. The plant is reported to show sedative, antiepileptic, vasoconstrictor and anti-inflammatory activity (Handa, 1998). Its antioxidant properties and its ability to balance super oxide dismutase (SOD) and catalase levels were postulated to account for this effect. (Sairam, 2001). It has been reported that the plant contains tetracyclic triterpenoid saponins, bacosides A and B, hersaponin, alkaloids viz. herpestine and Bacopin and flavonoids (Jobin *et al* ., 2010; Handa., 1998; Kiritikar & Basu., 1994).

Bacopa monnieri (Brahmi) is recommended in formulations for the management of a range of mental conditions including anxiety, poor cognition, lack of concentration and epilepsy. Pharmacologically, it is understood that Brahmi has an unusual combination of constituents that are beneficial in mental inefficiency and illnesses and useful in the management of convulsive disorders like epilepsy (Jobin *et al*., 2010).

Bacoside A, a triterpenoid saponin, is a major constituent isolated from the plant *Bacopa monnieri* Linn. Used as a memory herb and for mental enhancement. This substance appears to have antioxidant and brain protective

potential. Bacoside A is the active ingredient in bacopa herb along with bacoside B. Besides, Bacoside A also exhibits vasodilatory, calcium antagonistic, muscle relaxant, mast cell stabilizing and antiulcer properties (Sumathi *et al*., 2011). But so far there has been no study reporting the role of *Bacopa monnieri* and Bacoside A treatment on the functional regulation of dopamine receptors.

At present, our understanding of the effects of hypoglycemia on the developing brain is incomplete. We also do not know the extent of impact of a hypoglycemic shock which triggers brain injury during development. To address these issues, we examined the susceptibility of the developing brain to acute hypoglycemia involving DAD1 and DAD2 receptor functional regulation. In the present study a detailed investigation of the alterations of dopamine receptors in the brain regions of insulin induced hypoglycemic neonatal rats were carried out, using glucose, *Bacopa monnieri* and Bacoside A as treatment options. The molecular studies on the various brain regions through dopaminergic receptors will elucidate the corrective measures for hypoglycemia induced brain damage. This study makes the point that - a plan for rational intervention, will result in the reduction in the incidence of lifelong disabilities like epilepsy, and behavioural and learning disorders. Many adult diseases have their origins in prenatal or early postnatal life, and hence delineating the vulnerability of the developing CNS to diverse insults, will lead to new therapeutic interventions.

OBJECTIVES OF THE PRESENT STUDY

In the present work we studied the potential of *Bacopa monnieri* and Bacoside A treatment to enhance the antioxidant system and support the neuronal survival in the hypoglycemic neonatal brain. For achieving the aim, DAD1 and DAD2 receptors functional regulation, gene expression of growth factors, neuronal survival and apoptotic factors during insulin induced hypoglycemic neonatal brain in rats were studied. The objectives are

1. To induce hypoglycemia in neonatal rats using intra peritoneal injection of insulin.
2. To study the anti- hypoglycemic activity of *Bacopa monnieri* and Bacoside A in the insulin induced hypoglycemic neonatal rat.
3. To study the Dopamine D1 and D2 receptor binding parameters in the brain regions of control and hypoglycemic neonatal rats.
4. To study the dopamine signalling through the gene expression of Dopamine D1, Dopamine D2 receptor subtypes, PLC and CREB.
5. To study the cAMP and IP3 content in the brain regions of control and hypoglycemic neonatal rats, using second messenger assays.
6. To study the GLUT 3 gene expression in the brain regions of control and hypoglycemic neonatal rats, using Real Time PCR.
7. To study neuronal survival factors using NF-κB, GDNF, and BDNF gene expression in the brain regions of control and hypoglycemic neonatal rats, using Real Time PCR.
8. To study antioxidant property of *Bacopa monnieri* and Bacoside A using SOD and GPx gene expression in brain regions of control and hypoglycemic neonatal rats, using Real Time PCR.
9. To study apoptotic pathway using Akt -1, TNF - α, Bax and Caspase 8 gene expression in the brain regions of control and hypoglycemic neonatal rats, using Real Time PCR.

10. To study localization and expression status of Dopamine D1 and D2 receptors in the brain slices of control and hypoglycemic neonatal rats, using confocal microscopy.

Literature Review

Hypoglycemia is the most common metabolic problem occurring in newborns. In majority of cases, it merely reflects a normal process of adaptation to extra uterine life. The term hypoglycemia refers to a reduction in the glucose concentration of the circulating blood. 'Hypoglycemia is not a medical condition in itself, but a feature of illness or of failure to adapt from the fetal state of continuous transplacental glucose consumption to the extra uterine pattern of intermittent nutrient supply'. Hypoglycemia is known to be associated with brain dysfunction and neuromotor developmental retardation in both symptomatic and asymptomatic cases (Williams, 1997). The initial phase of neuronal damage occurs within minutes of hypoglycemic shock and the energy failure gives rise to selective neuronal necrosis (Auer & Siesjo., 1988).Hypoglycemia in the newborn is a surrogate marker for central nervous system energy deficiency. Hyperinsulinemic hypoglycemia produces profound central nervous system energy deficiency leading to long-term central nervous system damage (Straussman & Lynn, 2010). The importance of early identification, prevention, and treatment of low glucose concentrations in certain conditions, such as genetic defects in specific metabolic pathways (eg, fatty acid oxidation disorders) or defects in the regulation of insulin secretion leading to hyperinsulinemic hypoglycemia, is generally agreed upon. However, transient, asymptomatic low glucose concentrations may herald metabolic disorders that can cause serious injury (Rozance & Hay., 2012).

Incidence

The overall incidence has been estimated at 1 to 5 per 1,000 live births, but it is higher in at-risk populations. 8% of large-for-gestational-age infants (primarily infants of diabetic mothers) and 15% of preterm infants and infants who have intrauterine growth retardation have been reported as having hypoglycemia;

the incidence in the entire population of "high-risk" infants may be as high as 30% (McGowan., 1999).

The World Health Organization (WHO) has predicted that between 1995 and 2025, there will be a 35% increase in the worldwide prevalence of diabetes (Ang *et al*., 2005). Moreover, women born in Asian countries display the highest prevalence of Gestational Diabetes Mellitus (GDM), with up to 17% of women likely to develop GDM, in comparison to 4% of European and white American women (Ferrara, 2007; Sesiah *et al*., 2008). The prevalence of gestational diabetes in southern India was recently found to be 17.8% in urban women, 13.8% in semi urban and 9.9% in rural women (Ramachandran *et al*., 1994). Infants of diabetic mothers have a higher incidence of neonatal complications than those born to non-diabetic women (Nasrat *et al*., 1993). The incidence in Kerala is approx. 41/1000 live births. (Sasidharan CK *et al*., 2004).

Symptoms

When plasma glucose levels approach 3.6–3.8 mmol/L (64.8–68.4 mg/dL), clinical signs such as nervousness, tremors, cardiac palpitations, and weakness have been observed in humans, rats, mice, rabbits, cats, dogs, and monkeys. When plasma glucose levels fall below 1 mmol/L (18 mg/dL), seizure activity, severe brain damage, coma, and death can occur as a result of neuronal dysfunction and cell death (Rudloff & Franklin., 2009).

Hypoglycemia in neonates can be symptomatic and asymptomatic. The most common symptoms such as jitteriness, convulsions, apathy, hypotonia, coma, refusal to feed, cyanosis, high pitched cry, hypothermia are very nonspecific and especially in small sick infants, these symptoms may be easily missed. Therefore hypoglycemia must always be confirmed biochemically and by response to treatment (Charles *et al*, 2005; Kleigman, 2005; Barbara & Robert., 2004).

Infant populations at Risk

Infants of diabetic mothers can sustain long-term brain injury if there is a period of severe hypoglycemia in the first 12 h after birth (Vannucci & Vannucci., 2001). In young children with diabetes mellitus, short-term episodes of hypoglycemia are associated with long-term learning problems (Kaufman *et al*., 1999; Rovet & Ehrlich., 1999).

Variables which strongly and independently predict the risk of neonatal hypoglycemia are prematurity and low birthweight, maternal diabetes mellitus, delay in initiation of breastfeeding for more than 2 h postnatally, maternal pre-eclampsia and eclampsia, birth asphyxia, hypoxia, cold stress or hypothermia and maternal oligohydramnios (Rudloff & Franklin., 2009).

Impact of neonatal hypoglycemia on the Brain

The brain of a human neonate is more vulnerable to hypoglycemia than that of pediatric and adult patients (Liu *et al*., 2013). The mechanisms that induce neuronal injury following glucose deprivation are more complex than simply depriving neurons of their primary energy source (Auer & Siesjo., 1993). Hypoglycemia often occurs not in isolation but in combination with other disorders affecting brain metabolism (Tam *et al*., 2008). Insulin induced hypoglycemia does not generate uniform injuries throughout the brain (Dasgupta *et al*., 2013). Moderate hypoglycemia is known to have significant impact on functions of the CNS, and any differential effect of hypoglycemia on the peripheral nervous system may offer insights into the metabolic requirements of central and peripheral neurons (Antony *et al*., 2010).

The most vulnerable areas of hypoglycemia-related brain injuries are the involvement of hippocampus, basal ganglia, and cortical and subcortical areas in adults (Sarnat, 2004). Repetitive and profound hypoglycemia (RPHN) affects occipital cortex and hippocampal activities, neurotransmitter transition, energy metabolism, and other metabolic equilibria in newborn rats; these effects are further aggravated when the newborn rats develop into adolescence (Liu *et al*., 2013). It is recognized that there can be occipital injury after neonatal

hypoglycemia. Parietooccipital lobe diffusion restriction and cortical visual deficits are observed after a hypoglycemic insult (Tam *et al*., 2008).

Brain stem neurons are remarkably resistant to irreversible injury by hypoglycemia (Sarnat, 2004). Glucoreceptive sites exist within the hindbrain, and it has been suggested that glucoreceptors exist only in the hindbrain. The hindbrain does not appear to be vulnerable to the CNS adaptive mechanisms that impair counter regulatory responses under conditions of recurrent hypoglycemia (Sanders *et al*., 2007). The cerebellum suffers a lesser metabolic insult due to the greater efficiency of the cerebellar glucose transporter, explaining the relative resistance of the cerebellum to hypoglycemic brain damage (Auer, 2004).

Animal studies, using models of both type 1- and type 2 diabetes, show deleterious dose-dependent effects of hyperglycemia in the mature brain resulting in reversible pathoanatomical changes in the hippocampal regions of the brain as well as impaired cognitive function in the adult diabetic animals . Unfortunately, there are no animal studies which investigate the potential adverse impact of intrauterine hyperglycemia on offspring cognitive function (Clausen *et al*., 2013).

BRAIN NEUROTRANSMITTER CHANGES DURING NEONATAL HYPOGLYCEMIA

The brain of a human neonate is more vulnerable to hypoglycemia than that of pediatric and adult patients. During the neonatal period RPHN causes brain damage and leads to severe neurologic sequelae (Liu *et al*., 2013). Severe hypoglycemia has been shown to alter brain structure (Puente et al., 2010) and cause significant cognitive damage (Suh *et al*., 2007) also. Changes in the metabolism of neurotransmitter system are seen to be an adaptive measure of the CNS in response to RPHN (Liu *et al*., 2013).

The neuronal death ensuing from hypoglycemia is not a simple result of energy failure but instead results from a sequence of events initiated by hypoglycemia. These events have been enumerated as activation of neuronal glutamate receptors (Nellgard & Wieloch 1992), production of ROS, neuronal

zinc release, activation of poly(ADP-ribose) polymerase–1, and mitochondrial permeability transition (Suh *et al*. 2007).

The brain is normally dependent on glucose for metabolism and function. Hyperinsulinemic hypoglycemia leads to extensive deterioration of cerebral energy state and neuronal cell damage and follows an excitotoxic mechanism of neuronal necrosis. Cerebral glutamate is derived solely from endogenous sources; mainly from α - ketoglutarate, which is a product of the Krebs cycle. The neuronal glutamate acts as a neurotransmitter, resulting in excitatory interaction between neurons. Excitotoxicity results due to an excessive activation of neuronal amino acid, glutamate which is the key mechanism implicated in the mediation of neuronal death in hypoglycemia (Leighton *et al*., 2001). Hypoglycemic brain damage thus falls into the newly defined class of 'excitotoxic' neuropathologies, where neurons are selectively killed by an extracellular overflow of excitatory amino acids produced by the brain itself (Aurer & Seisjo., 1993).

Cerebellar cholinergic neurotransmission is impaired during hyperglycemia and hypoglycemia and the hypoglycemia causes more prominent imbalance in cholinergic neurotransmission which is suggested to be a cause of cerebellar dysfunction associated with hypoglycemia (Antony *et al*., 2010).

Hypoglycemia plays an important contributory role in regulation of adenosine 2A receptors in different areas of the brain (Haas & Selbach, 2000). Studies have found significantly higher distribution of these receptors in the striatal areas in comparison to the brain stem, cortex and cerebellum (Buyng *et al*., 2001) and furthermore, attributed excessive ROS and glutamate production in the basal ganglia to high concentration of adenosine 2A receptors in striatal region (Golembiowska & Dziubina., 2012).

The brain generally functions through the interaction of multiple regions, the hippocampus, cortex, and striatum are unique in that DA, through interactions with glutamate, is required for neuronal potentiation in each area (Bales *et al*., 2010). The widespread disruption of neuronal projections at the time of a hypoglycemic shock has implications for all neurotransmitter systems, including DA.

Dopaminergic System

Brain damage is closely related to neurochemical alterations caused by hypoglycemia in both immature and mature rats. When hypoglycemia is profound and recurrent, brain damage and severe neurological sequelae, including epilepsy, abnormal cognitive abilities, and speech and language delays, inevitably develop (Liu *et al*., 2013). The DA receptors are widely expressed in the central nervous system because they are involved in the control of locomotion, cognition, emotion and affect neuro-endocrine secretion (Missale *et al*., 1998). DA represents a unique signalling system within the CNS due to its role as a neurotransmitter and neuromodulator. It is important in regulating neuronal growth and development. The DA receptors, expressed in the CNS, are involved in the control of locomotion, cognition, memory, emotion and affect neuro-endocrine secretion. DA receptors are G protein coupled receptors (GPCR) mediating slow neurotransmission. At the cellular level, DAD1 /D5 receptor agonists regulate neuronal excitability by altering ion channel activity. In addition, there is evidence that DAD1 -like receptors modulate various forms of synaptic plasticity, including long-term potentiation and long-term depression in neocortex (Gurden *et al*., 2000; Otani *et al*., 1998).

Dopamine Pathways

DA is the predominant catecholamine neurotransmitter in the mammalian brain. This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function and gastrointestinal motility (Missale *et al*., 1998). DA containing neurons arise mainly from DA cell bodies in the substantia nigra (SN) and ventral tegmental area in mid-brain region (Carlsson, 1993; Tarazi *et al*., 1997[a,b], 1998). Dopaminergic system is organized into four major subsystems:

(i) the *nigrostriatal* system involving neurons projecting from the substantia nigra, pars compacta to the caudate-putamen of the basal ganglia. This

is the major DA system in the brain as it accounts for about 70% of the total DA in the brain, and its degeneration makes a major contribution to the pathophysiology of Parkinson's disease;

(ii) *the mesolimbic system* that originates in the midbrain tegmentum and projects to the nucleus accumbens septi and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus and the entorhinal cortex, all of which are considered components of the limbic system and so are of particular interest for the patho-physiology of idiopathic psychiatric disorders;

(iii) the *mesocortical* system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions;

(iv) the *tuberinfundibular* pathway, which is a neuroendocrinological pathway arising from the arcuate and other nuclei of the hypothalamus and ending in the median eminence of the inferior hypothalamus. DA released in this system exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin.

DA is involved in the control of both motor and emotional behaviour. Despite the large number of crucial functions it performs, this chemical messenger is found in a relatively small number of brain cells. In fact, while there are a total of 10 billion cells in the cerebral cortex alone, there are only one million dopaminergic cells in the entire brain (Missale *et al*., 1998).

Dopamine receptors and its subtypes

DA mediates its actions via membrane receptor proteins. DA receptors are found on postsynaptic neurons in brain regions that are DA enriched. In addition, they reside presynaptically on DA neuronal cell bodies and dendrites in the midbrain as well as on their terminals in the forebrain. DA receptors belong to a family of large peptides that are coupled to G-proteins which are modified by attached carbohydrate, lipid-ester or phosphate groups. The topologies of the five DA receptors are predicted to be the same as all the other GPCR. They are characterized by having seven hydrophobic transmembrane-spanning regions

(Hartman & Civelli., 1997). The third intracytoplasmic loop is functionally critical and interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects (Carlsson, 1993; Tarazi *et al*., 1997 [a, b], 1998). In their putative transmembrane domains, the DAD1 and D5 receptors are 79% identical to each other, while they are only 40–45% identical to the DAD2, D3 and D4 receptors. Conversely, the DAD2, D3, and D4 receptors are between 75% and 51% identical to each other.

Classification of Dopamine Receptors

DA receptors are divided into two families on the presence or absence of ability of DA to stimulate adenylyl cyclase and produce the second messenger molecule cyclic-Adenosine monophosphate (cAMP) (Civelli *et al.*, 1993; O'Dowd, 1993). This classification is based on similarities in structure, pharmacology, function and distribution. DAD1 like receptors are characterized initially as mediating the stimulation of cAMP production. DAD2 like receptors inhibits the production of cAMP (Seeman, 1980).

Applications of molecular genetics have greatly facilitated the isolation and characterisation of novel DA receptors**,** DAD3, D4 and D5 with different anatomical localisation from traditional DAD1 or DAD2 receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families**:** the DAD1 like family which includes DAD1 and D5 receptors. The DAD2 like family includes DAD2, D3 and D4 receptors (Schwartz *et al*., 1992; Grandy *et al*., 1993; Sibley *et al*., 1993). The genomic organisations of the DA receptors demonstrate that they are derived from the divergence of two gene families that mainly differ in the absence or the presence of introns in their coding sequences. DAD1 like receptors genes do not contain introns in their coding regions, a characteristic shared with most GPCR. The genes encoding the DAD2 like receptors are interrupted by introns (Gingrich & Marc, 1993). Furthermore, most

of the introns in the DAD2-like receptor genes are located in similar positions (Hartman & Civelli, 1997).

Dopamine D1-like family

The DAD1 receptor is the most abundant DA receptor in the CNS. The DAD1 like receptors are characterized by a short third loop as in many receptors coupled to Gs protein (Gs alpha subunit) (Civelli *et al*., 1993; Gingrich & Canon *et al*., 1993; O'Dowd, 1993). The DAD1 like receptors have short third intracellular loops and long carboxy terminal tails. The DAD1 like receptors are classified into DAD1 and D5. In the DAD1 and D5 receptor third intracellular loop and the carboxy terminus are similar in size but divergent in their sequence. In contrast, the small cytoplasmic loops 1 and 2 are highly conserved so that any difference in the biology of these receptors is probably related to the third cytoplasmic loop and the carboxy terminal tail (Civelli *et al*., 1993, Gingrich & Canon *et al*., 1993; O'Dowd, 1993). The external loop between transmembrane domain (TM) TM4 and TM5 is considerably different in the two receptor subtypes, being shorter (27 amino acids) in the D1 receptor than in the D5 receptor (41 amino acids). The amino acid sequence of this loop is divergent in the DAD5 receptor (Marc *et al*., 1998).

DAD1 receptors are found at high levels in the typical DA regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercles (Cadet *et al*., 2010). DAD1 receptor seems to mediate important actions of DA to control movement, cognitive function and cardiovascular function. In humans, DAD1 receptor gene has been localized to chromosome 5 (Sunahara *et al.,* 1990). The DAD1 receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5- trisphosphate (IP3) and diacylglycerol (DAG) *via* the activation of phospholipase c (PLC) (Monsma *et al.,* 1990; Sibley *et al.,* 1990)**.** DAD1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. DAD1 receptors messenger ribonucleic acid (mRNA) is colocalized in striatal neurons of the basal ganglia with mRNA for DA receptor phosphor protein (DARPP-32; KD) which is a DA

and cAMP-regulated phosphoprotein. DRPP contributes to the actions of DAD1 receptor (Hemmings & Greengard, 1986; Greengard, *et al*., 1987).

Dopamine D2 like family

DAD2 like receptors belongs to the GPCR and has 400 amino acid residues. DAD2-like receptors are characterized by a long extracellular amino terminus which has several glycosylation sites and a shorter carboxy terminal tail with putative phosphorylation sites. The function of sugar moieties is unclear (Sibley, 1999). It is generally believed that the membrane enclosed part of the amino-acid chain of GPCR is folded into seven a-helices. The transmembrane helices consist primarily of hydrophobic amino-acid residues. The unique feature of DAD2 like receptors family is that they posses a bigger third cytoplasmic (intracellular) loop in common, which is the site where the G-protein couples. Between the different DA receptors, the third loop also displays the greatest variability in amino-acid sequence. This has consequences for their respective second messenger systems. The DAD2-like receptors are coupled to Gi-protein and inhibit the formation of cAMP. The DAD2 receptors tertiary structure is stabilized by two cysteine disulphide bridges.

The DAD2 receptor gene encodes a protein that extends for 415 amino acids. Similar to other GPCR, the DAD2 receptor has seven transmembrane segments, but in contrast to DAD1 -like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. Unlike the DAD1 -like receptor genes, the DAD2 receptor gene contains seven introns that are spliced out during mRNA transcription (Fischer *et al*., 1989).

Pharmacologically, both isoforms exhibit nearly similar profiles in terms of their affinities to different DAD2 selective agents and inhibit adenylyl cyclise activity. However, these isoforms display an opposite regulatory effect (Sibley *et al*., 1993). These isoforms have the same pharmacological profile, even though a marginal difference in the affinity of some substituted response to DA treatment is reported: DA induces the up regulation of DAD2L isoform of DAD2 receptors.

When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc *et al*., 1998; Sibley, 1999).

Dopamine D3 receptors

DAD3 receptor gene contains five introns and encodes a 446 amino acid protein (Schwartz *et al*., 1992). The gene encoding this receptor resides on chromosome 3 (Giros *et al*., 1990). The DAD3 receptors bear close structural and pharmacological similarities to the DAD2 receptors. DAD3 mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz *et al*., 1992). Distribution of DAD3 receptor mRNA is distributed and expressed mainly in subcortical limbic regions including islands of Calleja, nucleus accumbens septi and olfactory tubercle, with low levels of expression in the basal ganglia. D3 receptor mRNA has also been found in neurons of the cerebellum, which regulate eye movements (Levesque *et al*., 1992). The status of the DAD3 molecular entity as a functional receptor remains uncertain since it neither couples to G-proteins nor consistently transduces an effector mechanism. However, the structural similarity with DAD2 receptor raises the possibility that DAD3 receptor also inhibit adenylyl cyclase activity in its normal cellular setting.

Dopamine D4 receptors

DAD4 receptor gene contains four introns and encodes a 387 amino acid protein (Van Tol *et al*., 1991). The overall homology of the DAD4 receptor to the DAD2 and D3 receptors is about 41% and 39% respectively, but this homology increases to 56% for both receptors when only the transmembrane spanning segments are considered. The gene encoding the human DAD4 protein is located at the tip of the short arm of chromosome 11 (Civelli & Bunzow., 1993; Missale *et al*., 1998). DAD4 receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes. The stimulation of DAD4 receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Misalle *et al*., 1998). In humans, DAD4 receptor occurs in several genomic polymorphic variants that contain two to eleven repeats of a 48 base pair

segment that is expressed in the third cytoplasmic domain (Van Tol *et al*., 1992; Misalle *et al*., 1998). These are called the DAD4 alleles which are represented as DAD4.2, D4.4 and D4.7. This contributes to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind., 1994).

Dopamine D5 receptors

The DAD5 receptor gene is intron less and encodes a protein that extends for 47 amino acids (George *et al.,* 1991). This protein has an overall 50% homology with DAD1 receptor and 80% if only the seven transmembrane segments are considered. The gene encoding the human DAD5 protein is located at the short arm of chromosome 4, the same region where the Huntington disease gene has been located. Two DAD5 receptor pseudogenes having 154 amino acids have been identified with 90% homology (Gusella, 1989). These pseudogenes, however, contain stop codons in their coding regions that prevent them from expressing functional receptors.

DAD5 receptor mRNA expression is unique and limited to the hippocampus and parafascicular nucleus of the thalamus (Civelli *et al*., 1992). It is involved in the thalamic processing of painful stimuli (Giesler *et al*., 1979). DAD5 receptors appear to interact with G-proteins and can stimulate adenylyl cyclase, with relatively high affinity for DA and DAD1 -selective agonists (George *et al*., 1991).

DOPAMINE AND ITS RECEPTOR ALTERATIONS DURING HYPOGLYCEMIA

The dopaminergic system with its D1 and D2 type DA receptors and coupling to second messengers beginning day 17 of embryonic age and completing maturation on day 14 after birth (Money & Stanwood., 2013); and the DA vesicular transport maturing as early as day 8 after birth (Leroux-Nicollet *et al*., 1990). DA controls the expression of certain genes such as c- fos involved in

neuronal transduction during development in critical periods such as the first 15 days after birth in the striatum (Arnauld *et al*.,1995)

As the blood glucose levels progressively drop in hypoglycemia to the range of 1–2 mM, changes in the brain monoamines dopamine, noradrenaline and serotonin already occur at this stage, explaining the changes in cerebration that occur in the early, pre-coma stages of hypoglycemia (Aurer, 2004). DA, like glutamate, can also be a potent excitotoxic agent. High levels of DA in the synaptic cleft can be rapidly oxidized to form DA semiquinone/quinine. In addition, oxidized DA *via* monoamine oxidase activity or redox cycling can induce the generation of hydrogen peroxide and superoxide causing significant oxidative stress (Bales *et al*., 2010). It was suggested by Milusheva *et al*., 2006 that in response to hypoxia combined with hypoglycemia there is a massive release of glutamate due to the increased firing rate which in turn releases dopamine from the axon terminals through stimulation of presynaptic NMDA receptors.

DA signalling has been implicated in the control of food intake and body weight. In particular, DA is important in the control of meal size and number and is thought to mediate the response to metabolic deprivation states (Benoit *et al*, 2003). Both appetitive and consummatory responses to glucose deprivation are controlled and coordinated by multilevel terminations of the catecholamine neurons. the catecholamine neurons themselves are glucoreceptive (Ritter *et al*., 2006).

In brain slices, dopamine neurons are seen to undergo hyperpolarization during hypoxia and hypoglycemia, which results in silencing of the neurons (Singh *et al*., 2007). Following striatal hypoxic ischemica injury, there was also a reduction in the number of dopaminergic neurons. (Burke *et al*., 1992). As dopamine and dopaminergic neurocircuitries are extremely sensitive to hypoxia and ischemia at birth (Klawitter *et al*., 2007), perinatal or postnatal hypoxic ischemia brain injury is associated with dopaminergic neuronal loss, and resulted in cognitive and motor impairments (Andreeva *et al*., 2001; Meng *et al*., 2006).

And hence, the dopamine neurotransmission is looked into in great detail in our study giving importance to one member each of the DAD1 like family and DAD2 i.e. DAD1 and DAD2 respectively.

Signal transduction by Dopaminergic activation

Physiological levels of DA promotes neuronal plasticity and exerts a neuroprotective role though receptor-dependent mechanism (Schapira, 2002; Yao *et al*., 2008). Changes in multiple transmitter systems underlie major mechanisms leading to the dopaminergic neurodegenerative disorders of neonatal hypoglycemia, and in many cases these dysfunctions precede the onset of mental retardation. It is when these pathways converge with other biological or environmental influences, leading to DA hyperfunction. DA receptors have been shown to regulate pathways through G protein-mediated signalling. G-proteins transduce the activation of neurotransmitter receptors into alterations in intracellular levels of second messengers in target neurons (Beaulieu & Gainetdinov., 2011)**.** Prominent second messengers in brain include cAMP, cyclic GMP (cGMP), calcium, the major metabolites of phosphatidyl inositol (PI), IP3 and diacyl glycerol (DAG) and of arachidonic acid, and nitric oxide (NO).

SECOND MESSENGER CHANGES DURING NEONATAL HYPOGLYCEMIA

Second messengers relay signals received at receptors on the cell surface to target molecules in the cytosol and/or nucleus. Three major classes of second messengers are (1) cyclic nucleotides (e.g., cAMP and cGMP), (2) IP3 and DAG, (3) calcium ions (Ca^{2+}). The signal transduction in metabotrophic neurotransmitters occur through activation of second messengers, whereas ionotrophic neurotransmitters act through ligand gated ion channels. The changes in neurotransmitter level and its receptor should agree with a concomitant change in second messenger for effective signal transduction.

Cyclic adenosine monophosphate (cAMP)

cAMP, cyclic AMP or 3'-5'-cyclic adenosine monophosphate is a second messenger, important in many biological processes. cAMP is derived from adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway (Abramovitch *et al*., 2004). cAMP is synthesized from ATP by adenylate cyclase located on the inner side of the plasma membrane. Adenylate cyclase is activated by a range of signalling molecules through the activation of adenylate cyclase stimulatory GPCR (G_s) and inhibited by agonists of adenylate cyclase inhibitory GPCR (G_i) (Dumas *et al*., 2006). cAMP is used for intracellular signal transduction, such as transferring into cells the effects of hormones , which cannot pass through the cell membrane. It is involved in the activation of protein kinases and regulates the effects of adrenaline and glucagon. cAMP and its associated kinases, function in biochemical processes including the regulation of glycogen, glucose and lipid metabolism. cAMP also binds to and regulates the function of ion channels (Simpson *et al*., 1996).

cAMP is involved in neuronal survival and protection. D1 GPCR receptors down regulation inactivates adenylyl cyclase through G-protein alpha(olf) (Golf alpha) subunits leading to decreased cAMP levels and inactivation of protein kinase A (PKA). DA binding to D2 GPCR receptors furthur lowers cAMP levels by inhibiting adenylyl cyclase through G-protein alpha(i) (Gαi) subunits (Kim *et al*., 2004). This leads to the closing of the voltage dependant Ca^{2+} channels via a cAMP independent mechanism thus altering the normal cellular signal transduction.

Studies by Wang *et al*., 2007, suggests that cAMP affects the function of higher-order thinking in the prefrontal cortex through its regulation of ion channels called hyperpolarization - activated cyclic nucleotide-gated channels (HCN). When cAMP stimulates the HCN, the channels open, closing the brain cell to communication and thus interfering with the function of the cortex.

Although the precise dynamics of memory/learning processing remains a little-understood process, it involves dopaminergic neurons of the mesolimbic pathway that release the neurotransmitter into the presynaptic space in response to a motivation- related action potential. DA then binds and activates dopamine receptors, such as the DA receptor D1, GPCR that modulates the cAMP second messenger to produce a cellular response ultimately triggering an action potential by opening plasmalemmal ion channels (Rössger *et al*., 2013).

Inositol 1,4,5-trisphosphate (IP3)

IP3 receptors (IP3R) are the IP3 gated intracellular Ca^{2+} channels that are mainly present in the endoplasmic reticulum (ER) membrane. Many biological stimuli, such as neurotransmitters and hormones, activate the hydrolysis of PIP2, generating IP3. The IP3 mediates Ca^{2+} release from intracellular Ca^{2+} stores by binding to IP3 receptors. The IP3 induced Ca^{2+} signalling plays a crucial role in the control of diverse physiological processes such as contraction, secretion, gene expression and synaptic plasticity (Berridge, 1989).

IP3 diffuses through the cytosol to bind to IP3 receptors, particularly, calcium channels in the smooth endoplasmic reticulum. This causes the cytosolic concentration of calcium to increase, causing a cascade of intracellular changes and activity. In addition, calcium and DAG together work to activate protein kinase C, which goes on to phosphorylate other molecules, leading to altered cellular activity (Alberts *et al*, 2002).The conventional mechanism by which GPCRs stimulate Ca^{2+} release involves stimulation of Gq/11 and resultant phospholipase C β (PLC β) catalyzed hydrolysis of PIP2 to IP3 (phospholipase C β (PLC β) catalyzed hydrolysis of PIP2 to IP3 (Qin, 2000). Binding of IP3 to IP3 receptors localized on vesicular stores opens these channels, resulting in increased cytosolic Ca^{2+} levels (Berridge *et al*. 1998).

In mammalian cells, there are three IP3R subtypes, type 1 (IP3R1), type 2 (IP3R2), and type 3 (IP3R3), which are expressed to varying degrees in individual

cell types (Taylor *et al.*, 1999) and form homotetrameric or heterotetrameric channels (Monkawa *et al*., 1995).

IP3 receptors and calcium release channels in the ER membrane, play a key role in regulating intracellular calcium concentration. IP3R type 1, a major neuronal type of IR_3R, is expressed ubiquitously and is involved in diverse biological processes. Increased cytosolic free Ca^{2+} and lowered mitochondrial transmembrane potential after ethanol exposure in the developing neonatal brain is seen to significantly decrease the expression of anti-apoptotic protein (Bcl-2), increase expression of proapoptotic protein Bax, and stimulate the release of cytochrome-c from mitochondria in primary rat cortical neurons (Nandhu *et al*., 2011).

Glucose homeostasis in humans is an important factor for the functioning of the nervous system. A decrease in glucose content below a minimal level or hypoglycemia is dangerous for cells of the central and peripheral nerve system. Studies by Anu *et al*., 2010, showed that at the second messenger level, the IP3 content and IP3 receptors were enhanced in the cerebellum of both hypoglycemic and diabetic rats. Enhanced glutamate content in the brain activates NMDA receptors, which increases the IP3 content mediating Ca^{2+} overload in cells, thus causing cell damage and neurodegeneration. Hypoxic insult to neonatal rats is also seen to increase the brain cAMP, cGMP and IP3 levels as an adaptive response (Anju *et al*., 2010).

SIGNAL TRANSDUCTION IN DOPAMINE NEUROTRANSMISSION

Phospholipase C (PLC)

PLC belongs to a class of enzymes that cleave phospholipids just before the phosphate group. Thirteen kinds of mammalian PLC are classified into six isotypes (β, γ, δ, ε, ζ, η) according to structure. Receptors that activate this pathway are mainly GPCR coupled to the $G_{\alpha q}$ subunit, include 5-HT_2 serotonergic receptors, α_1 (Alpha-1) adrenergic receptors, Calcitonin receptors, H_1 histamine receptors, Metabotropic glutamate receptors Group I , M_1, M_3, and M_5 muscarinic

receptors, Thyroid Releasing Hormone receptor in anterior pituitary gland. PLC regulates various cellular processes by catalyzing the formation of IP3 and DAG from PIP2. Lee *et al.*, 2002, demonstrated that the co-activation of co-expressed D1R and D2R results in a PLC-mediated increase in intracellular Ca^{2+} levels that is independent of Ca^{2+} priming.

Phosphoinositide-specific PLC C-g1 is an important signalling regulator involved in various cellular processes. In brain, PLC-g1 is highly expressed and participates in neuronal cell functions mediated by neurotrophins. It is involved in development of brain and synaptic transmission. Significantly, abnormal expression and activation of PLC-g1 appears in various brain disorders such as epilepsy, depression, Huntington's disease and Alzheimer's disease. Thus, PLC-g1 has been implicated in brain functions as well as related brain disorders (Jang *et al*., 2013).It is also seen that during brain injury, significant rise in PLC activity is responsible for generating DAG that is source of free arachidonic acid that stimulates prostaglandin synthesis. These changes account for the rise in brain prostaglandin levels that occur after brain injury, aggravating damage (Wei *et al*., 1982).

cAMP response element-binding protein (CREB)

CREB is a cellular transcription factor (Mioduszewska *et al.,* 2003). It binds to certain DNA sequences called cAMP response elements (CRE), thereby increasing or decreasing the transcription of the downstream genes. CREB was first described in 1987 as a cAMP-responsive transcription factor regulating the somatostatin gene. Genes whose transcription is regulated by CREB include: c-fos, the neurotrophin BDNF (Brain-derived neurotrophic factor), tyrosine hydroxylase, and many neuropeptides (such as somatostatin, enkephalin, Vasular growth Factor, and corticotropin-releasing hormone) (Wang *et al*., 2009). CREB is closely related in structure and function to cAMP response element modulator and ATF-1 proteins.

CREB has long been implicated in neuronal function, with much recent interest centered on its role in the maintenance of long-term memory. several studies involving overexpression of dominant-negative CREB suggested a role for CREB as a survival factor in various cellular models, possibly acting downstream of the AKT/PKB survival pathway (Mantamadiotis *et al*., 2002).

CREB proteins are expressed in many animals, including humans. CREB is a transcription factor that has been shown to be integral in the formation of spatial memory (Silva, 1998). CREB possess therapeutic potential for patients that have Alzheimer's disease. CREB has a well-documented role in neuronal plasticity and long-term memory formation in the brain. Glycogenolysis and astrocytic lactate transporters in the brain were shown to be critical for the induction of molecular changes required for memory formation, including the induction of phosphor CREB, activity-regulated cytoskeletal-associated protein, and phospho-cofilin (Suzuki *et al*., 2011).

Impaired GABA receptor and CREB expression along with motor function deficit were prominent in hypoglycemic rats which neuronal damage at molecular level. These molecular changes observed during hypoglycemia contribute to motor and learning deficits which has clinical significance in diabetes treatment (Antony *et al*., 2010). Studies by Thorn *et al*., 2012 showed that sheep fetuses with intrauterine growth restriction and hypoglycemia, have increased hepatic phosphorylation of CREB thus supporting the concept that increased counter-regulatory hormone-mediated cAMP activation drives activation of glucose production. Hence, the early induction of gluconeogenesis in fetal life produces persistent and detrimental effects across the life span.

GLUCOSE TRANSPORT ACROSS THE BLOOD – BRAIN BARRIER

Glucose transporter 3 (GLUT -3)

The brain has high energy requirements. About 20% of the oxygen and 25% of the glucose consumed by the human body are dedicated to cerebral functions, yet the brain represents only 2% of the total body mass. Maintenance and restoration of ion gradients dissipated by signalling processes such as

postsynaptic and action potentials, as well as uptake and recycling of neurotransmitters, are the main processes contributing to the high brain energy needs (Attwell & Laughlin, 2001; Alle *et al*., 2009).

Glucose transporters (GLUT) are a wide group of membrane proteins that facilitate the transport of glucose over plasma membrane. Glucose enters cells trough specific glucose transporters and is phosphorylated by hexokinase to produce glucose-6-phosphate. As in other organs, glucose 6-phosphate can be processed via different metabolic pathways, the main ones being (1) glycolysis (leading to lactate production or mitochondrial metabolism), (2) the pentose phosphate pathway (PPP), and (3) glycogenesis. Overall, glucose is almost entirely oxidized to CO_2 and water in the brain (Sokoloff, 1999).

Facilitative glucose transporters are a family of structurally related membrane-spanning glycoproteins that mediate transport of glucose. across lipid bilayers (Thorens & Mueckler, 2010). Of the 14 isoforms, GLUT 1 and GLUT 3 play a significant role in trans-placental glucose transport and embryonic development (Carruthers *et al*., 2009). Both are expressed in the mammalian trophectoderm and brain with GLUT 1 being expressed in the blood–brain barrier and GLUT 3 in neurons. GLUT 3, expressed in mammalian neurons and trophoblasts, mediates glucose transport and availability for fueling oxidative metabolism (Simpson *et al*., 2008).

Mouse brain expresses three principal glucose transporters. GLUT 1 is an endothelial marker and is the principal glucose transporter of the blood-brain barrier. GLUT 3 and GLUT 6 are expressed in glial cells and neural cells (Stuart *et al*., 2011). GLUT 3 expression upregulation causes inhibition on neuronal apoptosis (Xue *et al*, 2010). During the neonatal period, when parenchymal cellular proliferation is at a peak, GLUT 3 is localized not only to the microvasculature but also to certain cells which express glial morphological characteristics (Carruthers *et al*., 2009).

Hypoglycemia induces progressive reduction in cerebral glycogen and glucose, which is due to an increase in gene expression of GLUT 3, the glucose

transporter rather abundant in the brain. Alteration of expression of GLUT 3 in the cerebral cortex in hypoglycemia is the evidence for impairment of neuronal glucose transport during glucose deprivation. The impaired transport and utilization of neuronal glucose in hypoglycemia is likely to be an important factor contributing to an increase of neuronal vulnerability. The disturbances of neuronal glucose transport and metabolism in hyperglycemia are similar to those in hypoglycemia and also induce neuronal damages and CNS disorders (Antony *et al*., 2010).

Recent Studies by Carayannopoulos *et al*., 2014, showed that impaired embryonic GLUT 3 expression is associated with increased apoptosis and an associated decline in growth potential, ultimately leading to embryo demise, in GLUT 1 knock down mice. In addition, brain development is deranged suggesting that the presence of normal concentrations of GLUT 1 expression is not sufficient to save the GLUT 3 morphants from developing such phenotypic changes.

NEURONAL SURVIVAL FACTORS

Neuronal viability is maintained through a complex interacting network of signalling pathways that can be perturbed in response to a multitude of cellular stresses. A shift in one or more of these signalling pathways can alter the fate of a neuron resulting in cell death or continued survival. The nature of the stresses affecting neurons, the duration of the stresses, the developmental stage of the neuron and a variety of other factors influence the signalling pathways that are ultimately affected. These diverse parameters also regulate the temporal response as well as the final disposition of the affected neurons. Neurotrophins have also been reported to protect central neurons (e.g., hippocampal and neocortical) during hypoglycemia and other stresses *in vitro* and *in vivo* (Chaverneff & Barrett, 2009).

Levi-Montalcini and Cohen (Levi-Montalcini and Hamburger, 1966; Cohen *et al*.,1960) were first to describe the nerve growth factor (NGF) and for that discovery they won the Nobel Prize in Physiology or Medicine in 1986. Afterwards, several novel structurally homologous neurotrophic factors belonging to the nerve growth factor family termed neurotrophins were discovered in

vertebrates. These were BDNF, NT-3, NT-4/5 (Barde *et al*., 1982, Phillips *et al*., 1990, Ibáñez *et al*., 1993). Other members of neurotrophins neurotrophin-6 (NT-6) and neurotrophin-7 (NT-7) were only cloned from some teleost species (Götz *et al*., 1994, Lai *et al*., 1998) and are not expressed in other vertebrate thanteleost fishes.

The mechanism(s) underlying the protective effects of neurotrophins during hypoglycemic stress remains to be determined; one mechanism appears to involve reduction of stress-induced increases in intracellular Ca^{2+} (Cheng & Mattson, 1994). The ability of NGF protects cholinergic function of neurons after hypoglycemic stress explains the finding of Svendsen *et al*. (1994), that NGF increased the number of cholinergic neurons survival. BDNF and NGF increased survival of cholinergic neurons and choline acetyl transferase (ChAT) activity after a hypoglycemic stress (Antony *et al*., 2010).

Glia - derived neurotrophic factor (GDNF)

GDNF is well known to be a potent neurotrophic factor supporting the survival of dopaminergic neurons of the SN *in vitro* and *in vivo*. Many studies with *in vitro* and *in vivo* models have shown that GDNF supports neuritic outgrowth or survival of mesencephalic dopaminergic neurons (Connor & Dragunow., 1998), cranial nerve and spinal cord motor neurons, brain stem noradrenergic neurons (Arenas *et al.*, 1995), basal forebrain cholinergic neurons, Purkinje cells and certain groups of dorsal ganglion and sympathetic neurons (Siegel & Chauhan., 2000). The binding of GDNF to GFRα receptors activates a transmembrane tyrosine kinase, c-Ret and induces further downstream signalling *via* multiple pathways including the MAP kinase (Mitogen-activated protein kinase) pathway and PLCγ pathway. GDNF also induces responses through c-Ret-independent mechanisms such as the activation of Src family tyrosine kinases and interaction of the receptor complex with neural cell adhesion molecule (Sariola & Saarma., 2003). GDNF has been shown to exert neurotrophic effects both at the level of the cell bodies in the substantia nigra and at the level of the axon terminals

in the striatum. Intrastriatal administration of GDNF appears to be a particularly effective site for induction of axonal sprouting and regeneration accompanied by recovery of spontaneous sensorimotor behaviours in the chronically lesioned nigrostriatal DA system (Björklund *et al.*, 1997). GDNF treatment of cultured human fetal ventral mesencephalon nearly doubles the DA neuron survival while halving the rate of apoptosis from 6% to 3% (Clarkson *et al.*, 1997).

The GDNF was first identified as a survival factor for midbrain dopaminergic neurons, but additional studies provided evidences for a role as a trophic factor for other neurons of the central and peripheral nervous systems. GDNF regulates cellular activity through interaction with glycosyl-phosphatidylinositol-anchored cell surface receptors, GDNF family receptor-α1, promotes cell survival, neurite outgrowth, and synaptogenesis. neuroprotection by GDNF was observed when administered after the ischemic injury in the neonatal brain (Duarte *et al*., 2012).

Brain-derived neurotrophic factor (BDNF)

NGF family includes BDNF, which is primarily expressed in the brain but are also expressed in peripheral tissues, demonstrating interactions between peripheral and central systems. It plays an important role in the survival, function and adaptive plasticity of neurons in the adult brain. Regulation of AKT has been associated with the action of insulin, insulin related peptides and neurotrophins (i.e. BDNF) that exert their biological function by stimulating receptor tyrosine kinase. AKT activation results in the up-regulation of BDNF. BDNF was the first neurotrophin to be isolated after the discovery of nerve growth factor and was found to not only support the survival of neurons but also promote the outgrowth of fibers (Chen & Russo-Neustadt., 2009)

BDNF signalling occurs via two major classes of receptors (Huang & Reichardt., 2003). The first class of receptors is the tropomyosin-related kinase (Trk) family of receptor TK (Tyrosine Kinase), specifically TrkB. Through TrkB, BDNF activates Ras, phosphatidyl inositol-3 (PI3)-kinase, PLC and consequently, the MAP kinases. Additionally, BDNF activates the p75 neurotrophin receptor

(p75NTR), resulting in the activation of the nuclear factor-κB (NF-κB) and Jun kinase. BDNF mRNA is expressed widely throughout the SN, hippocampus, cortex, cerebellum and basal forebrain (Wong *et al.*, 1997) and its receptor TrkB is expressed within the striatum and the SN (Wong *et al.*, 1997). BDNF protects against apoptotic death by inhibiting caspase activation following neuronal injury (Kim *et al*., 2002).

The highest levels of BDNF are observed perinatally, and then it declines with age, although the proform remains detectable in adulthood. This provides the reason that brains of newborns and infants are more fragile to ischemia stroke due to low-frequency neuronal activities and the lack of an adequate amount of mature BDNF in the CNS. BDNF promotes neuronal survival via TrkB, preferentially activating p75 to mediate neuronal cell death, particularly apoptosis. Therefore, the amount of pro-BDNF is critical in neuronal cell death (Chen *et al*., 2013).

Nuclear Factor B (NFκB)

NF-*κ*B is a transcriptional factor that plays an important role in regulating the transcription of a number of genes, especially those involved in producing mediators involved in local and systemic inflammation, such as cytokines, chemokines, cell adhesion molecules and apoptotic factors. The transcription factor NF-κB is a dimeric complex of proteins of the Rel-family that regulate several cellular functions upon activation by a variety of extracellular stimuli (Piette *et al.*, 1997). In the nervous system, NF-*κ*B is found in both glia and neurons (O'Neil & Kaltschmidt., 1997) and its activation is considered as a regulator of cell-stress response particularly oxidative stress.

NF-*κ*B plays a vital role as a critical regulator of cell death (Barkett & Gilmore., 1999). The inhibition of NF-*κ*B activity participates in the neuroprotective effect of melatonin and nor-melatonin against H_2O_2 insult (Lezoualc'h *et al.*, 1998a).

The anti-apoptotic function of NF-*κ*B is supported by several studies showing that the activation of NF-*κ*B protects primary neuronal cells against β-

amyloid (Mattson *et al.*, 1997), mediates NGF-promoted survival of PC12 cells (Taglialatela *et al.*, 1997; Foehr *et al.*, 2000) and increases the resistance of neuronal cells against H_2O_2 (Lezoualc'h *et al.*, 1998[b]). Therefore, NF-κB activation corresponds to a protective mechanism against deleterious effects of these neurotoxins (i.e. oxidative stress) (Cassarino *et al.*, 2000; Park *et al.*, 2004; Hu *et al.*, 2010). NF-κB activation modulates the occurrence of cell death and would be an interesting potential target site for neuroprotection. In neonatal hypoglycemia, NF-κB, potentiate injury by promoting the synthesis of proinflammatory mediators at the site of injury (Rao *et al*., 2009).

OXIDATIVE STRESS AND NEONATAL HYPOGLYCEMIA

The brain is particularly susceptible to the attack of ROS/RNS due to several factors like an enriched content of polyunsaturated fatty acid chains in cell membranes, a high demand of oxygen and a considerable dependence on a redox metabolism with limited antioxidant defense system (Ozben, 1998). Neuronal cells in the brain are highly sensitive to oxidative stress due to their large dependence on oxidative phosphorylation for energy as compared to other cells (Moura *et al*., 2010). The human body is exposed to exogenous and endogenous free radicals. The cells necessitate oxygen to produce the energy. During mitochondrial respiration, the cells take in oxygen, burn it, release energy and free radicals are produced. Oxidative stress occurs when the antioxidant production is decreased or free radical production exceeds the body's ability to neutralize them. Oxidative stress is defined as a tissue injury induced by increase in reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$), and hydroxyl radical ($^{\bullet}OH$). Oxidative damage to various brain regions constitutes into the long term complications, morphological abnormalities and memory impairments (Fukui *et al.,* 2003).

The reactive oxygen intermediates produced in mitochondria, peroxisomes and the cytosol are scavenged by cellular defending systems including enzymatic (eg. superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase and catalase) and nonenzymatic antioxidants (ex.

glutathione G-SH, thioredoxin, lipoic acid, ubiquinol, albumin, uric acid, flavonoids, vitamins A, C and E). Antioxidants are located in cell membranes, cytosol and in the blood plasma (Maritim *et al*., 2003).

Superoxide Dismutase (SOD)

The brain and nervous system is especially prone to oxidative damage for a number of reasons (Ozben, 1998): the membrane lipids are especially rich in polyunsaturated fatty acid side-chains, which are prime targets for free radicals attack; the brain has only moderate amounts of CAT, SOD and GPx.

DA can cause oxidative stress and significant cellular dysfunction when either depleted or over-expressed, and also plays an important role in central nervous system inflammation (Bales *et al*., 2010). Neonatal hypoglycemia has been reported to increase SOD activity within the brain which in turn makes the brain more vulnerable to damage (Kadekaro *et al.*, 1988). During prolonged periods of stress, exhaustion of neuronal defense mechanisms, such as anti-oxidant enzymes, reported to increase neuronal vulnerability to the point where neuronal adaptation shifts from neuronal plasticity towards neuronal damage (Reagan *et al.*, 1999).

Glutathione peroxidase (GPx)

Cells have an enzymatic antioxidant pathway against ROS which are generated during oxidative metabolism: firstly, SOD catalyzes the formation of hydrogen peroxide from superoxide radicals, which is removed by a reaction catalyzed by CAT and GPx (Michel *et al*., 1994). The neonatal brain is especially at risk of free radical mediated injury because neuronal membranes are rich in polyunsaturated fatty acids and the human newborn has a relative deficiency of brain superoxide dismutase and glutathione peroxidise (Buonocore *et al*., 2001). Normally, various antioxidant enzymes protect the body from these free radicals, but in hypoxic situations, there is explosive free radical production leading to swamping of the enzyme systems and as a result free radicals escape inactivation

(Chawla & Lavaniya., 2001). It is seen that hypoglycemia rather than hyperglycemia cause more damage in the developing brain of newborn rats.

NEONATAL HYPOGLYCEMIA AND APOPTOSIS

The brain is mainly a glucose-dependent organ, which can be damaged by hyper- as well as by hypoglycemia (Scheen, 2010). The disturbances of neuronal glucose transport and metabolism in hyperglycemia induce neuronal damages and CNS disorders. Synaptic defects undoubtedly contribute to the memory and cognitive defects that accompany neurodegeneration. However, the overwhelming feature of most neurodegenerative disorders is excessive neuronal cell death.

Cell death occurs by necrosis or apoptosis (Wyllie *et al.,* 1980; Martin, 2001; Kanduc *et al.,* 2002). These two mechanisms have distinct histologic and biochemical signatures. In necrosis, the stimulus of death (e.g., ischemia) is itself often the direct cause of the demise of the cell. In apoptosis, by contrast, the stimulus of death activates a cascade of events that orchestrate the destruction of the cell. Unlike necrosis, which is a pathologic process, apoptosis is part of normal development (physiologic apoptosis) however, it also occurs in a variety of diseases (aberrant apoptosis).

Apoptotic cell death also known as programmed cell death, can be a feature of both acute and chronic neurologic diseases (Martin, 2001). In apoptosis, a biochemical cascade activates proteases that destroy molecules that are required for cell survival and others that mediate a program of cell suicide. The major executioners in the apoptotic program are proteases known as caspases (cysteine-dependent, aspartate-specific proteases). Caspases directly and indirectly orchestrate the morphologic changes of the cell during apoptosis. Caspases exist as latent precursors, which, when activated, initiate the death program by destroying key components of the cellular infrastructure and activating factors that mediate damage to the cells (Friedlander, 2003).

AKT -1

AKT is a member of the PI3K signal transduction of the enzyme family, which regulate cellular activation, inflammatory responses, chemotaxis and apoptosis. It is a serine/threonine kinase regulated through PI mediated signalling. PI3 K/AKT pathway is a pro-survival signalling system in neurons. Alterations in the upstream and downstream pathways of AKT have been found in many psychiatric disorders. AKT modulates cell survival and growth and has been reported to play a role in cell survival pathway.

Reduced activity of PI3K/AKT signalling pathway leads to the the cognitive impairment, synaptic morphologic abnormality, neuronal atrophy and dysfunction of neurotransmitter signalling in neonatal brain exposed to stress. Reduced levels of AKT increases the effects of risk factors on neurodevelopment attenuate the effects of growth factors on neurodevelopment. AKT regulates a number of downstream signalling cascades that impact on a variety of cellular activities including survival, differentiation, proliferation, migration, polarity and metabolism (Greene *et al.*, 2011). A number of *in vitro* studies have shown that activation of AKT is both necessary and sufficient to maintain survival of a range of different neuron types as well as of other cell types and that AKT mediates the neuronal survival-promoting activities of a variety of neurotrophic factors (; Duronio, 2008). AKT promotes neurite outgrowth (Read & Gorman., 2009) and increases axonal branching and regeneration (Namikawa *et al.*, 2000; Grider *et al.*, 2009). AKT signalling is important in mechanistic actions of dopaminergic receptors (Beaulieu *et al.*, 2007) and homeostatic regulation of dopaminergic transporters (Garcia *et al.*, 2005). Drugs targeting the dopaminergic system, used to treat PD, have been shown to be neuroprotective *via* AKT activation (Yu *et al.*, 2009).

Tumor necrosis factor alpha (TNF α)

Over the past decades, inflammation has been recognized as an important contributor to acute CNS injury in both neonates and adults. Inflammation can

cause injury or increase the vulnerability of the brain. Acute inflammation may fail to resolve and be shifted to a chronic inflammatory state and/or adversely affect brain development. Hypothetically, such harmful effects on the CNS could have longterm consequences and increase the risk of a variety of neurological disorders including CP, autism spectrum disorders, multiple sclerosis, schizophrenia, Alzheimer's and Parkinson's disease (Hagberg & Gressens., 2012).

TNF-α is a potent pro-inflammatory molecule, which upon engagement with its cognate receptors on target cells, triggers downstream signalling cascades that control a number of cellular processes related to cell viability, gene expression, ion homeostasis and synaptic integrity (Park & Bowers., 2010). There is conflicting evidence on the role of TNF-α in the injured brain, showing its potential effect in both processes of repair and of damage (Shohami *et al*., 1999).

Under normal conditions, the blood-brain barrier restricts soluble immune mediators and although lymphocyte immune surveillance does proceed, effective antigen presentation and costimulation are attenuated in brain creating a state of relative immune privilege. Thus, resident macrophages (microglia) have a special role not only in combating infections, but also in neonatal pathologies such as ischemia. TNF-α is one of the mediators that lead to the activation, proliferation and hypertrophy of mononuclear, phagocytic cells and to gliosis (Shohami *et al*., 1999).

Studies conducted by Guo *et al*., 2012, showed that in response to the ischemic injury in the developing brain, glial cells quickly become activated and undergo morphological transformations, and are accompanied by functional changes, such as increasing expression of cytokines: interleukins (IL-1β, IL-4, IL-6, IL-10), TNFα, interferons and chemokines . The accumulaiton of pro-inflammatory factors should further induce ischemic damages. ischemic insult resulted in over-activation of astrocytes and microglia, and thereby robustly elevated the mRNA expressions of TNFα and IL-1β in the brain.

TNF-α exposure during neonatal period can alter brain and behavior development in a dose and sex-dependent manner in mice (Babri *et al*., 2014). Pro-inflammatory cytokine expression within the brain, especially IL-1b and TNF-

α, is implicated in perinatal brain damage induced by pathogen components and/or ischemia both in experimental model and the human newborn brain (Brochu *et al*., 2013). Microglia elaborates TNF-α which triggers neuronal death cascades and exacerbate edema after CNS insults (Drabek *et al*., 2014).

Bax

In the central nervous system, programmed cell death or apoptosis is considered to be an important phenomenon that is related to neuron vulnerability to stress condition. Bax is a protein, identified as regulating molecules for programmed cell death. The Bax protein, a 21-kD proapoptotic member of Bcl-2 family, appears to be particularly critical to neurotoxicity during brain development in early periods (Heaton *et al*., 2013). A possible relationship between the localization and expression of Bax protein and the cell vulnerability in central nervous system is reported (Hara *et al.*, 2008).

In the developing brain there are reports that hypoxia–ischemia triggers a number injurious events in the immature brain: release of excitatory amino acids, increased intracellular calcium, accumulation of NO and other reactive oxygen species, loss of trophic factor support, and induction of c-jun kinases. At a certain threshold level, these upstream "stressors" will increase the pro- versus antiapoptotic Bcl-2 family protein balance, which will induce mitochondrial outer membrane permeabilization. Cyclophilin D- dependent opening of the mitochondrial membrane permeability transition plays a marginal role in the setting of the immature brain response to injury. Instead Bax- dependent mitochondrial outer membrane permeabilization appears to be the predominant mechanism of proapoptotic protein release, which agrees with earlier studies showing that Bax gene deletion reduces immature brain injury (Wang *et al*., 2010). One mechanism pertaining to the death of immature neurons is the accumulation of Bax, which is highly expressed in the immature brain, to mitochondria, where it cleaves pro-caspase-3 resulting in the activation of caspase-3.

Bax is one of the key proteins that turn on the apoptotic cascade. The expression of Bax in brain regions is taken as an index of the brain damage caused by hypoglycemic stress (Curton – Meyers *et al*., 2000). DA is carefully regulated by the CNS and alterations can lead to significant cellular dysfunction and/or death (Calabresi *et al*., 2000).

Caspase 8

Activation of the cellular suicide program results in a characteristic type of cell death called apoptosis. This program is intrinsic to all cells that make up vertebrate and invertebrate multicellular animals including nematodes, insects and mammals. The program has been conserved during the evolution of the animal kingdom and the final executioners are invariably caspases (Yakovlev & Faden, 2001).

The apoptotic component of acute CNS injury involves caspase activation, in particular the 'executioner' caspase 3 – a key effector of apoptosis (Springer *et al*., 1999; Eldadah & Faden, 2000). Indeed, selective caspase 3 inhibition may improve recovery after trauma or stroke (ChenG *et al*., 1998).

Caspases can be divided into two groups according to their structure and function: "initiator caspases" and "effector caspases". The former have characteristic protein-protein interaction domains that facilitate binding to adaptor proteins. Caspase-8 and caspase-10 (the latter is present in humans but not mice) have two death effector domains (DED) through which they interact with the adaptor protein FADD that has a single DED motif (Thornberry & Lazebnik., 1998).

Caspase 8 and caspase 9 are regarded as 'initiator' caspases, activated at a relatively early stage in apoptosis (Minano *et al*., 2003). Caspase 8 is typically associated with the 'extrinsic' or receptor-activated pathway of apoptosis, while caspase 9 mediates the mitochondrial apoptotic pathway following cytochrome C release. Caspase 3 is a key effector caspase thought to be central to neurodegenerative processes in the CNS (Citron *et al*., 2008).

Caspase-9 through its CARD domain and by way of a homotypic interaction is able to bind the adaptor Apaf-1 which also has a CARD motif. Adaptor molecules function to aggregate the initiator caspase zymogens and the induced proximity allows their low-level enzymatic activity to effect autocatalytic processing and throw the death switch on. The autoactivated initiator caspases are then able to process and activate effector caspases including caspases-3, -6 and -7. This sets in train a series of cascading and amplifying activation subroutines that proceed inexorably to cell collapse as critical cellular proteins are proteolytically destroyed. Caspase after caspase becomes activated and in turn inactive enzymes like CAD (caspase activated DNase) are liberated from their shackled state to become active and destructive proteins that, in the case of CAD, chew up the instruction code library of life, DNA (Kuida *et al.*, 1998).

Various sensors in the cell that detect intracellular perturbations or external death signals initiate the apoptotic program. Mammals have two distinct pathways that converge and feed into the central apoptotic processing unit with its effector caspases: the extrinsic and the intrinsic pathways. The two pathways utilize different adaptor molecules and different initiator caspases (Kuida *et al.*, 1996).

CURRENT TREATMENTS FOR NEONATAL HYPOGLYCEMIA

Following a hypoglycemic injury to immature brains, neurons begin to die over the following days. Preventing the neuronal death has been a focus for developing new therapeutic strategies.There are many treatments for neonatal hypoglycemia (Cornblath & Schwartz., 1976; Jones & Roberton., 1984): glucose infusion is one but it induce complications such as hyperglycaemia, rebound hypoglycemia after interruption of the infusion and hypersecretion of insulin which can induce recurrence of hypoglycemia; it also inhibit the compensative gluconeogenesis (Kalhan *et al.*, 1986) and ketogenesis. Corticoids are useful to prevent hypoglycemia but it limit the peripheral uptake of glucose (Sann *et al.*,

1983), an effect which results in an increased incidence of neurological and electro-encephalographic abnormalities (Jones & Roberton., 1984).

Ketone bodies, d-3-Hydroxybutyrate (3DHB) is used as an alternative energy substrate for the brain during hypoglycemia, especially in infancy. 3OHB treatment delays the onset of clinical and burst-suppression coma during hypoglycemia, but the prolonged duration of hypoglycemia is associated with increased mortality after resuscitation and cellular white matter injury (Schutz *et al*., 2011).

Bacopa monnieri

Treatment with herbal drugs has been in use since ancient times and herbs have been an effective source of treatment regimens for different diseases. In modern medicine, medicinal herbs are an integral part of alternative therapy. *Bacopa monnieri* L. (Fam. Scrophulariaceae) is a creeping, glabrous, succulent herb, rooting at nodes, distributed throughout India in all plain districts, ascending to an altitude of 1,320 m. The plant is reported to show sedative, antiepileptic, vasoconstrictor and anti-inflammatory activity (Handa, 1998). Its antioxidant properties and its ability to balance SOD and CAT levels were postulated to account for this effect (Sairam, 2001). It has been reported that the plant contains tetracyclic triterpenoid saponins, bacosides A and B, hersaponin, alkaloids viz. herpestine and Bacopin and flavonoids (Jobin *et al*., 2010; Handa, 1998).

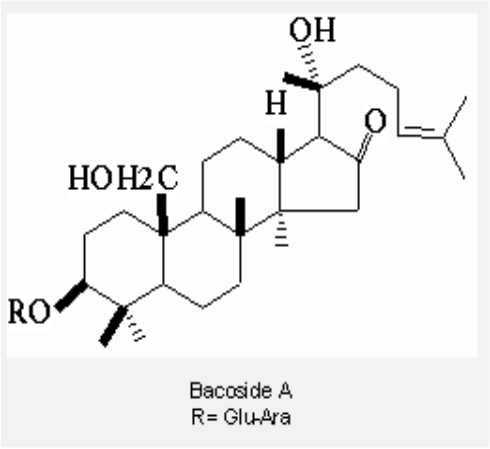
Bacopa monnieri (Brahmi) is recommended in formulations for the management of a range of mental conditions including anxiety, poor cognition, lack of concentration and epilepsy. Pharmacologically, it is understood that Brahmi has an unusual combination of constituents that are beneficial in mental inefficiency and illnesses and useful in the management of convulsive disorders like epilepsy (Gupta *et al*., 2013). Therapeutic uses include improving intellect on consciousness and mental activity, calming the mind and promoting relaxation by increasing protein synthesis and activity in brain cells, improving memory, mental clarity and longevity and also decreasing anxiety, restlessness & senility.

Bacoside A

Bacosides are the putative bioactive component of the Indian medicinal plant *Bacopa monnieri* which was placed second in the most important medicinal plants' list by the Export-Import Bank of India. Among the bacoside components, bacoside A was found to be more pharmacologically active than bacoside B. Traditionally, *Bacopa* has been used in ayurvedic medicines as a cure for mental disorders and loss of memory. Later on, other pharmacological properties like antioxidant, antidepressant, antiulcer, hepatoprotective, anticancerous, vasodilator, smooth muscle relaxant, mast cell stabilizer, and various other functions are revealed. Increasing clinical trials indicate the potential role of bacosides even in Alzheimer's disease and in epilepsy (Jobin *et al*., 2000). Bacosides attribute to the neuroprotective function mainly through modulating antioxidant enzymes, namely, SOD, CAT, etc. Bacosides also regulate the levels of different neurotransmitters in the brain. Interestingly, bacosides do not exert any side effects as proven both in animal models and in human volunteers. These features render *B. monnieri* as well as bacosides pharmacologically immensely important (Sukanya *et al*., 2013).

The drug is characteristically designated on the basis of its total bacosides content which are tetra cyclic triterpenoid saponins. These are Bacoside A and Bacoside B. Bacoside A, a triterpenoid saponin, is a major constituent isolated from the plant *Bacopa monniera* Linn. Used as a memory herb and for mental enhancement. This substance appears to have antioxidant and liver protective potential. Bacoside A is thought to be the active ingredient in bacopa herb along with bacoside B. There are various standardized extracts of bacopa containing different percentages of bacosides. Two common bacopa extracts include bacosides at 20 percent and 40 percent. Bacoside A protects the brain from the oxidative damage through its antioxidant potential (Anbarasi *et al*., 2006). Bacoside administration was seen to enhance learning ability in rats along with augmentation in memory retrieval and prevention of dendritic atrophy following hypoxic exposure. In addition, it decreased oxidative stress, plasma corticosterone

levels and neuronal degeneration. Bacoside administration also increased cytochrome c oxidase activity along with a concomitant increase in ATP levels. Hence, administration of bacosides could be a useful therapeutic strategy in ameliorating hypobaric hypoxia induced cognitive dysfunctions and other related neurological disorders (Kunte & Kuna, 2013).



Bacoside A
R= Glu-Ara

Significance

Currently, our understanding on the effects of and the extent of impact of hypoglycemic shock in triggering brain injury in the developing brain is incomplete. Prompt recognition, specific diagnosis, and aggressive treatment, therefore, are essential if central nervous system damage is to be prevented. This is an important area of study given the significant motor and cognitive impairment that may arise from neonatal hypoglycemia if proper treatment is not implemented.

Thus we have worked on the *Bacopa monnieri* whole plant extract and Bacoside A - one of the active components present in *Bacopa*. This is to validate the function of this plant extract for immediate medical use. *Bacopa monnieri* is currently recognized as being effective in the treatment of mental illness and epilepsy (Russo *et al*., 2003). But so far there has been no study reporting the role of *Bacopa monnieri* treatment on the functional regulation of DA neurotransmission. Hence, early recognition of hypoglycemia and emergency

therapeutic intervention is vital for early return of neuronal function and decreasing the risks for permanent neuronal damage.

Materials and Methods

CHEMICALS USED IN THE STUDY AND THEIR SOURCES

Biochemicals

Sulpiride, SCH 23390, dextrose, bovine serum albumin, ethylene diamine tetra acetic acid (EDTA), sucrose, magnesium chloride, calcium chloride, pargyline, ascorbic acid, Tris HCl, foetal calf serum (heat inactivated), D-glucose and paraformaldehyde (PFA) were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade purchased locally from SRL, India. Tissue freezing medium, Jung was purchased from Leica Microsystems Nussloch GmbH, Germany.

Radiochemicals

[^{3}H]SCH 23390 (Sp. activity 83Ci/mmol) and [^{3}H]YM-09151-2 (*cis–N-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide* Sp. activity - 85.0Ci/mmol) were purchased from NEN Life Sciences Products, Inc. Boston, USA. The [^{3}H]IP_3 and [^{3}H]cAMP were purchased from American Radiolabelled Chemicals, USA .

Molecular Biology Chemicals

Tri-reagent kit was purchased from Sigma Chemical Co., St. Louis, USA. ABI PRISM High Capacity cDNA Archive kit and Taqman probes for Real-Time PCR were purchased from Applied Biosystems, Foster City, CA, USA.

Dopamine D1 (Rn_02043440), Dopamine D2 (Rn_00561126), CREB (Rn_ 00578826), Bax (Rn_01480160), SOD (Rn01477289), GPx (Rn_00577994), Akt 1 (Rn00583646), NF-κB (Rn01399583), Caspase-8 (Rn00574069), BDNF (Rn01484924), GDNF (Rn00569510), GLUT 1

(Rn_00567331), phospholipase C (Rn_01647142) and TNF α (Rn00562500_m1) primers were used for the gene expression studies using Real-Time PCR.

Confocal Dyes

Rat specific primary antibody for Dopamine D1 (No: NRG 01691597 Millipore), Dopamine D2. (No: LV 1583420 Millipore) and secondary antibody of FITC (No: AB7130F, Chemicon) were used for the immunohistochemistry studies using confocal microscope.

Animals

Wistar neonatal (postnatal day, P7) rats weighing 10.0–12.0 g were used for all experiments. They were purchased from Kerala Agriculture University, Mannuthy, India and Amrita Institute of Medical Sciences, Kochi, India. All groups of neonatal rats were maintained with their mothers under optimal conditions—12-h light and 12-h dark periods—and were fed standard food and water *ad libitum*. Adequate measures were also taken to minimize pain and discomfort of the animals. All animal care procedures were in accordance with Institutional, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA – Reg. No: 383/01/a/CPSCEA) and the National Institute of Health guidelines.

Plant Material

Specimens of *Bacopa monnieri* were collected from Cochin University area. The plants were taxonomically identified and authenticated by Mr. K.P. Joseph, Head of the Dept. of Botany (Retd), St. Peter's College, Kolencherry and voucher specimens are deposited at the herbarium of the Centre for Neuroscience, Dept. of Biotechnology, Cochin University of Science and Technology, Cochin, Kerala.

Preparation of *Bacopa monnieri* Plant Extract

Crude whole plant extract was used to study the anti-hypoglycemic effect in insulin induced neonatal hypoglycemia. *Bacopa monnieri* plant extract was prepared by the procedure of Paulose *et al*., (2008). Fresh, whole *Bacopa monnieri* plant (6–8 months old) was collected (in the month of March) and washed. Leaves, roots and stems of *Bacopa monnieri* plant were cut into small pieces and dried in shade. About 100 g fresh plant dried in shade yielded 15 g powder. Homogenate was extracted at required concentration (300 mg fresh plant/Kg body weight) by dissolving 450 mg of dried powder in 80 ml distilled water and used to study the anti-hypoglycemic effect in insulin induced neonatal hypoglycemia.

Preparation of Bacoside A

Bacoside A was a generous gift from the Natural Remedies Pvt Ltd. Veerasandra Industrial Area, Bangalore, India and the extraction procedure was follows. Bacoside A was extracted according to the protocol of Pal & Sarin, 1992.

EXPERIMENTAL DESIGN

Determination of Anti- hypoglycemic Potential of *Bacopa monnieri* and Bacoside A

Experimental Animals were divided into following Groups:

i. Control (C)
ii. Neonatal Hypoglycemia (H)
iii. Neonatal Hypoglycemia treated with Glucose (H+G)
iv. Neonatal Hypoglycemia treated with Bacoside A (H+D)
v. Neonatal Hypoglycemia treated with *Bacopa monnieri* (H+B)
vi. Neonatal Hypoglycemia treated with Glucose and Bacoside A (H+G+D)
vii. Neonatal Hypoglycemia treated with Glucose and *Bacopa monnieri* (H+G+B)

Each group consisted of 4-6 animals.

Induction of neonatal hypoglycemia

The control neonatal rats were intra-peritoneally injected with saline (Oliver *et al.* 1999) and hypoglycemia was induced in the experimental groups (P7) using human regular insulin (Actrapid) in a dose of 10 IU/kg intra peritoneally followed by fasting for 240 min. The target blood glucose was <40 mg/dL, a value conventionally used to define hypoglycemia in newborn infants (Burns *et al.* 2008). Recurrent hypoglycemia followed by treatment was induced for 10 days (P7 – P16).

Treatment

Glucose (500 mg/kg body wt) was intra-peritoneally (i.p.), administered to the hypoglycemic neonatal rats, a dose that corrects brain glucose concentration in hypoglycemic newborn rats (Vannucci & Vannucci, 1997), Bacoside A (50 mg/kg body wt) and *Bacopa monnieri* (100 mg/kg body wt) was orally administered (Amee *et al.,* 2009) to the hypoglycemic neonatal rats.

Determination of Body Weight

Body weight of all experimental group of rats were determined gravimetrically with animal weighing balance (Essae Teraoka, India) on 0^{th} and 10^{th} day of the experiment.

Determination of Blood Glucose

The hypoglycemic state of animals was assessed by measuring blood glucose concentrations at 3 hours after insulin treatment. The rats with a blood sugar level < 40 mg/dl were selected as hypoglycemic rats.

Sacrifice and Tissue Preparation

The control and experimental rats were sacrificed on the 11^{th} day (P17) by decapitation. The brain regions –cerebral cortex, corpus striatum, cerebellum and brain stem were dissected out quickly over ice according to the procedure of

Glowinski and Iversen, (1966). The blood samples were collected and plasma was separated by centrifugation. The tissue samples and plasma were kept at -80° C until experiment.

DOPAMINE D1 AND DOPAMINE D2 RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS

Dopamine D1 receptor binding studies using [^{3}H] SCH 23390

Dopamine D1 receptor binding assay using [^{3}H] SCH 23390 in the brain regions were done according to the modified procedure of Mizoguchi *et al*., (2000). The tissues were weighed and homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer, along with 1mM EDTA, 4mM $MgCl_2$, 1.5mM $CaCl_2$, 5mM KCl, pH. 7.4. The homogenate was centrifuged at 40,000 x g for 30min. The pellet was washed and centrifuged with 50 volumes of the buffer at 40,000 x g for 30min. This was suspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.5 - 5.0nM of [^{3}H]SCH 23390 in 50mM Tris-HCl buffer, along with 1mM EDTA, 4mM $MgCl_2$, 1.5 mM $CaCl_2$, 5mM KCl with 12µM pargyline and 0.1% ascorbic acid in a total incubation volume of 250µl containing 100-200µg protein with 50µM unlabelled SCH 23390.

Tubes were incubated at 25°C for 60 min. and filtered rapidly through GF/B filters. The filters were washed quickly by three successive washing with 5.0ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Perkin Elmer Tri-Carb 2810 TR liquid scintillation analyser.

Dopamine D2 receptor binding studies using [^{3}H] YM-09151-2

Dopamine D2 receptor binding assay was done according to the modified procedure of Unis *et al*., (1998). The dissected brain tissues were weighed and homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM MgCl2, 1.5mM CaCl2, 120mM NaCl, 5mM KCl, pH 7.4. The

homogenate was centrifuged at 40,000 x g for 30min. The pellet was washed and centrifuged with 50 volumes of the buffer at 40,000 x g for 30min. This was suspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.1 - 2.0nM of [^{3}H]YM-09151-2 in 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM MgCl2, 1.5mM CaCl2, 120mM NaCl, 5mM KCl with 10µM pargyline and 0.1% ascorbic acid in a total incubation volume of 250µl containing 100-200µg of protein. Specific binding was determined using 5.0µM unlabelled sulpiride. Tubes were incubated at 25°C for 60 min. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 5.0ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Perkin Elmer Tri-Carb 2810 TR liquid scintillation analyser.

Protein determination

Protein was measured by the method of Lowry *et al*., (1951) using bovine serum albumin as standard. The intensity of the purple blue colour formed was proportional to the amount of protein which was read in a spectrophotometer (Shimadzu UV-1700) at 660nm.

ANALYSIS OF THE RECEPTOR BINDING DATA

Linear regression analysis for Scatchard plots

The data was analysed according to Scatchard, (1949). The specific binding was determined by subtracting non-specific binding from the total. The binding parameters, maximal binding (B_{max}) and equilibrium dissociation constant (K_d), were derived by linear regression analysis by plotting the specific binding of the radioligand on X-axis and bound/free on Y-axis. The maximal binding is a measure of the total number of receptors present in the tissue and the equilibrium

dissociation constant is the measure of the affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity.

GENE EXPRESSION STUDIES IN DIFFERENT BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS

Preparation of RNA

RNA was isolated from the different brain regions - cerebral cortex, corpus striatum, cerebellum and brain stem of control and experimental rats using Tri reagent from Sigma Chemical Co., St. Louis, USA.

Isolation of RNA

Tissue (25-50 mg) homogenates were made in 0.5ml Tri Reagent and was centrifuged at 12,000 x *g* for 10 minutes at 4°C. The clear supernatant was transferred to a fresh tube and it was allowed to stand at room temperature for 5 minutes. 100µl of chloroform was added to it, mixed vigorously for 15 seconds and allowed to stand at room temperature for 15 minutes. The tubes were then centrifuged at 12,000 x *g* for 15 minutes at 4°C. Three distinct phases appear after centrifugation. The bottom red organic phase contained protein, interphase contained DNA and a colourless upper aqueous phase contained RNA. The upper aqueous phase was transferred to a fresh tube and 250 µl of isopropanol was added and the tubes were allowed to stand at room temperature for 10 minutes. The tubes were centrifuged at 12,000 x *g* for 10 min at 4ºC. RNA precipitated as a pellet on the sides and bottom of the tube. The supernatants were removed and the RNA pellet was washed with 500µl of 75% ethanol, vortexed and centrifuged at 12,000 x *g* for 5 min at 4°C. The pellets were semi dried and dissolved in minimum volume of DEPC-treated water. 2µl of RNA was made up to 1ml and absorbance was measured at 260 nm and 280 nm in spectrophotometer (Shimadzu

UV-1700). For pure RNA preparation the ratio of absorbance at 260/280 was ≥ 1.7. The concentration of RNA was calculated as one absorbance $_{260}$ = 42µg.

cDNA Synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA Archive kit in 0.2ml microfuge tubes. The reaction mixture of 20µl contained 0.2µg total RNA, 10X RT buffer, 25X dNTP mixture, 10X Random primers, MultiScribe RT (50U/µl) and RNase free water. The cDNA synthesis reactions were carried out at 25°C for 10 minutes and 37°C for 2 hours using an Eppendorf Personal Cycler. The primers and probes were purchased from Applied Biosystems, Foster City, CA, USA designed using Primer Express Software Version (3.0).

Real-Time PCR Assay

Real Time PCR assays were performed in 96-well plates in ABI 7300 Real Time PCR instrument (Applied Biosystems). To detect gene expression, a TaqMan quantitative 2-step reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantify mRNA levels. First-strand cDNA was synthesized with use of a TaqMan RT reagent kit, as recommended by the manufacturer. PCR analyses were conducted with gene-specific primers and fluorescently labeled TaqMan probe (designed by Applied Biosystems). Endogenous control, β-actin, was labelled with a reporter dye (VIC). All reagents were purchased from Applied Biosystems. The probes for specific gene of interest were labeled with FAM at the 5' end and a quencher (Minor Groove Binding Protein - MGB) at the 3' end. The real-time data were analyzed with Sequence Detection Systems software version 1.7. All reactions were performed in duplicate.

The TaqMan reaction mixture of 20µl contained 25ng of total RNA-derived cDNAs, 200nM each of the forward primer, reverse primer and TaqMan probes, endogenous control (β-actin) and 12.5µl of TaqMan 2X Universal PCR Master Mix (Applied Biosystems). The volume was made up with RNase free water. Each run contained both negative (no template) and positive controls.

The thermocycling profile conditions were as follows:

50ºC -- 2 minutes	--- Activation	
95ºC -- 10 minutes	--- Initial Denaturation	
95ºC -- 15 seconds	--- Denaturation	40 cycles
50ºC -- 30 seconds	--- Annealing	
60ºC -- 1 minute	--- Final Extension	

Fluorescence signals measured during amplification were considered positive if the fluorescence intensity was 20-fold greater than the standard deviation of the baseline fluorescence. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control β-actin in the same samples ($\Delta CT = CT_{Target} - CT_{\beta\text{-actin}}$). It was further normalized with the control ($\Delta\Delta CT = \Delta CT - CT_{Control}$). The fold change in expression was then obtained ($2^{-\Delta\Delta CT}$).

cAMP CONTENT IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS *IN VIVO*

Brain tissues (cerebral cortex, corpus striatum, cerebellum and brain stem) were homogenised in a polytron homogeniser with cold 50mM Tris-HCl buffer, pH 7.4, containing 1mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15min and the supernatant was transferred to fresh tubes for cAMP assay using [^{3}H] cAMP Biotrak Assay System kit.

Principle of the assay

cAMP assay kit was used. The assay is based on the competition between unlabelled cAMP and a fixed quantity of [^{3}H] cAMP for binding to a protein which has a high specificity and affinity for cAMP. The amount of labeled protein -cAMP complex formed is inversely related to the amount of unlabelled cAMP

present in the assay sample. Measurement of the protein-bound radioactivity enables the amount of unlabelled cAMP in the sample to be calculated.

	Free [^{3}H] cAMP		Bound [^{3}H] cAMP-binding protein
+	Binding cAMP protein	=	+ cAMP-binding protein

Separation of the protein bound cAMP from unbound nucleotide is achieved by adsorption of the free nucleotide on to a coated charcoal followed by centrifugation. An aliquot of the supernatant is then removed for liquid scintillation counting. The concentration of unlabelled cAMP in the sample is then determined from a linear standard curve.

Assay Protocol

The tubes were placed on a water bath at 0°C. The assay mixture consisted of different concentrations of standard, [^{3}H] cAMP and binding protein in case of standards; buffer, [^{3}H] cAMP and binding protein for zero blank and unknown samples. The mixture was incubated at 2°C for 2h. Cold charcoal reagent was added to the tubes and the tubes were immediately centrifuged at 12,000 x g for 2min at 2°C. Aliquots of the supernatant was immediately transferred to scintillation vials and mixed with cocktail-T and counted in a liquid scintillation counter (Perkin Elmer Tri-Carb, 2810).

C_o/C_x is plotted on the Y-axis against picomoles of inactive cAMP on the X- axis of a linear graph paper, where C_o is the counts per minute bound in the absence of unlabelled cAMP and C_x is the counts per minute bound in the presence of standard or unknown unlabelled cAMP. From the C_o/C_x value for the sample, the number of picomoles of unknown cAMP was calculated.

IP_3 CONTENT IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS *IN VIVO*

Brain tissues (cerebral cortex, corpus striatum, cerebellum and brain stem) were homogenised in a polytron homogeniser in 50mM Tris-HCl buffer, pH 7.4, containing 1mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15 minutes and the supernatant was transferred to fresh tubes for IP_3 assay using [^{3}H] IP_3 Biotrak Assay System kit.

Principle of the assay

The assay was based on competition between [^{3}H] IP_3 and unlabelled IP_3 in the standard or samples for binding to a binding protein prepared from bovine adrenal cortex. The bound IP_3 was then separated from the free IP_3 by centrifugation. The free IP_3 in the supernatant was then discarded by simple decantation, leaving the bound fraction adhering to the tube. Measurement of the radioactivity in the tube enables the amount of unlabelled IP_3 in the sample to be determined.

Assay Protocol

Standards, ranging from 0.19 to 25 pmoles/tube, [^{3}H] IP_3 and binding protein were added together and the volume was made up to 100µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. The tubes were then vortexed and incubated on ice for 15min and they were centrifuged at 2000 x g for 10min at 4°C. The supernatant was aspirated out and the pellet was resuspended in water and incubated at room temperature for 10min. The tubes were then vortexed and decanted immediately into scintillation vials. The radioactivity in the suspension was determined using liquid scintillation counter (Perkin Elmer Tri-Carb, 2810).

A standard curve was plotted with %B/Bo on the Y-axis and IP_3 concentration (pmoles/tube) on the X-axis of a semi-log graph paper. $\%B/B_o$ was calculated as:

$$\frac{\text{(Standard or sample cpm – NSB cpm)}}{\text{(B_0 cpm – NSB cpm)}} \times 100$$

NSB- non specific binding and B_0 - zero binding. IP_3 concentration in the samples was determined by interpolation from the plotted standard curve.

DAD1 AND DAD2 EXPRESSION STUDIES IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS USING CONFOCAL MICROSCOPE

Anaesthetized animals were transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (pH 7.4). After perfusion, the brain was dissected and immersion fixed in 4% paraformaldehyde for 1 hr and then equilibrated with 30% sucrose solution in 0.1M PBS. 30 µm sections were cut using Cryostat (Leica, CM1510 S). The sections were treated with PBST (PBS in 0.05% Triton X-100) for 20 min. Brain sections were blocked with 5% normal goat serum for 4 hours. Brain sections were then incubated overnight at 4 °C with either rat specific primary antibody for Dopamine D1 (No: NRG 01691597 Millipore, 1: 500 dilution in a 1X PBS solution containing 5% normal goat serum) or Dopamine D2. (No: LV 1583420 Millipore, 1: 500 dilution in a 1X PBS solution containing 5% normal goat serum). After overnight incubation brain sections washed with PBS and then incubated for 1 hour with secondary antibody conjugated with FITC (No: AB7130F, Chemicon, 1:1000 dilution in a 1X PBS solution containing 5% normal goat serum) in brain regions. After the incubations brain sections were washed with PBS. Remove the excess PBS off from the slides and mount cover glass with anti-fade mounting media. The sections were observed and photographed using confocal imaging system (Leica SP 5).

Expressions were analysed using pixel intensity method. Quantification was done using Leica application suit advanced fluorescence (LASAF) software by considering the mean pixel intensity of the image. The fluorescence obtained depends on the number of receptors specific to the added primary antibody. The mean pixel intensity was directly related to the fluorescence emitted from the sections and calculated with the LASAF software. All the imaging parameters in the confocal imaging system like PMT, pinhole and zoom factor were kept same for imaging the sections of all experimental groups.

STATISTICS

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme. Linear regression Scatchard plots were made using Sigma Plot software (version 2.0, Jandel GmbH, Erkrath, Germany). Relative Quantification Software was used for analyzing Real-Time PCR results.

Results

BODY WEIGHT

In the beginning of the experiment, the body weight of all the rats was within the normal range. Body weight of hypoglycemic neonatal rats was decreased significantly ($p<0.001$) in 10^{th} day when compared to control. After treatment with glucose, *Bacopa monnieri* and Bacoside A alone and in combination for 10 days, the body weight was significantly reversed ($p<0.001$) when compared with hypoglycemic neonatal rats (Table-1).

BLOOD GLUCOSE LEVEL

Blood glucose levels of all rats before insulin administration and treatment was within the normal range (100 – 120 mg/dl). 10 days after treatment, hypoglycemic neonatal rats showed a significant decrease ($p<0.001$) in blood glucose when compared to control (Table-2a).

Blood glucose level was monitored from day 1 to day 10. Blood glucose level of all rats at time 0 min, before insulin administration, was within the normal range. Insulin administration in rats led to a significant decrease ($p<0.001$) in blood glucose level 240 min after administration, when compared to control group. Glucose, *Bacopa monnieri* and Bacoside A treatments alone and in combination were significantly reversed ($p<0.001$) the increased blood glucose level when compared to hypoglycemic neonatal group (Table-2b).

CEREBRAL CORTEX

Real Time PCR amplification of Dopamine D1 receptor mRNA in the cerebral cortex of control and experimental rats

Gene expression of Dopamine D1 receptor subtype mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of hypoglycemic neonatal rats and rats treated with glucose when compared to control. In H+B, H+D, H+G+B and H+G+D groups, there was significant up regulation ($p<0.05$, $p<0.01$, $p<0.001$) In all the treatment groups, H+G, H+B, H+D, H+G+B and H+G+D groups there was a significant reversal of the gene expression to near control ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$) when compared with hypoglycemic neonatal and glucose treated group (Figure-1, Table-3).

Real Time PCR amplification of Dopamine D2 receptor mRNA in the cerebral cortex of control and experimental rats

Gene expression of Dopamine D2 receptor subtype mRNA showed significant up regulation ($p<0.001$) in the cerebral cortex of all the experimental groups and an up regulation of when compared to control. H+G showed significant reversal ($p<0.001$) when compared to the neonatal hypoglycemic group. In H+B, H+D, H+G+B and H+G+D groups there was a significant reversal of the gene expression ($p<0.001$) when compared with both the H and H+G group (Figure-2, Table-4).

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the cerebral cortex of control and experimental rats

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH 23390 in the cerebral cortex of hypoglycemic neonatal rats and H+G groups showed a significant ($p<0.001$) decrease in B_{max} compared to control rats. H+G+D showed no difference when compared to control. This showed decreased Dopamine D1 receptor density in the cerebral cortex of

hypoglycemic neonatal rats. K_d showed no significant difference. Significant reversal in the B_{max} was observed in treatment groups: H+B, H+D, H+G+B and H+G+D ($p<0.001$ when compared with glucose treatment and neonatal hypoglycemic groups (Figure-3,4 Table-5, 6).

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in cerebral cortex of control and experimental rats

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 binding against sulpiride in the cerebral cortex of hypoglycemic neonatal rats and H+G groups showed a significant ($p<0.001$) increase in B_{max} compared to control rats. K_d showed no significant difference. Significant reversal in the B_{max} was observed in all the other treatment groups: H+B, H+D, H+G+B and H+G+D ($p<0.001$) when compared with glucose treatment and neonatal hypoglycemic groups (Figure-5,6 Table-7, 8).

Dopamine D1 receptor subtype antibody staining in control and experimental groups of rats using confocal microscope

Dopamine D1 subtype specific antibody staining in the cerebral cortex showed a significant decrease ($p<0.001$) in mean pixel value in the hypoglycemic neonatal rats and H+G treated group when compared to control. H+B and H+D groups significantly reversed ($p<0.001$) the mean pixel value when compared with hypoglycemic neonatal rats and H+G treated group. H+G+B and H+G+D treatment showed prominent down regulation ($p<0.05$) when compared when compared to control and a significant reversal ($p<0.001$) when compared to hypoglycemic neonatal rats and glucose treated rats (Figure-7, Table-9).

Dopamine D2 receptor subtype antibody staining in control and experimental groups of rats using confocal microscope

Dopamine D2 subtype specific antibody staining in the cerebral cortex showed a significant increase ($p<0.001$) in mean pixel value in the hypoglycemic neonatal rats and H+G treated group when compared to control. H+B and H+D

($p<0.05$, $p<0.001$) groups showed significant up regulation when compared to control and significantly ($p<0.001$) reversed mean pixel value when compared with the H and H+G groups. H+G+B and H+G+D treatment showed no significant change when compared to control but, showed prominent reversal ($p<0.001$) when compared with the H and H+G groups (Figure-8, Table-10).

cAMP content in the cerebral cortex of control and experimental rats

cAMP content showed significant decrease ($p<0.001$) in the cerebral cortex of hypoglycemic neonatal rats and compared to control rats. H+G treated group showed no reversal when compared to neonatal hypoglycemic group. There was a significant reversal ($p<0.001$) in cAMP content of the treatment groups: H+B, H+D, H+G+B and H+G+D to near control levels when compared with H and H+G groups. (Figure -9, Table-11).

IP3 content in the cerebral cortex of control and experimental rats

IP3 content showed significant decrease ($p<0.001$) in the cerebral cortex of hypoglycemic neonatal rats compared to control rats. H+G treated group showed no reversal when compared to neonatal hypoglycemic group. There was a significant reversal ($p<0.001$) in IP3 content in all the treatment groups H+B, H+D, H+G+B and H+G+D to near control levels when compared with neonatal hypoglycemic rats and glucose treated rats. (Figure -10, Table -12).

Real time PCR amplification of Phospholipase C mRNA in the cerebral cortex of control and experimental rats

Gene expression of Phospholipase C mRNA showed significant up regulation ($p<0.001$) in the cerebral cortex of hypoglycemic neonatal rats and H+G group when compared to control. In H+B group and H+D groups there was an up regulation of the gene expression ($p<0.05$) when compared control and showed significant reversal ($p<0.001$) when compared to the H and H+G groups. In the combination treatment groups, H+G+B and H+G+D there was no significant change when compared to control, but showed significant reversal of

the gene expression levels to near control ($p<0.001$) when compared with the H and H+G groups (Figure -11, Table -13).

Real time PCR amplification of CREB mRNA in the cerebral cortex of control and experimental rats

Gene expression of CREB mRNA showed significant ($p<0.001$) up regulation in the cerebral cortex of hypoglycemic neonatal rats and H+G group when compared to control. In H+B and H+D groups there was a significant up regulation ($p<0.001$) when compared to control and significant ($p<0.001$) reversal of the gene expression to near control levels when compared with hypoglycemic neonatal and the glucose treated groups. In the combination treatment groups, H+G+B and H+G+D there was a up regulation of the gene expression levels ($p<0.001$) when compared to control and a significant reversal ($p<0.001$) when compared with H and H+G groups (Figure-12, Table-14).

Real Time PCR amplification of GLUT 3 mRNA in the cerebral cortex of control and experimental rats

Real-time PCR gene expression of GLUT 3 showed significant decrease ($p<0.001$) in the cerebral cortex of hypoglycemic neonatal rats when compared to control group. H+G group showed a marked up regulation ($p<0.001$) compared to control and hypoglycemic neonatal groups. H+B, H+D, H+G+B and H+G+D treatment showed a significant ($p<0.001$) up regulation when compared to control. These groups showed a prominent reversal of gene expression ($p<0.001$) when compared to the H and H+G groups (Figure -13, Table-15).

Real Time PCR amplification of Akt -1 mRNA in the cerebral cortex of control and experimental rats

Real time PCR gene expression of Akt -1 showed significant ($p<0.001$) up regulation in the cerebral cortex of hypoglycemic neonatal rats when compared to control and H+G group. In H+B, H+D and H+G+D treated groups showed a significant ($p<0.001$) reversal of the gene expression to near control levels when

compared with hypoglycemic neonatal group. In the combination treatment group, H+G+B, there was no significant change with respect to control and showed a marked reversal ($p<0.001$) of the gene expression levels when compared with H and H+G groups (Figure-14, Table-16).

Real Time PCR amplification of TNF-α mRNA in the cerebral cortex of control and experimental rats

Real-time PCR gene expression of TNF-α in the cerebral cortex of neonatal hypoglycemic rats showed a significant down regulation ($p<0.001$) when compared to the control and H+G groups. The other treatment groups: H+B, H+D, H+G+B and H+G+D showed a significant down regulation ($p<0.001$) when compared to control and also significantly reversed ($p<0.001$) the altered gene expression when compared with H and H+G groups to near control levels (Figure-15, Table-17).

Real Time PCR amplification of GDNF mRNA in the cerebral cortex of control and experimental rats

Real-time PCR gene expression of GDNF showed significant down regulation ($p<0.001$) in the cerebral cortex of hypoglycemic neonatal rats and and all the other treatment compared to control rats. The GDNF gene expression was significantly reversed ($p<0.001$) in H+B, H+D, H+G+B and H+G+D treatment groups when compared with hypoglycemic and glucose treatment groups (Figure-16, Table-19).

Real Time PCR amplification of BDNF mRNA in the cerebral cortex of control and experimental rats

Real-time PCR gene expression of BDNF showed significant down regulation ($p<0.001$) in the cerebral cortex of hypoglycemic neonatal rats and all the other treatment compared to control rats. There was a significant reversal ($p<0.001$) of BDNF gene expression in the H+B, H+D, H+G+B and H+G+D groups when compared to the H and H+G groups. (Figure -17, Table -19).

Real Time PCR amplification of NF-ƙB mRNA in the cerebral cortex of control and experimental rats

Real-time PCR gene expression of NF-ƙB showed significant down regulation ($p<0.001$) in the cerebral cortex of all the groups of neonatal rats when compared to control rats. The H+B, H+D and the combination treatment groups H+G+B and H+G+D groups, showed a marked ($p<0.001$) reversal of the gene expression when compared to both the hypoglycemic and H+G groups (Figure-18; Table-20).

Real Time PCR amplification of SOD mRNA in the cerebral cortex of control and experimental rats

Real-time PCR gene expression of SOD showed significant decrease ($p<0.001$) in the cerebral cortex of H and H+G group, and a significant ($p<0.001$) increase in the H+B, H+D, H+G+B and H+G+D groups when compared to control. The treatment groups, H+B, H+D, H+G+B and H+G+D groups showed a significant ($p<0.001$) reversal of the SOD gene expression when compared to hypoglycemic neonatal rats and glucose treated group (Figure-19; Table-21).

Real Time PCR amplification of GPx mRNA in the cerebral cortex of control and experimental rats

Real-time PCR gene expression of GPx showed significant down regulation ($p<0.001$) in the cerebral cortex of hypoglycemic neonatal rats, H+G, H+D and H+B groups and the H+G+B and H+G+D groups showed a significant ($p<0.001$) up regulation when compared to control rats. There was a significant reversal ($p<0.001$) in GPx gene expression in the following treatment groups: H+B, H+D, H+G+B and H+G+D, when compared to H and H+G groups (Figure-20; Table-22).

Real Time PCR amplification of Bax mRNA in the cerebral cortex of control and experimental rats

Real-time PCR gene expression of Bax showed significant up regulation ($p<0.001$) in the cerebral cortex of H, H+G, H+B and H+D groups when compared to control rats. H+G+B and H+G+D groups showed no significant change when compared to control. There was a significant reversal ($p<0.01$) in Bax gene expression in H+B, H+D, H+G+B and H+G+D groups compared to H and H+G (Figure-21, Table-23).

Real Time PCR amplification of caspase 8 mRNA in the cerebral cortex of control and experimental rats

Real-time PCR gene expression of caspase 8 showed significant up regulation ($p<0.001$, $p<0.001$, $p<0.01$) in the cerebral cortex of hypoglycemic neonatal rats, H+G and H+B group compared to control rats. The H+D group showed no change when compared to control There was a significant reversal ($p<0.001$) in Caspase 8 gene expression in H+D, H+G+B and H+G+D groups when compared to H and H+G groups (Figure-22, Table-24).

CORPUS STRIATUM

Real Time PCR amplification of Dopamine D1 receptor mRNA in the corpus striatum of control and experimental rats

Gene expression of Dopamine D1 receptor subtype mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of hypoglycemic neonatal rats and H+G groups when compared to control. H+B and H+D groups and the combination treatment groups, H+G+B and H+G+D showed a marked reversal ($p<0.001$) of the gene expression to near control levels, when compared with hypoglycemic neonatal group and H+G group (Figure-23, Table-25).

Real Time PCR amplification of Dopamine D2 receptor mRNA in the corpus striatum of control and experimental rats

Gene expression of Dopamine D2 receptor subtype mRNA showed significant up regulation ($p<0.001$) in the corpus striatum of all the other experimental groups when compared to control. In H+B, H+D, H+G+B and H+G+D groups there was a significant reversal ($p<0.001$) of the gene expression when compared with hypoglycemic neonatal group and H+G group (Figure-24, Table-26).

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the corpus striatum of control and experimental rats

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH 23390 in the corpus striatum of hypoglycemic neonatal rats and H+G groups showed a significant ($p<0.001$) decrease in B_{max} compared to control rats. K_d showed no significant change. Significant ($p<0.001$) reversal in the B_{max} was observed in treatment groups: H+B, H+D H+G+B and H+G+D groups when compared to glucose treatment and neonatal hypoglycemic groups (Figure- 25, 26 Table- 27, 28).

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in corpus striatum of control and experimental rats

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 binding against sulpiride in the corpus striatum of hypoglycemic neonatal rats and H+G groups showed a significant ($p<0.001$) increase in B_{max} compared to control rats. Significant reversal in the B_{max} was observed in treatment groups: H+B, H+D, H+G+B and H+G+D ($p<0.001$) when compared with glucose treatment and neonatal hypoglycemic groups (Figure- 27,28 Table- 29, 30).

Dopamine D1 receptor subtype antibody staining in the corpus striatum of control and experimental groups of rats using confocal microscope

Dopamine D1 subtype specific antibody staining in the corpus striatum showed a significant decrease ($p<0.001$) in mean pixel value in the hypoglycemic neonatal rats, H+G, H+B and H+D treated groups when compared to control. The treatment groups, H+B, H+D, H+G+B and H+G+D significantly ($p<0.001$) reversed mean pixel value when compared with the H and H+G groups (Figure-29, Table-31).

Dopamine D2 receptor subtype antibody staining in the corpus striatum of control and experimental groups of rats using confocal microscope

Dopamine D2 subtype specific antibody staining in the corpus striatum showed a significant increase ($p<0.001$) in mean pixel value in the hypoglycemic neonatal rats, H+G groups when compared to control. The treatment groups H+B, H+D, H+G+B and H+G+D treatment showed prominent reversal when compared with both the H and H+G treated rats (Figure-30, Table-32).

cAMP content in the corpus striatum of control and experimental rats

cAMP content showed significantly decreased ($p<0.001$) in the corpus striatum of hypoglycemic neonatal rats, H+G, H+B and H+D groups when compared to control. Both H+G+B and H+G+D groups showed no prominent change when compared to control. There was a significant reversal ($p<0.001$) in

cAMP content in H+B, H+D, H+G+B and H+G+D treatment groups when compared with the neonatal hypoglycemic rats and glucose treated rats (Figure-31, Table-33).

IP3 content in the corpus striatum of control and experimental rats

IP3 content showed significantly increased ($p<0.001$) in the corpus striatum of hypoglycemic neonatal rats, H+G and H+D group when compared to control rats. H+B, H+G+B and H+G+D treatment groups showed no marked change when compared to control. H+B, H+G+B and H+G+D treatment showed prominent reversal to near control levels when compared to both the H and H+G groups. (Figure -32, Table -34).

Real time PCR amplification of Phospholipase C mRNA in corpus striatum of control and experimental rats

Gene expression of Phospholipase C mRNA showed significant up regulation ($p<0.001$) in the corpus striatum of hypoglycemic neonatal rats and H+G group when compared to control. The groups, H+B and H+D also showed a significant ($p<0.01$, $p<0.05$) up regulation, whereas the H+G+B and H+G+D groups showed no apparent change when compared to control. In H+B, H+D, H+G+B and H+G+D groups, there was a significant reversal of the gene expression levels to near control ($p<0.001$) when compared with hypoglycemic neonatal group and H+G group (Figure -33, Table -35).

Real time PCR amplification of CREB mRNA in the corpus striatum of control and experimental rats

Gene expression of CREB mRNA showed significant ($p<0.001$) up regulation in the corpus striatum in the groups H when compared to control. H+G showed no prominent change when compared to hypoglycemic group. Also the group H+G+D showed no prominent change when compared to control. It was observed that in the groups, H+B, H+D, H+G+B and H+G+D the gene expression

levels were significantly ($p<0.001$) brought down when compared with H and H+G groups (Figure -34, Table -36).

Real Time PCR amplification of GLUT 3 mRNA in the corpus striatum of control and experimental rats

Real-time PCR gene expression of GLUT 3 showed significant down regulation ($p<0.001$) in the corpus striatum of hypoglycemic neonatal rats when compared to control rats. H+G group showed a marked up regulation ($p<0.001$) compared to both the neonatal hypoglycemic and the control rats. All the other treatment groups, H+B, H+D, H+G+B and H+G+D prominent up regulation ($p<0.05$, $p<0.05$, $p<0.01$, $p<0.01$) in GLUT 3 gene expression when compared to control. The treatment groups, H+B, H+D, H+G+B and H+G+D showed a marked reversal ($p<0.001$) when compared to both the H and H+G groups (Figure-35, Table-37).

Real Time PCR amplification of Akt -1 mRNA in the corpus striatum of control and experimental rats

Real time PCR gene expression of Akt -1 showed significant ($p<0.001$) up regulation in the corpus striatum of all the groups H, H+G, H+B, H+D, H+G+B and H+G+D groups when compared to control. It was also observed that in H+B, H+D, H+G+B and H+G+D groups there was a marked reversal of the gene expression levels ($p<0.001$) when compared with H and H+G groups (Figure-36, Table-38).

Real Time PCR amplification of TNF-α mRNA in the corpus striatum of control and experimental rats

Real-time PCR gene expression of TNF-α showed significant down regulation ($p<0.001$) in the corpus striatum of H, H+G and H+D groups when compared to control. The groups H+B, H+G+B and H+G+D showed no significant change when compared to control. In H+B, H+D, H+G+B and H+G+D treated hypoglycemic neonatal rats, there was significant ($p<0.001$) reversal of

TNF-α gene expression when compared to hypoglycemic neonatal and H+G rats respectively (Figure-37, Table-39).

Real Time PCR amplification of GDNF mRNA in the corpus striatum of control and experimental rats

Real-time PCR gene expression of GDNF showed significant down regulation ($p<0.001$) in the corpus striatum of hypoglycemic neonatal rats and H+G group compared to control rats. The GDNF gene expression was significantly reversed ($p<0.001$) in H+B, H+D, H+G+B and H+G+D treatment groups of rats when compared with hypoglycemic neonatal rats and glucose treatment groups (Figure-38, Table-40).

Real Time PCR amplification of BDNF mRNA in the corpus striatum of control and experimental rats

Real-time PCR gene expression of BDNF showed significant down regulation ($p<0.001$) in the corpus striatum of of all the groups of rats compared to control rats. There was a significant reversal ($p<0.001$) in BDNF gene expression in H+B, H+D, H+G+B and H+G+D groups to near control levels when compared to the H and H+G groups (Figure-39, Table-41).

Real Time PCR amplification of NF-ƙB mRNA in the corpus striatum of control and experimental rats

Real-time PCR gene expression of NF-ƙB showed significant down regulation ($p<0.001$) in the corpus striatum of hypoglycemic neonatal rats when compared to control rats. The treatment groups H+G, H+B, H+D, H+G+B and H+G+D showed a significant ($p<0.001$) up regulation in the gene expression levels when compared to control and hypoglycemic neonatal rats. There was a significant reversal ($p<0.001$) in NF-ƙB gene expression in H+B, H+D, H+G+B and H+G+D ($p<0.001$) groups when compared to hypoglycemic neonatal rats and neonatal hypoglycemic group treated with glucose (Figure-40, Table-42).

Real Time PCR amplification of SOD mRNA in the corpus striatum of control and experimental rats

Real-time PCR gene expression of SOD showed significant down regulation ($p<0.001$) in the corpus striatum of neonatal rats group. No significant change was observed in the H+G group compared to neonatal hypoglycemic group. There was a marked reversal ($p<0.001$) in SOD gene expression in hypoglycemic neonatal rats treated with *Bacopa monnieri* and Bacoside A and the combination treatments, H+G+B and H+G+D groups, when compared to hypoglycemic and H+G group (Figure-41, Table-43).

Real Time PCR amplification of GPx mRNA in the corpus striatum of control and experimental rats

Real-time PCR gene expression of GPx showed significant down regulation ($p<0.001$) in the corpus striatum of hypoglycemic neonatal rats, H+G, H+B and H+D groups when compared to control rats. The groups H+G+B and H+G+D also showed an apparent down regulation ($p<0.05$, $p<0.01$) when compared to the control group. There was a significant reversal ($p<0.001$) in GPx gene expression in hypoglycemic neonatal rats treated with *Bacopa monnieri* and Bacoside A alone and H+G+B and H+G+D groups when compared to H and H+G groups (Figure-42, Table-44).

Real Time PCR amplification of Bax mRNA in the corpus striatum of control and experimental rats

Real-time PCR gene expression of Bax showed significant up regulation ($p<0.001$) in the corpus striatum of all the groups when compared to control rats. There was seen to be a significant reversal ($p<0.01$) in Bax gene expression in hypoglycemic neonatal rats treated with *Bacopa monnieri* and Bacoside A alone, and the combination treatment groups, H+G+B and H+G+D compared to hypoglycemic neonatal rats and the glucose treated group (Figure-43, Table-45).

Real Time PCR amplification of caspase 8 mRNA in the corpus striatum of control and experimental rats

Real-time PCR gene expression of caspase 8 showed significant up regulation ($p<0.001$) in the corpus striatum of hypoglycemic neonatal rats, H+G, H+B and H+D groups compared to control rats. There was a prominent reversal ($p<0.001$) in Caspase 8 gene expression in H+B, H+D, H+G+B and H+G+D groups compared to H and H+G groups (Figure-44, Table-46).

CEREBELLUM

Real Time PCR amplification of Dopamine D1 receptor mRNA in the cerebellum of control and experimental rats

Gene expression of Dopamine D1 receptor subtype mRNA showed significant down regulation ($p<0.001$) in the cerebellum of hypoglycemic neonatal rats, H+G, H+B, H+D, H+G+B and H+G+D groups when compared to control. Both the individual treatment groups, H+B and H+D and the combination treatment groups, H+G+B and H+G+D there was a significant reversal of the gene expression levels ($p<0.001$) when compared with hypoglycemic neonatal group and H+G group (Figure-45, Table-47).

Real Time PCR amplification of Dopamine D2 receptor mRNA in the cerebellum of control and experimental rats

Gene expression of Dopamine D2 receptor subtype mRNA showed significant up regulation ($p<0.001$) in the cerebellum of hypoglycemic neonatal rats and the treatment groups H+G, H+B and H+D when compared to control. There was no significant up regulation of the gene expression ($p<0.001$) in the H+G+B and H+G+D groups when compared to control. H+D, H+G+B and H+G+D ($p<0.01$, $p<0.001$, $p<0.001$) showed a significant decrease of the gene expression levels when compared with hypoglycemic neonatal group and H+G group (Figure-46, Table-48).

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the cerebellum of control and experimental rats

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH 23390 in the cerebellum of hypoglycemic neonatal rats showed a significant decrease in B_{max} ($p<0.001$) and increase in K_d($p<0.001$) compared to control rats. This showed decreased Dopamine D1 receptor density in the cerebellum of hypoglycemic neonatal rats. No Significant reversal in the B_{max} was observed. K_d showed significant was observed in all the other treatment

groups: H+B ($p<0.001$) and H+G+B ($p<0.001$) when compared with control groups (Figure- 47,48 Table- 49, 50).

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in cerebellum of control and experimental rats

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 binding against sulpiride in the cerebellum of hypoglycemic neonatal rats, H+G, H+B, H+D and H+G+D groups showed a significant ($p<0.001$) increase in B_{max} and K_d compared to control rats. This showed an increased Dopamine D2 receptor density in the cerebellum. H+G showed a significant reversal ($p<0.001$) compared to hypoglycemic group. The treatment groups: H+B, H+D, H+G+B and H+G+D groups showed prominent reversal ($p<0.001$) in B_{max} and K_d when compared with glucose treatment and neonatal hypoglycemic groups (Figure-49,50 Table-51, 52).

cAMP content in the cerebellum of control and experimental rats

cAMP content showed significant decrease ($p<0.001$) in the cerebellum of all groups of rats compared to control. The individual treatment groups, H+B and H+D and combination treatment groups H+G+B and H+G+D treatment showed prominent reversal to near control levels when compared with both the neonatal hypoglycemic rats and the glucose treated rats (Figure-51, Table-53).

IP3 content in the cerebellum of control and experimental rats

IP3 content showed significant increase ($p<0.001$) in the cerebellum of hypoglycemic neonatal rats, H+G, H+B and H+A treated groups compared to control rats. There was no significant change observed in IP3 content in H+G+B and H+G+D treatment compared to control. A prominent reversal to near control levels was observed in H+B, H+D, H+G+B and H+G+D when compared with H and H+G groups. (Figure -52, Table -54).

Real time PCR amplification of Phospholipase C mRNA in cerebellum of control and experimental rats

Gene expression of Phospholipase C mRNA showed significant up regulation ($p<0.001$) in the cerebellum of all groups except H+G+B and H+G+D when compared to control. In the treatment groups, H+B, H+D, H+G+B and H+G+D there was a marked reversal of the gene expression levels to near control ($p<0.001$) when compared with hypoglycemic neonatal group and H+G group (Figure -53, Table -55).

Real time PCR amplification of CREB mRNA in the cerebellum of control and experimental rats

Gene expression of CREB mRNA showed significant ($p<0.001$) down regulation in the cerebellum of H, H+G, H+B and H+D groups when compared to control. Combination treatment groups, H+G+B and H+G+D showed no significant down regulation when compared to control group. In H+B, H+D, H+G+B and H+G+D groups there was a significant reversal of the gene expression to near control levels ($p<0.001$) when compared with hypoglycemic neonatal group H+G groups (Figure -54, Table -56).

Real Time PCR amplification of GLUT 3 mRNA in the cerebellum of control and experimental rats

Real-time PCR gene expression of GLUT 3 showed a significant upregulation ($p<0.001$) in the cerebellum of all groups of rats when compared to control rats. H+G group showed a marked increase ($p<0.001$) compared to neonatal hypoglycemic rats. All the other treatments with *Bacopa monnieri* and Bacoside A: H+B, H+D, H+G+B and H+G+D significantly ($p<0.001$) increased the GLUT 3 gene expression when compared to the hypoglycemic neonatal rats (Figure-55, Table-57).

Real Time PCR amplification of Akt -1 mRNA in the cerebellum of control and experimental rats

Real time PCR gene expression of Akt -1 showed significant down regulation in the cerebellum of both hypoglycemic neonatal rats ($p<0.001$) and H+G group ($p<0.001$) whereas the H+B, H+D, H+G+B and H+G+D showed a significant up regulation ($p<0.001$) when compared to control. In H+B, H+D H+G+B and H+G+D groups there was an significant increase of the gene expression levels ($p<0.001$) when compared with H and H+G groups (Figure-56, Table-58).

Real Time PCR amplification of TNF-α mRNA in the cerebellum of control and experimental rats

Real-time PCR gene expression of TNF-α showed significant up regulation ($p<0.001$) in the cerebellum of all the neonatal groups of rats compared to control. Treatment using *Bacopa monnieri* and Bacoside A: H+B, H+D, H+G+B and H+G+D significantly reversed ($p<0.001$) the altered gene expression when compared with hypoglycemic neonatal rats and glucose treated rats (Figure-57, Table-59).

Real Time PCR amplification of GDNF mRNA in the cerebellum of control and experimental rats

Real-time PCR gene expression of GDNF showed significant down regulation ($p<0.001$) in the cerebellum of hypoglycemic neonatal rats and H+G group and in the H+B and H+D groups ($p<0.05$) compared to control rats whereas H+G+B and H+G+D treatment showed no marked change when compared to control. The GDNF gene expression was significantly ($p<0.001$) reversed in H+B, H+D, H+G+B and H+G+D treatment to near control levels when compared with hypoglycemic and glucose treatment groups (Figure-58, Table-60).

Real Time PCR amplification of BDNF mRNA in the cerebellum of control and experimental rats

Real-time PCR gene expression of BDNF showed significant down regulation ($p<0.001$) in the cerebellum of all the groups of rats when compared to control. There was a significant reversal ($p<0.001$) in BDNF gene expression in the treatment groups: H+B, H+D, H+G+B and H+G+D groups when compared to H and H+G groups (Figure-59, Table-61).

Real Time PCR amplification of NF-ƙB mRNA in the cerebellum of control and experimental rats

Real-time PCR gene expression of NF-ƙB showed significant down regulation ($p<0.001$) in the cerebellum of hypoglycemic neonatal rats and H+G group compared to control rats. In H+B, H+D and H+G+D groups also there was seen to be a significant down regulation ($p<0.05$) compared to control. The gene expression was significantly ($p<0.001$) reversed in H+B, H+D, H+G+B and H+G+D groups when compared to hypoglycemic and H+G groups (Figure-60, Table-62).

Real Time PCR amplification of SOD mRNA in the cerebellum of control and experimental rats

Real-time PCR gene expression of SOD showed significant down regulation ($p<0.001$) in the cerebellum of hypoglycemic neonatal rats, H+G, H+B, H+D, H+G+B and H+G+D groups compared to control. There was a significant reversal ($p<0.001$) in SOD gene expression in hypoglycemic neonatal rats treated with *Bacopa monnieri* and Bacoside A and the combination treatments, H+G+B and H+G+D groups, ($p<0.001$) when compared to hypoglycemic neonatal group. Also a marked ($p<0.001$) reversal was observed in groups H+D, H+G+B and H+G+D compared to H+G group. H+B showed no prominent change when compared to H+G (Figure-61, Table-63).

Real Time PCR amplification of GPx mRNA in the cerebellum of control and experimental rats

Real-time PCR gene expression of GPx showed significant down regulation ($p<0.001$) in the cerebellum of all the experimental groups except H+G+B, compared to control rats. There was a significant reversal ($p<0.001$) in GPx gene expression in hypoglycemic neonatal rats treated with H+B, H+D, H+G+B and H+G+D groups when compared to hypoglycemic neonatal group. A significant ($p<0.001$) reversal was observed in groups H+**B**, H+G+B and H+G+D compared to H+G group. H+D is shown to no prominent change when compared to H+G (Figure-62, Table-64).

Real Time PCR amplification of Bax mRNA in the cerebellum of control and experimental rats

Real-time PCR gene expression of Bax showed significant up regulation ($p<0.001$) in the cerebellum of all the experimental groups except in H+G+D when compared to control rats. There was a significant reversal ($p<0.001$) in Bax gene expression in H+G, H+B, H+D, H+G+B and H+G+D groups compared to neonatal hypoglycemic group. In H+D, H+G+B and H+G+D groups, a significant ($p<0.001$) reversal in Bax gene expression was observed when compared to H+G group. (Figure-63, Table-65).

Real Time PCR amplification of caspase 8 mRNA in the cerebellum of control and experimental rats

Real-time PCR gene expression of caspase 8 showed significant down regulation ($p<0.001$) in the cerebellum of hypoglycemic neonatal rats and H+G group compared to control rats. There was a significant reversal ($p<0.001$) in Caspase 8 gene expression in the treatment groups H+B, H+D, H+G+B and H+G+D groups to near control levels compared to H and H+G groups (Figure-64, Table-66).

BRAIN STEM

Real Time PCR amplification of Dopamine D1 receptor mRNA in the brain stem of control and experimental rats

Gene expression of Dopamine D1 receptor subtype mRNA showed significant down regulation ($p<0.001$) in the brain stem of all experimental groups when compared to control. In H+B, H+D, H+G+B and H+G+D there was a significant reversal of the gene expression levels ($p<0.001$) when compared with hypoglycemic neonatal group and H+G group (Figure-65, Table-67).

Real Time PCR amplification of Dopamine D2 receptor mRNA in the brain stem of control and experimental rats

Gene expression of Dopamine D2 receptor subtype mRNA showed significant up regulation ($p<0.001$) in the brain stem of all the experimental groups when compared to control. In the treatment groups, H+B, H+D H+G+B and H+G+D there was a significant reversal ($p<0.001$) of the gene expression levels when compared with hypoglycemic neonatal group. The dopamine D2 receptor mRNA expression showed a significant reversal in H+B ($p<0.01$), H+D ($p<0.001$), H+G+B ($p<0.001$) and H+G+D ($p<0.001$) when compared to H+G group (Figure-66, Table-68).

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the brain stem of control and experimental rats

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH 23390 in the brain stem of hypoglycemic neonatal rats showed a significant ($p<0.001$) decrease in B_{max} and significant increase in K_d ($p<0.01$) compared to control rats. This showed decreased Dopamine D1 receptor density in the brain stem of hypoglycemic neonatal rats. Significant reversal ($p<0.05$) in the B_{max} was observed in treatment groups: H+B, H+D, H+G+B and H+G+D when compared with glucose treatment and neonatal hypoglycemic groups (Figure- 67,68, Table- 69, 70).

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in brain stem of control and experimental rats

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 binding against sulpiride in the brain stem of hypoglycemic neonatal rats and H+G groups showed a significant ($p<0.001$) increase in B_{max} compared to control rats. K_d showed no significant change. This showed increased Dopamine D2 receptor density in the brain stem of hypoglycemic neonatal rats. Significant reversal in the B_{max} was observed in treatment groups: H+B, H+D, H+G+B and H+G+D groups showed prominent reversal ($p<0.001$) when compared with glucose treatment and neonatal hypoglycemic groups (Figure- 69,70, Table- 71, 72).

cAMP content in the brain stem of control and experimental rats

cAMP content showed significant decrease ($p<0.001$) in the brain stem of all experimental groups except H+G+D group when compared to control rats. There was a significant reversal ($p<0.001$) in cAMP content in hypoglycemic neonatal rats treated with *Bacopa monnieri* and Bacoside A and H+G+B and H+G+D to near control levels when compared to hypoglycemic neonatal groups glucose treated rats (Figure-71, Table-73).

IP3 content in the brain stem of control and experimental rats

IP3 content showed significant increase ($p<0.001$) in the brain stem of all experimental groups except H+G+D group when compared to control rats. There was a significant reversal ($p<0.001$) in IP3 content in H+B, H+D, H+G+B and H+G+D groups to near control levels when compared with glucose treated rats (Figure -72, Table -74).

Real time PCR amplification of Phospholipase C mRNA in brain stem of control and experimental rats

Gene expression of Phospholipase C mRNA showed significant up regulation ($p<0.001$) in the brain stem of all experimental groups except H+G+D

group when compared to control rats. In the goups, H+B, H+D, H+G+B and H+G+D there was a significant reversal of the gene expression ($p<0.001$) when compared with hypoglycemic neonatal group and H+G group (Figure -73, Table -75).

Real time PCR amplification of CREB mRNA in the brain stem of control and experimental rats

Gene expression of CREB mRNA showed significant up regulation in the brain stem of all the experimental groups when compared to control. In the individual treatment groups, H+B and H+D and In the combination treatment groups, H+G+B and H+G+D there was a significant reversal of the gene expression levels ($p<0.001$) when compared with H and H+G groups (Figure -74, Table -76).

Real Time PCR amplification of GLUT 3 mRNA in the brain stem of control and experimental rats

Real-time PCR gene expression of GLUT 3 showed significant decrease ($p<0.001$) in the brain stem of hypoglycemic neonatal rats, H+G+B and H+G+D groups when compared to control rats. H+G, H+B and H+D groups showed a marked up regulation ($p<0.001$) compared to control rats. H+G, H+B, H+D, H+G+B and H+G+D showed a significant ($p<0.001$) reversal when compared to the neonatal hypoglycemic group. (Figure-75, Table-77).

Real Time PCR amplification of Akt -1 mRNA in the brain stem of control and experimental rats

Real time PCR gene expression of Akt -1 showed significant($p<0.001$) down regulation in the brain stem of hypoglycemic neonatal rats, H+G, H+B and H+D groups when compared to control. In the treatment with *Bacopa monnieri* and Bacoside A, H+B and H+D groups and in the combination treatment groups, H+G+B and H+G+D there was a significant reversal of the gene expression levels ($p<0.001$) when compared with H and H+G groups (Figure-76, Table-78).

Real Time PCR amplification of TNF-α mRNA in the brain stem of control and experimental rats

Real-time PCR gene expression of TNF-α showed significant down regulation ($p<0.001$) in the brain stem of H, H+G and H+B groups when compared to control. Treatment using All the treatment groups, H+B, H+D, H+G+B and H+G+D treatment significantly reversed ($p<0.001$) the altered gene expression to near control levels when compared with hypoglycemic neonatal group and H+G group (Figure-77, Table-79).

Real Time PCR amplification of GDNF mRNA in the brain stem of control and experimental rats

Real-time PCR gene expression of GDNF showed significant down regulation ($p<0.001$) in the brain stem of all the experimental groups compared to control rats. The GDNF gene expression was significantly reversed ($p<0.001$) in H+B, H+D, H+G+B and H+G+D treatment when compared with hypoglycemic and glucose treatment groups (Figure-78, Table-80).

Real Time PCR amplification of BDNF mRNA in the brain stem of control and experimental rats

Real-time PCR gene expression of BDNF showed significant down regulation ($p<0.001$) in the brain stem of all the experimental groups compared to control rats. There was a significant reversal ($p<0.001$) in BDNF gene expression in H+B, H+D, H+G+B and H+G+D groups, the gene expression was reversed back when compared with hypoglycemic and glucose treatment groups (Figure-79, Table-81).

Real Time PCR amplification of NF-ƙB mRNA in the brain stem of control and experimental rats

Real-time PCR gene expression of NF-ƙB showed significant down regulation ($p<0.001$) in the brain stem of all the experimental groups compared to

control rats. There was a significant reversal to near control levels ($p<0.001$) in NF-ƙB gene expression in hypoglycemic neonatal rats treated with *Bacopa monnieri* and Bacoside A alone: H+B, H+D and in combination: H+G+B and H+G+D groups, when compared to hypoglycemic and H+G group (Figure-80, Table-82).

Real Time PCR amplification of SOD mRNA in the brain stem of control and experimental rats

Real-time PCR gene expression of SOD showed significant down regulation ($p<0.001$) in the brain stem of H, H+G, H+B, H+D, H+G+B and H+G+D groups. In the treatment groups: H+B, H+D, H+G+B and H+G+D groups, the gene expression was significantly reversed ($p<0.001$) when compared to hypoglycemic and H+G group (Figure-81, Table-83).

Real Time PCR amplification of GPx mRNA in the brain stem of control and experimental rats

Real-time PCR gene expression of GPx showed significant down regulation in the brain stem of hypoglycemic neonatal rats ($p<0.001$), H+G ($p<0.001$) and H+B ($p<0.01$) group compared to control rats. H+D and H+G+D showed a significant ($p<0.01$) up regulation compared to control. There was a significant reversal ($p<0.001$) in GPx gene expression in hypoglycemic neonatal rats treated with *Bacopa monnieri* and Bacoside A alone and in combination, H+G+B and H+G+D when compared to H and H+G groups (Figure-82, Table-84).

Real Time PCR amplification of Bax mRNA in the brain stem of control and experimental rats

Real-time PCR gene expression of Bax showed significant up regulation ($p<0.001$) in the brain stem of all the experimental groups compared to control rats. H+B, H+D, H+G+B and H+G+D groups showed significant reversal

($p<0.001$) of the Bax mRNA levels compared to H and H+G groups (Figure-83, Table-85).

Real Time PCR amplification of caspase 8 mRNA in the brain stem of control and experimental rats

Real-time PCR gene expression of caspase 8 showed significant up regulation ($p<0.001$) in the brain stem of hypoglycemic neonatal rats, H+G, H+B, H+D groups compared to control rats. There was a significant reversal ($p<0.001$) in Caspase 8 gene expression in H+B, H+D, H+G+B and H+G+D groups when compared to H and H+G groups (Figure-84, Table-86).

Table-1
Body Weight of control and
Experimental Groups of Neonatal Rats

Experimental groups	Body weight (g)	
	Initial Day of Experiment	Final Day of Experiment
C	8.56 ± 0.29	12.16 ± 0.15
H	7.35 ± 0.54	9.22 ± 0.55[a]
H+G	7.12 ± 0.25	12.11 ± 0.62[d]
H+B	7.85 ± 0.45	13.14 ± 0.32 [d]
H+D	7.33 ± 0.18	13.31± 0.35 [d]
H+G+B	7.12 ± 0.25	13.52 ± 0.15 [d]
H+G+D	7.03 ± 0.15	13.42 ± 0.13 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Table-2a
Blood glucose level of control and experimental groups of rats on the initial Day and final Day of treatment

Experimental groups	Initial Blood Glucose Level before treatment (mg/dl) (Initial Day)	Blood Glucose levels 24 hrs after final treatment (mg/dl) (10^{th} Day)
C	110 ± 1.32	120.23 ± 1.74
H	120 ± 2.84	83.52± 0.58 [a]
H+G	110 ± 2.48	138.55 ± 1.15 [a,d]
H+B	115 ± 1.22	113.21 ± 2.15 [d]
H+D	120 ± 1.31	110.37 ± 1.38 [d]
H+G+B	110 ± 2.15	129.05 ± 1.25 [d]
H+G+D	115 ± 2.77	123.59 ± 1.03 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Table-2b
Blood Glucose level of control and Experimental Groups of Rats

Experimental groups	Blood Glucose levels (mg/dl)	
	0 Min (Before Insulin Injection)	240 Min after Insulin Injection
C	120±1.32	120.23 ± 1.74
H	115± 2.84	48.52 ± 0.58 [a]
H+G	118 ± 3.48	53.55 ± 1.15 [a]
H+B	117± 2.22	73.21 ± 2.15 [a,d,g]
H+D	120± 2.31	80.37 ± 1.38 [a,d,g]
H+G+B	114±2.15	89.05 ± 1.25 [a,d,g]
H+G+D	115 ± 3.77	83.59 ± 1.03 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-1
Real Time PCR amplification of Dopamine D1 receptor mRNA in the cerebral cortex of control and experimental rats

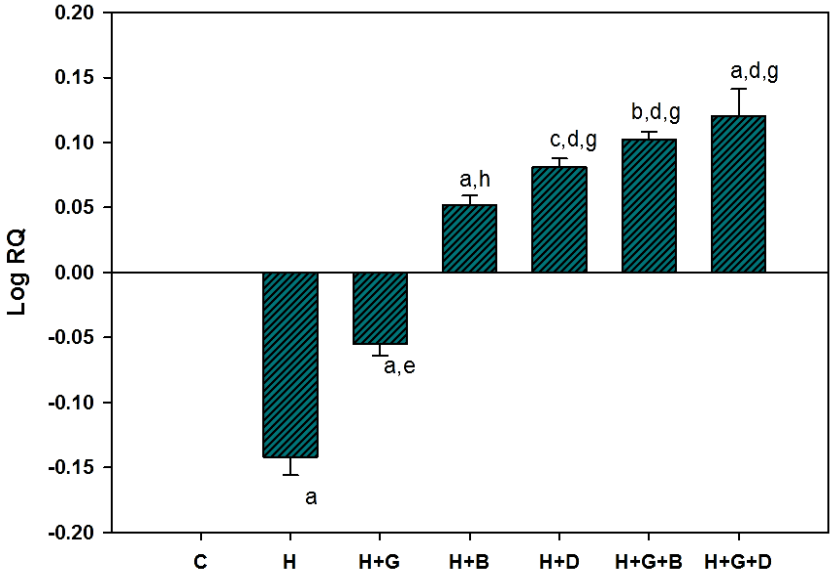


Table-3
Real Time PCR amplification of Dopamine D1 receptor mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**-0.14 ± 0.083** [a]
H+G	**-0.05 ± 0.005**[a,e]
H+B	**0.05 ± 0.004**[a,h]
H+D	**0.08 ± 0.003**[c,d,g]
H+G+B	**0.10 ± 0.004** [b,d,g]
H+G+D	**0.12 ± 0.007** [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 2
Real Time PCR amplification of Dopamine D2 receptor mRNA in the cerebral cortex of control and experimental rats

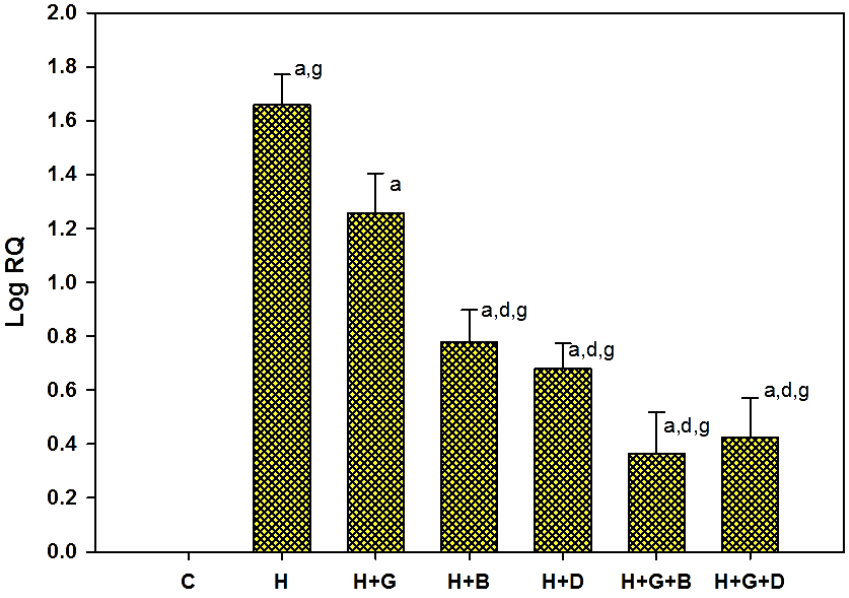


Table- 4
Real Time PCR amplification of Dopamine D2 receptor mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**1.66 ± 0.12** [a]
H+G	**1.26 ± 0.09** [a,d]
H+B	**0.78 ± 0.13** [a,d,g]
H+D	**0.68 ± 0.05** [a,d,g]
H+G+B	**0.36 ± 0.09** [a,d,g]
H+G+D	**0.42 ± 0.14** [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure- 3
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the cerebral cortex of control and experimental rats

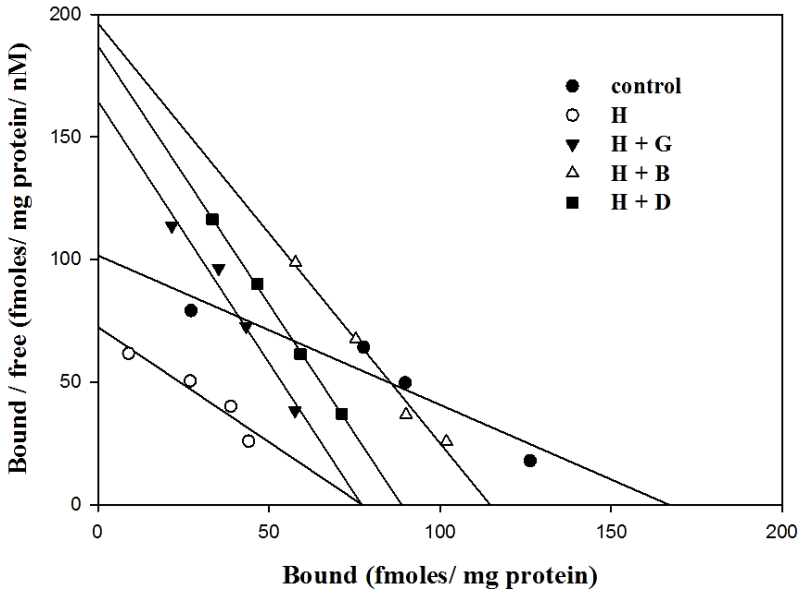


Table- 5
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 against SCH 23390 in cerebral cortex of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	164.90 ± 12.55	1.61± 0.03
H	75.15 ± 14.01 [a]	1.25 ± 0.12
H+G	76.80 ± 12.50 [a]	1.12 ± 0.01
H+B	113.61 ± 11.55 [a,d,g]	1.41 ± 0.05
H+D	89.16 ± 13.05 [a,d,g]	1.30 ± 0.04

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-4
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the cerebral cortex of control and experimental rats

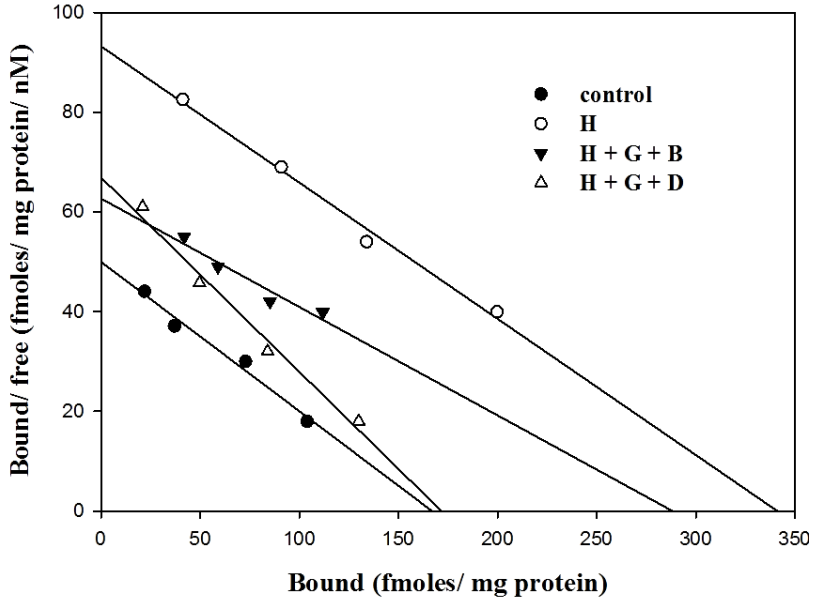


Table- 6
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 against SCH 23390 in cerebral cortex of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	**164.90 ± 12.55**	**1.60 ±0.10**
H	**75.15 ± 14.01** [a]	**1.25 ± 0.12**
H+G+B	**288.10 ± 12.60** [b, d,g]	**1.50 ± 0.10**
H+G+D	**171.17 ± 12.50** [d,g]	**1.70 ± 0.12**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-5
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in cerebral cortex of control and experimental rats

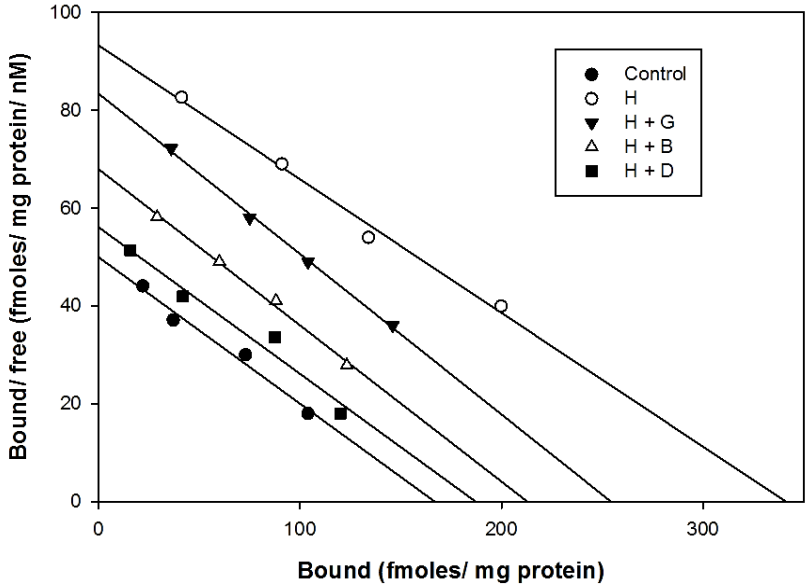


Table- 7
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 binding against sulpiride in the cerebral cortex of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	**166.31 ± 11.88**	**3.40 ± 0.04**
H	**340.52 ± 12.05** [a]	**3.77 ± 0.08**
H+G	**253.10 ± 12.66** [a,d]	**2.90 ± 0.05**
H+B	**212.20 ± 12.90** [a,d,g]	**3.60 ± 0.15**
H+D	**185.91 ± 12.45** [a,d,g]	**3.31 ± 0.01**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c] p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f] p<0.05 when compared to Hypoglycemic group.
[g] p<0.001, [h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure- 6
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in cerebral cortex of control and experimental rats

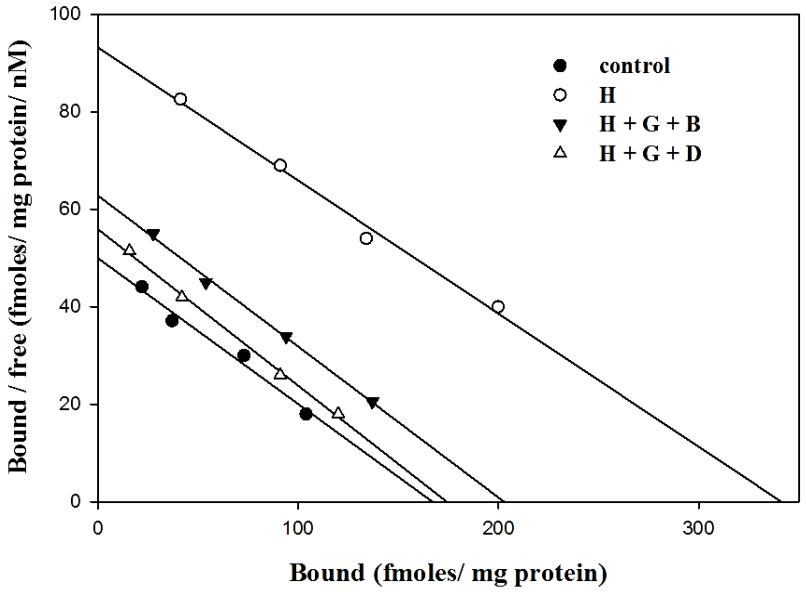


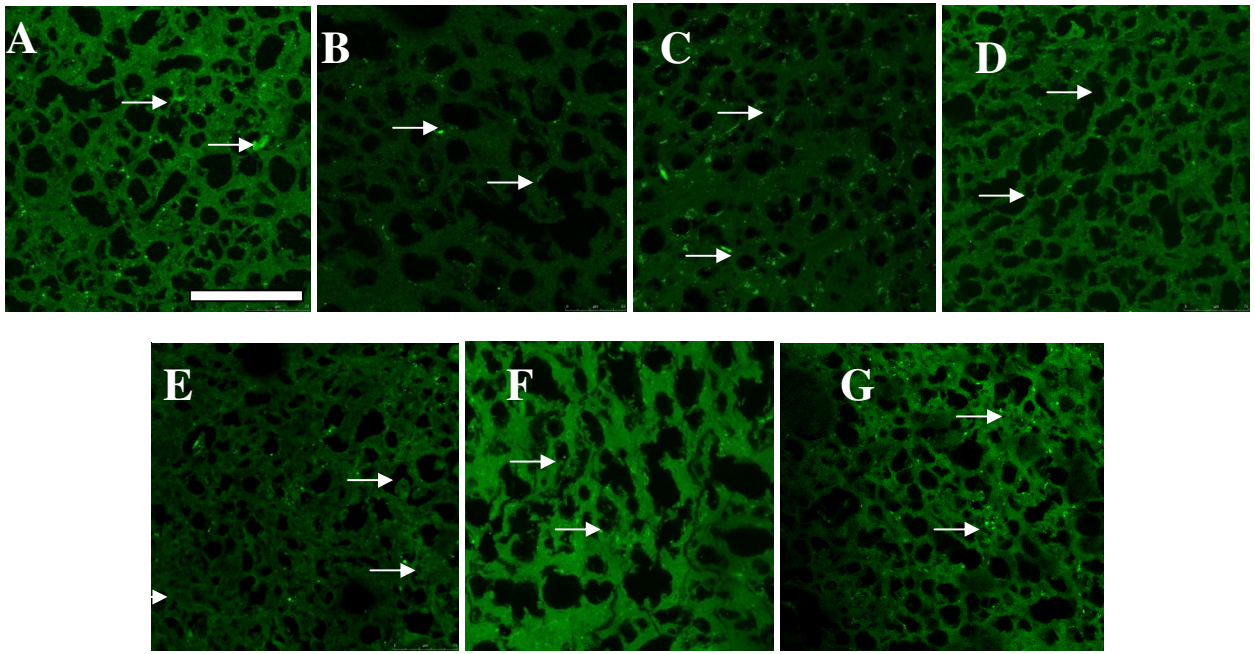
Table- 8
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 binding against sulpiride in the cerebral cortex of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	**166.31 ± 11.88**	**3.40 ± 0.04**
H	**340.50 ± 12.05** [a,g]	**3.77 ± 0.08**
H+G+B	**205.51 ±12.12** [a,d,g]	**4.10 ± 0.12**
H+G+D	**171.80 ± 12.15** [c,d,g]	**3.10 ± 0.13**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 7

Dopamine D1 receptor expression in the cerebral cortex of control and experimental rats



A- Control, B- Hypoglycemia, C- Hypoglycemia treated with glucose, D - Hypoglycemia treated with *Bacopa monnieri*. E- Hypoglycemia treated with Bacoside A, F- Hypoglycemia treated with Glucose and *Bacopa monnieri*. G- Hypoglycemia treated with Glucose and Bacoside A. The scale bar represents 75 μm.

Table - 9

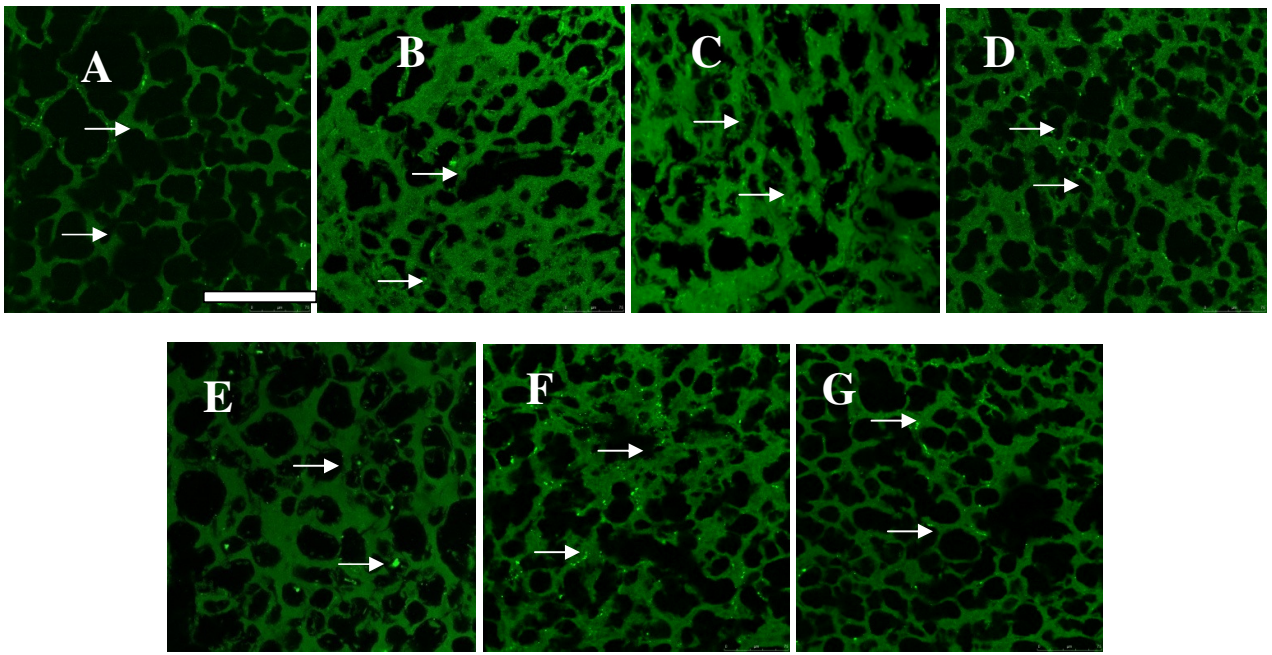
Dopamine D1 receptor expression in the cerebral cortex of control and experimental rats

Experimental groups	Mean pixel intensity
Control	**79.50 ± 3.50**
H	**32.20 ± 3.00** [a]
H + G	**34.45 ± 2.50** [a]
H + B	**51.25 ± 3.05** [a,d,g]
H + D	**47.54 ± 2.55** [a,d,g]
H + G + B	**70.65 ± 5.00** [c,d,g]
H + G + D	**72.50 ± 3.50** [c,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control. [d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group. [g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose. C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 8

Dopamine D2 receptor expression in the cerebral cortex of control and experimental rats



A- Control, B- Hypoglycemia, C- Hypoglycemia treated with glucose, D - Hypoglycemia treated with *Bacopa monnieri*. E- Hypoglycemia treated with Bacoside A, F- Hypoglycemia treated with Glucose and *Bacopa monnieri*. G- Hypoglycemia treated with Glucose and Bacoside A. The scale bar represents 75 µm.

Table - 10

Dopamine D2 receptor expression in the cerebral cortex of control and experimental rats

Experimental groups	Mean pixel intensity
Control	**32.50 ± 3.50**
H	**79.20 ± 3.00** [a]
H + G	**74.45 ± 2.50** [a]
H + B	**41.25 ± 3.05** [c,d,g]
H + D	**47.54 ± 2.55** [a,d,g]
H + G + B	**37.65 ± 5.00** [d,g]
H + G + D	**33.50 ± 3.50** [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control. [d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group. [g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose. C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 9

cAMP content in the cerebral cortex of control and experimental rats

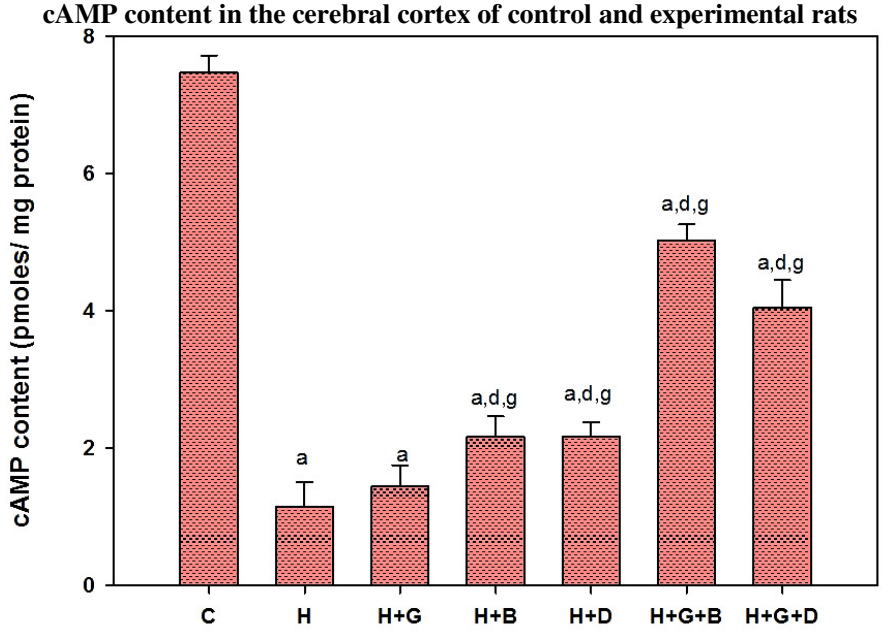


Table- 11

cAMP content in the cerebral cortex of control and experimental rats

Experimental groups	cAMP content (pmoles/mg protein)
C	**7.45 ± 0. 2**
H	**1.14 ± 0.36** [a]
H+G	**1.44 ± 0.31** [a]
H+B	**2.16 ± 0.30** [a,d,g]
H+D	**2.17 ± 0.16** [a,d,g]
H+G+B	**5.03 ± 0.23** [a,d,g]
H+G+D	**4.04 ± 0.40** [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D-Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure – 10

IP3 content in the cerebral cortex of control and experimental rats

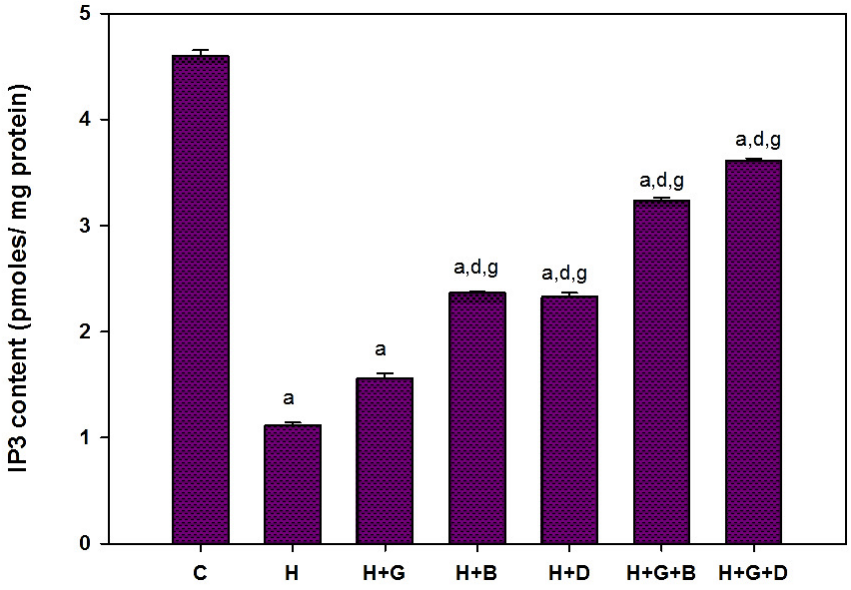


Table- 12

IP3 content in the cerebral cortex of control and experimental rats

Experimental groups	IP3 content (pmoles/mg protein)
C	**4.60 ± 0.05**
H	**1.12 ± 0.03** [a]
H+G	**1. 56 ± 0.05** [a,d]
H+B	**2.37 ± 0.015** [a,d,g]
H+D	**2.33 ± 0.04** [a,d,g]
H+G+B	**3.24 ± 0.02** [a,d,g]
H+G+D	**3.61 ± 0.02** [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 11
Real Time PCR amplification of Phospholipase C mRNA in the cerebral cortex of control and experimental rats

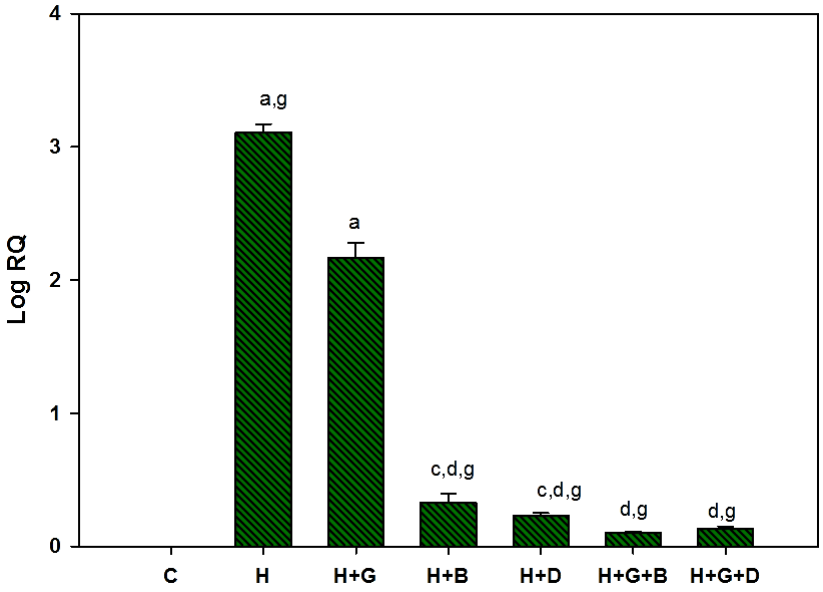


Table- 13
Real Time PCR amplification of Phospholipase C mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	0
H	3.11 ± 0.06 [a,g]
H+G	2.16 ± 0.11 [a]
H+B	0.33 ± 0.07 [c,d,g]
H+D	0.23 ± 0.02 [c,d,g]
H+G+B	0.10 ± 0.01 [d,g]
H+G+D	0.13 ± 0.01 [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 12

Real Time PCR amplification of CREB mRNA in the cerebral cortex of control and experimental rats

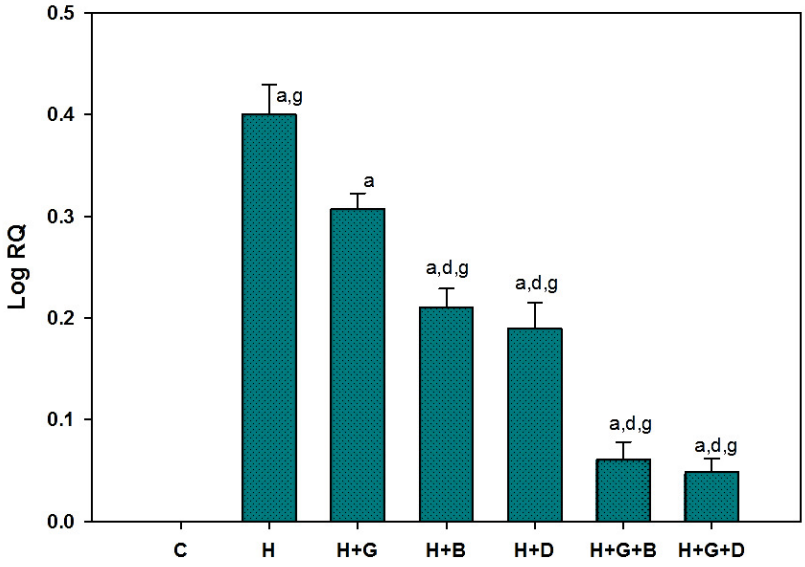


Table- 14
Real Time PCR amplification of CREB mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	0
H	0.40 ± 0.11 [a,g]
H+G	0.31 ± 0.05 [a]
H+B	0.21 ± 0.08 [a,d,g]
H+D	0.19 ± 0.04 [a,d,g]
H+G+B	0.06 ± 0.01[a,d,g]
H+G+D	0.05 ± 0.01[a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 13
Real Time PCR amplification of GLUT 3 mRNA in the cerebral cortex of control and experimental rats

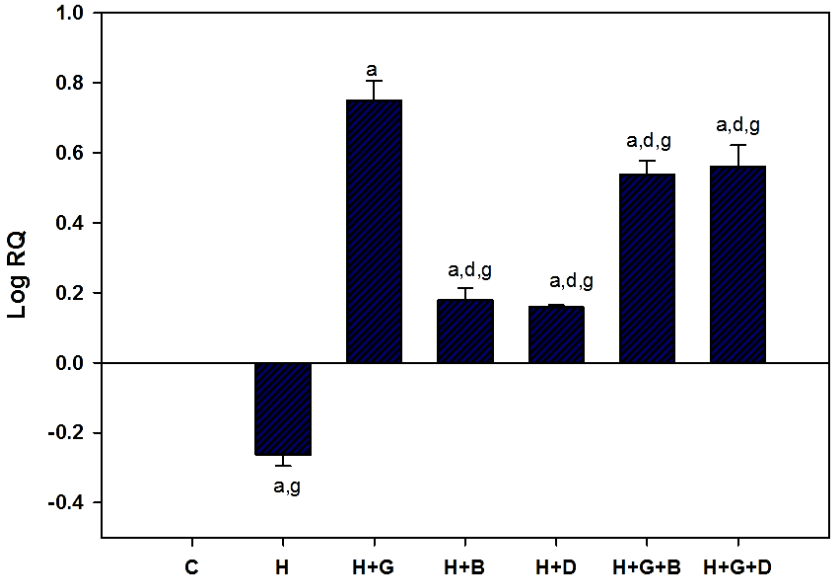


Table- 15
Real Time PCR amplification of GLUT 3 mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.26 ± 0.03[a,g]
H+G	0.75 ± 0.05 [a]
H+B	0.18 ± 0.03 [a,d,g]
H+D	0.16 ± 0.01 [a,d,g]
H+G+B	0.54 ± 0.04 [a,d,g]
H+G+D	0.56 ± 0.06 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose andu *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 14

Real Time PCR amplification of Akt -1 mRNA in the cerebral cortex of control and experimental rats

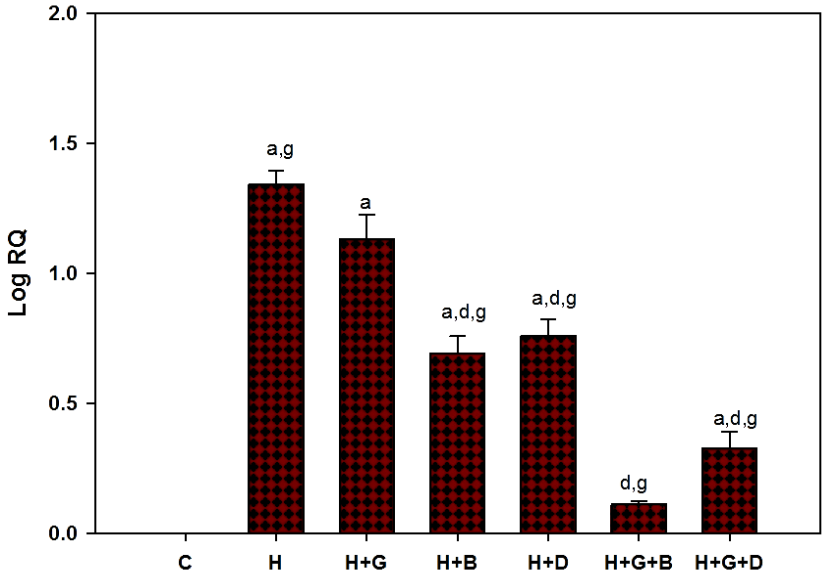


Table- 16
Real Time PCR amplification of Akt-1 mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	0
H	1.34 ± 0.05 [a,g]
H+G	1.13 ± 0.09 [a]
H+B	0.69 ± 0.07 [a,d,g]
H+D	0.76 ± 0.06 [a,d,g]
H+G+B	0.11 ± 0.01 [d,g]
H+G+D	0.33 ± 0.06 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 15
Real Time PCR amplification of TNF-α mRNA in the cerebral cortex of control and experimental rats

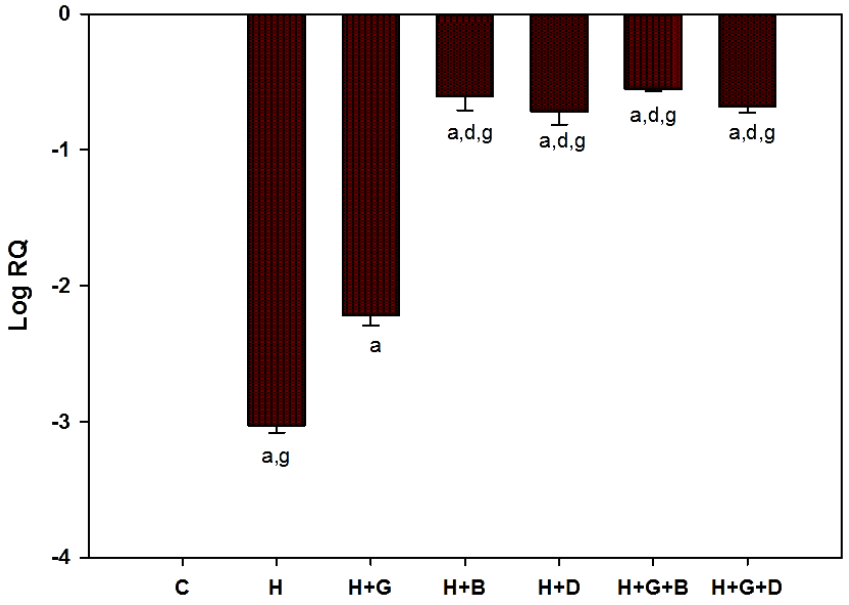


Table- 17
Real Time PCR amplification of TNF-α mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	0
H	-3.03 ± 0.05[a,g]
H+G	-2.22 ± 0.07[a]
H+B	-0.06 ± 0.10 [a,d,g]
H+D	-0.72 ± 0.09 [a, d,g]
H+G+B	-0.55 ± 0.01 [a,d,g]
H+G+D	-0.68 ± 0.04 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 16

Real Time PCR amplification of GDNF mRNA in the cerebral cortex of control and experimental rats

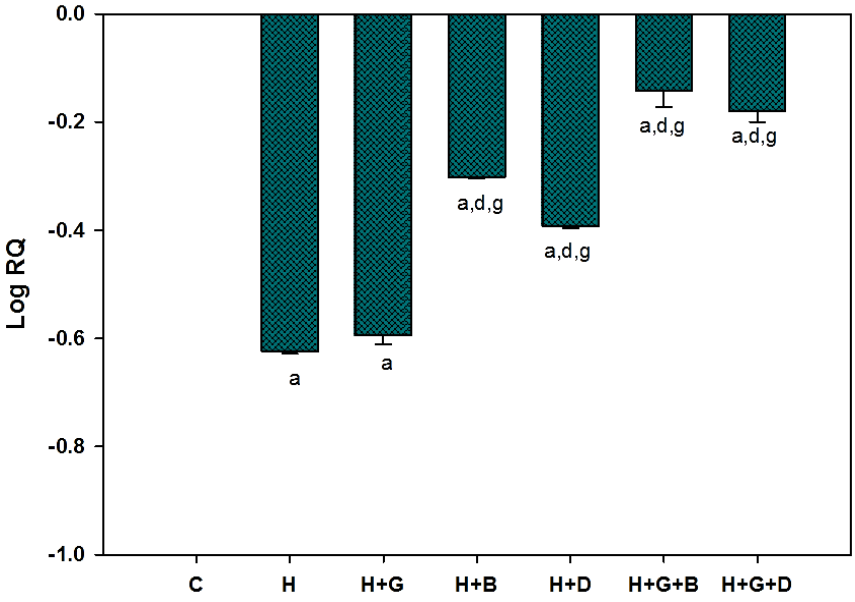


Table- 18
Real Time PCR amplification of GDNF mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**-0.62 ± 0.01** [a]
H+G	**-0.59 ± 0.01** [a]
H+B	**-0.40 ± 0.01** [a,d,g]
H+D	**-0.39 ± 0.01** [a,d,g]
H+G+B	**-0.24 ± 0.03** [a,d,g]
H+G+D	**-0.15 ± 0.02** [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 17
Real Time PCR amplification of BDNF mRNA in the cerebral cortex of control and experimental rats

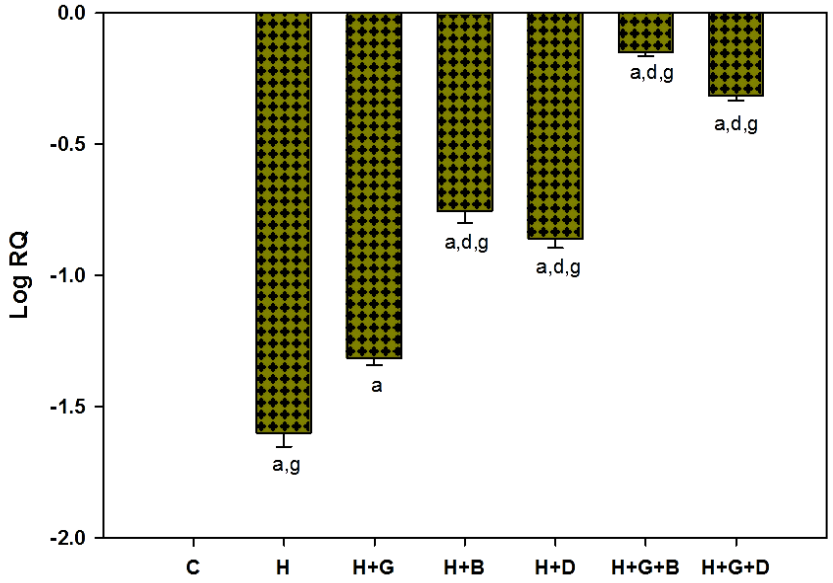


Table- 19
Real Time PCR amplification of BDNF mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	0
H	-1.60 ± 0.05 [a,g]
H+G	-1.32 ± 0.03 [a]
H+B	-1.06 ± 0.04 [a,d,g]
H+D	-0.86 ± 0.04 [a,d,g]
H+G+B	-0.14 ± 0.01 [a,d,g]
H+G+D	-0.31 ± 0.02 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 18

Real Time PCR amplification of NF-ƙB mRNA in the cerebral cortex of control and experimental rats

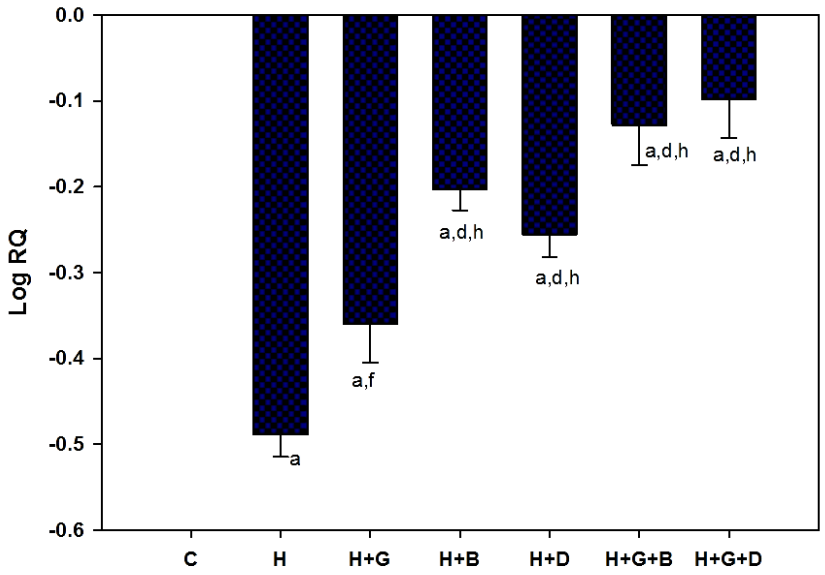


Table- 20
Real Time PCR amplification of NF-ƙB mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**-0.49 ± 0.05[a]**
H+G	**-0.36 ± 0.05[a,f]**
H+B	**-0.20 ± 0.04[a,d,h]**
H+D	**-0.25 ± 0.06 [a,d,h]**
H+G+B	**-0.13 ± 0.09[a,d,h]**
H+G+D	**-0.09 ± 0.08[a,d,h]**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 19
Real Time PCR amplification of SOD mRNA in the cerebral cortex of control and experimental rats

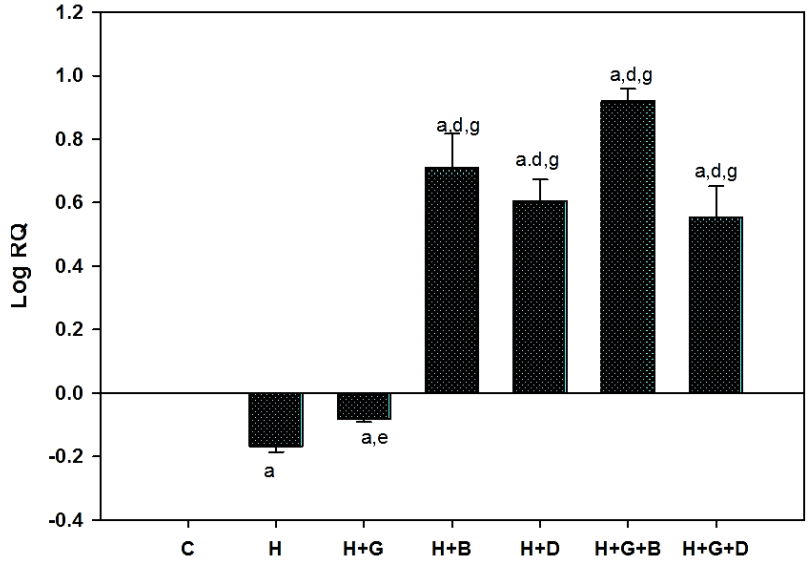


Table-21
Real Time PCR amplification of SOD mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.17 ± 0.01[a]
H+G	-0.08 ± 0.01 [a,e]
H+B	0.71 ± 0.06 [a,d,g]
H+D	0.60 ± 0.04 [a,d,g]
H+G+B	0.91 ± 0.02 [a,d,g]
H+G+D	0.55 ± 0.06 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 20

Real Time PCR amplification of GPx mRNA in the cerebral cortex of control and experimental rats

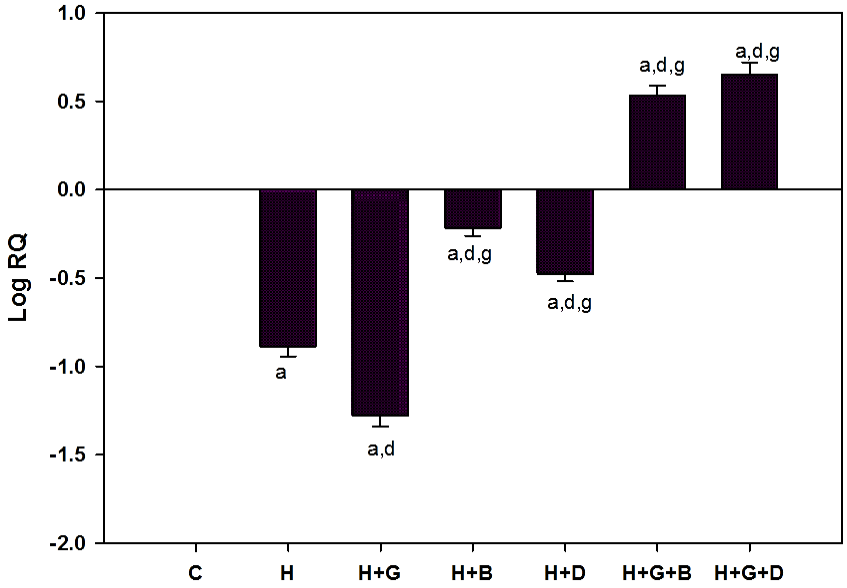


Table- 22
Real Time PCR amplification of GPx mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.89 ±0.06 [a]
H+G	-1.27 ±0.06 [a,d]
H+B	-0.22 ±0.05 [a,d,g]
H+D	-0.48 ±0.04 [a,d,g]
H+G+B	0.53 ±0.06 [a,d,g]
H+G+D	0.65 ±0.07 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 21

Real Time PCR amplification of Bax mRNA in the cerebral cortex of control and experimental rats

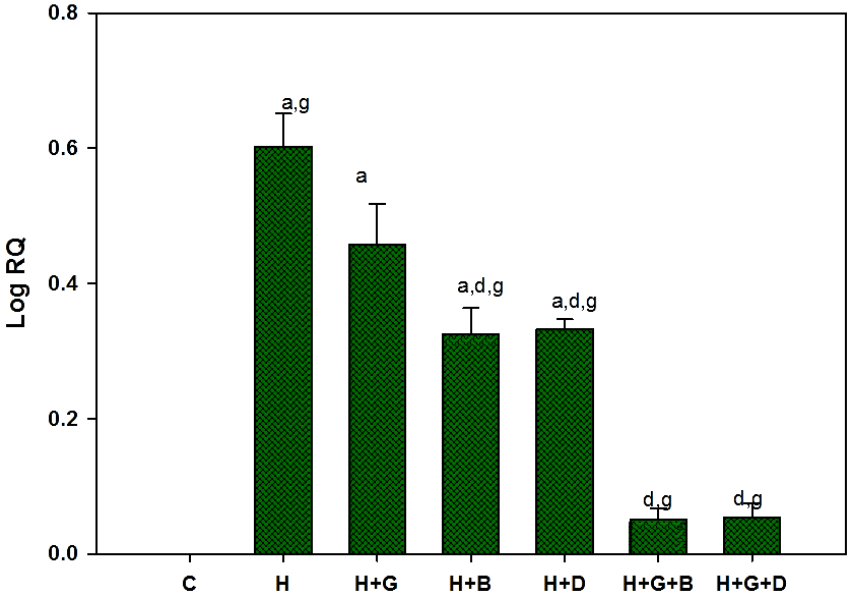


Table-23
Real Time PCR amplification of Bax mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**0.60 ± 0.09** [a,g]
H+G	**0.46 ± 0.09**[a]
H+B	**0.33 ± 0.02** [a,d,g]
H+D	**0.34 ± 0.08** [a,d,g]
H+G+B	**0.05 ± 0.01**[d,g]
H+G+D	**0.05 ± 0.01**[d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 22

Real Time PCR amplification of caspase 8 mRNA in the cerebral cortex of control and experimental rats

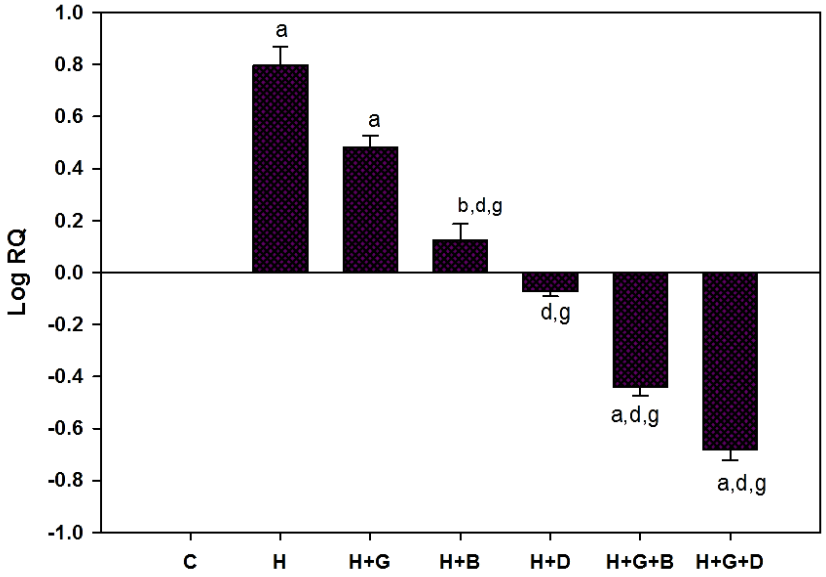


Table- 24
Real Time PCR amplification of caspase 8 mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
C	0
H	0.80 ± 0.02^{a}
H+G	0.48 ± 0.08^{a}
H+B	$0.12 \pm 0.06^{b,d,g}$
H+D	$-0.07 \pm 0.01^{d,g}$
H+G+B	$-0.44 \pm 0.04^{a,d,g}$
H+G+D	$-0.68 \pm 0.04^{b,d,g}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-23
Real Time PCR amplification of Dopamine D1 receptor mRNA in the corpus striatum of control and experimental rats

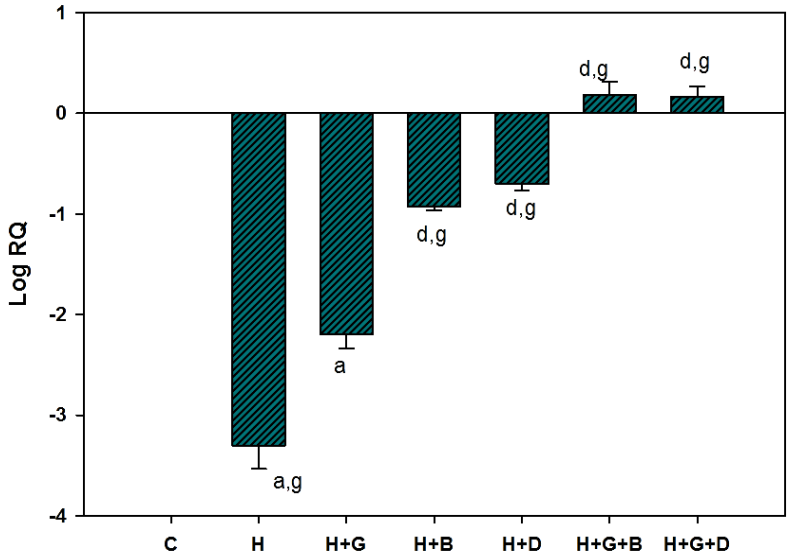


Table-25
Real Time PCR amplification of Dopamine D1 receptor mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-3.30 ± 0.23[a,g]
H+G	-2.19 ± 0.14[a]
H+B	0.93 ± 0.04 [a d,g]
H+D	0.70 ± 0.06 [a d,g]
H+G+B	1.54 ± 0.13[d,g]
H+G+D	2.36 ± 0.31[d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 24
Real Time PCR amplification of Dopamine D2 receptor mRNA in the corpus striatum of control and experimental rats

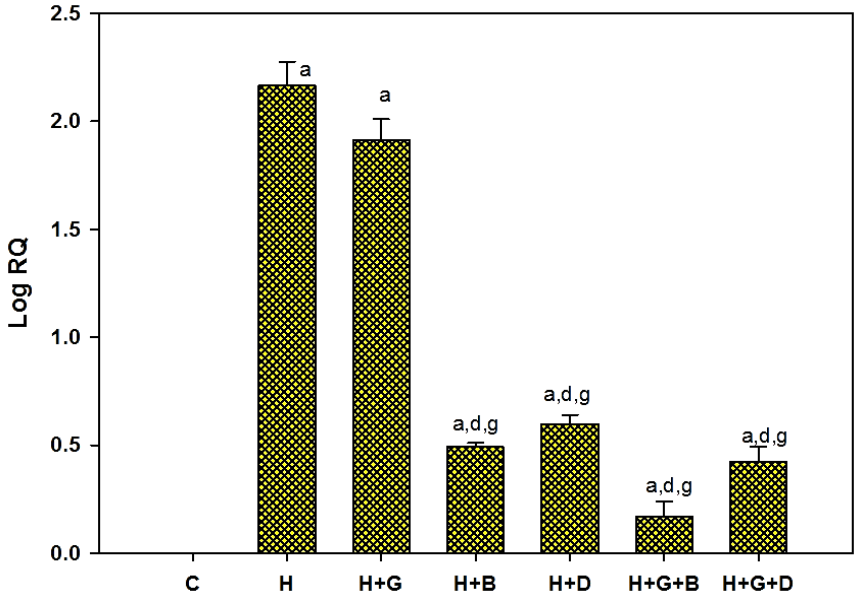


Table- 26
Real Time PCR amplification of Dopamine D2 receptor mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	2.16 ± 0.11[a]
H+G	1.91 ± 0.09[a]
H+B	0.49 ± 0.02[a,d,g]
H+D	0.30 ± 0.04[a,d,g]
H+G+B	1.57 ± 0.07[a,d,g]
H+G+D	1.14 ± 0.07[a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-25

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the corpus striatumof control and experimental rats

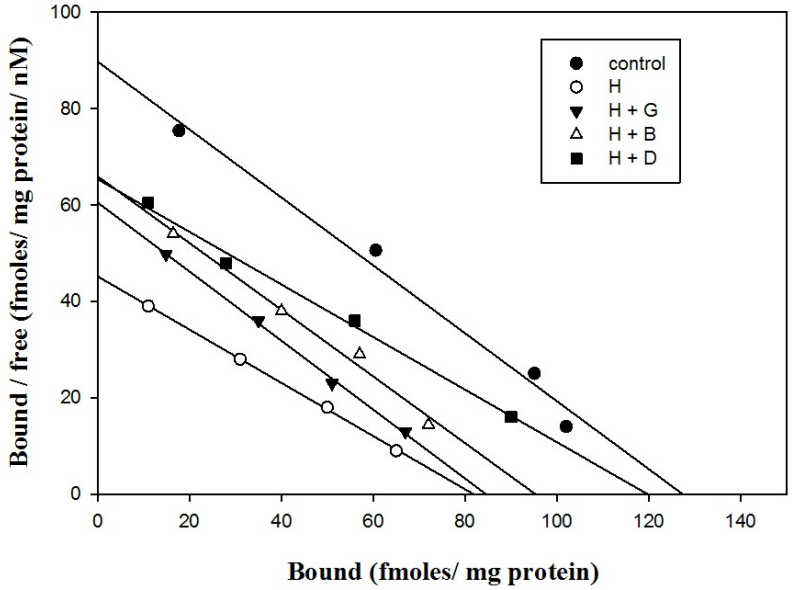


Table-27
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the corpus striatum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	126.8 ± 12.61	1.3 ± 0.20
H	80.94 ± 11.52 [a]	1.9 ± 0.02
H+G	84.69 ± 10.61 [a,d,g]	1.7 ± 0.12
H+B	95.31 ± 7.02 [a,d,g]	1.5 ± 0.20
H+D	119.30 ± 11.02 [a,d,g]	1.3 ± 0.34

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-26
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the corpus striatumof control and experimental rats

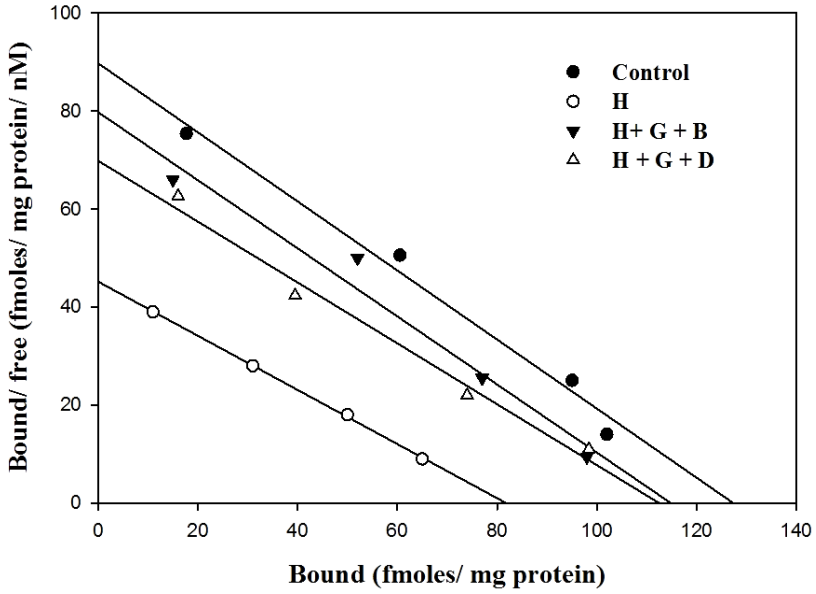


Table-28
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the corpus striatum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	126.80 ± 12.61	1.31 ± 0.12
H	80.94 ± 11.52 [a]	1.90 ± 0.13
H+G+B	114.90 ± 12.60 [a,d,g]	1.21 ± 0.15
H+G+D	111.60 ± 14.51 [a,d,g]	1.22 ± 0.12

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-27

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in corpus striatum of control and experimental rats

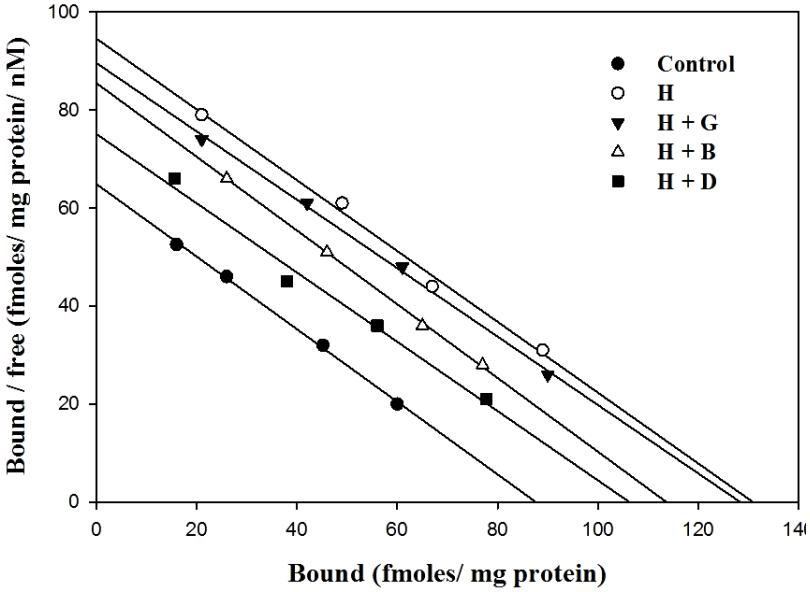


Table-29

Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in corpus striatum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	87.50 ± 5.01	1.51 ± 0.10
H	130.30 ± 13.51 [a]	1.30 ± 0.09
H+G	127.70 ± 11.20 [a]	1.21 ± 0.11
H+B	113.80 ± 10.51 [a,d,g]	1.42 ± 0.16
H+D	105.50 ± 12.01 [a,d,g]	1.20 ± 0.12

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-28
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in corpus striatum of control and experimental rats

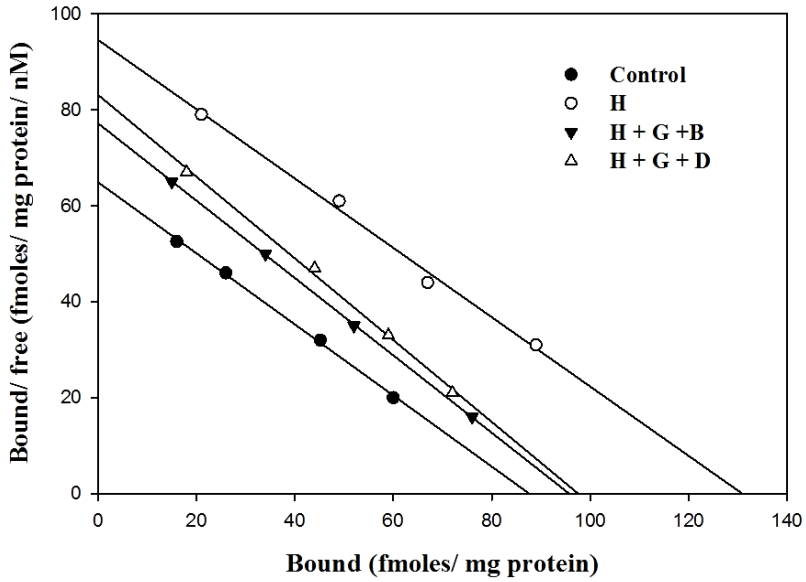


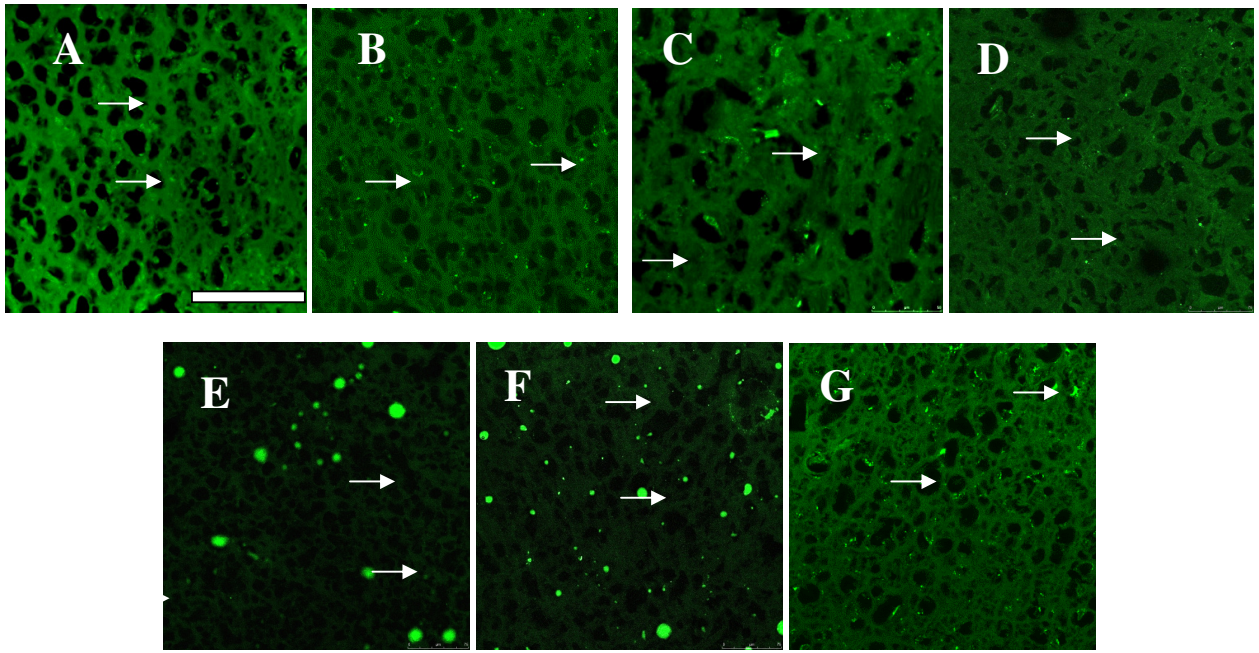
Table-30
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in corpus striatum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	87.50 ± 15.00	1.51 ± 2.50
H	130.30 ± 16.50 [a]	1.30 ± 1.40
H+G+B	114.90 ± 11.50 [a,d,g]	1.20 ± 1.10
H+G+D	112.30 ± 12.00 [b,d,g]	1.42 ±1.50

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 29

Dopamine D1 receptor expression in the corpus striatum of control and experimental rats



A- Control, B- Hypoglycemia, C- Hypoglycemia treated with glucose, D - Hypoglycemia treated with *Bacopa monnieri*. E- Hypoglycemia treated with Bacoside A, F- Hypoglycemia treated with Glucose and *Bacopa monnieri*. G- Hypoglycemia treated with Glucose and Bacoside A. The scale bar represents 75 µm.

Table - 31

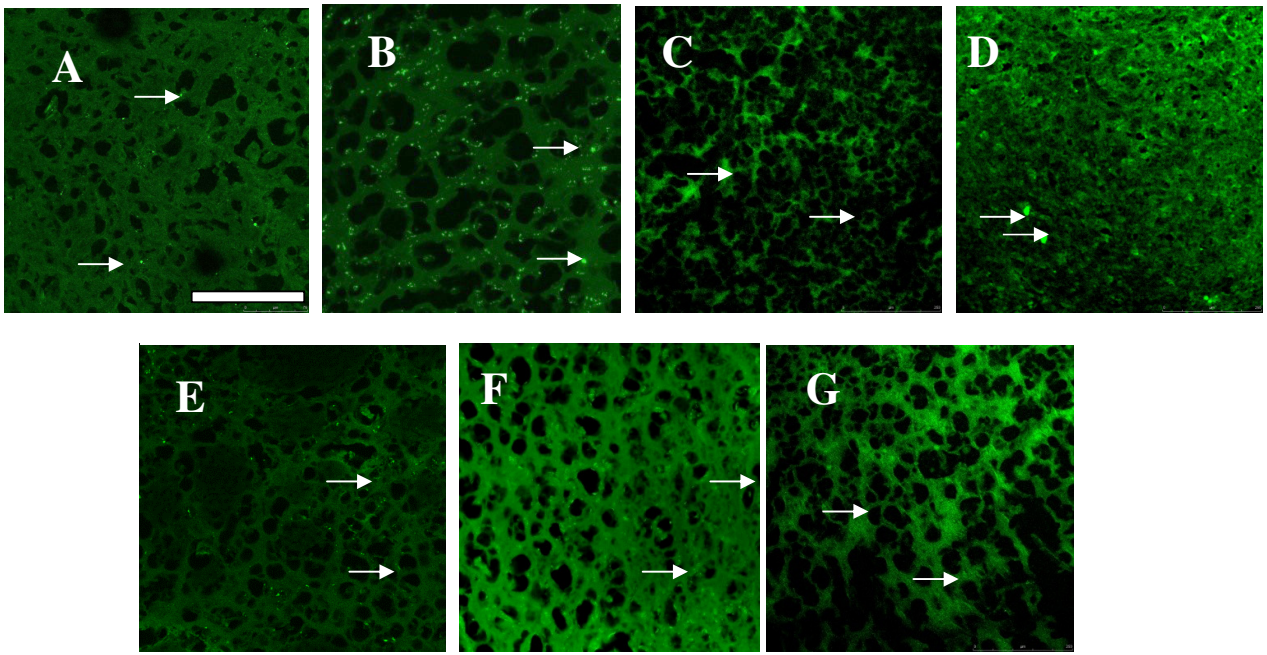
Dopamine D1 receptor expression in the corpus striatum of control and experimental rats

Experimental groups	Mean pixel intensity
Control	**82.50 ± 3.50**
H	**39.20 ± 3.00** [a]
H + G	**34.45 ± 2.50** [a]
H + B	**51.25 ± 3.05** [a,d,g]
H + D	**67.54 ± 2.55** [a,d,g]
H + G + B	**72.65 ± 5.00** [d,g]
H + G + D	**79.50 ± 3.50** [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control. [d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group. [g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose. C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 30

Dopamine D2 receptor expression in the corpus striatum of control and experimental rats



A- Control, B- Hypoglycemia, C- Hypoglycemia treated with glucose, D - Hypoglycemia treated with *Bacopa monnieri*. E- Hypoglycemia treated with Bacoside A, F- Hypoglycemia treated with Glucose and *Bacopa monnieri*. G- Hypoglycemia treated with Glucose and Bacoside A. The scale bar represents 75 µm.

Table - 32

Dopamine D2 receptor expression in the corpus striatum of control and experimental rats

Experimental groups	Mean pixel intensity
Control	**52.50 ± 3.50**
H	**87.20 ± 3.00** [a]
H + G	**84.45 ± 2.50** [a]
H + B	**41.25 ± 3.05** [d,g]
H + D	**47.54 ± 2.55** [d,g]
H + G + B	**49.65 ± 5.00** [d,g]
H + G + D	**50.50 ± 3.50** [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control. [d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group. [g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose. C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 31

cAMP content in the corpus striatum of control and experimental rats

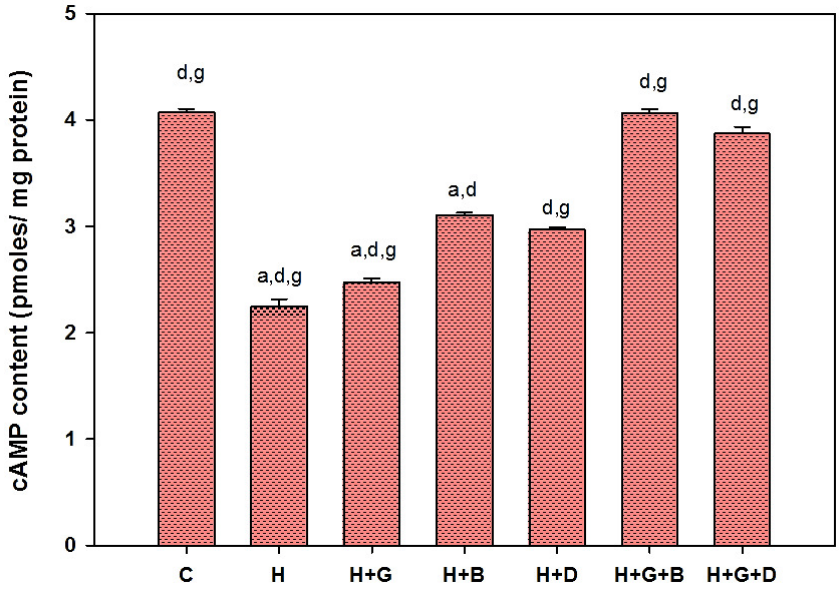


Table- 33
cAMP content in the corpus striatum of control and experimental rats

Experimental groups	cAMP content (pmoles/mg protein)
C	**4.07± 0.03**
H	**2.45± 0.06[a,d,g]**
H+G	**2.75± 0.04[a,d,g]**
H+B	**3.11± 0.02[a,d]**
H+D	**3.97± 0.02[d,g]**
H+G+B	**4.06± 0.04[d,g]**
H+G+D	**3.87± 0.06[d,g]**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 32
IP3 content in the corpus striatum of control and experimental rats

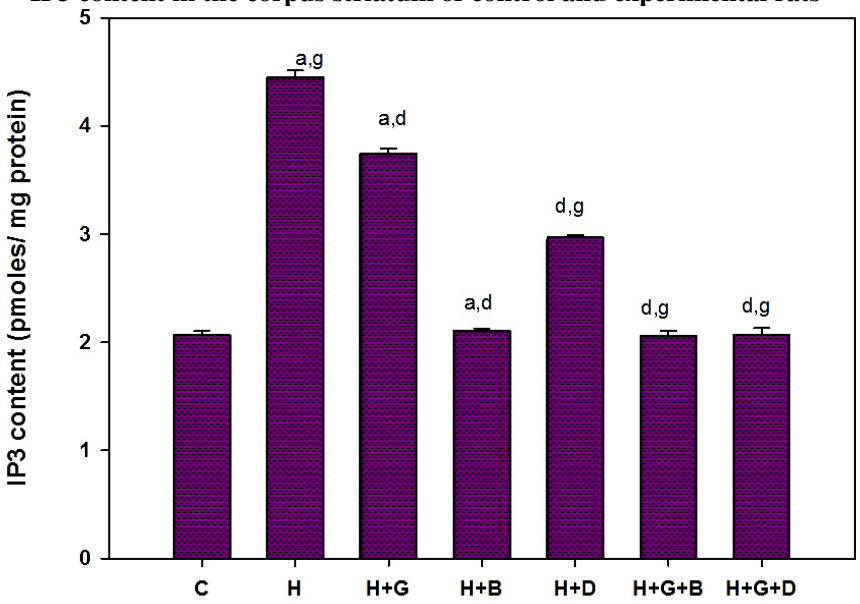


Table- 34
IP3 content in the corpus striatum of control and experimental rats

Experimental groups	IP3 content (pmoles/mg protein)
C	**2.07± 0.03**
H	**4.45± 0.06[a,g]**
H+G	**3.75± 0.04[a,d]**
H+B	**2.11± 0.02[a,d]**
H+D	**2.97± 0.02[d,g]**
H+G+B	**2.06± 0.04[d,g]**
H+G+D	**2.07± 0.06[d,g]**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 33

Real Time PCR amplification of PhospholipaseC mRNA in the corpus striatum of control and experimental rats

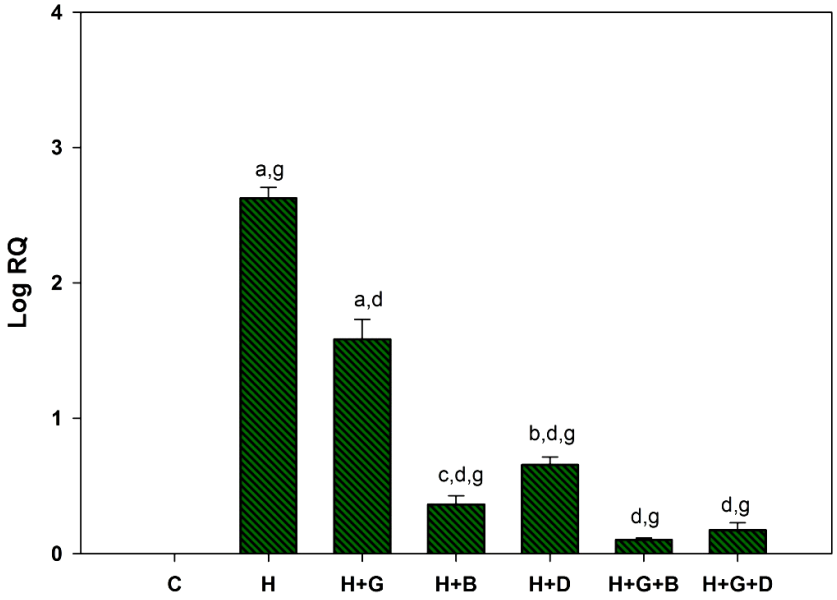


Table- 35
Real Time PCR amplification of PLC mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	2.63± 0.08[a,g]
H+G	1.59± 0.15[a,d]
H+B	0.36± 0.06[c,d,g]
H+D	0.66± 0.06[b,d,g]
H+G+B	0.10± 0.01[d,g]
H+G+D	0.17± 0.05[d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure -34
Real Time PCR amplification of CREB mRNA in the corpus striatum of control and experimental rats

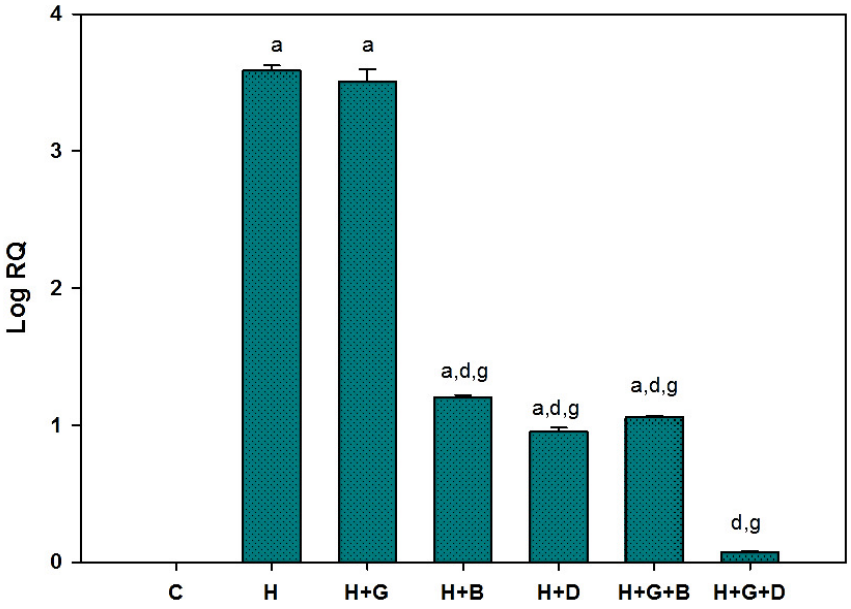


Table- 36
Real Time PCR amplification of CREB mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**3.59±0.036** [a]
H+G	**3.51±0.09** [a]
H+B	**1.20±0.02** [a,d,g]
H+D	**0.95±0.03** [a,d,g]
H+G+B	**1.06±0.01** [a,d,g]
H+G+D	**0.07±0.01** [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 35

Real Time PCR amplification of GLUT-3 mRNA in the corpus striatum of control and experimental rats

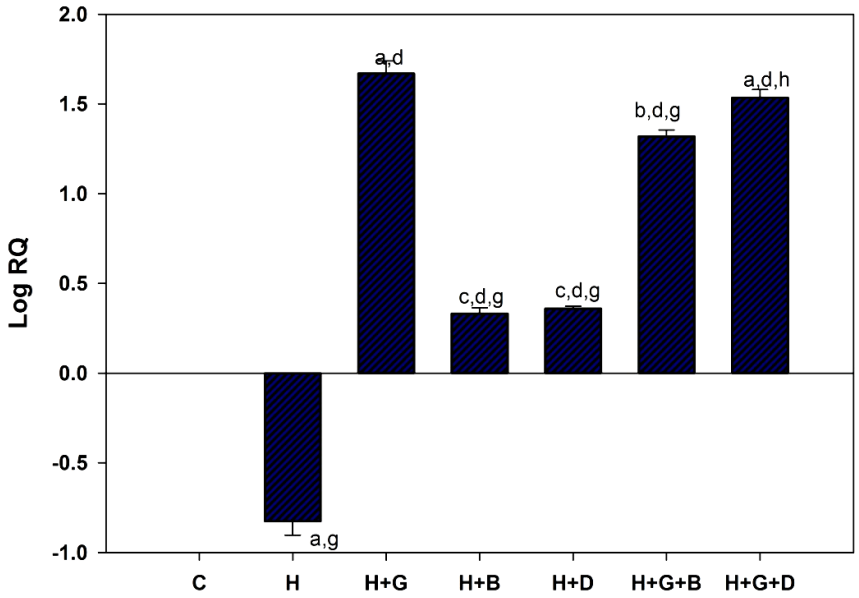


Table- 37
Real Time PCR amplification of GLUT-3 mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.83± 0.07[a,g]
H+G	1.67± 0.07[a,d]
H+B	0.33± 0.03[c,d,g]
H+D	0.36± 0.01[c,d,g]
H+G+B	1.32± 0.04[b,d,g]
H+G+D	1.54± 0.05[a,d,h]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 36
Real Time PCR amplification of Akt -1 mRNA in the corpus striatum of control and experimental rats

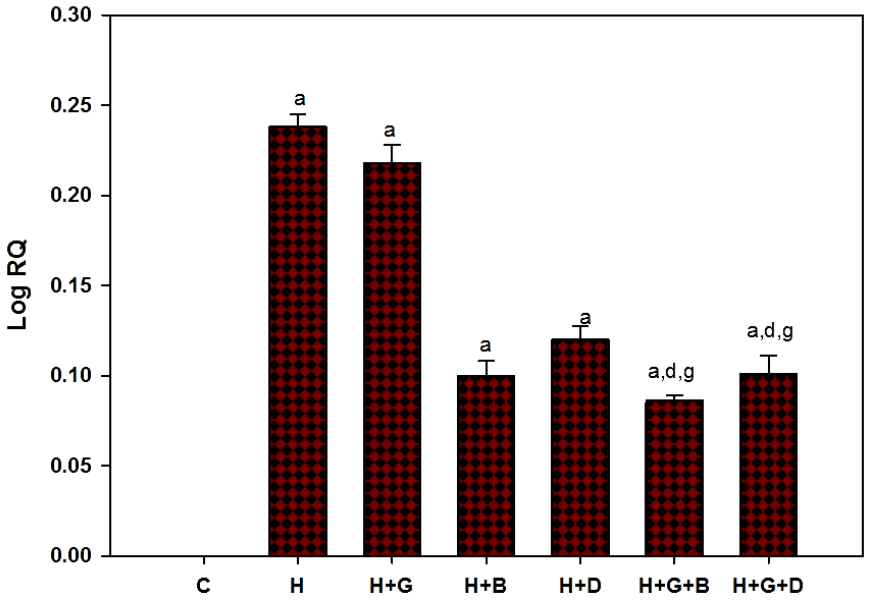


Table- 38
Real Time PCR amplification of Akt-1 mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	0.24±0.007 [a]
H+G	0.24±0.010 [a]
H+B	0.18±0.008 [a]
H+D	0.21±0.008 [a]
H+G+B	0.09±0.003 [a,d,g]
H+G+D	0.10±0.020 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 37
Real Time PCR amplification of TNF-α mRNA in the corpus striatum of control and experimental rats

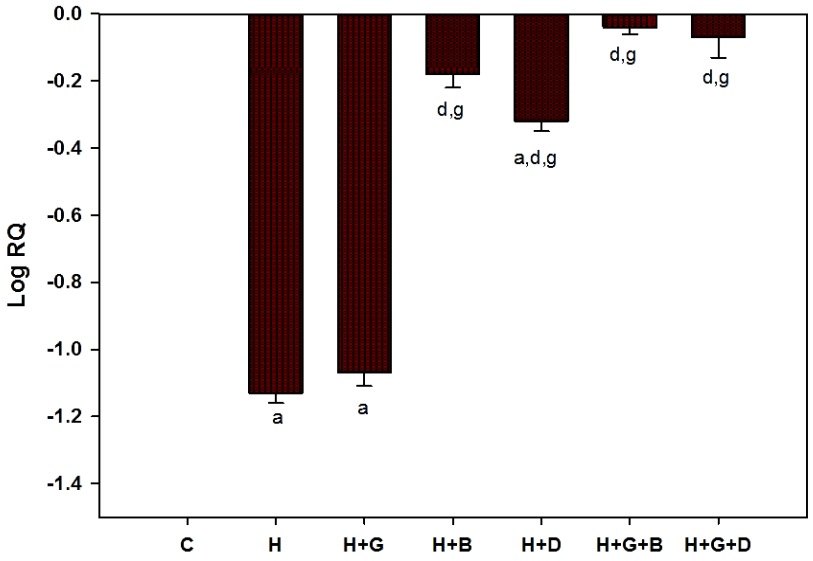


Table- 39
Real Time PCR amplification of TNF-α mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-1.13 ±0.03 [a]
H+G	-1.07 ±0.04 [a]
H+B	-0.18 ±0.04 [d,g]
H+D	-0.32 ±0.03 [a,d,g]
H+G+B	-0.04 ±0.02 [d,g]
H+G+D	-0.07 ±0.06 [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 38

Real Time PCR amplification of GDNF mRNA in the corpus striatum of control and experimental rats

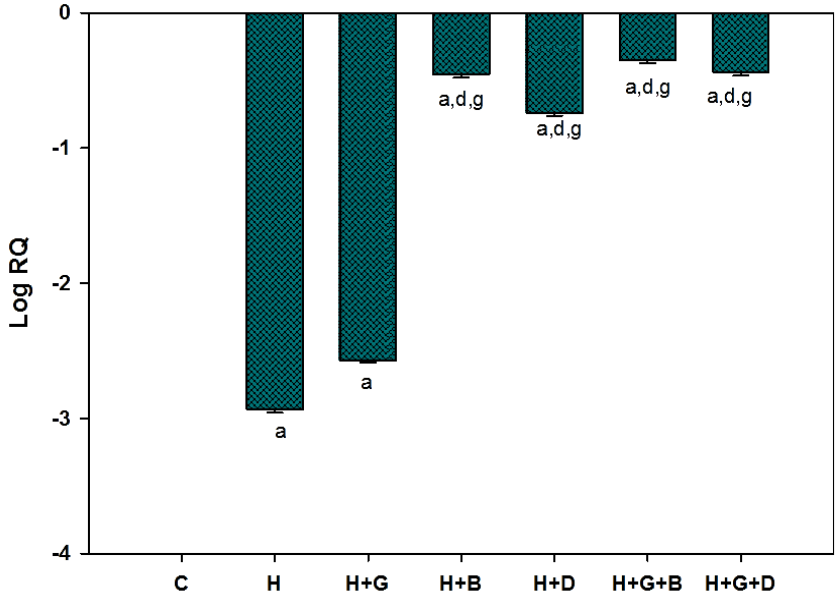


Table- 40
Real Time PCR amplification of GDNF mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-2.93± 0.02[a]
H+G	-2.57± 0.02[a]
H+B	-0.46± 0.02[a,d,g]
H+D	-0.74± 0.02[a,d,g]
H+G+B	-0.35± 0.02[a,d,g]
H+G+D	-0.44± 0.03[a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 39
Real Time PCR amplification of BDNF mRNA in the corpus striatum of control and experimental rats

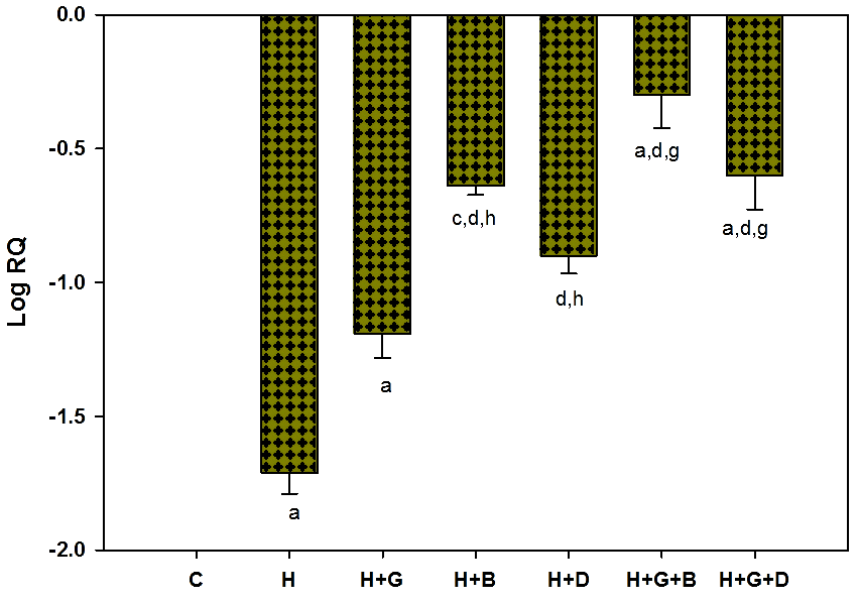


Table- 41
Real Time PCR amplification of BDNF mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-1.71± 0.07[a]
H+G	-1.19±0.09 [a]
H+B	-0.64± 0.03[c,d,h]
H+D	-0.90± 0.07[d,h]
H+G+B	-0.30± 0.12[a,d,g]
H+G+D	-0.60± 0.13[a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 40
Real Time PCR amplification of NF-ƙB mRNA in the corpus striatum of control and experimental rats

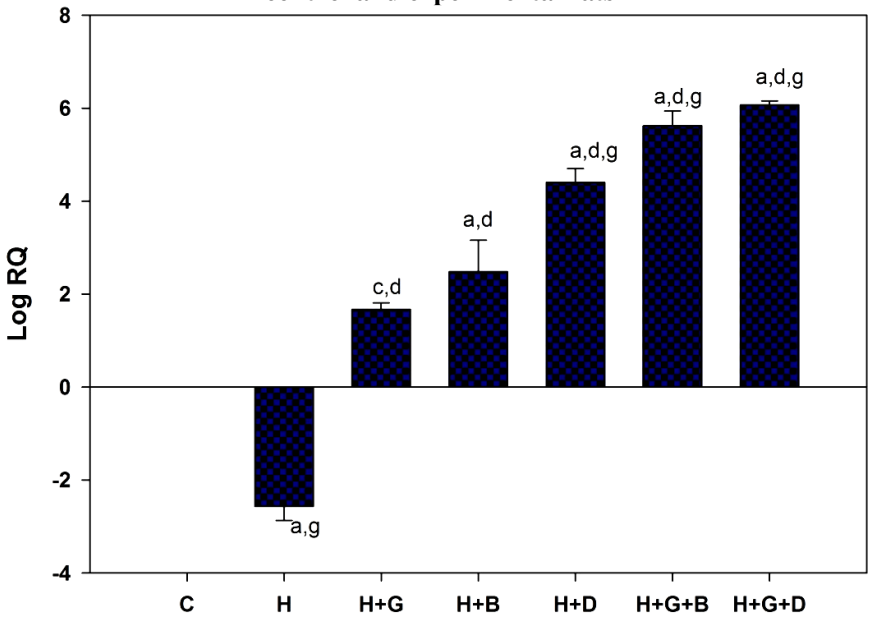


Table- 42
Real Time PCR amplification of NF-ƙB mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**-2.57 ±0.30** [a,g]
H+G	**1.67 ±0.14** [c,d]
H+B	**0.20 ±0.68** [a,d]
H+D	**0.73 ±0.30** [a,d,g]
H+G+B	**0.83 ±0.32** [a,d,g]
H+G+D	**1.07 ±0.09** [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri.* H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 41

Real Time PCR amplification of SOD mRNA in the corpus striatum of control and experimental rats

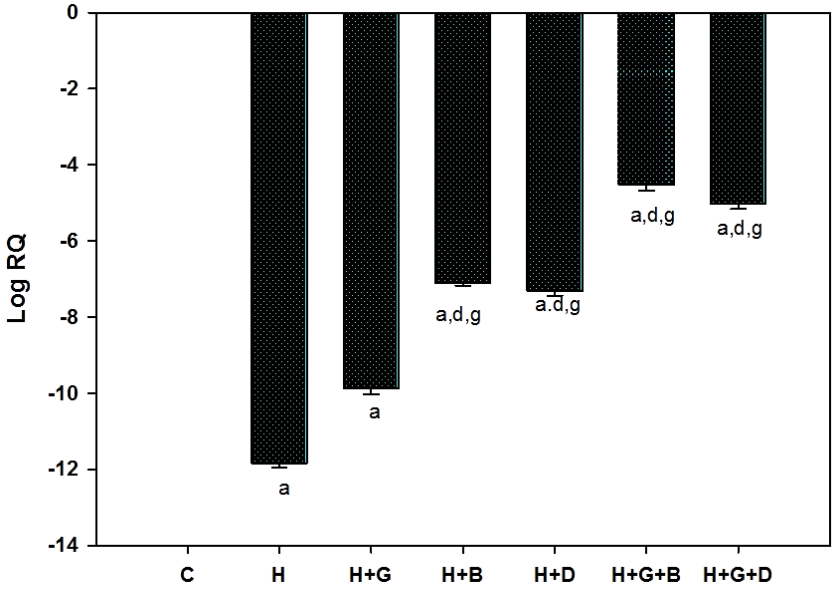


Table-43
Real Time PCR amplification of SOD mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**-11.84±0.11** [a]
H+G	**- 9.87±0.17** [a]
H+B	**-7.10±0.081** [a,d,g]
H+D	**-7.30±0.12** [a,d,g]
H+G+B	**-4.53±0.15** [a,d,g]
H+G+D	**-5.03±0.14** [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 42
Real Time PCR amplification of GPx mRNA in the corpus striatum of control and experimental rats

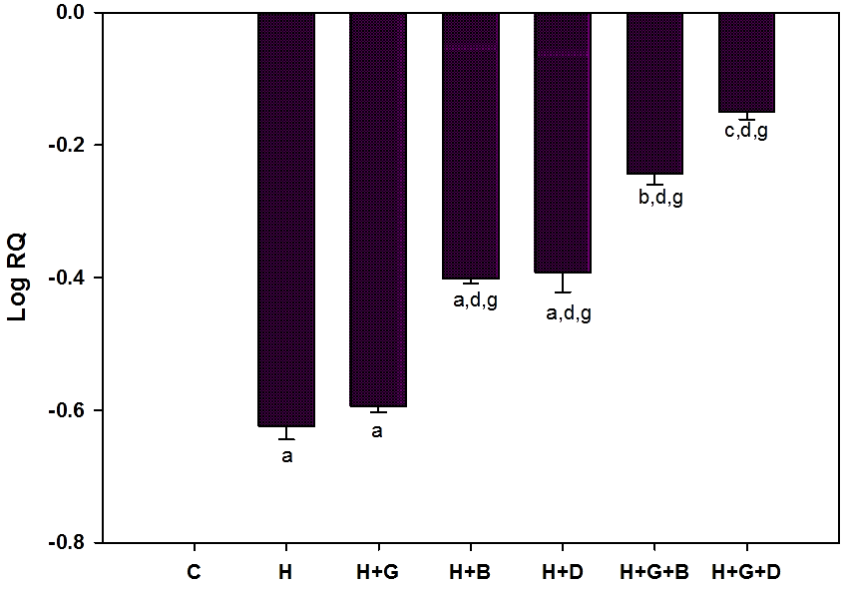


Table- 44
Real Time PCR amplification of GPx mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.62± 0.02[a]
H+G	-0.59± 0.01[a]
H+B	-0.40± 0.01[a,d,g]
H+D	-0.39± 0.03[a,d,g]
H+G+B	-0.24± 0.02[b,d,g]
H+G+D	-0.15± 0.01[c,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 43
Real Time PCR amplification of Bax mRNA in the corpus striatum of control and experimental rats

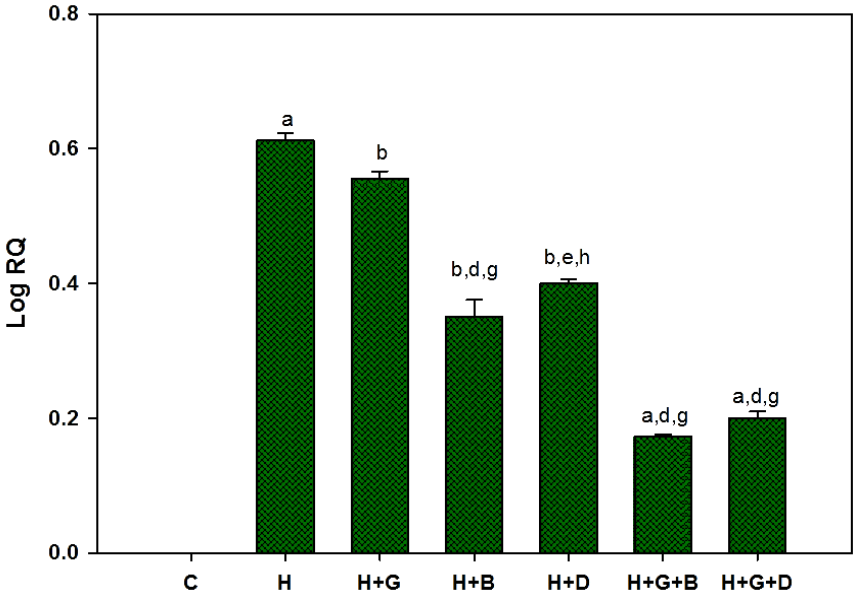


Table- 45
Real Time PCR amplification of Bax mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	0.61±0.01 [a]
H+G	0.56±0.01 [b]
H+B	0.35±0.01 [b,d,g]
H+D	0.40±0.01 [b,e,h]
H+G+B	0.17±0.01 [a,d,g]
H+G+D	0.20±.01 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 44

Real Time PCR amplification of caspase 8 mRNA in the corpus striatum of control and experimental rats

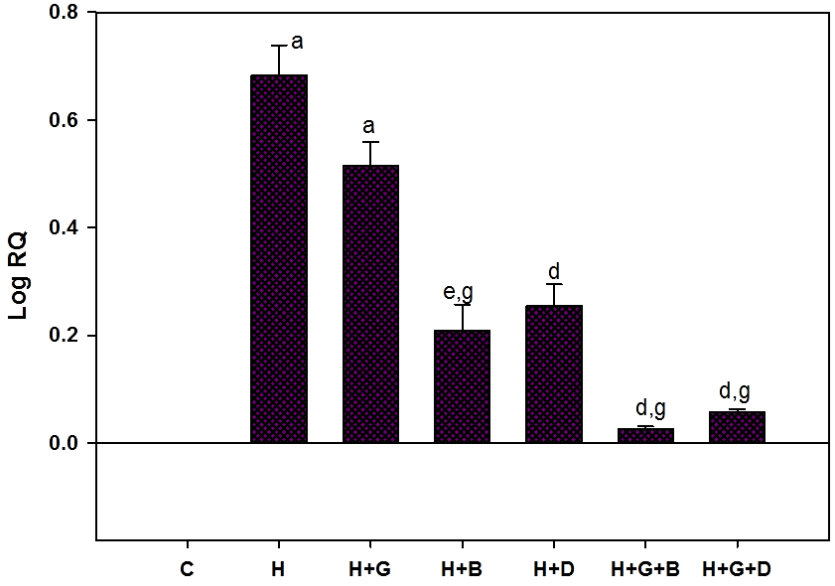


Table- 46
Real Time PCR amplification of caspase 8 mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
C	0
H	0.68±0.06[a]
H+G	0.52±0.04 [a]
H+B	0.21±0.05 [e,g]
H+D	0.25±0.10 [d]
H+G+B	0.03±0.01 [d,g]
H+G+D	0.06±0.01 [b,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure- 45

Real Time PCR amplification of Dopamine D1 receptor mRNA in the cerebellum of control and experimental rats

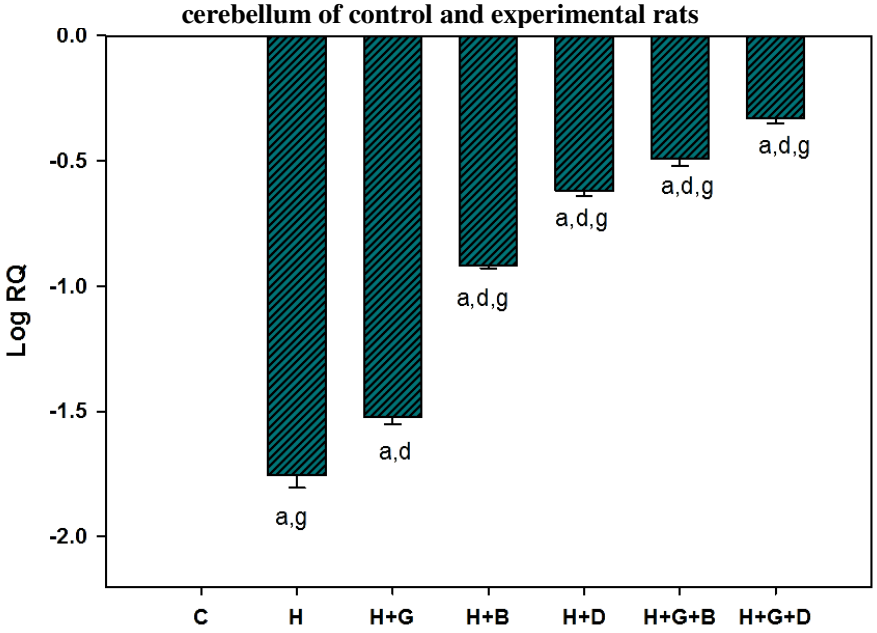


Table- 47
Real Time PCR amplification of Dopamine D1 receptor mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-1.71 ± 0.05[a,g]
H+G	-1.52 ± 0.03[a,d]
H+B	-0.91 ± 0.01[a,d,g]
H+D	-0.62 ± 0.02[a,d,g]
H+G+B	-0.49 ± 0.03[a,d,g]
H+G+D	-0.33 ± 0.03[a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 46

Real Time PCR amplification of Dopamine D2 receptor mRNA in the cerebellum of control and experimental rats

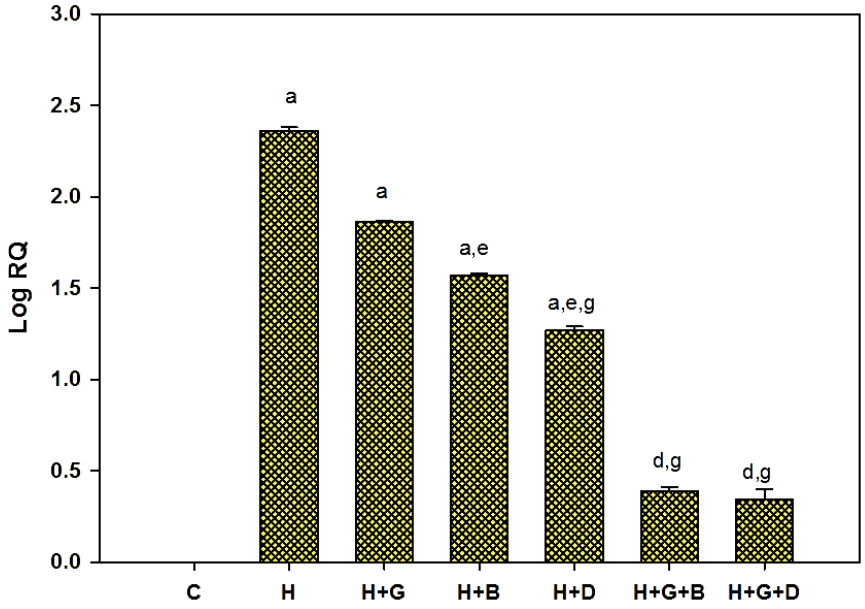


Table- 48
Real Time PCR amplification of Dopamine D2 receptor mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**2.36 ± 0.02[a]**
H+G	**1.86 ± 0.01[a]**
H+B	**1.56 ± 0.01[a,e]**
H+D	**1.28 ± 0.02[a,e,g]**
H+G+B	**0.39 ± 0.01[d,g]**
H+G+D	**0.34 ± 0.06[d.g]**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-47

Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in cerebellum of control and experimental rats

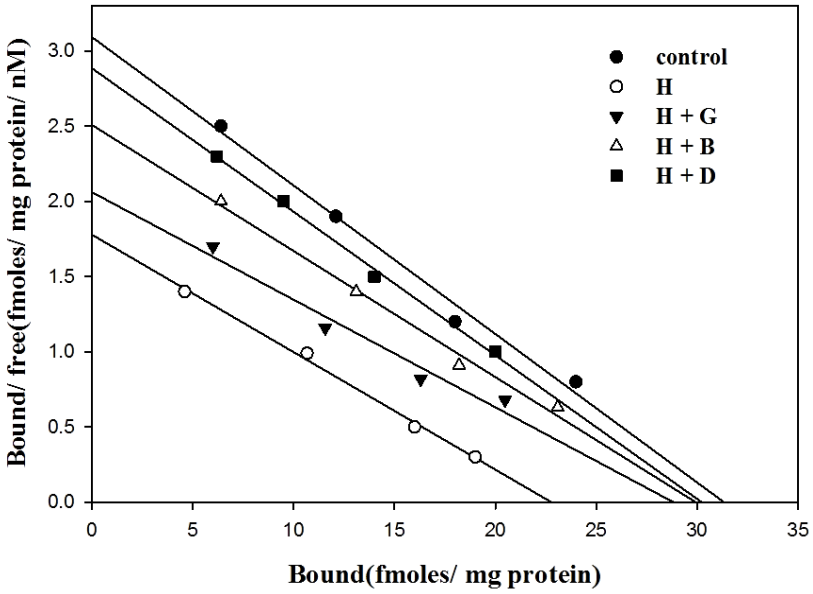


Table-49
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in cerebellum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	31.20 ± 3.20	10.42 ± 0.53
H	22.71 ± 1.25 [a]	12.61 ± 0.44 [a]
H+G	28.62 ± 2.80	14.50 ± 0.62 [a]
H+B	29.71 ± 2.15	11.31 ± 0.45 [b]
H+D	30.10 ± 1.65	9.02 ± 0.24

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c] p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f] p<0.05 when compared to Hypoglycemic group.
[g] p<0.001, [h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-48
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the cerebellum of control and experimental rats

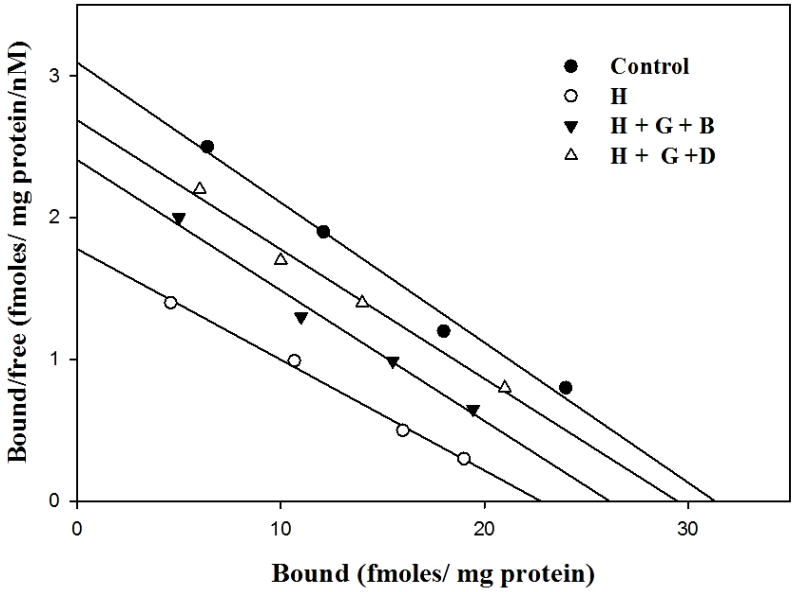


Table-50
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in the cerebellum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	31.20 ± 3.20	10.40 ± 0.03
H	22.71 ± 1.25 [c]	12.60 ± 0.04[a]
H+G+B	26.11 ± 2.61	11.81 ± 0.01[c]
H+G+D	29.20 ± 4.52	10.81 ± 0.02

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-49
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in cerebellum of control and experimental rats

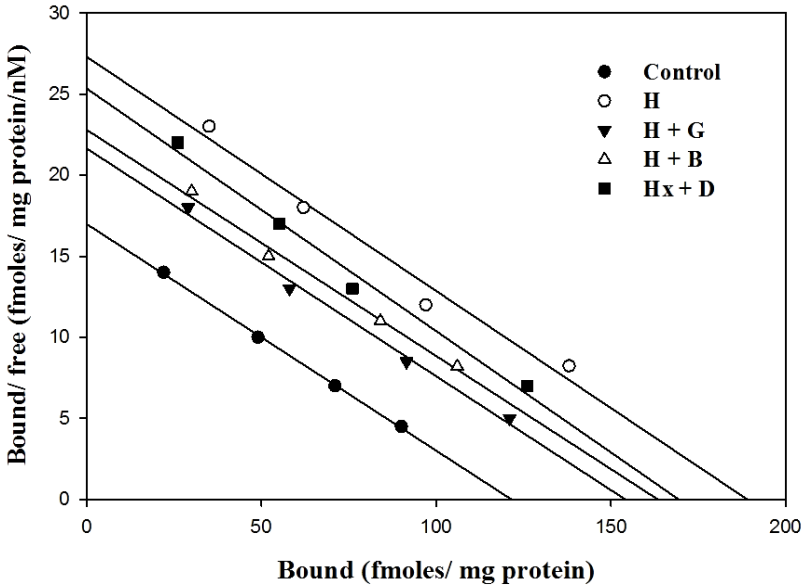


Table-51
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in cerebellum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	120.08 ± 11.50	7.61 ± 0.01
H	187.90 ± 10.67 [a]	8.16 ± 0.05 [a]
H+G	153.31 ± 12.25 [a,d]	6.90 ± 0.05 [a,d]
H+B	162.83 ± 10.74 [a,d,g]	7.42 ± 0.04 [a,d,g]
H+D	169.51 ± 11.55 [a,d,g]	6.71 ± 0.03 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-50
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in cerebellum of control and experimental rats

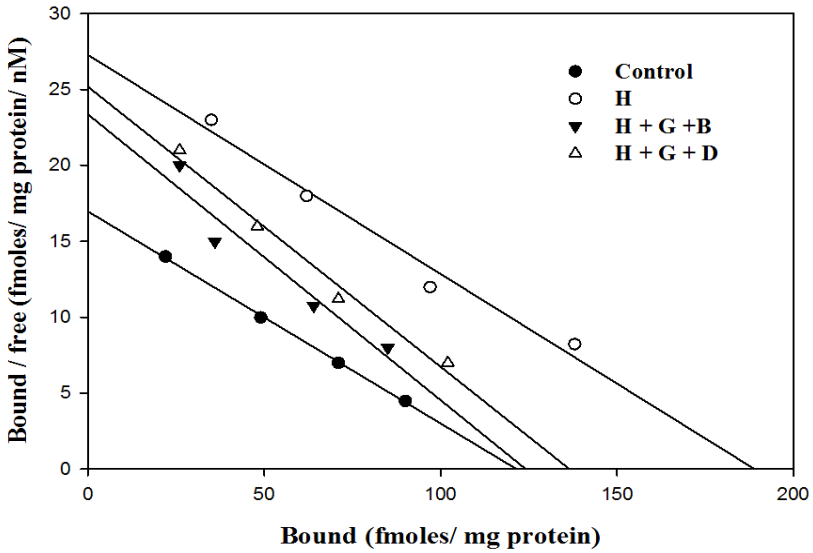


Table-52
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in cerebellum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	120.80 ±11.50	7.60 ± 0.01
H	187.91 ± 10.67 [a]	8.16 ± 0.05 [a]
H+G+B	122.93 ±12.55 [d,g]	5.20 ± 0.05 [a,d,g]
H+G+D	132.61 ±10.60 [a,d,g]	5.73 ± 0.06 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 51

cAMP content in the cerebellum of control and experimental rats

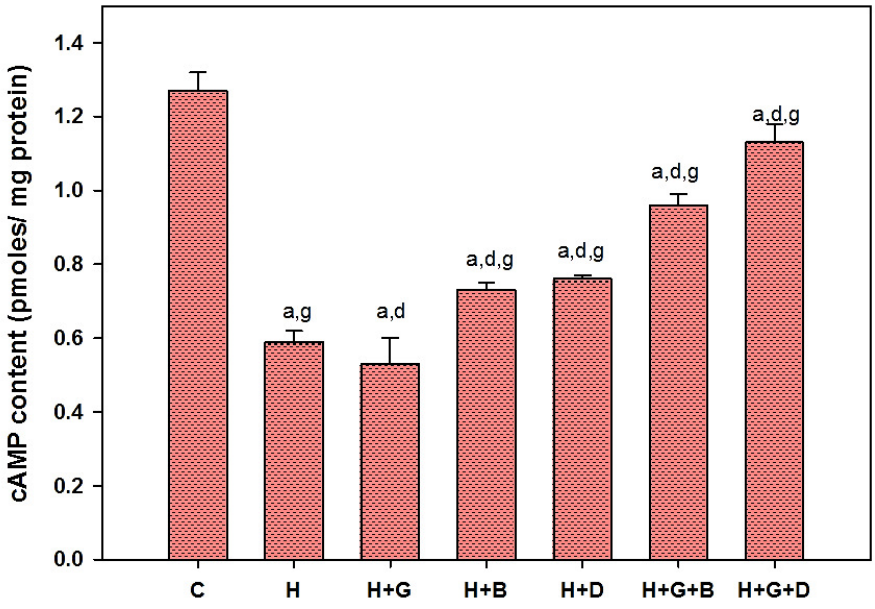


Table- 53

cAMP content in the cerebellum of control and experimental rats

Experimental groups	cAMP content (pmoles/mg protein)
C	**1.27 ± 0.05**
H	**0.59 ± 0.03** [a,d]
H+G	**0.53 ± 0.07** [a,g]
H+B	**0.73 ± 0.02** [a,d,g]
H+D	**0.76 ± 0.01** [a,d,g]
H+G+B	**0.96 ± 0.03** [a,d,g]
H+G+D	**1.13 ± 0.05** [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*. H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 52

IP3 content in the cerebellum of control and experimental rats

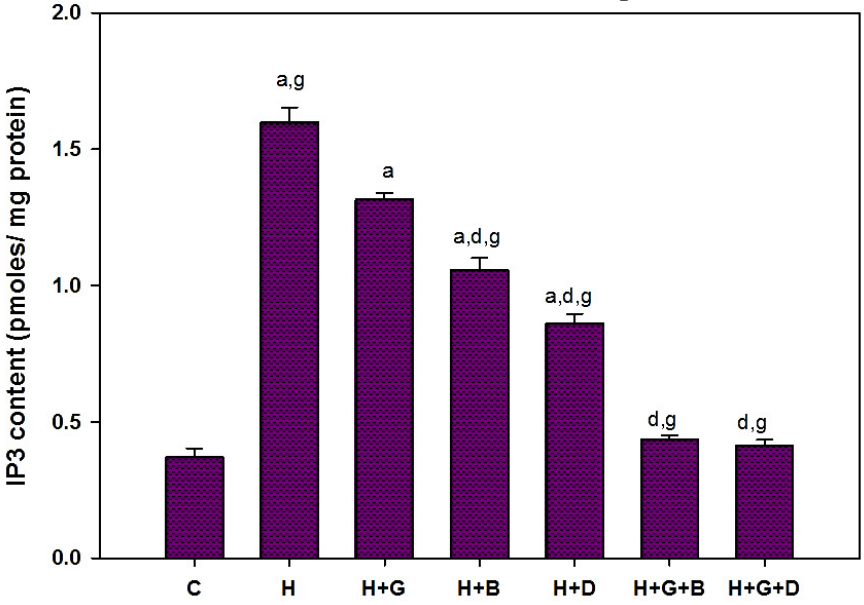


Table- 54
IP3 content in the cerebellum of control and experimental rats

Experimental groups	IP3 content (pmoles/mg protein)
C	**0.37 ± 0.03**
H	**1.60 ± 0.05** [a,g]
H+G	**1.32 ± 0.02** [a]
H+B	**1.06 ± 0.05** [a,d,g]
H+D	**0.86 ± 0.03** [a,d,g]
H+G+B	**0.44 ± 0.01** [d,g]
H+G+D	**0.41 ± 0.02** [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 53

Real Time PCR amplification of Phospholipase C mRNA in the cerebellum of control and experimental rats

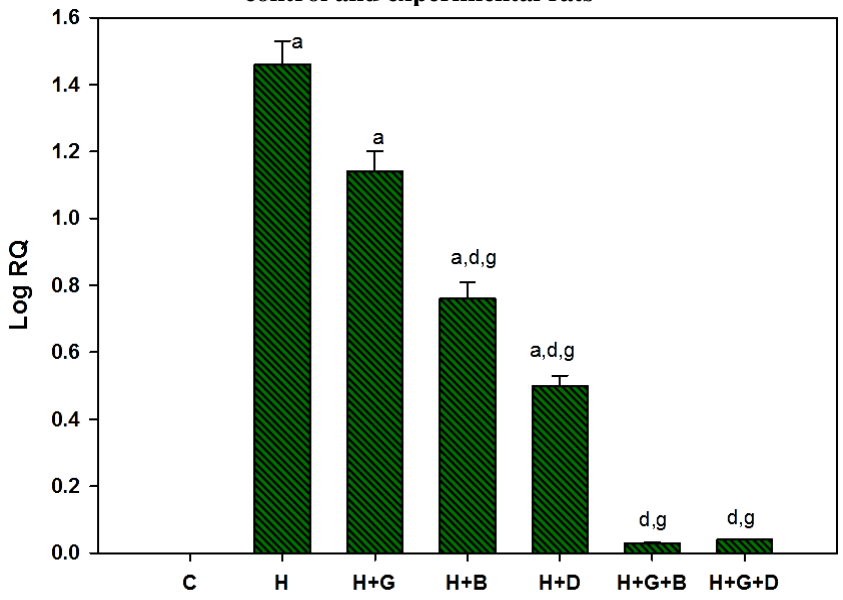


Table- 55
Real Time PCR amplification of Phospholipase C mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	1.46 ± 0.07 [a]
H+G	1.14 ± 0.06 [a]
H+B	0.76 ± 0.05 [a,d,g]
H+D	0. 05 ± 0.03 [a,d,g]
H+G+B	0.30 ± 0.001[d,g]
H+G+D	0.40 ± 0.002 [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure -54

Real Time PCR amplification of CREB mRNA in the cerebellum of control and experimental rats

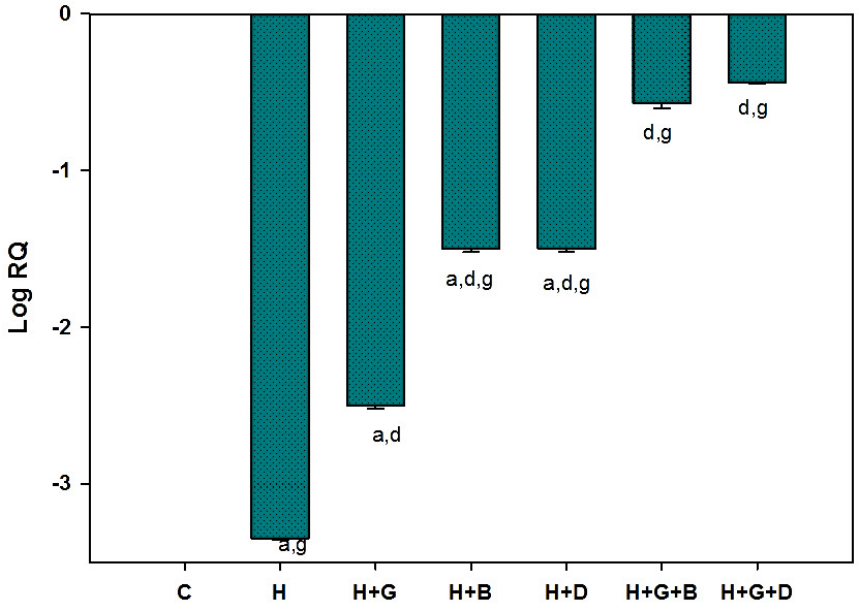


Table- 56
Real Time PCR amplification of CREB mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-3.35 ± 0.01 [a,g]
H+G	-2.50 ± 0.02 [a,d]
H+B	-1.50 ± 0.02 [a,d,g]
H+D	-1.50 ± 0.02 [a,d,g]
H+G+B	-0.57 ± 0.03 [d,g]
H+G+D	-0.44 ± 0.01 [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 55

Real Time PCR amplification of GLUT 3 mRNA in the cerebellum of control and experimental rats

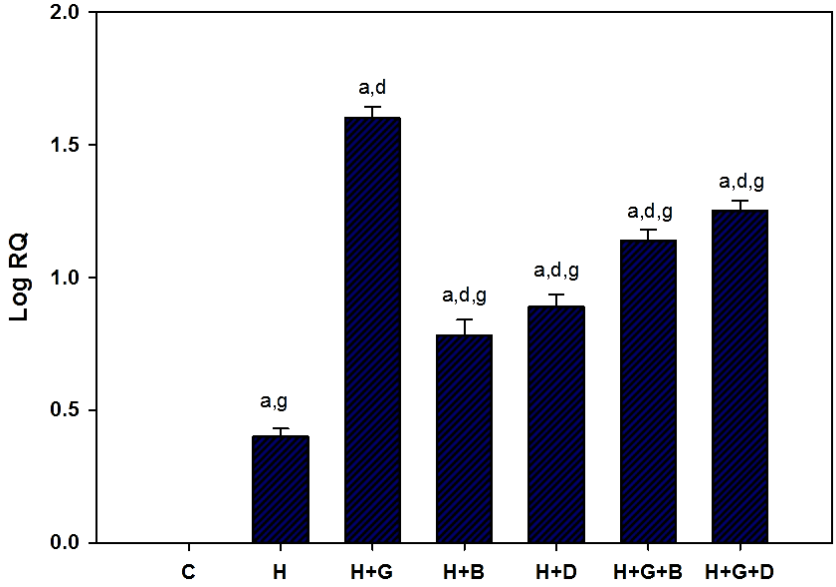


Table- 57
Real Time PCR amplification of GLUT 3 mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	0.40 ± 0.03 [a,g]
H+G	1.67 ± 0.04 [a,d]
H+B	0.78 ± 0.06 [a,d,g]
H+D	0.89 ± 0.05 [a,d,g]
H+G+B	1.14 ± 0.04 [a,d,g]
H+G+D	1.25 ± 0.04 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 56

Real Time PCR amplification of Akt -1 mRNA in the cerebellum of control and experimental rats

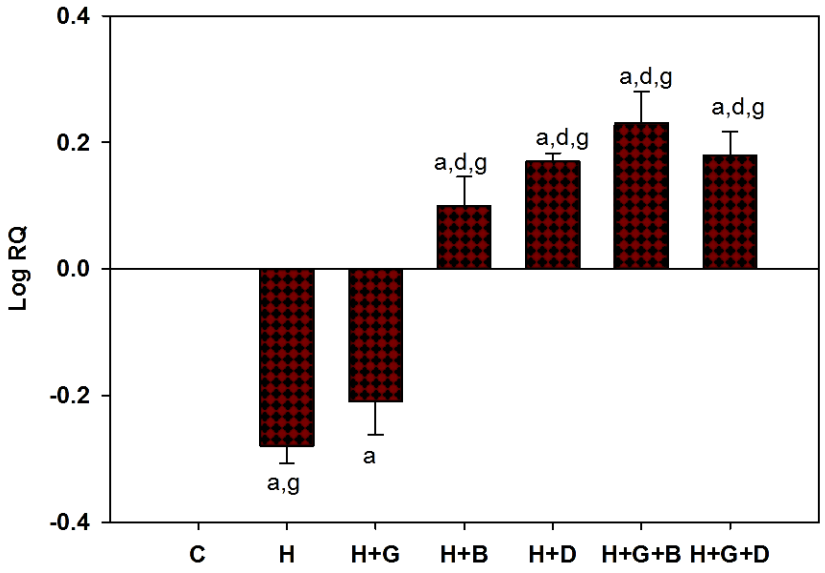


Table- 58
Real Time PCR amplification of Akt-1 mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.28 ± 0.027 [a,g]
H+G	-0.21± 0.052 [a]
H+B	0.10 ± 0.046 [a,d,g]
H+D	0.17 ± 0.013 [a,d,g]
H+G+B	0.23 ± 0.050 [a,d,g]
H+G+D	0.18 ± 0.037 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure -57

Real Time PCR amplification of TNF-α mRNA in the cerebellum of control and experimental rats

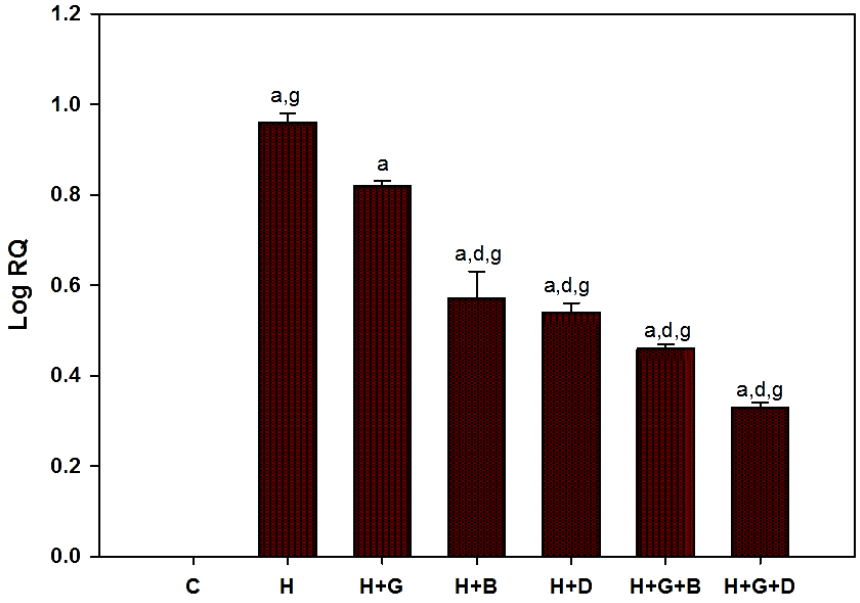


Table- 59
Real Time PCR amplification of TNF-α mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	0.96 ± 0.02 [a,g]
H+G	0.83 ± 0.01 [a]
H+B	0.57 ± 0.06 [a,d,g]
H+D	0.54 ± 0.02 [a,d,g]
H+G+B	0.46 ± 0.01 [a,d,g]
H+G+D	0.33 ± 0.01 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 58
Real Time PCR amplification of GDNF mRNA in the cerebellum of control and experimental rats

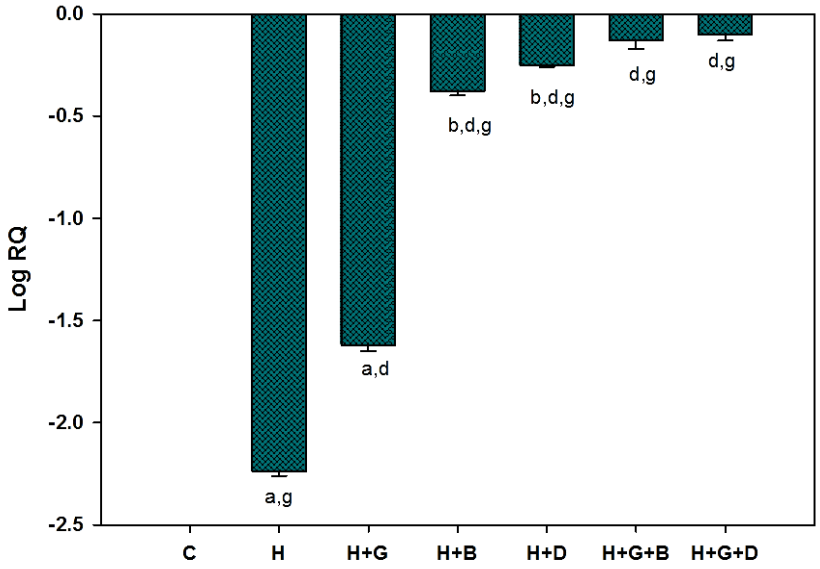


Table- 60
Real Time PCR amplification of GDNF mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-2.24 ± 0.02[a,g]
H+G	-1.62 ± 0.03[a,d]
H+B	-0.38 ± 0.02[b,d,g]
H+D	-0.25 ± 0.01[b,d,g]
H+G+B	-0.13 ± 0.04[d,g]
H+G+D	-0.10 ± 0.03[d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 59

Real Time PCR amplification of BDNF mRNA in the cerebellum of control and experimental rats

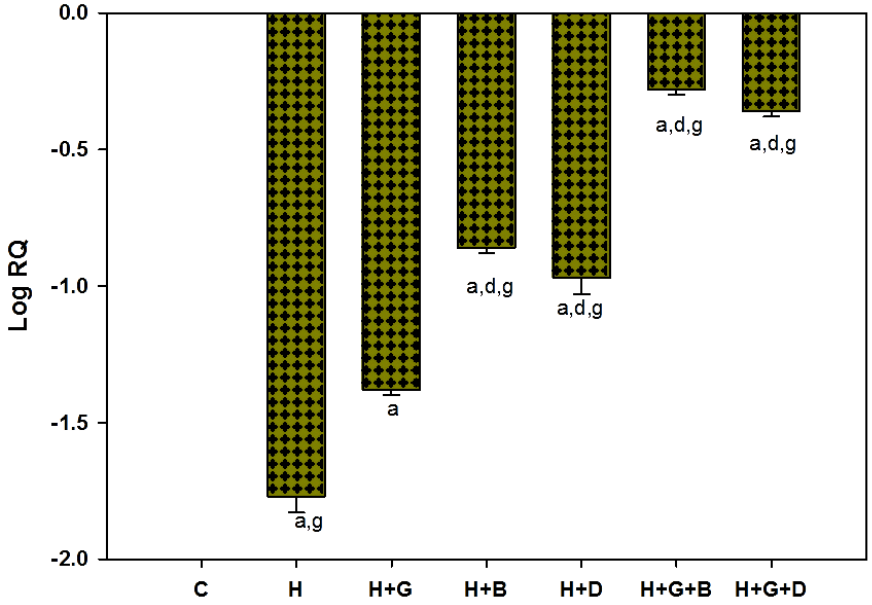


Table- 61
Real Time PCR amplification of BDNF mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-1.77 ± 0.06 [a,g]
H+G	-1.38 ± 0.02 [a]
H+B	-0.86 ± 0.02 [a,d,g]
H+D	-0.97 ± 0.06 [a,d,g]
H+G+B	-0.28 ± 0.02 [a,d,g]
H+G+D	-0.36 ± 0.02 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 60

Real Time PCR amplification of NF-ƙB mRNA in the cerebellum of control and experimental rats

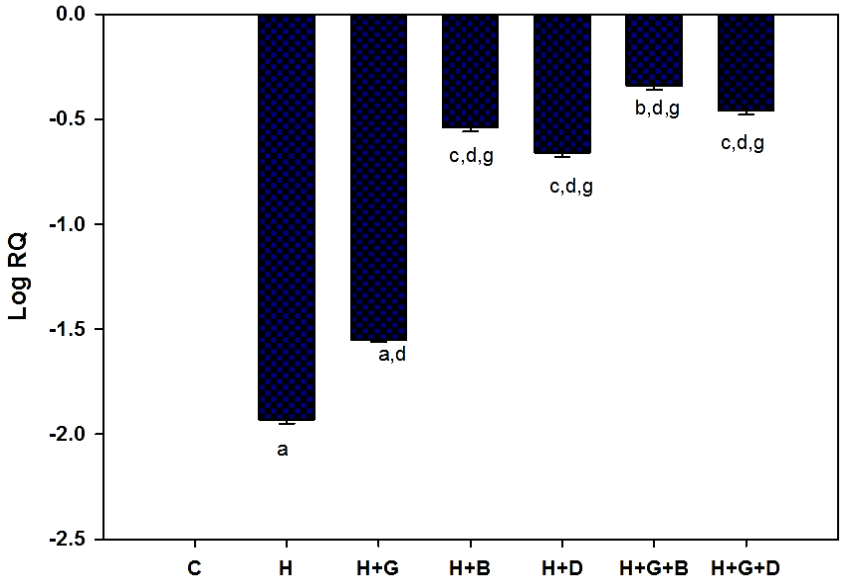


Table- 62
Real Time PCR amplification of NF-ƙB mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-1.93 ± 0.02 [a]
H+G	-1.55 ± 0.01 [a,d]
H+B	-0.54 ± 0.03 [c,d,g]
H+D	-0.66 ± 0.01 [c,d,g]
H+G+B	-0.34 ± 0.02 [b,d,g]
H+G+D	-0.46 ± 0.02 [c,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 61
Real Time PCR amplification of SOD mRNA in the cerebellum of control and experimental rats

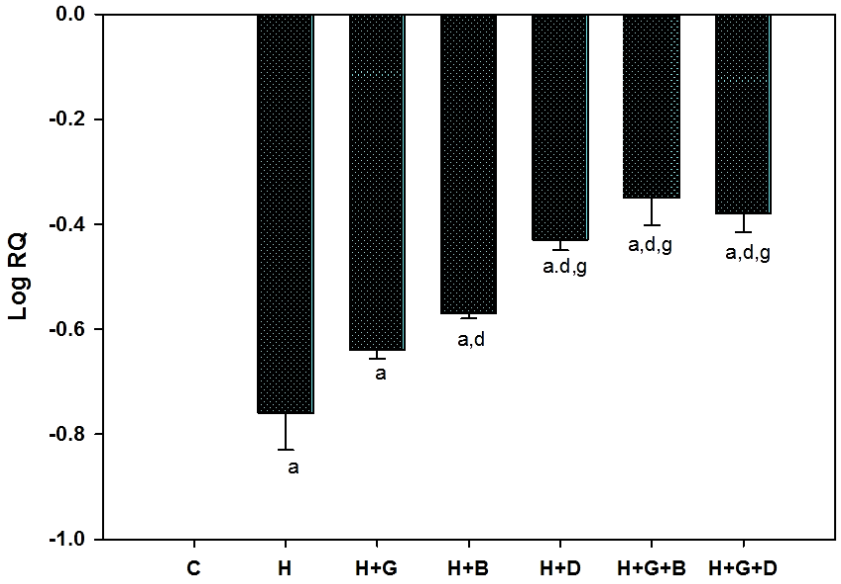


Table-63
Real Time PCR amplification of SOD mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.76 ± 0.07 [a]
H+G	-0.64 ± 0.01 [a]
H+B	-0.57 ± 0.01 [a,d]
H+D	-0.43 ± 0.01 [a,d,g]
H+G+B	-0.35 ± 0.01 [a,d,g]
H+G+D	-0.38 ± 0.04 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 62

Real Time PCR amplification of GPx mRNA in the cerebellum of control and experimental rats

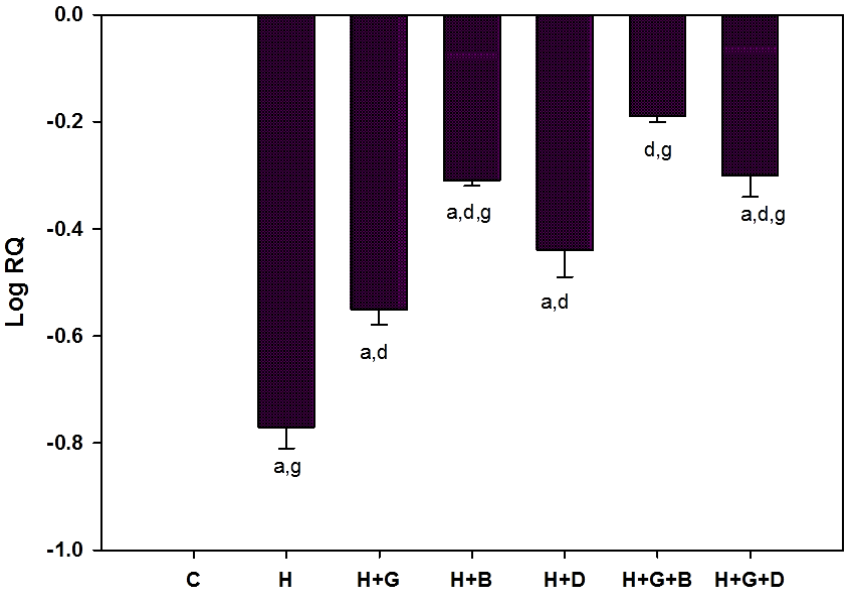


Table- 64
Real Time PCR amplification of GPx mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.77 ± 0.04 [a,g]
H+G	-0.55 ± 0.03 [a,d]
H+B	-0.31 ± 0.01 [a,d,g]
H+D	-0.44 ± 0.05 [a,d,g]
H+G+B	-0.19 ± 0.01 [d,g]
H+G+D	-0.30 ± 0.04 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 63

Real Time PCR amplification of Bax mRNA in the cerebellum of control and experimental rats

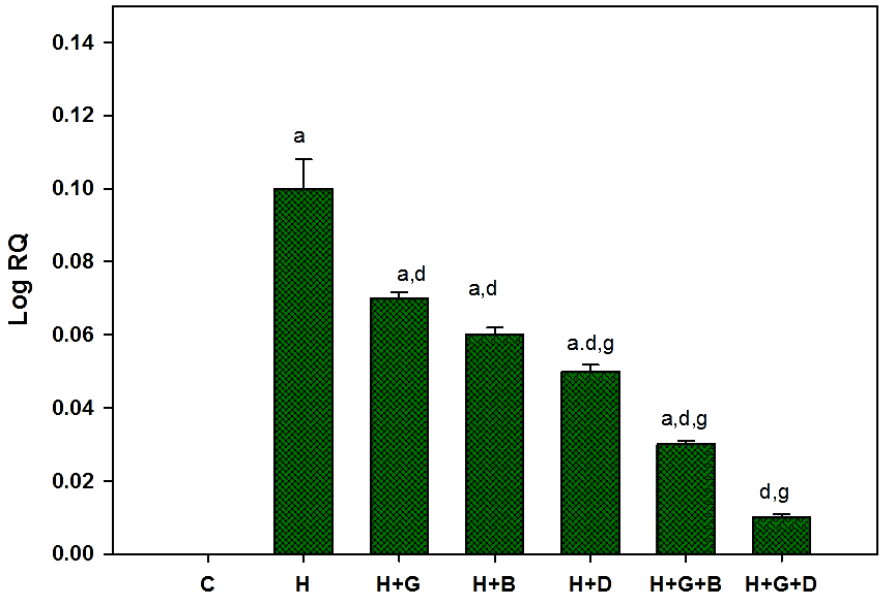


Table- 65

Real Time PCR amplification of Bax mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0
H	0.10 ± 0.008 [a]
H+G	0.07 ± 0.001 [a,d]
H+B	0.06 ± 0.002 [a,d]
H+D	0. 50 ± 0.001 [a,d,g]
H+G+B	0.03 ± 0.001 [a,d,g]
H+G+D	0.01 ± 0.001 [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 64

Real Time PCR amplification of caspase 8 mRNA in the cerebellum of control and experimental rats

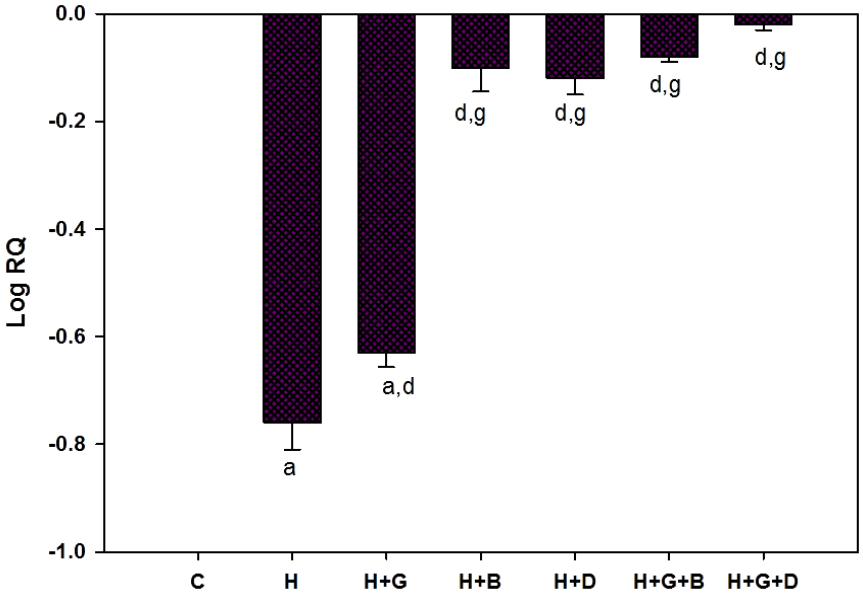


Table- 66
Real Time PCR amplification of caspase 8 mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
C	0.0000
H	-0.76 ±0.05 [a]
H+G	-0.63 ±0.04 [a]
H+B	-0.10 ±0.04 [d,g]
H+D	-0.12 ±0.10 [d,g]
H+G+B	-0.08 ±0.04 [d,g]
H+G+D	-0.02 ±0.04 [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-65

Real Time PCR amplification of Dopamine D1 receptor mRNA in the Brain Stem of control and experimental rats

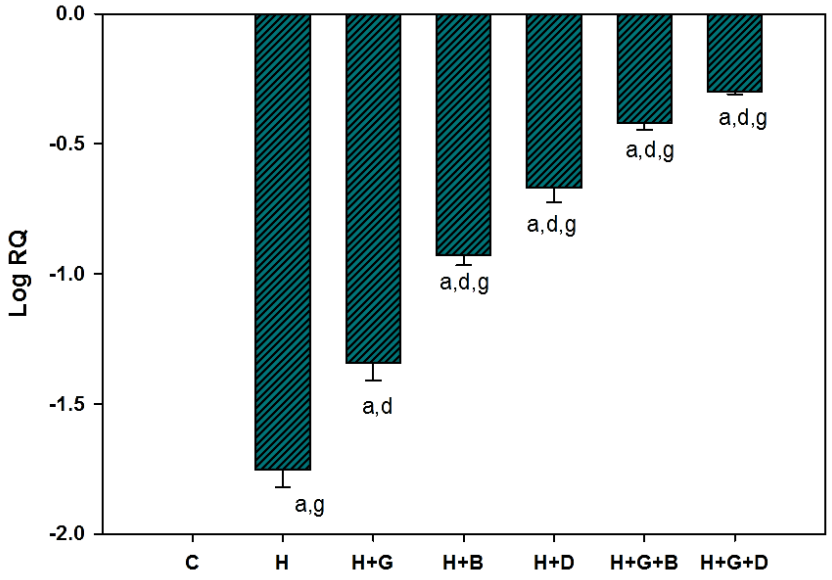


Table-67
Real Time PCR amplification of Dopamine D1 receptor mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0
H	-1.76 ±0.07 [a,g]
H+G	-1.34 ±0.07 [a,d]
H+B	-0.93 ±0.04 [a,d,g]
H+D	-0.67 ±0.06 [a,d,g]
H+G+B	-0.42 ±0.03 [a,d,g]
H+G+D	-0.30 ±0.01 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure -66

Real Time PCR amplification of Dopamine D2 receptor mRNA in the Brain Stem of control and experimental rats

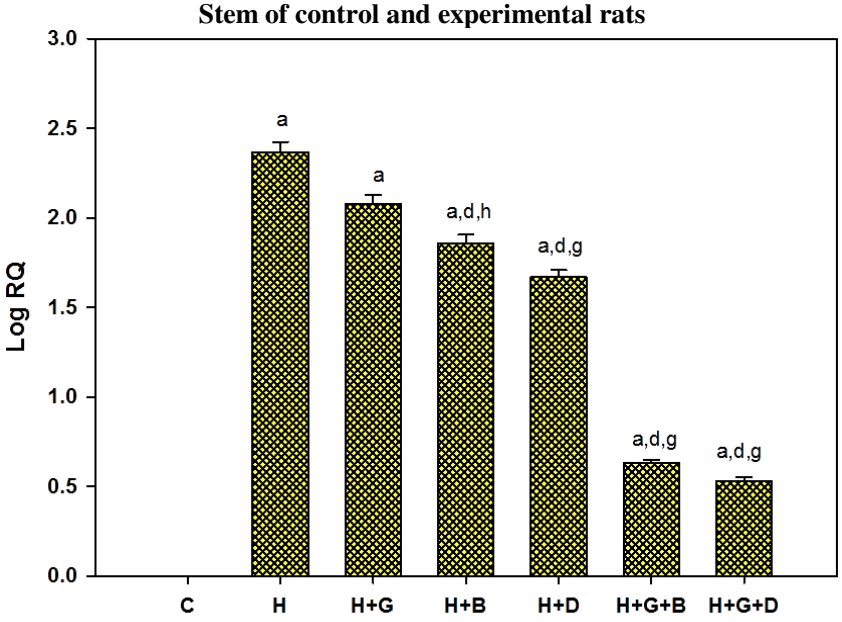


Table- 68
Real Time PCR amplification of Dopamine D2 receptor mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0
H	2.37 ±0.06 [a]
H+G	2.08 ±0.05 [a]
H+B	1.86 ±0.05 [a,d,h]
H+D	1.67 ±0.04 [a,d,g]
H+G+B	0.63 ±0.02 [a,d,g]
H+G+D	0.53 ±0.02 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-67
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in brain stem of control and experimental rats

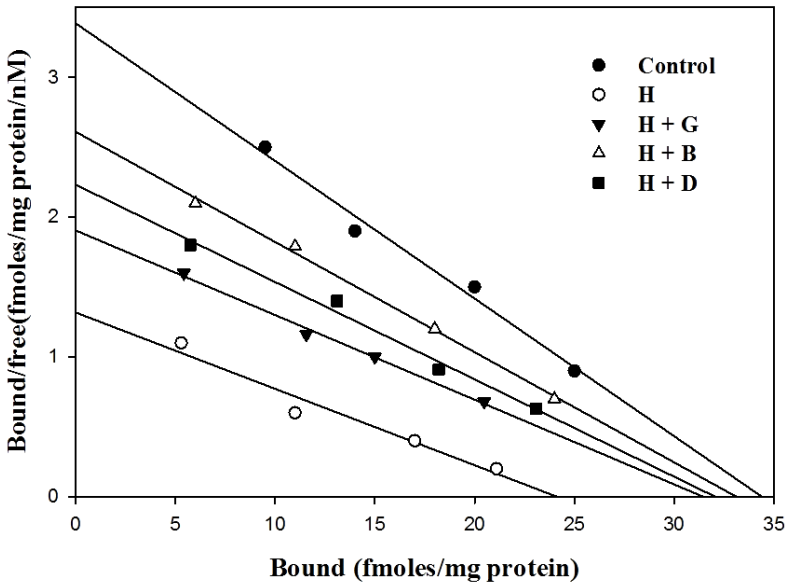


Table- 69
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 the brain stem of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	34.30 ± 2.16	9.80 ±1.20
H	23.91 ± 1.05[a]	15.07 ± 1.02 [c]
H+G	30.92 ± 2.60[f]	16.27 ± 2.12 [c]
H+B	32.80 ± 3.10[f]	13.24 ± 2.00 [e,h]
H+D	31.61 ± 2.10[f]	9.10 ± 1.34 [e,h]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-68
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in brain stem of control and experimental rats

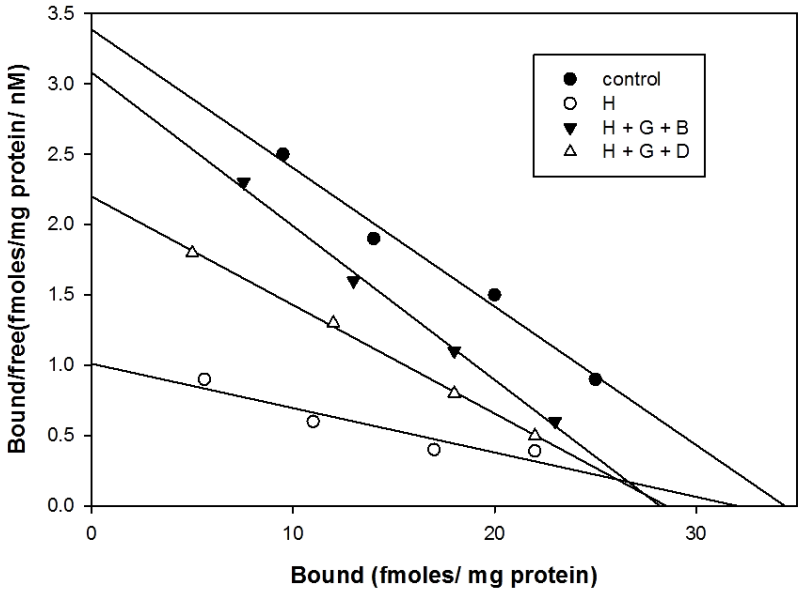


Table- 70
Scatchard analysis of Dopamine D1 receptor using [^{3}H] SCH 23390 binding against SCH23390 in brain stem of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	**34.30 ± 2.16**	**9.81 ±1.2**
H	**23.91 ± 1.05** [a]	**15.07 ± 1.02** [a]
H+G+B	**28.30 ± 2.61** [f]	**9.50 ± 1.85**
H+G+D	**28.42 ± 4.50** [f]	**12.05 ± 1.20**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Hypoglycemic group.
[g] $p<0.001$, [h] $p<0.01$ when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure- 69
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in brain stem of control and experimental rats

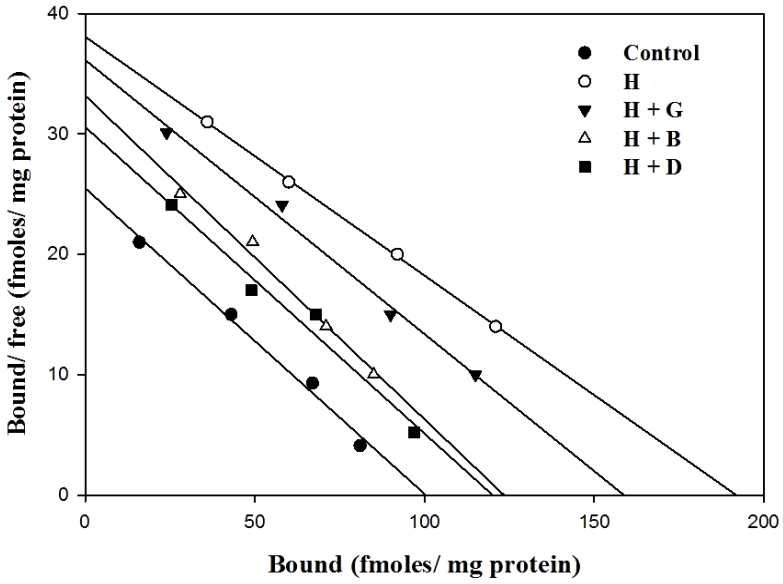


Table- 71
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in brain stem of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	99.86 ± 14.10	2.60 ± 0.05
H	191.30 ± 16.15 [a,g]	5.21 ± 0.04
H+G	157.81 ± 14.02 [a]	4.27 ± 0.05
H+B	123.50 ± 15.25 [a,d,g]	3.81 ± 0.05
H+D	118.42 ± 14.10 [a,d,g]	3.90 ± 0.01

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure-70
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in brain stem of control and experimental rats

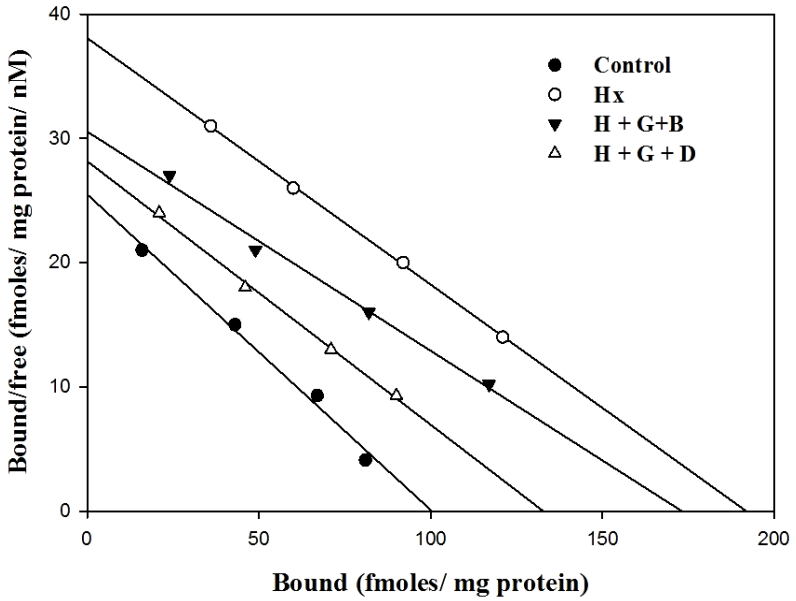


Table-72
Scatchard analysis of Dopamine D2 receptor using [^{3}H] YM-09151-2 against sulpiride in brain stem of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
C	99.86 ± 14.10	2.60 ± 0.05
H	191.30 ± 16.15 [a,d,g]	5.21 ± 0.04
H+G+B	171.81 ± 17.50 [a,d,g]	5.52 ± 0.10
H+G+D	132.41 ± 15.02 [a,d,g]	4.80 ±0.05

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 71
cAMP content in the Brain Stem of control and experimental rats

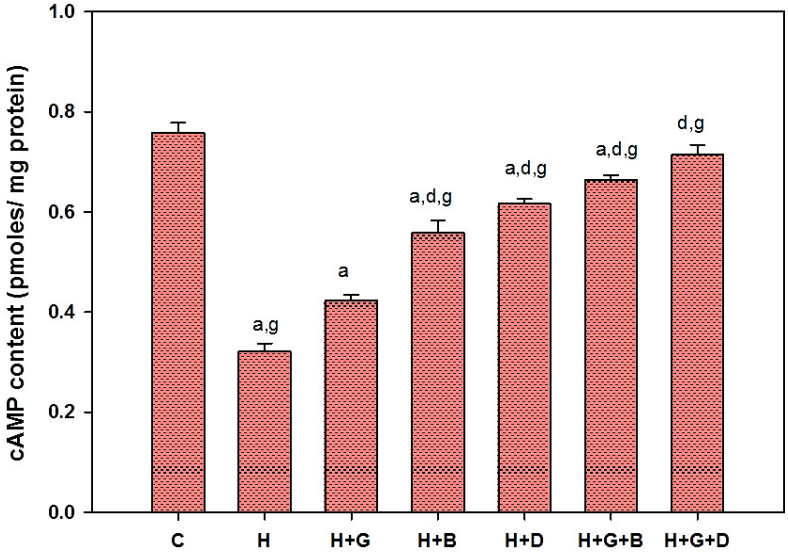


Table- 73
cAMP content in the Brain Stem of control and experimental rats

Experimental groups	cAMP content (pmoles/mg protein)
C	0.77 ±0.03
H	0.32±0.05 [a,g]
H+G	0.42±0.03 [a]
H+B	0.56±0.05 [a,d]
H+D	0.62±0.04 [a,d,g]
H+G+B	0.66±0.02 [a,d,g]
H+G+D	0.71±0.02 [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 72
IP3 content in the Brain Stem of control and experimental rats

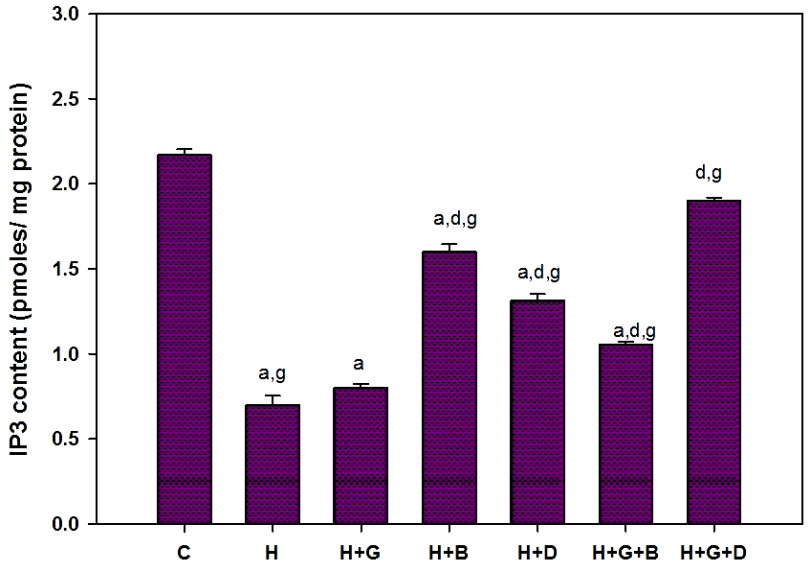


Table- 74
IP3 content in the Brain Stem of control and experimental rats

Experimental groups	IP3 content (pmoles/mg protein)
C	**2.17 ± 0.03**
H	**0.70 ± 0.05** [a]
H+G	**0.82 ± 0.03** [a]
H+B	**1.06 ± 0.05** [a,d,g]
H+D	**1.36 ± 0.04** [a,d,g]
H+G+B	**1.04 ± 0.02** [a,d,g]
H+G+D	**1.91 ± 0.02** [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 73

Real Time PCR amplification of Phospholipase C mRNA in the Brain Stem of control and experimental rats

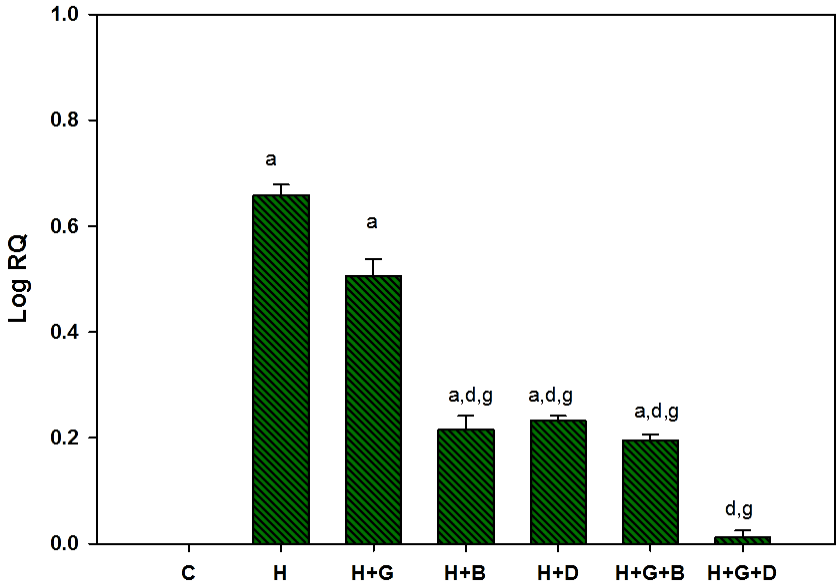


Table- 75
Real Time PCR amplification of Phospholipase C mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0
H	0.68 ±0.02 [a]
H+G	0.51 ±0.03 [a]
H+B	0.21 ±0.01 [a,d,g]
H+D	0.23 ±0.01 [a,d,g]
H+G+B	0.20 ±0.01 [a,d,g]
H+G+D	0.12 ±0.01 [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 74

Real Time PCR amplification of CREB mRNA in the Brain Stem of control and experimental rats

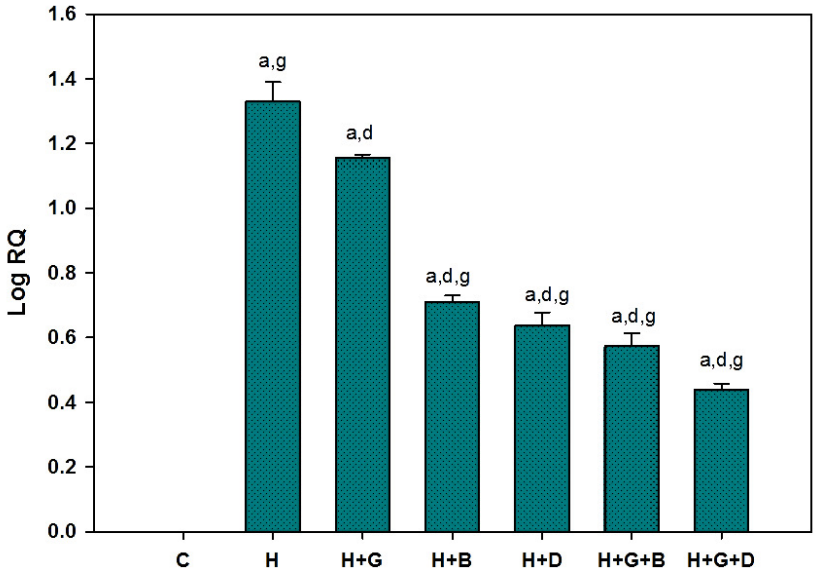


Table- 76
Real Time PCR amplification of CREB mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**1.33 ± 0.11** [a,g]
H+G	**1.15 ± 0.05** [a,d]
H+B	**0.71 ± 0.08** [a,d,g]
H+D	**0.64 ± 0.04**[a,d,g]
H+G+B	**0.57 ± 0.09**[a,d,g]
H+G+D	**0.44 ± 0.05**[a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 75

Real Time PCR amplification of GLUT 3 mRNA in the Brain Stem of control and experimental rats

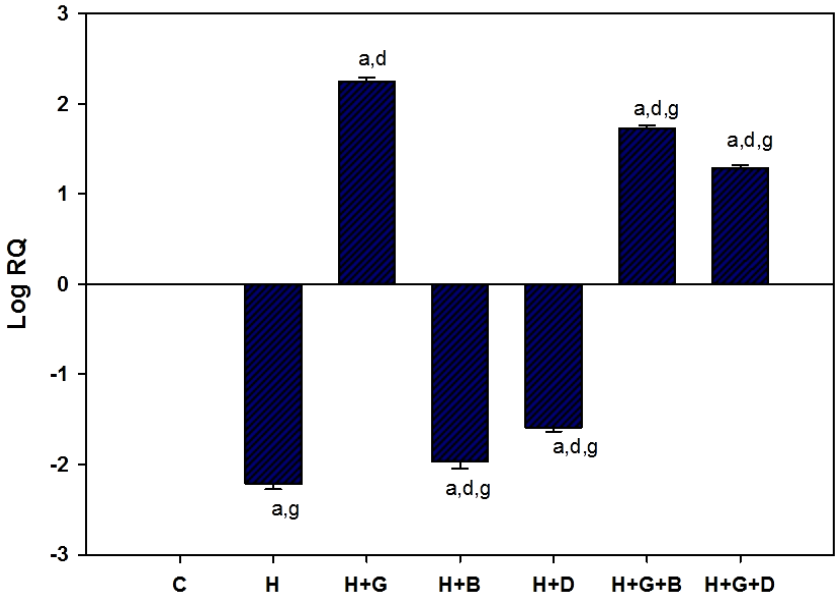


Table- 77
Real Time PCR amplification of GLUT 3 mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0
H	-2.22± 0.06[a,g]
H+G	2.25± 0.04[a,d]
H+B	-1.97± 0.08[a,d,g]
H+D	-1.59± 0.05[a,d,g]
H+G+B	1.73± 0.03[a,d,g]
H+G+D	1.29± 0.03[a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 76

Real Time PCR amplification of Akt-1 mRNA in the Brain Stem of control and experimental rats

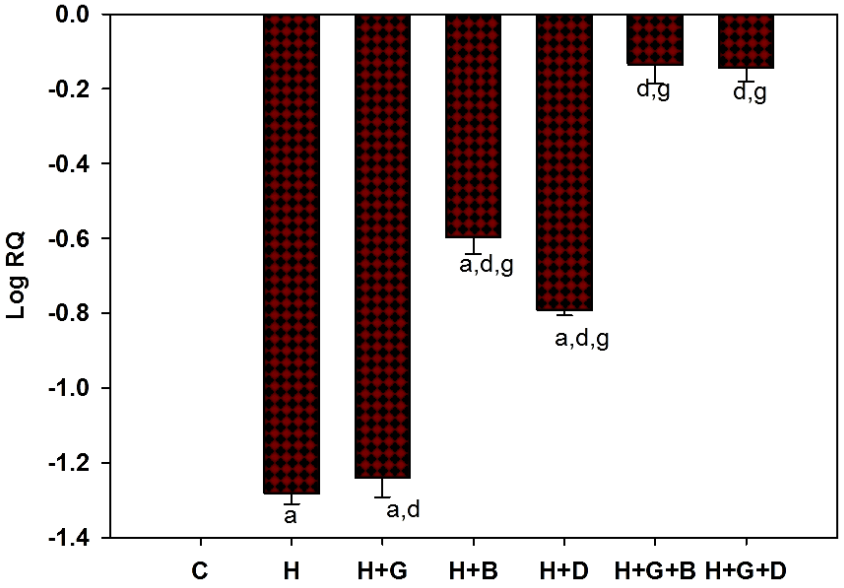


Table- 78
Real Time PCR amplification of AKT-1 mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**-1.28 ±0.03** [a]
H+G	**-1.24 ±0.05** [a,d]
H+B	**-0. 60 ±0.05** [a,d,g]
H+D	**-0.79 ±0.01** [a,d,g]
H+G+B	**-0.14 ±0.05**[d,g]
H+G+D	**-0.14 ±0.06** [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 77

Real Time PCR amplification of TNF-α mRNA in the Brain Stem of control and experimental rats

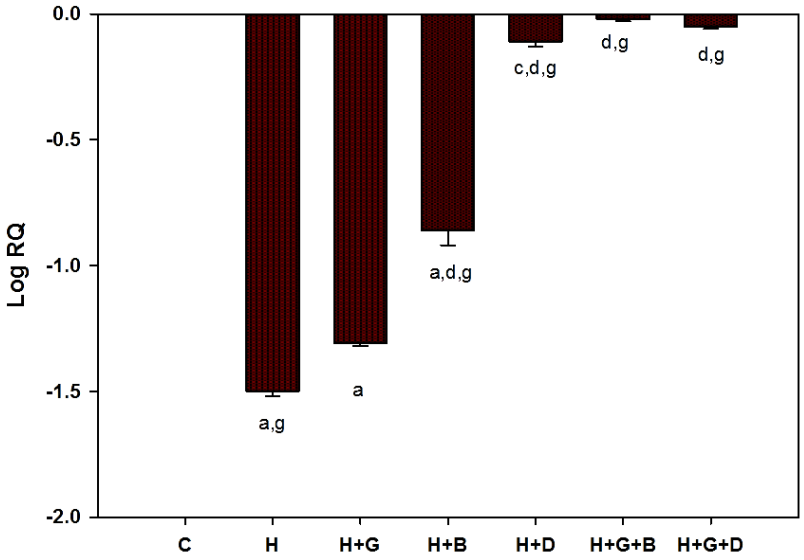


Table-79
Real Time PCR amplification of TNF-α mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0
H	-1.50 ± 0.02[a,g]
H+G	-1.31 ± 0.01[a]
H+B	-0.86 ± 0.06[a,d,g]
H+D	-0.11 ± 0.02[c,d,g]
H+G+B	-0.02 ± 0.01[d,g]
H+G+D	-0.05 ± 0.01[d,,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 78

Real Time PCR amplification of GDNF mRNA in the Brain Stem of control and experimental rats

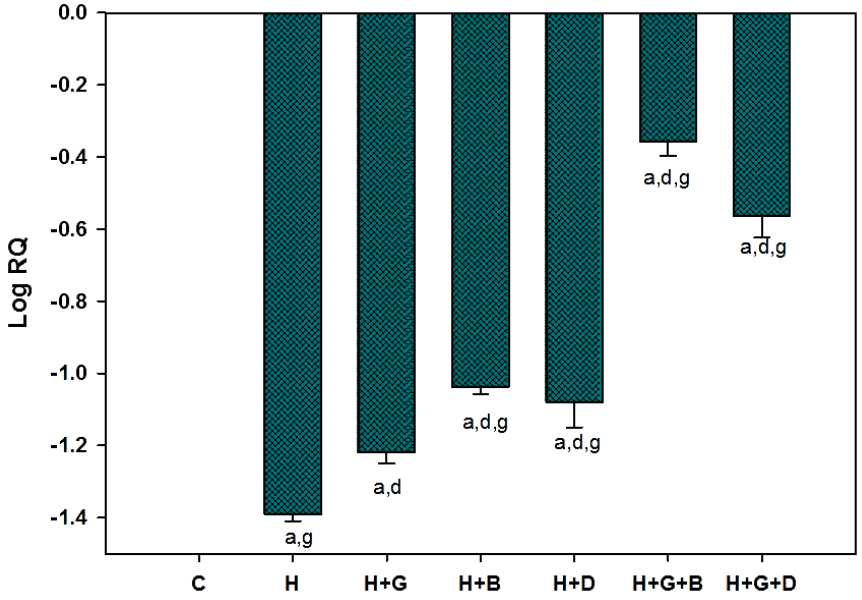


Table- 80
Real Time PCR amplification of GDNF mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0
H	-1.39±0.02 [a,g]
H+G	-1.22±0.03 [a,d]
H+B	-1.04±0.02 [a,d,g]
H+D	-1.08±0.07 [a,d,g]
H+G+B	-0.36±0.04 [a,d,g]
H+G+D	-0.56±0.06 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 79

Real Time PCR amplification of BDNF mRNA in the Brain Stem of control and experimental rats

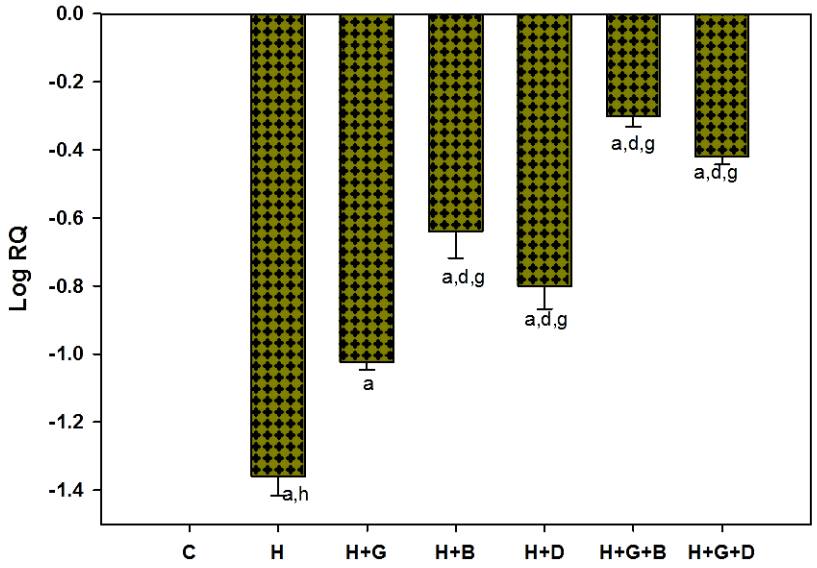


Table- 81
Real Time PCR amplification of BDNF mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0.000
H	-1.36±0.05 [a,h]
H+G	-1.02±0.02 [a]
H+B	-0.64±0.07 [a,d,g]
H+D	-0.80±0.06 [a,d,g]
H+G+B	-0.30±0.03 [a,d,g]
H+G+D	-0.42±0.02 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 80

Real Time PCR amplification of NF-ƙB mRNA in the Brain Stem of control and experimental rats

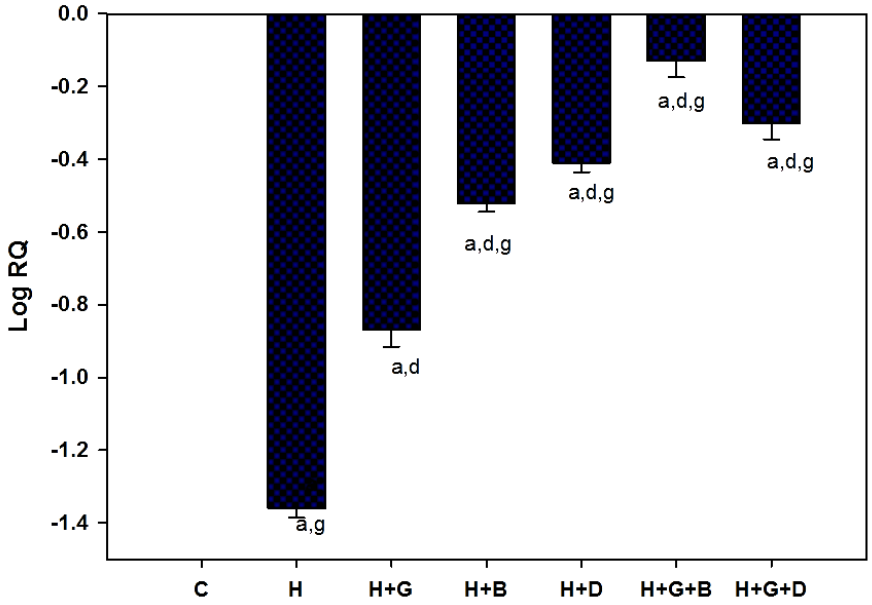


Table-82
Real Time PCR amplification of NF-ƙB mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**-1.36 ± 0.03[a,g]**
H+G	**-0.87 ± 0.04[a,d]**
H+B	**-0.52 ± 0.05 [a,d,g]**
H+D	**-0.41 ± 0.03 [a,d,g]**
H+G+B	**-0.13 ± 0.04[a,d,g]**
H+G+D	**-0.30 ± 0.04[a,d,g]**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 81

Real Time PCR amplification of SOD mRNA in the Brain Stem of control and experimental rats

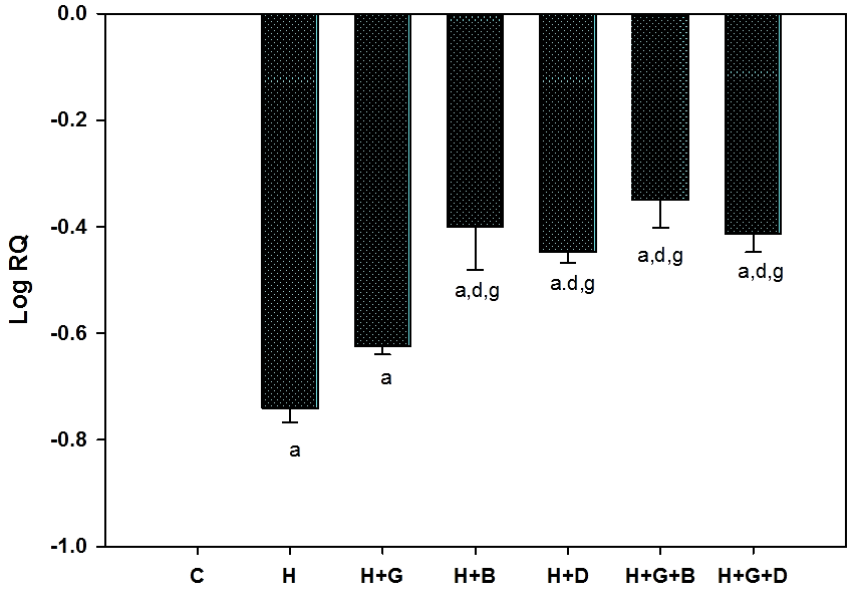


Table-83
Real Time PCR amplification of SOD mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.74 ± 0.02[a]
H+G	-0.62 ±0.01 [d]
H+B	-0.40 ± 0.08 [a,d,g]
H+D	-0.45 ± 0.02 [a,d,g]
H+G+B	-0.35 ± 0.05 [a,d,g]
H+G+D	-0.41 ± 0.04[a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 82

Real Time PCR amplification of GPx mRNA in the Brain Stem of control and experimental rats

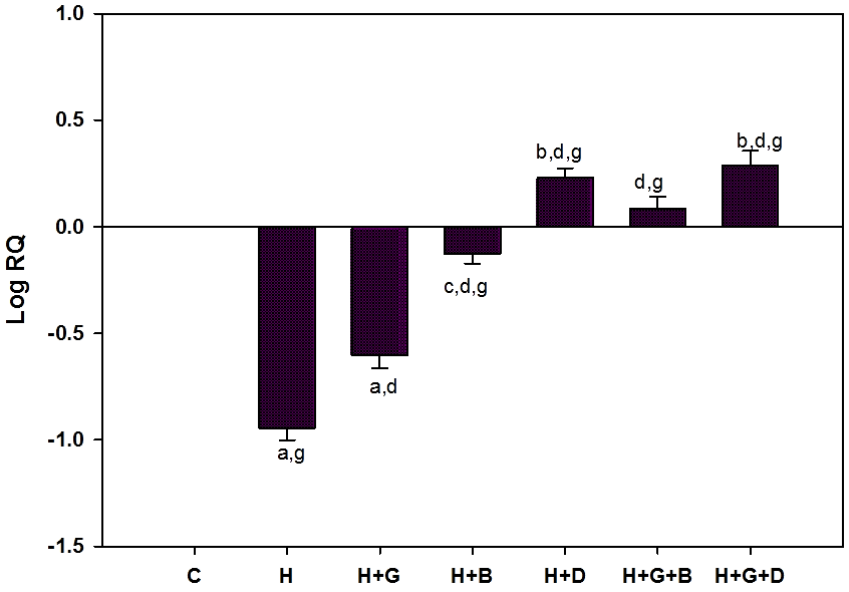


Table- 84
Real Time PCR amplification of GPx mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0
H	-0.95 ±0.05 [a,g]
H+G	-0.60 ±0.06 [a,d]
H+B	-0.13 ±0.04 [c,d,g]
H+D	0.23 ±0.04 [a,d,g]
H+G+B	0.09 ±0.05 [d,g]
H+G+D	0.29 ±0.06 [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 83

Real Time PCR amplification of Bax mRNA in the Brain Stem of control and experimental rats

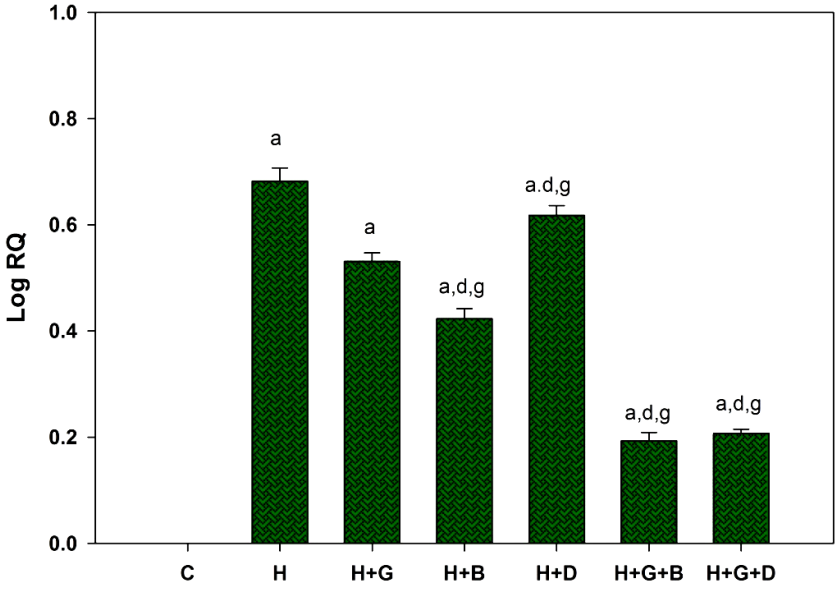


Table- 85

Real Time PCR amplification of Bax mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	**0**
H	**0.68 ±0.03** [a]
H+G	**0.53 ±0.02** [a]
H+B	**0.42 ±0.02** [a,d,g]
H+D	**0.62 ±0.02** [a,d,g]
H+G+B	**0.19 ±0.02** [a,d,g]
H+G+D	**0.21 ±0.01** [a,d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Figure - 84

Real Time PCR amplification of caspase 8 mRNA in the Brain Stem of control and experimental rats

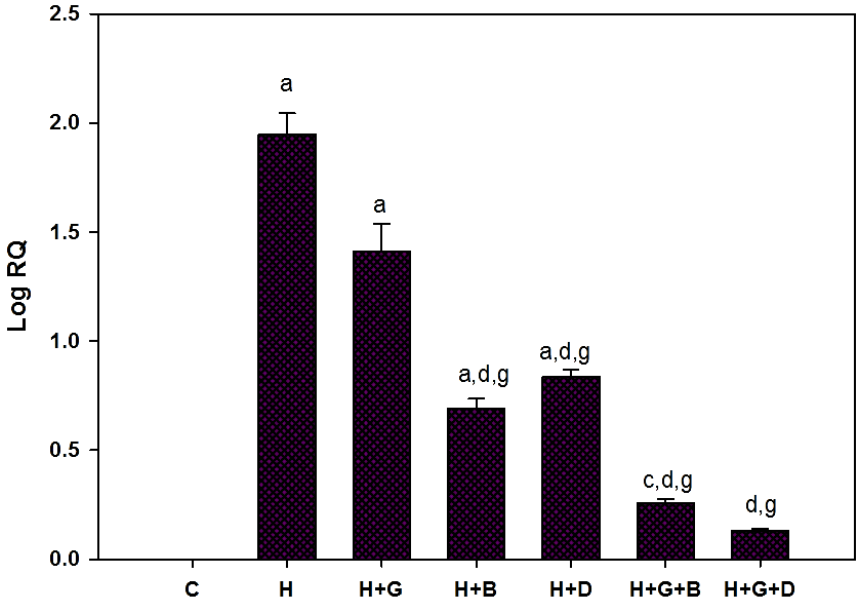


Table- 86
Real Time PCR amplification of caspase 8 mRNA in the Brain Stem of control and experimental rats

Experimental groups	Log RQ
C	0
H	1.95 ±0.09 [a]
H+G	1.41 ±0.13 [a]
H+B	0.69 ±0.045 [a,d,g]
H+D	0.84 ±0.03 [a,d,g]
H+G+B	0.26 ±0.02 [c,d,g]
H+G+D	0.13 ±0.01 [d,g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 4-6 rats.
[a] p<0.001, [b] p<0.01, [c]p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f]p<0.05 when compared to Hypoglycemic group.
[g] p<0.001,[h] p<0.01 when compared to Hypoglycemic group treated with glucose.
C- Control, H- Hypoglycemia, H+G- Hypoglycemia treated with glucose, H+B- Hypoglycemia treated with *Bacopa monnieri*. H+D- Hypoglycemia treated with Bacoside A, H+G+B- Hypoglycemia treated with Glucose and *Bacopa monnieri*.
H+G+D- Hypoglycemia treated with Glucose and Bacoside A.

Discussion

Glucose is the major source of energy for organ function. Although all organs can use glucose, the human brain uses it almost exclusively as a substrate for energy metabolism. Because cerebral glycogen stores are limited, maintenance of adequate glucose delivery to the brain is an essential physiologic function. The high brain-to-bodyweight ratio in the newborn results in a proportionately higher demand for glucose compared with the capacity for glucose production. It is higher than that encountered in the adult, with cerebral glucose use accounting for as much as 90% of total glucose consumption (McGowan, 1999). Although alternate fuels, such as lactate and ketone bodies, can be used as a substrate for energy production, the newborn's immature counter regulatory response limits the availability of these molecules. Thus, neonates are extremely susceptible to any condition that impairs the establishment of normal glucose homeostasis during the transition from intrauterine to independent extrauterine life (Kalhan *et al*., 2000). Hypoglycemia is associated with acute neurologic dysfunction, and it has been associated with long-term neurodevelopmental impairment (Burns *et al*., 2008).

BLOOD GLUCOSE AND BODY WEIGHT

The insulin induced neonatal hypoglycemic rat serves as an excellent model to study the molecular, cellular and morphological changes in brain induced by stress during neonatal hypoglycemia. Before birth, the fetus receives glucose through the materno-placental circulation at a daily amount of 7 g/kg (Sann, 1989). When the umbilical cord is clamped, the neonate must meet several metabolic challenges, two of which are the maintenance of adequate circulating levels of glucose or alternate fuels to the brain and other organs, and adaptation to intermittent milk feedings. If these processes fail to occur, neonatal hypoglycemia develops (Hawdon *et al*., 1994). Enzymes for gluconeogenesis are present by 3rd month of gestation. Insulin starts

production by 12 Wks and increases by the third trimester and fetal glycogen deposits begins by the ninth week and increases by third trimester. In the first week of life, the neonate is very dependent on gluconeogenesis for glucose production (Haymond, 1989).

There was a significant decrease in blood glucose level of neonatal hypoglycemic rats when compared to control group. Glucose, *Bacopa monnieri* leaf extracts and Bacoside A treatment significantly increased the blood glucose level.

Previous reports confirmed the glucose increasing activity of *Bacopa monnieri* leaf extract and Bacoside A. Bacopa monnieri contains the alkaloids herpestine, brahmin, bacosine, bacosterols, monnierin, bacoside-A and bacoside-B. Monnierin, on hydrolysis, gave glucose, arabinose and aglycone. Bacosides A and B give glucose, arabinose and bacogenines A, A2, A3 and A4 (Gohil & Patel, 2010).This provides the slow release of glucose moieties into the blood and prevents the rapid shift from hypoglycaemia to hyperglycemia.

Neonatal hypoglycemic rats also have showed a significant decrease in body weight. Hypoglycemia and decreased body weight during neonatal hypoglycemia are similar with previous reports. The availability of glucose and glycolytic intermediates glucose 6 phosphate and lactate were decreased during hypoglycemia (Vanucci & Vanucci, 2001) leading to low body weight. Earlier studies have have shown that modulation of DAD2 receptor activity profoundly affects energy homeostasis in humans and animals and drugs that block DAD2 receptor enhance appetite and induce weight gain in animals and humans (Baptista, 1999). Our results have have shown an increase in DAD2, which correlates with the loss of weight seen in the neonatal hypoglycemic rats.

Treatment with Glucose, *Bacopa monnieri* and Bacoside A significantly reduced body weight loss in neonatal hypoglycemic rats. Supplementation with glucose increased the availability of glucose that was decreased during hypoglycemic condition, thus reversing the loss of body weight. *Bacopa monnieri* and Bacoside A

reduces the neonatal hypoglycemic complications by decreasing the DAD2 receptor expression thus enhancing appetite and induce weight gain.

CNS DOPAMINERGIC SYSTEM

DA is intimately involved in the regulation of energy balance. DA plays an important role in the complex physiology driving meal initiation and termination. Moreover, DAergic neurotransmission profoundly affects glucose and lipid metabolism (Cincotta & Meier, 1996). DAD2 receptor agonists improve glucose and lipid metabolism in patients with hyper prolactinemia and acromegaly (Yavuz *et al.,* 2003). Short term administration of DAD2 receptor agonist-bromocriptine ameliorates various metabolic anomalies in obese humans without affecting body weight and longer term treatment improves glycemic control and serum lipid profiles (Cincotta *et al.,* 1993).

DOPAMINERGIC RECEPTOR EXPRESSIONS

CEREBRAL CORTEX

Cerebral cortex is comprised of layers of neurons exhibiting distinct morphologies and synaptic connections (McConnell., 1991). The mechanisms that induce neuronal injury following glucose deprivation are more complex than simply depriving neurons of their primary energy source (Auer & Siesjo., 1993). As in brain injury associated with ischemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycemic brain injury (Aral *et al*., 1998; Auer, 1991; Auer & Siesjo., 1988). It results from the release of excitatory amino acids, such as glutamate (McGowan *et al.,* 2002) or other neurochemicals, such as adenosine (Calabresi *et al.,* 1997), which influence neuronal function and survival (Dunwiddie & Masino, 2001; Turner *et al.,* 2002[a,b]).

In the central nervous system, the cortical DAergic system is important in regulating neuronal growth and development. The DA are widely expressed in the

central nervous system because they are involved in the control of locomotion, cognition, emotion and affect neuro-endocrine secretion (Missale *et al*., 1998).The DAergic innervation of the cerebral cortex has been well characterized and DA receptor-mediated effects have also been demonstrated in the cortex (Bannon *et al.,* 1982). Prefrontal cortex is a cortical area involved in selecting and retaining information to produce complex behaviours (Arianna *et al*., 2007). Somatosensory cortex has important role in food intake and obesity. (Tataranni *et al.,* 1999). DAergic modulation of pre frontal cortex neural activity is regulated by multiple DA receptor subtypes. In animals, the meso prefrontal DAergic system is particularly vulnerable to stress. Any disturbance in the central DAergic function will affect the normal memory processing and cognition. DAergic innervations of the dorsal and medial prefrontal cortex appear to be sensitive to stress and relatively low intensity levels of stress are capable of disrupting functions like 'working memory' and attention. DAD1 receptor is a metabotropic G- protein coupled receptor which leads to the activation of cAMP metabolism. Hypoglycemic stress induced activation of apoptotic signalling cascade further augment the cognitive impairment. Food consumption increases brain DA levels in animals and humans (Small *et al.,* 2003).

Cerebral cortical DA metabolism is reported to decrease because decreased glucose during neonatal hypoglycemia affects the DAergic activites such as working, memory and stress response. Our results have showed that in neonatal hypoglycemic group and hypoglycemic group treated with glucose, DAD1 receptor binding parameter - B_{max} was significantly decreased with an down regulation in DAD1 receptor gene expression, whereas DAD2 receptor binding parameter - B_{max} was significantly increased and gene expression was up regulated. Confocal imaging studies of DAD1 and D2 receptor expressions in cerebral cortex demonstrated the decreased expression DAD1 receptors and increased expression of DAD2 receptors in neonatal hypoglycemic condition and treatment groups have showed significant reversal. Confocal imaging studies confirm the receptor binding results and gene

expression data. DA activity is related to the density of DA receptors. in the present study increased DA level in the cerebral cortex augmented the expression of DAD2 receptors.

Altered levels of DA D1and DAD2 in the present study indicated dysfunction in the functional regulation of the DA receptors of the cerebral cortex in neonatal rats exposed to hypoglycemic insult. During glucose treatment, significant decrease in DAD1 receptor number and gene expression and significant increase in DAD1 receptor number and gene expression in the cerebral cortex was observed. Supplementation of glucose to already excited system results in its hyper activity and it affects the balance of various neurotransmitters. DAD1 stimulation optimizes physiological signaling in prefrontal neurons engaged by working memory in, whereas too little DAD1 stimulation resulted in diminished neuronal activation (Williams & Goldman-Rakic., 1995). Higher DAD2 receptor density is associated with poorer performance on cognitive tasks involving corticostriatal pathways. It also suggests that dopamine dysregulation is also a trait phenomenon related to psychosis vulnerability (Hirvonen *et al*., 2005).

Our results have showed that treatment with *Bacopa monnieri* and Bacoside A reversed the altered DA receptor expressions when compared with neonatal hypoglycemic rats. Neonatal hypoglycemia and its complications are related with oxidative stress. Cognitive deficits do not have shown consistent improvement with presently available resuscitation with glucose alone. *Bacopa monnieri* and Bacoside A is seen to to provide optimal stimulation of cortical DAD1 sites as well as to antagonize DAD2 receptors. Both the decrease in receptor number and receptor affinity was restored back to near control levels by Bacoside A and *Bacopa monnieri* supplementation. Previous studies suggest that *Bacopa monnieri* and Bacoside A treatment normalises oxidative stress (Gupta *et al.,* 2014). Although, the mechanism of action of *Bacopa monnieri* is not established, its antioxidant property accounts for the restoration of DAergic receptor expressions. In Bacoside A supplemented group,

improved mitochondrial function and maintenance of ATPases contributes to decrease in antioxidant levels (Sumathi *et al*., 2011).

CORPUS STRIATUM

The striatum is a subcortical part of the forebrain. It is the major input station of the basal ganglia system (Bear *et al*., 2001). The corpus striatum is a large subcortical structure in the mammalian brain that is involved in motor coordination, cognitive functions and complex processes associated with adaptive behaviours (Schultz *et al.*, 2000; Zhou *et al.*, 1995). Several intrinsic neurotransmitters interact to regulate its function, including DA from the nigrostriatal pathway and 5-HT from afferent raphe projections (Walker *et al.*, 1991). DA modulates a variety of functions including striatal synaptic plasticity (Gerfen & Surmeier, 2011).The striatum, in turn, gets input from the cerebral cortex. In rat striatum severe hypoglycemia causes an irreversible nerve cell injury, which does not become manifest until during the post-insult recovery period (Liden *et al*., 1998).

Dopamine exerts a powerful effect on the central regulation of motor activity and behavior by the striatum. The importance of this neurochemical in the CNS is emphasized by the fact that abnormalities in dopamine transmission have been implicated in a wide range of striatal disorders including Parkinson's disease, schizophrenia, and drug addiction (Paul *et al*., 1992). DA is involved in the regulation of energy balance. Studies suggest that excessive release of DA is responsible for the development of ischemic cell damage in the striatum (Globus *et al*., 1988). Glucose deprivation increases metabolic stress on neurophysiology, including dopamine release. Hypoglycemia was associated with varied neurophysiological effects, including increased cerebral blood flow (Bryan *et al*., 1994) and increased striatal concentrations of conjugated HVA, a dopamine metabolite (Cottet-Emard & Peyrin, 1982). DAD2 plays significant role in receptor-mediated mechanisms in striatal excitotoxicity (Garside *et al*., 1996).

The present study have showed decreased expression of DAD1 receptors and increased expression of DAD2 receptors in the striatum in neonatal hypoglycemic condition. The firing of DAD1 neurons projecting from the substantia nigra to the striatum is reported to be rapidly suppressed by hyperglycemia leading to the hypofunction of DAD1 receptors (Sailer & Chiodo, 1980). DAD2 receptor expression increased in the striatum during hypoglycemia as a result of the decreased transmission of DA. Hypoglycemia depresses the dopaminergic function. Therefore a decreased dopaminergic activity is always suggested to increase the DAD2 receptors. An increase in the expression of DAD2 receptors gene results in the increased receptor number. The metabolic abnormalities during disturbances in the striatum cause alterations in DAergic neurons by decreasing their firing rate (Sailer & Chiodo, 1980). DAD2 receptor up regulation observed in the present study is a compensatory mechanism to meet decreased DAD1 receptor expression. DAD2 receptors are reported to regulate the release of DA from dopaminergic neurons originating in the ventral tegmental area as well as in the substantia nigra (Plantje *et al.,* 1987), Insulin induced hypoglycaemia damages the DAD1 receptors, decreasing the DA related functions in the striatum and other brain regions. The two DA receptor subtypes interact in a synergistic way (Paul *et al*., 1992) to adapt to the alterations in glucose metabolism. During DA depletion there is increased activity of indirect pathway neurons (striatopallidal neurons, which express mainly DAD2 receptor) and decreased activity of direct pathway neurons (striatonigral neurons, which express predominantly DAD1 receptor). DA D2-type receptors have higher affinity for DA than DAD1 receptors and D1-mediated effects emerge earlier during DA depletion and small changes in DA levels could affect preferentially the activation of low affinity versus high affinity receptors, while DAD2 receptor mediated effects become more prominent as the disease progresses (Richfield *et al.,* 1989).

Glucose treatment also have showed altered DA D1, D2 receptor mRNA expressions and increased DAD2 receptor binding sites in the striatum. High levels of

glucose causes a shift from hypoglycaemia to hyperglycemia, which aggrevates cell death (Moley & Mueckler, 2000), which in turn results in decreased DAD1 and increased DAD2 receptor status. *Bacopa monnieri* and Bacoside A treatment significantly increased the binding sites of total DA receptors, DAD1 receptors and reversed the DAD2 receptor binding parameters and mRNA expression of both receptor subtypes compared to neonatal hypoglycemic condition. Confocal imaging of DA D1, D2 receptor expression studies are also in accordance with real time and receptor assay data. Confocal imaging studies in striatum have showed decreased DAD1 receptor expression and increased DAD2 receptor expression in neonatal hypoglycemic condition and have showed reversal in treatment groups. Although the processes and mechanisms underlying the neuroprotective effects of Bacoside A on DA receptor expressions remain to be elucidated, several reports suggest, the antioxidant properties of Bacoside A in this process. Studies suggest that bacoside A protects the brain from the oxidative damage through its antioxidant potential (Anbarasi *et al*., 2006).

CEREBELLUM

Cerebellum is a region of the brain that plays an important role in the integration of sensory perception, memory consolidation, coordination and motor control. In order to coordinate motor control, there are many neural pathways linking the cerebellum with the cerebral motor cortex and the spino-cerebellar tract (Roberta & Peter, 2003). There is currently enough anatomical, physiological and theoretical evidence to support the hypothesis that cerebellum is the region of the brain for learning, basal ganglia for reinforcement learning and cerebral cortex for unsupervised learning (Doya, 1999). There is increasing evidence that the cerebellum is involved in cognition, behaviour and emotion (Schmahmann & David, 2006). Cerebellar dysfuncton is associated with poor fine motor skills, hypotonia (Wassmer *et al*., 2003). Studies from our laboratory have demonstrated that cerebellum is susceptible

to hypoglycemia (Joseph *et al*., 2007, 2008). Studies have have shown that in the developing brain, Cerebellum significantly differs with respect to ischemia and hypoxia, this response being directly related to the duration and intensity of the injury. Cerebellar hypoxia is responsible for important aspects of cognitive deterioration and motor disturbances in neurological disorders, such as stroke, vascular dementia and neurodegeneration (Rodrigo *et al*., 2004). Hypoxia retards the development of neuronal processes, resulting in a smaller cerebellum (Yu & Wan-Hua Yu, 1980). Studies confirmed that presence of dopaminergic innervations in the cerebellum and suggests there is a small dopaminergic element, whose properties are similar to the well characterized system of striatum (Giompres & Delis, 2005). The cerebellum has generally been suggested to be involved in the control and integration of motor processes, as well as cognitive functions. Studies by Botez-Marquard, 2001, originally suggested a cognitive role for the cerebellum. Since then, many studies have confirmed that the cerebellum contributes to cognitive and other non-motor functions.

Gene expression studies have showed that the mRNA level of DAD1 and receptors in the cerebellum of hypoglycemic neonatal rats substantially increased compared to control rats. Also, the binding parameters B_{max} of DAD1 were decreased in experimental rats. Immunohistochemistry study using confocal microscope confirmed a similar expression pattern in localization of DAD1 receptors in the cerebellum of experimental rats. Expression studies on DAD2 have shownwed a marked increase, substantiated by an increased B_{max}. Our results have shown that dopaminergic transmission in the cerebellar circuitory is affected during hypoglycemia and exacerbated by glucose treatment increasing the susceptibility of cerebellar purkinje cells to neuronal damage. These dopaminergic receptor alterations were reversed by *Bacopa monnieri* and Bacoside A supplementation. Hence the decreased DA content and the altered DAD1 and DAD2 are suggested to play a critical role in motor and cognitive functions dysfunction if not resuscitated during the

critical period. *Bacopa monnieri* and Bacoside A supplementation proves beneficial to reverse the hypoglycemic damage to dopaminergic function in the cerebellum. These neurofunctional deficits are one of the key contributors to motor deficits and cellular stress associated with hypoglycemia treated with glucose caused aggrevated damage at molecular level than hypoglycemia.

BRAINSTEM

Chronic changes in glycemia induce alterations in brain glucose metabolism in rodents (McCall *et al.,* 1982; Nagy *et al.,* 1994). Brain stem reticular formation has been considered to play an important role in generating behavioural states as well as in the modulation of pain sensation (Paré & Steriade 1993, Steriade, 1996). These reticular functions originate from interacting neuronal groups in the brain stem, including cholinergic, adrenergic and serotoninergic neurons (Steriade, 1996). Hypoglycemia result primarily from a lowered glucose level in the brain and it adversely affects the central and autonomic nervous systems (Charles *et al*., 2005). Brainstem is an important part of the brain in monitoring the glucose status and the regulation of feeding (Guillod *et al*., 2003). Also, it serves as one of the key centres of the central nervous system for regulating body homeostasis.The brain stem is a complex rostral continuation of the spinal cord and contains several collections of cell bodies. Brain stem receives sensory information from the face and contains the motor nuclei innervating the muscles of the face, eyes and the cranial parasympathetic system. In addition, much of the specialized sensory information originating from the cochlea and vestibular labyrinth, eyes, taste buds, cardiovascular, respiratory and digestive systems directly reaches the brain stem, where it is further processed. The sensory input and motor output of the brain stem is carried by cranial nerves.

In the central nervous system, glucose regulates the activity of glucose-sensitive neurons present in the brain stem and the hypothalamus. Glucose sensing neurons have a critical role in regulating glucose and energy homeostasis through

secretion of endocrine pancreas hormones, regulation of liver glucose production, feeding behaviour and energy expenditure (Marty *et al.,* 2007). These are glucose-excited neurons, which increase their firing rate with elevation in extracellular glucose concentrations and glucose-inhibited neurons, which are activated by a decrease in extracellular glucose concentration or by cellular glucose deprivation (Routh, 2002). Both types of neurons are widely distributed in the brain stem regions, in particular in the nucleus of the solitary tract, the area postrema and the dorsal motor nucleus of the vagus and it is involved in the control of energy homeostasis and food intake (Yettefti *et al.,* 1997).

Our results have showed differential expression pattern DA receptor subtypes in brain stem of neonatal hypoglycemic rats. D1 receptor binding sites are significantly decreased with a significant decrease in affinity whereas D2 receptor binding sites are increased with an increase in affinity. Real time PCR analysis demonstrated the down regulation of DAD1 receptor gene expression and up regulation of DAD2 receptor gene expression in the brain stem of neonatal hypoglycemic rats. Adverse effects of hypoglycemia on brain function are not limited to higher centers but also involve the brain stem (Jones *et al.*, 1990). Our previous studies demonstrated adrenergic, serotonergic and dopaminergic, muscarinic and receptor functional alterations in the brainstem of hypoglycemic rats (Robinson *et al*., 2009; Joseph *et al*., 2009; Antony *et al.,* 2010). These results indicate imbalance in DAergic receptor expressions and suggest impaired DAergic activity and DA related functions in the brain stem of hypoglycemic rats. Our results have showed that treatment with *Bacopa monnieri* and Bacoside A reversed the altered DA receptor expressions when compared with neonatal hypoglycemic rats and rats treated with glucose. Resetting of doapminergic tone and activity in the striatum and cerebral cortex by *Bacopa monnieri* and Bacoside A treatment has a significant role in the modulation of DAergic receptor expressions in the brain stem.

SECOND MESSENGERS IN BRAIN REGIONS

cAMP content in brain

cAMP is produced when G-proteins activate adenylyl cyclase in the plasma membrane. cAMP produced by adenylyl cyclase, activates PKA by binding to the regulatory subunits in ways that result in the release and nuclear translocation of active catalytic subunits (Meinkoth *et al.,* 1993). cAMP stimulates the proliferation of many cell types, but in some cases cAMP inhibits cellular proliferation (Dugan *et al.,* 1999; Wang *et al.,* 2000). cAMP regulate the activity of neurons throughout the central nervous system, controlling metabolic processes, electrical signaling and synaptic physiology (Nathanson, 1977).The cyclic nucleotides cAMP are involved in a number of intracellular processes such as signal transduction, gene transcription, activation of kinases and regulation of channel function (Burns *et al*., 1996).

In the present study cAMP content was significantly decreased in cerebral cortex, striatum, cerebellum and brain stem of neonatal hypoglycemic rats. This corresponds to the decreased DAD1 signaling. Reports suggest that decrease of D1-like family receptors is coupled to the G protein $G_{s\alpha}$, which subsequently deactivates adenylyl cyclase, decreasing the intracellular concentration of the cAMP (Corvol *et al.,* 2004). In the present study neonatal hypoglycemic associated DAergic depletion and imbalance in the expression pattern of DA D1, D2 receptors attributed to the decreased the content of cAMP. DA and cAMP regulate a diverse array of neuronal functions ranging from ion conductance and synaptic plasticity to gene expression (Cooper, 2003). cAMP regulate the activity of neurons throughout the central nervous system, controlling metabolic processes, electrical signaling and synaptic physiology (Nathanson, 1977). In the present study *Bacopa monnieri* and Bacoside A treatment significantly increased cAMP content suggesting its significant role in ameliorating the disturbances in dopaminergic signalling.

IP3 content in brain

IP3 is a ubiquitous second messenger that functions by binding to IP3 receptors on the endoplasmic reticulum membrane to cause liberation of sequestered Ca $^{2+}$ (Berridge, 1998, 2002). The resultant cytosolic Ca^{2+} transients serve numerous signaling functions in neurons, including modulation of membrane excitability synaptic plasticity and gene expression (Stutzmann *et al.,* 2003). IP3 functions by binding to receptors (IP3Rs) on the endoplasmic reticulum (ER) membrane to cause liberation of sequestered Ca $^{2+}$ (Berridge, 1998, 2002). In the phosphoinositide signaling pathway, agonist-induced interaction of cell surface receptors with G proteins activates the enzyme phosphoinositol-specific PLC, which catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate into IP3 and DAG (Berridge, 2002).

In the present study IP3 contents have showed differential expression pattern. IP3 content were significantly increased in brain regions- corpus striatum and brain stem, whereas IP3 content were significantly decreased in cerebral cortex and cerebellum of neonatal hypoglycemic rats. Decreased expression pattern of DAD2 receptors observed in the present study, have played an important role in the down regulation of phospholipase-C with a decrease in IP3 content. Increased gene expression of phospholipase- C enzyme observed in different brain regions is responsible for the increased content of IP3 in neonatal hypoglycemic rats. IP3 mobilizes Ca^{2+} from intracellular sources after binding with IP3 receptors; DAG activates the phosphorylating enzyme protein kinase C (Berridge *et al.,* 1998). These events mediate cellular activation and subsequent biological responses such as neurotransmitter release, cell growth, differentiation, neuronal development and gene expression (Berridge, 1998). The neuronal intracellular calcium has an important role in the regulation of synaptic plasticity (Barbara, 2002). Moreover, disruptions in this pathway are implicated in neurodegenerative disorders (Mattson *et al.,* 2000).

Increased IP3 observed in the brain of neonatal hypoglycemic rats corresponds to increased DAD2 signalling. Reports also have shown that under abnormal conditions, excessive phospholipase activation, along with a decreased ability to resynthesize membrane phospholipids, lead to the generation of free radicals, excitotoxicity, mitochondrial dysfunction and apoptosis/necrosis *Bacopa monnieri* and Bacoside A treatment significantly decreased IP3 content in cerebral cortex, corpus striatum, brain stem and cerebellum. Thus it is seen that, *Bacopa monnieri* and Bacoside A has a crucial role in the modulation of second messenger cascade in neonatal hypoglycemic rats.

PLC expression in brain

In the phospho inositol signaling pathway, agonist-induced interaction of cell surface receptors with G proteins activates the enzyme phospho inositol-specific phospholipase C, which catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate into IP3 and DAG (Berridge, 2002). PLC has been categorized into three major families: PLC β, PLC δ, and PLC γ (Cockcroft &Thomas, 1992). All Phospholipase C isozymes recognize PIP_2 as a substrate and carry out Ca^{2+} dependent hydrolysis of inositol lipids; however, these isozymes are differentially regulated and expressed (Cockcroft &Thomas, 1992). In the CNS, neurotransmitter receptor coupling to phospholipase C has been extensively documented in [^{3}H] inositol-labeled tissue slices and synaptosomes obtained from animal brains (Chandler & Crews, 1990).

In the present study, we observed, up regulation of phospholipase C gene expression in cerebral cortex, corpus striatum, brain stem and cerebellum of neonatal hypoglycemic rats. Up regulation of the Phospholipase C in brain during neonatal hypoglycemia contribute to the impaired signal transduction of G-protein coupled neurotransmitter receptors. Altered phospholipase C expression fails to modulate the activity of downstream proteins important for cellular signaling. Defective expression

of phospholipase C results in high levels of IP3 causing the impaired release of Ca^{2+} and bring up the level of intracellular calcium and thus causing excitotoxicity, affecting the normal neuronal function in brain regions. Reports suggest that, neuronal Ca^{2+} accumulation and mitochondrial membrane depolarization in response to glutamate is a requirement for the progression to cell death (Vaarmann *et al*., 2013). Glucose treatment aggrevates the neuronal death caused. glucose infusion is one but it induce complications such as hyperglycemia, rebound hypoglycaemia after interruption of the infusion and hypersecretion of insulin which can induce recurrence of hypoglycaemia; it also inhibit the compensative gluconeogenesis (Kalhan *et al*., 1986)

Bacopa monnieri and Bacoside A treatment significantly reversed the expression pattern compared with neonatal hypoglycemic condition. These results suggest neuromodulatory role of *Bacopa monnieri* and Bacoside A in neonatal hypoglycemic condition.

CREB expression in brain

CREB is a critical factor in many important functions in the nervous system, including neurogenesis and neuronal survival, development and differentiation, as well as neuroprotection, axonal outgrowth and regeneration, synaptic plasticity (Barco & Kandel, 2006)**.** Genes whose transcription is regulated by CREB include: c-fos, BDNF, tyrosine hydroxylase and neuropeptides such as somatostatin, enkephalin, VGF and corticotropin-releasing hormone (Lauren, 2005). It is a crucial neurotrophic factor and possess pro-survival and/or differentiation effects on several neuronal populations and synaptic plasticity (Thoenen, 2000). CREB is a transcription factor which is a downstream target of cyclic AMP signaling.

Our results demonstrated the up regulation of CREB in cerebral cortex, corpus striatum, brain stem and a down regulation in the cerebellum of insulin-induced neonatal hypoglycemia rats. In majority of the brain regions, there is an abnormal

metabolic environment including low glucose, and high insulin. Accumulation of ROS has been reported in severe hypoglycemic condition (Shin *et al.,* 2010). There is a suggestion in the neuronal literature that oxidative stress interferes with acute signaling to CREB. Glucose homeostasis mechanisms are highly regulated in the hindbrain (Scott *et al*., 2012), which accounts or the cerebellum remaining protected by the ROS production.

Up regulation of CREB in cerebral cortex, corpus striatum, brain stem and cerebellum of neonatal hypoglycemic rats treated with glucose is seen. High glucose can increase intracellular ROS by numerous mechanisms including activation of PKC, advanced glycation end products (AGE) accumulation and release from mitochondria (Nishikawa *et al.,* 2000). The neuronal cell death, which arises after hypoglycemia, is not simply a result of energy failure resulting from lack of glucose, but is instead the result of a cell death program that is initiated by the re-introduction of glucose after a period of hypoglycemia (Suh et al, 2007).

Our results have showed that *Bacopa monnieri* and Bacoside A treatment significantly decreased the gene expression of CREB. This study demonstrated that *Bacopa monnieri* and Bacoside A possess regulatory effect in the CREB expression, which is crucial in maintaining the normal neuronal functions and neuroplasticity.

GLUT 3 expression

Glucose is the principle energy source for the mammalian brain. Delivery of glucose from the blood to the brain requires transport across the endothelial cells of the blood-brain barrier and into the neurons and glia (Vanucci *et al*., 1997). The brain responds to large changes in plasma glucose and initiates compensatory responses to maintain glucose homeostasis. The families of facilitative GLUT proteins are responsible for the entry of glucose into cells throughout the periphery and the brain (Vannucci *et al.,* 1997). The expression, regulation and activity of glucose transporters play an essential role in neuronal homeostasis, because glucose represents the primary

energy source for the brain (Lund & Anderen, 1979). Although many isoforms of glucose transporters have been identified in the brain, GLUT 3, the neuron-specific glucose transporter, is solely responsible for the delivery of glucose into neurons in the central nervous system. Our study investigated the effect of brain glucose utilization in neonatal hypoglycemia associated neuronal damage.

The results of the current study have showed decreased expression of GLUT 3 in the cerebral cortex, striatum and brainstem, whereas an increased expression in cerebellum of neonatal hypoglycemic rats. GLUT 3 mRNA expression was decreased as a response to decreased glucose utilization in neonatal hypoglycemic condition. The reductions in GLUT3 were is a potential cause of the deficits in glucose metabolism (Simpson *et al*., 1994). the transporter contributes to brain glucose utilization, as part of overall metabolism and metabolic interactions among cells and alterations in energetic demand and/or substrate supply affect glucose transporter expression Vannucci *et al.,* 1997. Glucose homeostasis mechanisms play a highly significant role the cerebellum, thus, maintaining the GLUT 3 gene expression (Scott *et al*., 2012).

Glucose, *Bacopa monnieri* and Bacoside A significantly improved the glucose transport system in brain regions of neonatal hypoglycemic rats by the modulation of GLUT 3 gene expressions in cerebral cortex striatum, cerebellum and brain stem. The maintenance of glucose level by Glucose, *Bacopa monnieri* and Bacoside A treatment accounts for the increased expression of GLUT 3. Our findings suggest a modulation of GLUT 3 expression in the brain regions with *Bacopa monnieri* and Bacoside A supplementation which consecutively normalise the glucose transport in CNS.

NEURONAL SURVIVAL FACTORS

GDNF and BDNF gene expression in brain regions

GDNF is a potent neurotrophic factor for survival of dopaminergic neurons (Xing *et al.*, 2010). GDNF has been have shown to exert neuroprotective and

restorative effect on the nigral DA system (Ding *et al.*, 2004). GDNF promotes the survival and affects the proliferation, migration, and differentiation of a number of neuronal populations within the central and peripheral nervous. Several studies have revealed a neuroprotective influence of GDNF against various toxic challenges (Krieglstein *et al*., 1995). It has also been suggested that GDNF could also modulate neuronal death induced by acute brain injury (Wang *et al*., 1997). After binding to its specific receptor complex, GDNF activates several downstream intracellular pathways, including MAP) (Worby *et al*., 1996) and PI-3 kinase (Soler *et al*., 1999), resulting in long-term changes in gene expression (Messer *et al*., 1999).The neuroprotective activity of GDNF against ischemia-induced neuronal death is now well established (Abe *et al*., 1997; Kitagawa *et al*., 1998),

BDNF is important in differentiation, survival and plasticity of the CNS. BDNF belongs to the family of neutotrophins. The cellular actions of BDNF are mediated through TrkB tyrosine kinase receptor and by p75 neurotrophin receptor (Chao, 2006). The Ras pathway regulates neuronal survival and differentiation through downstream signaling. BDNF signaling through PI_3K plays an important role in survival of neurons and the downstream signaling includes serine/threonine kinases 3, phosphoinositide dependent kinase-1 (PDK1) and Akt (Vanhaesebroeck & Alessi, 2000). Akt activated by PDK1 in turn activates substrates involved in neuronal survival such as Bcl-2, Caspase-9 and IκB kinase (Roux & Barker, 2002). PLC-γ activation leads to increased levels of IP3 and DAG (Vetter *et al.,* 1991). BDNF is a survival gene contains cAMP response element. It is a crucial neurotrophic factor and possess pro-survival and/or differentiation effects on several neuronal populations and synaptic plasticity (Thoenen, 2000). CNS neurons are supported by several neurotrophic and transcription factors. BDNF is a potent trophic factor supports striatal cells and promotes survival and/or differentiation of GABAergic neurons *in vitro* (Mizuno *et al.,* 1994; Ventimiglia *et al.,* 1995).

GDNF and BDNF expression was significantly down regulated in cerebral cortex, corpus striatum, brain stem and cerebellum of neonatal hypoglycemic rats. Studies have suggested that enteric neuropathy is induced by decreased GDNF levels. This change may be mediated, via a reduction its main downstream signalling pathway PI3K/Akt, which is a survival signal for enteric neurons (Du et al., 2009). GDNF exerts a selective neuroprotective activity against NMDA-induced neuronal death. This neuroprotective activity of GDNF is caused by a reduction of the NMDA-induced Ca^{2+} influx; and the neuroprotective effect of GDNF involves the activation of ERKs (Nicole *et al*., 2001). Activation of apoptotic factors -TNF-α, caspase-8 and NFκB resulted in the decreased expression of BDNF in the cerebral cortex, corpus stiatum, cerebellum and brain stem of neonatal hypoglycemic rats. BDNF is known to regulate synaptic plasticity, neurogenesis and neuronal survival in the adult brain. Impaired BDNF signaling is central to depression and anxiety disorders, but could also play important roles in the pathogenesis of several age-related disorders, including insulin resistance syndrome, Alzheimer's disease and Huntington's disease (Mattson *et al*., 2004). This has showed a marked decrease in neuronal survival, leading to increased neuronal loss. It is suggested that there is neonatal hypoglycemic associated neuronal complication and neuronal loss in the brain.

Glucose supplementation has caused a significant decrease in GDNF and BDNF. This is due to the rapid shift from hypo to hyperglycemia in the brain aggrevating brain injury and neuronal damage in the developing brain. GDNF supports the survival of dopaminergic midbrain neurons and motor neurons (Milbrandt *et al*., 1998). Loss of dopaminergic neurons is consistent with the decrease in GDNF levels.

Bacopa monnieri and Bacoside A treatment significantly reduced the expression of GDNF and BDNF. Antioxidant properties of *Bacopa monnieri* and Bacoside A played a crucial role in protecting the dopaminergic neurons and thus ameliorating the defects in dopamine signalling. Treatment groups have showed

significant reversal in BDNF expression and suggests neuroprotective role of *Bacopa monnieri* and Bacoside A. Thus, enhancement of BDNF signaling may be a key mechanism whereby cognitive stimulation preserve brain function during developmental stages of the brain.

NF-κB gene expression in brain regions

NF-κB is ubiquitously expressed in peripheral and brain cells and regulates the expression of a wide variety of genes involved in cell survival, growth, stress responses, immune and inflammatory processes (Baldwin, 1996; Weih & Caamañ, 2003). NFκB controls the expression of genes that regulate a broad range of biological processes in the CNS such as synaptic plasticity, neurogenesis and differentiation (Ghosh & Hayden, 2008). NFκB is widely expressed in the central nervous system. NFκB is a ubiquitous transcription factor comprising at least five DNA binding protein subunits. Classic NFκB consists of two subunits, p50 and p65, which can bind to DNA as a homo- or heterodimer (Baeuerle & Henkel, 1994). At least two different cellular forms of NFκB exist: an inactive, non-DNA binding cytoplasmic form that is bound by inhibitor proteins IκBa and IκBf3 and an activated form that appears when IκB is released. Activated NFκB translocates to the nucleus where it interacts with DNA-binding sites to regulate gene expression (Baldwin *et al.,* 1996). After activation, NFκB induces the expression of genes encoding cytokines, inflammatory enzymes, cell adhesion molecules, cell surface receptors and acute-phase proteins.

In the present study NFκB gene expression in cerebral cortex, striatum, cerebellum and brain stem of neonatal hypoglycemic rats was down regulated. NFκB is also a modulator of apoptosis and ROS production. Basal levels of NFκB activity are normally present within neuronal nuclei in the cerebral cortex, although white matter is almost completely devoid of activated NFκB (Botchkina *et al*., 1999). Our

results indicate a reduction in cortical NFκB expression in neonatal hypoglycemic condition. Eventhough the possibility of sensitization of brain cells to the cytotoxicity due to declined NFκB function is high, the reduced expression of TNF-α observed in our study rules out that possibility. A mechanism of NFκB function reported by Lesoualc'h *et al.,* (1998) points to increased NFκB levels in neurons resistant to oxidative cell death and an inhibition of NFκB activity reversing this resistance thereby potentiating cell death.

Thus, in the present context the severe oxidative stress and declined antioxidant system in the cortical region resulted in the decreased NFκB expression. This along with the reduced TNF-α expression is suggested to be a defensive mechanism of central nervous system to counteract the fight against apoptosis or oxidative brain damage to prevent or prolong the onset of neonatal hypoglycemic encephalopathy. Reports suggest that impaired insulin-signaling activity acts unfavorably on the expression and translocation of NFκB and CREB with effects on proinflammatory factors and apoptosis (Francis *et al.,* 2008). Thus in our study the modulatory role of Bacoside A is suggested to alter the expression pattern of NFκB in neonatal hypoglycemic rats.

ANTIOXIDANT ENZYME EXPRESSION IN THE BRAIN REGIONS

GPx and SOD gene expression in brain

Neonatal hypoglycemia causes a variety of functional and structural disorders in the central and peripheral nervous systems (Biessels *et al.,* 1994). Low and high glucose levels produce ROS as a result of glucose auto-oxidation, metabolism and the development of advanced glycosylation end products. The level of antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress

and is associated with the development of complications in neonatal hypoglycemia (Baynes, 1991).

In the present study GPx gene expression significantly up regulated whereas SOD gene expression down regulated in neonatal hypoglycemic condition. SOD has ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, it stimulates several antioxidant enzymes that increase its efficiency as an antioxidant. In the present study SOD gene expression was significantly down regulated to compensate with the oxidative stress induced by hypoglycemia. Decrease in SOD activity leads to an increased production of superoxide (O_2^-) which has been implicated in cell dysfunction. Decreased level of SOD attributes to the free radical accumulation in neonatal hypoglycaemia which in turn confirmed the cell death (Junqing *et al*., 2005).

Glucose supplementation causes a decrease in the antioxidant enzymes. High levels of glucose cause a shift from hypoglycemia to hyperglycamia, which mediates apoptotic pathways (Moley & Mueckler., 2000). Research suggests that hypoglycemic induced oxidative stress and neuronal death may be attributed to the activation of NADPH oxidase during glucose reperfusion. Suh *et al*. 2007, have showed increased oxidative injury to neurons receiving glucose supplementation after a hypoglycemic event. Following hypoglycemia, the production of ROS was excessive when glucose was returned to normal or supranormal levels. While oxidative injury still occurred in neurons exposed to hypoglycemia alone, this study may support the clinical relevance of goal-directed therapy to subnormal glucose levels.

Bacopa monnieri and Bacoside A treatment significantly modulated the expression of SOD and GPx in the brain of neonatal hypoglycemic rats. They help to increase the production of SOD to overcome the stress caused due to free radical accumulation. Antioxidant properties of *Bacopa monnieri* and Bacoside A are

responsible for this modulation. Reports suggest that the antioxidant phytochemical such as flavonoids, alkaloids, sterols, tannins, phlobotannins and flavonoid glycosides present in the leaf extract possess free radical scavenging activity. Bacosine and monnierin compounds in *Bacopa monnieri* have independently have shown their activity against oxidative stress. The presence of Bacosine, a compound in *Bacopa monnieri* that is not one of the standard Bacosides (A and B) has insulin mimetic properties. The total phenolic content, flavonoid content and the presence of bacosine confer anti-oxidant capabilities to *Bacopa monnieri*. It is have shown increase anti-oxidant defence enzymes (Catalase by 26%, Glutathoine Peroxidase by 22%, SOD by 21%) and reduces levels of pro-oxidant compounds like MDA (15-21%) ROS (18-25%) and hydroperoxide (34-40%); these reductions in oxidants occurred in the cytosol and the mitochondria of neurons (Gohil & Patel., 2010). In Bacoside A supplemented group, improved mitochondrial function and maintenance of ATPases contributes to decrease in antioxidant levels (Sumathi *et al*., 2011).The present study suggests antioxidant properties of *Bacopa monnieri* and Bacoside A in protecting the brain regions from neonatal hypoglycemia associated oxidative stress.

APOPTOTIC FACTORS EXPRESSION IN THE BRAIN REGIONS

Akt-1 gene expression in brain regions

Akt is implicated in cellular processes such as cell survival, proliferation and growth, glucose metabolism, apoptosis, angiogenesis, transcription and migration (Scheid &Woodgett, 2003). Akt consists of three homologous members known as Akt-1, Akt-2 and Akt-3 (Robertson, 2005). Binding of phosphoinositide 3-OH kinase (PI_3K) products results in Akt translocation to the plasma membrane where it is activated *via* phosphorylation by upstream kinases such as the PDK1. Akt is an important mediator of the physiological effects of several growth and survival factors and promotes cell survival through the inhibition of apoptosis (Datta *et al.,* 1999).

Within the nucleus, Akt controls expression of genes involved in cell survival *via* the transcription factors- Forkhead, NFκB and CREB (Brunet *et al.,* 2001).

In the present study, the transcriptional profile of Akt-1 have showed up regulation in cerebral cortex and corpus striatum, and a down regulation in the cerebellum and brain stem of neonatal hypoglycemic rats. Up regulation of Akt-1 and activated expression of apoptotic factors in the brain suggests neonatal hypoglycemia associated neuronal loss. Reports suggest that, the neuronal death resulting from hypoglycemia is not a straight forward result of energy failure but instead results from a sequence of events. These events include activation of neuronal glutamate receptors, production of ROS, neuronal zinc release, activation of poly(ADP-ribose) polymerase–1 and mitochondrial permeability transition. The strong counterregulatory mechanisms in the hinb brain is seen to protect it from the oxidative damage produced. Glucose supplementation is seen to aggrevate the cell death process by the up regulation of AKT. This is in correspondence with reports which suggest that correction of plasma glucose concentration alone does not interrupt this cell death process (Su *et al*., 2007).*Bacopa monnieri* and Bacoside A treatment has a modulatory role in the expression of Akt-1. Akt is a central player in insulin and growth factor signaling and a regulator of several cellular functions including cell growth and apoptosis (Hanada *et al.,* 2004). PI3K/Akt pathway promots the anti-apoptotic signals and also inhibit proapoptotic gene (Nakamura *et al.,* 2001).

TNF-α expression in brain regions

Emerging evidence suggests impairment of neuronal function or loss of neurons in neonatal hypoglycemia. Oxidative stress seems to play a central role in neuronal damage (Bonnefont-Rousselot, 2002). Brain injury appears to result from a number of processes that are initiated when blood glucose concentration is altered. Many cytokines have been suggested to participate in neurodegeneration and

neurotoxicity. TNF-α is a major mediator of apoptosis and inflammation (Chen & Goeddel, 2002; Wajant *et al.,* 2003). Increased expression of TNF-α have been observed before neuronal death (Little & O'Callagha, 2001). TNF- α, the most widely studied cytokine, plays many roles as a signaling and as an effector molecule in both physiology and pathophysiology of the central nervous system (Munoz-Fernandez & Fresno, 1998). TNF- α is released during various inflammatory diseases of the CNS, being synthesized by microglia, astrocytes and some populations of neurons (Lieberman *et al.,* 1989; Chung *et al.,* 2005).

TNF-α expression was significantly down regulated in cerebral cortex, corpus striatum and brain stem and up regulated in the cerebellum of neonatal hypoglycemic rats. Decreased TNF-α expression in brain regions suggests neuronal damage caused by dysfunctional TNF-α signaling in neurodegeneration and it is supported by previous reports. hypoglycemia induces proinflammatory changes including an increase in the plasma concentration of interleukin (IL)-6 and increases in other proinflammatory mediators, including leucocytosis, ROS generation, lipid peroxidation, and levels of TNFα, IL-1β, and IL-8 . Even though the neurodegenerative activity of TNF-α was documented in many studies, several reports emphasise the neuroprotective role of the same (Fontaine *et al*., 2002). TNF-α induces neuroprotection against excitotoxic damage in primary cortical neurons *via* sustained NF-κB activation (Dolga *et al.*, 2008). A significant reduction of TNF- α levels in cerebellum observed in our study, indicate the spatial variation in the vulnerability of brain parts to hypoglycemia and its related brain complications. Glucose supplementation has caused a marked increase in TNF alpha. This is due to the rapid shift from hypo to hyperglycemia in the brain, leading to the release of pro inflammatory cytokines, aggrevating brain injury and neuronal damage in the developing brain. Our results correspond with the studies conducted by Desouza *et al*., 2010, which suggest that hyperglycemia is associated with increased

proinflammatory cytokines (TNF-alpha, IL-1beta, IL-6, and IL-8), markers of lipid peroxidation, ROS, and leukocytosis. *Bacopa monnieri* and Bacoside A treatment significantly reduced the expression of TNF- α and rendered neuroprtotective effect. Previous studies suggest antioxidant and neuroprotective role of Bacoside A and *Bacopa monnieri* in normalizing neonatal hypoglycemic related oxidative stress (Majumdar *et al.,* 2012). Antioxidant properties of *Bacopa monnieri* and Bacoside A played a crucial role in this modulation.

Bax and Caspase 8 expression in the brain regions

Bax, a member of the Bcl-2 family proteins, are distinct regulators of early stages of the apoptotic process by forming oligomers onto the mitochondrial outer membrane and creating a channel for the release of cytochrome *C* and other apoptotic substances (Antonsson *et al*., 2001). Studies also suggested that Bax can bind to the voltage-dependent anion channel and promote the release of cytochrome *C* from it, (Vander Heiden et al., 1997). Bax translocation onto the mitochondrial membrane therefore becomes one of the important indicators for the onset of mitochondria-mediated apoptosis. A critical role in initiation and progression of apoptosis has been attributed to members of the caspase family (Alnemri *et al.,* 1996). The major executioners in the apoptotic program are proteases known as caspases (cysteine-dependent, aspartate-specific proteases (Friedlander, 2003). Upstream caspases are activated by the cell-death signal (e.g., TNF α) and have a long N-terminal prodomain that regulates their activation. These upstream caspases activate downstream caspases, which directly mediate the events leading to the demise of the cell. Downstream caspases have a short N-terminal prodomain (Hengartner, 2000). In caspase dependent cell death, caspase-8 activation is the most upstream event, and caspase-3 activation is the critical downstream event. Caspase-8 is prototypical initiator caspase of the extrinsic cell death pathway.

In the present study, we examined whether gene expression of pro-apoptotic Bax is altered or affected by hypoglycemia. Results have shown that hypoglycemia induces an up regulation in Bax and Caspase 8 expression in the brain regions, except cerebellum where caspase 8 has have shown a down regulation. The up regulated Bax expression is a marker of high apoptotic cell death in the brain regions. Increased TNF-α expression, observed in the neonatal hypoglycemic condition has contributed to the increased caspase-8 expression. Activation of the caspases represents a pivotal step in the cell death signaling cascade. The cell death in the brain regions in the early stage of development can drastically affect the memory and cognition in the later stages of life. Cerebellum have showed inhibition in the expression of caspase-8 expression, owing to its strong glucose homeostatic mechanisms (Scott *et al*., 2012). Hypoglycemia further up regulated the increased mRNA expression during glucose supplementation. In hypoglycemia, starvation of the cell takes place since there is deprivation of glucose. Up regulation of Bax and Caspase 8 mRNA and protein has been reported for CA1 neurons after global brain ischemia in rat (Campagne *et al*., 1998) and is associated with DNA damage (Levine *et al*., 1997).Thus in both hypoglycemia and glucose treated rats,, the cells are starved which is suggested to up regulate the pro - apoptotic protein expression.

The Bacoside A and *Bacopa monnieri* supplementation helps to overcome the high level of Bax expression. *Bacopa monnieri* contains the alkaloids herpestine, brahmine, bacosine, bacosterols, monnierin, bacoside -A and bacoside –B. Monnierin, on hydrolysis, gave glucose, arabinose and aglycone. Bacosides A and B give glucose, arabinose and bacogenines A, A2, A3 and A4 (Gohil & Patel, 2010). This prevents the rapid shift from hypoglycemia to hyperglycemia thus minimizing the neuronal death in the brain regions. Reduction in Bax expression suggests the anti apoptotic modulation of *Bacopa monnieri* and Bacoside A in reducing neonatal hypoglycemia related complications. Inhibition of caspase activation protects against neuronal loss in several animal models of brain diseases involving hypoxic ischaemia, brain trauma

and Parkinsons's disease (Schulz *et al.,* 1998, Depino *et al.,* 2003). Supplementation with antioxidants allows a delay in the appearance or in the development of neuronal complications (Hayoz *et al.,* 1998).

Thus our results have showed that neuronal damage inflicted by oxidative stress is one of the key mechanisms that results in severe neurological implications in neonatal hypoglycemia. This neurological damage is aggrevated during glucose supplementation, resulting in a rapid shift from hypo to hyperglycemia. The treatment programme we have put forth, suggesting *Bacopa monnieri* and Bacoside A alone and in combination with glucose, ameliorates the negative effects of oxidative stress with its neuroprotective and antioxidant ability. It therefore plays a neuroprotective role in the brain regions by maintaining glucose homeostasis and aiding in the dopamine functional recovery.

Summary

1. Insulin induced neonatal hypoglycemic rats were used as model to study the expression patterns of dopamine receptor subtypes DA D1 and DA D2, status of second messengers- cAMP and IP3, transcription factor - CREB, second messenger enzyme phospholipase- C, GLUT 3, neuronal survival factors - NFκB, GDNF and BDNF , antioxidant enzymes- GPx and SOD and apoptotic factors - Akt-1, TNF- α, Bax and caspase-8. Experiments were designed to study the neuroprotective role of *Bacopa monnieri* and Bacoside A in neonatal hypoglycemia management.

2. Antihypoglycemic activity of *Bacopa monnieri* and Bacoside A were evaluated by the measurement of blood glucose in experimental rats. Neonatal hypoglycemic rats showed decreased blood glucose level. Glucose, *Bacopa monnieri* and Bacoside A treatments to neonatal hypoglycemic rats significantly reversed the blood glucose level.

3. Dopamine D1 and D2 receptor binding studies were done in cerebral cortex, corpus striatum, cerebellum and brain stem of control and experimental rats.

4. Dopamine D1 and D2 receptor subtypes showed differential expression pattern. Dopamine D1 receptors were decreased in cerebral cortex, corpus striatum, cerebellum and brain stem in neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. The gene expression studies of dopamine D1 receptor showed a significant down regulation in cerebral cortex, corpus striatum, cerebellum and brain stem. *Bacopa monnieri* and

Bacoside A treatment reversed the altered expression of dopamine D1 receptor number and gene expression.

5. Dopamine D2 receptors were increased in cerebral cortex, corpus striatum, cerebellum and brain stem of neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. The gene expression studies of dopamine D2 receptors showed a significant up regulation in cerebral cortex, corpus striatum, cerebellum and brain stem of neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. *Bacopa monnieri* and Bacoside A treatment reversed the altered expression of dopamine D2 receptor number and gene expression.

6. cAMP contents were significantly decreased in cerebral cortex, corpus striatum, cerebellum and brain stem of neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. *Bacopa monnieri* and Bacoside A treatment reversed cAMP contents in neonatal hypoglycemic rats.

7. IP3 contents showed differential expression pattern. IP3 content were significantly increased in brain regions- corpus striatum and brain stem, whereas IP3 content were significantly decreased in cerebral cortex and cerebellum of neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. *Bacopa monnieri* and Bacoside A treatment reversed the IP3 content in cerebral cortex, corpus striatum, cerebellum and brain stem.

8. Second messenger enzyme - phospholipase C showed an increased expression in hypoglycemic brain regions - cerebral cortex, corpus striatum, cerebellum and brain stem. Treatment groups, *Bacopa monnieri* and Bacoside A showed significant reversal when compared with hypoglycemic group.

9. Gene expressions of CREB showed differential expression pattern. CREB expression was up regulated in cerebral cortex, corpus striatum and brain stem, whereas in cerebellum, CREB mRNA showed decreased expression in neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. *Bacopa monnieri* and Bacoside A treatment to neonatal hypoglycemic rats significantly reversed the differential expression pattern.

10. GLUT 3 mRNA expressions were studied in cerebral cortex, corpus striatum, cerebellum and brain stem. GLUT 3 mRNA showed differential expression pattern. GLUT 3 was up regulated in brain regions- corpus striatum and cerebellum, and down regulated in the cerebral cortex and brain stem of neonatal hypoglycemic rats compared to control. Glucose, *Bacopa monnieri* and Bacoside A treatment reversed the disrupted GLUT 3 gene expression in brain regions.

11. Akt-1 showed differential expression patterns in cerebral cortex, corpus striatum, cerebellum and brain stem. Akt-1 gene expression was up regulated in cerebral cortex and corpus striatum, whereas in cerebellum and brain stem it was seen to be down regulated in neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. *Bacopa monnieri* and Bacoside A treatment to neonatal hypoglycemic rats showed significant reversal in brain regions.

12. TNF- α mRNA showed differential expression patterns in cerebral cortex, corpus striatum, cerebellum and brain stem. TNF- α mRNA was decreased in cerebral cortex, corpus striatum and brain stem, whereas cerebellum showed

increased expression of TNF- α in neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. *Bacopa monnieri* and Bacoside A treatment to neonatal hypoglycemic rats showed significant reversal in brain regions.

13. Gene expression of neuronal survival factors - GDNF, BDNF and NFκB was down regulated in cerebral cortex, corpus striatum, cerebellum and brain stem of neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. Treatment groups *Bacopa monnieri* and Bacoside A showed significant reversal in GDNF, BDNF and NFκB expression.

14. Anti oxidant enzymes - GPx and SOD showed differential expression pattern in cerebral cortex, corpus striatum, cerebellum and brain stem of neonatal hypoglycemic rats and hypoglycemic rats treated with glucose. GPx gene expression was down regulated whereas SOD gene expression was up regulated in neonatal hypoglycemic group. Treatment groups, *Bacopa monnieri* and Bacoside A showed significant reversal in GPx and SOD gene expressions.

15. Apoptotic marker- Bax, gene expression in cerebral cortex, corpus striatum, cerebellum and brain stem was up regulated in hypoglycemic condition and hypoglycemic rats treated with glucose compared to control. *Bacopa monnieri* and Bacoside A treatment to neonatal hypoglycemic rats showed significant reversal in Bax gene expression.

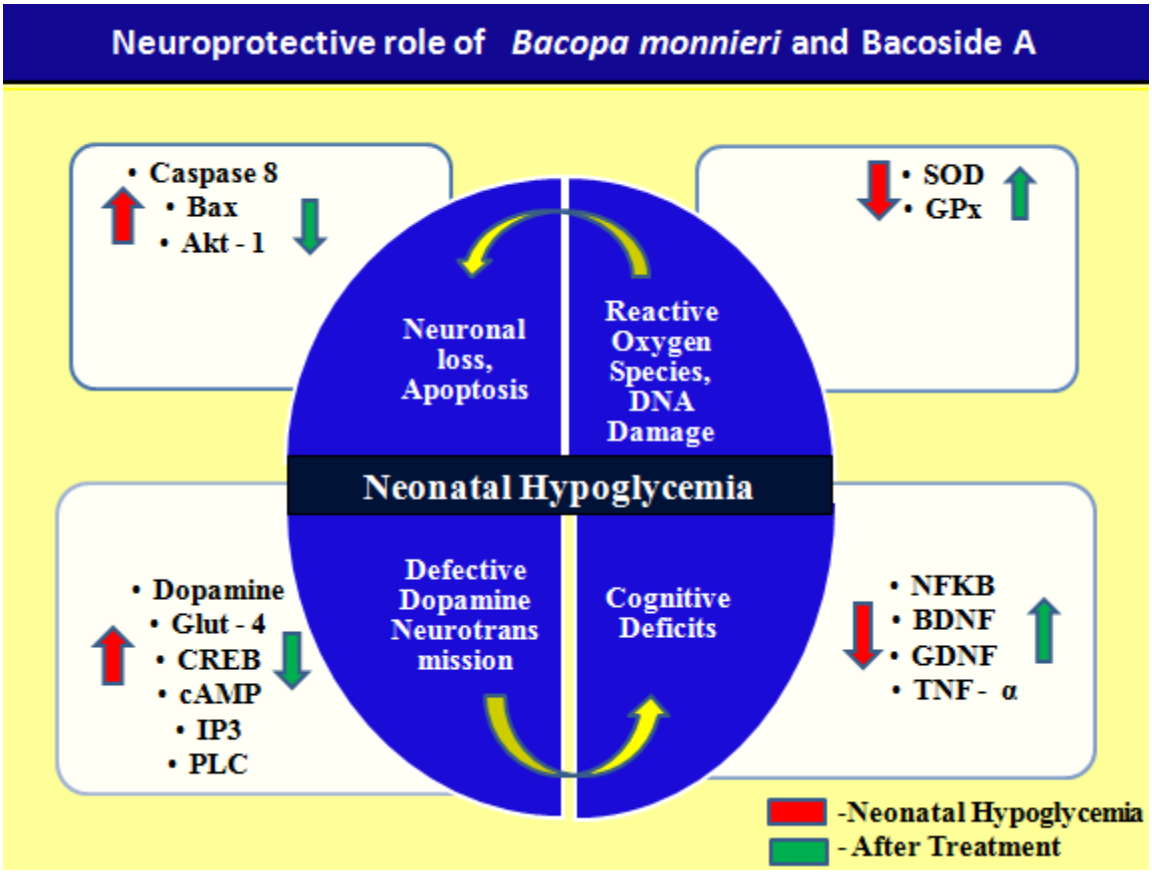
16. Caspase 8 showed differential expression pattern in cerebral cortex, corpus striatum, cerebellum and brain stem. Caspase 8 gene expression in cerebral

cortex, corpus striatum and brain stem was up regulated, whereas in the cerebellum it was down regulated in hypoglycemic condition and hypoglycemic rats treated with glucose compared to control. *Bacopa monnieri* and Bacoside A treatment to neonatal hypoglycemic rats showed significant reversal in caspase 8 gene expression.

In the present study, we summarize, that dopaminergic receptor subtypes showed differential expression pattern in brain regions and it has significant role in glucose metabolism. Glucose transporter- GLUT 3 was altered in hypoglycemic condition. Transcription factor- CREB, Phospholipase- C, second messengers, antioxidant enzymes and apoptotic factors alterations were seen in neonatal hypoglycemic rats. *Bacopa monnieri and* Bacoside A treatment decreased the expression of apoptotic factors and increased the expression of neuronal survival factors in neonatal hypoglycemic rats. The adverse effects of glucose infusion alone as a resuscitation method were also evident from the present study. Our results showed that glucose administration along with *Bacopa monnieri* and Bacoside A treatment as a resuscitation method will be of tremendous advantage in neonatal care. Thus the results suggest the therapeutic role of *Bacopa monnieri* and Bacoside A in ameliorating CNS dysfunctions, to overcome neonatal hypoglycemia and for a better intellect in the later stages of life.

Conclusion

Repetitive and profound hypoglycemia in newborn infants can result in irreversible brain damage and lead to severe neurologic sequelae and motor and developmental abnormalities. Our results showed imbalance in the expression pattern of dopaminergic receptor subtypes in neonatal hypoglycemia and its role in glucose regulation. Disrupted dopaminergic signalling and increased hypoglycemic stress in neonatal hypoglycemia contributed to the neuronal loss. Neuronal loss in neonatal hypoglycemic rats is mediated through the expression of pattern of second messengers- cAMP and IP3, transcription factor- CREB, second messenger enzyme- PLC and Glucose transporter- GLUT 3. Decrease in the expression of neuronal survival factors – NFκB, GDNF, BDNF and antioxidant enzymes – SOD, GPx and activation of apoptotic factors - Akt-1, TNF-α, Bax and caspase-8 clearly indicated the extent of brain damage inflicted by both hypoglycemia and glucose infusion induced hyperglycemia. We observed that the adverse effects of neonatal hypoglycemia are a result of oxidative stress in the brain tissue which inflicts irreversible tissue damage. In our study, we have suggested a resuscitation program using a neuroprotectant, *Bacopa monnieri* crude extract and its active component, Bacoside A. Treatment of neonatal hypoglycemic rats with *Bacopa monnieri* and Bacoside A significantly reversed the altered dopaminergic neurotransmission, second messenger signalling and activation of apoptotic factors. The present study confirmed the increased antioxidant protection offered by *Bacopa monnieri* and Bacoside A, there by decreasing neuronal damage and providing effective functional recovery of dopaminergic system. We have thus put forth a promising herbal therapy which ameliorates the risks for permanent neuronal damage and reduces the incidence of lifelong disabilities.


Neuroprotective role of *Bacopa monnieri* and Bacoside A
• Caspase 8
• Bax
• Akt - 1
• SOD
• GPx
Neuronal loss, Apoptosis
Reactive Oxygen Species, DNA Damage
Neonatal Hypoglycemia
Defective Dopamine Neurotrans mission
Cognitive Deficits
• Dopamine
• Glut - 4
• CREB
• cAMP
• IP3
• PLC
• NFKB
• BDNF
• GDNF
• TNF - α
-Neonatal Hypoglycemia
-After Treatment

Abe K, Hayashi T, Itoyama Y. (1997). Amelioration of brain edema by topical application of glial cell line-derived neurotrophic factor in reperfused rat brain. Neurosci. Lett., 231:37– 40.

Abramovitch R, Tavor E, Jacob-Hirsch J, Zeira E, Amariglio N, Pappo O, *et al.* (2004). A pivotal role of cyclic amp-responsive element binding protein in tumor progression. Cancer Res., 64: 1338–1346.

Alberts B, Lewis J, Raff M, Roberts K, Walter P. (2002). Molecular biology of the cell (4th ed.). New York: Garland Science., ISBN 0-8153-3218-1.

Alkalay AL, Sarnat HB, Sarnat LF, Simmons CF. (2005). Neurologic aspects of neonatal hypoglycaemia., IMAJ7:188-192

Alle H, Roth A, Geiger JR. (2009). Energy-efficient action potentials in hippocampal mossy fibers. Science., 325, 1405–1408.

Anbarasi K, Vani G, Balakrishna K, Devi CS. (2006). Effect of bacoside A on brain antioxidant status in cigarette smoke exposed rats. Life Sci., 78(12):1378-84.

Ang C, Howe D, Lumsden M, James DK, Steer PJ, Weiner CP, *et al*. (2005). Diabetes High Risk Pregnancy Management options, Philadelphia. Saunders: 986-1004.

Anju TR, Nandhu MS, Jes P, Paulose CS. (2010). Insulin and triiodothyronine regulation of neonatal hypoxia: Role of glucose, oxygen and epinephrine supplementation. Fetal and Pediatric Pathology. 30(5): 338-349.

Antonsson B, Montessuit S, Sanchez B, Martinou JC. (2001). Bax is present as a high molecular weight oligomer/complex in the mitochondrial membrane of apoptotic cells. J. Biol. Chem., 276: 11615–11623.

Antony S, Kumar TP, Mathew J, TR Anju , Paulose CS. (2010). Hypoglycemia induced changes in cholinergic receptor expression in the cerebellum of diabetic rats. J. Biomed. Sci.,17:7

Antony S, KT Peeyush, G Naijil, R. Chinthu, CS Paulose. (2010). Hypoglycemia induced behavioural deficit and decreased GABA receptor, CREB expression in the cerebellum of streptozoticin induced diabetic rats. Brain Res. Bull., 83(6): 360–366

Anu J, Peeyush Kumar T, Nandhu MS, Paulose CS. (2010). Enhanced NMDAR1, NMDA2B and mGlu5 receptors gene expression in the cerebellum of insulin induced hypoglycaemic and streptozotocin induced diabetic rats. Eur. J. Pharmacol., 630(1-3): 61-68.

Aral YZ, Gucuyener K, Atalay Y, Hasanoglu A, Turkyilmaz C, Sayal A, *et al*. (1998). Role of excitatory aminoacids in neonatal hypoglycaemia. Acta Paediatr. Jpn., 40: 303–306.

Arenas E, TruppM, Akerud P, Ibanez C. F.(1995). GDNF prevents degeneration and promotes the phenotype of brain noradrenergic neurons in vivo. Neuron., 15:1465-1473.

Arianna R, Silvia M, Alberto O, Andrea M. (2007). D1 and D2 Receptor Antagonist Injections in the Prefrontal Cortex Selectively Impair Spatial Learning in Mice. Neuropsychopharmacology., 32: 309-319.

Arie AL, Sarnat HB, Sarnat LF, Simmons CF. (2005). Neurologic aspects of neonatal hypoglycaemia. IMAJ., 7: 188-192.

Arnauld E, Arsaut J, Tafani JA, Demotes-Mainard J. (1995). Dopaminergic control of gene transcription during striatal ontogeny: c-fos induction by D1 receptor activation in the developing striosomes. Brain Res. Mol. Brain Res., 30:223–232.

Attwell D , Buchan A M , Charpak S , Lauritzen M, MacVicar BA, Newman EA. (2010). Glial and neuronal control of brain blood flow. Nature., 468, 232–243.

Auer RN, Siesjo BK (1988) Biological differences between ischemia, hypoglycemia, and epilepsy. Ann. Neurol., 24: 699–707

Auer RN, Siesjo BK. (1993). Hypoglycaemia: Brain neurochemistry and neuropathology. Bailliere's Clinical Endocrinology and Metabolism, 7(3): 611–625.

Auer RN. (1991). Excitotoxic mechanisms and age-related susceptibility to brain damage in ischemia, hypoglycemia and toxic mussel poisoning. Neurotoxicology., 12: 541–546.

Auer RN. (2004). Hypoglycemic brain damage. Metab. Brain Dis., 19(3–4):169–175

Babri S, Doosti MH, Salari AA. (2014). Tumor necrosis factor-alpha during neonatal brain development affects anxiety- and depression-related behaviors in adult male and female mice. Behav. Brain Res., 261: 305–314.

Baeuerle P, Henkel T. (1994). Function and activation of NF-kappa B in the immune system. Annu. Rev. Immunol., 12: 141-179.

Baldwin AS Jr. (1996). The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev. Immunol., 14: 649–683.

Bales JW, Kline AE, Wagner AK, Dixon CE. (2010). Targeting dopamine in acute traumatic brain injury. Open Drug Discov., 2: 119-128.

Baloh R H, Tansey M G, Lampe P A, Fahrner T J, Enomoto H, Simburger K S, *et al.(*1998). A Novel Member of the GDNF Ligand Family,Supports Peripheral and Central Neurons and Signals through the GFRa3–RET Receptor Complex. Neuron, Vol. 21: 1291–1302.

Bannon M J, Reinhard JF, Bunney B S, Roth RH. (1982). Nature (London)., 296: 444- 446.

Baptista T. (1999). Body weight gain induced by antipsychotic drugs: mechanisms and management. Acta Psychiatr. Scand., 100: 3–16.

Barbara JG. (2002). IP3-dependent calcium-induced calcium release mediates bidirectional calcium waves in neurones: functional implications for synaptic plasticity. Biochim. Biophys. Acta., 1600: 12–18.

Barbara JS, Robert MK, Behrman RE, Kliegman RM, Jenson HB. (2004). The Endocrine System. Nelson Text Book of Pediatrics., 17: 614-616.

Barco A, Kandel ER. (2006). The Role of CREB and CBP in Brain Function.Weinheim, Germany: Wiley-VCH Verlag., 207.

Barde YA, Edgar D, Thoenen H. (1982). Purification of a new neurotrophic factor from mammalian brain. EMBO J., 1: 549–553.

Barkett M, Gilmore TD. (1999). Oncogene., 18: 6910-6924.

Baynes JW. (1991). Perspectives in diabetes: role of oxidative stress in development of complications in diabetes. Diabetes., 40: 405-412.

Beaulieu J, Tirotta E, Sotnikova T D, Masri B, Salahpour A, Gainetdinov R R, *et al*.(2007). Regulation of Akt Signaling by D2 and D3 Dopamine Receptors *In Vivo*. J. Neurosci., 27(4):881– 885

Benoit SC, McQuade JA, Clegg DJ, Xu M, Rushing PA, Woods SC, *et al*. (2003). Altered feeding responses in mice with targeted disruption of the dopamine-3 receptor gene. Behav. Neurosci., 117(1): 46-54.

Bermanzohn PC, Siris SG, Akinesia. (1992). A syndrome common to parkinsonism, retarded depression, and negative symptoms of schizophrenia. Compr. Psychiatry., 33: 221-32.

Berridge MJ, Irvine RF. (1989). Inositol phosphates and cell signaling. Nature., 341:197–205.

Berridge MJ. (1993). Inositol trisphosphate and calcium signalling. Nature., 361: 315–325.

Berridge MJ. (1998). Neuronal calcium signaling. Neuron., 21(1): 13-26.

Berridge MJ. (2002). The endoplasmic reticulum: a multifunctional signalling organelle. Cell Calcium., 32(5-6): 235-49.

Biessels GJ, Kappelle AC, Bravenboer B, Erkelens DW, Gispen WH. (1994). Cerebral function in diabetes mellitus. Diabetologia., 37: 643–650.

Georgievska B, Kirik D, Bjo¨rklund A. (2004). Overexpression of Glial Cell Line- Derived Neurotrophic Factor Using a Lentiviral Vector Induces Time- and Dose-Dependent Downregulation of Tyrosine Hydroxylase in the Intact Nigrostriatal Dopamine System. J. Neurosci., 24(29):6437– 6445.

Blanca AF, Irene CP, Rigobelo E. (2011). Neonatal Hypoglycemia - Current Concepts, Hypoglycemia - Causes and Occurrences. ISBN: 978-953-307-657-7.

Bonnefont-Rousselot D. (2002). Glucose and reactive oxygen species. Curr. Opin. Clin. Nutr. Metab. Care., 5(5): 561-568.

Botchkina GI, Geimonen E, Bilof ML,Villarreal O, Tracey KJ. (1999). Loss of NF-cB Activity during Cerebral Ischemia and TNF Cytotoxicity. Mol. Med., 5(6): 372-381.

Brochu ME, Girard S, Lavoie K, Sébire A. (2011). Developmental regulation of the neuroinflammatory responses to LPS and/or hypoxia-ischemia between preterm and term neonates: An experimental study. J. Neuroinflammation. 8:55

Brunet A, Datta SR, Greenberg ME. (2001). Transcription-dependent and - independent control of neuronal survival by the PI3K-Akt signaling pathway. Curr. Opin. Neurobiol., 11: 297–305.

Buonocore G, Perrone S, Bracci R. (2001). Free radicals and brain damage in the newborn. Biol. Neonate., 79: 180 –186.

Burke RE, Macaya A, DeVivo D, Kenyon N, Janec EM. (1992). Neonatal hypoxic-ischemic or excitotoxic striatal injury results in a decreased adult number of substantia nigra neurons. Neuroscience., 50(3):559-69.

Burns CM, Rutherford MA, Boardman JP, Cowan FM. (2008). Patterns of cerebral injury and neuro developmental outcomes after symptomatic neonatal hypoglycemia. Pediatrics., 122: 65–74.

Byung CS, TD Kim, Kim KT. (2011). Pharmacological characterization of adenosine receptors in PGT-β mouse pineal gland tumour cells., 134(1): 132-142.

Cadet JL, Jayanthi S, McCoy MT, Beauvais G, Cai NS. (2010). Dopamine D1 receptors, regulation of gene expression in the brain, and neurodegeneration. CNS Neurol Disord Drug Targets., 9(5): 526–538.

Calabresi P, Centonze D, Pisani A, Bernardi G. (1997). A possible mechanism for the aglycemia-induced depression of glutamatergic excitation in the striatum. Journal of Cerebral Blood Flow and Metabolism., 17: 1121–1126

Calabresi P, Gubellini P , Centonze D, Picconi B, Bernardi G , Chergui K, *et al.* (2000). Dopamine and cAMP-regulated phosphoprotein 32 kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic plasticity. J. Neurosci., 20: (22) 8443–8451.

Callahan B, Yuan J, Stover G, Hatzidimitriou G, Ricaurte G. (1998). Effects of 2- deoxy-D-glucose on methamphetamine-induced dopamine and serotonin neurotoxicity.J. Neurochem.,70:190-7.

Campagne V, Lookeren M, Gill R. (1998). Increased expression of cyclin G1 and p21 in neurons following transient forebrain ischemia: Comparison with early DNA damage. J. Neurosci. Res., 53: 279–296.

Carlsson A. (1993). Thirty years of dopamine research. Adv. Neurol., 60:1-10.

Carruthers A, DeZutter J, Ganguly A, Devaskar SU. (2009). Will the original glucose transporter isoform please stand up!. Am. J. Physiol. Endocrinol. Metab., 297 (4) E836–E848.

Chandler LJ, Crews FT. (1990).Calcium-versus G protein-mediated phosphoinositide hydrolysis in rat cerebral cortical synaptoneurosomes. J. Neurochem., 55: 1022-1030.

Chao MV, Rajagopal R, Lee FS. (2006). Neurotrophinsignalling in health and disease. Clin. Sci. (Lond)., 110:167–173.

Chaverneff F, Barrett J. (2009). Casein kinase II contributes to the synergistic effects of BMP7 and BDNF on Smad 1/5/8 phosphorylation in septal neurons under hypoglycemic stress. J Neurochem., 109(3): 733–743.

Chawla A, Lavania AK. (2001). Oxygen toxicity. MJAFI., 57: 131–133.

Chen A, Xiong LJ, Tong Y, Mao M. (2013). The neuroprotective roles of BDNF in hypoxic ischemic brain injury. Biomedical Reports., 1: 167-176.

Chen G, Goeddel DV. (2002). TNF-R1 signaling: a beautiful pathway. Science., 296:1634 –1635.

Chen MJ, Russo-Neustadt AA. (2009). Running exercise-induced up-regulation of hippocampal brainderived neurotrophic factor is CREB-dependent. Hippocampus, 19(10): 962–972. Civelli O, Bunzow J. (1993). Molecular diversity of the dopamine receptor. Ann. Rev Pharmacol Toxicol., 32: 281-307.

Cheng B, Mattson MP. (1994). NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. Brain Res., 607:275–285.

Cheng Y, Deshmukh M, D'Costa A, Demaro J, Gidday J, Shah A, *et al.* (1998). Caspase inhibitor affords neuroprotection with delayed administration in a rat model of neonatal hypoxic-ischemic brain injury. J. Clin. Invest., 101: 1992–1999.

Chung CY, Seo H, Sonntag KC, Brooks A, Lin L, *et al*. (2005). Cell type-specific gene expression of midbrain dopaminergic neurons reveals molecules involved in their vulnerability and protection. Hum. Mol. Genet., 14: 1709–1725.

Cincotta AH, MacEachern TA, Meier AH. (1993). Bromocriptine redirects redirects metabolism and prevents seasonal onset of obese hyperinsulinemic state in Syrian hamsters. Am. J. Physiol., 264: E285–E293.

Cincotta AH, Meier AH. (1996). Bromocriptine (Ergoset) reduces body weight and improves glucose tolerance in obese subjects. Diabetes Care., 19: 667 – 670.

Citron BA, Arnold PM, Sebastian C, Qin F, Malladi S, Ameenuddin S, et al. (2000).Rapid upregulation of caspase-3 in rat spinal cord after injury: mRNA, protein, and cellular localization correlate with apoptotic cell death. Exp. Neurol., 166:213–226.

Clarkson ED, Zawada WM, Freed CR. (1997). GDNF improves survival and reduces apoptosis in human embryonic dopaminergic neurons in vitro. Cell Tissue Res., 289:207–210.

Clausen TD, Mortensen EL, Schmidt L, Mathiesen PR, Hansen T, Jensen DM, Damm P. (2013). Cognitive Function in Adult Offspring of Women with Gestational Diabetes. The Role of Glucose and Other Factors. DOI: 10.1371/journal.pone.0067107.

Cohen S. (1960). Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. Proc. Natl. Acad. Sci., 46: 302–311.

Connor B, Dragunow M. (1998).The role of neuronal growth factors in neurodegenerative disorders of the human brain. Brain Res Rev., Jun;27(1):1-39.

Cooper DM. (2003). Molecular and cellular requirements for the regulation of adenylatecyclases by calcium. Biochem. Soc. Trans., 31(5): 912-915.

Cornblath M, Joassin G, Weisskopf B, Swiatek KR. (1996). Hypoglycemia in the newborn. Pediatrics Clinic of North America., 13:905-930.

Cornblath M, Schwartz R (1976). Disorders of carbohydrate metabolism in infancy. Philadelphia., 155-217.

Corvol JC, Muriel MP, Valjent E, Fe´ger J, Nai¨maHanoun, Girault JA, *et al*. (2004). Persistent Increase in Olfactory Type G- Protein α Subunit Levels May Underlie D1 Receptor Functional Hypersensitivity in Parkinson Disease. J. Neurosci., 24(31):7007–7014

Cottet-Emard JM, Peyrin L. (1982). Conjugated HVA increase in rat urine after insulin-induced hypoglycemia: Involvement of central dopaminergic structures but not of adrenal medulla. J. Neural Transm.,55:121–138.

Dandona P, Chaudhuri A, Dhindsa S (2010). Proinflammatory and Prothrombotic Effects of Hypoglycemia. Diabetes Care., Vol. 33(7): 1686-1687.

Dasgupta A, Pal M, Ghosh T, Roy UK, Konar S, Biswas S, *et al*. (2013). Differential effects of insulin induced hypoglycaemia upon redox balance in distinct rat brain areas. Orient. Pharm. Exp. Med., 13: 279–287.

Datta SR, Brunet A, Greenberg ME. (1999) .Cellular survival: a play in three Akts. Genes Dev., 13:2905–2927.

de Courten-Myers GM, Xi G, Hwang JH, Dunn RS, Mills AS, Holland SK, Wagner KR, Myers RE. (2000). Journal of Cerebral Blood Flow & Metabolism., 20, 82–92.

Depino AM, Kaczmarczyk E, Ferrari C, *et al*. (2003). Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease.Eur. J. Neurosci., 18: 2731–2742.

Desouza CV, Bolli G B, Fonseca V (2010). Hypoglycemia, Diabetes, and Cardiovascular Events. Diabetes Care, Vol. 33(6): 1389- 194.

Dhananjaya CD, Kiran B. (2011). Clinical profile of hypoglycemia in newborn babies in a rural hospital setting. Int J Biol Med Res., 2(4): 1110 – 1114

Ding YM, Jaumotte JD, Signore AP, Zigmond MJ. (2004). Effects of 6-hydroxydopamine on primary cultures of substantianigra:specific damage to dopamine neurons and the impact of glial cell line-derived neurotrophic factor. J. Neurochem., 89, 776–787.

Dolga AM, Granic I, Blank T, Knaus HG, Spiess J, Luiten PG.et al. (2008). TNF-alpha-mediates neuroprotection against glutamate-induced excitotoxicity via NF-kappaB-dependent up-regulation of K2.2 channels. J. Neurochem., 107(4):1158- 1167.

Doya K. (1999). What are the computations of the cerebellum, the basal ganglia and the cerebral cortex? Neural Network, 12: 961–974.

Drabek T, Janata A, Wilson CD, Stezoski J, Feldman KJ, Tisherman SA, Foley LM, Verrier JD, Kochanek PM. (2014). Minocycline attenuates brain tissue levels of TNF-α produced by neurons after prolonged hypothermic cardiac arrest in rats. Resuscitation, 85(2): 284–291.

Du F, Wang L, Qian W, Liu S. Loss of enteric neurons accompanied by decreased expression of GDNF and PI3K/Akt pathway in diabetic rats

Duarte EP, Curcio M, Canzoniero LM, Duarte CB. (2012). Neuroprotection by GDNF in the ischemic brain. Growth Factors, 30(4): 242-257.

Dumaz N, Hayward R, Martin J, Ogilvie L, Hedley D, Curtin JA, Bastian BC, Springer C, Marais R. (2006). In Melanoma, RAS Mutations Are Accompanied by Switching Signaling from BRAF to CRAF and Disrupted Cyclic AMP Signaling. Cancer Res., 66: (19). 9483- 9491.

Dugan LL, Kim JS, Zhang Y, Bart RD, Sun Y, Holtzman DM, Gutmann DH. (1999). Differential effects of cAMP in neurons and astrocytes: Role of B-raf. J. Biol. Chem., 274: 25842–25848.

Dunwiddie TV, Masino SA. (2001). The role and regulation of adenosine in the central nervous system. Ann. Rev. of Neurosci., 24: 31–55.

Duronio V. (2008). The life of a cell: apoptosis regulation by the PI3K/PKB pathway. Biochem. J., 415: 333-44,

Dutta AK, Aggarwal A. (2000). Neonatal Hypoglycemia-Controversies. Paediatrics Today., 3:740-42.

E. Milusheva, M. Doda, E. Pasztor, A. Lajtha, H. Sershen, E. S. Vizi. (2006). Regulatory interactions among axon terminals affecting the release of different transmitters from rat striatal slices under hypoxic and hypoglycemic conditions. Journal of neurochemistry, DOI: 10.1111/j.1471-4159.1992.tb08335.

Eldadah BA, Faden AI (2000) Caspase pathways, neuronal apoptosis,and CNS injury. J Neurotrauma 17:811–829

Ertl AC, Davis SN. (2004). Evidence for a vicious cycle of exercise and hypoglycaemia in type 1 diabetes mellitus. *Diabetes Metab. Res. Rev*., 20: 124-1230.

Ferrara A. (2007). Increasing prevalence of gestational diabetes mellitus: a public health perspective. Diabetes Care., 302;S141-6.

Fischer HD, Wustmann C, Rudolph E, Yuv Z, YuS B, Schmidt J. (1989). The antihypoxic effect of ethymisole: a comparison with other nootropic drugs. Biomed Biochim Acta., 48: 843-847.

Foehr E D, Bohuslav J, Chen L, DeNoronha C, Geleziunas R, Lin X, O'Mahony A, Greene W C (2000). The NF- B-inducing kinase NIK induces PC12 cell differentiation and prevents apoptosis. J Bio Chem.,1-26

Fontaine V, Mohand-Said S, Hanoteau N, Fuchs C, Pfizenmaier K, Eisel U. (2002). Neurodegenerative and Neuroprotective Effects of Tumor Necrosis Factor (TNF) in Retinal Ischemia: Opposite Roles of TNF Receptor 1 and TNF Receptor 2. J. Neurosci., 22: RC216

Francis GJ, Martinez JA, Liu WQ, Xu K, Ayer A, Fine J, Tuor UI, Glazner G, Hanson LR, Frey WH, Toth C. (2008). Intranasal insulin prevents cognitive decline, cerebral atrophy and white matter changes in murine type I diabetic encephalopathy. Brain, 131:3311–3334.

Friedlander RM, (2003). Apoptosis and Caspases in Neurodegenerative Diseases. The New England journal of medicine., 348(14): 1365-1375.

Fukui T, Rahman M, Hayashi K, Takeda K, Higaki J, Sato T. (2003). Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial of cardiovascular events in high-risk hypertensive patients: rationale, design, and methods. Hypertens Res., 26(12): 979-90.

Garside S, Furtado JCS, Mazurek M. (1996). Dopamine–glutamate interactions in the striatum: behaviourally relevant modification of excitotoxicity by dopamine receptor-mediated mechanisms. Neuroscience., 75: 4,065–1074.

George SR, Roldan L, Haas DA. (1990). Adrenergic regulation of hypothalamic pro enkephalin neurons: evidence for opposite effects in subpopulations terminating in median eminence and neurointermediate pituitary. Neuroendocrinology., 52: 191-195.

Gerfen CR, Surmeier DJ. (2011). Modulation of striatal projection systems by dopamine. *Annu Rev Neurosci* .34:441–466.

Ghosh S, Hayden MS. (2008). New regulators of NF-kappaB in inflammation. Nat Rev Immunol. 8(11): 837-848.

Giesler GJ, Menetrey D, Basbaum AI. (1979). Differential origins of spinothlamic tract projections to medial and lateral thalamus in the rat. J Comp Neurol., 184: 107-126.

Gingrich JA, Marc GC. (1993). Recent advances in the molecular biology of dopamine receptors. Annu Rev Neurosci.,16: 299-321.

Giros B, Sokoloff P, Martres MP, Riou JF, Emorine LJ, Schwartz JC. (1990). Cloning of the human D3 dopaminergic receptor and chromosome identification. C R Acad Sci Paris Serie III., 311: 501-508.

Globus YT, Busto R, Dietrich WD, Martinez E, Valdes I,Ginsberg MD. (2006). Effect of Ischemia on the In Vivo Release of Striatal Dopamine, Glutamate, and γ-Aminobutyric Acid Studied by IntracerebralMicrodialysis. J.Neurochem., 51:5.1455–1464, 1988.

Glowinski J, Iversen LL. (1966). Regional studies of catecholamines in the rat brain, the disposition of [^{3}H] Norepinephrine, [^{3}H] DOPA in various regions of the brain. J. Neurochem, 13:655-669.

Gohil KJ, Patel JA. (2010). A review on Bacopa monniera: Current research and future prospects. Int. J. Green Pharm. 4: 1-9.

Gołembiowska K, Dziubina A. (2012). Effect of Adenosine A2A Receptor Antagonists and l-DOPA on Hydroxyl Radical, Glutamate and Dopamine in the Striatum of 6-OHDA-Treated Rats. Neurotoxicity Research., 21(2): 222

Götz R, Köster R, Winkler C, Raulf F, Lottspeich F, Schartl M, Thoenen H. (1994). Neurotrophin-6 is a new member of the nerve growth factor family. Nature., 372: 266–269.

Grandy DK, Gelernter J, Kennedy JL, Zhou QY, Civelli O, Pauls DL, Pakstis A, Kurlan R, Sunahara RK, Niznik HB. (1993). Exclusion of close linkage of Tourette's syndrome to D1 dopamine receptor. Am J Psychiatry., 3: 449-453.

Greene LA, Levy O, Malagelada C (2011). Akt as a victim, villain and potential hero in Parkinson's disease pathophysiology and treatment. Cell Mol Neurobiol., 31:969–978

Greengard P, Browning MD, McGuinness TL, Llinas R. (1987). Synapsin I, a phosphoprotein associated with synaptic vesicles: possible role in regulation of neurotransmitter release. Adv Exp Med Biol., 221: 135-153.

Grider MH, Park D, Spencer DM, Shine HD. (2009). Lipid raft-targeted Akt promotes axonal branching and growth cone expansion via mTOR and Rac1, respectively. J Neurosci Res., 87: 3033–3042.

Guo RB, Wang GF, Zhao AP, Gu J, Sun XL, Hu G. (2012). Paeoniflorin Protects against Ischemia-Induced Brain Damages in Rats via Inhibiting MAPKs/NF-kB-Mediated Inflammatory Responses. Plos One., 7(11): e49701.

Gupta P, Khatoon S, Tandon PK, Rai V. (2013). Effect of Cadmium on Growth, Bacoside A, and Bacopaside I of *Bacopa monnieri* (L.), a Memory Enhancing Herb. The Scien. World J., Doi 10.1155/2014/824586

Gurden H, Takita M, Jay TM. (2000). Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal prefrontal cortex synapses in vivo. J. Neurosci 106(20), 1–5.

Gusella JF. (1989). Location cloning strategy for characterizing genetic defects in Huntington's disease and Alzheimer's disease. FASEB., 3: 2036-2041.

Haas H.L, Selbach O. (2000). Functions of neuronal adenosine receptors. Naunyn Schmiedebergs Arch. Pharmacol., 362:375–381.

Haces ML, Montiel T, Massieu L. Selective vulnerability of brain regions to oxidative stress in a non–coma model of insulin–induced hypoglycemia. (2010). Neuroscience, 165: 28–38.

Hagberg H, Gressens P, Mallard C. (2012). Inflammation during Fetal and Neonatal Life: Implications for Neurologic and Neuropsychiatric Disease in Children and Adults. Ann Neurol., 71: 444–457.

Hanada M, Feng J, Hemmings BA. (2004). Structure, regulation and function of PKB/AKT–a major therapeutic target. Biochim. Biophys. Acta, 1697: 3-16.

Handa SS .(1998). Indian Herbal Pharmacopoeia., Vol. I, IDMA, Mumbai. 30 -37.

Hara T, Takamura A, Kishi C, Iemura S, Natsume T, Guan JL, Mizushima N. (2008). A ULK-interacting protein, is required for autophagosome formation in mammalian cells. J. Cell Biol., 181:497–510.

Hartman DS, Civelli O. (1997). Dopamine receptor diversity: molecular and pharmacological perspectives. Prog Drug Res., 48: 173–194.

Hawdon JM. (1999). Hypoglycaemia and the neonatal brain. Eur J Pediatr., 158 Supp l 1: S9–S12.

Haymond MW. (1989). Hypoglycemia in infants and children. Endocrinol Metab Clin North Am., 18:211–52.

Hayoz D, Ziegler T, Brunner HR, Ruiz J. (1998). Diabetes mellitus and vascular lesions. Metabolism, 47(12): 16-9.

Heaton MB, Marsiglio KS, Madorsky V. (2013). Ethanol Influences on Bax Associations with Mitochondrial Membrane Proteins in Neonatal Rat Cerebellum. Dev Neurobiol., 73(2): 127–141.

Hemmings H, Greengrade P. (1986). DARPP-32, a dopamine and 3'-5' monophosphate-regulated phospho protein: regional, tissue and phylogenetic distribution. J Neurosci., 6: 1469-1481.

Hengartner MO. (2000). The biochemistry of apoptosis. Nature, 407:770-6.

Hernandez-Lopez S, Tkatch T, Perez-Garci E, Galarraga E, Bargas J, Hamm H, Surmeier DJ (2000) D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca^{2+} currents and excitability *via* a novel PLC [beta] 1-IP3-calcineurin-signaling cascade. J Neurosci 20: (24) 8987–8995.

Hirvonen J, Theo GM, Van E, Huttunen J, Aalto S, Någren K, Huttunen M, Lönnqvist J, Kaprio J, Hietala J, Cannon TD. (2005). Increased Caudate Dopamine D2 Receptor Availability as a Genetic Marker for Schizophrenia. Arch Gen Psychiatry., 62,371-378.

Hu YL, Baud V, Delhase M, Zhang PL, Deerinck T, Ellisman M, Johnson R, Karin M (1999). Abnormal morphogenesis but intact IKK activation in mice lacking the IKKa subunit of IkB kinase. Science, 284:316–320

Huang EJ, Reichardt LF. (2003). Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem., 72: 609-642.

Hypoglycemic Brain Injury: Potentiation From Respiratory Depression and Injury Aggravation From Hyperglycemic Treatment Overshoots

I.A. Simpson, D. Dwyer, D.Malide, K.H.Moley, A. Travis, S.J. Vannucci. (2008). The facilitative glucose transporter GLUT3: 20 years of distinction, Am. J. Physiol. Endocrinol. Metab. 295 (2) E242–E253.

Ibáñez CF, Ernfors P, Timmusk T, Ip NY, Arenas E, Yancopoulos GD, Persson H. (1993). Neurotrophin-4 is atarget-derived neurotrophic factor for neurons of the trigeminal ganglion. Development., 117: 1345–1353.

Jackson DM, Westlind DA. (1994). Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmacol Ther., 64: 291-370.

Jang HJ, Yang YR , Kim JK, Choi JH, Seo YK , Lee YH, Lee JE, Ryu SH, Suh PG. (2013). Phospholipase C-γ1 involved in brain disorders. Adv. in Bio. Reg., 53: 51–62.

Jean-Martin Beaulieu , Raul R. Gainetdinov. (2011). The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev., 63: 182–217.

Jobin M, Jes P, Nandhu MS, Paulose CS. (2010). Bacopa monnieri and Bacoside-A for ameliorating epilepsy associated behavioral deficits. Fitoterapia., 8(1): 5315-322.

Jones PF, Jakubowicz T, Pitossi FJ, Maurer F, Hemmings BA. (1991). Molecular cloning and identification of a serine/threonine protein kinase of the secondmessenger subfamily. Proc Natl Acad Sci U S A, 88:4171–7175.

Jones RAK, Roberton NRC (1984). Problems of the small-fordates baby. Clin Obstet Gynecol., 11: 499-524.

Joseph A, Remya R, Paulose CS. (2007). Enhanced [3H] Glutamate Binding in the Cerebellum of Insulin Induced Hypoglycaemic and Streptozotocin Induced Diabetic Rats. Cellular and Molecular Neurobiology, 27: 1085–1095.

Joseph A, Sherin B, Paulose CS. (2008). Increased glutamate receptor gene expression in the cerebral cortex of insulin induced hypoglycaemic and streptozotocin induced diabetic rats. Neuroscience, 156: 298–304.

Junqing W, Junhui W, Zhaoming Z. (2005) Oxidative Stress in Mouse Brain Exposed to Lead. Ann. Occup. Hyg., 50: (4)405–409.

Kadekaro M, Ito M, Gross PM. (1988). Local cerebral glucose utilization is increased in acutely adrenalectomized rats. Neuroendocrinology., 47: 329–334.

Kalhan S, Parimi P. (2000). Gluconeogenesis in the fetus and neonate. Sem Perinatol., 24:94–106.

Kalhan SC, Oliven A, King KC, Lucero C (1986) Role of glucose in the regulation of endogenous glucose production in the human newborn. Pediatr Res 20: 49-51

Kanduc D, Mittelman A, Serpico, R. (2002). Cell death: apoptosis versus necrosis. Int J Oncol., 21: 165-170.

Kaufman FR, Epport K, Engilman R, Halvorson M. (1999). Neurocognitive functioning in children diagnosed with diabetes before age 10 years. J Diabetes Complications 13: 31–38.

Kim AN, Jeremy KS, Heather TD. (2004). Dopamine receptor signaling. Journal of Receptors and Signal Transduction. 24(31): 65–205.

Kim SH, Won SJ, Sohn S. (2002). Brain-derived neurotrophic factor can act as a pronecrotic factor through transcriptional and translational activation of NADPH oxidase. J Cell BioL., 159: 821-831.

Kiritikar K R, Basu BD. (1994). Indian Medicinal Plants. Vol. I, 1816-1817.

Kitagawa H, Sasaki C, Sakai K, Mori A, Mitsumoto Y, Mori T, Fukuchi Y, Setoguchi Y, Abe K. (1999). Adenovirus-mediated gene transfer of glial cell line-derived neurotrophic factor prevents ischemic brain injury after transient middle cerebral artery occlusion in rats. J. Cereb Blood Flow Metab., 19:1336 –1344.

Klawitter V. (2006). Plasticity of the central nervous system (CNS) following perinatal asphyxia: Does nicotinamide provide neuroprotection? Amino Acids., 31(4): p. 377-84.

Kleigman RM. Problems In Metabolic Adaptation: Glucose, Calcium and Magnesium. In: Care Of The High Risk Neonate. Klaus MH & Faranoff AA. 5th ed. Philadelphia : Saunders, 2000; 304-309.

Krieglstein K, Suter-Crazzolara C, Fischer WH, Unsicker K (1995) TGF-beta superfamily members promote survival of midbrain dopaminergic neurons and protect them against MPP1 toxicity. EMBO J 14:736 –742.

Kuida K. et al. (1996) .Nature., 384:368.

Kuida K. et al. (1998). Cell., 94:325.

Kunte KB, Kuna Y. (2013). Neuroprotective effect of Bacopa monniera on memory deficits and ATPase system in Alzheimer's disease (AD) induced mice. J Scien and Inn Res., 2 (4): 719-735

Lai KO, Fu WY, Ip FC, Ip NY. (1998). Cloning and expression of a novel neurotrophin, NT-7, from carp. Mol. Cell Neurosci.,11: 64–76.

Lauren S. (2005). Opening Skinner's Box: Great Psychological Experiments of the Twentieth Century. New York: W. W. Norton & Company: 86-90.

Lee FJ, Xue S, Pei L, Vukusic B, Che´ry N, Wang Y, Wang YT, Niznik HB, Yu XM, Liu F. (2002). Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell., 111: 219 –230.

Leroux-Nicollet I, Darchen F, Scherman D, Costentin J. (1990). Postnatal development of the monoamine vesicular transporter in mesencephalic and telencephalic regions of the rat brain: a quantitative autoradiographic study with [^{3}H]dihydrotetrabenazine. Neurosci Lett.,117: 1–7.

Lesoualc'h F, Sagara Y, Holsboer F, Behl C. (1998). High constitutive NF-kappa B activity mediates resistance to oxidative stress in neuronal cells. J. Neurosci., 18(9): 3224-3232

Lévesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, Schott D, Morgat JL, Schwartz JC, Sokoloff P. (1992). Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-N,N-di-n-propyl-2-aminotetralin. Proc. Natl. Acad. Sci. USA., 89: 8155-8159.

Levi-Montalcini, R. (1966). The nerve growth factor: Its mode of action on sensory and sympathetic nerve cells. Harvey Lect., 60: 217–259.

Levine AJ. (1997). p53, the cellular gatekeeper for growth and division. Cell, 88: 323–331.

Lieberman AP, Pitha PM, Shin HS, Shin ML. (1989). Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. Proc. Natl. Acad. Sci. USA, 86: 6348–6352.

Little AR, O'Callagha JP. (2001). Astrogliosis in the adult and developing CNS: is there a role for proinflammatory cytokines. Neurotoxicology, 5:607-618.

Liu K, Ye XJ , Hu WY, Zhang GY, Bai GH , Zhao LC, He JW, Zhu JW, Shao JB , Yan ZH ,Gao HC. (2013). Neurochemical Changes in the Rat Occipital Cortex and Hippocampus after Repetitive and Profound Hypoglycemia During the Neonatal Period: An Ex Vivo 1H Magnetic Resonance Spectroscopy Study. Mol. Neurobiol.,48:729–736

Lowry OH, Roserbrough NJ, Farr AL, Randall RJ. (1951). Protein measurements and folin phenol reagent. J. Biol. Chem., 193: 265-275.

Lund-Anderen H. (1979). Transport of glucose from blood to brain. Physiol. Rev, 59: 305-310.

M.O. Carayannopoulos, Xiong F, Jensena P, Rios-Galdamez Y, Huang H, Lin S, Devaskar SU. (2014). GLUT3 gene expression is critical for embryonic growth, brain development and survival. Mol. Genet. Metab., doi:10.1016/j.ymgme.2014.01.013

Majumdar S, Basu A, Paul P, Halder M, Jha S (2013). Bacosides and Neuroprotection. Nat Products, 3639-3660.

Mantamadiotis T, Lemberger T, Bleckmann SC, Kern H, Kretz O, Villalba AM, Tronche F, Kellendonk C, Gau D, Kapfhammer J, Otto C, Schmid W, Schütz G. (2002). Disruption of CREB function in brain leads to neurodegeneration. Nature Genetics, 31: 47-54.

Marc RE, Murry RF, Fisher SK, Linberg KA, Lewis GP. (1998). Amino acid signatures in the detached cat retina. Invest. Ophthalmol. Vis. Sci., 39: 1694-1702.

Maritim AC, Sanders RA, Watkins JB. (2003). Diabetes, Oxidative stress and antioxidants: A review. J. Biochem. Mol. Toxicol., 17: 24-38.

Mark LP, Prost RW, Ulmer JL, Smith MM, Daniels DL, Strottmann JM, Brown WD, Bey LH. (2011). Pictorial Review of Glutamate Excitotoxicity: Fundamental Concepts for Neuroimaging. Am. J. Neuroradiology., 22:1813–1824.

Martin LJ. (2001). Neuronal cell death in nervous system development, disease, and injury. Int. J. Mol. Med.,7: 455-478.

Mattson M P, Maudsley S, Martin B (2004). BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci., 27(10): 589–594

Mattson M, LaFerla F, Chan S, Leissring M, Shepel P, Geiger J. (2000). Calcium signaling in the ER: its role in neuronal plasticity and neurodegenerative disorders. Trends Neurosci., 23: 222–229.

Mattson MP, Barger SW, Furukawa K, Bruce AJ, Wyss-Coray T, Mark RJ, Mucke L (1997) Cellular signaling roles of TGF beta, TNF alpha and beta APP in brain injury responses and alzheimer's disease. Brain Res. Brain Res. Rev., 23:47–61.

McCall AL, Millington WR, Wurtman RJ. (1982).Metabolic fuel and amino acid transport into the brain in experimental diabetes mellitus. Proc. Natl. Acad. Sci. USA, 79(17): 5406–5410.

McConnell SK (1991). The generation of neuronal diversity in the central nervous system. Annu. Rev. Neurosci., 14: 269-300.

McGowan JE, Zanelli SA, Haynes-Laing AG, Mishra OP, Delivoria-Papadopoulos M. (2002). Modification of glutamate binding sites in newborn brain during hypoglycemia. Brain Res., 92:780–86.

McGowan JE. (1999). Neonatal hypoglycemia. NeoReviews. e6-e15.

Meinkoth JL. (1993). Signal transduction through the cAMP-dependent protein kinase. Mol. Cell Biochem., 127–128:179–186.

Messer CJ, Son JH, Joh TH, Beck KD, Nestler EJ. (1999). Regulation of tyrosine hydroxylase gene transcription in ventral midbrain by glial cell line-derived neurotrophic factor. Synapse, 34:241–243.

Michel C, Raes M, Toussaint O, Remacle. (1994). J. Free Radic. Biol. Med., 17:235–248.

Miñano A, Caballero-Benítez A, Lluch M, Morán J, Rodríguez-Alvarez J. (2008). C2-ceramide mediates cerebellar granule cells apoptosis by activation of caspases-2, -9, and -3. J. Neurosci. Res.,86(8):1734-47.

Mioduszewska B, Jaworski J, Kaczmarek L. (2003). Inducible cAMP early repressor (ICER) in the nervous system: a transcriptional regulator of neuronal plasticity and programmed cell death. J. Neurochem., 87: 1313–1320.

Mishra PK , Sharma B.(1977). Hypoglycemia in Newborns-A Prospective Study. Indian Pe; 14: 129-132.

Missale C, Nash SR, Robinson SW, Jaber MC.(1998). Dopamine receptors: from structure to function. Physiol Rev., 78:(1)189–225.

Mitanchez D. (2008). Ontogenesis of glucose regulation in neonate and consequences in neonatal management. Archives de Pediatriecs 15: 164-74.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. (2000). Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. J Neurosci, 20: 1568-74

Mizuno K, Carhahan J, Nawa H. (1994). Brain-derived neurotrophic factor promotes differentiation of striatal GABAergic neurons. Dev. Biol, 165: 243–256.

Moley KH, Mueckler MM. (2000). Glucose transport and apoptosis. Apoptosis.,5: 99–105.

Money KM, Stanwood GD. (2013). Developmental origins of brain disorders: roles for dopamine. Front. Cell Neurosci.,7(260): 1-14.

Monkawa T, Miyawaki A, Sugiyama T, Yoneshima H, Yamamoto-Hino M, Furuichi T, Saruta T, Hasegawa M, Mikoshiba K. (1995). Heterotetrameric complex formation of inositol 1,4,5-trisphosphate receptor subunits. J. Biol. Chem., 270: 14700-14704.

Monsma F, Mahan L, McVittie L, Gerfen C, Sibley D. (1990). Molecular cloning and expression of a D1 dopamine receptor linked to adenylyl cyclase activation. Proc. Natl. Acad. Sci., 87: 6723-6727.

Moura MB, Santos LSD, Houten BV. (2010). Mitochondrial dysfunction in neurodegenerative diseases and cancer. Env and Mol Mutagenesis., 51 (5):391–405.

Munoz-Fernandez MA, Fresno M. (1998).The role of tumour necrosis factor, interleukin 6, interferon-gamma and inducible nitric oxide synthase in the development and pathology of the nervous system. Prog. Neurobiol, 56: 307–340.

Nakamura T, Sakamoto K. (2001). Reactive oxygen species up-regulates cyclooxygenase-2, p53 and Bax mRNA expression in bovine luteal cells. Biochem. Biophy. Res. Comm. 284: 203–210.

Nandhu MS, Paul J, Kuruvilla KP, Malat A, Romeo C, Paulose CS. (2011). Enhanced glutamate, IP3 and cAMP activity in the cerebral cortex of Unilateral 6-hydroxydopamine induced Parkinson's rats: Effect of 5-HT, GABA and bone marrow cell supplementation. J. Biomed. Sci., 18:5

Nasrat HA, Salleh M, Ardawi M, Ghafouri H. (1993). Outcome of pregnancy in diabetic mothers. Int.J. Gynecol. Obstet., 43:29-34.

Nathanson JA. (1977). Cyclic nucleotides and nervous system function. Physiol. Rev., 57(2): 157–256.

Nicole O, Ali C, Docagne F, Plawinski L, MacKenzie E T, Vivien D, Buisson A (2001). Neuroprotection Mediated by Glial Cell Line-Derived Neurotrophic Factor: Involvement of a Reduction of NMDA-Induced Calcium Influx by the Mitogen-Activated Protein Kinase Pathway. J. Neurosci., 21(9):3024–3033

Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature, 404:787–790.

Nogueira CR, Machado UF, Curi R, Carpinelli AR. (1994). Modulation of insulin secretion and 45Ca2+ efflux by dopamine in glucose-stimulated pancreatic islets. Gen. Pharmacol., 25: 909-16.

O'Neil LAJ, Kaltschmidt C. (1997) NF-κB: a crucial transcription factor for glial and neuronal cell function. Trends Neurosci., 20:252–258.

O'dowd BF. (1993). Structure of dopamine receptors. J. Neurochem., 60: 804-814.

Oliver FJ, Menissier-de Murcia J, Nacci C, Decker P, Andriantsitohaina R, *et al*. (1999) Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly (ADP-ribose) polymerase-1 deficient mice. EMBO J., 18: 4446–4454.

Otani S, Blond O, Desce JM, Crepel F. (1998). Dopamine facilitates long-term depression of glutamatergic transmission in rat prefrontal cortex. Neuroscience, 85: 669–676.

Ozben T. (1998). Mechanisms involved in neuronal damage. In: Free radicals, oxidative stress and antioxidants, NATO ASI series, Plenum Press, New York, London, 163–189.

Pal R, Dwivedi AK, Singh S,Kulshrestha DK. (1998). High performance liquid chromatographic (HPLC) quantification of bacosides in Bacopa monnieri extracts. Indian J. Pharm. Sci., 60: 328-329.

Park KM, Bowers WJ. (2010). Tumor necrosis factor-alpha mediated signaling in neuronal homeostasis and dysfunction. Cellular signalling., 22(7):977-83.

Paul ML, Graybiel AM, David JC, Robertson HA. (1992). D1-like and D2-like dopamine receptors synergistically activate rotation and c-fos expression in the dopamine-depleted striatum in a rat model of Parkinson's disease. J. Neurosci., 12(10):3729-42.

Paulose CS, Chathu F, Khan RS, Krishnakumar A. (2008). Neuroprotective role of Bacopa monnieri extract in epilepsy and effect of glucose supplementation during hypoxia: glutamate receptor gene expression. Neurochem. Res., 33: 1663-1671.

Phillips HS, Hains JM, Laramee GR, Rosenthal A, Winslow JW. (1990). Widespread expression of BDNF but not NT3 by target areas of basal forebrain cholinergic neurons. Science., 250: 290–294.

Piette J, Piret B, Bonizzi G, Schoonbroodt S, Merville MP, Legrand-Poels S, Bours V. (1997). Multiple redox regulation in NF-kappaB transcription factor activation. Biol. Chem., 378:1237–1245.

Plantje JE Steinbush HWM, Schipper J, Dijcks FA, Verheijden PFHM, Stoof JC. (1987). D-2 dopamine-receptors regulate the release of [3H] dopamine in rat cortical regions showing dopamine immunoreac- tive fibers. Neurosci., 20: 157-l 68.

Puente EC, Silverstein J, Fisher SJ. (2010). Recurrent Moderate Hypoglycemia Ameliorates Brain Damage and Cognitive Dysfunction Induced by Severe Hypoglycemia. Diabetes., 59 (4): 1055 –1062.

Qin S, Stadtman ER, Chock PB. (2000). Regulation of oxidative stress-induced calcium release by phosphatidylinositol 3-kinase and Bruton's tyrosine kinase in B cells. PNAS., 97(13): 7118–7123

Ramachandran A, Snehalatha C, Shyamala P, Vijay V, Viswanathan M. (1994). Prevalence of diabetes in pregnant women—a study from southern India. Diabetes Res. Clin. Pract., 25:71-74.

Read D E, Gorman A M. (2009). Involvement of Akt in neurite outgrowth. Cell. Mol. Life Sci., 66:2975–2984.

Reagan LP, Magariños AM, Lucas LR, van Bueren A, McCall AL, McEwen BS. (1999). Regulation of GLUT-3 glucose transporter in the hippocampus of diabetic rats subjected to stress. Am. J. Physiol., 276: E879-886.

Richfield EK, Penney JB, Young AB. (1989). Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience , 30:767–777.

Ritter S, Dinh TT, Li AJ. (2006). Hindbrain catecholamine neurons control multiple glucoregulatory responses. Physiol Behav., 89(4):490-500.

Roberta MK, Peter LS. (2003). Cerebellar Loops with Motor Cortex and Prefrontal Cortex of a Nonhuman Primate. J. Neurosci., 23: 8432–8444.

Robertson GP. (2005). Functional and therapeutic significance of AKT deregulation in malignant melanoma. Cancer and metastasis reviews, 24: 273-285.

Rodrigo J, Fernández–Vizarra P, Castro–Blanco S, Bentura ML, Nieto M, Gómez– Isla T, Martínez–Murillo R, MartInez A, Serrano J, Fernández AP. (2004). Nitric oxide in the cerebral cortex of amyloid precursor protein (SW) Tg2576 transgenic mice. Neuroscience, 128: 73–89.

Rössger K, Charpin-El GH, Fusseneggera M. (2013). Reward-based hypertension control by a synthetic brain–dopamine interface. PNAS, 110(45): 18150–18155.

Routh VH. (2002). Glucose-sensing neurons: Are they physiologically relevant?. Physiology & Behavior, 76(3): 403-413.

Roux PP, Barker PA. (2002). Neurotrophin signaling through the p75 neurotrophin receptor. Prog. Neurobiol., 67:203–233.

Rovet JF, Ehrlich RM.(1996). The effect of hypoglycemic seizures on cognitive function in children with diabetes: a 7-year prospective study. J. Pediatr., 134: 503–506, 1999.

Rozance PJ, William WH. (2012). Neonatal hypoglycemia—answers, but more questions. J. Pediatr. . 161(5): 775–776.

Rudloff E, Franklin RK. (2009). Hypoglycemia. Standards of care: emergency and critical care medicine, 11: 7-11.

Russo A, Izzo AA, Borrelli F, Renis M, Vanella A. (2003). Free radical scavenging capacity and protective effect of Bacopa monnieri L. on DNA damage. Phytot Res., 17: 46–54.

Ryan C, Vega A, Drash A (1985) Cognitive deficits in adolescents who developed diabetes early in life. Pediatrics, 75921–927.

Sailer CF, Chiodo LA. (1980). Glucose Supresses basal firing and haloperidol – induced Increases in the firing rate of Central dopaminergic neurons. Science, 210: 1269-1271.

Sairam K, Rao CV, Babu MD, Goel RK. (2001). Prophylactic and curative effects of Bacopa monniera in gastric ulcer models. Phytomed., 84: 23-30.

Sanders NM, Taborsky GJ, Figlewicz DP. (2007). Antecedent Hindbrain Glucoprivation Does Not Impair the Counterregulatory Response to Hypoglycemia. Diabetes., 56(1): 217-223.

Sann L, Morel Y, Lasne Y. (1983). Effects of hydrocortisone on intravenous glucose tolerance in small-for-gestational-age infants. Helv. Paediatr. Acta., 38: 475-482.

Sariola H, Saarma M (2003). Novel functions and signalling pathways for GDNF. J. Cell Sci., 116: 3855-3862

Sarnat HB. (2004). Watershed infarcts of the fetal and neonatal brain stem. An aetiology of the central hypoventilation, dysphagia, Mobius syndrome and micrognathia. Eur.Pediar.Neurol., 8:71-87.

Sasidharan CK, Gokul E, Sabitha S. (2004). Incidence and risk factors for neonatal hypoglycaemia in Kerala, India. Ceylon Med. J.;49(4):110-3.

Scatchard G. (1949). The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci., 51: 660–672.

Scheen AJ. (2010). Central nervous system: a conductor orchestrating metabolic regulations harmed by both hyperglycaemia and hypoglycaemia. Diabetes Metab., 3: S31-38.

Scheid MP, Woodgett JR. (2003). Unravelling the activation mechanisms of protein kinase B/AKT. FEBS Lett., 546: 108-112.

Schmahmann JD, David Caplan. (2006). Cognition, emotion and the cerebellum. Brain, 129: 290–292.

Schultz W. (2000). Multiple reward signals in the brain. Nat. Rev. Neurosci, 1:199–207.

Schulz JB, Weller M, Matthews RT et al. (1998). Extended therapeutic window for caspase inhibition and synergy with MK-801 in the treatment of cerebral histotoxic hypoxia. Cell Death Differ., 5: 847–857.

Schutz PW, Wong PKH, O'Kusky J, Innis SM, Stockler S. (2011). Effects of d-3-hydroxybutyrate treatment on hypoglycemic coma in rat pups. Exp. Neurol., 227(1):180–187.

Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D Jr. (1992). Insulin in the brain: a hormonal regulator of energy balance. Endocr. Rev., 13: 387-414.

Scott MM, Perello M, Chuang JC, Sakata I, Gautron L, Lee CE, Lauzon D, Elmquist J K, Zigman JM. (2012). Hindbrain Ghrelin Receptor Signaling Is Sufficient to Maintain Fasting Glucose. 7(8): e44089.

Seeman P. (1980). Brain dopamine receptors. Pharmacol. Rev., 32: 229-313.

Seshiah V, Balaji V, Balaji MS, Paneerselvam A, Arthi T, Thamizharasi M. (2008). Prevalence of gestational diabetes mellitus in South India (Tamil Nadu) – A community based study. J. Assoc Physicians India. 56:329-33.

Shohami E, Ginis I, Hallenbeck JM. (1999). Dual role of tumor necrosis factor alpha in brain injury. Cytokine Growth Factor Rev., 10(2): 119-30.

Sibley D, Monsama F, Shen Y. (1993). Molecular neurobiology of dopaminergic receptors. Intl. Rev. Neurobiol., 35: 391-415.

Siegel GJ, Chauhan NB. (2000). Neurotrophic factors in Alzheimer's and Parkinson's disease brain. Brain Res. Rev., 33:199–227

Silva AJ, Jeffrey HK, Paul WF, Satoshi K. (1998). Creb and Memory. Annu. Rev. Neurosci, 21: 127–148.

Simpson BJB, Ramage AD, MJ Hulme, Burns DJ, Katsaros D, Langdon SP, Miller WR. (1996). Cyclic adenosine 3', 5'-monophosphate-binding proteins in human ovarian cancer: Correlations with clinicopathological features. Clin. Cancer Res., 2: 201-206.

Simpson I A, Chundu K R, Davies-Hill T, Honer W G, Davies P(1994). Decreased concentrations of GLUT1 and GLUT3 glucose transporters in the brains of patients with Alzheimer's disease. Ann of Neuro, Vol. 35(5): 546–551.

Singh V, Carman M, Roeper J, Bonci A. (2007). Brief ischemia causes long-term depression in midbrain dopamine neurons. Eur. J. Neurosci., 26(6):1489-99.

Small DM, Jones-Gotman M, Dagher A. (2003). Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. NeuroImage., 19:1709–1715.

Sokoloff L. (1999). Energetics of functional activation in neural tissues. Neurochem Res., 24(2):321–329.

Soler RM, Dolcet X, Encinas M, Egea J, Bayascas JR, Comella JX. (1999). Receptors of the glial cell line-derived neurotrophic factor family of neurotrophic factors signal cell survival through the phosphatidylinositol 3-kinase pathway in spinal cord motoneurons. J. Neurosci., 19:9160 – 9169.

Springer JE, Azbill RD, Knapp PE. (1999). Activation of the caspase-3 apoptotic cascade in traumatic spinal cord injury. Nat. Med., 5:943–946.

Steriade M. (1996). Arousal: revisiting the reticular activating system. Science, 272: 225-6.

Straussman, Sharon, Levitsky, Lynne L. (2010). Neonatal hypoglycemia. Current Opinion in Endocrinology, Diabetes & Obesity, 17(1): 20–24.

Stuart CA , Ross IR, Howell MEA, McCurry MP, Wood TG, Ceci JD, Kennel SJ, Wall J. (2011). Brain glucose transporter (Glut3) haploinsufficiency does not impair mouse brain glucose uptake. Brain Res., 1384: 15–22.

Stutzmann GE, LaFerla FM, Parker I. (2003). Ca2+ signaling in mouse cortical neurons studied by two-photon imaging and photoreleased inositol triphosphate. J. Neurosci., 23: 758–765.

Suh SW, ET Gum, AM Hamby, PH Chan, RA Swanson. 2007. Hypoglycemic neuronal death is triggered by glucose reperfusion and activation of neuronal NADPH oxidase. The J Clin. Inves. 117(4): 910-918.

Suh SW, Hamby AM, Swanson RA. (2007). Hypoglycemia, brain energetics, and hypoglycemic neuronal death. Glia., 55(12):1280–1286

Sukanya M, Basu A, Paul P, Halder M, Jha S (2013). Bacosides and Neuroprotection. Nat.Products. 3639-3660.

Sumathi T, Nathiya VC, Sakthikumar M. (2011). Protective effect of bacoside-A against morphine-induced oxidative stress in rats. Indian J. Pharm Sci., 73(4): 409–415.

Sunahara RK, Niznik HB, Weiner DM, Stormann TM, Brann MR, Kennedy JL,Gelernter JE, Rozmahel R, Yang YL, Israel Y, et al. (1990). Human dopamine D1 receptor encoded by an intronless gene on chromosome 5. Nature., 347: 80-83.

Suzuki A, Stern SA, Alberini CM. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. Cell., 144(5): 810–823.

Svendsen CN, Kew JNC, Staley K, Sofroniew MV. (1994). Death of developing septal cholinergic neurons following NGF withdrawal *in vitro*: protection by protein synthesis inhibition. J Neurosci.,14:75– 87.

Tam EW, Widjaja E, Blaser SI, Macgregor DL, Satodia P, Moore AM (2008) Occipital lobe injury and cortical visual outcomes after neonatal hypoglycemia. Pediatrics 122(3):507–512.

Tarazi FI, Florijn WJ, Creese I. (1997). Differential regulation of dopamine receptors after chronic typical and atypical antipsychotic drug treatment. Neuroscience, 78: 985-996.

Tarazi FI, Kula NS, Baldessarini RJ. (1997). Regional distribution of dopamine D4 receptors in rat forebrain. Neuroreport., 8: 3423-3426.

Tarazi FI, Tomasini EC, Baldessarini RJ. (1998). Postnatal development of dopamine D4-like receptors in rat forebrain regions: comparison with D2-like receptors. Brain Res. Dev. Brain Res., 110: 227-233.

Tataranni PA, Gautier JF, *et al*. (1999). Neuro anatomical correlates of hunger and satiation in humans using positron emission tomography. Proc. Natl. Acad. Sci. USA., 96: 4569–4574.

Taylor CW, Genazzani AA, Morris SA. (1999). Expression of inositol trisphosphate receptors. Cell Calcium., 26: 237-251.

Thoenen H. (2000). Neurotrophins and activity-dependent plasticity. Prog. Brain Res., 128: 183–191.

Thorens B, Mueckler M. (2010). Glucose transporters in the 21st Century, Am. J. Physiol. Endocrinol. Metab., 298 (2) E141–E145.

Thorn SR, Sekar SM, Lavezzi JR, O'Meara MC, Brown LD, Hay WW, Rozance PJ. (2012). Am J Physiol. Regul. Integr. Comp. Physiol., 303(8): R861–R869.

Thornberry NA, Lazebnik Y. (1998). Science., 281:1312.

Turner CP, Pulciani D, Rivkees SA. (2002). Reduction in intracellular calcium levels induces injury in developing neurons. Exp. Neurol., 17: 821–32.

Turner CP, Yan H, Schwartz M, Othman T, Rivkees SA. (2002). A1 adenosine receptor activation induces ventriculomegaly and white matter loss. Neuroreport., 13: 1199–1204.

Unis AS, Roberson MD, Robinette R, Ha J, Dorsa DM. (1998). Ontogeny of human brain dopamine receptors. I. Differential expression of [3H]-SCH23390 and [3H]-YM09151-2 specific binding. Dev. Brain Res., 106: 109- 117.

Vaarmann A, Kovac S, Holmstro¨m KM, Gandhi S, Abramov AY. (2013). Dopamine protects neurons against glutamate-induced excitotoxicity. Cell Death and Disease., 4: e455

Van Tol HHM, Bunzow JR, Guan HC, Sunahara, RK, Seeman P, Niznik HB. (1991). Cloning of a human dopamine D4 receptor gene with high affinity for the antipsychotic clozapine. Nature., 350: 614-619.

Vander Heiden MG, Chandel NS, Williamson EK, Schumacker PT, Thompson CB. (1997). Bcl–xL regulates the membrane potential and volume homeostasis of mitochondria. Cell, 91: 627–637.

Vanhaesebroeck B, Alessi DR. (2000). The PI3K-PDK1 connection: more than just a road to PKB. Biochem. J., 346(Pt 3): 561–576.

Vannucci RC, Vannucci SJ. (2001). Hypoglycemic brain injury. Semin. Neonatol, 6: 147–155.

Vannucci S J, Maher F, Simpson I A (1997). Glucose transporter proteins in brain: Delivery of glucose to neurons and glia. Glia, Vol. 21(1): 2–21

Vannucci SJ, Maher F, Simpson IA. (1997). Glucose transporter proteins in brain: delivery of glucose to neurons and glia. Glia, 21: 2–21.

Ventimiglia R, Mather PE, Jones BE *et al*. (1995) The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. Eur. J. Neurosci. 7:213–222.

Vetter ML, Martin-Zanca D, Parada LF, Bishop JM, Kaplan DR. (1991). Nerve growth factor rapidly stimulates tyrosine phosphorylation of phospholipase C gamma 1 by a kinase activity associated with the product of the trk protooncogene. Proc. Natl. Acad. Sci. USA, 88: 5650–5654.

Wajant H, Pfizenmaier K, Scheurich P. (2003). Tumor necrosis factor signaling. Cell Death Differ., 10: 45–65.

Walker PD, Riley LA, Hart RP, Jonakait GM. (1991). Serotonin regulation of neostriatal tachykinins following neonatal 6-hydroxydopamine lesions. Brain Res., 557, 31–36.

Wang L, Liu F, Adamo ML. (2000). Cyclic AMP Inhibits Extracellular Signal regulated Kinase and Phosphatidylinositol 3-Kinase/Akt Pathways by Inhibiting Rap1. J. Biol. Chem., 276: 37242–37249.

Wang M, Ramos BP, Paspalas CD, Shu Y, Simen A, Duque A, *et al*. (2007). Alpha 2A-adrenoceptors strengthen working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. Cell., 129, 397–410.

Wang X, Han W, Du X, Zhu C, Carlsson Y, Mallard C, Jacotot E, Hagberg H. (2010). Neuroprotective Effect of Bax-Inhibiting Peptide on Neonatal Brain Injury. Stroke.,41: 2050-2055.

Wang Y, Ghezzi A, Yin JCP, Atkinson NS. (2009). CREB regulation of BK channel gene expression underlies rapid drug tolerance. Genes Brain Behav. 8(4): 369–376.

Wassmer E, Davies P, William P, Whitehouse, Stuart H. (2003). Clinical spectrum associated with cerebellar hypoplasia. Pediatr. Neurol., 28: 347–351.

Wei EP, Lamb RG, Kontos HA. (1982). Increased phospholipase C activity after experimental brain injury. J. Neurosurg., 56(5): 695-8.

Weih F, Caamaño J. (2003). Regulation of secondary lymphoid organ development by the nuclear factor-kappaB signal transduction pathway. Immunol. Rev., 195: 91−105.

Wieloch T. (1985). Hypoglycemia-induced neuronal damage prevented by an N-methyl -D - aspartate antagonist. Science 230: 681–683

Williams AF. (1997). Hypoglycemia in newborns: a review. Bull.WHO.,75: 261-90.

Williams GV, Goldman-Rakic PS. (1995). Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature. 376: 572–575.

Williams L. (2001). Parkinson's Disease site from Evergreen State College; Bear, M. F., Connors, Paradiso M.AWest Camden St., Baltimore. Neuroscience: Exploring the Brain, Second Edition.

Wong JY, Liberatore GT, Donnan GA, Howells DW. (1997). Expression of brain-derived neurotrophic factor and TrkB neurotrophin receptors after striatal injury in the mouse. Exp Neurol., 148:83–91.

Worby CA, Vega QC, Zhao Y, Chao HHJ, Seasholtz AF, Dixon JE. (1996). Glial cell line-derived neurotrophic factor signals through the RET receptor and activates mitogen-activated protein kinase. J. Biol. Chem., 271: 23619 –23622.

Wyllie AH, Kerr JF, Currie AR. (1980). Cell death: the significance of apoptosis. Int. Rev. Cytol., 68: 251-306.

Xing B, Xin T, Zhao L, Hunter RL, Chen Y, Bing G. (2010). Glial cell line-derived neurotrophic factor protects midbrain dopaminergic neurons against lipopolysaccharide neurotoxicity. J Neuroimmunol., 225(1-2):43-51.

Xue Y, Kuok C, Xiao A, Zhu Z, Lin S, Zhang B. (2010). Identification and expression analysis of mical family genes in zebrafish, J. Genet. Genomics., 37 (10) 685–693.

Yakovlev A, Faden A. (2001). Caspase-dependent apoptotic pathways in CNS injury. Mol. Neurobiol., 24:131–144.

Yao WD, Gainetdinov R, Arbuckle M, Sotnikova T, Cyr M, Beaulieu JM, Torres G, Grant S, Caron M. (2004). Identification of PSD-95 as a regulator of dopamine mediated synaptic and behavioral plasticity. Neuron., 41:625–638

Yavuz D, Deyneli O, Akpinar I, *et al*. (2003). Endothelial function, insulin sensitivity and inflammatory markers in hyper prolactinemic pre-menopausal women. Eur. J. Endocrinology., 149(3):187–193.

Yettefti K, Orsini JC, Perrin J. (1997). Characteristics of glycemia-sensitive neurons in the nucleus tractus solitarii: possible involvement in nutritional regulation. Physiol. Behav., 61: 93–100.

Yu MC, Yu Wan–Hua A. (1980). Effect of hypoxia on cerebellar development: Morphologic and radioautographic studies. Exp. Neurol., 70: 652–664.

Yu X, Riley T, Levine AJ. (2009). The regulation of the endosomal compartment by p53 the tumor suppressor gene. FEBS J 276:2201–2212.

Zhou QY, Palmiter RD. (1995). Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell., 83:1197-1209.

List of Publications

1. **Roshni Baby Thomas,** Shilpa Joy, M. S. Ajayan, C. S. Paulose. "Neuroprotective potential of *Bacopa monnieri* and Bacoside A against Dopamine receptor dysfunction in the cerebral cortex of neonatal hypoglycaemiac rats."Cell Mol Neurobiol. (**2013**). (DOI: 10.1007/s10571-013-9973-0)

2. **Roshni Baby Thomas,** T.R.Anju, Shilpa Joy and C.S.Paulose**.** Role of Curcumin as a Nutritional Supplement to Enhance Cell Proliferation and survival in hypoxia Induced Hepatocyte Injury-*in vitro* model.(**2012**), Ist Edition, Bloomsbury Publishing India Pvt.Ltd. ISBN:978-93-82563-27-3.

3. J. Shilpa , **B. T. Roshni** , R. Chinthu ,C. S. Paulose. Role of GABA and serotonin coupled chitosan nanoparticles in enhanced hepatocyte proliferation. J Mater Sci: Mater Med (**2012**) 23:2913–2921. DOI 10.1007/s10856-012-4754-8

4. Anju T R, Naijil G, Shilpa J, **Roshni T**. Neonatal hypoxic insult mediated cholinergic disturbances in the brain stem:Effect of glucose, oxygen and epinephrine resuscitation. Neurological Sciences. **(2012)**

5. Sherin A, Anu J, Peeyush KT, Smijin S, Anitha M, **Roshni BT** and C S Paulose**.** Cholinergic and GABAergic Receptor Functional Deficit in the Hippocampus of Insulin Induced Hypoglycemic and Streptozotocin Induced Diabetic Rats. J. neuroscience. **(2011)** 202:69-76.

Abstracts Presented

1. Ajayan M S, Anju T R, **Roshni B T**, Paulose C S. Gymnemic acid mediated neuronal survival in the cerebral cortex of streptozotocin induced diabetic rats-Muscarinic Receptor subtypes functional regulation. National Conference on Current Advances in Biotechnology & Annual Meeting of Society Of Biotechnologist (India). Sant Gadge Baba Amravati University, Amravati (Maharashtra). November 25-26, 2013.

2. **Roshni Baby Thomas**, Shilpa Joy, Naijil George, C.S. Paulose. "Dopamine D1 Receptor Down Regulation And Enhanced Bax Expression In Neonatal Hypoglycemic Rat Cerebral Cortex: Glucose, Bacoside A, *Bacopa monnieri*–Resusitation and Functional Recovery". National seminar on emerging trends in Biotechnology and Annual meeting of Society for Biotechnologists, India , Acharya Nagarjuna University, Guntur (Sept. 24 – 26, 2011)

3. Shilpa Joy, **Roshni Baby Thomas**, C. S. Paulose. SOD, Bax and MAT 2A functional regulation in partially hepatectomised rats: GABA - chitosan nanoparticles induced hepatocyte proliferation. Cochin Nano, IMA hall, Cochin (August 14-17, 2011)

4. **Roshni Baby Thomas**, Anju T.R., Chinthu Romeo, C.S. Paulose. 'Cortical muscarinic receptor subtypes down regulation and enhanced Bax expression in hypoxic neonatal rats: An induced cognitive impairment'. Innovations in Biotechnology, SRM University, Chennai, (October, 2010).