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 centrally 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-inflammmatory 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.