

**Introduction**

Diabetes mellitus is a common metabolic disorder characterised by hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (DeFronzo, 2004). According to World Health Organisation (WHO), diabetes is currently growing at a fast rate throughout the world and is the 9th leading cause of global mortality. Increased risk for diabetes is primarily associated with age, ethnicity, family history of diabetes, smoking, obesity and physical inactivity. Diabetes related complications including cardiovascular disease, nephropathy, neuropathy, blindness and lower-extremity amputation are a significant cause of increased morbidity and mortality among people with diabetes.

The most common form of diabetes is type 2 diabetes, in which resistance to insulin is accompanied by an inadequate compensation in the secretion of insulin. Type 1 diabetes is caused by an absolute shortage in the production of insulin due to the destruction of pancreatic β cells (American Diabetes Association, 2002). This type of diabetes, which was previously defined as Insulin Dependent Diabetes Mellitus, is not as common as type 2 diabetes; only 5–10% of the patients with diabetes have type 1 diabetes.

A great number of anatomical, functional and biochemical alterations have been described in the nervous system of diabetic animals (Tomlinson *et al.*, 1992; Ozturk *et al.*, 1996). These variety of alterations (generally named as diabetic neuropathy) affects the brain, spinal cord and peripheral nerves. Diabetic patients have increased risk for developing central nervous system (CNS) dysfunctions like impaired learning and memory, neurodegeneration and loss of synaptic plasticity. In the CNS, diabetes reduces brain weight and neocortical volume, which is associated with a reduction of the number of cortical neurons (Jakobsen *et al.*, 1987).

The CNS neurotransmitters play an important role in the regulation of glucose homeostasis. These neurotransmitters mediate rapid intracellular communications not only within the CNS but also in the peripheral tissues.
Neurotransmitters have been reported to show significant alterations during hyperglycaemia resulting in altered functions causing neuronal degeneration (Bhardwaj et al., 1999). Chronic hyperglycaemia during diabetes mellitus is a major initiator of diabetic micro-vascular complications like retinopathy, neuropathy and nephropathy (Sheetz & King, 2002; Monnier et al., 2009). The autonomic nervous system plays a prominent role in the regulation of insulin secretion. It has been proposed that neuronal afferent signals delivered to the pancreatic β cell through the vagus are responsible for the cephalic phase of insulin secretion (Lausier et al., 2010). In pancreatic β-cells, IP3 mobilizes Ca²⁺ from intracellular stores, resulting in an elevation of the intracellular concentration of Ca²⁺ and allowing activation of Ca²⁺/calmodulin. Diacylglycerol (DAG) on the other hand, activates Protein kinase C (PKC) (Nishizuka, 1995; Renstrom et al., 1996; Shawl et al., 2009). PKC, like Ca²⁺/calmodulin, accelerates exocytosis of insulin granules (Nakano et al., 2002). Chronic hyperglycaemia is strongly implicated in the development of vascular complications of diabetes, including gradual damage to the CNS (Brands et al., 2004).

Glutamate is the primary excitatory neurotransmitter of the CNS and glutamate receptors play important roles in many CNS functions, including learning, memory, development and synaptogenesis (Collingridge & Lester 1989). Glutamate receptors have been classified into two major categories: ionotropic receptors (iGluRs) and metabotropic receptors (mGluRs). The ionotropic GluRs possess intrinsic cation-permeable channels and include N-methyl-d-aspartate (NMDA), kainate (KA) and α--amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Excessive stimulation of glutamate receptors can be neurotoxic, a phenomenon known as excitotoxicity that causes neuronal damage (Gill & Pulido, 2001). Glutamic acid decarboxylase (GAD) is the rate limiting enzyme in the decarboxylation of glutamate to GABA; deactivation of GAD can trigger synaptic glutamate overload (Roberts & Kuriyama, 1968). Various studies reported the glutamate excitotoxicity mediated neuronal damage in diabetic
complications like diabetic retinopathy and diabetic neuropathy (Chabot et al., 1997; Delyfer et al., 2005; Gowda et al., 2011).

Clearance of extracellular glutamate from the synaptic cleft is carried out by specific high-affinity sodium-dependent excitatory amino acid transporters (EAAT), which can be modulated by the redox status of the cells (Trotti et al., 1998). Therefore, higher oxidative stress associated with loss of activity of antioxidant enzymes and glutamate transporters aggravate cell damage in the brain. The astrocytic sodium dependent glutamate transporters - glutamate aspartate transporter EAAT1 (GLAST) and EAAT2 (GLT-1) stabilize the concentration of extracellular excitatory amino acids and are responsible for removal of more than 90% of the extracellular glutamate. This buffers the glutamate level, thus avoiding excessive stimulation of neuronal glutamate receptors and protecting neurons from glutamate toxicity (Dunlop, 2006).

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes mellitus (Brownlee 2001; Rosen et al., 2001; Bonnefont-Rousselot 2002; Ceriello 2003; Yang et al., 2011). Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins. The increase in the level of reactive oxygen species (ROS) in diabetes could be due to their increased production and/or decreased destruction by nonenzymic and enzymic catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) antioxidants (Chen et al., 2012). Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance (Hansen et al., 1999; Urakawa et al., 2003; Furukawa et al., 2004; Houstis et al., 2006). Therefore, treatment with antioxidants or over expression of antioxidant enzymes can, at least partially, prevent oxidative stress induced insulin resistance. Glutathione peroxidases (GPxs) are members of the family of antioxidant enzymes that scavenge hydrogen peroxide in the presence of reduced GSH (Drevet et al., 2006).
Nutritional therapy is important in preventing diabetes, managing existing diabetes and preventing or at least slowing the rate of development of diabetic complications. Antioxidant agents from diet have a significant therapeutic influence on various neurodegenerative disorders associated with diabetes and oxidative stress (González-Burgos & Gómez-Serranillos, 2012). The significance of Curcuma longa Linn (Turmeric) in health and nutrition has changed considerably since the discovery of the anti-oxidant properties of naturally occurring phenolic compounds. Curcuminoids, the active polyphenols of C. longa rhizomes, contain curcumin, demethoxycurcumin and bis-de-methoxycurcumin, which were shown to have a wide spectrum of pharmacological actions (Chattopadhyay et al., 2004). Curcumin, a yellow pigment from Curcuma longa, is a major component of turmeric and exhibits powerful anti-oxidant, anti-diabetic, anti-inflammatory and anti-cancer properties (Miller, 2001; Surh et al., 2001; Reddy et al., 2010; Meng et al., 2012). In diabetes, curcumin was shown to perform a multitude of activities including reduction in glycemic level, elevation in antioxidant status of pancreatic β-cells and attenuation of the mechanisms involved in diabetic encephalopathy (Arun & Nalini 2002; Kuhad & Chopra, 2007). A number of experimental studies have demonstrated curcumin's antioxidant and neuroprotective potential (Bala et al., 2006; Kuhad & Chopra, 2007; Huang et al., 2012). Studies from our lab showed that curcumin treatment ameliorates the altered muscarinic expression in the brain regions of diabetic rats (Peeyush et al., 2011).

Vitamin D is made in the epidermis from 7-dehydrocholesterol in response to sunlight exposure and is obtained from the diet (Lips 2001; Mathieu et al., 2005; Holick 2005; Hart 2012). The major dietary sources of vitamin D are oily fish, eggs and meat. Even in countries where certain foods are fortified with vitamin D, dietary intake of vitamin D alone is usually insufficient to maintain adequate serum levels of 25-hydroxyvitamin D (Nowson et al., 2002; Holick et al., 2003). The biological actions of Vitamin D3 are mediated through binding to the vitamin D receptor (VDR), a member of the nuclear steroid hormone receptor family. An increased prevalence of diabetes has been described in vitamin D-
deficient individuals (Chiu et al., 2004). The VDR can be viewed as a master regulator of transcription. VDRs are present in pancreatic β-cells and vitamin D is essential for normal insulin secretion (Jessica et al., 2010). An increased prevalence of type 2 diabetes has been described in vitamin D-deficient individuals (Boucher et al., 1995; Isaia et al., 2001; Chiu et al., 2004).

The present study was designed to investigate the protective effect of curcumin and vitamin D₃ in the functional regulation of glutamatergic NMDA and AMPA receptors in streptozotocin (STZ) induced diabetic rats. Alterations in glutamatergic neurotransmission in the brain were evaluated by analyzing the glutamate content, glutamate receptors - NMDA and AMPA receptors binding parameters and gene expression, GAD and GLAST gene expression. Immunohistochemistry studies using confocal microscope were carried out to confirm receptor density and gene expression results of NMDA and AMPA receptors. The role of glutamatergic receptors in pancreas was studied using the following parameters; glutamate content, GLAST expression, glutamate receptors - NMDA and AMPA receptor binding and gene expression. Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes. In the present study SOD assay and GPx gene expression were done to evaluate the activity of antioxidant enzymes in the brain regions and pancreas. NeuroD1 and Pdx1 gene expression were performed in pancreas of experimental rats to evaluate pancreatic islet survival. Gene expression profiles of caspase 8, Bax, and Akt in brain regions and pancreas were studied to understand the possible mechanism behind curcumin and vitamin D₃ mediated neuroprotection and islet survival. Gene expression studies of vitamin D₃ receptor localisation in the pancreas was done to understand the mechanism of vitamin D₃ in insulin secretion. Curcumin and vitamin D₃ mediated insulin secretion via Ca²⁺ release were studied using confocal microscope.
OBJECTIVES OF THE PRESENT STUDY

1. To study the anti-hyperglycemic activity of curcumin and vitamin D₃ in STZ-induced diabetic rat model.

2. To measure the circulating insulin concentration of control, diabetic, insulin, curcumin and vitamin D₃ treated diabetic rats.

3. To quantify glutamate content in the cerebral cortex, hippocampus, brain stem, cerebellum and pancreas of experimental rats.

4. To study GAD gene expression in the cerebral cortex, hippocampus, brain stem and cerebellum of experimental rats using Real-Time PCR.

5. To study the transport of glutamate using GLAST gene expression in the cerebral cortex, hippocampus, brain stem, cerebellum and pancreas of experimental rats using Real-Time PCR.

6. To study AMPA and NMDA receptors binding parameters in cerebral cortex, hippocampus, brain stem, cerebellum and pancreas of experimental rats.

7. To study NMDA R1, NMDA 2B, AMPA (GluR2), AMPA (GluR4) receptor subunits gene expression in cerebral cortex, hippocampus, brain stem and cerebellum of experimental rats using Real-Time PCR.

8. To study AMPA (GluR2), AMPA (GluR4) receptor subunits gene expression in pancreas of experimental rats using Real-Time PCR.

9. To measure the second messenger IP3 levels in the cerebral cortex, hippocampus, brain stem, cerebellum and pancreas of experimental rats.
10. To study antioxidant potential of curcumin and vitamin D₃ using SOD assay and GPx gene expression in cerebral cortex, hippocampus, brain stem, cerebellum and pancreas of experimental rats.

11. To study the expression of NMDA R1, NMDA 2B, AMPA (GluR2), AMPA (GluR4) receptor subunits by immunofluorescent specific antibodies in the brain slices of experimental rats using confocal microscope.

12. To study the gene expression of apoptotic factors Bax and caspase 8 in the cerebral cortex, hippocampus, brain stem, cerebellum and pancreas of experimental rats using Real-Time PCR.

13. To study the gene expression of anti-apoptotic factor Akt-1 in the cerebral cortex, hippocampus, brain stem and cerebellum of experimental rats using Real-Time PCR.

14. To study the gene expression of pancreatic β cell survival transcription factors Pdx-1 and NeuroD1 in experimental rats using Real-Time PCR.

15. To study the expression of insulin, AMPA (GluR2), AMPA (GluR4), IP3 receptor and vitamin D₃ receptor in the pancreatic islets of experimental rats using confocal microscope.

16. To study the calcium release in pancreatic β cells of curcumin and vitamin D₃ treated streptozotocin induced diabetic rats.