Discussion

Diabetes mellitus, a major endocrine disorder, has become a severe health problem in the world. The disease is one of the most severe metabolic disorders in humans characterised by hyperglycaemia due to relative or an absolute lack of insulin or the action of insulin on its target tissue or both. Prolonged exposure to chronic hyperglycaemia in diabetes can lead to vascular disorder, retinopathy, altered immune functions, changes in the intestinal function, peripheral neuropathy and dysfunctions of the CNS (Biessels *et al.,* 2004; McNay & Sherwin 2004). The neurological consequences of diabetes mellitus in the CNS are now receiving greater attention. Studies reported that diabetic patients are vulnerable to neurodegenerative diseases (Gasparini *et al.,* 2002; Matsuzawa *et al.,* 2012). Both type 1 and type 2 diabetes can cause impaired learning, memory, mental flexibility and cognitive functions (Umegaki *et al.,* 2012; McCrimmon *et al.,* 2012; Kalalian-Moghaddam *et al.,* 2012). The various neurotransmitter systems including serotonergic, cholinergic, dopaminergic and GABAergic undergo a significant change in diabetes (Gireesh *et al.,* 2008; Antony *et al.,* 2010; Kumar *et al.,* 2010; Anitha *et al.,* 2012).

BLOOD GLUCOSE, INSULIN LEVEL AND BODY WEIGHT

In the present study STZ-induced rats were used as an experimental model for diabetes, since they provide a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycaemia (Low *et al.,* 1997). The STZ diabetic rat serves as an excellent model to study the molecular, cellular and morphological changes in brain induced by stress during diabetes (Aragno *et al.,* 2000). There was an increase in blood glucose level and a decrease in circulating insulin level in diabetic rats when compared to control group. The increased blood glucose level is due to the decreased circulating insulin level. Decreased circulating insulin level in diabetic rats is a result of marked destruction of insulin secreting pancreatic islet β-cells by STZ (Junod *et al.,* 1969; Ahmadi *et al.,* 2010). Treatment using curcumin, insulin and Vitamin D_3 showed restorative effect on

blood glucose level by increasing the insulin level in the serum. Previous reports showed that curcumin has the potential to protect pancreatic islet cells against STZ-induced death (Meghana *et al.,* 2007) and elevated plasma insulin level in diabetic mice (Seo *et al.,* 2008). Previous studies reported that Vitamin D deficiency in rabbits and mice lead to impaired insulin secretion and supplementation with vitamin D corrects the defect (Cade & Norman, 1986). The increased insulin secretion in curcumin treated rats is due to the activation of β-cell survival factors Pdx-1 and Neuro D in this group. Vitamin D_3 treatment modulates the altered AMPA receptor subunit expression leads to increased intracellular Ca^{2+}, which enhances exocytosis of insulin granules (Fujimoto *et al.,* 1995; Shimono *et al.,* 2005).

Diabetic rats showed a significant decrease in body weight when compared with control. Hyperglycemia and decreased body weight during diabetes are in agreement with the previous reports. (Junod *et al.,* 1969; Kumar *et al.,* 2010; Willsky *et al.,* 2011). The decreased body weight in the diabetic rats is due to the excessive breakdown of tissue proteins (Salahuddin *et al.,* 2010; Poongothai *et al.,* 2011). Treatment of diabetic rats with insulin, curcumin and Vitamin D_3 improved body weight significantly which indicate prevention of muscle tissue damage due to hyperglycemic condition. Evidence has shown that NMDA receptors mediate some aspects of eating and satiety (Duva *et al.,* 2005). It has also been shown that stimulation of eating by intra hypothalamically injected neuropeptide Y is dependent upon NMDA receptor activation (Lee & Stanley, 2005). These findings suggest that several central and peripheral glutamatergic circuits are involved in feed intake regulation. Present study showed an altered NMDA receptor expression and density in the brain regions of diabetic rats. These alterations in NMDA receptor subunit might have affected the feeding habit of diabetic rats. Increased oxidative stress in diabetic rats promotes the skeleton muscle damage leading to weight loss (Aragno *et al.,* 2005). Insulin, curcumin and vitamin D_3 treatment significantly reversed the body weight when compared with the control group. A more prominent reversal in body weight was observed in curcumin treated diabetic rats than the other treatment groups. This can be

explained as an effect of the antioxidant activity of curcumin which helped in preventing skeletal muscle damage and also its ability to ameliorate the altered NMDA receptor expression in brain regions thereby maintaining the feed intake. Previous studies reported that curcumin treatment can suppress body weight loss in diabetic db/db mice (Seo *et al.,* 2008).

GLUTAMATERGIC RECEPTOR ALTERATIONS AND FUNCTIONAL REGULATION IN CONTROL AND EXPERIMENTAL RATS

Diabetes mellitus is a metabolic disorder that not only causes a decrease in efficiency of the pancreatic β-cells to secrete insulin but also is accompanied by altered monoamine levels and their turnover rates in the CNS (Garris, 1990; Lackovic *et al.,* 1990; Bhattacharya & Saraswathi, 1991; Manni *et al.,* 2012). Complications of the peripheral nervous system also are known to be very common in diabetic patients (Dyck *et al.,* 1993; Ametov *et al.,* 2003) and a substantial body of evidence has demonstrated that diabetes have negative impacts on the CNS (Gispen & Biessels, 2000; Ryan & Geckle, 2000; Biessels *et al.,* 2002). People with diabetes, especially older adults, apparently face a greater risk of vascular dementia, with large population studies detecting an association between diabetes mellitus, depression and Alzheimer's disease (Leibson *et al.,* 1997; Ott *et al.,* 1999; Anderson *et al.,* 2001; Gasparini *et al.,* 2002).

Glutamate is involved in most aspects of normal brain function including cognition, memory and learning. Brain tissue contains large amounts of glutamate, around 5-15 mmol per kg depending on the region (Schousboe, 1981; Zaganas *et al.,* 2012). The extracellular concentrations are kept low and are in the order of a few micromolar (Hamberger *et al.,* 1983), or even lower (Herman & Jahr, 2007). The highest glutamate concentrations are found intracellularly in glia cells, nerve terminals and synaptic vesicles (in increasing order) (Ottersen *et al.,* 1992). Glutamate concentration of more than 60 mM was reported inside synaptic vesicles (Shupliakov *et al.,* 1992). Excessive activation of ionotropic glutamate receptors (NMDAR, AMPA-R and Kainate-R) induces a massive Ca^{2+} influx into

the cell which can trigger neuronal death in the CNS. There is strong evidence suggesting the involvement of this glutamate excitotoxicity in acute injury to the CNS and many chronic neurodegenerative disorders

Cerebral cortex

The cerebral cortex is the seat of our highest forms of intelligence. It plays a central role in many complex brain functions including memory, attention, perceptual awareness, thought, language and consciousness. Besides autonomic and peripheral neuropathy, diabetes is also associated with gradually developing end-organ damage in the CNS (Brands *et al.,* 2004) and leads to impairment in cognitive functions and electrophysiological changes (Allen *et al.,* 2004). L-Glutamate is regarded as the major excitatory neurotransmitter in the mammalian CNS. All three ionotropic glutamate receptors exhibit a ubiquitous distribution in the brain, the NMDA receptors being particularly abundant in the forebrain (Ozawa *et al.,* 1998). Although all receptors have pivotal roles in brain functions, the NMDA receptors have received special attention in development and aging. They are involved in cell migration, growth and differentiation in the developing brain (Vallano, 1998; Unezaki *et al.,* 2012).

In addition to being the most important excitatory neurotransmitter in the brain, glutamate is a potent neurotoxin and is considered the primary cause of neuronal death during acute insults to the brain and in neurodegenerative diseases. GAD catalyzes the decarboxylation of glutamate yielding CO_2 and GABA. Our findings reported a decreased gene expression of GAD mRNA in the cerebral cortex of diabetic rat. Decreased gene expression of GAD leads to increased glutamate content in the diabetic rats. The extracellular concentration of the excitatory neurotransmitter L-glutamate in the CNS must be kept low to ensure a high signal to noise ratio during synaptic activation (Katagiri *et al.,* 2001) and to prevent excitotoxicity due to excessive activation of glutamate receptors (Mangano & Schwarcz, 1983; Wang *et al.,* 1998). Curcumin and vitamin D_3 treatment significantly reversed the GAD expression near to control which helps in the decarboxylation of glutamate to GABA and CO_2. This conversion of

glutamate to GABA helps to reduce the glutamate content in the cerebral cortex. Decreased glutamate in the postsynaptic neuron helped in inhibition of hyper excitability of glutamate and reduces glutamate induced excitotoxicity. Over activation of glutamate receptors can damage the neurons leading to impairment in the motor function and co-ordination in hyperglycaemic rats (Anu *et al.,* 2010). Previous reports showed that dysregulation of glutamate signaling in the brain regions leads to neuronal damage and causes a number of neuropsychiatric diseases (Rahn *et al.,* 2012) and neurodegenerative diseases like Alzheimer's disease (Hynd *et al.,* 2004). Population-based study estimated that the risk of Alzheimer's disease increased with diabetes mellitus (Leibson *et al.,* 1997; Exalto *et al.,* 2012). Glutamate excitotoxicity in diabetic brain is a reason for neuronal injury leading to neurodegenerative disorders.

To evaluate the role of curcumin and vitamin D_3 in cortical NMDA receptor kinetics, radio receptor assay was done in the cerebral cortex of control and experimental rats and it was observed that the NMDA receptor number was significantly increased in the diabetic group when compared to control. The gene expression analysis of NMDA R1 and NMDA 2B receptor subunits supported the NMDA receptor binding data. Curcumin and vitamin D_3 treatments significantly reversed the altered NMDA receptor density and gene expression in the cerebral cortex to near control. The increased B_{max} observed showed the increased receptor number whereas the K_d value signifies that the receptor affinity remained unaltered. The immunohisochemical studies using confocal microscope confirmed the binding parameters and gene expression of NMDA receptor subunits in cerebral cortex of control and experimental rats.

AMPARs mediate the majority of the fast excitatory transmission in the CNS of vertebrates. These receptors are concentrated at postsynaptic densities of excitatory synapses, although large pools of AMPARs are also present in the cytoplasm of neuronal somata and dendrites (Petralia, 1997; Beckerman & Glass, 2011). One subtype of glutamate receptor that is thought to play a central role in excitotoxic injury is the AMPA (Beattie *et al.,* 2010). The results from our study suggest that AMPA receptor number significantly increased in the cerebral cortex

of diabetic rats with no change in the receptor affinity. We observed a different pattern of gene expression with AMPA receptor subunits. AMPA GluR4 receptor gene expression showed a significant up regulation while AMPA GluR2 receptor subunit expression was significantly down regulated in the cerebral cortex of diabetic rats. Studies showed that neuronal cells preferentially expressing the GluR4 subunit of AMPA receptors are particularly vulnerable to AMPA-induced toxicity (Page & Everitt, 1995). Homomeric complexes made of the GluR1, GluR3 or GluR4 proteins form channels that are permeable to Ca^{2+}, Mg^{2+} and Ba^{2+} (Hollmann *et al.,* 1991). A key subunit in determining the ion channel properties of AMPA receptors is GluR2 (Lee *et al.,* 2010). When this subunit is present in a receptor complex, the AMPA receptors exhibit a linear relationship between voltage applied to the membrane and the current conducted through the receptor channels (Nakanishi *et al.,* 1990; Hume *et al.,* 1991). These receptor complexes also have very low permeability to Ca^{2+}, i.e. they resemble most native AMPA receptors in CNS neurons, although extrapolations to native receptor structure is imprecise as neuronal receptors with linear conductance are also permeable to Ca^{2+} (Schneggenburger *et al.,* 1993; Li *et al.,* 2012).

The results from our study indicate that the change in AMPA receptor subunit composition in diabetic condition makes AMPA receptor more permeable to Ca^{2+}. The over stimulation of GluR4-containing AMPA receptor leads to excessive calcium accumulation in neurons; ultimately leading to their death (Choi *et al.,* 1988). Treatment using curcumin and vitamin D_3 reversed the receptor number and gene expression in cerebral cortex of the diabetic rats and it could prevent the neuronal death as evident from the decreased apoptotic factors expression in our study. The immunohistochemical studies using confocal microscope confirmed the gene expression of AMPA receptor subunits in cerebral cortex of control and experimental rats. We also reported an increase in intracellular IP3 content in cerebral cortex of diabetic rats. Inositol phosphates are known to regulate AMPA receptor trafficking, intracellular Ca^{2+} homeostasis, particularly the release of stored Ca^{2+} through IP3 receptors (Miyazaki, 1995). This leads to excess Ca^{2+} release through IP3 receptor mediated Ca^{2+} channel

leading to neuronal damage. The treatment with curcumin and vitamin D_3 has resulted in reversal of enhanced IP3 content. Vitamin D_3 treatment showed a prominent reversal in the IP3 content when compared with insulin treated rats.

In diabetes, oxidative stress cause an increased production of free radicals and a sharp reduction in antioxidant defenses (Giugliano *et al.*, 1995). Diabetes also induces an increase in lipid peroxidation products (Sies *et al.*, 1985) and a decrease in SOD, CAT (Wohaieb & Godin, 1987) and GSH levels (Miranda *et al.*, 2006). GPx is a soluble selenoprotein which reduces H_2O_2 and organic peroxides to H_2O and corresponding stable alcohols using reduced GSH as an essential co-substrate thus inhibiting the formation of free radicals. Our study showed a decreased gene expression of GPx and decreased SOD activity in cerebral cortex of diabetic rats compared to control. Decreased SOD activity and GPx gene expression leads to high oxidative stress in diabetic rats. The high oxidative stress in the cerebral cortex resulted in a decreased GLAST gene expression which in turn leads to reduced reuptake of extracellular glutamate. GLAST contains functional cysteine residues that are sensitive to oxidative formation of cysteine bridges leading to inhibition of glutamate flux through the transporters (Trotti *et al.*, 1998). Hydrogen peroxide, nitric oxide, superoxide anion and peroxynitrite anion can inhibit glutamate uptake through GLAST (Zeng *et al.*, 2010). GPx catalyzes the reduction of hydrogen peroxide and hydro peroxides formed from fatty acids, thereby effectively removing toxic peroxides from living cells. It plays the important role of protecting cells from potential damage by free radicals, formed by peroxide decomposition (Mannervik 1985; Ursini 1995). We observed that the treatment using insulin, curcumin and vitamin D_3 increased GPx gene expression and SOD activity thereby reducing the oxidative stress. GLAST gene expression was significantly reversed to control when compared with diabetic rats. Curcumin being a potent antioxidant showed a more prominent reversal in SOD activity and GPx gene expression than the insulin treated rats.

Our results showed that the glutamate content, NMDA and AMPA receptor number in the cerebral cortex of the diabetic rats were increased with decreased GLAST and GAD gene expression. Previous studies reported that

NMDA R1 and GluR4-containing AMPA receptor play a primary role in triggering intracellular cascades that lead to glutamate mediated neuronal apoptosis (Hollmann *et al.,* 1991; Santos *et.al*., 2006). To find out whether these changes in the glutamate pathway cause any neuronal damage due to apoptosis we studied the expression of apoptotic factors like caspase 8 and Bax. Bax is a proapoptotic factor which act as apoptosis executers (Reed, 1998; Cory & Adams, 2002; Polster & Fiskum, 2004; Ward *et al.,* 2004). In the present study, the gene expression of apoptotic factors, caspace 8 and Bax was up regulated in diabetic groups. Suppression of antioxidant enzyme GPx gene expression and reduced SOD activity in diabetic condition could lead to the increased oxidative stress and directly activates apoptotic pathways. The treatment using curcumin and vitamin D_3 reversed the altered GPx gene expression and SOD activity to near control and glutamate mediated excitotoxicity by reversing the altered NMDA and AMPA receptors. Akt-1 gene expression was down regulated in the diabetic group. Akt-1 or serine threonine kinase is a member of an anti-apoptotic cascade of neurons (Endo *et al.,* 2006). The constitutively active Akt-overexpressing neurons could survive potential cellular distresses (Namikawa *et al.,* 2000; Narayanan *et al.,* 2009). In the present study only vitamin D_3 treatment activated the Akt mediated survival pathway.

In conclusion our study showed that curcumin and vitamin D_3 treatment provides neuroprotection by acting as an antioxidant and modulator of glutamatergic neurotransmission. The result of this study has demonstrated that the supplementation of curcumin and vitamin D_3 to STZ-induced diabetic rats has beneficial effects in reducing the alterations in glutamatergic receptors, oxidative stress and imbalanced glutamate metabolism in the cerebral cortex.

Hippocampus

Hippocampus is based on recent or declarative memory and plays important roles in long-term memory and spatial navigation (Squire *et al.,* 1992). Many organs and organ systems are adversely affected by diabetes, including the brain, which undergoes changes that increase the risk of depression and cognitive

decline (Greenwood & Winocur, 2005; Messier, 2005). In the hippocampus excitatory transmission is mediated by glutamate acting on ionotropic NMDA and non-NMDA receptors as well as on metabotropic receptors (Hollmann & Heinemann, 1994). Glutamate receptors are implicated in physiological functions like neuronal plasticity, learning and memory (Ekonomou & Angelatou, 1999; Lynch, 2004). Moderate disturbances of learning and memory and complex information processes have been reported in both type 1 and 2 diabetic patients (Biessels & Gispen 2005, Cukierman *et al*., 2005, Biessels *et al*., 2006; Haider *et al.,* 2012).

Alterations in the hippocampal glutamate receptor can cause impaired cognitive function, learning and memory (Smijin *et al.,* 2012). Our results showed that NMDA and AMPA receptors were increased in diabetic rats compared to control. Gene expression studies showed up regulation of NMDA R1, NMDA 2B and AMPA GluR4 receptor subunits mRNA in the hippocampus of diabetic rats. AMPA GluR2 receptor subunit showed significant down regulation in the diabetic rats when compared to control. Immunohistochemical studies also showed an increased NMDA R1, NMDA 2B, AMPA GluR4 and decreased GluR2 receptor subunits expression in diabetic rats. The altered AMPA subunits expression in the hippocampus of diabetic rats makes AMPA receptor more permeable to Ca^{2+} and causes neuronal damage through the activation of apoptotic factors Bax and caspase 8. Presence of GluR2 subunit determines the Ca^{2+} permeability of the AMPA receptor (Isaac *et al.,* 2007; Liu & Zukin, 2007). NMDA receptor channels are highly permeable to Ca^{2+} ions. Hyper activity of NMDA receptors can lead Ca^{2+} influx and neuronal damage in the hippocampus of diabetic rats (Arundine & Tymianski, 2003). Recent reports suggest that both hypoglycaemia and hyperglycaemia have adverse effects on the brain neuronal structural changes and impaired long-term spatial memory (Malone *et al.,* 2008). Long-term potentiation of neuronal activity in the hippocampus is thought to be a substrate for learning and memory. Gasparova *et al.,* (2008) revealed that prolonged exposure to hypoglycaemic state influenced induction of LTP in the hippocampus and that it had deleterious effects on learning and memory. Insulin, curcumin and vitamin D_3

treatment significantly reversed the altered NMDA and AMPA receptor number and receptor subunits gene expression when compared with diabetic group. Curcumin and vitamin D_3 treatment showed prominent reversal in the NMDA and AMPA receptor subunits when compared with insulin. Immunohistochemical analysis also showed a significant reversal in the NMDA and AMPA receptor subunits expression in the treatment group. While reversing the altered NMDA, AMPA receptors subunit gene expression and receptor number, it prevents the hyperactivation of the receptor leading to excitotoxicity. Second messenger IP3 content was increased in the hippocampus of diabetic rats .The increased levels of IP3 in hippocampus can cause enhanced Ca^{2+} levels resulting in activation of protein kinase C leading to series of events culminating in internalisation of AMPA receptors (Ruiz *et al.,* 2009).

Increased glutamate content was observed in the hippocampus of diabetic rats mainly due to the down regulation of GAD and GLAST mRNA. Decreased GAD gene expression indicates the reduced decarboxylation of glutamate to GABA. GLAST is the glutamate transporter which transports 90% of the glutamate from synapse to glial cells (Kim *et al.,* 2011). The decreased expression of glutamate transporter leads to the impaired clearance of glutamate from the extracellular space and high glutamate content in the hippocampus of diabetic rats compared with control. Up regulation of GAD gene expression and down regulation of glutamate transporter gene expression can lead to altered synaptic glutamate levels (Lyon *et al.,* 2008). Treatment using insulin, curcumin and vitamin D_3 significantly reversed the altered GAD and GLAST gene expression when compared to diabetic group. Curcumin and vitamin D_3 treatment showed prominent reversal in GAD and GLAST gene expression when compared to insulin treatment. This reversed GAD and GLAST gene expression in the curcumin and vitamin D_3 treated rats maintain the glutamate content in the synapse than insulin treatment. The hyperactivation of glutamate receptor can cause increased oxidative stress (Parfenova *et al.,* 2005).

Oxidative stress is a key participant, along with metabolic compromise and excitotoxicity, in apoptotic neurodegenerative process (Alexi *et al.,* 2000).

The brain is particularly vulnerable to oxidative injury because of its high rate of oxygen consumption, intense production of reactive radicals and high levels of transition metals, such as iron, that catalyze the production of reactive radicals. In the animal models of diabetes, several brain alterations have been described, such as increased hippocampal astrocytic reactivity, impaired synaptic plasticity, vascular changes, decreased dendritic complexity and disturbed neurotransmission (Magariños *et al.,* 2000). Many studies reported that excitotoxicity can cause increased oxidative stress (Trudeau *et al.,* 2004; Melo *et al.,* 2011). In the present study, diabetic group showed a significant decrease in the SOD activity and GPx gene expression when compared with control. Decreased antioxidant enzymes in the diabetic group can lead to a state of oxidative stress and the activation of apoptotic factors, Bax and caspase 8. Treatment using curcumin and vitamin D_3 significantly enhances the SOD activity and GPx gene expression when compared with diabetic group. Curcumin treatment showed prominent reversal in antioxidant system when compared to insulin treatment because of its antioxidant activity. We observed a significant up regulation in the Bax and caspase 8 gene expressions in the hippocampus of diabetic group indicating increased apoptosis. Insulin, curcumin and vitamin D_3 treatment significantly reverse the Bax and caspase 8 gene expression by reversing the altered glutamatergic neurotransmission and antioxidant enzyme status.

In conclusion the diabetic rats showed alterations in the glutamatergic transmission in the hippocampus leading to increased oxidative stress and activation of apoptotic factors. The altered glutamate transmission in the hippocampus can lead to impaired cognitive function, learning and memory. Curcumin and vitamin D_3 treatment reversed the altered glutamate transmission and reduced the expression of apoptotic factors than insulin treatment.

Brain Stem

Brain Stem is a part of the brain located beneath the cerebrum and in front of the cerebellum. It connects the spinal cord to the rest of the brain. 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). Brain stem along with hypothalamus serves as the key centre of the central nervous system regulating the body homeostasis (Araújo & Martel, 2012). Stimulation of the peripheral vagus nerve leads to an increase in circulating insulin levels. Anatomical studies suggest that the origin of these vagal efferent fibres is nucleus ambiguus and dorsal motor nucleus directly innervating pancreas (Bereiter *et al.,* 1981). Brain stem along with hypothalamus serves as one of the key centers of the central nervous system regulating body homeostasis (Araújo & Martel, 2012). Stimulation of the peripheral vagus nerve leads to an increase in circulating insulin levels. The dorsal motor nucleus of the vagus nerve is located in the brain stem. It is connected to the endocrine pancreas exclusively through vagal fibers and has a role in neural mediated insulin release (Costoli *et al.,* 2005). Nucleus ambiguous stimulation has been reported to increase plasma insulin levels in rats (Bereiter *et al.,* 1981; Moreno *et al.,* 2008).

Neurotransmitter alterations are reported in the brain stem of STZ induced diabetic rats (Carndall *et al.,* 1981; Gireesh *et al.,* 2008; Abraham *et al.,* 2010). Present study showed an increase in NMDA and AMPA receptor binding in the brain stem of diabetic rats when compared with control with no change in affinity. Insulin, curcumin and vitamin D_3 treatment significantly reversed the changes when compared with diabetic group. The increased NMDA receptor activity observed in diabetic group from the Scatchard plot was supported by the gene expression study of NMDA R1 and NMDA 2B subunits. AMPA receptor subunits showed differential expression. AMPA GluR4 subunit expression was significantly increased and AMPA GluR2 subunit expression was decreased in diabetic group when compared with control. The immunohistochemical studies using confocal microscope confirmed the binding parameters and gene expression of NMDA and AMPA receptor subunits in brain stem of control and experimental rats. This subunit variation in the diabetic group make AMPA receptors more permeable to Ca^{2+} and leads to glutamate mediated excitotoxicity (Santos *et al.,* 2006). The increased levels of second messenger, IP3 in diabetic rats indicates enhanced Ca^{2+} levels (Miyakawa *et al.,* 1999; Rahman 2012).

Glutamate content in the brain stem was significantly increased in the diabetic rats due to the altered gene expression of GAD mRNA. Decreased GLAST gene expression was observed in the brain stem indicating the altered glutamate transport in the diabetic group. The elevated NMDA and AMPA receptor in the presence of increased glutamate content and altered glutamate transport in diabetic brain leads to the neuronal apoptosis through glutamate mediated excitotoxicity. Apoptotic factors, Bax and caspase 8 were up regulated in the brain stem indicating apoptosis. Insulin curcumin and vitamin D_3 treatment significantly reversed the altered glutamate receptor subunit and GAD gene expression to near control and prevent the Ca^{2+} influx and excitotoxic cell death through the over-activation of NMDA and AMPA receptors. Studies have shown that vitamin D_3 has regulatory benefits in neuronal Ca^{2+} homeostasis and protects neurons from excess Ca^{2+} entry in the brain (Brewer *et al.,* 2001). Insulin treatment was found to alter glutamate receptor activation (Liu *et al.*, 1995) and interact with AMPA receptor trafficking between the plasma membrane and the intracellular compartment in neuronal cell culture (Man *et al.,* 2000) indicating that mechanisms underlying diabetic neuropathies could be initiated in the early stages of the disease, as a consequence of abnormal glutamate receptor properties. This is relevant to the clinical situation because excessive activation of glutamate receptors is a characteristic feature of brain damage during stroke and ischemia (McCall, 1992; La Via *et al.,* 2012).

The antioxidant enzyme GPx gene expression and SOD activity was decreased in the diabetic rats indicating increased state of oxidative stress in the diabetic brain stem. The increased Ca^{2+} influx due to hyper activation of NMDA and AMPA receptors can cause increased oxidative stress (Bondy *et al.,* 1993). This increased state of oxidative stress led to neuronal death which is indicated by enhanced expression of caspase 8 and Bax. The treatment using curcumin and vitamin D_3 restores antioxidant status and reduce the expression of apoptotic factors. Curcumin treatment showed prominent reversal in GPx gene expression and SOD activity due to its antioxidant properties (Menon & Sudheer, 2007). The up regulation of Akt-1 gene expression indicates the activation of neuronal

survival pathway (Brunet *et al.,* 2001) in the brain stem of vitamin D_3 treated rats. Active Akt has vital roles in cell survival, metabolism and neuronal function (Guo *et al.,* 2012).

Cerebellum

Experimental evidence indicate the involvement of cerebellum in a variety of human mental activities including language (Fiez *et al.,* 1996), attention (Allen *et al.,* 1997), cognitive affective syndromes (Schmahmann & Sherman, 1998), fear and anxiety caused by threats of pain (Ploghause *et al.,* 1999), thirst sensation and fear for air, hunger (Parsons *et al.,* 2001) and motor relearning (Stoodley & Schmahmann, 2009; Strick *et al.,* 2009). Cerebellar activity is often detected in neuroimaging studies of pain (Moulton *et al.,* 2010) and other studies evaluating emotional processing (Fusar-Poli *et al.,* 2009), even in the absence of a motor task. The cerebellar vermis integrates and processes the inputs from the vestibular, visual and proprioceptive systems to coordinate muscle timing as a result of which the centre of gravity stays within the limits of stable upright standing (Diener *et al.,* 1989). Damage to the cerebellum, in particular the vermis (Baloh *et al.,* 1998) results in more postural sway than in control subjects (Ho *et al.,* 2004, Marvel *et al.,* 2004). Decreased postural stability would correspond with abnormalities of the vermis observed in autistic subjects (Gowen & Miall, 2005). Unlike explicit memory such as recognition memory and spatial memory, motor learning is characterized by slow development, without the requirement of conscious recall and in general being lifetime-lasting (Llinas & Welsh, 1993; Tulving & Markowitsch, 1998; Eichenbaum, 2000). Based on the role of the cerebellum in motor activities such as fine motor movement and motor coordination as well as the computational network within the neural circuitries, cerebellar motor learning was first postulated by Marr (1969) and Albus (1971). Studies have indicated that the cerebellum is involved in generalized emotional perception (Murphy *et al.,* 2003; Konarski *et al.,* 2005), including aversive picture perception (Lane *et al.,* 1997; Paradiso *et al.,* 1999; Bermpohl *et al.,* 2006). Studies from our laboratory showed that neurotransmitter receptor alterations in cerebellum during diabetes

can cause impaired motor learning and coordination (Joseph *et al.,* 2007; Peeyush *et al.,* 2010)

The present study showed that glutamate content is significantly increased in the cerebellum of diabetic rats with decreased gene expression of GAD and GLAST. The decreased production of GAD resulted in the decreased decarboxylation of glutamate yielding CO_2 and GABA. This blockage in the glutamate content leads to glutamate accumulation in the synapse of diabetic rats. Our previous studies reported that GDH enzyme activity enhanced during diabetes and did not completely reverse even after insulin administration leading to increased glutamate content (Preetha *et al*. 1996; Aswathy *et al*. 1998; Biju & Paulose, 1998). Clearance of extracellular glutamate from the synaptic cleft is carried out by specific high-affinity sodium-dependent excitatory amino acid transporters, GLAST. Glutamate aspartate transporter (EAAT1) stabilizes the concentration of extracellular excitatory amino acids 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). The decreased expression of GAD and GLAST mRNA in the cerebellum result in the elevated extracellular glutamate levels and lead to abnormalities in glutamatergic neurotransmission. The extracellular concentration of the excitatory neurotransmitter L-glutamate in the CNS must be kept low to ensure a high signal to noise ratio during synaptic activation (Katagiri *et al.,* 2001) and to prevent excitotoxicity due to excessive activation of glutamate receptors (Wang *et al.,* 1998). Treatment using insulin, curcumin and vitamin D_3 significantly reversed the altered GAD and GLAST gene expression when compared with diabetic group. Curcumin and vitamin D_3 treatment showed prominent reversal when compared to insulin treated group. The improved GAD and GLAST gene expression in the curcumin and vitamin D_3 treated rats helped in the normal conversion and transportation of glutamate and its helps to maintain the reduced glutamate content in the synapse than insulin treated rats.

AMPARs mediate the majority of the fast excitatory transmission in the CNS of vertebrates. These receptors are concentrated at postsynaptic densities of excitatory synapses, although a large pool of AMPARs is also present in the cytoplasm of neuronal somata and dendrites (Petralia, 1999). One subtype of glutamate receptor that is thought to play a central role in excitotoxic injury is the AMPA. The results from our study showed that AMPA receptor number was significantly increased in the cerebellum with out change in affinity. The elevated AMPA receptor number in the presence of increased glutamate content resulted in hyper activation of AMPA receptor. GluR2 and GluR4 subunits showed a different pattern of gene expression. GlurR2 gene expression was significantly down regulated in the cerebellum of diabetic rats when compared with control. The relative presence of the GluR2 subunit determines the functional properties of AMPA receptors. The GluR2 subunit in most neurons expresses at a high level, which renders these cells impermeable for Ca^{2+} influx through AMPA receptors. Studies showed that reduction of GluR2 subunit levels enhance glutamate excitotoxicity (Friedman *et al.,* 1998; Bogaert *et al.,* 2012). The immunohisochemical studies using confocal microscope confirms the gene expression of GluR2 AMPA receptors in cerebellum of control and experimental rats. GluR4 receptor subunit gene expression was significantly up regulated in the cerebellum of diabetic rats. Studies reported that neuronal cells expressing the GluR4 subunit of AMPA receptors are particularly vulnerable to AMPA-induced toxicity (Page & Everitt, 1995). The over stimulation of GluR4 AMPA receptor leads to excessive Ca^{2+} accumulation in neurons ultimately leading to their death (Choi, 1988). The immunohisochemical studies showed an increased expression of AMPA GluR4 receptor and decreased expression of AMPA GluR2 in diabetic group when compared to control. Treatment using curcumin and vitamin D_3 reversed the altered AMPA receptor number and subunit gene expression when compared to diabetic group. Vitamin D_3 treatment showed prominent reversal in the AMPA receptor number when compared to insulin treatment. Both curcumin and vitamin D_3 treatment showed prominent reversal in AMPA GluR4 and AMPA GluR2 receptor subunit gene expression when compared to insulin treated group.

IP3 content in the cerebellum is significantly increased in the diabetic rats compared to control. Inositol phosphates are known to regulate AMPA receptor trafficking, intracellular Ca^{2+} homeostasis, particularly the release of stored Ca^{2+} through IP3 receptors (Miyazaki, 1995; Bogaert *et al.,* 2012). Treatment using curcumin and vitamin D_3 significantly reversed the IP3 content in the cerebellum when compared to diabetic rats.

Recent studies have shown the involvement of NMDA receptor subunits-NMDAR1, NMDA2B in the cerebellum in motor learning in mouse (Jiao *et al.,* 2008). Receptor binding studies in cerebellum showed an increased B_{max} without change in K_d value in the diabetic rats when compared to control. This increased B_{max} observed indicates the increased receptor number with no change in the affinity of the receptors as shown from the K_d value. Linear regression data by Scatchard plot was supported by the gene expression and immunohistochemical studies of NMDAR1 and NMDA 2B subunits. The elevated NMDA receptor number in the presence of increased glutamate content resulted in the hyper activation of NMDA receptor. NMDA receptors possess several unique properties that distinguish them from other ionotropic glutamate receptors. In addition to monovalent cations, NMDA receptors are also highly permeable to the divalent cation Ca^{2+}, which has numerous important intracellular functions. Over activation of NMDA receptors also result in cellular dysfunction and contribute to the symptoms of many disorders of the nervous system (Rothman & Olney, 1986). Treatment using vitamin D_3 significantly reversed the altered NMDA receptors when compared with diabetic without change in affinity. The reduction in the glutamate content helped to reduce the elevated NMDA receptors in synapse. Treatment using insulin, curcumin and vitamin D_3 reversed the altered NMDA receptor number and subunit gene expression when compared with diabetic group. Curcumin and vitamin D_3 treatment showed prominent reversal in the NMDA receptor number and NMDA R1 when compared to insulin treatment. Vitamin D_3 treatment showed prominent reversal in NMDA 2B receptor subunit gene expression when compared with insulin treated group.

Cerebellum of diabetic rats showed a significant increase in the glutamatergic receptor activity, up regulated glutamatergic activity mediated neurodegeneration through excitotoxicity. Excitotoxicity can cause increased oxidative stress (Nguyen *et al.,* 2011). Our study showed a decreased SOD activity and GPx gene expression in the cerebellum of diabetic rats. Decrease in the antioxidant enzymes SOD and GPx indicates increased oxidative stress in the diabetic rats. Treatment using curcumin showed a significant reversal in the SOD activity but insulin and vitamin D_3 did not show any significant change when compared with diabetic rats. Insulin, curcumin and vitamin D_3 treatment showed a significant reversal in the GPx gene expression when compared with diabetic group. Curcumin treatment showed a significant reversal when compared with insulin treated rats. Curcumin is a potent antioxidant and this antioxidant activity of curcumin helps to protect the cerebellum from further damages. Glutamate mediated excitotoxicity and increased oxidative stress in the diabetic cerebellum can activate the apoptotic factors (Fonfría *et al.,* 2002). In the present study apoptotic factors caspase 8 and Bax gene expression in the cerebellum was significantly up regulated when compared with control rats. Increased gene expression of caspase 8 and Bax indicates increased apoptosis in the diabetic group. Treatment using curcumin and vitamin D_3 reversed caspase 8 and Bax expression and showed neuroprotective effect on diabetic cerebellum through revising altered glutamatergic neurotransmission and antioxidant system.

Pancreas

Plasma glucose levels are regulated by the action of insulin, a hormone that is produced and secreted by the pancreatic islet β-cells in response to nutrients. Diabetes mellitus, which comprises a heterogeneous group of hyperglycaemic disorders, results from inadequate mass and function of β -cells (Prentki & Nolan, 2006). Insulin secretion from the pancreatic islets is controlled by the central nervous system through sympathetic and parasympathetic nerves (Burr *et al.,* 1976; Campfield & Smith, 1980; Ahren, 2000). Studies from our laboratory described the regulatory role of the sympathetic and parasympathetic

systems in pancreatic regeneration (Renuka *et al.,* 2004, 2005; Mohanan *et al.,* 2005). Pancreatic islets receive innervations from both divisions of the autonomic nervous system and pancreatic endocrine secretion is partly controlled by the autonomic nervous system (Liu *et al.,* 2001). Anatomical studies suggest that the vagal efferent fibers originating from the nucleus ambiguus and dorsal motor nucleus of the brain stem directly innervate the pancreas (Bereiter *et al.,* 1981) and have a role in neural mediated insulin release (Azmitia & Gannon, 1986).

Reports suggest that amino acids can, under appropriate conditions, enhance insulin secretion from primary islet cells and β-cell lines (Charles & Henquin 1983; Smith *et al.,* 1997; Brennan *et al.,* 2002; Dixon *et al.,* 2003). L-glutamine release from skeletal muscle modulates glucagon release from pancreatic α-cells (Chang & Goldberg, 1978), which subsequently influence insulin secretion from β -cells. Glutamate, a major excitatory neurotransmitter in the central nervous system, is also found in pancreatic islets (Gonoi *et al.,* 1994; Inagaki *et al.,* 1995; Muroyama *et al.,* 2004) and is released from α cells (Yamada *et al.,* 2001; Hayashi *et al.,* 2003). Various ionotropic glutamate receptors are found in insulinoma cells and pancreatic islets (Inagaki *et al.,* 1995; Muroyama *et al.,* 2004). Studies suggest that cytoplasmic glutamate concentration can influence insulin production (MacDonald & Fahien, 2000; Hoy *et al.,* 2002; Maechler *et al.,* 2002).

In the present study, the glutamate content was significantly increased in diabetic group when compared to control. It was reported that enhanced GDH can produce glutamate, a second messenger of insulin secretion (Anno *et al.,* 2004). The claim that glutamate potentiated insulin secretion (Maechler & Wollheim, 1999; Rubi *et al.,* 2001; Hoy *et al.,* 2002) was based on the observation that a rise in cytoplasmic glutamate concentration correlated with increased insulin release, but this claim was later contradicted (Bertrand *et al.,* 2002) when it was found that insulin release did not always correlate with the total islet glutamate concentration. Curcumin and vitamin D_3 treatments showed a significant reversal in glutamate content of pancreas when compared with diabetic group. Insulin treatment did not show any significant change in the glutamate content. Increased glutamate content

in the pancreas can cause damage to insulin producing β-cells (Choi *et al.,* 2010). Insulin treatment did not show any significant change in the glutamate content while the treatment using curcumin and vitamin D_3 significantly reversed the changes when compared to diabetic rats.

Glutamate acts through two classes of receptors, ligand gated ion channels (ionotropic receptors) and G-protein coupled (metabotropic) receptors. Many studies showed that iGluRs expressed in β cells can modulate the level of insulin release (Weaver *et al.,* 1995; Moriyama & Hayashi, 2004). All three ionotropic receptors are present in the pancreas (Inagaki *et al.,* 1995). Our study showed that NMDA receptor number did not show any significant change in the diabetic and treatment group when compared with control. Bertrand *et al.,* (1992) reported that NMDA receptors did not have a significant role in insulin secretion. Various reports suggest that glutamate stimulates insulin release in rat pancreas, by acting on an excitatory amino acid receptor of AMPA subtype (Bertrand *et al.,* 1992; Wu *et al.,* 2012). Our study showed that the AMPA receptor number was significantly increased in the diabetic group when compared with control. AMPA GluR4 receptor subunit showed a significant down regulation and AMPA GluR2 receptor subunit showed a significant up regulation when compared with diabetic rats. This change in subunit variation can change the AMPA receptor function. GluR2 subunit determines the functional properties of AMPA receptors; presence of GluR2 subunit makes cells impermeable for Ca^{2+} influx through AMPA receptors (Friedman *et al.,* 1998). The reduction in the Ca^{2+} influx can affect the insulin secretion (Chen *et al.,* 2010). Treatment using insulin, curcumin and vitamin D_3 significantly reversed the receptor number when compared with diabetic rats. Altered AMPA GluR4 and GluR2 receptor subunit expression was significantly reversed in curcumin and vitamin D_3 treatment. Insulin treatment did not show any significant reversal in the GluR4 receptor subunit when compared with diabetic group. Vitamin D_3 treatment showed more prominent reversal in the AMPA GluR2 receptor subunit gene expression when compared with insulin treated group. To understand the role of AMPA receptor subunits expression in the insulin producing pancreatic islets, the double immunohistochemical analysis was

done. Insulin staining is localized to the insulin granules of the islet β cells, thereby considering it as a specific marker for pancreatic β cells. Insulin-AMPA GluR2 subunit co-labelling study showed that in diabetic condition, AMPA GluR2 receptor expression was significantly increased in the insulin positive cells when compared with control. AMPA GluR4 receptor expression was significantly decreased in the insulin positive cells when compared with control. Increased expression of AMPA GluR2 receptor subunit during diabetes makes AMPA receptor more impermeable to Ca^{2+}. Immunohistochemical analysis showed that curcumin and vitamin D_3 treatment significantly reversed the altered AMPA receptor subunits expression in the pancreatic islets.

Inositol phosphates are known to regulate AMPA receptor trafficking, intracellular Ca^{2+} homeostasis, particularly the release of stored Ca^{2+} through IP3 receptors (Miyazaki, 1995). Our study showed that IP3 content was significantly decreased in the pancreas of the diabetic rats when compared with control rats. Treatment using insulin, curcumin and vitamin D_3 reversed the decreased IP3 content to near control. Immunohistochemical analysis showed a decreased expression of IP3 receptor subtype 3 (IP3R-3) in the pancreatic islets of diabetic rats when compared to control. Decreased IP3R3 in the pancreatic islets can affect the Ca^{2+} release (Wong *et al.,* 2006). Immunolocalization studies indicated that IP3R3 receptor is present in secretory granules of the β cell (Blondel *et al.,* 1994; Ravazzola *et al.,* 1996). Treatment using curcumin and vitamin D_3 reversed the decreased IP3 receptor to near control.

In recent years, several reports suggested that the endocrine pancreas is also a target tissue for the hormonally active form of vitamin D_3, 1,25-(OH)2-D3, along with the classical vitamin D target organs: the intestine, bone and kidney (Norman *et al.,* 1982). The biological actions of vitamin D_3 are mediated through binding to the VDR, a member of the nuclear steroid hormone receptor family (McGrath *et al.,* 2001). Immunohistochemical analysis showed a decreased expression of vitamin D receptor in the pancreatic islets of diabetic rats when compared to control. Decreased expression of vitamin D receptor in the diabetic rats affect the vitamin D_3 mediated pathways. Reduced expression of VDR can

cause vitamin D_3 deficiency. Reports suggest that vitamin D_3 deficiency decreases Ca^{2+} uptake by islets (Chertow *et al.,* 1986) 1, 25(OH) $2D_3$ enhances Ca^{2+} entry into islets (Billaudel *et al.,* 1993). Previous studies have indicated that the pancreas has receptors specific for Vitamin D_3 and that Vitamin D_3 increases insulin secretion in vitamin D-deficient rats (Norman *et al.,* 1980). Treatment using vitamin D_3 reversed the altered expression of VDR to near control. Insulin and curcumin treatment did not show any significant reversal. In our *in vitro* Ca^{2+} release studies in pancreatic β cell using Fluo 4-AM, decreased Ca^{2+} release from pancreatic islets observed in hyperglycemic condition. Elevation of ATP is necessary for the membrane-dependant increase in cytosolic Ca^{2+}, the main trigger of insulin exocytosis (Maechler & Wollheim, 2000). In the presence of reduced Ca^{2+} release in diabetic rats islets fail to produce insulin through exocytosis. Vitamin D_3 treatment showed more prominent increase in Ca^{2+} release in the presence of AMPA when compared with other treatment groups.

Oxidative stress is produced under diabetic conditions and is likely involved in progression of pancreatic β-cell dysfunction found in diabetes (Kajimoto & Kaneto, 2004). In the present study the antioxidant enzyme SOD activity was decreased in the diabetic rats when compared with control group. Reduction in the SOD activity is due to increased oxidative stress (Matsunami *et al.,* 2010). Superoxide dismutase administration showed protection against STZ-induced diabetes (Robbins *et al.,* 1980). Treatment using curcumin and vitamin D_3 showed significant reversal in the SOD activity when compared to diabetic group. Insulin treatment did not show any significant change in the SOD activity. Real-Time PCR amplification studies showed that GPx gene expression in diabetic pancreas was down regulated indicating increased oxidative stress. Insulin, curcumin and vitamin D_3 treatment showed prominent reversal in GPx gene expression when compared to diabetic group. The reduction in the antioxidant enzyme makes pancreas vulnerable to oxidative stress and leads to the destruction of pancreatic β-cells through the activation of apoptotic factors, Bax and caspase 8. We observed that Bax and caspase 8 gene expressions were significantly up regulated in the pancreas of diabetic rats when compared with

control. Treatment using insulin, curcumin and vitamin D_3 showed a significant reversal in the caspase 8 and Bax gene expression when compared to diabetic rats.

Insulin gene transcription is regulated by the cooperation of a group of glucose-sensitive transcription factors expressed in a tissue-restricted manner (Ohneda *et al.,* 2000, Aramata *et al.,* 2005). Among the most important of these transcription factors are NeuroD1 and the homeodomain transcription factor pancreatic duodenal homeobox 1 (Pdx1) which activate the insulin gene promoter synergistically and are essential for glucose-stimulated insulin gene transcription. Pdx-1 is required for pancreas development in mice and in humans (Jonsson *et al.,* 1994; Ahlgren *et al.,* 1996; Offield *et al.,* 1996). Pdx1 plays critical role in insulin gene transcription, insulin secretion as well as β cell survival (Babu *et al.,* 2007). Genetic studies in which Pdx1 is conditionally inactivated in mice suggest that *Pdx1* gene dosage is critical both for development of the endocrine and exocrine pancreas and for the maintenance of adult β cells (Hale *et al.,* 2005; Holland *et al.,* 2005; Fujitani *et al.,* 2006). In the present study Pdx1 gene expression was significantly down regulated in the diabetic rats when compared with control. Animal models suggest that down regulation of Pdx1 expression in the β-cell may underlie the pathogenesis of β-cell failure and type 2 diabetes (Weir *et al.,* 1997). Treatment using curcumin and vitamin D_3 significantly reversed and up regulated the Pdx 1 expression when compared with both diabetic and control. The up regulation of Pdx 1 indicates β-cell survival in treatment group (Claiborn *et al.,* 2010). Report suggests that the Pdx 1 expression is downregulate during chronic oxidative stress (Kaneto *et al.,* 2005). The reduced oxidative stress in the treatment group may favor the expression of Pdx 1 resulting in reversing pancreatic dysfunction.

NeuroD is a basic helix-loop-helix (bHLH) transcription factor that is crucial for development of the pancreas (Naya *et al.,* 1997; Huang *et al.,* 2002; Chae *et al.,* 2004; Chao *et al.,* 2007). NeuroD null mice die of severe diabetes shortly after birth; and their β cells are poorly differentiated, islets fail to form, and the majority of β cells are lost (Naya *et al.,* 1997). In the presence study, NeuroD1 gene expression is significantly down regulated in the diabetic rats when

compared with control. NeuroD has been shown to be critical for insulin gene expression *in vitro* (Naya *et al.,* 1995; Qiu *et al.,* 2002). Islets lacking NeuroD respond poorly to glucose and display glucose metabolic profile similar to immature β cells (Gu *et al.,* 2010). Treatment using curcumin and vitamin D_3 showed significant reversal in NeuroD1 gene expression when compared with diabetic group. Insulin treatment did not show any significant change in the NeuroD1 gene expression. The increased expression of NeuroD1 indicates the β cell survival in the treatment group.

In conclusion, the altered AMPA receptor subunit expression in the pancreas of diabetic rats affects AMPA receptor mediated insulin release. Treatment using curcumin and vitamin D_3 showed beneficial effect through ameliorating the alterations in AMPA receptor and increases the Ca^{2+} release in pancreatic islets leading to restored insulin secretion. The decrease in antioxidant enzymes SOD and GPx indicates oxidative stress in the pancreas of diabetic rats leading to the activation of pro apoptotic factors Bax and caspase 8 resulting in β cell death. The down regulation of transcription factors Pdx1 and NeuroD1 indicates reduced insulin production and β cell function. The treatment using curcumin and vitamin D_3 enhanced the anti oxidant enzyme status leading to a state of abridged oxidative stress thereby restricting the expression of Bax and caspase 8. The increased expression of Pdx1 and NeuroD1 in treatment group indicates β cell survival leading to increased insulin production.