SEROTONIN- 5-HT_{2A} AND GLUTAMATE RECEPTORS GENE EXPRESSION IN STREPTOZOTOCIN INDUCED DIABETIC RATS: EFFECTS OF PYRIDOXINE AND *AEGLE MARMELOSE* LEAF EXTRACT

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BY

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JANUARY 2010

Dedicated To My Beloved Parents…

CERTIFICATE

This is to certify that the thesis entitled **"Serotonin- 5-HT_{2A} and Glutamate Receptors Gene Expression in Streptozotocin induced diabetic rats: Effects of pyridoxine and *Aegle marmelose* leaf extract"** is a bonafide record of the research work carried out by Ms. Pretty Mary Abraham, 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.

Cochin - 682 022 (C. S. Paulose)

January 30, 2010

DECLARATION

I hereby declare that the thesis entitled **"Serotonin- 5-HT_{2A} and Glutamate Receptors Gene Expression in Streptozotocin induced diabetic rats: Effects of pyridoxine and *Aegle marmelose* leaf extract"** is authentic record of the research work carried out by me for my doctoral degree, under the supervision and guidance of **Dr. C. S. Paulose,** Professor & Head, Department of Biotechnology, Director, Centre for Neuroscience, Cochin University of Science and Technology, Cochin and that no part thereof has been presented for the award of any other degree, diploma, associateship or other similar titles or recognition.

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They would be too many to recount"

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ABRREVIATIONS

5-HT	5-Hydroxy tryptamine
5-HTP	5-Hydroxy trptophan
5-HTT	5-HT transporter
7-OH DPAT	7-Hydroxy-2 (di-n-popylamino)-tetralin
AC	Adenylate cyclase
ACh	Acetylcholine
AChR	Acetylcholine receptor
AChE	Acetylcholine esterase
AD	Alzheimers disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
BBB	Blood brain barrier
B_{max}	Maximal binding
CA	Cornu Ammonis
CB	Cerebellum
CC	Cerebral cortex
cAMP	Cylic adenosine monophosphate
cAPK	cAMP-dependent protein kinase
cDNA	Complementary deoxy ribonucleic acid
cGMP	Cyclic guanosine monophosphate
CNS	Central Nervous System
CREB	cAMP regulatory element binding protein
CSF	Cerebrospinal fluid
CT	Crossing threshold
DA	Dopamine
DAG	Diacylglycerol
DBH	Dopamine β hydroxylase
DEPC	Di ethyl pyro carbonate

DG	Dentate Gyrus
DNA	Deoxy ribonucleic acid
EAA	Excitatory amino acids
EDTA	Ethylene diamine tetra acetic acid
EEG	Electroencephalogram
EPI	Epinephrine
ER	Endoplasmic reticulum
EPSCs	Excitatory postsynaptic current
FCS	Fetal calf serum
GABA	Gamma amino butyric acid
GAD	Glutamic acid decarboxylase
GDH	Glutamate dehydrogenase
GFP	Green fluorescent protein
GLAST	Glutamate/aspartate transporter
GLT	Glutamate transporter
GLUR2	Glutamate Receptor-2
GOD	Glucose oxidase
GPCR	G-protein-coupled receptors
Gq PRC	Gq Protein coupled receptors
GTP	Guanosine triphosphate
GPx	Glutathione peroxidase
HBSS	Hank's Balanced Salt Solution
HFS	High frequency stimulation
IDDM	Insulin dependent diabetes mellitus
IIH	Insulin induced hypoglycaemia
iGluRs	Ionotropic glutamate receptors
INSR	Insulin Receptor
IPI	Initial Precipitating Injury
IP3	Inositol 1,4,5-triphosphate
KA	Kainate

K_d	Dissociation constant
LTD	Long term depression
LTP	Long term potentiation
MCI	Mild cognitive impairment
mGLU	Metabotropic Glutamate
(+) MK-801	(+)5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate
MRI	Magnetic resonance imaging
mRNA	Messenger Ribonucleic acid
NADH	Nicotinamide adenine dinucleotide, reduced form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NE	Norepinephrine
NGF	Nerve growth factor
NIDDM	Non-insulin dependent diabetes mellitus
NMDA	N-methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
NOS	Nitric-oxide synthase
NPY	Neuropeptide Y
NSB	Non specific binding
O.D.	Optical density
p	Level of significance
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline Triton X- 100
PCP	Phencyclidine
pCREB	Phosphorylated cAMP regulatory element binding protein
PEG	Poly ethylene glycol
PFC	Prefrontal cortex
Pi	Inorganic phosphate
PIP2	Phospatidyl 4,5-bisphosphate
PKC	Protein kinase C

PKG	Protein kinase G
PLC	Phospolipase C
POD	Peroxidase
PHHI	Persistent Hyperinsulinemia with Hypoglycaemia of infant
RIA	Radioimmunoassay
ROS	Reactive oxygen species
RT-PCR	Reverse-transcription-polymerase chain reaction
S.E.M	Standard error of mean
SERT	Serotonin transporter
SMOCCs	Second messenger operated calcium channels
SOD	Superoxide dismutase
STZ	Streptozotocin
T3	Triiodothyronine
T4	Thyroxine
TCA	Tricarboxylic acid
TM	Transmembrane
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone
VICCs	Voltage insensitive calcium channels
VOCC	Voltage sensitive calcium channels

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Introduction

Diabetes and its chronic complications lead to extensive quality of life and economic burdens that are shared across the world (Wild *et al.,* 2004; Ettaro *et al.,* 2004). It is indicative of inadequate or resistance of insulin for glucose metabolism. Insulin signalling regulates a large number of cellular processes (Shepherd *et al.,* 1998). Sustained periods of hyperglycaemia are considered a contributing factor in the development of diabetic complications, including retinopathy, nephropathy and neuropathy. Diabetic patients are also at increased risk for developing central nervous system (CNS) dysfunction (Biessels *et al.,* 1999; Ryan *et al.,* 2003; Allen *et al.,* 2004), including impaired central motor conduction (Tchen *et al.,* 1992; Abbruzzese *et al.,* 1993) and, on rare occasions, hemichorea-hemiballismus associated with nonketotic hyperglycaemia (Lee *et al.,* 2002). Diabetes associated complications are major reason for morbidity and mortality associated with this disease and is a major factor causing ramifications in life of diabetic patients. Even though insulin secretion is mainly regulated by changes in circulating concentrations of glucose and other metabolic fuels, stimuli such as neurotransmitters and gastrointestinal hormones make an important contribution to the overall regulation of pancreatic β cell function.

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. They exert their function through receptors present in both neuronal and non-neuronal cell surface that trigger second messenger signaling pathways (Julius *et al.,* 1989). 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. In pancreatic β-cells, IP_3 mobilizes Ca^{2+} from intracellular stores, resulting in an elevation of the intracellular concentration of Ca^{2+} and allowing activation of Ca^{2+}/calmodulin. DAG on the other hand, activates PKC (Nishizuka, 1995; Renstrom *et al.,* 1996). PKC, like Ca^{2+}/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).

Approaches to the control and prevention of hyperglycaemia are central to the management of diabetes (Herman & Crofford, 1997). The development of new dietary adjuncts and novel anti-diabetic agents, which reinstate a normal metabolic environment, thereby reducing the long term complications associated with diabetes. Such agents would both ideally stimulate the secretion and improve the action of insulin (Bailey & Flatt, 1995; Bashan *et al.,* 2009). It has been widely accepted that peripherally synthesized insulin can be transported into the brain *via* the cerebrospinal fluid. Recent molecular biological evidence suggests that it can also be synthesized *de novo* by neurons, because the presence of preproinsulin I and II mRNA or insulin receptor mRNA was observed in cultured neurons. Moreover, insulin immunoreactivity occurs in the endoplasmic reticulum and Golgi apparatus *in vivo* (Schechter *et al.,* 1998; Zhao *et al.,* 1999).

In the CNS, insulin seems to play an important role, particularly in the complications caused by diabetes, involving the regulation of brain metabolism (Shah *et al.,* 1993; Santos *et al.,* 1999), neuronal growth and differentiation (Schechter *et al.,* 1998; Gerozissis *et al.,* 2003), or neuromodulation (Gerozissis *et al.,* 2003; Rhoads *et al.,* 1984). Insulin also protects against brain damage, induced by stress conditions,

such as oxidative stress or ischemia (Santos *et al.,* 1999; Duarte *et al.,* 2003). Brain glucose utilization and metabolism are essential to cognitive functions, and a disturbance in both or in the desensitization of brain insulin receptors are involved in the intellectual decline in Alzheimer's disease and related neurodegenerative disorders (Biessels *et al.,* 2002; Hoyer, 2002), in which excitotoxicity and oxidative stress have been shown to occur (Bohr, 2002).

Traditional medicinal plants treatment for diabetes exists and therein lays a hidden wealth of potentially useful natural products for diabetes control (Bailey & Day, 1989**;** Gray & Flatt, 1997**;** Swanston-Flatt *et al.,* 1991). Despite this, few anti-diabetic medicinal plants have received scientific or medical scrutiny and the World Health Organization (1980) recommended that this area warrants further attention. Medicinal plants provide a potential source of anti-hyperglycaemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes. There are probably several contributing factors, including changes in the epidemiology of diabetes that attributes to its control. Gray & Flatt (1987) gathered scientific validation for the use of certain traditional anti-diabetic plants and this has encouraged botanical exploration in the quest for new anti-diabetic drugs. Additionally there is the wider appeal of 'natural' dietary adjuncts as functional foods through which patients can gain added benefits to the management of their disease (Swanston-Flatt *et al.* 1991). Many traditional anti-diabetic plants probably act at least in part through their fibre, vitamin or mineral content. Mineral deficiencies are common in diabetes and can exacerbate insulin resistance. Several of these minerals are co-factors for signalling intermediaries of insulin action and key enzymes of glucose metabolism.Several medicinal plants have anti-hyperglycaemic agents in the Indian system of medicines, including Ayurveda. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reports occur in numerous scientific journals (Mukherjee *et al.,* 2006).

Aegle marmelose Corr. (Rutaceae) commonly called as 'Koovalam' in Malayalam and 'Bael' in Hindi is indigenous to India. Preliminary reports indicate *Aegle marmeloes* leaf extract exhibits anti-diabetic action in glucose-induced hyperglycaemic rats (Sachdewa *et al.,* 2001) and in alloxan induced diabetic rats (Ponnachan *et al.,* 1993). *Aegle marmelose* extract, which is being used in the traditional medicine to reduce the serum glucose level, has significant antioxidant activity in vitro (Sabu & Kuttan, 2000). Diabetes has been shown to damage islet cells of pancreas by the liberation of oxygen radicals (Halliwell & Gutteridge, 1985). *Aegle marmelose* leaf extract is found to reduce blood sugar levels and markers of oxidative stress i.e. lipid peroxidation, conjugated diene and hydroperoxide levels in serum and catalase, glutathione and superoxide dismutase in blood and liver of rats (Maxwell *et al.,* 1997; Wen-Chi *et al.,* 2009). Natural antioxidants strengthen the endogenous antioxidant defences from reactive oxygen species (ROS) and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, *A. marmelose* is rightly mentioned as a medicinal plant of varied properties (Halliwell & Gutteridge, 1985).

Pyridoxine (Vitamin B_6) a water-soluble vitamin that exists in three major chemical forms: pyridoxine, pyridoxal and pyridoxamine (Leklem, 1999; Bender, 1989). It performs a wide variety of functions in your body and is essential for good health. Vitamin B_6 is needed for more than 100 enzymes involved in protein and red blood cell metabolism. Body needs vitamin B_6 to make hemoglobin which carries oxygen from red blood cells to tissues (Bender, 1994). Vitamin B_6 also helps increase the amount of oxygen carried by hemoglobin. Vitamin B_6 deficiency can result in iron deficiency anaemia (Allen & Kollas, 1997). Through its involvement in protein metabolism and cellular growth, is important to the immune system. It helps maintain

the health of lymphoid organs (thymus, spleen and lymph nodes) that make your white blood cells (Gerster, 1996). Animal studies show that a vitamin B_6 deficiency can decrease your antibody production and suppress immune response (Chandra & Sudhakaran, 1990). Vitamin B_6 helps maintain your blood glucose within a normal range. Beaton & Goodwin (1954) found that in vitamin B_6 deficient rats there was a significant decrease in fasting blood sugar, pyruvic acid, lactic acid and glycogen levels. Improper diet leads to deficient vitamin B_6 to help convert stored carbohydrate or other nutrients to glucose to maintain normal blood glucose levels (Huang & Wang, 1999).

Pyridoxine has been implicated for many years in the metabolism of proteins, amino acids and fat. Pyridoxine is used for relieving headaches and depression associated with low dose oral contraceptives (Villegas ***et al.,*** 1997). The nervous and immune systems need vitamin B_6 to function efficiently and it is also needed for the conversion of tryptophan (an amino acid) to niacin (a vitamin). Beaton ***et al.,*** (1956) found that pyridoxine deficient rats given daily injections of insulin were able to maintain an amount of body fat similar to their respective controls. Huber ***et al.,*** (1902) reported that insulin-like activity in the serum and pancreas of pyridoxine deficient rats was significantly lowered, compared to control rats. Pyridoxine functions in decarboxylation system as a coenzyme of amino acid decarboxylase. Snell (1958) reviewed and indicated that all amino acid decarboxylases in animal tissues and in bacteria were pyridoxal phosphate dependent. Neurotransmitters are the products of metabolic process. Pyridoxal phosphate (PLP), the major co-enzyme form of pyridoxine, inhibits GDH through Schiff's base formation with an amino group of a lysine residue (Anderson ***et al.,*** 1996; Cho ***et al.,*** 1996). The ratio of pyridoxal phosphate to pyridoxal in the plasma was decreased by streptozotocin (STZ) injection (Okada ***et al.,*** 1997). Pyridoxine deficient rat is showed a significant increase in glutamate concentration (Nayeemunnisa ***et al.,*** 1977).

Serotonergic control is suggested to exert different effects on insulin secretion according to the activation of different receptors subclasses (Pontiroli *et al.,* 1975, Schilman *et al.,* 2010). Our previous studies reported increased monoamine content in the plasma and platelet of diabetic patients (Jackson *et al.,* 1997). In addition to this mechanism, the secretion of insulin is dependent on the turnover ratio of endogenous 5-hydroxy tryptophan (5-HTP) to 5-HT in the pancreatic islets (Jance *et al.,* 1980). CNS is the pathway triggered by decrease in the brain 5-HT content brought about by a decrease in transport of tryptophan across the blood-brain-barrier (BBB). This transport of tryptophan across the BBB depends on the circulating tryptophan and decreased uptake of it into the brain leading to decreased brain 5-HT synthesis (Fernstorm & Fernstorm, 1995; Fernstorm, 1991; Fernstorm, 1979; Biggio *et al.,* 1974). This decreased brain 5-HT stimulates the over expression of 5-HT_{2A} receptors in the brain regions, which lead to sympathetic stimulation that inhibits insulin release (Jackson *et al.,* 2004). Once the circulating insulin content is reduced, it leads to an increase in large neutral amino acids, which compete with tryptophan for uptake into brain. Diabetes is a peculiar case because it is influenced by glutamate receptors present outside of the central nervous system and it also influences glutamate receptors in the central nervous system. Diabetes an endocrine disorder, induces cognitive impairment and defects of long-term potential in the hippocampus, interfering with synaptic plasticity. Defects of long-term potential in the hippocampus are due to abnormal glutamate receptors, specifically the malfunctioning NMDA glutamate receptors during early stages of the disease (Trudeau *et al.,* 2004).

Research is being done to address the possibility of using hyperglycaemia and insulin treatment to regulate these receptors and restore cognitive functions. Pancreatic islets regulating insulin and glucagon levels also express glutamate receptors (Weaver *et al.,* 1996). Metabotropic glutamate receptors (mGluRs)

indirectly activate ion-channels on the plasma membrane through a signaling cascade that involves G proteins. Ionotropic receptors tend to be quicker in relaying information but metabotropic are associated with a more prolonged stimulus. The increase in GDH activity in the diabetic group may be the cause for the increase in glutamate content (Nayeemunnisa ***et al.,*** 1977). Treatment using pyridoxine and insulin reversed the enzyme activity to control level (Aswathy ***et al.,*** 1998). Glutamate binding to the extracellular region of an mGluR causes G proteins bound to the intracellular region to be phosphorylated, affecting multiple biochemical pathways and ion channels in the cell (Platt, 2007). Because of this, mGluRs can both increase or decrease the exitability of the post synaptic cell, thereby causing a wide range of physiological effects. Over stimulation of glutamate receptors causes neuronal degradation and death through a process called excitotoxicity. Excessive glutamate, or excitotoxins acting on the same glutamate receptors, overactivates glutamate receptors, causing high levels of calcium ions (Ca^{2+}) to influx into the postsynaptic cell (Dubinsky, 1993). High Ca^{2+} concentrations activate a cascade of cell degradation processes involving proteases, lipases, nitric oxide synthase and a number of enzymes that damage cell structures often to the point of cell death (Manev ***et al.,*** 1989). Ingestion or exposure to excitotoxins that act on glutamate receptors induce excitotoxicity and cause toxic effects on the CNS (Meldrum, 1993).

Peripheral control of insulin secretion occurs directly within the pancreatic β-cells. During diabetes the amount of 5-HT within the pancreatic islets increases. This excess 5-HT binds and down regulates nuclear receptors and directly alter the transcription of insulin gene from the β-cells. The present work is to understand the alterations of serotonin, subtype $5\text{-}HT_{2A}$ receptors in brain regions and pancreatic islets of streptozotocin induced diabetic rats. Pharmacologically, Ayurveda, Aegle ***marmelose*** has a combination of constituents that are beneficial in the management of

diabetic stress. Glucose consumption in the brain is required to meet the energy demands of brain cells for metabolic and physiologic processes (Sokoloff ***et al.,*** 1977). Due to pyridoxine deficiency there is an impairment leading to brain cognitive functional activities in diabetes (Wei ***et al.,*** 1999). The work focuses on the evaluation of the anti-hyperglycaemic activity of pyridoxine alone and in combination with insulin and ***Aegle marmelose*** leaf extract and the changes in the Serotonin, subtype 5-HT_{2A}, glutamate receptors kinetics, gene expression and immunohistochemical studies during diabetes and regulation of insulin secretion. This will help to elucidate the role of serotonin, subtype 5-HT_{2A} and glutamate receptors in diabetes and the regulatory activity of this treatment on insulin secretion.

OBJECTIVES OF THE PRESENT STUDY

1. To study the anti-hyperglycaemic activity of pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extracts in streptozotocin induced diabetic animal model.

2. To measure the circulating insulin level, T3 concentration of pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract treated streptozotocin induced diabetic rats.

3. To quantify serotonin in the brain regions - cerebral cortex (CC), brain stem (BS), cerebellum (CB) and Hippocampus (Hippo) of pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract treated streptozotocin induced diabetic rats.

4. To study the serotonin, 5-HT_{2A} subtype receptor binding parameters in brain regions and pancreas of pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract treated streptozotocin induced diabetic rats.

5. To quantify glutamate content in the brain regions – brain regions and pancreas of pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract treated streptozotocin induced diabetic rats.

6. To study the Glutamate receptor binding parameters in brain regions and pancreas of pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract treated streptozotocin induced diabetic rats.

7. To study the expression of 5-HT_{2A}, 5-HT transporter, mGluR5 glutamate receptor subtype and GLAST glutamate transporter gene expression in the brain regions and pancreas of pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract treated streptozotocin induced diabetic rats using Real Time PCR.

8. To study the expression of insulin receptors and status of antioxidants-superoxide dismutase and glutathione peroxidase gene expression in the brain regions and pancreas of pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract treated streptozotocin induced diabetic rats using Real Time PCR.

9. To study the localization of 5-HT_{2A}, 5-HT transporter, mGluR5 glutamate receptor subtypes using confocal microscope by immunofluorescent receptor specific antibodies in the brain slices of CC, BS, CB, Hippo and pancreatic islets of pyridoxine alone and in combination with insulin and ***Aegle marmelose*** leaf extract treated streptozotocin induced diabetic rats.

10. To study the intracellular calcium release in pancreatic islets of pyridoxine alone and in combination with insulin and ***Aegle marmelose*** leaf extract treated streptozotocin induced diabetic rats.

11. To study the behavioural changes in Control and Experimental rats using Rotarod test, Elevated Plus-Maze and Beam walk test.

Literature Review

Diabetes mellitus is a metabolic disorder that either arrives during the early years of growth (Juvenille diabetes) or later in life or is called as maturity onset diabetes. It is observed as the body's inability to effectively regulate the sugar balance which leads to severe complications such as hyperglycaemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporosis and coma leading to death. Pancreatic damage resulting in the dysfunction of α and β cells causes disordered glucose homeostasis. In diabetic individuals the regulation of glucose levels by insulin is defective, either due to defective insulin production which is called as Insulin Dependent Diabetes Mellitus (IDDM) or due to insulin resistance that is termed as Non Insulin Dependent Diabetes Mellitus (NIDDM).

Diabetes and Brain

Glucose is the only fuel that the neuronal tissue can use for energy under normal circumstances (Sokoloff, 1981). The brain can neither synthesize nor store more than a few minutes worth of glucose; thus a continuous systemic supply is essential for normal cerebral metabolism (Pardridge, 1983). Chronic changes in the antecedent level of glycaemia (either sustained hyperglycaemia or hypoglycaemia) induce alterations in brain glucose metabolism in rodents (McCall *et al.,* 1982; Boyle *et al.,* 1994). Diabetes mellitus is a major global health problem that affects more than 185 million people around the world (Amos *et al.,* 1997; Zimmet, 1999; Zimmet *et al.,* 2001). This disease is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycaemia (Kumar & Clarke, 2002**;** Dunne *et al.,* 2004). The number of diabetic patients is expected to reach 300 million by the year 2025. The projected increase in the number of diabetic patients will strain the capabilities of healthcare providers the world over (Adeghate *et al.,* 2006). The pancreatic hormones

have an important role in the regulation of glucose metabolism. The secretion of insulin by ß-cells of the endocrine pancreas is regulated by glucose and other circulating nutrients. It is also modulated by several hormones and neurotransmitters, among which serotonin and glutamate plays a prominent role. DM is accompanied by an altered monoamine neurotransmission in the brain, mostly manifested by an increase in content and a decrease in turnover rate (McCall, 1992; Biessels *et al.,* 1994). However, the changes are not generalized, but are regionally specifically distributed, with the appearance dependent on the duration of diabetes (Bitar *et al.,* 1985; Chu *et al.,* 1986; Oliver *et al.,* 1989; Lackovié *et al.,* 1990; Ramakrishnan & Namasivayam, 1995). The specific regional character of these changes, particularly in the hypothalamus and the striatum, suggests functional correlations with the distinctive behavioural disorders like increased feeding. Speculation that an adaptation in the CNS might exist in patients with diabetes, depending upon antecedent glycaemia, appeared nearly a decade ago (Cryer & Gerich, 1985; Cryer, 2003). Hormonal defects with unawareness of symptoms can be induced in patients with diabetes (Hepburn *et al.,* 1991; Dagogo *et al.,* 1993) and non - diabetics (Davis & Shamoon, 1991; Heller & Cryer, 1991; Veneman *et al.,* 1993). Sleep, submissive and avoidance behaviour, depression, decreased sexual and aggressive behaviour, spontaneous locomotor activity, and cognitive dysfunctions that are observed in diabetes (Leedom & Mechan, 1989; Lustman et aI., 1992; Biessels *et al.,* 1994).

β-cells ability to proliferate in response to rising blood glucose concentrations is remarkably well preserved during severe, chronic beta-cell autoimmunity. Control of the destructive immune response after disease manifestation allows spontaneous regeneration of sufficient β-cell mass to restore normal glucose homeostasis (Pechhold *et al.,* 2009*b*). Declining glucose levels in the brain stimulate the autonomic nervous system, causing epinephrine and nor epinephrine to be released from the adrenal medulla. Nor epinephrine and acetylcholine from the sympathetic nervous

system is also involved in glucose control. Symptoms occur as these hormones and neurotransmitters simultaneously stimulate α-cells in the pancreas to release glucagon, which consequently induces new glucose production in the liver (Cryer, 1999, 2002 a, b, 2003). In this homeostatic mechanism, rising blood glucose levels shut down the neoglucogenesis activities of autonomic nervous system (Towler ***et al.,*** 1993; Cryer, 1997; McAulay ***et al.,*** 2001, Charles & Goh, 2005).

Diabetes, Oxidative Stress and Antioxidants

Oxidative stress is produced during normal metabolic process in the body as well as induced by a variety of environmental factors and chemicals. Oxidative stress has been shown to have a significant effect in the cause of diabetes as well as diabetes related complications in human beings (Wilson, 1998). Oxidative stress plays a central role in the pathogenesis of metabolic diseases like diabetes mellitus and its complications (like peripheral neuropathy) as well as in neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease (PD) (Biörkhem ***et al.,*** 2009; Eggers, 2009). Oxidative stress in diabetes has shown to co-exist with a reduction in the antioxidant status. (Trevisan ***et al.,*** 2001; Waden ***et al.,*** 2009). Oxidative stress has shown to produce glycation of proteins, inactivation of enzymes, alterations in structural functions of collagen basement membrane (Boynes, 1991). Oxidative stress may have significant effect in the glucose transport protein (GLUT) or in insulin receptor (Jacqueline ***et al.,*** 1997). Scavengers of oxidative stress may have an effect in reducing the increased serum glucose level in diabetes and may alle***via***te the diabetes as well as reduce its secondary complications. ***Aegle marmelose*** extract, which is being used in the traditional medicine to reduce the serum glucose level has significant antioxidant activity ***in vitro*** (Sabu & Kuttan, 2000). Alloxan, which is an accepted model for the induction of diabetes, has been shown to damage islet cells of pancreas by the liberation of oxygen radicals (Halliwell & Gutteridge, 1985).

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. (Kawamura *et al.,* 1992; Morgan *et al.,* 2002). Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms leads to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance. Plasma oxidized LDL, a commonly used marker for oxidative stress, is involved in the development of diabetes- and obesity-related traits (Wen-Chi Hsueh *et al.,* 2009). These consequences of oxidative stress can promote the development of complications of diabetes mellitus. Changes in oxidative stress biomarkers, including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipid peroxidation, nitrite concentration, nonenzymatic glycosylated proteins, and hyperglycemia in diabetes, and their consequences (Yan & Harding, 1997; Maritim *et al.,* 2003). The insulin signaling cascade constitutes a complex signaling network, adequately activated, in the induction of diverse biological functions. Insulin resistance is the reduced capacity of insulin to induce its biological actions in its target organs. Oxidants are commonly generated by various potential inducers of insulin resistance (Bashan *et al.,* 2009). Even in a healthy population, variations in insulin sensitivity are related to lipid hydroperoxyde levels, reduced catalase and Vitamin E levels (Facchini *et al.,* 2000). ROS oxidize various types of biomolecules, finally leading to cellular lesions by damaging DNA or stimulating apoptosis for cell death (Kaneto *et al.,* 1994).

Oxidative stress is produced during normal metabolic process in the body as well as induced by a variety of environmental factors and chemicals. Oxidative stress has been shown to have a significant effect in the cause of diabetes as well as diabetes

related complications in human beings (Wilson, 1998). Oxidative stress in diabetes has been shown to co-exist with a reduction in the antioxidant status. The exact role of oxidative stress in the etiology of human diabetes is however not known. Oxidative stress has been shown to produce glycation of proteins, inactivation of enzymes, alterations in structural functions of collagen basement membrane (Boynes, 1991). Oxidative stress have significant effect in the glucose transport protein (GLUT) or in insulin receptor (Jacqueline ***et al.,*** 1997). Scavengers of oxidative stress have an effect in reducing the increased serum glucose level in diabetes and alle***via***te the diabetes as well as reduce its secondary complications. ***Aegle marmelose*** extract, which is being used in the traditional medicine to reduce the serum glucose level has significant antioxidant activity invitro (Sabu & Kuttan, 2000). Alloxan, which is an accepted model for the induction of diabetes, has been shown to damage islet cells of pancreas by the liberation of oxygen radicals (Halliwell & Gutteridge, 1985).

β-Cell function: Physiology and Pathophysiology

Islets of Langerhans are microscopic organelles scattered diffusely throughout the pancreas. Each islet contains approximately 2000 cells, which include four types: α, β, δ, and PP cells. The major secretory products of these cells are glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. The α-cell secretes glucagon primarily in response to hypoglycemia, but also to amino acids. The β-cell secretes insulin in response to elevated glucose levels. Insulin response to intravenous glucose are time-dependent and referred to as first- and second-phase responses. The δ-cell releases somatostatin in response to glucose. The PP cell releases pancreatic polypeptide in response to hypoglycemia and secretin. The functions of these hormones are distinctly different. Glucagon stimulates glycogenolysis in the liver to increase blood glucose levels. Insulin decreases hepatic glucose production and increases glucose entry into muscle and fat cells. Somatostatin inhibits the secretion of

many hormones, including insulin and glucagon and likely is an intra islet paracrine regulator of α and β cells. The function of pancreatic polypeptide in humans remains unclear (Robertson & Harmon, 2006; Nakatsuji *et al.,* 2009).

The endocrine pancreas is richly innervated, but the abundance and organization of these innervations are highly variable between species (Kobayashi & Fujita, 1969). Most of the nerve fibers enter the pancreas along the arteries (Woods & Porte, 1974; Miller, 1981). Unmyelinated nerve fibers are found in the neighborhood of all islet cell types at the periphery and within the islet. At some distance from the islets, glial Schwann cells often form a thin sheet around nerve fibers on their travel toward and within the islet. In the vicinity of islet cells, however, it is not rare to see some nerve fibers lacking this glial protection and coming close to or ending blindly 20–30 nm from the endocrine cells (Legg, 1967; Watari, 1968; Kobayashi & Fujita, 1969; Shorr & Bloom, 1970; Fujita & Kobayashi, 1979; Bock, 1986; Radke & Stach (a), 1986; Radke & Stach (b), 1986). Alterations in induced and spontaneous autoimmune diabetes became apparent at diabetes onset, and differed markedly within islets compared with sub-islet-sized endocrine cell clusters and among pancreatic lobes. These changes are adaptive in nature, possibly fueled by worsening glycemia and regenerative processes (Pechhold *et al.,* 2009*a*). The autonomic innervation of the endocrine pancreas has several origins. The autonomic nervous system uses two interconnected neurons to control effector functions and is divided into two systems, the sympathetic and the parasympathetic nervous systems, according to the location of the preganglionic cell bodies. However, there are indications suggesting that these two systems are not always independent of each other, but display anatomical interactions (Berthoud & Powley, 1993) or share similar neurotransmitters (Sheikh *et al.,* 1988; Verchere *et al.,* 1996; Liu *et al.,* 1998).

The parasympathetic innervation

The preganglionic fibers of the parasympathetic limb originate from perikarya located in the dorsal motor nucleus of the vagus (Ionescu *et al.,* 1983; Luiten *et al.,* 1984; Ahrén *et al.,*1986; Rinaman & Miselis, 1987; Louis-Sylvestre, 1987; Berthoud *et al.,* 1990; Berthoud & Powley, 1991; Chen *et al.,* 1996) and possibly also in the nucleus ambiguus (Weaver, 1980; Sharkey & Williams, 1983; Sharkey *et al.,* 1984; Luiten *et al.,* 1984; Luiten *et al.,*1986) which are both under the control of the hypothalamus. They are organized in well separated branches travelling within the vagus nerves (cranial nerve X), and through the hepatic, gastric (Berthoud *et al.,* 1990; Berthoud & Powley, 1991) and possibly celiac branches of the vagus (Kinami *et al.,* 1997). They reach intrapancreatic ganglia that are dispersed in the exocrine tissue. These ganglia send unmyelinated postganglionic fibers toward the islets (Woods & Porte Jr, 1974; Berthoud *et al.,* 1981; Berthoud & Powley, 1990). Preganglionic vagal fibers release ACh that binds to nicotinic receptors on intraganglionic neurons. Postganglionic vagal fibers release several neurotransmitters: ACh, Vasoactive Intestinal Peptide (VIP), gastrin-releasing peptide (GRP), nitric oxide (NO), and pituitary adenylate cyclase-activating polypeptide (PACAP) (Bloom& Edwards,1981; Bloom *et al.,*1983; Knuhtsen *et al.,* 1985; Ahrén *et al.,* 1986; Knuhtsen *et al.,* 1987; Ekblad *et al.,* 1994; Sha *et al.,* 1995; Havel *et al.,* 1997; Ahrén *et al.,* 1999; Love & Szebeni , 1999; Wang *et al.,* 1999; Ahren, 2000; Myojin *et al;*2000). Cholinergic terminals are found in the neighbourhood of all islet cell types at the periphery and within the islet (Coupland, 1958; Esterhuizen *et al.,* 1968; Stach & Radke, 1982; Radke & Stach, 1986; Van der Zee *et al.,* 1992; Love & Szebeni, 1999).

The sympathetic innervation

The sympathetic innervation of the pancreas originates from preganglionic perikarya located in the thoracic and upper lumbar segments of the spinal cord (Furuzawa *et al.,* 1996). The myelinated axons of these cells traverse the ventral roots to form the white communicating rami of the thoracic and lumbar nerves that reach the paravertebral sympathetic chain (Chusid, 1979). Preganglionic fibers communicate with a nest of ganglion cells within the paravertebral sympathetic chain or pass through the sympathetic chain, travel through the splanchnic nerves, and reach the celiac (Sharkey & Williams, 1983; Fox & Powley, 1986; Brunicardi *et al.,* 1995; Furuzawa *et al.,* 1996; Ahrén, 2000) and mesenteric ganglia (Furuzawa *et al.,* 1996). Ganglia within the paravertebral sympathetic chain, and the celiac and mesenteric ganglia, give off postganglionic fibers that eventually reach the pancreas. The existence of intrapancreatic sympathetic ganglia has also been reported (Liu *et al.,* 1984; Luiten *et al.,* 1986; Luiten *et al.,* 1998). Pancreatic islets are innervated by autonomic fibres. Sympathetic neural cell bodies are located in the superior mesenteric and celiac ganglia, the splanchnic nerve and parasympathetic innervation comes from the vagus nerve (Cabrera-Vásquez *et al.,* 2009). The preganglionic fibers release ACh that acts on nicotinic receptors on intraganglionic neurons, whereas the postganglionic fibers release several neurotransmitters: norepinephrine, galanin, (Ahrén & Taborsky, 1986; Dunning *et al.,* 1988; Ahrén, 2000; Myojin *et al.,* 2000). A rich supply of adrenergic nerves in close proximity of the islet cells has been observed in several mammalian species (Esterhuizen *et al.,* 1968; Ahrén *et al.,* 1981; Stach & Radke, 1982; Radke & Stach, 1986). Neurons found in the CNS and in the sympathetic nervous system serve as links between ganglia and the effected organs (Elseweidy *et al.,* 2009).

Brain neurotransmitter changes during diabetes

Neurotransmitters have been reported to show significant alterations during hyperglycaemia resulting in altered functions causing neuronal degeneration. Neuropathic pain and neurons develop hyperexcitability in diabetic rats, attributed to disturbances in neurotransmitters pattern (Elseweidy *et al.,* 2009). Neurotransmitters have been reported to show significant alterations during hyperglycaemia resulting in altered functions causing neuronal degeneration. A significant increase in the catecholamine contents and activity of metabolising enzymes has been reported in experimental diabetes (Gupta *et al.,* 1992). Norepinephrine has been reported to increase in several brain regions during diabetes (Oreland & Shasken, 1983; Fushimi *et al.,* 1984; Chu *et al.,* 1986; Wesselmann *et al.,* 1988; Chen & Yang, 1991; Tassava *et al.,* 1992), but a significant decrease in NE has been reported in hypothalamus (Ohtani *et al.,* 1997), pons and medulla (Ramakrishna & Namasivayam, 1995). EPI levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrishna & Namasivayam, 1995). Streptozotocin- induced diabetes and acute insulin deficiency were demonstrated to result in increased content of EPI in the supra chiasmatic nucleus. In addition to this, a decreased turnover of dopamine in the ventromedial nucleus in diabetes was found to be reversed by insulin treatment (Oliver *et al.,* 1989). These data indicate that experimental diabetes and acute insulin deficiency result in the rapid onset of detectable alterations in EPI and DA activity in specific hypothalamic nuclei. This can lead to the development of secondary neuroendocrine abnormalities known to occur in the diabetic condition. The DA content was increased in whole brain, (Lackovic *et al.,* 1990; Chen & Yang, 1991) corpus striatum (Chu *et al.,* 1986), cerebral cortex and hypothalamus of diabetic rats (Tassava *et al.,* 1992; Ohtani *et al.,* 1997). The plasma DA content was decreased in diabetic rats (Eswar *et al.,* 2006). Serotonin (5-HT) content is increased in the brain

regions and hypothalamic nuclei (Lackovic *et al.,* 1990; Chen & Yang, 1991), but there are reports suggesting a decrease in brain 5-HT content during diabetes (Sandrini *et al.,* 1997; Sumiyoshi *et al.,* 1997; Jackson & Paulose, 1999). Brain tryptophan was also reduced during diabetes (Jamnicky *et al.,* 1991). Insulin treatment was reported to reverse this reduced tryptophan content to normal (Jamnicky *et al.,* 1993). The cerebellar cortex, like all other motor structures, receives serotoninergic innervation in the form of a plexus of fine varicose fibers that do not face any differentiated postsynaptic element (Ungerstedt, 1971; Chan-Palay, 1975; Bishop and Ho, 1985; Trouillas & Fuxe, 1993). Serotonin is therefore acting in this structure as a paracrine agent, released through volume transmission. *In vivo,* local ionophoretic applications of serotonin have been shown to modify the spontaneous activity of the Purkinje cells, suggesting that serotonin is able to alter the input–output function of the cortex (Strahlendorf *et al.,* 1988; Darrow *et al.,* 1990; Kerr & Bishop, 1992). It has been reported that application of serotonin potentiates the inhibition of Purkinje cells by exogenous GABA (Strahlendorf *et al.,* 1989, 1991; Kerr & Bishop, 1992) and inhibits their excitation by exogenous non-NMDA glutamatergic agonists *in vivo* (Hicks *et al.,* 1989; Kerr & Bishop, 1992; Netzeband *et al.,* 1993). Serotonin also modulates the potassium conductance activated by depolarization (Wang *et al.,* 1992) as well as the cationic conductance activated by hyperpolarization (Li *et al.,* 1993) recorded from Purkinje cells in cerebellar slices. Finally, serotonin may affect the efficiency of excitatory transmission at mossy fiber terminals (Maura *et al.,* 1991; Lu & Larson-Prior, 1996) and parallel fiber synapses in the molecular layer (Raiteri *et al.,* 1986; Maura & Raiteri, 1996). In this work we have combined electrophysiological recordings in thin slices of the rat cerebellar cortex with cell reconstruction and immunohistochemical methods to identify a new site of action of serotonin. During exercise, blood glucose fell with placebo but, unexpectedly, rose with exenatide. Plasma adrenaline (epinephrine) and noradrenaline (norepinephrine),

cortisol concentrations increased to a greater extent during exercise after exenatide (Khoo *et al.,* 2010).

Insulin secretion regulating factors

Glucose

Glucose is an important regulator of various β-cell processes including insulin biosynthesis and release. Glucose, over short intervals stimulates insulin biosynthesis at the level of translation (Permut *et al.,* 1972). Studies have shown that preproinsulin mRNA levels rise 4-10 folds in response to glucose stimulation. Studies of insulin gene expression in primary cultures of rat islets transfected Insulin I gene 5 'flanking sequence suggested that metabolic signal from glucose influx is transmitted through the insulin enhancer (German *et al.,* 1990).

Phosphorylation of glucose to glucose-6-phosphate serves as the rate limiting step in glucose oxidation (Schuit, 1996). Glucokinase acts as sensor during this process. The entry of glucose into β-cells is followed by an acceleration of metabolism that generates one or several signals that close ATP-sensitive K^+ channels in the plasma membrane. The resulting decrease in K^+ conductance leads to depolarisation of the membrane with subsequent opening of voltage dependent Ca^{2+} channels. The rise in the cytoplasmic free Ca^{2+} eventually leads to the exocytosis of insulin containing granules (Dunne, 1991; Gembal *et al.,* 1992). Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of protein kinase C within the β-cell (Harris, 1996). It is suggested that PKC is tonically active and effective in the maintenance of the phosphorylated state of the voltage-gated L-type Ca^{2+} channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar, 1994).

Fatty acids

Short chain fatty acids and their derivatives are highly active stimulators of insulin release in sheep (Horino *et al.,* 1968). Exogenous saturated long chain fatty acids markedly potentiated glucose-induced insulin release and elevated long chain acyl-CoA esters in the clonal β-cell line, HIT (Prentki *et al.,* 1992). A novel ester of succinic acid 1, 2, 3-tri-(methyl-succinyl) glycerol ester displayed stimulation of insulin release and biosynthetic activity in pancreatic islets of Goto-Kakizaki rats (Laghmich *et al.,* 1997). A monomethyl ester of succinic acid along with D-glucose is required to maintain the β-cell response to D-glucose (Fernandez *et al.,* 1996).

Amino acids

Amino acids act as potent stimulators of insulin release. L-Tryptophan, which is the precursor of 5-Hydroxytryptamine (5-HT) act as a stimulator of insulin release (Bird *et al.,* 1980). L-Arginine also stimulates insulin release from pancreatic β-cells. Several *in vitro* studies have suggested the production of nitric oxides from islet nitric oxide system have a negative regulation of the L-arginine induced secretion of insulin in mice.

Substrates derived from nutrients

Substrates like pyruvate (Lisa, 1994), citrate, ATP (Tahani *et al.,* 1979), NADH and NADPH involve in the indirect reflux stimulation triggered by food intake or local islet stimulation through the production of metabolites. The NADH acts as an intracellular regulator of insulin secretion. Heterotrimeric GTP-binding protein G□i is involved in regulating glucose induced insulin release (Konrad *et al.,* 1995). GTP analogues are also important regulators of insulin secretion (Lucia *et al.,* 1987). Glucose induced insulin secretion is accompanied by an increase in the islet content of cAMP (Rabinovitch *et al.,* 1976).

Glucagon

Glucagon is the hormone secreted by pancreatic α-cells. It has been shown that glucagon has a striking stimulatory effect on insulin release in the absence of glucose (Sevi, 1966). Cellular JNK2- and JNK1/JNK2-deficiency divert glucose from oxidation to glycogenesis due to increased glycogen synthase (GS) activity and induction of Pdk4 (Vijayvargia ***et al.,*** 2010). The presence of specific glucagon receptors on isolated rat pancreatic β -cells as well as a subpopulation of α- and δ-cells shows the relevance of glucagon on regulation of insulin secretion. Intra-islet glucagon appears to be a paracrine regulator of cAMP ***in vitro*** (Schuit, 1996). Glucagon stimulates insulin release by elevating cAMP. cAMP through activation of protein kinase A, increases Ca^{2+} influx through voltage dependent L-type Ca^{2+} channels, thereby elevating Ca^{2+} and accelerating exocytosis (Carina, 1993). Protein phosphorylation by Ca^{2+}/Calmodulin and cAMP dependent protein kinase play a positive role in insulin granule movement which results in potentiation of insulin release from the pancreatic β-cell (Hisatomi, 1996).

Somatostatin

This hormone is secreted by the pancreatic δ-cells of the islets of Langerhans. Somatostatin inhibits insulin release. Its action is dependent on the activation of G-proteins but not associated with the inhibition of the voltage dependent Ca^{2+} currents or adenylate cyclase activity (Renstrom ***et al.,*** 1996). Reports from our lab showed that Long-term low dose somatotropin and insulin treatment in regulating cholinergic and glutamergic receptors subtypes in ageing rats and rejuvenation of brain function (Savitha ***et al.,*** 2010).

Pancreastatin

Pancreastatin is known to be produced in islet β-cells and to inhibit insulin secretion. Pancreastatin is a modulator of the early changes in insulin secretion after increase of glucose concentration within the physiological range (Ahren ***et al.,*** 1996). It is reported to increase Ca^{2+} in insulin secreting RINm5F cells independent of extracellular calcium (Sanchez ***et al.,*** 1992).

Amylin

Amylin is a 37-amino acid peptide hormone co-secreted with insulin from pancreatic β-cells. Amylin appears to control plasma glucose ***via*** several mechanisms that reduce the rate of glucose appearance in the plasma. Amylin limits nutrient inflow into the gut and nutrient flux from the gut to blood. It is predicted to modulate the flux of glucose from liver to blood by its ability to suppress glucagon secretion. Amylin is absolutely or relatively deficient in type I - diabetes and in insulin requiring type II - diabetes (Young, 1997). It inhibits insulin secretion ***via*** an autocrine effect within pancreatic islets. Amylin fibril formation in the pancreas cause islet cell dysfunction and cell death in type II - diabetes mellitus (Alfredo ***et al.,*** 1994).

Adrenomedullin

Adrenomedullin is a novel hypotensive adrenal polypeptide isolated from a human pheochromocytoma and is structurally related to calcitonin gene related peptide and amylin. It has been suggested that besides being an adrenal hypotensive peptide, adrenomedullin is a gut hormone with potential insulinotropic function (Mulder ***et al.,*** 1996).

Galanin

Galanin is a 29 amino acid neuropeptide localised in the intrinsic nervous system of the entire gastrointestinal tract and the pancreas of man and several animal species (Scheurink ***et al.,*** 1992). It inhibits insulin secretion in rat, mouse and also in isolated human islets and pig. In isolated rat and mouse islets galanin inhibits insulin secretion by increasing the K^+ permeability and interfering with activation of adenylate cyclase and the activity of protein kinase C and cAMP. Among other functions, galanin inhibits insulin release (Ahren ***et al.,*** 1991), probably ***via*** activation of G proteins (Renstrom, 1996) by the mediation of activated galanin receptors.

Macrophage migration inhibitory factor

Macrophage migration inhibitory factor (MIF), originally identified as cytokinesand are secreted by T lymphocytes. It was found recently to be both a pituitary hormone and a mediator released by immune cells in response to glucocorticoid stimulation. Recently it has been demonstrated that insulin secreting β-cells of the islets of Langerhans express MIF and its production is regulated by glucose in a time and concentration dependent manner. MIF and insulin were both present within the secretory granules of the pancreatic β-cells and once released, MIF appears to regulate insulin release in an autocrine fashion. MIF is therefore a glucose dependent islet cell product that regulates insulin secretion in a positive manner and play an important role in carbohydrate metabolism (Waeber ***et al.,*** 1997).

Nerve growth factor

Nerve growth factor (NGF) is a neurotropic growth factor that promotes neurite outgrowth during development. This growth factor is capable of modulating β-cell plasticity because it promotes neurite-like outgrowth in fetal and adult pancreatic β-cells from primary cultures (Vidaltamayo ***et al.,*** 1996) and in RINm5F

and insulinoma cells (Polak *et al.,* 1993). In adult rat β-cells, *in vitro* NGF stimulates glucose induced insulin secretion. The presence of the high affinity receptor for NGF has been described in insulinoma cell lines as well as in foetal and adult β-cells. The adult β-cells synthesise and secrete NGF in response to increasing extra cellular glucose concentration (Vidaltamayo *et al.,* 1996). The effect of NGF on insulin secretion is partly mediated by an increase in calcium current through calcium channels (Rosenbaum *et al.,* 2001).

Neuropeptides

Immunocytochemistry has revealed the presence of three neuropeptides in the nerve terminals of pancreatic ganglia and islets of different species: Vasoactive intestinal peptide (VIP), gastrin releasing peptide (GRP) and pituitary adenylate cyclase activating polypeptide (PACAP).

Gastrin releasing peptide

Gastrin releasing peptide (GRP) consists of a 27 amino acid residue. It is localised to pancreatic nerves, including islet nerve terminals of several species. GRP released from the pancreas after vagal nerve activation stimulates insulin secretion (Knuhtsen *et al.,* 1987; Sundler & Bottcher, 1991). In islets, activation by GRP receptors is coupled to PLC and phospholipase D (Wahl *et al.,* 1992; Gregersen & Ahren, 1996).

Vasoactive intestinal peptide

Vasoactive intestinal peptide (VIP) stimulates insulin secretion in a glucose dependent manner accompanied by increased action of adenylate cyclase with increased formation of cAMP (Klinteberg *et al.,* 1996). VIP increases activity of sympathetic system, including release of catecholamines from the adrenal medulla and

lead to the release of the pancreatic glucagon and inhibition of insulin release, by the activation of adrenergic receptors (Jarrhult & Holst, 1978).

Pituitary adenylate cyclase activating polypeptide

Pituitary adenylate cyclase activating polypeptide (PACAP) is localised to the parasympathetic nerves and released by the activation of the vagus nerve (Ahren, 2000). It exists in two forms consisting of 27 and 38 amino acids and show 68% homology (Arimura & Shioda, 1995). PACAP stimulates insulin secretion in a glucose dependent manner accompanied by increased action of adenylate cyclase with increased formation of cAMP (Klinteberg *et al.,* 1996).

Serotonin and serotonin transporter

Serotonin content is increased in the brain regions and hypothalamic nuclei (Chen & Yang, 1991); (Lackovic *et al.,* 1990), but there are reports suggesting a decrease in brain 5-HT content during diabetes (Sandrini *et al.,* 1997; Sumiyoshi *et al.,* 1997; Jackson & Paulose, 1999). Ohtani *et al.,* (1997) have reported a significant decrease in extracellular concentrations of NE, 5-HT and their metabolites in the ventro medial hypothalamus (VHM). The ratio of 5-HIAA/5-HT was increased. A similar observation was reported by (Ding *et al.,* 1992) with a decrease in 5-HT in cortex (19%) and 5-HT turnover (5-HIAA/5-HT) that was increased by 48%. Chu *et al.,* (1986) has reported lower 5-HT levels in both hypothalamus and brainstem but not in corpus striatum. Insulin treatment brought about an increase in the cerebral concentration of 5-HIAA and accelerated the cerebral 5-HT turnover (Juszkiewicz, 1985). The 5-HIAA concentration was reported to be approximately twice as high as the controls regardless of duration of treatment. Brain tryptophan, the precursor of 5-HT, was also reduced in brain regions during diabetes (Jamnicky *et al.,* 1991). Insulin treatment was reported to reverse this reduced tryptophan content to normal

(Jamnicky *et al.,* 1993). There was a significant increase in 5-HIAA observed at 2-6 hours after insulin administration (Kwok & Juorio, 1987).

Insulin partly reversed the changes observed in the STZ-treated rats. There was a decrease in the muscarinic receptor number and axonal transport of receptor-bound opiate in STZ induced hyperglycaemia suggesting that impaired axonal transport of receptors partly involved in the neurological disturbance which is seen in diabetic patients (Laduron & Janssen, 1986). It has long been recognized that 5-hydroxytryptamine (serotonin; 5-HT) and its biosynthetic precursor tryptophan, play an important role in regulating immune functions through non-5-HT receptor interactions involving circulating tryptophan and kynurenine levels (Mossner & Lesch, 1998; Schrocksnadel *et al.,* 2006; Muller & Schwarz, 2007). Individual serotonin receptors, however, are expressed in many immune-related tissues and interactions at specific receptors are also known to modulate aspects of the immune response and inflammation (Stefulj *et al.,* 2000; Kubera, *et al.,* 2005; Yu, *et al.,* 2008). Within the CNS, serotonin and serotonin receptors have been strongly associated with normal function. Certain neuropsychiatric disorders that include depression, bipolar disorder, OCD, anorexia and schizophrenia have been linked to dysregulation of CNS serotonin (Lucki, 1998; Geyer & Vollenweider, 2008). Indeed, therapeutics for these disorders often include inhibition of the serotonin transporter (SERT) with selective serotonin reuptake inhibitor (SSRI) medications, or blockade of specific serotonin receptor subtypes. SSRIs also show an efficacy in treating aspects of cardiovascular disease associated with depression (Halaris, 2009), and have been demonstrated in animal models to have an anti-inflammatory effect (Abdel-Salam *et al.,* 2004, Holmes *et al.,* 2010). The mechanisms underlying the protective effect of antidepressants are not precisely known, but are predicted by some researchers to involve activation of the pituitary-adrenocortical system *via* increased central serotonin levels (Bianchi *et al.,* 1994), by modulation of cytokine levels in peripheral tissues (Xia *et al.,* 1996;

Kubera ***et al.,*** 2004), and by suppression of platelet activation (Serebruany ***et al.,*** 2003). Furthermore, acute SSRI administration has been shown to have a vasodilatory effect on the coronary artery that is cardioprotective (Van Melle ***et al.,*** 2004). Interestingly, TNF-α, as well as certain other cytokines, have been shown to influence both expression and transport activity of the serotonin transporter. In neuronally derived cells and choriocarcinoma cells, TNF-α, INF-γ, and IL1β increases function (Ramamoorthy ***et al.,*** 1995; Mossner ***et al.,*** 1998; Zhu ***et al.,*** 2006), whereas in B lymphocytes, IL4 decreases function (Mossner ***et al.,*** 2001), and in intestinal epithelial derived Caco-2 cells, TNF- α has been found to decrease both expression and transport activity of SERT (Foley ***et al.,*** 2007).The nature of the influence of cytokines on SERT function (e.g., facilitation or repression) likely depends on the cytokine and tissue modulation of synaptic serotonin levels in various brain regions by inflammatory cytokines would certainly be anticipated to have some effect on neuronal function relevant to psychiatric disorders like depression. In summary, there appears to be a strong link between proper functioning and regulation of the serotonin system and factors underlying cardiovascular disease and neuropsychiatric disorders (Uçeyler ***et al.,*** 2010). We hypothesize that a particular aspect of the serotonin system, the 5-HT_{2A} receptor, is a common and contributing factor underlying aspects of normal cardiovascular and CNS function. The dysfunction of this receptor results in certain characteristics of cardiovascular and neuropsychiatric disorders. There are seven families of serotonin receptors comprised of fourteen distinct subtypes (Nichols & Nichols, 2008). With the exception of the 5-HT_3 receptor, which is a ligand-gated ion channel, all are seven transmembrane-spanning G-protein coupled receptors. Of all the serotonin receptors, the 5- HT_{2A} receptor has been the one most closely linked to complex behaviours and neuropsychiatric disorders (Binder ***et al.,*** 2009). The 5-HT_{2A} receptor is highly expressed within the frontal cortex, with lower expression levels throughout the brain (Nichols & Nichols, 2008). There has been extensive

research performed to establish the role of 5-HT_{2A} receptors within the brain, where they have been shown to participate in processes such as cognition and working memory (Williams *et al.,* 2004). This mediate the primary effects of hallucinogenic drugs (Nichols, 2004) and has been implicated in mechanisms underlying schizophrenia (Vollenweider *et al.,* 1998; Aghajanian & Marek, 2000). Furthermore, abnormal expression of 5-HT_{2A} receptors has also been linked to depression. For example, some studies have shown that receptor protein expression is increased in certain cortical areas of patients with major depression (Bhagwagar *et al.,* 2006; Shelton *et al.,* 2009), as well as suicide victims (Pandey *et al.,* 2002; Oquendo *et al.,* 2006). 5-HT_{2A} receptor expression decreases, however, have been found in brain limbic regions of patients with major depressive disorder (Mintun *et al.,* 2004). CNS receptor dysfunction results in or contributes to the development of neuropsychiatric disorders including depression, bipolar disease and psychosis. This dysfunction either come from alterations in regulation due to promoter polymorphisms or other regulatory mechanisms influencing expression, or polymorphisms or mutations affecting the protein itself that could influence responsiveness and downstream signal transduction pathways. Polymorphisms in the promoter region of the human HTR_{2A} locus have been shown to alter receptor expression levels (Myers *et al.,* 2007), and these same polymorphisms have been linked to response to antipsychotics and certain SSRIs (Choi, *et al.,* 2005; Benmessaoud, *et al.,* 2008), and in some studies positively associated with various CNS conditions including major depression, bipolar disorder, and schizophrenia (Chee *et al.,* 2001; Choi, *et al.,* 2004; Penas-Lled *et al.,* 2007; S´aiz *et al.,* 2007). Polymorphisms within the coding regions of the HTR_{2A} ocus have been found in some studies to be positively associated with neuropsychiatric disorders. There is significant opportunity for future research to investigate how 5-HT_{2A} receptor function mediates certain aspects of both neuropsychiatric and metabolic effects of atypical antipsychotics. If they did, then perhaps long-term therapy with these new

highly selective receptor antagonists would produce metabolic and cardiovascular disorders. The widespread expression and importance of the 5-HT_{2A} receptor, the knockout animal appears overly normal. There are, however, certain behavioural effects associated with loss of this receptor (Weisstaub *et al.,* 2006; Salomon *et al.,* 2007). Interestingly, some observed behaviours are opposite to the effects of receptor antagonists (Popa *et al.,* 2005), indicating that caution should be exercised in the interpretation of knockout studies using this model. Nevertheless, studies utilizing this mouse in models of cardiovascular related diseases will likely be of value. A better understanding of the relationship between 5-HT_{2A} receptor function and its roles in both the CNS and cardiovascular system should lead to development of improved therapeutics to treat diseases affecting each of these systems either separately or together.

Serotonin receptors in diabetes

5-HT receptors comprise a complex family. On the basis of their pharmacology, signal transduction mechanisms and molecular structure, more than a dozen types of 5-HT receptors have been identified (Hoyer *et al.,* 1994). Most of these receptors are coupled to various G proteins with the exception of the 5-HT3 receptor, which is a ligand gated cation channel (Derkach *et al.,* 1989; Maricq *et al.,* 1991; Jackson & Yakel 1995). Multiple 5-HT receptor subtypes are expressed in the cerebral cortex (Mengod *et al.,* 1997). In cerebral cortex, 5-HT3 receptors are only expressed in inhibitory neurons (Morales & Bloom 1997) whereas 5-HT2A receptors are heavily expressed in pyramidal cells and to a lesser extent in inhibitory neurons (Willins *et al.,* 1997; Hamada *et al.,* 1998; Jakab & Goldman-Rakic 1998). Since the 1960s, many experiments using *in vivo* microiontophoretic methods have characterized how 5-HT affects neuronal behaviour. The predominant effect of 5-HT on cerebral cortical pyramidal neurons is an inhibition of spontaneous spiking. (Phillis 1984; Reader &

Jasper 1984; Jacobs & Azmitia 1992). Intracellular studies in rat cortical slices suggested that 5-HT induces depolarization and action potential firing in pyramidal cells (Davies *et al.,* 1987; Araneda & Andrade 1991; Tanaka & North 1993). Furthermore, Aghajanian & Marek (1997) reported that 5-HT enhances spontaneous excitatory postsynaptic currents (sEPSCs) without significantly changing spontaneous inhibitory postsynaptic currents (sIPSCs) in frontal pyramidal neurons. These in vitro results suggest that 5-HT is mainly excitatory in cortical neuronal circuitry. 5-HT and a-methyl-5-HT had no effect on sEPSCs in l ayer I neurons. Even though sampling bias might have contributed to this observation, the fact that activation of 5-HT_{2A} receptors induced robust enhancement of sEPSCs in all pyramidal neurons tested suggests that this differential modulation of sEPSCs in the two cell types was real. 5-HT_{2A} receptor expression is high in pyramidal neuron proximal apical dendrites and low in distal parts (Willins *et al.,* 1997; Jakab & Goldman-Rakic, 1998). It is possible that activation of dendritic 5-HT_{2A} receptors induce dendritic transmitter release and/or release of retrograde messenger(s).

Hyperglycaemia induced by 5-HT_{2A} Receptor Stimulation

Administration of a selective 5-HT_{2A} receptor agonist DOI produced a rapid increase in blood glucose level. Administration of DOI is also accompanied by an increase in EPI concentration (Glennon, 1987). Pre-treatment of the animals with 5-HT_{2A} receptor antagonists i.e., ketanserin are able to reverse the increase in sympathetic nerve discharge produced by DOI (Hoyer 1988c; McCall & Hornis, 1988; Chaouloff *et al.,* 1990b). These findings show that the central 5-HT_{2A} receptor stimulates sympathetic nerve discharge which in turn increases EPI release from adrenal medulla similar to the 5-HT_{1A} receptor activation.

Administration of the 5-HT_{2A} receptor agonist, α-methyl-5-HT, also elicit hyperglycaemic effects which are blocked by 5-HT_{2A} antagonist ketaserin (Chaouloff *et al.,* 1990b). α-methyl-5-HT was able to suppress food intake by food-deprived rats and also inhibited 2-deoxy-D-glucose induced hyperphagia in rats. α-methyl-5-HT induced hyperphagia was antagonised by ketanserin (Sugimoto *et al.,* 1996; Yamada *et al.,* 1997) Intraperitonial administration of 5-HT brought about hyperglycaemia mediated through a dose dependent increase in plasma EPI level. 5-HT induced hyperglycaemia was abolished by pre-treatment with ketanserin and also adrenodemedullation (Yamada *et al.,* 1995). This suggests that the hyperglycaemic effects of 5-HT are closely related to the decrease of EPI from the adrenal gland, mediated by 5-HT_{2A} receptors. 5-HT_{2A} receptors are partly involved in the pharmacological effects of induction of hyperglycaemia induced by the 5-HT_4 receptor agonist 5-methoxytryptamine.

Glutamate receptors in diabetes

Diabetes mellitus induces cognitive impairment and defects of long-term potentiation in the hippocampus as indicated by behavioural and electrophysiological analysis. Considered to be an important mechanism of learning and memory in mammals, long-term potentiation is known to require regulation of the glutamate receptor properties. According to many studies, defects of long-term potentiation in the hippocampus of diabetic animals are due to abnormal glutamate receptors. Earlier studies explained that changes in glutamate receptors account for modifications of long-term potentiation in various models of diabetes mellitus. Deficits in long-term potentiation during chronic diabetes arise from dysfunction of glutamate receptors in early stages of the disease (Trudeau *et al.,* 2004). Previous studies demonstrated that disruption of glutamate homeostasis occurs in the diabetic retina (Qing & Donald, 2002). Binding properties of brain glutamate receptors of STZ induced rats and the possible role of AMPA receptors in cognitive deficits during diabetes is reported by

Gagne *et al.,* (1997). Altered glutamatergic neurotransmission and calcium homeostasis contribute to retinal neural cell dysfunction and apoptosis in diabetic retinopathy. Elevated glucose is reported to change the expression of ionotropic glutamate receptor subunits and impairs calcium homeostasis in retinal neural cells (Ana *et al.,* 2006). It is suggested that enzymes of the glutamate system respond differently towards diabetes or deprivation of food and diabetes affect the glutamate uptake system in glial cells (Galanopoulos *et al.,* 1988). Recent studies suggest that glutamate plays a pivotal role in the processing of sensory information in the spinal cord of patients with diabetic neuropathy. Abnormal expression of multiple glutamate receptors is involved in the development of diabetic neuropathy (Tomiyama *et al.,* 2005).

Studies reported that neurons impaired of energy metabolism are highly sensitive to excitotoxicity (Simon *et al.,* 1984; Wieloch, 1985; Monyer *et al.,* 1989; Cebers *et al.,* 1998). Pathophysiological mechanisms responsible for neuronal cell death in hypoglycaemia include the involvement of glutamate excitotoxicity.

Inositol 1, 4, 5-trisphosphate (IP3) and activation of calcium release

Cytosolic Ca^{2+} is a focal point of many signal transduction pathways and modulates a diverse array of cellular activities ranging from fertilization to cell death (Berridge *et al.,* 2000). In most cell types, the major internal [Ca^{2+}] stores are the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR). One mechanism for mobilizing such stores involves the phosphoinositide pathway. The binding of many hormones to specific receptors on the plasma membrane leads to the activation of an enzyme, phosphoinositidase C that catalyses the hydrolysis of phospholipids to produce the intracellular messenger. Although derived from a lipid, IP3 is water soluble and diffuses into the cell interior where it encounters IP3 receptors (IP3Rs) on the ER/SR. The binding of IP3 changes the conformation of IP3Rs such that an

integral channel is opened, thus allowing the [Ca^{2+}] stored at high concentrations in the ER/SR to enter the cytoplasm. A critical feature of IP3Rs is that their opening is regulated by the cytosolic Ca^{2+}concentration. This sensitivity to cytosolic [Ca^{2+}] allows them to act as Ca^{2+}-induced calcium release (CICR) channels that promote the rapid amplification of smaller trigger events.

Alterations of glucose transport during diabetes

In diabetes mellitus apart from raised blood glucose levels, disturbances in the metabolism of a number of other biomolecules such as glycogen, lipids, proteins and glycoproteins have also been reported (Randle *et al.,* 1963; Williamson *et al.,* 1968). Treatment with insulin generally rectifies these disturbances in diabetic state as it increases the peripheral utilisation of glucose by influencing key enzymes of glucose metabolic pathways (Exton *et al.,* 1966; Lenzen *et al.,* 1990). The liver plays a major role in insulin-regulated glucose homoeostasis through the balance between glucose utilization and glucose production, both processes being tightly coordinated (Nevado *et al.,* 2006). More recently, it has been shown that glucose uptake and release required a family of membrane facilitated-diffusion glucose transporters which are expressed in a tissue-specific manner. In muscle and fat, GLUT-4 is the main isoform of glucose transporters (Burant *et al.,* 1991). In adipose tissue the concentrations of GLUT-4 protein and mRNA are markedly decreased after 2-3 weeks of diabetes, and they are restored by insulin therapy (Berger *et al.,* 1989; Garvey *et al.,* 1989), whereas in skeletal muscle the concentrations of GLUT-4 protein and mRNA are marginally altered (Garvey *et al.,* 1989; Bourey *et al.,* 1990). In liver, GLUT-2 is the main isoform of glucose transporters (Thorens *et al.,* 1988). Much less information is available concerning the expression of GLUT-2 in liver of diabetic rats. Vitamin D3 functional regulation through dopaminergic, cholinergic and insulin receptors and

glucose transport mechanism through GLUT3 in the cerebellum of diabetic rats which play a major role in neuroprotection in diabetes which has clinical application ().

Electrophysiological changes during diabetes

Neuroelectrophysiological recordings represent a non-invasive and reproducible method of detecting central and peripheral nervous system alterations in diabetes mellitus (Morano *et al.,* 1996). Diabetes mellitus is associated with chronic complications such as nephropathy, angiopathy, retinopathy and peripheral neuropathy. In diabetic patients, hyperglycaemia may precipitate seizures, and in experimental diabetes, indications for an increased neuronal excitability have been found (Anderson *et al.,* 2006). Neurophysiological alterations have also been described in animal models of diabetes, in particular in rats. In the peripheral nervous system (PNS) of diabetic rats the time course of neurophysiological changes is well established. Deficits in both motor and sensory nerve conduction velocity (MNCV and SNCV, respectively) can be detected within weeks after the onset of diabetes and increase up to 2–3 months after diabetes onset, remaining relatively stable thereafter (Moore *et al.,* 1980; Cameron *et al.,* 1986; Brismar *et al.,* 1987; Kappelle *et al.,* 1993). Studies of MNCV and SNCV in diabetic rats have made important contributions to the elucidation of the pathogenesis of the effects of diabetes on the PNS, as well as in the development of putative pharmacotherapy. Neurophysiological alterations have also been reported in the CNS of diabetic rats. Less is known about the underlying mechanisms of alterations in the CNS in diabetic rats. Cerebral metabolic (Knudsen *et al.,* 1989; Kumar and Menon, 1993) and vascular (Duckrow *et al.,* 1987; Jakobsen *et al.,* 1990) disturbances have been demonstrated within weeks after diabetes induction. However, the severity of these disturbances appears to be limited compared with the PNS (Biessels *et al.,* 1994), possibly leading to a less hostile neuronal microenvironment.

Neurotransmitters alterations in diabetes

Diabetes mellitus is a metabolic disorder that either arrives during the early years of growth (Juvenille diabetes) or later in life called as maturity onset diabetes. It is observed as the body's inability to effectively regulate the sugar balance which leads to severe complications such as hyperglycaemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporesis and coma leading to death. Pancreatic damage resulting in the dysfunction of α and β cells causes disordered glucose homeostasis. In diabetic individuals the regulation of glucose levels by insulin is defective, either due to defective insulin production which is called as Insulin Dependent Diabetes Mellitus (IDDM) or due to insulin resistance that is termed as Non Insulin Dependent Diabetes Mellitus (NIDDM).

Diabetes mellitus has been reported to cause degenerative changes in neurons of the central nervous system (Garris, 1990; Lackovic *et al.,* 1990; Bhattacharya & Saraswathi, 1991). Our previous studies demonstrated adrenergic, serotonergic and dopamine D_2 receptor function alterations in the brain of diabetic rats (Abraham & Paulose, 1999; Padayatti & Paulose, 1999; Paulose *et al.,* 1999; Eswar *et al.,* 2007). The concentration of 5-HT, DA and NE increased in the brain regions of diabetic rats and accumulation of these monoamines is produced by inhibition of monoamine oxidase activity (Salkovic & Lackovic, 1992). Norepinephrine has been reported to increase in several brain regions during diabetes. Ohtani *et al.,* (1997) have reported a significant decrease in extracellular concentrations of NE, 5HT and their metabolites in the ventro medial hypothalamus (VMH). Epinephrine (EPI) levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrisha & Namasivayam, 1995). Diabetes is reported to cause a high level of degeneration in neurons in different regions of the brain. Strepozotocin -induced diabetes and acute

deficiency of insulin is reported to result in increased concentrations of EPI in the supra chiasmatic nucleus. It is also reported that β-adrenergic receptor populations were decreased in diabetes (Garris, 1995). 5-HT content in the brain is reported to be decreased during diabetes (Chu *et al.,* 1986; Sumiyoshi *et al.,* 1997 Jackson & Paulose, 1999). Garris, (1995) reported chronically elevated levels of NE in the brain regions of amydgala, hypothalamus and medulla of diabetic mice. This was proposed to be associated with the expression of the gene causing diabetes mellitus. Hyperglycaemia is reported to alter the noradrenergic and cholinergic nerve components (Akria *et al.,* 1994) with decrease in the Na^+ - K^+ ATPase activity in different brain regions (Gurcharan & Sukwinder, 1994). NE, DA and 5-HIAA are reported to be increased in the heart and adrenal gland in STZ rats. In the heart the initial changes in short-term diabetes included an increase in NE concentration but did not persist in the long term diabetic animals. In the adrenal gland there was an initial reduction followed by a steady increase in the concentration of NE and EPI (Cao & Morrison, 2001). Studies of Gireesh *et al.,* (2008a) showed that there is a decrease in total muscarinic and muscarinic M1 receptors during diabetes in the cerebral cortex. A decreased muscarinic M1 receptor gene expression in the hypothalamus, brainstem, and pancreatic islets of diabetic rats was also demonstrated by Gireesh *et al.,* (2008b).

Glucose in brain, supplies energy essential for maintenance of the nervous system. It is reported that 2-D Glucose augmented the turnover of NE, DA and 5-HT under the fasted condition. Insulin perfusion within the medial hypothalamic sites evoked a significant increase in the synthesis and release of DA from the sated rat, but did not alter its turnover. However, in the interval following insulin perfusion, DA and 5-HT turnover were enhanced while the efflux of 5-HT was suppressed.

Severe deterioration in cognitive function and personality in patients with long-standing diabetes as a complication of a consequence of insulin treatment is reported (Gold *et al.,* 1995). The diabetic hippocampus adapt to high circulating

glucose, with increased susceptibility to reductions in glucose availability. This is accompanied by alterations within the hippocampus, including both ECF glucose and lactate levels during cognitive testing and electrophysiological function.

Exposure to stress is known to precipitate or exacerbate many neuropsychiatric disorders such as depression, Parkinson's disease, schizophrenia, and others (Schwab & Zieper, 1965; Mazure, 1995). All these disorders involve a working memory deficit caused by prefrontal cortical (PFC) dysfunction (Mattes, 1980; Weinberger *et al.,* 1986; Deutch, 1993; Fibiger, 1995). This increase of DA from the dendrites of dopamine neurons is due to an alteration in GABA regulation of the dopamine neurons. As with noradrenergic systems, single or repeated exposures to stress potentiates the capacity of a subsequent stressor to increase DA function in the forebrain without altering basal DA turnover, suggesting that the receptors have been hyper-sensitized (Basso *et al.,* 1999). DA neurons are vulnerable to metabolic stress (Callahan *et al.,* 1998). The maintenance of normal energy metabolism in T1DM during hypoglycaemia effect glucose sensing in the brain and contribute to hypoglycaemia-associated autonomic failure (Bischof *et al.,* 2006).

The projected increase in the number of diabetic patients will strain the capabilities of healthcare providers the world over (Adeghate *et al.,* 2006). The pancreatic hormones have an important role in the regulation of glucose metabolism. The secretion of insulin by ß-cells of the endocrine pancreas is regulated by glucose and other circulating nutrients. The progression of diabetes is associated with an impaired ability of the neurons in the CNS to release neurotransmitters (Broderick & Jacoby, 1989). Neurotransmitters show significant alterations during hyperglycaemia and causes degenerative changes in neurons of the central nervous system (Garris, 1990; Lackovic *et al.,* 1990; Bhardwaj, *et al.,* 1999). Studies on STZ-induced diabetic rat models have shown similar results which exhibits morphological, behavioural and electrophysiological alterations on diabetes (Jakobsen *et al.,* 1987; Biessels *et al.,*

1996; Chabot *et al.,* 1997). Learning and memory deficits are associated with Type I and Type II diabetes mellitus (Gispen & Biessels, 2000) and brain morphological abnormalities have been found in diabetic patients, mainly in the cortical area (Dejgaard *et al.,* 1991). STZ-induced diabetes results in structural alterations of mAChRs in the brain (Latifpour *et al.,* 1991) which in turn alters cholinergic nerve components (Akria *et al.,* 1994) with decrease in the Na^+, K^+-ATPase activity (Gurcharan & Sukwinder, 1994). Studies of Latifpour and McNeill, (1984) on long-term STZ-induced diabetes reported large reduction in muscarinic receptor densities as compared with their age-matched controls. Diabetes is intimately related at a molecular level and hence diabetes is able to provide the link between disease treatment and the prevention of age-related diseases. If specific molecular pathways controlling the rate of ageing are modulated genetically, then perhaps they are modulated pharmacologically (Geesaman, 2006). These insights ultimately have an important impact on the discovery and development of drugs to both treat and prevent a wide range of diseases.

Factors affecting insulin regulation from pancreatic β-cells

D-Glucose is the major physiological stimulus for insulin secretion. The mechanism of glucose induced insulin release is not completely understood. Phosphorylation of glucose to glucose-6-phosphate serves as the rate limiting step in glucose oxidation (Schuit, 1996). Glucokinase acts as a glucose sensor during this process. An increased ATP/ADP ratio is believed to close K^+-ATP channel at the plasma membrane, resulting in decreased K^+ efflux and subsequent depolarisation of the β-cell (Dunne, 1991). Depolarisation activates voltage-dependent Ca^{2+} channels, causing an influx of extracellular Ca^{2+} (Liu *et al.,* 1998). Although intracellular Ca^{2+} activates protein kinases such as Ca^{2+} and calmodulin dependent protein kinase (Breen & Aschcroft, 1997), it remains unclear how increase in intracellular Ca^{2+} leads to

insulin release. Intracellular Ca^{2+}stores appear to regulate a novel plasma membrane current [Ca^{2+}] release activated non-selective cation current], whose activity control glucose activated secretion. Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of PKC within the β-cell (Harris *et al.,* 1996). It is suggested that PKC is tonically active and effective in the maintenance of the phosphorylated state of the voltage-gated L-type Ca^{2+} channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar *et al.,* 1994). Glucose is an important regulator of various β-cell processes including insulin biosynthesis and release. Glucose, over short intervals stimulates insulin biosynthesis at the level of translation (Permut & Kipnis, 1972). Studies have shown that preproinsulin mRNA levels raise 4-10 folds in response to glucose stimulation. Studies of insulin gene expression in primary cultures of rat islets transfected Insulin I gene 5 -flanking sequence suggested that metabolic signal from glucose influx is transmitted through the insulin enhancer (German *et al.,* 1990).

ROLE OF NEUROTRANSMITTERS IN INSULIN REGULATION

Epinephrine and Norepinephrine

Epinephrine and norepinephrine has an antagonistic effect on insulin secretion and glucose uptake (Renstrom *et al.,* 1996; Porte, 1967). They also inhibit insulin stimulated glycogenesis through inactivation of glycogen synthase and activation of phosphorylase with consequent accumulation of glucose-6-.phosphate. In addition, it has been reported that epinephrine enhances glycolysis through an increased activation of phosphofructokinase. EPI and NE at low concentrations bind and activate β-adrenergic receptors which in turn stimulate the insulin secretion from pancreatic islets and at high concentration they can bind to α_{2A} receptors and inhibit insulin secretion (Lacey *et al.,* 1993). Previous studies had shown that in diabetic

condition α_{2A} receptors are more activated which brought out the insulin inhibition and in turn hyperglycaemia (Lacey *et al.,* 1993). Rat islet cell membrane is equipped with α_{2A}-adrenoceptors (Filipponi *et al.,* 1986) which are linked to adenylyl cyclase inhibiting insulin secretion.

NE and EPI, the flight and fright hormones are released in all stress conditions and are the main regulators of glucose turnover in strenuous exercise (Simartirkis *et al.,* 1990). In severe insulin induced hypoglycaemia, a 15 to 40 -fold increase of epinephrine plays a pivotal role in increasing glucose production independently of glucagon (Gauthier *et al.,* 1980). In humans, adrenaline stimulates lipolysis, ketogenesis, thermogenesis and glycolysis and raises plasma glucose concentrations by stimulating both glycogenolysis and gluconeogenesis. It is already known that, when used in high doses *in vivo* or *in vitro*, EPI reduces the insulin response to stimulators (Malaisse, 1972). *In vitro* studies with yohimbine showed that the insulin secretion from the pancreatic islets increased significantly suggesting that when the α_2-adrenergic receptors are blocked, it enhances islet cell proliferation and insulin secretion. Our previous studies demonstrated the role of α and β-adrenergic receptors in the insulin secretion (Ani *et al.,* 2006a; b; c). We also reported the effect of NE in DA mediated insulin secretion (Eswar *et al.,* 2006).

Acetylcholine

Acetylcholine is the neurotransmitter of the parasympathetic system. Cholinergic receptors are classified as ionotropic nicotinic receptor and metabotropic muscarinic receptor. Acetylcholine increases insulin secretion through muscarinic receptors in pancreatic islet cells (Tassava *et al.,* 1992; Greenberg & Pokol, 1994). Muscarinic receptors are classified as M_1, M_2, M_3, M_4 and M_5. They are G protein coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions that interacts with G-proteins and other effector

molecules to mediate the physiological and neurochemical effects. Expression studies have revealed the presence of M_1 and M_3 receptors in the pancreas. Acetylcholine is reported to be involved in the activation of glucose transport in the chromaffin cells. The cholinergic activation affecting this process is coupled with calmodulin and protein kinase C (Serck-Hanssen *et al.,* 2002). It is reported that the role of acetylcholine in insulin secretion is mediated through M_1 and M_3 receptors (Renuka *et al.,* 2004; 2005; 2006).

γ-Aminobutyric acid

Gamma aminobutyric acid (GABA) is the major inhibitory neurotransmitter in central nervous system. GABA is reported to be present in the endocrine pancreas at concentrations comparable with those found in central nervous system. The highest concentration of GABA within the pancreatic islet is confined to β-cells (Sorenson *et al.,* 1991). Glutamate decarboxylase, the primary enzyme that is involved in the synthesis of GABA, has been identified as an early target antigen of the T-lymphocyte mediated destruction of pancreatic β-cells causing insulin-dependent diabetes mellitus (Baekkeskov *et al.,* 1990). The brain GABAergic mechanisms also play an important role in glucose homeostasis. Recent studies reported the regulatory role of GABA during pancreatic regeneration (Balaram *et al.,* 2007; 2008). Also, we reported the role of GABA in hepatocyte proliferation (Biju *et al.,* 2002). GABA through its receptors has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic α-cells and δ-cells respectively (Gaskins *et al.,* 1995). GABA which is present in the cytoplasm and in synaptic-like microvesicles is co-released with insulin from β-cells in response to glucose (Reetz *et al.,* 1991). GABA inhibits islet α and δ-cell hormonal secretion in a paracrine manner. GABA release is decreased in diabetes resulting in the enhancement of glucagon secretion from α-cells leading to hyperglycaemia. GABA is involved in the maintenance of glucose homeostasis and

inhibition of central $GABA_A$ receptors increasing the plasma glucose concentration (Lang, 1995). Thus, any impairment in the GABAergic mechanism in central nervous system and/or pancreatic islets is important in the pathogenesis of metabolic stress.

Serotonin

Brain serotonin content decreased during diabetes (Jackson & Paulose, 1999). This decrease is reported to be due to a decrease in uptake of tryptophan through the blood brain barrier (BBB) (Fernstrom & Wurtman, 1971; Fernstrom & Wurtman, 1972; Madras *et al.,* 1974) and a decrease in rate of 5-HT synthesis (Carndall *et al.,* 1981). The turnover rate of 5-HT to 5-HIAA in diabetic rats was also reported to be lower (Kwok & Juorio, 1987; Sandrini *et al.,* 1997). A decrease in brain 5-HT lead to an up regulation of $5\text{-}HT_{2A}$ receptors of cerebral cortex and brain stem which in turn inhibit insulin secretion due to increased sympathetic activity (Jackson & Paulose, 1999). Our earlier studies reported the role of serotonin in cell proliferation (Sudha & Paulose, 1998). The functional regulation of brain 5-HT during pancreatic regeneration is also reported (Mohanan *et al.,* 2005a, b; 2006).

Glutamate

Although the role of glutamate as a signaling molecule is well established in the central nervous system, a similar role in the periphery has only recently been suggested. Inagaki *et al.,* (1995) and Weaver *et al.,* (1996) have detected functional glutamate receptors in the pancreatic islets of Langerhans. Pancreas is composed of four major cell types: the insulin-secreting β-cell, the glucagon-secreting α-cell, the pancreatic polypeptide-secreting PP cell and the somatostatin-secreting delta cells. The electrically excitable β-cells are stimulated to secrete insulin in response to changes in serum glucose concentrations. Secretion of insulin and the three other major peptide hormones found in islets is also believed to be affected by other

metabolic and neuronal signals (Boyd, 1992; Ashcroft ***et al.,*** 1994). Bertrand ***et al.,*** (1992; 1993) have shown that AMPA receptor agonists can potentiate both insulin and glucagon secretion from a perfused pancreas preparation and that oral or intravenous glutamate can increase insulin secretion and glucose tolerance ***in vivo*** (Bertrand ***et al.,*** 1995).

The precise role of a glutamatergic signaling system in islet physiology or pathology is important. Glutamate also subserves communication between islets and the central nervous system. Glucose-stimulated insulin release is Ca^{2+}-dependent, perhaps because Ca^{2+} couples the process of stimulus recognition to that of insulin discharge (Douglas, 1968; Milner & Hales, 1970; Matthews, 1970; Malaisse, 1973; Malaisse ***et al.,*** 1974). Although several studies have indicated that glucose alters the state of Ca^{2+} in the pancreatic cells, the nature of the changes and the mechanisms by which they occur has to be understood (Hellman ***et al.,*** 1976).

Effect of insulin on glucose uptake and metabolism

The insulin receptor is a transmembrane receptor that is activated by insulin (Ward & Lawrence, 2009) . It belongs to the large class of tyrosine kinase receptors.
Two alpha subunits and two beta subunits make up the insulin receptor. The beta subunits pass through the cellular membrane and are linked by disulfide bonds. The alpha and beta subunits are encoded by a single gene (***INSR***). The insulin receptor has also recently been designated CD220 (cluster of differentiation 220).Insulin binds to its receptor which in turn starts many protein activation cascades. These include: translocation of Glut-4 transporter to the plasma membrane and influx of glucose.
Tyrosine kinase receptors, including the insulin receptor, mediate their activity by causing the addition of a phosphate group to particular tyrosines on certain proteins within a cell. The "substrate" proteins which are phosphorylated by the Insulin receptor include a protein called "IRS-1" for "insulin receptor substrate 1". IRS-1

binding and phosphorylation eventually leads to an increase in the high affinity glucose transporter (Glut4) molecules on the outer membrane of insulin-responsive tissues, including muscle cells and adipose tissue and therefore to an increase in the uptake of glucose from blood into these tissues. The glucose transporter Glut4 is transported from cellular vesicles to the cell surface, where it then can mediate the transport of glucose into the cell. The main activity of activation of the insulin receptor is inducing glucose uptake. Courses of glucose and insulin mechanism for production, elimination and homeostatic feedback, has been extensive to oral glucose provocations, meal tests and insulin administration (Silber *et al.,* 2009). For this reason "insulin insensitivity", or a decrease in insulin receptor signaling, leads to diabetes mellitus - the cells are unable to take up glucose and the result is hyperglycaemia (an increase in circulating glucose) and all the sequelae which result from diabetes.

Specific membrane transporters facilitate the movement of glucose into cells to reduce plasma glucose concentrations in response to insulin stimulation. The transported glucose is subsequently used as metabolic fuel or stored as the complex macromolecular structure, glycogen. Two major types of glucose transporters are known: Na^+-dependent and Na^+ -independent transporters. Only the Na^+-independent transporters possess an insulin responsive isoform. The Na^+-dependent glucose transporter has been identified in several tissues particularly in the small intestine epithelium and the proximal tubule cells of the kidney, as well as in other kidney tubule cells (Takayama *et al.,* 1988). These transporters are located on the lumenal side of intestinal and kidney cells and act to absorb glucose against its concentration gradient by coupling the movement of glucose into these cells with the concomitant movement of Na^+ into the cell. Since Na^+ is moving down its electrochemical gradient this energy can be used to co-transport glucose into the cells. Thus, this

transporter is dependent on the concentrations of extracellular and intracellular sodium which are maintained by a Na^+/K+-ATPase ion pump.

The Na^+-independent glucose transporter family consists of several isoforms which facilitate the movement of glucose down its concentration gradient across a plasma membrane. Although seven isoforms have been identified (Glut1-7) (Brozinick *et al.,* 2003) only one will be discussed in detail here, Glut4, because it is the transporter that is in highest concentration in insulin-sensitive tissues such as, skeletal muscle, fat and cardiac muscle (Cai & Helke, 2003). Glut4, and to a lesser extent Glut1 enable these cells to increase their glucose uptake, thereby lowering circulating glucose levels. Because the intracellular concentration of glucose is low due to the rapid phosphorylation of glucose to glucose-6-phosphate and its dissimulation to other metabolic products, the presence of active transporters in the plasma membrane favours the movement of glucose into cells.

Insulin enhances glucose uptake by increasing the number of transporters in the plasma membrane of cells. Insulin stimulation of cells mobilize transporters from intracellular compartments to the plasma membrane to facilitate glucose transport. This translocation of receptors to the plasma membrane has been demonstrated to occur within 30 seconds of insulin stimulation (Hill *et al.,* 2001) and as the stimulus dissipates the decrease in the number of plasma membrane receptors declines coincident with a decline in glucose transport (Puro & Agardh, 1984).

The impaired ability of insulin to signal Glut4 translocation from intracellular stores is currently believed to be an important contributory factor to postprandial hyperglycaemia in diabetes (Song *et al.,* 2003). In fact, decreased insulin levels in diabetic animals have been shown to, not only decrease transporter translocation but diminish expression of Glut4 (Brussee *et al.,* 2004). Thus, it appears that insulin serves not only to acutely increase glucose transporter translocation, but also to maintain a basal level of expression of transporters in cells. Thus one mechanism by

which diabetes characterized by either low insulin levels, as in type 1 diabetes, or insulin resistance, as in type 2 diabetes, could cause pathologically high plasma glucose levels is *via* loss of regulation and expression of transmembrane glucose transporters. Several authors have also proposed that Glut2 on the β-cell membrane is relevant in regulating insulin secretion from islets (Yi *et al.,* 2005).

Triiodotyhronine (T3) regulation in diabetes

Diabetes mellitus and thyroid diseases are the two common endocrinipathies seen in the adult population. Insulin and thyroid hormones being intimately involved in cellular metabolism and excess/ deficit of either of these hormones could result in the functional derangement of the other. In euthyroid individuals with diabetes mellitus, the serum T3 levels, basal TSH levels and TSH response to thyrotropin releasing hormone (TRH) is influenced by the glycemic status (Schlienger *et al.,* 1982). Alterations in serum T3 levels have been described in association with energy deprivation (Vagenakis *et al.,* 1975; Eisenstein *et al.,* 1978) wasting illnesses (Burke & Eastman, 1974) the neonatal period (Larsen, 1972) and the use of such drugs as propylthiouracil (Oppenheimer *et al.,* 1972), dexamethasone (Chopra *et al.,* 1975) and propranolol (Roszkowska *et al.,* 1974; Tevaarwerk & Boyd, 1977; Tevaarwerk *et al.,* 1978). Fasting appears to inhibit 5'-monodeiodination, causing a decrease in the rate of conversion of T4 to T3 and an increase in the reverse T3 Concentration (Monnier *et al.,* 2009).. Poorly controlled diabetes, both Type 1 and Type 2, induce a "Low T3 state" characterized by low serum total and free T3 levels, increase in reverse T3 (rT3) but near normal serum T4 and TSH concentrations. Low serum T3 is due to reduced peripheral conversion of thyroxine (T4) to T3 *via* 5' monodeiodination reaction. Studies indicate that long term diabetic control determines the plasma T3 levels. TSH responses and low T3 state normalized with improvement in glycaemic status but even with good diabetes control, the normal nocturnal TSH peak is not restored in C-

peptide negative patients i.e., those with totally absent pancreatic β cell function (Coiro ***et al.,*** 1997). Studies show decreased insulin secretion (Ahren ***et al.,*** 1985) as well as normal or increased levels of insulin is reported in the peripheral and portal circulation in hyperthyroidism (Dimitriadis ***et al.,*** 1985). Long term thyrotoxicosis has been shown to cause beta cell dysfunction resulting in reduced pancreatic insulin content, poor insulin response to glucose and decreased rate of insulin secretion (Bech ***et al.,*** 1996).

In hyperthyroidism, the endogenous glucose production is greatly increased by a variety of mechanisms: (a) an increase in the availability of gluconeogenic precursors in the form of lactate, glutamine and alanine from skeletal muscles and glycerol from adipose tissue, (b) an increase in the concentration of plasma FFA stimulating hepatic gluconeogenesis (Dimitriadis & Raptis, 2001); (c) an increase in glycogenolysis due to inhibition of glycogen synthesis resulting in hepatic glucose output even in fed state (Holness & Sugden, 1987); (d) an up regulation of GLUT-2 glucose transporters protein expression in the hepatocyte plasma membrane. This permits increased glucose efflux to occur without intracellular glucose accumulation which would limit hepatic glucose production (Mokuno ***et al.,*** 1990); and (e) an increased secretion and exaggerated effects of glucagon and adrenaline on liver cells (Dimitriadis & Raptis, 2001). In skeletal muscle, there is a preferential increase in glucose uptake and lactate formation relative to glucose oxidation and storage in hyperthyroid state. This is due to increase in both basal and insulin stimulated GLUT1 and GLUT-4 transporters (Haber ***et al.,*** 1995), increased responsiveness of glycogenolysis to beta adrenergic stimulation (Dimitriadis & Raptis, 2001), increased activity of hexokinase and 5- phosphofructokinase and decreased sensitivity of glycogen synthesis to insulin (Dimitriadis ***et al.,*** 1997). In hypothyroidism, the synthesis and release of insulin is decreased (Ahren ***et al.,*** 1985). The rate of hepatic glucose output is decreased probably due to reduced gluconeogenesis. A post receptor

defect has been proposed to explain the decrease in insulin stimulated glucose utilization in peripheral tissues (Dimitriadis & Raptis, 2001).

Calcium imaging

The Langerhans' islet is another example of the presence of peripheral glutamatergic systems (Satin & Kinard, 1998). Intracellular free Ca^{2+} concentration plays a pivotal role in the regulation of various cellular functions as an intracellular messenger system. After stimulation of islets with AMPA or kainate, intracellular Ca^{2+} increased by way of activation of voltage-gated Ca^{2+} channels (Inagaki *et al.,* 1995; Weaver *et al.,* 1999), resulting in an elevated level of insulin secretion through increased exocytosis of insulin granules in β-cells (Bertrand *et al.,* 1992). Since the development of digital video imaging of Ca^{2+} novel findings including Ca^{2+} oscillations (Berridge & Galione, 1988; Berridge, 1991) and Ca^{2+} waves (Berridge, 1993) have been described in many different cultured cell types. Ca^{2+} spots were reported as an elementary Ca^{2+} influx event through mechanosensitive channels directly coupled with the initial step in mechanotransduction in cultured endothelial (Ohata *et al.,* 2001a, b; Tanaka & Takamatsu, 2001) and cultured lens epithelial cells (Ohata *et al.,* 2001b, c). The Ca^{2+} spots, which develop sporadically, exhibit a spatiotemporal pattern distinct from Ca^{2+} sparks, the elementary Ca^{2+} release events from intracellular stores (Cheng *et al.,* 1993; Nelson *et al.,* 1995).

Medicinal Plants as antidiabetic agents

Antidiabetic plants have often been used by practitioners of herbal medicine in treating individuals with non-insulin-dependent diabetes. In such cases patient response must be carefully monitored and significant benefit can be gained from such therapies. While hypoglycemic herbs offer promise in the treatment of diabetes in their combined effect with insulin, treatment is inherently disruptive and extreme

caution must be exercised in order to promote a smooth transition, maintain suitable blood sugar levels and avoid insulin shock. Plants still remain a major source for drug discovery inspite of the development of synthetic molecules. Consequently, the uses of traditional plant extract in the treatment of various diseases have been flourished (Fabricant & Farnsworth, 2001). According to the World Health Organisation (WHO), more than 150 plants are known to be used for the treatment of diabetes mellitus and the study of hypoglycemic plants is then encouraged (Marles & Farnsworth, 1995). The ethnobotanical information reports about 800 plants that possess anti-diabetic potential (Alarcon-Aguilara ***et al.,*** 1998). Several such herbs have shown anti-diabetic activity when assessed using presently available experimental techniques.

A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of NIDDM (Ivorra ***et al.,*** 1988; Bailey & Day, 1989; Marles & Farnsworth, 1995). Among these are alkaloids, glycosides, galactomannan gun, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions. Even the discovery of widely used hypoglycemic drug, metformin came from the traditional approach of using Galega officinalis . Thus, plants are a potential source of anti-diabetic drugs but this fact has not gained enough momentum in the scientific community. The reasons may be many including lack of belief among the practitioners of conventional medicine over alternative medicine, alternative forms of medicine are not very well-defined, possibility of quacks practising such medicine providing alluring and magical cures and natural drugs vary tremendously in content, quality and safety (Grover ***et al.,*** 2002).

In modern medicine, no satisfactory effective therapy is still available to cure the diabetes mellitus. Though insulin therapy is also used for the management of diabetes mellitus but there are several drawbacks like insulin resistance (Piedrola ***et***

al., 2001), anorexia nervosa, brain atrophy and fatty liver (Yaryura-Tobias *et al.,* 2001) after chronic treatment. In recent years, there has been renewed interest in plant medicine (Dubey *et al.,* 1994; Prince *et al.,* 1998; Ladeji *et al.,* 2003) for the treatment against different diseases as herbal drugs are generally out of toxic effect (Geetha *et al.,* 1994; Rao *et al.,* 2003) reported from research work conducted on experimental model animal.

Aegle marmelose

Medicinal plants have formed the basis for Indian traditional medicine systems. *Aegle marmelose* Corr. (Rutaceae) commonly called as 'Koovalam' in Malayalam and 'Bael' in Hindi is indigenous to India. It is a medium sized, armed deciduous tree found in wild, especially in dry forests and is also cultivated throughout Indian subcontinent for its fruit. The fruit are globose with smooth, hard and aromatic rind. The ripe fruit is used for digestive and stomachic complications. Leaves, fruits, stem and roots of *Aegle marmelose* have been used in ethno medicine for several medicinal properties: astringent, antidiarrheal, antidysenteric, demulcent, antipyretic, antiscourbutic, haemostatic, aphrodisiac and as an antidote to snake venom (Kirtikar & Basu, 1935; Nandkarni, 1976). *Aegle marmelose* is also known as herbal medicine for the treatment of diabetes mellitus (Alam *et al.,* 1990; Prakash, 1992). Preliminary report indicates blood glucose lowering activity in green leaves of *Aegle marmelose* (Chakrabarti *et al.,* 1960). Oral administration of aqueous decoction of *Aegle marmelose* root bark (1 ml/100 g) showed hypoglycemic effect, which was maximum (44%) at 3 h in normal fasted rats. In addition, the same extract completely prevented peak rise of blood sugar at 1 h in OGTT (Karunanyake *et al.,* 1984). Ponnachan *et al*. (1993) have observed that the crude aqueous leaf extract (1 g/kg for 30 days) exhibit hypoglycemic effect in alloxan induced diabetic rats. Aqueous leaf extract reversed the increase in Km values of liver malate dehydrogenase enzyme

(Seema *et al.,* 1996) and improved histopathological alterations in the pancreatic and kidney tissues of streptozotocin (STZ) induced diabetic rats (Das *et al.,* 1996).

The aqueous extracts of fruits have also been reported to possess hypoglycemic activity (Kamalakkannan & Prince, 2003, 2004). Aqueous seed extract of *Aegle marmelose* possess antidiabetic and hypolipidemic effects in diabetic rats (Kesari *et al.,* 2006). *Aegle marmelose* extract effectively reduced the oxidative stress induced by alloxan and produced a reduction in blood sugar (Sabu *et al.,* 2004). Anandharajan *et al.,* (2006) reported that methanolic extracts of *Aegle marmelose* activate glucose transport in a PI3 kinase-dependent fashion. *Aegle marmelose* root extract treated animals showed significant inhibitory activity against castor oil induced diarrhea (Mazumder *et al.,* 2006). *Aegle marmelose* fruit extract exhibits protective effects on the pancreas of streptozotocin induced diabetic rats (Kamalakkannan & Prince, 2005).

Scopoletin (7-hydroxy-6-methoxy coumarin) was isolated from the leaves of *Aegle marmelose* and evaluated for its potential to regulate hyperthyroidism, lipid peroxidation and hyperglycaemia in levo-thyroxine-induced hyperthyroid rats. Scopoletin (1.0 mg/kg, p.o.) administered daily for 7 days to levo-thyroxine-treated animals decreased the levels of serum thyroid hormones and glucose as well as hepatic glucose-6-phosphatase activity, demonstrating its potential to regulate hyperthyroidism and hyperglycaemia (Panda & Kar, 2006).

The leaves of *Aegle marmelose* Correa were reported as a source of aegeline (Chatterjee *et al.,* 1959). An examination of the fruits by various workers has revealed the occurrence of a coumarin termed ‘marmelosin’ (Asima & Sudhangsu, 1949). There molecular aspects of *Aegle marmelose* therefore, on blood glucose and lipids in normal and streptozotocin induced diabetic rats has been investigated. *Aegle marmelose* on blood sugar levels and markers of oxidative stress, i.e. lipid peroxidation, conjugated diene and hydroperoxide levels in serum and catalase,

glutathione and superoxide dismutase in blood and liver in streptozotocin treated rats (Sabu & Kuttan, 2000). Natural antioxidants strengthen the endogenous antioxidant defences from reactive oxygen species (ROS) and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, *Aegle. marmelose* can rightly be mentioned as a plant of considerable interest.

Neurobiology of Pyridoxine

Pyridoxine is required for the production of the monoamine neurotransmitters serotonin, dopamine, norepinephrine and epinephrine, as it is the precursor to pyridoxal phosphate cofactor for the enzyme aromatic amino acid decarboxylase. This enzyme is responsible for converting the precursors 5-hydroxytryptophan (5-HTP) into serotonin and levodopa (L-DOPA) into dopamine, noradrenaline and adrenaline. It has been implicated in the treatment of depression and anxiety (Dakshinamurti *et al.,* 1990). Imbalance between dopamine and serotonin in the hypothalamus of the pyridoxine-deficient rat leads to severe neuroendocrine consequences. The decrease in pineal serotonin leads to a deficiency of melatonin (Yehuda *et al.,* 1984). The decrease in cerebral and cerebellar GABA content in the pyridoxine deficient rat is accompanied by a significant increase in the concentration of the excitatory amino acid, glutamic acid. Spontaneous or drug induced seizure activity in the pyridoxine-deficient rat is ascribed to the neurotransmitter imbalance (Dakshinamurti *et al.,* 1984). An overdose of pyridoxine cause a temporary deadening of certain nerves such as the proprioceptory nerves; causing a feeling of disembodiment common with the loss of proprioception (Jones, 1982). Although vitamin B_6 is a water-soluble vitamin and is excreted in the urine, high doses of pyridoxine over long periods of time results in painful neurological symptoms known as sensory neuropathy (Perry *et al.,* 2004).

Pyridoxine has a role in preventing heart disease. Without enough pyridoxine, a compound called homocysteine builds up in the body. Homocysteine damages blood vessel linings, setting the stage for plaque buildup when the body tries to heal the damage. Vitamin B6 prevents this buildup, thereby reducing the risk of heart attack. Pyridoxine lowers blood pressure and blood cholesterol levels and keeps blood platelets from sticking together (Perry ***et al.,*** 2007). All of these properties work to keep heart disease. Nutritional supplementation with high dose vitamin B_6 and magnesium is one of the most popular alternative medicine choices for autism (Angley ***et al.,*** 2007). Some studies suggest the B6-magnesium combination help attention deficit disorder, citing improvements in hyperactivity, hyperemotivity and aggressiveness (Mousain-Bosc ***et al.,*** 2006).

Materials and Methods

Chemicals used and their sources

Biochemicals used in the present study were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade purchased locally. HPLC solvents were of HPLC grade obtained from SRL and MERCK, India.

Biochemicals

(±)Norepinephrine, (±)epinephrine, 5-hydroxytrptophan, 5-hydroxy indole acetic acid, Homovanillic acid, sodium octyl sulfonic acid, Streptozotocin, Ketanserin, Glutamate, collagenase type XI and bovine serum albumin fraction V, ethylene diamine tetra acetic acid (EDTA), Tris HCl, calcium chloride, HEPES - [n′ (2-hydroxy ethyl)] piperazine-n′-[2-ethanesulfonic acid], glycine, foetal calf serum (heat inactivated), citric acid, RPMI-1640 medium and paraformaldehyde were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade purchased locally from SRL, India.

Radiochemicals

5-Hydroxy [G-^{3}H] tryptamine creatine sulphate ([^{3}H]5-HT, 18.4Ci/mmol) and [Ethylene-^{3}H]-Ketanserin Hydrochloride (Sp. Activity 63.3Ci/mmol) were purchased from Perkin Elmer Nen Life and Analytical Sciences, Boston, MA, USA. L- [G-^{3}H]Glutamic acid (Sp. Activity 49.0Ci/mmol) were purchased from Amersham Life Science, UK. Radioimmunoassay kits for insulin and Triiodothyronine (T3) assay were purchased from Bhabha Atomic Research Centre (BARC), Mumbai, India.

Molecular Biology Chemicals

Tri-reagent kit was purchased from Sigma Chemical Co., St. Louis, USA. ABI PRISM High Capacity cDNA Archive kit, Primers and TaqMan probes for Real-Time PCR were purchased from Applied Biosystems, Foster City, CA, USA. $5HT_{2A}$; Rn01468302_m1, 5HTT (Rn00564737_m1), mGluR5 (Rn00566628_m1) and GLAST (Rn00570130_m1) primers were used for the gene expression studies using real time PCR.

Confocal Dyes

Ca^{2+} fluorescent dye- fluo 4-AM (Molecular Probes, Eugene, OR, USA) were used for the calcium imaging. Primary antibody for 5-HT_{2A} (No: RA24288 BD PharmenginTM), 5-HTT (No: AB9726 Chemicon), mGluR5 (No: AB7130F, Chemicon) and secondary antibody of either FITC (No: AB7130F, Chemicon) were used immunohistochemistry studies using confocal microscope.

Animals

Male wistar adults rats were purchased from Amrita Institute of Medical Sciences, Cochin and used for all experiments. They were housed in separate cages under 12 hours light and 12 hours dark periods and were maintained on standard food pellets and water *ad libitum*. Male Wistar rats, weighing 200 to 250g body weight were housed for 1 to 2 weeks before experiments were performed. All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines.

Plant Material

Specimen of *Aegle marmelose* were collected from Cochin University area. The plants were taxonomically identified and authenticated by Prof. K. P. Joseph, Head of the Dept. of Botany (Retd), St. Peter's College, Kolenchery and voucher specimens are deposited at the herbarium of the Centre for Neuroscience,

Dept. of Biotechnology, Cochin University of Science and Technology, Cochin, Kerala.

PREPARTION OF PLANT EXTRACT

Fresh leaves of ***Aegle marmelose*** plant were air dried in shade and powdered. 10g of leaf powder was mixed with 100ml of distilled water and stirred for 2hr. It was kept overnight at 4ºC. The supernatant was collected and evaporated to dryness followed by lyophylization in Yamato, Neocool, Japan lyophilizer. This was used as the crude leaf extract to study the anti diabetic effect in streptozotocin induced diabetes.

INDUCTION OF DIABETES

Diabetes was induced in rats by intrafemoral injection of streptozotocin (Sigma chemicals Co., St. Louis, MO, U.S.A.) freshly dissolved in citrate buffer, pH 4.5 under anesthesia (Junod ***et al.,*** 1969). Streptozotocin was given at a dose of 55mg/Kg body weight (Hoheneggar & Rudas, 1971; Arison ***et al.,***1967).

DETERMINATION OF BLOOD GLUCOSE

The diabetic states of animals were assessed by measuring blood glucose concentration at 72 hours after streptozotocin treatment. The rats with a blood sugar level above 250 mg/dl were selected as diabetic rats.

DETERMINATION OF ANTI-DIABETIC POTENTIAL OF *AEGLE MARMELOSE* LEAF EXTRACT

Male Wistar rats, weighing 200 to 250g body weight were randomly divided into following groups. Each group consisted of 4-6 animals.

a) Group 1: Control (C)
b) Group 2: Diabetic (D)
c) Group 3: diabetic treated with insulin (D+I)

d) Group 4: Diabetic treated with insulin + pyridoxine (DIP)
e) Group 5: Diabetic treated with pyridoxine alone (D+P)
f) Group 6: Diabetic treated with *Aegle marmelose* leaf extract (D+A)
g) Group 7: Diabetic treated with *Aegle marmelose* leaf extract + pyridoxine (DAP)

The D+I and DIP groups received a daily dose (1 Unit/kg body weight) of Lente and Plain insulin (Abbott India) were given for the better control (Sasaki & Bunag, 1983). Aqueous extract of *Aegle marmelose* was given orally to the 6th and 7th group of diabetic rats in the dosage of 1g/Kg body weight (Ponnachan ***et al.,*** 1993) at 24 hour intervals. 100 mg/kg body weight of pyridoxine was injected subcutaneously to the 4th, 5th and 7th group. Blood samples were collected from the tail vein at 0 hours (Before the start of the experiment), 3rd day, 6th day, 10th day and 14th day the glucose levels were estimated. Changes in the body weight of animals were monitored 1st day (before the streptozotocin injection), 7th day and 15th day.

TISSUE PREPARATION

The animals were then sacrificed on 15th day by decapitation. The cerebral cortex, brain stem, cerebellum, hippocampus and pancreas were dissected out quickly over ice according to the procedure of Glowinski & Iversen, (1906). Hippocampus was dissected out quickly over ice according to the procedure of Heffner ***et al.,*** (1980). The blood samples were collected and plasma was separated by centrifugation. The tissue samples and plasma were kept at -70°C until assay.

ESTIMATION OF BLOOD GLUCOSE

Blood glucose was estimated by GOD-POD glucose estimation kit from Biolab Diagnostics Pvt. Ltd. The glucose was estimated at 0, 30, 60, 120, 180, 240

and 300 minutes after the insulin administration. The spectrophotometric method using glucose oxidase-peroxidase reactions was as follows:

Principle: Glucose oxidase (GOD) catalysed the oxidation of glucose in accordance with the following equation:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{(GOD)}} \text{Gluconic acid} + H_2O_2.$$

$$H_2O_2 + \text{Phenol} + \text{4- aminoantipyrene} \xrightarrow{\text{(PEROXIDASE)}} \text{Coloured complex} + H_2O$$

The hydrogen peroxide formed in this reaction reacted with 4-aminoantipyrine and 4-hydroxybenzoic acid in the presence of peroxidase (POD) to form N-(-4-antipyryl)-p-benzo quinoneimine. The addition of mutarotase accelerated the reactions. The amount of dye formed was proportional to the glucose concentration. The absorbance was read at 500nm in (Shimadzu UV-1700 pharmaSPEC) spectrophotometer.

Estimation of Circulating Insulin by Radioimmunoassay

Principle of the assay

The assay was done according to the procedure of BARC radioimmunoassay kit. The radioimmunoassay method was based on the competition of unlabelled insulin in the standard or samples and [^{125}I] insulin for the limited binding sites on a specific antibody. At the end of incubation, the antibody bound and free insulin were separated by the second antibody-polyethylene glycol (PEG) aided separation method. Measuring the radioactivity associated with bound fraction of sample and standards quantitates insulin concentration of samples.

Assay Protocol

Standards, ranging from 0 to 200µU/ml, insulin free serum and insulin antiserum (50µl each) were added together and the volume was made up to 250µl with assay buffer. Samples of appropriate concentration from experiments were

used for the assay. They were incubated overnight at 2°C. Then 50µl [^{125}I] Insulin was added and incubated at room temperature for 3 hrs. 50µl second antibody was added along with 500µl of PEG. The tubes were then vortexed and incubated for 20 minutes and they were centrifuged at 1500 x g for 20 minutes. The supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

Estimation of Circulating Triiodothyronine (T3) by Radioimmunoassay

Principle of the assay

The assay was done according to the procedure of BARC radioimmunoassay kit. The radioimmunoassay method was based on the competition of unlabelled endogenous T3 with [^{125}I]T3 for the limited binding sites on the antibody (Ab1) made specially for T3. The antibody was in the form of a complex with second antibody (Ab2). At the end of incubation, the T3 (Ag) bound to the antibody- second antibody complex (Ag-Ab1-Ab2) and free T3 was separated by the addition of PEG. The amount bound to the antibody complex in the assay tubes were compared with values of known T3 standards and the T3 concentration in the samples were calculated.

Assay Protocol

Standards, ranging from 0.15 to 2.5ng, T3 free serum, [^{125}I] T3 and antiserum complex were added together and the volume was made up to 275µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. They were incubated at 37°C for 45 minutes. The PEG was added to all tubes and they were centrifuged at 1500 x g for 20 minutes. The supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

T3 concentrations in the samples were determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland).

A standard curve was plotted with $\%B/B_o$ on the Y-axis and insulin concentration/ml on the X-axis of a log-logit graph. $\%B/B_o$ was calculated as:

$$\frac{\text{Corrected average count of standard or sample}}{\text{Corrected average count of zero standard}} \times 100$$

Insulin concentration in the samples was determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland).

Quantification of brain monoamines and their metabolites in the experimental groups of rats

The monoamines were assayed according to the modified procedure of Paulose *et al.,* (1988). The cerebral cortex, hippocampus, brain stem and cerebellum of experimental groups of rats was homogenised in 0.4N perchloric acid. The homogenate was then centrifuged at 5000xg for 10 minutes at 4°C in a Sigma 3K30 refrigerated centrifuge and the clear supernatant was filtered through 0.22 µm HPLC grade filters and used for HPLC analysis.

5-Hydroxy indole Acetic Acid (5-HIAA) and serotonin (5-HT) contents were determined in high performance liquid chromatography (HPLC) with electrochemical detector (ECD) (Waters, USA) fitted with CLC-ODS reverse phase column of 5 µm particle size. The mobile phase consisted of 50mM sodium phosphate dibasic, 0.03M citric acid, 0.1mM EDTA**,** 0.6mM sodium octyl sulfonate, 15% methanol. The pH was adjusted to 3.25 with orthophosphoric acid, filtered through 0.22 µm filter (Millipore) and degassed. A Waters model 515, Milford, USA, pump was used to deliver the solvent at a rate of 1 ml/minute. The neurotransmitters and their metabolites were identified by amperometric detection using an electrochemical detector (Waters, model 2465) with a reduction potential of +0.80 V. Twenty microlitre aliquots of the acidified supernatant were injected into the system for quantification. The peaks were identified by relative retention

times compared with external standards and quantitatively estimated using an integrator (Empower software) interfaced with the detector. Data from different brain regions of the experimental and control rats were statistically analysed and tabulated.

SEROTONIN RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS IN THE BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS

5-HT Receptor Binding Studies Using [^{3}H]-5-hydroxytryptamine

5-HT receptor assay was done using [^{3}H]-5-hydroxytryptamine binding in crude synaptic membrane preparations of cerebral cortex and brain stem by the modified method of Uzbekov *et al.,* (1979). Crude membrane preparation was suspended in 50 mM Tris-HCl buffer, pH 8.5, containing 1.0 µM paragyline. The incubation mixture contained 0.3-0.4 mg protein was used in each assay. In the saturation binding experiments, assays were done using different concentrations i.e., 1.0nM-30nM of [^{3}H] 5-HT was incubated with and without excess of unlabelled 10µM 5-HT. Tubes were incubated at 37^{o}C for 15 minutes. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris buffer, pH 8.5. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

5-HT$_{2A}$ Receptor Binding Studies Using [^{3}H]Ketanserin

5-HT$_{2A}$ receptor assay was done using [^{3}H] Ketanserin binding in crude synaptic membrane preparations of cerebellum by the modified method Leysen *et al.,* (1979). Crude membrane preparation was suspended in 50 mM Tris-HCl buffer, pH 7.6. The incubation mixture contained 0.3-0.4 mg protein. In the saturation binding experiments, assays were done using different concentrations i.e., 0.1nM-2.5nM of [^{3}H] Ketanserin which was incubated with and without

excess of unlabelled 10µM Ketanserin. Tubes were incubated at 37°C for 15 minutes. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris buffer, pH 7.6. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Quantification of Glutamate

Glutamate content in the brain regions - cerebral cortex, cerebellum, hippocampus and pancreas of control experimental rat were quantified by displacement method using modified procedure of Enna & Snyder, (1976). Tissues were homogenized in 20 volumes of 0.32 M sucrose, 10 mM Tris HCl and 1 mM $MgCl_2$ buffer, pH 7.4, with a polytron homogenizer. The homogenate was centrifuged twice at 27,000 x g for 15 minutes. The supernatant was pooled and used for the assay. The incubation mixture contained 1 nM [^{3}H] Glutamate with and without glutamate at a concentration range of 10^{-9} M to 10^{-4} M. The unknown concentrations were determined from the standard displacement curve using appropriate dilutions and calculated for nmoles/g wt. of the tissue.

GLUTAMATE RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS IN THE BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS

Glutamate Receptor Binding Studies Using [^{3}H]Glutamate

Membranes were prepared according to the modified method of Timothy *et al.,* (1984). The brain regions - cerebral cortex, cerebellum, hippocampus and pancreas were homogenized in 20 volumes of 0.32 M sucrose, 10 mM Tris-HCl and 1 mM $MgCl_2$ buffer, pH 7.4, with a polytron homogenizer. The homogenate was centrifuged twice at 1,000 x g for 15 minutes at 4°C and the pellets were discarded. The supernatants were pooled and centrifuged at 27,000 x g for 15 minutes. The resulting pellet was lysed in a 10 mM Tris-HCl buffer, pH 7.4, for

30 minutes and centrifuged at 27,000 x g for 15 minutes. The pellet was washed three times in 10 mM Tris-HCl buffer, pH 7.4, and centrifuged at 27,000 x g for 15 minutes. All steps were carried out at 4°C.

Membranes were incubated in 0.25 ml reaction mixture containing 25 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$ and 20 nM to 350 nM of [^{3}H]Glutamate containing 0.2 mg to 0.3 mg protein concentrations. Incubation was carried out at 30°C for 15 minutes and the reaction was stopped by centrifugation at 27,000 x g for 15 minutes. The pellet and the wall of the tube were quickly and carefully washed with ice-cold distilled water. 0.1% SDS and scintillation fluid were added to the dry pellet and radioactivity incorporated was determined with a Wallac scintillation

counter. All the assays were carried out in triplicate. Nonspecific binding was determined by adding 350 µM nonradioactive glutamate to the reaction mixture in a parallel assay. Specific binding was considered to be the difference between total and nonspecific binding.

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 Spectrophotometer at 660nm.

ANALYSIS OF THE RECEPTOR BINDING DATA

Linear regression analysis for Scatchard plots

The data were 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 OF 5-HT RECEPTOR SUBTYPES – 5-HT_{2A}, 5-HT TRANSPORTER, GLUTAMATE RECEPTOR SUBTYPE - mGluR5, GLAST, INSULIN RECEPTOR, STATUS OF ANTIOXIDANTS - SOD AND GPx IN DIFFERENT BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS

Preparation of RNA

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

Isolation of RNA

Tissue (25-50 mg) homogenates were made in 0.5 ml 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 minutes 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 500 µl of 75% ethanol, vortexed and centrifuged at 12,000 x *g* for 5 minutes at 4°C. The pellets were semi dried and dissolved in minimum volume of DEPC-treated water. 2 µl of RNA was made up to 1 ml and absorbance was measured at 260 nm and 280 nm in spectrophotometer (Shimadzu UV-1700 pharmaSPEC). For pure RNA preparation the ratio of absorbance at 260/280 was ≥ 1.7. The concentration of RNA was calculated as 1 OD at 260 = 42 µg.

cDNA Synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA Archive kit in 0.2 ml microfuge tubes. The reaction mixture of 20 µl contained 0.2 µg 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 an 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. 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 20 µl contained 25 ng of total RNA-derived cDNAs, 200 nM each of the forward primer, reverse primer and PCR analyses were conducted with gene-specific primers and fluorescently labelled TaqMan probes of 5-HT_{2A} (Rn01468302_m1), 5-HTT (Rn00564737_m1), mGluR5 (Rn00566628_m1), GLAST (Rn00570130_m1), superoxide dismutase (SOD; Rn01477289) and glutathione peroxidise (GPx; Rn00577994). Endogenous control, β-actin was labeled with a reporter dye, VIC. 12.5 µl of TaqMan 2X Universal PCR Master Mix was taken and 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 minutes	--- 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 ($2^{-\Delta\Delta CT}$).

ROTAROD TEST

Rotarod has been used to evaluate motor coordination by testing the ability of rats to remain on revolving rod (Dunham & Miya, 1957). The control

and experimental adult and old rats were subjected to rotarod test after 10 days of hypoglycaemic shock once daily for 15 days. Rotarod has been used to evaluate motor co-ordination by testing the ability of rats to remain on revolving rod. The apparatus has a horizontal rough metal rod of 3 cm diameter attached to a motor with variable speed. This 70 cm long rod was divided into four sections by wooden partitions. The rod was placed at a height of 50 cm to discourage the animals to jump from the rotating rod. The rate of rotation was adjusted to allow the normal rats to stay on it for five minutes. Each rat was given five trials before the actual reading was taken. The readings were taken at 10, 25 and 50 rpm after 10 days of treatment in all groups of rats.

ELEVATED PLUS MAZE

The elevated plus-maze is a widely used animal model of anxiety that is based on two conflicting tendencies; the rodent's drive to explore a novel environment and its aversion to heights and open spaces. Four arms were arranged in the shape of a cross. Two arms had side walls and an end wall ("closed arms") - the two other arms had no walls ("open arms"). The open arms were surrounded by small ledges to prevent the animal from falling from the maze. The maze was fastened to a light-weight support frame. Thus "anxious" animals spent most of the time in the closed arms while less anxious animals explored open areas longer.

Procedure – Animals were placed individually into the center of elevated plus-maze consisting of two open arms (38L x 5W cm) and two closed arms (38L x 5W x 15H cm), with a central intersection (5cm x 5cm) elevated 50 cm above the floor. Behaviour was tested in a dimly lit room with a 40W bulb hung 60 cm above the central part of the maze. The investigator sitting approximately 2m apart from the apparatus observed and detected the movements of the rats for a total of 5 minutes. The experimental procedure was similar to that described by Pellow ***et al.,*** (1985). During the 5 minutes test period the following parameters were measured to analyze the behavioural changes of the experimental rats using

elevated plus-maze: open arm entry, closed arm entry, percentage arm entry, total arm entry, time spent in open arm, time spent in closed arm, percentage of time spent in open arm (Espejo, 1997; Holmes & Rodgers,1998). An entry was defined as entering with all four feet into one arm. A decrease in open arm entries and decrease in time spent in the open arms is indicative of anxiogenic activity shown by experimental rats.

BEAM WALK TEST

Rats were trained to traverse on a 1 m long wooden beam with a diameter of 1.2 cm was used for the study. (Taylor *et al.,* 2001, 2005). The animals were recorded for three trials per session on 1 day, 7day and 14 day after post-STZ injections. A foot slip was counted if either the left or right hind paw slipped off the beam. The number of foot slips was averaged.

ISOLATION OF PANCREATIC ISLETS

Pancreatic islets were isolated from male Wistar rats by standard collagenase digestion procedures using aseptic techniques (Howell & Taylor, 1968). The islets were isolated in HEPES-buffered sodium free Hanks Balanced Salt Solution (HBSS) (Pipeleers *et al.,* 1985) with the following composition: 137 mM Choline chloride, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM KH_2PO_4, 14.3 mM $KHCO_3$ and 10 mM HEPES**.** Autoclaved triple distilled water was used in the preparation of the buffer.

The pancreas from the rats were aseptically dissected out into a sterile petridish containing ice cold HBSS and excess fat and blood vessels were removed. The pancreas was cut into small pieces and transferred to a sterile glass vial containing 2 ml collagenase type XI solution (1.5 mg/ml in HBSS, pH 7.4). The collagenase digestion was carried out for 15 minutes at 37°C in an environmental shaker with vigorous shaking (300 rpm/minute). The tissue digest was filtered through 500 μm nylon screen and the filtrate was washed with three successive centrifugations and resuspensions in cold HBSS. The pancreatic islet

preparation having a viability of >90% as assessed by Trypan Blue exclusion which was chosen for experiments.

CALCIUM IMAGING STUDIES USING CONFOCAL MICROSCOPE

Pancreatic islets were prepared from control and experimental rats by collagenase digestion method as mentioned earlier. The isolated islets were incubated for 4 hours at room temperature in 1 ml of calcium free RPMI medium containing 5 μM of Ca^{2+} fluorescent dye, fluo 4-AM (Molecular Probes, Eugene, OR) to monitor the changes in the intracellular Ca^{2+}. After incubation cells were washed twice in indicator free RPMI medium to remove excess dye that was non-specifically associated with the cell surface and then incubated for further 30 minutes to allow complete de-esterification of intra- cellular AM esters. The 35 mm plates, containing pancreatic islet cells were placed on the stage of a Leica TCS SP5 laser scanning confocal microscope equipped with a HC PL FLUOTAR 20.0 x 0.50 dry objective (NA 0.5). Fluo 4-AM was excited with 514 nm laser lines from an argon laser, with laser intensity set at 38% of available power. For visualization of Fluo 4-AM, the emission window was set at 508.4 nm – 571.5 nm. analyse the intracellular Ca^{2+} release from the pancreatic islet cells in experimental conditions.

SEROTONIN RECEPTOR SUBTYPE 5-HT_{2A}, SEROTONIN TRANSPORTER 5-HTT, GLUTAMATE RECEPTOR SUBTYPE mGluR5 RECEPTOR EXPRESSION STUDIES IN THE CEREBRAL CORTEX, BRAIN STEM, CEREBELLUM, HIPPOCAMPUS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS USING CONFOCAL MICROSCOPE

Control and experimental rats were deeply anesthetized with ether. The rat was transcardially perfused with PBS, pH- 7.4, followed by 4% paraformaldehyde in PBS (Chen *et al.,* 2007). After perfusion the brains were dissected and immersion fixed in 4% paraformaldehyde for 1 hr and then equilibrated with 30%

sucrose solution in 0.1 M PBS, pH- 7.0. 40 µm sections were cut using Cryostat (Leica, CM1510 S). The sections were treated with PBST (PBS in 0.01% Triton X-100) for 20 minutes. Brain slices were incubated overnight at 4°C with either rat primary antibody for 5-HT_{2A} (No: RA24288 BD PharmenginTM, diluted in PBST at 1: 500 dilution) and 5HTT (No: AB9726 Chemicon Temecula, diluted in PBST at 1: 500 dilution) and mGluR5 (No: AB7130F, Chemicon, diluted in PBST at 1: 500 dilution) (polyclonal or monoclonal). After overnight incubation, the brain slices were rinsed with PBST and then incubated with appropriate secondary antibody of either FITC (No: AB7130F, Chemicon, diluted in PBST at 1:1000 dilution). The sections were observed and photographed using confocal imaging system (Leica SP 5).

STATISTICS

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme. Linear regression Scatchard plots were made using SIGMA PLOT (Ver 2.03). Sigma Plot software (version 2.0, Jandel GmbH, Erkrath, Germany). Competitive binding data were analysed using non-linear regression curve-fitting procedure (GraphPad PRISM™, San Diego, USA). Relative Quantification Software was used for analyzing Real-Time PCR results.

Results

BODY WEIGHT OF CONTROL AND EXPERIMENTAL RATS

The body weight was significantly decreased ($p<0.001$) in the diabetic rats when compared to control group. After treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine supplementation for 14 days, the body weight was reversed significantly ($p<0.001$) to near the initial level (Table-1; Figure-1).

BLOOD GLUCOSE LEVEL OF CONTROL AND EXPERIMENTAL RATS

Blood glucose level of all rats before streptozotocin administration was within the normal range. Streptozotocin administration led to a significant increase ($p<0.001$) in blood glucose level of diabetic group when compared to control. Treatment using insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine was able to significantly reduced ($p<0.001$) the increased blood glucose level to near control when compared to diabetic rats (Table-2; Figure-2).

CIRCULATING INSULIN LEVEL IN THE PLASMA OF CONTROL AND EXPERIMENTAL RATS

Diabetic rats showed a significant decrease ($p<0.001$) in circulating insulin level compared to control. Treatment using insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine significantly increase ($p<0.001$) the plasma insulin level to near control when compared to diabetic rats (Table-3; Figure-3).

TRIIODOTHYRONINE (T3) CONCENTRATION IN SERUM OF CONTROL AND EXPERIMENTAL RAT

Diabetic rats showed a significant decrease ($p<0.001$) in T3 concentration compared to their respective controls. Diabetes rats treated with pyridoxine alone significantly increased ($p<0.05$) the plasma insulin level to near control when compared to diabetic rats. Treatment using insulin and *Aegle marmelose* leaf extract alone and in combination with insulin and *Aegle marmelose* leaf extract significantly increased ($p<0.001$) the plasma insulin level to near control when compared to diabetic rats (Table-4; Figure-4).

SEROTONIN AND ITS METABOLITES CONTENT IN DIFFERENT BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS

Cerebral Cortex

There was a significant decrease ($p<0.01$) in 5-HT content in the cerebral cortex of diabetic compared to control. The decreased 5-HT content was significantly reversed ($p<0.01$) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract. The 5-HIAA in the cerebral cortex was significantly decreased ($p<0.05$) in diabetic rats compared to control. The decreased 5-HIAA content was significantly reversed ($p<0.05$) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract (Table-5).

Brain stem

There was a significant decrease ($p<0.05$) in 5-HT content in brain stem of diabetic rats compared to control rats. The decreased 5-HT content was significantly reversed ($p<0.05$) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract. The 5-HIAA in the brain stem was significantly decreased ($p<0.05$) in diabetic rats compared to control. The decreased 5-HIAA content was significantly reversed ($p<0.05$) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract (Table-6).

Cerebellum

There was a significant decrease ($p<0.05$) in 5-HT content in the cerebellum of diabetic rats compared to control rats. The decreased 5-HT content was ($p<0.05$) reversed to near control in diabetic rats treated pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract. The 5-HIAA in the cerebellum was significantly decreased ($p<0.05$) in diabetic rats compared to control. The decreased 5-HIAA content was significantly reversed ($p<0.05$) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract (Table-7).

Hippocampus

There was a significant decrease ($p<0.05$) in 5-HT content in hippocampus of diabetic rats compared to control rats. The decreased 5-HT content was significantly reversed ($p<0.05$) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract. The 5-HIAA in the hippocampus was significantly decreased ($p<0.05$) in diabetic rats compared to

control. The decreased 5-HIAA content was significantly reversed ($p<0.05$) to near control in diabetic rats treated with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract (Table-8).

GLUTAMATE CONTENT IN DIFFERENT BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS

Cerebral Cortex

Our results showed a significant increase ($p<0.001$) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats significantly reversed ($p<0.001$) the glutamate content to near control compared to diabetic group (Table-9).

Brain stem

Our results showed a significant increase ($p<0.001$) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats significantly reversed ($p<0.001$) the glutamate content to near control compared to diabetic group (Table-10).

Cerebellum

Our results showed a significant increase ($p<0.05$) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats significantly

reversed ($p<0.001$) the glutamate content to near control compared to diabetic group (Table-11).

Hippocampus

Our results showed a significant increase ($p<0.001$) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats significantly reversed ($p<0.001$) the glutamate content to near control compared to diabetic group (Table-12).

Pancreas

Our results showed a significant increase ($p<0.001$) in the glutamate content of diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats significantly reversed ($p<0.001$) the glutamate content to near control compared to diabetic group (Table-13).

SEROTONIN, 5-HT_{2A}, GLUTAMATE RECEPTORS, 5-HT_{2A}, 5-HTT, mGluR5 AND GLAST GENE EXPRESSION CHANGES IN THE BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS

Cerebral Cortex

Scatchard analysis of Serotonin receptors using [^{3}H] 5-HT against 5-HT

Scatchard analysis of serotonin receptors showed that the B_{max} decreased significantly ($p<0.001$) in the cerebral cortex of diabetic rats with a significant

increase ($p<0.001$) in the affinity when compared to control. Treatment groups D+I ($p<0.01$), D+P ($p<0.01$), DIP ($p<0.001$), D+A ($p<0.01$) and DAP ($p<0.001$) significantly reversed the B_{max} and K_d ($p<0.01$) to near control compared to diabetic group (Table-14, 15; Figure-5, 6).

Scatchard analysis of 5-HT_{2A} receptors using [^{3}H] Ketanserin against ketanserin

Scatchard analysis showed no significant change in B_{max} when compared to control. The K_d was significantly decreased ($p<0.001$) in diabetic group. Treatment groups D+I ($p<0.001$), D+P ($p<0.05$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the K_d to near control compared to diabetic group (Table-16, 17; Figure-7, 8).

Scatchard analysis of Glutamate receptors using [^{3}H] Glutamate against glutamate

Scatchard analysis showed that the B_{max} increased significantly ($p<0.001$) in the cerebral cortex of diabetic rats with a significant increase ($p<0.001$) in the affinity. Treatment groups D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} and K_d to near control compared to diabetic group (Table-18, 19; Figure- 9,10).

Real-Time PCR analysis of 5-HT_{2A} receptors

Real Time-PCR analysis showed that the 5-HT_{2A} receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats and it was significantly reversed ($p<0.001$) to near control in diabetic rats treated with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine. Diabetic rats treated with pyridoxine alone was also significantly reversed ($p<0.05$) to near control (Table-20; Figure-11).

Real-Time PCR analysis of 5-HTT transporter

Real Time-PCR analysis showed that the 5-HTT receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats and it was significantly reversed ($p<0.001$) to near control in diabetic rats treated with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine. Diabetic rats treated with pyridoxine treated alone was also ($p<0.001$) reversed to near control (Table-21; Figure-12).

Real-Time PCR analysis of mGluR5 receptors

Real Time-PCR analysis of mGluR5 receptor mRNA was significantly up regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control in diabetic rats treated with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine (Table-22; Figure-13).

Real-Time PCR analysis of GLAST glutamate transporter

Real Time-PCR analysis of GLAST receptor mRNA showed a significant down regulation ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control in diabetic rats treated with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine (Table-23; Figure-14).

Brain stem

Scatchard analysis of Serotonin receptors using [^{3}H] 5-HT against 5-HT

Scatchard analysis showed that the B_{max} increased significantly ($p<0.001$) in the brain stem of diabetic rats with a significant decrease ($p<0.001$) in the affinity. Treatment groups D+I ($p<0.001$), D+P ($p<0.01$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) reversed the B_{max} and D+I ($p<0.001$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) reversed the K_d to near control compared to diabetic group (Table-27,28; Figure-18,19).

Scatchard analysis of 5-HT_{2A} receptors using [^{3}H] Ketanserin against ketanserin

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in the brain stem of diabetic rats with no change in the affinity. Treatment groups D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} to near control compared to diabetic group (Table-29,30; Figure-20,21).

Scatchard analysis of Glutamate receptors using [^{3}H]Glutamate against glutamate

Scatchard analysis showed that the B_{max} increased significantly ($p<0.001$) in the brain stem of diabetic rats with no change in the affinity. Treatment groups D+I ($p<0.001$), DIP ($p<0.001$), D+P ($p<0.01$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} to near control compared to diabetic group (Table-31, 32; Figure-22,23).

Real-Time PCR analysis of 5-HT_{2A} receptors

Real Time-PCR analysis showed that the 5-HT_{2A} mRNA was significantly up regulated ($p<0.001$) in diabetic rats compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine treated diabetic rats (Table-33; Figure-24).

Real-Time PCR analysis of 5-HTT transporter

Real Time-PCR analysis showed that the 5-HTT mRNA was significantly up regulated ($p<0.001$) in diabetic rats compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine treated diabetic rats. Diabetic rats treated with pyridoxine alone treated alone was reversed ($p<0.001$) to near control (Table-34; Figure-25).

Real-Time PCR analysis of mGluR5 receptors

Real Time-PCR analysis showed that the mGluR5 receptor mRNA was significantly up regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-35; Figure-26).

Real-Time PCR analysis of GLAST glutamate transporter

Real Time-PCR analysis showed that the GLAST receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and

it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-36; Figure-27).

Cerebellum

Scatchard analysis of Serotonin receptors using [^{3}H] 5-HT against 5-HT

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in the cerebellum of diabetic rats with a significant increase ($p<0.001$) in the affinity. Treatment groups D+I ($p<0.001$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} and K_d ($p<0.001$) to near control compared to diabetic group (Table-40, 41; Figure-31, 32).

Scatchard analysis of 5-HT_{2A} receptors using [^{3}H] Ketanserin against ketanserin

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in the cerebellum of diabetic rats with no change in affinity. Treatment groups D+I ($p<0.001$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} to near control compared to diabetic group (Table-42, 43; Figure-33, 34).

Scatchard analysis of Glutamate receptors using [^{3}H]Glutamate against glutamate

Scatchard analysis showed that the B_{max} increased significantly ($p<0.001$) in the cerebellum of diabetic rats with no change in the affinity. Treatment groups D+I ($p<0.001$), D+P ($p<0.01$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} to near control compared to diabetic group (Table-44, 45; Figure-35, 36).

Real-Time PCR analysis of 5-HT_{2A} receptors

Real Time-PCR analysis showed that the 5-HT_{2A} receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control level on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.001$) to near control (Table-46; Figure-37).

Real-Time PCR analysis of 5-HTT transporter

Real Time-PCR analysis showed that the 5-HTT mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone was also ($p<0.001$) reversed to near control (Table-47; Figure-38).

Real-Time PCR analysis of mGluR5 receptors

Real Time-PCR analysis showed that the mGluR5 receptor mRNA was significantly up regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-48; Figure-39).

Real-Time PCR analysis of GLAST glutamate transporter

Real Time-PCR analysis showed that the GLAST receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.01$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.05$) to near control (Table-49; Figure-40).

Hippocampus

Scatchard analysis of Serotonin receptors using [^{3}H] 5-HT against 5-HT

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in the hippocampus of diabetic rats with a significant increase ($p<0.001$) in the affinity. Treatment groups D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} and DIP ($p<0.001$) and DAP ($p<0.001$) reversed the K_d to near control compared to diabetic group (Table-53, 54; Figure-44, 45).

Scatchard analysis of 5-HT$_{2A}$ receptors using [^{3}H] Ketanserin against ketanserin

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in the hippocampus of diabetic rats with a significant decrease ($p<0.05$) in the affinity. Treatment groups D+I ($p<0.001$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} and ($p<0.05$) reversed the K_d to near control compared to diabetic group (Table-55, 56; Figure-46, 47).

Scatchard analysis of Glutamate receptors using [^{3}H] Glutamate against glutamate

Scatchard analysis showed that the B_{max} increased significantly ($p<0.001$) in the hippocampus of diabetic rats with no change in the affinity. Treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine reversed ($p<0.001$) the B_{max} to near control compared to diabetic group (Table-57, 58; Figure-48, 49).

Real-Time PCR analysis of 5-HT_{2A} receptors

Real Time-PCR analysis showed that the 5-HT_{2A} receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-59; Figure-50).

Real-Time PCR analysis of 5-HTT transporter

Real Time-PCR analysis showed that the 5-HTT mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.001$) to near control (Table-60; Figure-51).

Real-Time PCR analysis of mGluR5 receptors

Real Time-PCR analysis showed that the mGluR5 mRNA was significantly up regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-61; Figure-52).

Real-Time PCR analysis of GLAST glutamate transporter

Real Time-PCR analysis showed that the GLAST mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.01$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.05$) to near control level (Table-62; Figure-53).

Pancreas

Scatchard analysis of Serotonin receptors using [^{3}H] 5-HT against 5-HT

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in the pancreas of diabetic rats with a significant increase ($p<0.001$) in the affinity. Treatment groups D+I ($p<0.001$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} and K_d to near control compared to diabetic group (Table-66, 67; Figure-57, 58).

Scatchard analysis of 5-HT_{2A} receptors using [^{3}H] Ketanserin against ketanserin

Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in the pancreas of diabetic rats with no change in the affinity. Treatment groups D+I ($p<0.001$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} to near control compared to diabetic group (Table-68, 69; Figure-59, 60).

Scatchard analysis of Glutamate receptors using [^{3}H]Glutamate against glutamate

Scatchard analysis showed that the B_{max} increased significantly ($p<0.001$) in the pancreas of diabetic rats with a significant increase ($p<0.001$) in the affinity. Treatment groups D+I ($p<0.01$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) significantly reversed the B_{max} and D+I ($p<0.01$), DIP ($p<0.001$), D+A ($p<0.01$) and DAP ($p<0.001$) reversed the K_d to near control compared to diabetic group (Table-70, 71; Figure-61, 62).

Real-Time PCR analysis of 5-HT_{2A} receptors

Real Time-PCR analysis showed that the 5-HT_{2A} receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic

rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.001$) to near control level (Table-72; Figure-63).

Real-Time PCR analysis of 5-HTT transporter

Real Time-PCR analysis showed that the 5-HTT mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone reversed also ($p<0.01$) to near control (Table-73; Figure-64).

Real-Time PCR analysis of mGluR5

Real Time-PCR analysis showed that the mGluR5 mRNA was significantly up regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-74; Figure-65).

Real-Time PCR analysis of GLAST glutamate transporter

Real Time-PCR analysis showed that the GLAST mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.01$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats.

Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.05$) to near control (Table-75; Figure-66).

GENE EXPRESSION STUDIES OF INSULIN RECEPTORS AND STATUS OF ANTIOXIDANTS- SOD AND GPx IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS

Cerebral Cortex

Real-Time PCR analysis of Insulin receptors

Real Time-PCR analysis showed that the insulin mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-24; Figure-15).

Real-Time PCR analysis of SOD

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-25; Figure-16).

Real-Time PCR analysis of GPx

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-26; Figure-17).

Brain stem

Real-Time PCR analysis of Insulin receptors

Real Time-PCR analysis showed that the insulin receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-37; Figure-28).

Real-Time PCR analysis of SOD

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-38; Figure-29).

Real-Time PCR analysis of GPx

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control level on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-39; Figure-30).

Cerebellum

Real-Time PCR analysis of Insulin receptors

Real Time-PCR analysis showed that the insulin receptor mRNA was significantly up regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.05$) to near control (Table-50; Figure-41).

Real-Time PCR analysis of SOD

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.01$) to near control level (Table-51; Figure-42).

Real-Time PCR analysis of GPx

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.001$) to near control (Table-52; Figure-43).

Hippocampus

Real-Time PCR analysis of Insulin receptors

Real Time-PCR analysis showed that the insulin mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-63; Figure-54).

Real-Time PCR analysis of SOD

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-64; Figure-55).

Real-Time PCR analysis of GPx

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-65; Figure-56).

Pancreas

Real-Time PCR analysis of Insulin receptors

Real Time-PCR analysis showed that the insulin receptor mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats. Diabetic rats treated with pyridoxine treated alone also reversed ($p<0.05$) to near control (Table-76; Figure-67).

Real-Time PCR analysis of SOD

Real Time-PCR analysis showed that the SOD mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-77; Figure-68).

Real-Time PCR analysis of GPx

Real Time-PCR analysis showed that the GPx mRNA was significantly down regulated ($p<0.001$) in diabetic rats when compared to control and it was significantly reversed ($p<0.001$) to near control on treatment with insulin and *Aegle marmelose* leaf extract alone and in combination with pyridoxine in diabetic rats (Table-78; Figure-69).

BEHAVIOURAL STUDIES IN CONTROL AND EXPERIMENTAL RATS

Rotarod Performance of control and experimental groups of rats

Rotarod experiment at 10 ($p<0.001$), 25 ($p<0.001$) and 50 ($p<0.001$) revolutions per minute (rpm) showed a significant decrease in the retention time on the rotating rod in diabetic group compared to control. Diabetic rats treated with insulin and *Aegle marmelose* alone and in combination therapy significantly reversed D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) the retention time to near control (Table-79).

Beam walk test

Beam-walk test was used to assess sensorimotor ability. For a slip to be counted, the foot had to lose contact with the balance beam with the leg extended. The diabetic rats displayed a significantly higher ($p<0.001$) number of footslips. Treatment with insulin and *Aegle marmelose* alone and in combination therapy in diabetic rats significantly reversed D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) the footslips to near control (Figure-72).

Elevated plus maze experiment in the control and experimental Rats

(i) Behavioral response in streptozotocin induced diabetic rats: Open and closed arm entry in elevated plus- maze test in control and experimental rats

The experimental groups showed a significant increase ($p<0.001$) in the attempt taken for open arm entry in diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* alone and in combination therapy in diabetic rats significantly reversed D+I ($p<0.001$), D+P ($p<0.01$), DIP ($p<0.001$) and DAP ($p<0.001$) treated groups showed the open arm entry to near control (Figure-70).

There was a significant increase ($p<0.001$) in the number of entries made into closed arm by diabetic rats compared to control. Treatment with insulin and *Aegle marmelose* alone and in combination therapy in diabetic rats significantly reversed D+I ($p<0.001$), D+P ($p<0.01$), DIP ($p<0.001$) and DAP ($p<0.001$) treated groups showed the closed arm entry to near control (Figure-70, Figure-71).

(ii) *Behavioral response in streptozotocin induced diabetic Rats: Time spent in open and closed arms in Elevated plus-maze test in control and experimental rats*

There was a significant decrease in time spent in open arm by diabetic rats ($p<0.001$) compared to control (Figureure-6). Time spent in closed arm showed a significant increase in diabetic rats ($p<0.001$) when compared to control. Treatment with insulin and *Aegle marmelose* alone and in combination therapy in diabetic rats significantly reversed D+I ($p<0.001$), D+P ($p<0.01$), DIP ($p<0.001$) DAP ($p<0.001$) treated groups showed the time spent in open and closed arms near to control (Figure-71).

CONFOCAL STUDIES

Cerebral Cortex

5-HT$_{2A}$ receptor antibody staining in control and experimental groups of rats

The 5-HT$_{2A}$ receptor antibody staining in the cerebral cortex showed significant decrease ($p<0.001$) in the 5-HT$_{2A}$ receptor in diabetic rats compared to control. There was a significant reversal of 5-HT$_{2A}$ receptor to near control in D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of with pyridoxine alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-80; Figure-73).

5-HT transporter antibody staining in control and experimental groups of rats

The 5-HTT antibody staining in the cerebral cortex showed significant increase ($p<0.001$) in the 5-HTT in diabetic rats compared to control. There was a significant reversal to near control in expression of D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of 5-HTT on treatment with insulin and *Aegle marmelose* alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-81; Figure-74).

mGluR5 antibody staining in control and experimental groups of rats

The mGluR5 receptor antibody staining in the cerebral cortex showed significant increase ($p<0.001$) in diabetic rats compared to control. There was a significant reversal to near control in expression of D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of mGluR5 on treatment with insulin and *Aegle*

marmelose alone and in combination therapy with pyridoxine compared to diabetic rats (Table-82; Figure-75).

Brain stem

5-HT$_{2A}$ receptor antibody staining in control and experimental groups of rats

The 5-HT$_{2A}$ receptor antibody staining in the brain stem showed a significant increase ($p<0.001$) in the 5-HT$_{2A}$ receptor in diabetic rats compared to control. There was a significant reversal of 5-HT$_{2A}$ receptor to near control in D+I ($p<0.001$), D+P ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of 5-HT$_{2A}$ receptors on treatment with pyridoxine alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-83; Figure-76).

5-HT transporter antibody staining in control and experimental groups of rats

The 5-HTT antibody staining in the brain stem showed a significant decrease ($p<0.001$) in the 5-HTT in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of 5-HTT on treatment with insulin and *Aegle marmelose* alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats. (Table-84; Figure-77).

mGluR5 antibody staining in control and experimental groups of rats

The mGluR5 receptor antibody staining in the brain stem showed a significant increase ($p<0.001$) in diabetic rats compared to control. There was a significant reversal to near control in expression of D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of mGluR5 on treatment with insulin and *Aegle*

marmelose alone and in combination therapy with pyridoxine compared to diabetic rats (Table-85; Figure-78).

Cerebellum

5-HT$_{2A}$ receptor antibody staining in control and experimental groups of rats

The 5-HT$_{2A}$ receptor antibody staining in the cerebellum showed a significant decrease ($p<0.001$) in the 5-HT$_{2A}$ receptor in diabetic rats compared to control. There was a significant reversal of 5-HT$_{2A}$ receptor to near control in D+I ($p<0.001$), D+P ($p<0.05$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) on treatment with pyridoxine alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-86; Figure-79).

5-HT transporter antibody staining in control and experimental groups of rats

The 5-HTT antibody staining in the cerebellum showed a significant decrease ($p<0.001$) in the 5-HTT in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of 5-HTT on treatment with insulin and *Aegle marmelose* alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats. (Table-87; Figure-80).

mGluR5 receptor antibody staining in control and experimental groups of rats

The mGluR5 receptor antibody staining in the cerebellum showed a significant increase ($p<0.001$) in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of mGluR5 receptors on treatment with insulin and

Aegle marmelose alone and in combination therapy with pyridoxine compared to diabetic rats (Table-88; Figure-81).

Hippocampus

5-HT$_{2A}$ receptor antibody staining in control and experimental groups of rats

The 5-HT$_{2A}$ receptor antibody staining in the hippocampus showed a significant decrease ($p<0.001$) in the 5-HT$_{2A}$ receptor in diabetic rats compared to control. There was significant reversal of 5-HT$_{2A}$ receptor to near control level in D+I ($p<0.001$), D+P ($p<0.05$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of 5-HT$_{2A}$ receptors on treatment with pyridoxine alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-89; Figure-82).

5-HT transporter antibody staining in control and experimental groups of rats

The 5-HTT antibody staining in the hippocampus showed a significant decrease ($p<0.001$) in the 5-HTT in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of 5-HTT on treatment with insulin and *Aegle marmelose* alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats. (Table-90; Figure-83).

mGluR5 receptor antibody staining in control and experimental groups of rats

The mGluR5 receptor antibody staining in the hippocampus showed a significant up regulation ($p<0.001$) in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I ($p<0.001$), DIP ($p<0.001$), D+A ($p<0.001$) and DAP ($p<0.001$) of mGluR5 receptors on treatment

with insulin and *Aegle marmelose* alone and in combination therapy with pyridoxine compared to diabetic rats (Table-91; Figure-84).

Pancreas

5-HT$_{2A}$ receptor antibody staining in control and experimental groups of rats

The 5-HT$_{2A}$ receptor antibody staining in the pancreas showed a significant decrease ($p<0.001$) in the 5-HT$_{2A}$ receptor in diabetic rats compared to control. There was a significant reversal of 5-HT$_{2A}$ receptor to near control level in D+I ($p<0.01$), D+P ($p<0.05$), DIP ($p<0.001$), D+A ($p<0.01$) and DAP ($p<0.001$) of 5-HT$_{2A}$ receptors on treatment with pyridoxine alone and in combination therapy with insulin and *Aegle marmelose* compared to diabetic rats (Table-92; Figure-85).

mGluR5 receptor antibody staining in control and experimental groups of rats

The mGluR5 receptor antibody staining in the pancreas showed a significant increase ($p<0.001$) in diabetic rats compared to control. There was a significant reversal to near control level in expression of D+I ($p<0.01$), DIP ($p<0.001$), D+A ($p<0.01$) and DAP ($p<0.001$) of mGluR5 receptors on treatment with insulin and *Aegle marmelose* alone and in combination therapy with pyridoxine compared to diabetic rats (Table-93; Figure-86).

CALCIUM IMAGING

Calcium release from pancreatic islets using Fluo-4

The Fluo-4 staining showed a significant ($p<0.001$) increased calcium release from the pancreatic islets in diabetic rats compared to control. Treatment was significantly reversed to near control level in D+I ($p<0.05$), DIP ($p<0.001$), D+A ($p<0.05$) and DAP ($p<0.001$) with insulin and *Aegle marmelose* alone and in combination therapy with Pyridoxine compared to diabetic rats (Table-94; Figure-87).

Discussion

Diabetes mellitus poorly controlled results in structural and functional changes in brain regions. Streptozotocin is a toxic agent selective to pancreatic β-cells that induces diabetes by causing the β-cell destruction (Like & Rossini, 1976; Paik *et al.,* 1980). Increased blood glucose and decreased body weight during diabetes is similar with previous reports as a result of the marked destruction of insulin secreting pancreatic islet β-cells by streptozotocin (Junod *et al.,* 1969). Hyperglycaemia occurs as a result of increased glycogenolysis, decreased glycogenesis, increased gluconeogenesis, impaired glucose transport across membranes and almost complete suppression of the conversion of glucose into fatty acids. Hyperglycaemic state during diabetes is due to the increased gluconeogenic pathway, which is physiologically less sensitive to the inhibition by insulin (Burcelin *et al.,* 1995). During diabetes there is decrease in body weight as a result of altered metabolic function. It is well recognized that the glucose level is the primary determinant of the hormonal and metabolic counter regulatory responses to insulin induced hyperglycaemia. The glucose lowering activity of *Aegle marmelose* leaf extract confirmed the previous reports (Ponnachan *et al.,* 1993). Previous studies at molecular level showed diabetes-induced changes of the cholinergic activity and the regulatory role of insulin on binding parameters and gene expression of total and muscarinic M1 receptors (Gireesh *et al.,* 2009). It is also reported that there is decrease in total muscarinic and muscarinic M1 receptors during diabetes which is up regulated by insulin and *Aegle marmelose* leaf extract treatment (Gireesh *et al.,* 2008). The results suggest that the mode of action of the plant extract is probably mediated by an enhanced secretion of insulin and enhanced tissue glucose utilization. The decreased body weight in the diabetic rats is due to excessive breakdown of tissue proteins. Treatment of diabetic rats with pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf

extracts improved body weight significantly which indicate prevention of muscle tissue damage due to hyperglycaemic condition.

There was a significant decrease in the circulating insulin level of diabetic rats when compared to control group. The increase in insulin levels in pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract treated diabetic rats attribute to the stimulation of the surviving β-cells by the extract, which in turn exerts an anti-hyperglycaemic action. Reports are available to show that anti-diabetic plants are known to increase circulating insulin levels (Lamela *et al.,* 1985). Thus, it can be suggested that the treatment induce the release of insulin thereby potentiating its effect. A possible mechanism of action is that the *Aegle marmelose* stimulate the residual pancreatic β-cell function or produced the anti-hyperglycaemia through mechanism increasing peripheral utilization of glucose. This data confirmed the anti-hyperglycaemic activity of pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extracts.

Serotonin Content

The identification of improvements 5-HT receptor subtypes activation illustrates a new mechanistic strategy to treat diabetes (Zhou *et al.,* 2007). They have proved that the deletion of 5-HT receptors leads to insulin resistance. Brain serotonin content decreases in cerebral cortex and brain stem during diabetes. This decrease is reported to be due to a decrease in the rate of 5-HT synthesis (Crandall *et al.,* 1981). Changes in serotonin (5-hydroxytrptamine; 5-HT) neurotransmission have demonstrated to reduce 5-HT and 5-HIAA concentrations (Kwok & Juorio, 1987; Sandirini *et al.,* 1997). In cerebral cortex, inactive decarboxylation reaction due to lack of pyridoxal phosphate decreased the conversion of 5-HT. Also, serotonin is metabolised to 5-HIAA by the mitochondrial enzyme monoamine oxidase (MAO, primarily MAO-A) which is known to regulate insulin secretion (Pizzinat *et al.,*

1999). The decrease in 5-HT content in brain stem is brought about by significant increase in the rate of synthesis of 5-HT and its breakdown to 5-HIAA. This in turn inhibits insulin secretion by direct sympathetic stimulation and mediates diabetes induced stress. This shows that sympathetic tone plays a major regulatory role in insulin secretion. A decrease in brain 5-HT content leads to an up regulation of 5-HT receptors which in turn inhibit insulin secretion due to increased sympathetic activity (Paulose & Dakshinamurti, 1985). The crucial role played by pyridoxine in the nervous system is evident from the fact that the putative neurotransmitters 5-HT is the product of pyridoxal phosphate (PLP)-dependent enzymatic decarboxylation (Dakshinamurti, 1982; Dakshinamurti & Paulose, 1985). The role of insulin as a regulator for cell proliferation has already been established (Miyata ***et al.,*** 2007). Earlier studies, from our laboratory have proved the functional regulation of the central neurotransmitter receptor subtypes during diabetes, pancreatic regeneration, cell proliferation and insulin secretion (Paulose et al. 1988; Sudha and Paulose 1998; Abraham and Paulose CS 1999; Biju 2003; Mohanan et al. 2005; Kaimal et al. 2007; Gireesh et al.2008). Administration of insulin to the diabetic rat restores brain tryptophan concentrations to normal, but no changes in 5-HT or 5-HIAA occurs (MacKenzie & Trulson, 1978). Moreover 5-HT is a key modulator neurotransmitter and has implicated in the pathophysiology and treatment of diabetic stress. Pyridoxine resulted in restoring the serotonin content of the brain regions in diabetic rats. Insulin and *Aegle marmelose* leaf extract treated alone and in combination with pyridoxine to diabetic rats increased the 5-HT content in diabetic rats.

Glutamate content

Glutamate is essential for synaptic communication in the CNS, but inadequate regulation of extracellular glutamate and glutamate receptor agonists cause toxicity in the nervous system (Olney, 1989; Choi, 1992; Coyle & Puttfarcken, 1993; Greene &

Greenamyre, 1996; Doble, 1999) leading to neurodegenerative disorders. In the brain of diabetic, the glutamate content is reported to increase (Santos ***et al.,*** 1999). The increase in GDH activity in the diabetic group cause the increase in glutamate content (Nayeemunnisa ***et al.,*** 1977). Treatment using pyridoxine and insulin reversed the enzyme activity to control level (Aswathy ***et al.,*** 1998). Our previous studies reported increased monoamine content in the plasma and platelet of diabetic patients (Jackson ***et al.,*** 1997). We observed an increase in the glutamate content in the cerebral cortex, cerebellum, hippocampus and pancreas of diabetic rats. Treatment using pyridoxine along with insulin and ***Aegle marmelose*** leaf extract has reversed the glutamate content significantly. The data obtained in the present study support that a direct effect on glutamate transport system is the mechanisms responsible for the neuroprotective role of treatment in oxidative stress conditions, suggested occurring in diabetes and neurodegenerative diseases.

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. Serotonin affects the glucose homeostasis independently. Therapeutic application is one strategy to elucidate critical and physiologically relevant serotonin receptors. Influence of serotonin in the CNS, on glucose homeostasis is likely to be centrally mediated. The arcuate glucose-excited pro-opiomelanocortin (POMC) neurons are target to circulating hormones and nutrients such as glucose and as loss of these signals leading to impaired glucose tolerance (Balthasar ***et al.,*** 2004). Zhou et al. (2007) demonstrated that activation of POMC neurons is required for the improved glucose tolerance. Glucose sensing, when impaired ***via*** a disruption of K_{ATP} channels, leads to glucose intolerance regulation (Parton ***et al.,*** 2007) by serotonergic inputs, which in turn are known to be critical to

energy balance. 5-HT receptors effects on glucose tolerance and leads to insulin resistance showing that these effects are directly mediated by 5-HT receptors in diabetic rats (Zhou *et al.,* 2007). Our investigation revealed a down regulation of insulin receptor mRNA in cerebral cortex. Treatment using pyridoxine along with insulin and *Aegle marmelose* leaf extract reversed the insulin receptor to near control. Epidemiological and clinical studies reported that diabetes is related to psychiatric disorders, including depression (Anderson *et al.,* 2001; Lustman *et al.,* 2002). Serotonergic responses to stress are mediated by different serotonin receptor subtypes. 5-HT_{2A} receptor subtypes plays special role in serotonergic responses to stress and has been suggested to involve in affective disorders, anxiety disorders and depression (Mikuni *et al.,* 1991; Hoyer *et al.,* 1986). Treatment with pyridoxine to diabetic rats caused a reversal in the B_{max} of 5-HT, affinity of 5-HT_{2A} and insulin receptor expression to near control. Also, it is evident that pyridoxine along with insulin and *Aegle marmelose* leaf extracts have neuroprotective action mediated through the 5-HT transporter at the transcription level. Deletion of 5-HT receptors leads to insulin resistance. Alterations in these receptors play a major role in the pathogenesis of major stress and anxiety (Meltzer *et al.,* 1987) and diabetes has been reported to be a major risk factor (Lustman *et al.,* 1992; Popkin *et al.,* 1988).

The dysfunction of stress-elicited 5-HT release causes the increased expression of fear-related behaviour in diabetic rats (Miyata *et al.,* 2007; Leigh & Kramer, 1984; Mazze *et al.,* 1984). Investigation of its effects on elevated plus maze and spontaneous alternation in behaviour paradigm is a measure of anxiety. Our results showed that the diabetic rats exhibit significant alterations in its behavioural response due to cortical neuronal as a result of diabetes. Administration of pyridoxine and insulin significantly increased the percentage of the total number of entries in the open. Hence the treatment has pharmacological and neurobiological bases of arms and the number of total entries anxiety. Immunohistochemical studies for 5-HT_{2A}, 5-HTT

and mGluR5 receptors using confocal microscope in the rat cerebral cortex confirm the receptor and gene expression studies.

Our findings report treatment using pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract reversed the increase in total glutamate receptors function in the cerebral cortex with no significant change in K_d. This increased B_{max} observed showed the increased number of receptors with no change in the affinity of the receptors which was shown from the K_d. The increased receptor acitivity observed from the Scatchard plot was supported by the gene expression studies of mGluR5 glutamate receptor subtypes. Brain dysfunction limits intensified therapy in patients with insulin dependent diabetes mellitus, despite evidence that such therapy reduces the risk of chronic complications of the disease (Maran *et al.,* 1994). Our studies on the cerebral cortex showed that the increased glutamate content increased brain damage during hyperglycaemia which is suggested to contribute to cognitive and memory function (Joseph *et al.,* 2008). The mGluR5 is reported to mediate a G-protein-dependent release of intracellular stores (Valenti *et al.,* 2002). Yu *et al.,* (1997) pointed out that mGlu5 mediate direct inhibition *via* G-protein. We observed an increased expression of the mGluR5 receptor gene expression in the cerebral cortex of diabetic rats. Treatment using pyridoxine alone and in combination with insulin and *Aegle marmelose* leaf extract were reversed to near control. Hence, it is likely that G-protein-dependent release of intracellular Ca^{2+} is through mGluR5 activation. 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; Rosenberg & Aizenman, 1989; Rosenberg *et al.,* 1992; Rothstein *et al.,* 1996; Tanaka *et al.,* 1997; Wang *et al.,* 1998) and this function is served by glutamate transporter proteins. Glutamate uptake into neurons and glial cells is important for the termination of

glutamatergic transmission. They are essential for the maintenance of low extracellular levels of glutamate (Lo′pez Bayghen ***et al.,*** 2003). We observed a reduced expression of GLAST receptor transporter in diabetic rats. The decreased glutamate transporter GLAST expression reduces the reuptake of the extracellular glutamate which was confirmed from the glutamate content analysis. Thus we show evidence for the dysfunction of diabetic cerebral cortex that is a reflection for manifestation of abnormal behavioural patterns due to cellular proliferation. Treatment with insulin and ***Aegle marmelose*** leaf extract alone and in combination with pyridoxine restored the impairment to near control.

Brain contains large amounts of enzymes to protect brain against oxidative damage (Tayarani ***et al.,*** 1987). Living cells possess protective enzymes, SOD and GPx (Del Boccio ***et al.,*** 1990), to help the cells against a variety of oxidative stress (Hammond ***et al.,*** 1985). Decrease in the activity, mRNA level of SOD and GPx was evident in free radical metabolism in cerebral cortex of diabetic rats (Asplund ***et al.,*** 1984). In hyperglycaemia, glucose undergoes auto oxidation and produces free radicals. Pyridoxine treated alone and in combination with insulin and ***Aegle marmelose*** leaf extract strengthens the endogenous antioxidant defenses from reactive oxygen species (ROS) and restore optimal balance by neutralizing the reactive species (Sabu ***et al.,*** 2004). The receptor analysis and gene expression studies along with the behavioural data implicate a role of 5-HT, 5-HT_{2A} receptor in the manifestation of diabetes stress and mGluR5 for the cognitive deficits associated with diabetic rats. It is evident that neuroprotective and pharmacological role of pyridoxine along with insulin and ***Aegle marmelose*** leaf extract involved in the interaction of 5-HT, 5-HT_{2A} and glutamate receptors with modulation in 5-HT_{2A} , 5-HT transporter, mGluR5 and GLAST gene expression at the mRNA level.

Brain stem

We found a decrease in the serotonin content in the brain stem of diabetic rats when compared to control. Treatment has reversed these changes to near control. Brain stem is an important part of the brain in monitoring the glucose status and the regulation of feeding (Pénicaud *et al.,* 2002). The dorsal motor nucleus of the vagus nerve is located in the brain stem. It is connected to the endocrine pancreas exclusively *via* vagal fibres and has a role in neurally mediated insulin release. The cell bodies of the 5-HT containing neurons are confined primarily to the raphe nucleus of the brain stem (Duan *et al.,* 1989; Jocobs & Azimitia, 1992) where the extracellular 5-HT is determined (Sharp *et al.,* 1990). In the brain stem of diabetic rats, the decrease in 5-HT content was brought about by a significant increase in the rate of synthesis of 5-HT and the breakdown to 5-HIAA. The observed reduction in 5-HT synthesis will be associated with reduced transmitter release (Carndall *et al.,* 1981). A gradual decrease in the level of monoamines, a decrease in the content of their metabolites and alterations in monoamine receptor density in the brain stem of diabetic rats have been reported (Crandall *et al.,* 1981; Lozovsky et aI., 1981; Trulson & Himmel, 1985; Kolta *et al.,* 1986; Trulson *et al.,* 1986; Bitar & Desouza, 1990; Lackovié *et al.,* 1990; Salkovié & Lackovié, 1992). 5-HT transporter proteins, which pump monoamines from the synaptic cleft into the presynaptic terminal (Graham & Langer, 1992; Uhl, 1992), are one of the modulators of monoamine synaptic transmission. 5-HT transporters in the CNS response are impaired in diabetic animals (Massol *et al.,* 1989). Altered expression of 5-HT transporter genes contribute to dysfunction in monoamine neurotransmission during diabetes. Consistent with the regional monoamine content changes in the brain in diabetes, our results demonstrated that streptozotocin induced diabetes in rats is associated with altered expression of 5-HTT mRNAs in localized brain regions. Our results showed an up regulation of 5-HTT which was reversed to near control.

Insulin and ***Aegle marmelose*** leaf extract treatment was able to significantly increase the 5-HT content. It has been documented that long term hyperglycaemia in diabetic animals lead to chronic hypofunction of central 5-HT neurons leading to decrease brain tryptophan, 5-HT and 5-HIAA (Sandrini ***et al.,*** 1997; Kwok & Juorio, 1987). This decrease in brain 5-HT is due to decreased availability of the co-enzyme pyridoxine in brain that promote decarboxylation reaction. Treatment of diabetic rats with pyridoxine along with Insulin and ***Aegle marmelose*** leaf extract reversed the 5-HT content, 5-HT, 5-HT_{2A} receptor and 5-HT transporter in the brain stem of diabetic rats. Serotonin synthesis is increased, possibly as a result of desensitization of auto receptors (Lesch ***et al.,*** 1991) and thereby modifying synthesis and release of serotonin. 5-HT receptor increased its affinity to bind to available 5-HT which in turn regulates the transcription of the 5-HTT. This inhibits insulin secretion by direct sympathetic stimulation which mediates diabetes induced depression. This shows that sympathetic tone plays a major regulatory role in insulin secretion. A decrease in brain 5-HT content leads to an up regulation of 5-HT receptors which in turn inhibit insulin secretion due to increased sympathetic activity (Gispen & Biessels, 2000). Present study indicates a decreased serotonin receptor activity in brain stem with altered affinity of receptor during diabetes, bring about decrease in sympathetic nerve discharge and thereby decreasing the circulating serotonin level. Brain stem serves as one of the key regions of CNS regulating the body homeostasis. The crucial role played by pyridoxine in the nervous system is evident from the fact that the putative neurotransmitters 5-HT is the product of pyridoxal phosphate (PLP)-dependent enzymatic decarboxylation (Dakshinamurti, 1982; Dakshinamurti ***et al.,*** 1985). Pyridoxine resulted in restoring the serotonin content of brain stem in diabetic rats. The data obtained in the present study support that a direct effect on glutamate transport system is the mechanisms responsible for the neuroprotective role of treatment in oxidative stress conditions. It is suggested to occur in diabetes and

neurodegenerative diseases. In the brain of diabetic rats the glutamate content is reported to increase (Santos ***et al.,*** 1999). The reversal of hyperglycaemic condition in the Pyridoxine treated alone and in combination with insulin and ***Aegle marmelose*** leaf extract is due to the effect of insulin, pyridoxine and ***Aegle marmelose*** leaf extract on pancreatic β-cell regeneration. The role of insulin as a regulator for cell proliferation has already been established (Paul ***et al.,*** 1996).

In brain stem of diabetic rats, we observed an increased expression of mGlu-5 mRNA and up regulation of glutamate receptors without any change in affinity when compared to control. Treatment has reversed the changes to near control. Our results suggest that glutamate receptor alterations found in the brain regions contribute to cognitive and memory deficits during diabetes. Studies have shown that regulation of glutamate receptor properties contribute to learning and memory (Massicotte, 2000). Activation of this neurotransmitter system is also involved in neurodegeneration following a wide range of neurological insults, including ischemia, trauma and epileptic seizures (Hollmann & Heinemann, 1994; Lipton & Rosenberg, 1994). Insulin treatment was found to alter glutamate receptor activation (Liu ***et al.,*** 1995) as well as to 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 ischaemia (McCall, 1992), conditions that are exacerbated by hyperglycaemic states. The increase in GDH activity in the diabetic group is the cause for the increase in glutamate content (Nayeemunnisa ***et al.,*** 1977). Treatment using pyridoxine and insulin reversed the enzyme activity to control (Aswathy ***et al.,*** 1998).

Pyridoxine resulted in restoring the glutamate content of brain stem in diabetic rats. Glutamate levels reflect their intrasynaptic release. This is indicated by the response of the B_{max} of glutamate receptor binding to its ligand. Treatment of diabetic rats with pyridoxine reflects the synthesis and secretion of the neurotransmitter glutamate (Dakshinamurti *et al.,* 1990). Defects in glutamate because of a faulty transporter lead to neurotoxic levels of extracellular glutamate and thus be pathogenic. After synaptic release, glutamate is taken up by the nerve terminal *via* a plasma membrane-bound protein EAAT3. In the nerve terminal, glutamate is pumped into synaptic vesicles by different vesicular glutamate transporter proteins (VGLUT1-3), which represent unique markers for glutamatergic neurons. Using immunohistochemistry by confocal microscope the gene expression studies were confirmed. Oxidative stress plays an important role in tissue damage caused by diabetes, which results in deterioration in glucose homeostasis caused by these metabolic disorders. Severe energy deprivation following diabetes, cause mitochondrial free radicals scavenger system down regulated, which leads to reactive oxygen species (ROS) generation. High levels of ROS in turn activate the processes leading to DNA damage. Pyridoxine along with insulin and *Aegle marmelose* leaf extract involve in the interaction of 5-HT, 5-HT_{2A} and glutamate receptors, 5-HT_{2A}, 5-HT transporter, mGluR5 and GLAST gene expression at the mRNA level which regulate glucose homeostasis in the brain stem of diabetic rats.

Cerebellum

Experimental evidence indicate the involvement of the cerebellum in 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 learning (Imazumi *et al.,* 2004; Hermann *et al.,* 2004; Jiao *et al.,* 2008). Some of the most frequent signs of cerebellar

hypoplasia include poor fine motor skills, hypotonia and autistic features (Wassmer *et al.,* 2003). 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).

5-HT is released in the cerebellar cortex where mossy fibers afferents are active. The serotonin-induced disability consequently allows the cerebellar cortex to control the motor system. Studies demonstrates that fibers from cerebellar cortex and nuclei control various motor performances by different neural networks and also controls different types of motor performances (Eccles, 1973). This is responsible for better characterization of cerebellar nuclear functions. Evidence exists that 5-HT and their receptor subtypes are widely distributed throughout the cerebellar cortex and nuclei (Bishop & Ho, 1985; Pazos & Cortes, 1985). The neurons excited by 5-HT are located in cerebellar nuclei projecting to thalamus and cortex, whereas the nuclei projecting to the motor periphery are almost totally depressed by an increase of 5-HT levels. A possibility exists that 5-HT evoked excitations are mediated by receptors like 5-HT_{1A} or 5-HT_{2A} in the cerebellum (Geurts *et al.,* 2002). 5-HT_{2A} receptors are involved in a diversity of physiological functions such as the control of nociception, motor behaviour, endocrine secretion, thermoregulation, modulation of appetite and the control of exchanges between the central nervous system and the cerebrospinal fluid. Electrophysiolocal studies have reported that serotonergic agonists affect directly the firing of cerebellar neurons and are able to modulate the effect of excitatory amino acids. The cerebellum is known to play an important part in sensorimotor processing. The cerebellar system is also implicated in memory

impairment leading to neurodegerative disorders. Reciprocal interactions between the motor system and the serotonergic modulatory system are well documented (Jacobs & Fornal, 1997). 5-HT is a monoamine neurotransmitter synthesized by the aromatic amino acid decarboxylase using 5-HTP as a substrate. It was shown that 5-HT and its precursor have powerful antioxidant properties (Munoz ***et al.,*** 2006). The production of the neurotransmitter 5-HT is increased after administration of insulin and pyridoxine with an increase in transport of L-tryptophan from the plasma across the blood-brain barrier. In the brain, L-tryptophan is converted to 5-HT in the presence of the enzyme and the co-enzyme pyridoxine (Calderón-Guzmán ***et al.,*** 2004). 5-HT decrease has been reported in hypothyroidism and hypertension (Dakshinamurti ***et al.,*** 1985, 1990; Paulose ***et al.,*** 1988). Our results showed the effects of aqueous leaf extract of ***Aegle marmelose*** and pyridoxine on the cerebellar 5-HT through $5HT_{2A}$ subtype and glutamate receptors, status of antioxidants SOD, GPx, serotonin subtype $5HT_{2A}$, 5-HTT, mGluR5 and GLAST gene expression and immunohistochemical studies and motor function by Rotarod test in diabetic rats.

A decrease in the rate of 5-HT synthesis and changes in serotonin neurotransmission have demonstrated to reduce 5-HT concentrations (Crandall ***et al.,*** 1981; Kwok & Juorio, 1987; Sandrini ***et al.,*** 1997). Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins. High levels of free radicals with decline of antioxidant defence mechanisms lead to damage of energy disbursement, cellular organelles and insulin resistance (Maritim ***et al.,*** 2003). In the brain, serotonergic fibres acts on specific receptors to modulate the activity on autonomic pathways and affects energy expenditure and motor behaviour regulated by 5-HT receptors. 5-HT released, appears to regulate energy homeostasis through peripheral mechanisms.

Serotonin affect on energy balance leads to glucose homeostasis and insulin resistance. Serotonergic pathways also directly affect glucose homeostasis through regulation of autonomic efferents and action on peripheral tissues. Serotonergic compounds have been evaluated for clinical use in the treatment of diabetes (Daniel &, Lora, 2007).

The cerebellum, like most sensorimotor areas of the brain, receives serotonergic innervations from neurons of the reticular formation. It is established that local application of 5-HT modulates the firing rate of cerebellar Purkinje cells and the mechanisms by which 5-HT affects the cerebellar function. Interactions of 5-HT with other neurotransmitters have been reported to increase or decrease the firing frequency of Purkinje cells. There is an evidence for a modulation of excitatory and inhibitory synapses by 5-HT in the cerebellar cortex. The mechanisms of the serotonergic modulation of the cerebellar cortex are of clinical relevance, as abnormal 5-HT metabolism has been observed in animal models and pathological cases of motor disorders involving the cerebellum (Romero ***et al.,*** 1991; Reiter, 1995; Stéphane Dieudonné, 2001). The cerebellum are exposed to oxidative stress. SOD and GPx is an expressed antioxidant enzyme present in the cytosol and mitochondria. It is involved in the detoxification of hydrogen and lipid peroxides (Brigelius-Flohe ***et al.,*** 2003; Colak ***et al.,*** 2005). In the absence of this antioxidant enzyme, a buildup of ROS ensues that is known to damage DNA, proteins, and lipids (Sies ***et al.,*** 1997). There is a potential role for GPx in diabetes (Colak ***et al.,*** 2005). In diabetes, GPx activity was found to decrease and a parallel decrease in reduced GPx content suggests its direct chemical interaction with various free radical entities during prolonged diabetes stress and disturbs the cellular redox status. Decrease in the activity, mRNA level of SOD was evident in free radical metabolism in cerebellum of diabetic rats (Asplund ***et al.,*** 1984). In hyperglycaemia, glucose undergoes auto oxidation and produces free radicals. Pyridoxine treated alone and in combination with insulin and ***Aegle***

marmelose leaf extract strengthens the endogenous antioxidant defenses from reactive oxygen species (ROS) and restore optimal balance by neutralizing the reactive species (Sabu & Ramadasan, 2004).

Diabetes has been reported to be accompanied by a number of behavioural and enzymatic abnormalities, including reduced locomotor activity (Marchall ***et al.,*** 1976). Majczynski ***et al.,*** (2005) reported that restitutions of locomotor abilities and are brought about by serotonergic innervations and is mediated through 5-HT_{2A} receptors. Therapeutic application is of physiological relevance with 5-HT receptors. Glucose sensing, when impaired ***via*** a disruption of K_{ATP} channels, leads to glucose intolerance regulation (Parton ***et al.,*** 2007) by serotonergic inputs, which in turn are known to be critical to energy balance. Loss of coordination of motor movement, inability to judge distance and timing, incapacity to perform rapid alternating movements and hypotonia has been reported during cerebellar damage (Gowen & Miall, 2005). Poor limb–eye coordination in patients with cerebellar dysfunction has been earlier reported (Donkelaar & Lee, 1994). Rotarod test has been used to examine the motor in-coordination (Cendelin ***et al.,*** 2008) which demonstrates the impairment in the motor function and coordination in the diabetic rats. Diabetes rats showed lower fall off time from the rotating rod when compared to control suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture. They also do not adjust their limb movements on the metallic rod which is indicative of cerebellar dysfunction. Treatment using pyridoxine alone and in combination with insulin and ***Aegle marmelose*** leaf extract to diabetic rats reversed the decreased fall off time from the rod compared to diabetic rats. Treatments alle***via***te their stress level which assist in lowering their time for spatial recognition and helps to maintain their posture during movement on the rod.

The decrease in brain tryptophan alone is observed to decrease 5-HT synthesis rate. The decrease in brain tryptophan in diabetic rats appears to be due to decrease in

its plasma concentration. Also, there is increase in the branched chain amino acids that compete with tryptophan for uptake into the brain (Fernstrom & Wurtman, 1971). This leads to an altered 5-HT synthesis. The changes in brain 5-HT synthesis rate in diabetic rats are related to the various behavioural and psychological changes. The psychological changes observed in diabetes appear to persist even when the diabetic state is well-controlled with insulin administration (Michael *et al.,* 1986). 5-HT induced inhibition of neurons of the cerebellar nuclei is mediated by the activation mediated through 5-HT_{2A} receptors. This suggest that in multiple locations within the CNS, excitation of subpopulations of interneurons by 5-HT *via* 5-HT_{2A} receptors gives rise to indirect inhibitory effects (Cumming-Hood *et al.,* 1994). Previous reports showed a decrease in 5-HT in brain regions during diabetes (Sumiyoshi *et al.,* 1997; Tarui *et al.,* 1987; Sandrini *et al.,* 1997). In cerebellum, inactive decarboxylation reaction due to lack of pyridoxal phosphate decreased the conversion of 5-HT. Treatment of rats with moderate doses of pyridoxine results in an increment in brain 5-HT indicating that the tissue 5-HTP-decarboxylation responds to the pyridoxine status of the animal (Dakshinamurti *et al.,* 2003). Present study indicates a decreased 5-HT and 5-HT_{2A} receptor binding with increase in affinity in cerebellum of diabetic rats. This decreases the sympathetic nerve discharge and thereby decreasing the circulating 5-HT level. The pharmacological modulation of 5-HT receptor activity was an effective approach to treat diabetes. 5-HT plays an important role as a neurotransmitter in the CNS and as a mediator of peripheral signals. Pharmacological modulators of specific 5-HT receptor isoforms have been developed to treat various conditions that are associated with its functional role. The crucial role played by pyridoxine in the nervous system is evident from the fact that the putative neurotransmitters 5-HT is the product of pyridoxal phosphate (PLP)-dependent enzymatic decarboxylation (Dakshinamurti, 1982; 1985). Pyridoxine resulted in restoring the synthesis of 5-HT in cerebellum of diabetic rats. 5-HT levels reflect their

intrasynaptic release is indicated by the response of the B_{max} of 5-HT receptor binding to its ligand. The results indicate that the pyridoxal phosphate level in cerebellum regulates the extent of decarboxylation of the 5-HTP, the precursor of 5-HT. Treatment of diabetic rats with pyridoxine reflected the synthesis and secretion into the synaptic cleft of the neurotransmitter 5-HT (Dakshinamurti *et al.,* 1990; Paulose & Dakshinamurti, 1985). 5-HT synthesis is increased, possibly as a result of desensitization of auto receptors (Lesch *et al.,* 1991) and thereby modifying synthesis and release of 5-HT.

5-HTT regulates the entire serotoninergic system and its receptors *via* modulation of its expression and function. In brain, 5-HTT is situated both in presynaptic membranes of nerve terminals in proximity to serotonin-containing cell bodies (Dennis *et al.,* 2004). 5-HTT mediates rapid removal and recycling of released 5-HT following neuronal stimulation. Thus, it has a critical role in the homeostatic regulation of the signals reaching 5-HT receptors. 5-HTT is important in emotion regulation and social behaviour, drawing from an interdisciplinary perspective of behavioural genetics and cognitive neuroscience. Integration of these findings suggests that the impact of the 5-HTT gene on behaviour and have a role in social cognition (Turhan & Peter, 2007). 5-HT is packaged into vesicles for synaptic exocytosis. Extracellular 5-HT signals through $5\text{-}HT_{2A}$ receptors. Synaptic 5-HT signaling are terminated by uptake of $5\text{-}HT_{2A}$ from the synapse by the 5-HTT.

Administration of pyridoxine along with insulin serves as a control measure for diabetes, regulating GDH activity and glucose level were observed (Aswathy *et al.,* 1998). The reversal of hyperglycaemic condition in DIP is due to the effect of pyridoxine and insulin on pancreatic β cells. The role of insulin as a regulator for cell proliferation has already been established (Paul *et al.,* 1996). Further, PLP is known to function as co-factor to ornithine decarboxylase that catalyses the conversion of ornithine to putrescine, the precursor of other polyamines. Treatment with pyridoxine

to diabetic rats caused a reversal in the B_{max} of 5-HT_{2A} receptors to near control. Also, it is evident that pyridoxine along with insulin has neuroprotective action mediated through the 5-HTT at the transcription level.

Aegle marmelose leaf extract was comparable to insulin in reversing blood glucose to normal levels. Anandharajan ***et al.,*** (2006) reported that *Aegle marmelose* activate glucose transport in PI3 kinase-dependent fashion. The leaf extract treated animals appeared healthier and were less prone to the frequent hypoglycaemic condition observed in their insulin treated counterparts. Treatment with pyridoxine alone and in combination with pyridoxine and *Aegle marmelose* leaf extract to diabetic rats caused a reversal in the B_{max} of 5-HT_{2A} receptors, 5-HT_{2A} and 5-HTT gene expression to near control level. Also, it is evident that *Aegle marmelose* leaf extract has antioxidant and neuroprotective action mediated through the 5-HTT at the transcription. This study demonstrates the involvement of 5-HT_{2A} receptor which has modulating effect on the diabetes and associated motor defects. Administration of pyridoxine alone and in combination with *Aegle marmelose* and insulin significantly decreased the motor disability. Hence, the treatment has pharmacological and neurobiological bases of motor deficit.

The 5-HT_{2A} receptor analysis, SOD, GPx, 5-HT_{2A} and 5-HTT gene expression studies along with the rotarod test implicate a role for 5-HT receptors in the manifestation of motor defects associated with diabetic rats. The treatment of pyridoxine and *Aegle marmelose* leaf extract normalizes the 5-HT_{2A} receptor, SOD, GPx, 5-HT_{2A} and 5-HTT gene expression and diabetic related motor dysfunctions to control. Thus it is suggested that pyridoxine treated alone and in combination with insulin and *Aegle marmelose* leaf extract have a role through 5-HT_{2A} receptors functional regulation in the cerebellum which has clinical significance in the management of diabetes.

Metabotropic glutamate (mGlu) regulate synaptic glutamate release both in ***in vitro*** (Herrero ***et al.,*** 1994) rat brain slices (Croucher ***et al.,*** 1997) and ***in vivo*** (Patel & Croucher, 1997). We also observed an increase in the gene expression of mGluR5 receptor subtypes. Activation of mGluRs modulates synaptic transmission and activity-dependent synaptic plasticity (Pin & Duvoisin, 1995; Conn & Pin, 1997; Nicoletti ***et al.,*** 1999). Glutamate which caused excitotoxic neuronal damage, increased calcium influx in post synaptic neurons, leading to phospholipase A_2 mediated arachidonic acid release (Miriam ***et al.,*** 1996). Our previous studies on cerebellum also reported that GDH enzyme activity enhanced during diabetes and did not completely reverse after insulin administration (Preetha ***et al.,*** 1996; Aswathy ***et al.,*** 1998). Studies using diabetic rats clearly revealed that in cerebellum GDH activity regulation is essential to avoid diabetic associated brain glutamate toxicity (Biju & Paulose, 1998). Increased number of glutamate receptor activity leading to glutamate excitotoxicity and neuronal degeneration were reported from our lab (Joseph ***et al.,*** 2007).

One of the major causes of neuronal death in neurodegenerative disease is excitotoxicity from the neurotransmitter glutamate. This form of cell death arises from either excess levels of glutamate due to decreased astrocyte clearance or due to increased susceptibility. Several glutamate transporters have been characterized, the Na^+–dependent glutamate/ aspartate transporter, GLAST being the major uptake system within the cerebellum (Danbolt, 2001). We also observed a decrease in the GLAST glutamate transporter expression in both adult and old diabetic and hypoglycaemic rats. Previous studies from our lab showed the increased glutamate production ***via*** increased glutamate dehydrogenase enzyme activity (Joseph ***et al.,*** 2007). The present study showed the increased glutamate content, receptor number and gene expression in the cerebellum. Mitosek and his colleagues (2008) reported the protective role of GLAST glutamate transporter in the multiple sclerotic cerebellum.

Our findings suggest dysfunction of the diabetic cerebellum that is a reflection of cerebellar glutamatergic abnormality. The receptor analysis and gene expression studies along with the behavioural data implicate a role for glutamate and serotonergic system in the modulation of neuronal network excitability ***via*** their transporters. These neurofunctional deficits are one of the key contributors to motor deficits and stress associated with diabetes. Treatment with pyridoxine along with insulin and ***Aegle marmelose*** leaf extract has reversed these alterations to near control.

Hippocampus

Hippocampus is based on recent or declarative memory and plays important roles in long-term memory and spatial navigation. Rats treated with streptozotocin have reduced insulin and show hyperglycaemia, increased corticosterone and impairments in hippocampal neurogenesis, synaptic plasticity and learning. We also observed a significant down regulation in hippocampal insulin levels in diabetic rats. This confirms the possibility that insulin signalling pathways might be impaired in diabetes. Specifically, intrahippocampal insulin (Moosavi ***et al.,*** 2007) or activation of insulin signalling pathways (Revest ***et al.,*** 2005) block the effects of stress on learning and memory. Exposure to elevated corticosterone reduces insulin receptor signalling in many somatic tissues, including the brain (Piroli ***et al.,*** 2007). Therefore, it is possible that the negative effect of diabetes on hippocampal plasticity attribute to interaction between elevated insulin receptor signaling. Treatment has reversed the gene expression of insulin receptors mRNA to near control.

We observed a decrease in the serotonin content in the hippocampus of diabetic rats when compared to control. Studies of the serotonergic modulation of hippocampal function have been complicated by the marked heterogeneity of 5-HT receptor subtypes, with atleast 14 different subtypes expressed in the central nervous system. Psychological stress activates serotonergic neurons in the hippocampus and

amygdale through cortical associated areas and through ascending catecholaminergic neurons from the brain stem (Feldman & Weidenfeld, 1998; Knob & Heinrichs, 1999). Serotonergic neurotransmission exerts a considerable influence on hippocampus. This structure is influenced powerfully by serotonergic projections from midbrain raphe nuclei (Tecott ***et al.,*** 1998) which modulate hippocampal electrical activity, hippocampal dependent behaviours and long term potentiation (LTP), a form of hippocampal plasticity that has been implicated in memory formation (Vanderwolf & Baker, 1986). Gross analyses of 5-HT metabolism do not yield information on 5-HT release and suggest that the prevalence of diabetes among patients suffering from lack of hippocampal serotonergic response to aversive stimuli. The level of serotonin decreases the monoamine release from the hippocampus in diabetic rats. There is good evidence that noradrenaline and 5-HT interact to influence neuroplasticity in the brain (Delgado, 2004). In this study, we focused on the 5-HT_{2A} receptor which is expressed in the hippocampus. We observed a significant decrease in the B_{max} and K_d of 5-HT_{2A} receptors in the hippocampus of diabetic rats compared to control. 5-HT_{2A} receptor gene expression were confirmed the receptor binding data. 5-HTT was also down regulated in diabetes rats. Treatment with pyridoxine alone and in combination with ***Aegle marmelose*** and insulin significantly reversed the receptor alterations in B_{max} and K_d to near control. Treatment with pyridoxine along with insulin and ***Aegle marmelose*** leaf extract has been demonstrated to regulate serotonin levels, receptor alterations and reversed activity of 5-HTT in hippocampus.

The hippocampal formation contains a rich glutamatergic and GABA-ergic input, GABA-ergic interneurones containing peptide co-transmitters and the glutamatergic perforant pathway interconnects with entorhinal cortex, subiculum, CA1, CA3 fields and dentate gyrus (Ottersen & Storm-Mathisen, 1984). Potentiation, defined as an increase in synaptic efficacy, is readily induced by high frequency stimulation (HFS) of the synapses between the Schaffer collaterals and the pyramidal

cells in the hippocampus CA1 area (Collingridge & Bliss, 1995; Malenka & Nicoll, 1999). The excitatory synapse in the stratum radiatum of the CA1 area of the hippocampus has a number of features that have been attributed to various aspects of memory encoding (Martin *et al.,* 2000). In this study, we focused on the glutamate receptor, which is abundantly expressed throughout the hippocampal formation. Our results showed increased glutamate content in the hippocampus of diabetic rats. Hippocampal neurons receive a rich glutamergic innervation and evidence suggests that hypoglycaemic injury in these neurons is precipitated almost entirely by sustained glutamate receptor activation (Auer *et al.,* 1985). Tanaka *et al.,* (2008) reported that absence of glucose, insulin accelerated the neuronal cell death both in the CA1 and DG. They also concluded that insulin has a double-edged effect on the neuronal cell death dependent on glucose concentration and that the CA1 and the DG have a different sensitivity to insulin in terms of cell survival. Recent reports suggest that 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. Based on extensive supportive experimental data, the release of high levels of glutamate by neurons is thought to be the underlying mechanism for the initiation of neurodegeneration. Quintana *et al.,* (2006) reported that transient hypoglycaemia is associated with a marked enhancement of excitatory transmission with an increased synthesis of excitatory receptor subunits in organotypic hippocampal slice cultures. Our experimental results support the earlier reports. The immunohistochemical studies using confocal microscope in the present work confirmed the gene expression studies of mGluR5 receptors. This up regulation will increase the glutamate receptor activity and molecular cascades inside the cells.

Our experiments also demonstrated decreased expression of GLAST glutamate transporter in the hippocampus of experimental rats compared to control. This decreased expression of glutamate transporter will lead to the decreased clearance of glutamate from the extracellular space and we report in our present study that glutamate content is high in the hippocampus of experimental group compared to control. It was found that GLAST glutamate transporter down regulation is involved in cell swelling in hippocampus during diabetes (Han ***et al.,*** 2004). To summarize our results in hippocampus we observed an increased glutamate receptor activity. A decreased glutamate transporter expression and increased glutamate content was observed. Treatment with pyridoxine along with insulin and ***Aegle marmelose*** leaf extract has been demonstrated to regulate glutamate levels, receptor alterations and reversed activity of GLAST in hippocampus.

Pancreas

Previous studies demonstrated that these amino acid precursors are taken up by the islets by an active energy requiring process (Mahony & Feldman, 1977). They are then converted to their respective amine by the aromatic amino acid decarboxylase of the pancreatic islets (Lebovitz & Downs, 1973). The predominant enzyme found in islets that inactivates monoamines is monoamine oxidase (Feldman ***et al.,*** 1979). 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.,*** 2005a, b). Pancreatic islets receive innervations from both divisions of the autonomic nervous system and pancreatic endocrine secretion which is partly controlled by the autonomic nervous system (Liu ***et al.,*** 2001). Treatment with pyridoxine to diabetic rats caused a reversal in the B_{max} of 5-HT,

affinity of 5-HT_{2A} and insulin receptor expression to near control. Also, it is evident that pyridoxine along with insulin and *Aegle marmelose* leaf extracts have neuroprotective action mediated through the 5-HT transporter at the transcription level. Deletion of 5-HT receptors leads to insulin resistance. Alterations in these receptors play a major role in the pathogenesis of major stress and anxiety (Meltzer *et al.,* 1987) and diabetes has been reported to be a major risk factor (Lustman *et al.,* 1992; Popkin *et al.,* 1988).

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 neurally mediated insulin release (Azmitia & Gannon, 1986). Our laboratory has reported that dopamine differentially regulates glucose induced insulin secretion in the pancreatic islets, an effect mediated by pancreatic DA D_2 receptors (Eswar *et al.,* 2006). Studies from our laboratory also confirmed that the down regulation of DA D_2 receptors influence the regulation of insulin secretion by releasing epinephrine and norepinephrine from the adrenal medulla, which leads to the inhibition of insulin secretion in the pancreas (Eswar *et al.,* 2007).

GDH produced glutamate, a second messenger of insulin secretion (Anno *et al.,* 2004). Glutamate receptor agonists induce various cellular responses outside the CNS, such as a rise in intracellular calcium concentration in rat pituitary cells and stimulation of growth hormone secretion (Lindstrom & Ohlsson, 1992), stimulation of insulin and glucagons secretion from rat endocrine pancreas (Bertrand *et al.,* 1992; Bertrand *et al.,* 1993) and contractions of the myenteric plexus-longitudinal muscle of guinea pig ileum (Shannon & Sawyer, 1989). These pharmacological studies suggest the presence of glutamate receptors in peripheral tissues, including endocrine tissues. Two important findings were reported regarding a relationship between glutamate metabolism and insulin secretion. A new form of persistent hyperinsulinemia with

Hypoglycaemia of the infant (PHHI) was demonstrated to be caused by an excessive activity of glutamate dehydrogenase, which produces glutamate (Stanley *et al.,* 1998; Stanley *et al.,* 2000; Macmullen *et al.,* 2001). Second, glutamate produced *via* α-ketoglutarate from glucose was reported to enhance insulin secretion under conditions of clamped cytosolic Ca^{2+} and ATP at high levels (Macmullen *et al.,* 2001). Although the direction of metabolic flux between glutamate and α-ketoglutarate upon stimulation with glucose has been controversial in β-cells (Gao *et al.,* 1999; MacDonald & Fahien, 2000), these results raised a novel postulation that glutamate play a role in transducing secretory signals from glucose metabolism to secretory vesicles and that this pathway involve in modulation of secretory vesicle pH, the acidity of which is thought to be generated mainly by vacuolar-type H^+-ATPase (Hutton & Peshavaria, 1982; Hutton, 1989; Bode *et al.,* 1996; Nelson & Harvey, 1999). An increase in β-cell glutamate is an important messenger in the amplification of insulin secretion by glucose (Bertrand *et al.,* 2002). We observed an increase in the gene expression of mGluR5 receptors in the pancreas of diabetic compared to their control rats. Treatment with pyridoxine along with insulin and *Aegle marmelose* leaf extract reversed the mGluR5 receptor expression to near control.

Molnár *et al.,* (1995) and Inagaki *et al.,* (1995) reported the presence of glutamate receptor ligands increased insulin secretion. Studies have reported that glutamate, transmitted from α-cells and neurons, stimulates insulin secretion through activation of ionotropic glutamate receptors in β-cells (Nakanishi, 1992b; Inagaki *et al.,* 1995). Glutamate acts as an intracellular messenger that couples glucose metabolism to insulin secretion (Maechler & Wollheim, 1999). Glutamate produced *via* α-ketoglutarate from glucose was reported to enhance insulin secretion under conditions of clamped cytosolic Ca^{2+} and ATP at high levels (Macmullen *et al.,* 2001). Our experiments showed that the glutamate content and receptor activity were increased in diabetic condition which was reversed to near control in treatment

groups. The GLAST glutamate transporter expression was decreased in diabetic condition. Previous studies reported that an increased islet content of L-glutamate is necessary, but not sufficient, to allow its net conversion into 2-oxoglutarate and its further metabolism in the Krebs cycle or the GABA shunt. This and the subsequent stimulation of insulin secretion, requires activation of GDH by L-leucine (Li *et al.,* 2006). The insulin secretion stimulated by glutamate was blocked by an inhibitor of vacuolar type H^+-ATPase or by an inhibitor of vesicular glutamate transporter (Gao *et al.,* 1999; MacDonald *et al.,* 2000). Decreased GLAST activity during diabetes could account for the inhibition of insulin secretion. It was also reported that the enhanced glutamate activity during insulin induced hypoglyceamia in pancreas also enhanced glucagon release (Cabrera *et al.,* 2008). This report supports the gene expression of insulin receptor mRNA which is up regulated in diabetic rats. Treatment with pyridoxine along with insulin and *Aegle marmelose* leaf extract reversed the alterations to near control.

Signal-transduction in the pancreatic β-cell and thereby the insulin secretory process is regulated by a sophisticated interplay between glucose and a plethora of additional factors including other nutrients, neurotransmitters, islet generated factors and systemic growth factors. The coupling of glucose metabolism to electrical activity remains central in all models of β-cell stimulus-secretion coupling. The resting membrane potential of the β-cell is set by the ATP-sensitive potassium (K_{ATP}) channel (Ashcroft & Rorsman, 1990). Incubation of the pancreatic β-cells with stimulatory glucose concentrations leads to the activation of a cascade of reactions, which ends in the exocytosis of stored insulin. This complex of processes starts with the uptake of glucose by the β-cell high-K_m/low affinity glucose transporter GLUT2 and proceeds with the conversion of glucose into glucose-6-phosphate by the β-cell isoform of glucokinase (Randel, 1993; Matschinsky, 1996). Metabolism of glucose in glycolysis

and the Krebs cycle results in the generation of ATP. Elevation in the ATP/ADP ratio leads to closure of the K_{ATP}, which in turn results in depolarization of the plasma membrane. The subsequent opening of voltage-gated L-type Ca^{2+} channels leads to an increase in the cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_i$, which promotes insulin secretion (Berggren & Larsson, 1994). ***Aegle marmelose*** leaf extracts have a potential role in the insulin synthesis and secretion from the pancreatic β- cell, mediating its function through serotonergic and glutamatergic receptors. This has immense clinical significance in the management of diabetes.

Glutamate acts as an intracellular messenger that couples glucose metabolism to insulin secretion (Maechler & Wollheim, 1999). Studies reported that insulin secretion is under the control of mGlu5 receptors (Storto ***et al.,*** 2006). The role of glutamate on glucose induced insulin secretion by pancreatic islets is important. Our study showed that glutamate significantly increased Ca^{2+} release from pancreatic islets in hyperglycaemic condition. Elevation of ATP is necessary for the membrane-dependant increase in cytosolic Ca^{2+}, the main trigger of insulin exocytosis (Maechler & Wollheim, 2000). It has been demonstrated by Cabrera ***et al.,*** (2008) that glutamate acts on iGluRs, resulting in membrane depolarization, opening of voltage-gated Ca^{2+} channels, increase cytoplasmic free Ca^{2+} concentration. Our results suggest that regulation of insulin secretion is mediated through serotonergic and glutamergic receptor. Treatment has reversed the alterations to near control. This has therapeutic applications in diabetes.

Behavioural Alterations in Control and Experimental rats

Epidemiological and clinical studies reported that diabetes is related to psychiatric disorders, including depression (Anderson ***et al.,*** 2001; Lustman ***et al.,*** 2002). Serotonergic responses to stress are mediated by different serotonin receptor

subtypes. 5-HT_{2A} receptor subtypes plays a role in serotonergic responses to stress and has been suggested to involve in affective disorders, anxiety disorders and depression (Mikuni *et al.,* 1991; Hoyer *et al.,* 1986). Also, it is evident that pyridoxine along with insulin and *Aegle marmelose* leaf extracts have neuroprotective action. Alterations in these 5-HT receptors play a major role in the pathogenesis of major stress and anxiety (Meltzer *et al.,* 1987) and diabetes has been reported to be a major risk factor (Lustman *et al.,* 1992; Popkin *et al.,* 1988).

Anxiety is a neurological problem associated with diabetes mellitus. Kamei and colleagues revealed that in correlation between diabetic anxiety and serotonergic systems there is a decrease in the serotonergic response to stressful stimuli. The dysfunction of stress-elicited 5-HT release causes the increased expression of fear-related behaviour in diabetic rats (Miyata *et al.,* 2007; Leigh & Kramer, 1984; Mazze *et al.,* 1984). Investigation of its effects on elevated plus maze and spontaneous alternation in behaviour paradigm is a measure of anxiety. Our results showed that the diabetic rats exhibit significant alterations in its behavioural response due to cortical neuronal as a result of diabetes. Diabetic rats showed an increased percentage attempt made towards open arm entry and the animal also remained for longer period in closed arms of elevated plus-maze maze thereby causing hypo locomotion in diabetic rats. The treatment reversed the behavioural to control. The possible anxiolytic effect is related to its effect on serotonergic transmission (Razia, 2007). Thus perturbations of the 5-HT receptor system directly modulate stress susceptibility rendering them anxiogenic as well as depressive profile. A decrease in general exploratory activity in an open arena after restraint stress has been previously described (Kennett *et al.,* 1987). In the elevated plus-maze most studies found a decrease in the percentage of open arm entries and/or time spent in them (Guimarães *et al.,* 1993; Mendonça & Guimarães, 1996). This study demonstrates the involvement of 5-HT receptor which has modulating effect on the diabetes and associated motor defects. Administration of

pyridoxine and insulin significantly increased the percentage of the total number of entries in the open Hence the treatment has pharmacological and neurobiological bases of arms and the number of total entries anxiety.

The changes in brain 5-HT synthesis rate in diabetic rats are related to the various behavioral and psychological changes. The psychological changes observed in diabetes appear to persist even when the diabetic state is well-controlled with insulin administration (Michael ***et al.,*** 1986). Diabetes has been reported to be accompanied by a number of behavioral and enzymatic abnormalities, including reduced locomotor activity (Marchall ***et al.,*** 1976). Majczynski et al., (2005) reported that restitutions of locomotor abilities and are brought about by serotonergic innervations and is mediated through 5-HT_{2A} receptors. Therapeutic application is of physiological relevance with 5-HT receptors. Loss of coordination of motor movement, inability to judge distance and timing, incapacity to perform rapid alternating movements and hypotonia has been reported during cerebellar damage (Gowen ***et al.,*** 2005). 5-HTT is important in emotion regulation and social behaviour, drawing from an interdisciplinary perspective of behavioral genetics and cognitive neuroscience. Integration of these findings suggests that the impact of the 5-HTT gene on behaviour and have a role in social cognition (Turhan & Peter, 2007). Poor limb–eye coordination in patients with cerebellar dysfunction has been earlier reported (Van ***et al.,*** 1994). Rotarod test has been used to examine the motor in-coordination (Cendelin ***et al.,*** 2008) which demonstrates the impairment in the motor function and coordination in the diabetic rats. Diabetes rats showed lower fall off time from the rotating rod when compared to control suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture. They also do not adjust their limb movements on the metallic rod which is indicative of cerebellar dysfunction. Treatment using pyridoxine alone and in combination with insulin and ***Aegle marmelose*** to diabetic rats reversed the decreased fall off time from the rod compared

to diabetic rats. Treatments alleviate their stress level which assist in lowering their time for spatial recognition and helps to maintain their posture during movement on the rod. Administration of pyridoxine alone and in combination with *Aegle marmelose* and insulin significantly decreased the motor disability. Hence the treatment has pharmacological and neurobiological bases of motor deficit. Involvement of glutamate receptor which has modulating effect on the diabetes and associated motor defects. The beam-walk apparatus has been used to assess sensorimotor deficits following brain injury and other conditions resulting in altered gait, balance, proprioception (Fox et al., 1998; Sherbel et al., 1999; Ferrer et al., 2005). Baskin et al. (2003) suggested that the beam-walk task is effective in detecting sensorimotor deficits. These results suggest that Streptozotocin induced diabetic rats leads to a sensorimotor dysfunction that is detectable by the beam-walk apparatus. Administration of pyridoxine alone and in combination with *Aegle marmelose* and insulin significantly decreased the inability of motor functions.

Thus from our results we conclude that serotonin- 5-HT_{2A}, glutamate receptor functional balance and gene expression of 5-HT_{2A}, 5-HTT, mGluR5 and GLAST play an important role in the pathophysiology of diabetic rats. Insulin and *Aegle marmelose* leaf extract treated alone and in combination with pyridoxine showed a significant reversal of the serotonergic and glutamatergic dysfunction in the streptozotocin induced diabetic rats to near control. Pyridoxine is involved in the synthesis of neurotransmitters- serotonin from 5-Hydroxytryptophan and GABA from glutamate. Serotoninergic and glutamatergic controls insulin function and is found to be effective in glucose homeostasis from the present study at molecular level. Thus it is suggested that *Aegle marmelose* and pyridoxine treatment has a significant therapeutic role in diabetes management.

Summary

1. 5-HT and 5-HIAA contents were decreased in cerebral cortex, brain stem, cerebellum and hippocampus of diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

2. Serotonin and 5-HT_{2A} receptor subtype number were decreased in cerebral cortex, cerebellum and hippocampus whereas increased in brainstem of diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

3. Serotonin receptor was increased whereas 5-HT_{2A} receptor subtype number was decreased in pancreas of diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

4. 5-HT_{2A} serotonin subtype and 5-HTT serotonin transporter gene expression were down regulated in cerebral cortex, cerebellum, hippocampus and pancreas whereas up regulated in brainstem of diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

5. Glutamate content was increased in brain regions and pancreas of diabetic rats which was reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

6. Glutamate receptor number was increased in brain regions and pancreas of diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

7. mGluR5 glutamate receptor subtype gene expression was increased significantly in all brain regions and pancreas of diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine. Diabetes induces glutamate toxicity, modifies glutamate transporter GLAST and increases neuronal injury. Pyridoxine decreases intracellular levels of glutamate by increasing glutamic acid decarboxylase activity and decrease calcium influx through actions on cell surface calcium channels.

8. GLAST glutamate transporter gene expression was down regulated significantly in cerebral cortex, cerebellum, hippocampus and pancreas whereas it was up regulated in brainstem of diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

9. Insulin receptor gene expression was down regulated significantly in cerebral cortex, brain stem, hippocampus and pancreas whereas it was up regulated in cerebellum of diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

10. Status of antioxidants - SOD and GPx gene expression were down regulated significantly in all brain regions and pancreas of diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

11. Immunohistochemical study of serotonin receptor subtype $5HT_{2A,}$ 5-HTT serotonin transporter and mGluR5 glutamate receptor subtype in cerebral cortex, cerebellum and pancreas using confocal microscope showed an increased receptor expression in diabetic rats which were reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

12. Calcium imaging results showed increased calcium release from the pancreatic islets in diabetic rats. Treatment using insulin and *Aegle marmelose* treated alone and in combination with pyridoxine decreased the release to near control.

13. A prominent neurodegeneration of cerebellum was demonstrated by rotarod test in diabetic rats which was reversed to near control in insulin and *Aegle marmelose* treated alone and in combination with pyridoxine.

14. Treatment using insulin and *Aegle marmelose* treated alone and in combination with pyridoxine to diabetic rats showed a reversal of anxio-depressive behaviour to near control which is evident by the *Elevated Plus Maze Study*.

15. Streptozotocin induced diabetic rats lead to a sensorimotor dysfunction that was assessed by the beam-walk apparatus. Administration of pyridoxine alone and in combination with *Aegle marmelose* and insulin significantly increased the ability of motor functions. Hence the treatment has curative role in motor deficit.

Thus our studies showed insulin and *Aegle marmelose* treated alone and in combination with pyridoxine have anti-hyperglycaemic effect on serotonergic and glutamatergic receptors functions in streptozotocin induced diabetic rats. It is suggested that the corrective measures for the brain functional damage caused during

diabetes and anti-diabetic treatment, through serotonergic and glutamergic receptors, have therapeutic role in the management of diabetes.

Conclusion

Diabetes mellitus is caused by the deficiency or resistance to insulin in glucose homeostasis. Insulin regulates glucose utilisation and cellular metabolism. Glutamate toxicity causes neuronal damage. Glutamate transport system is also involved in diabetes induced oxidative stress. Serotonin affects insulin function. Pyridoxine is involved in the synthesis of neurotransmitters-serotonin from 5-Hydroxytryptophan and GABA from glutamate. Serotonergic and glutamatergic systems are impaired during diabetes. We observed an increase in the glutamate content in the cerebral cortex, brain stem, cerebellum, hippocampus and pancreas of streptozotocin induced diabetic rats. Treatment using pyridoxine along with insulin and *Aegle marmelose* leaf extract has reversed the glutamate content, insulin receptor gene expression, 5-HT, 5-HT_{2A} receptor binding parameters and gene expression of 5-HT_{2A} to near control. Also, it has neuroprotective action mediated through the 5-HT transporter and GLAST at the mRNA level. Confocal studies in pancreatic islets of experimental groups of rats showed that Ca^{2+} release regulates insulin secretion. Behavioural studies confirmed the serotonin, 5-HT_{2A}, glutamate receptor, gene expression data. Thus it is suggested that serotonin and glutamate receptor functional regulation controls glucose utilization at cellular level. *Aegle marmelose* and insulin treatment alone and in combination with pyridoxine have better therapeutic role in the management of diabetes.

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Figure-1

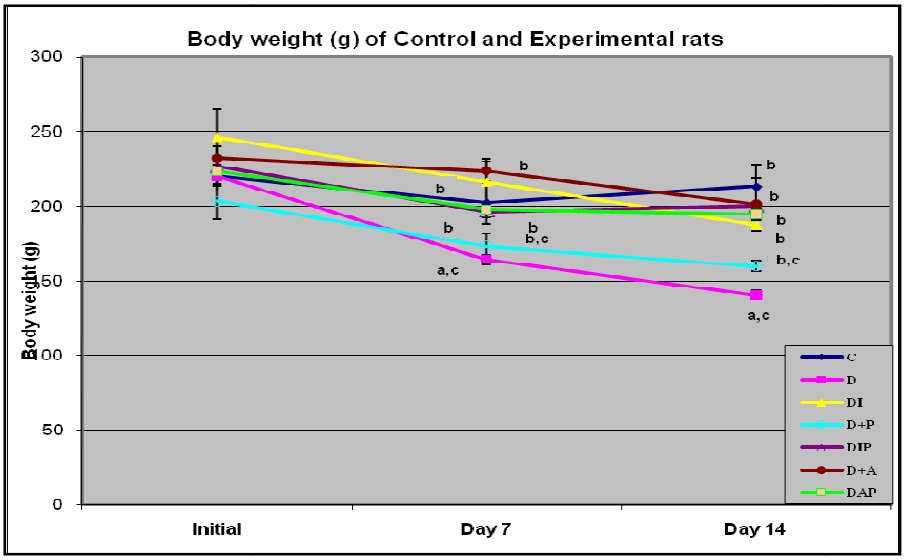


Table-1

Body weight (g) of Experimental rats

Animal status	Initial	7th day	14th day
Control	220.0 ± 10.0	202.5 ± 14.3	213.3 ± 14.5
Diabetic	220.0 ± 5.7	164.4 ± 3.3 [a,c]	140.5 ± 2.9 [a,c]
Diabetic + Insulin	246.3 ± 18.6	216.2 ± 14.0 [b]	187.2 ± 3.4 [b]
Diabetic + Pyridoxine	203.3 ± 12.0	173.3 ± 8.8 [b,c]	160.0 ± 3.5 [b,c]
Diabetic + Insulin + Pyridoxine	226.6 ± 13.3	196.6 ± 4.3 [b]	200.2 ± 2.0 [b]
Diabetic + *A.marmelose*	232.4 ± 17.6	223.7 ± 8.1 [b]	201.4 ± 17.6 [b]
Diabetic + *A.marmelose* + Pyridoxine	223.3 ± 8.8	197.8 ± 3.2 [b]	194.7 ± 2.3 [b]

Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control; [b] $p<0.001$ when compared with diabetic group; [c] $p<0.001$ when compared with initial weight.

Figure-2

Blood glucose (mg/dl) level in Control and Experimental rats

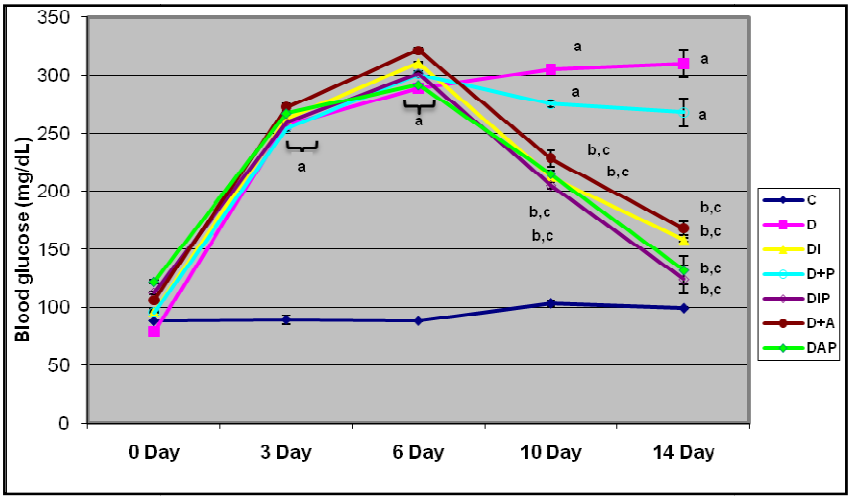


Table-2

Blood glucose (mg/dl) level in Control and Experimental rat

Animal status	0th day (Before STZ injection)	3rd day (Initial)	6th day	10th day	14th day (Final)
Control	88.25±1.1	89.33±4.5	88.41±0.8	103.26± 2.1	99.13±0.9
Diabetic	79.14±1.7	257.13±0.4 [a]	288.72±0.9 [a]	305.1±0.4 [a]	309.96±1.1[a]
D + I	96.09±0.7	262.36±4.8 [a]	310.41±0.7 [a]	212.8±1.3 [b,c]	158.0 ±1.6 [b,c]
D + P	95.61± 2.9	254.33±2.3 [a]	300.8±1.3 [a]	275.34±2.9 [a]	268.0±12.5 [a]
DIP	112.52±1.2	259.86±2.5 [a]	301.5±2.0 [a]	205.0±2.8 [b,c]	124.06±12.1 [b,c]
D + A	106.09±0.7	272.36±3.5 [a]	321.41±2.9 [a]	228.4±7.4 [b,c]	1680.0±5.6 [b,c]
DAP	122.2 ±1.8	267.16±1.7 [a]	291.5±3.0 [a]	215.0±2.8 [b,c]	132.06±12.1 [b,c]

Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control; [b] $p<0.001$ when compared to diabetic group; [c] $p<0.001$ when compared with initial reading.

Figure-3

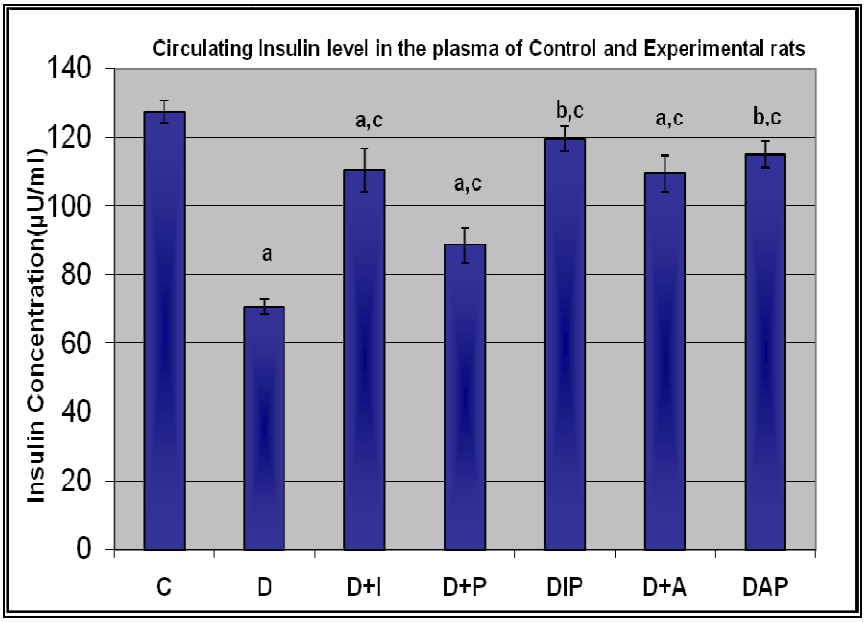


Table-3

Circulating Insulin level in the plasma of control and experimental rats

Experimental Groups	Insulin Concentration (µU/ml)
Control	127.5 ± 3.11
Diabetic	70.7 ± 2.17 [a]
Diabetic + Insulin	110.5 ± 6.31 [a,c]
Diabetic + Pyridoxine	88.5 ± 5.02 [a,c]
Diabetic + Insulin+ Pyridoxine	119.5 ± 3.72 [b,c]
Diabetic + *A. marmelose*	109.5 ± 5.16 [a,c]
Diabetic + *A. marmelose* + Pyridoxine	115.0 ± 3.93 [b,c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared with control rats; [c] $p<0.001$ when compared with diabetic rats.

Figure-4

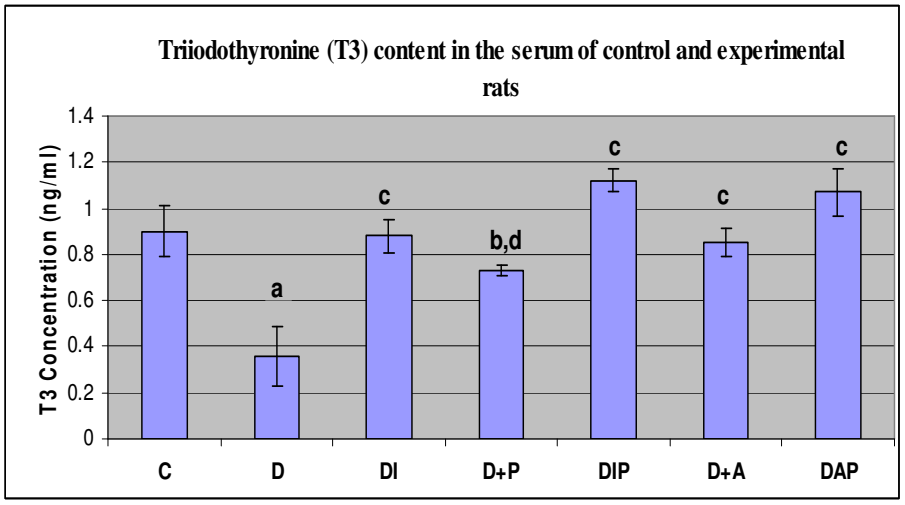


Table-4

Triiodothyronine (T3) content in the serum of control and experimental rats

Experimental Groups	Concentration (ng/ml)
Control	0.90 ± 0.11
Diabetic	0.36 ± 0.13 [a]
Diabetic + Insulin	0.88 ± 0.07 [c]
Diabetic + Pyridoxine	0.73 ± 0.02 [b,d]
Diabetic + Insulin+ Pyridoxine	1.12 ± 0.05 [c]
Diabetic + *A. marmelose*	0.85 ± 0.06 [c]
Diabetic + *A. marmelose* + Pyridoxine	1.07 ± 0.10 [c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared with control rats; [c] $p<0.001$, [d] $p<0.05$ when compared with diabetic rats.

Table-5

Serotonin and metabolites in the cerebral cortex of control and experimental rats			
Experimental Groups	**5-HT (nmoles/g wet wt. of tissue)**	**5-HIAA (nmoles/g wet wt. of tissue)**	**5-HT/ 5-HIAA**
Control	1.96 ± 0.06	1.42 ± 0.21	1.38 ± 0.06
Diabetic	0.95 ± 0.01 [b]	1.98 ± 0.24 [a]	0.47 ± 0.01 [b]
Diabetic+Insulin	1.36 ± 1.46 [d]	1.61 ± 0.11 [c]	0.84 ± 1.46 [d]
Diabetic+Pyridoxine	1.31 ± 0.04 [d]	1.04 ± 0.02 [c]	1.25 ± 0.04 [d]
Diabetic+Insulin+Pyridoxine	1.70 ± 0.05 [d]	1.21 ± 0.07 [c]	1.40 ± 0.05 [d]
Diabetic+*A. marmelose*	1.37 ± 0.25 [d]	1.48 ± 0.19 [c]	0.92 ± 0.25 [d]
Diabetic+*A. marmelose*+Pyridoxine	1.79 ± 0.14 [d]	1.31 ± 0.10 [c]	1.36 ± 0.14 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p < 0.05$, [b] $p<0.01$ when compared to control; [c] $p<0.05$, [d] $p<0.01$ when compared to diabetic group.

Table-6

Serotonin and metabolites in the brain stem of control and experimental rats			
Experimental Groups	**5-HT (nmoles/g wet wt. of tissue)**	**5-HIAA (nmoles/g wet wt. of tissue)**	**5-HT/ 5-HIAA**
Control	2.76 ± 0.08	2.98 ± 0.13	0.92 ± 0.10
Diabetic	2.15 ± 0.14 [a]	3.73 ± 0.16 [a]	0.57 ± 0.11 [a]
Diabetic+Insulin	2.21 ± 0.09 [b]	3.05 ± 0.10 [b]	0.72 ± 0.12 [b]
Diabetic+Pyridoxine	2.13 ± 0.21 [b]	2.91 ± 0.19 [b]	0.73 ± 0.18 [b]
Diabetic+Insulin+Pyridoxine	2.65 ± 0.11 [b]	2.89 ± 0.09 [b]	0.91 ± 0.08 [b]
Diabetic+*A. marmelose*	2.32 ± 0.10 [b]	3.20 ± 0.12 [b]	0.73 ± 0.11 [b]
Diabetic+*A. marmelose*+Pyridoxine	2.59 ± 0.13 [b]	2.86 ± 0.11 [b]	0.90 ± 0.15 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p < 0.001$ when compared to control; [b] $p<0.001$ when compared to diabetic group.

Table-7

Serotonin and metabolites in the cerebellum of control and experimental rats			
Experimental Groups	**5-HT (nmoles/g wet wt. of tissue)**	**5-HIAA (nmoles/g wet wt. of tissue)**	**5-HT/ 5-HIAA**
Control	2.76 ± 0.26	2.12 ± 0.21	1.30 ± 0.22
Diabetic	1.55 ± 0.21 [c]	2.98 ± 0.24 [c]	0.52 ± 0.26 [c]
Diabetic+Insulin	2.06 ± 0.26 [b]	2.11 ± 0.19 [d]	0.97 ± 0.22 [a]
Diabetic+Pyridoxine	2.00 ± 0.24 [b]	2.24 ± 0.22 [c]	0.89 ± 0.26 [b]
Diabetic+Insulin+Pyridoxine	2.50 ± 0.20 [e]	1.36 ± 0.27 [b,e]	1.83 ± 0.25 [e]
Diabetic+*A. marmelose*	1.87 ± 0.25 [b]	2.09 ± 0.29 [d]	0.89 ± 0.27 [a]
Diabetic+*A. marmelose*+Pyridoxine	2.29 ± 0.28 [e]	1.31 ± 0.20 [b,e]	1.74 ± 0.21 [e]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p < 0.05$, [b] $p<0.01$, [c] $p<0.001$ when compared to control; [d] $p<0.05$, [e] $p<0.001$ when compared to diabetic group.

Table-8

Serotonin and metabolites in the hippocampus of control and experimental rats			
Experimental Groups	**5-HT (nmoles/g wet wt. of tissue)**	**5HIAA (nmoles/g wet wt. of tissue)**	**5-HT/ 5-HIAA**
Control	1.56 ± 0.27	1.94 ± 0.22	0.80 ± 0.23
Diabetic	0.89 ± 0.29 [a]	2.93 ± 0.31 [a]	0.33 ± 0.28
Diabetic+Insulin	1.07 ± 0.19 [a,b]	2.29 ± 0.20 [a,b]	0.46 ± 0.20
Diabetic+Pyridoxine	0.98 ± 0.33 [a,b]	2.91 ± 0.36 [a]	0.33 ± 0.33
Diabetic+Insulin+Pyridoxine	1.45 ± 0.35 [c]	1.53 ± 0.29 [c]	0.94 ± 0.31
Diabetic+*A. marmelose*	1.10 ± 0.23 [a,b]	2.73 ± 0.24 [a,b]	0.40 ± 0.21
Diabetic+*A. marmelose*+Pyridoxine	1.59 ± 0.22 [c]	1.66 ± 0.22 [c]	0.95 ± 0.21

Values are mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p < 0.001$ when compared to control; [b] $p<0.01$, [c] $p < 0.001$ when compared to diabetic group.

Table-9

Glutamate content in the cerebral cortex of control and experimental rats

Experimental Groups	Glutamate (nmoles/g wet wt. of tissue)
Control	177.8 ± 0.12
Diabetic	1995.2 ± 0.19 [b]
Diabetic + Insulin	791.2 ± 0.22 [a,c]
Diabetic + Pyridoxine	1584.8 ± 0.08 [b]
Diabetic + Insulin+ Pyridoxine	223.8 ± 0.37 [c]
Diabetic + *A. marmelose*	707.9 ± 0.23 [a,c]
Diabetic + *A. marmelose* + Pyridoxine	251.1 ± 0.11 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.01$, [b] $p<0.001$ when compared to control;
[c] $p<0.001$ when compared to diabetic group.

Table-10

Glutamate content in the brainstem of control and experimental rats

Experimental Groups	Glutamate (nmoles/g wet wt. of tissue)
Control	198.2 ± 2.11
Diabetic	920.8 ± 5.03 [a]
Diabetic + Insulin	543.7 ± 6.13 [a,b]
Diabetic + Pyridoxine	881.2 ± 7.22 [a]
Diabetic + Insulin+ Pyridoxine	244.0 ± 5.16 [b]
Diabetic + *A. marmelose*	511.4 ± 7.13 [a,b]
Diabetic + *A. marmelose* + Pyridoxine	231.4 ± 8.01 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control;
[b] $p<0.001$ when compared to diabetic group.

Table-11

Glutamate content in the cerebellum of control and experimental rats

Experimental Groups	Glutamate (nmoles/g wet wt. of tissue)
Control	102.1 ± 6.2
Diabetic	351.4 ± 6.1 [a]
Diabetic + Insulin	223.2 ± 7.6 [b,c]
Diabetic + Pyridoxine	332.3 ± 12.4 [b]
Diabetic + Insulin+ Pyridoxine	143.7 ± 9.3 [a,c]
Diabetic + *A. marmelose*	231.8 ± 6.9 [b,c]
Diabetic + *A. marmelose* + Pyridoxine	159.6 ± 13.5 [a,c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats.[a] $p < 0.05$,[b] $p<0.001$ when compared to control;

[c] $p<0.001$ when compared to diabetic group

Table-12

Glutamate content in the hippocampus of control and experimental rats

Experimental Groups	Glutamate (nmoles/g wet wt. of tissue)
Control	223.6 ± 11.3
Diabetic	415.2 ± 8.7 [b]
Diabetic + Insulin	312.8 ± 10.1 [b,c]
Diabetic + Pyridoxine	401.5 ± 16.0 [b]
Diabetic + Insulin+ Pyridoxine	269.8 ± 11.2 [b,c]
Diabetic + *A. marmelose*	299.7 ± 6.7 [b,c]
Diabetic + *A. marmelose* + Pyridoxine	248.3 ± 9.2 [b,c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats.[a] $p<0.01$,[b] $p<0.001$ when compared to control;

[c] $p<0.001$ when compared to diabetic group.

Table-13

Glutamate content in the pancreas of control and experimental rats

Experimental Groups	Glutamate (nmoles/g wet wt. of tissue)
Control	119.9 ± 10.2
Diabetic	198.2 ± 8.2 [b]
Diabetic + Insulin	147.8 ± 8.6 [a, c]
Diabetic + Pyridoxine	172.3 ± 9.1 [b]
Diabetic + Insulin+ Pyridoxine	120.7 ± 5.9 [c]
Diabetic + *A. marmelose*	139.3 ± 12.1 [a, c]
Diabetic + *A. marmelose* + Pyridoxine	101.4 ± 16.0 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p < 0.05$, [b] $p < 0.001$ when compared to control;
[c] $p < 0.001$ when compared to diabetic group.

Figure-5

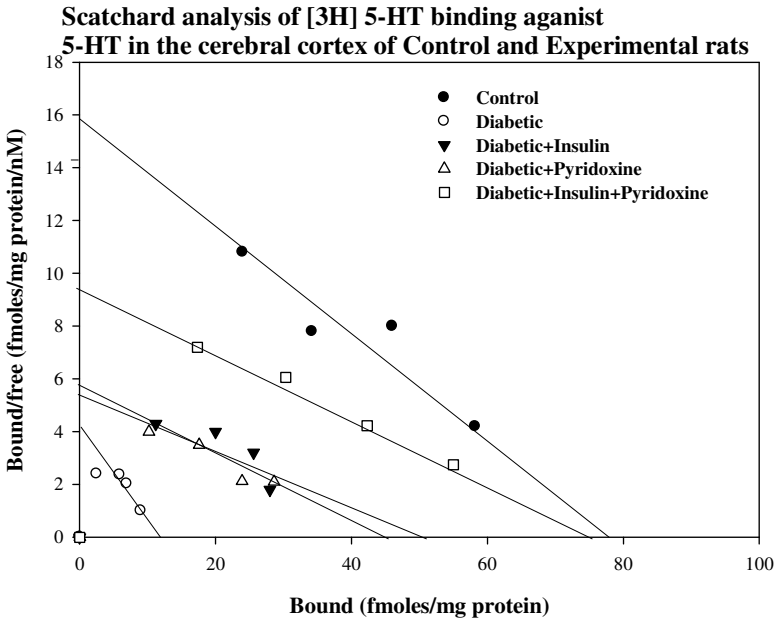


Table-14

[^{3}H] 5-Hydroxytryptamine binding parameters in the cerebral cortex of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	103.0 ± 8.03	10.16 ± 0.40
Diabetic	7.5 ± 3 .41 [b]	2.14 ± 0.92 [b]
Diabetic+Insulin	45.0 ± 12.15 [c]	8.89 ± 0.33 [d]
Diabetic+Pyridoxine	52.5 ± 2.56 [c]	10.50 ± 0.76 [d]
Diabetic+Insulin+Pyridoxine	75.0 ± 8.56 [d]	9.20 ± 0.40 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p < 0.05$, [b] $p<0.001$ when compared to control; [c] $p<0.01$, [d] $p<0.001$ when compared to diabetic group.

Figure-6

Scatchard analysis of [3H] 5-HT binding aganist 5-HTin the cerebral cortex of Control and Experimental rats

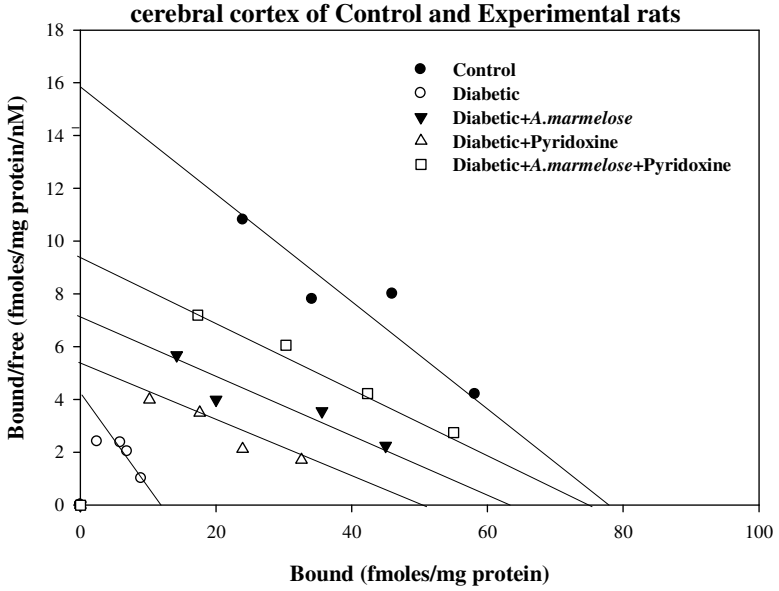


Table-15

[^{3}H] 5-Hydroxytryptamine binding parameters in the cerebral cortex of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	103.0 ± 8.03	10.16 ± 0.40
Diabetic	7.5 ± 3.41 [b]	2.14 ± 0.92 [b]
Diabetic+Pyridoxine	52.5 ± 2.56 [c]	10.50 ± 0.76 [d]
Diabetic+*A. marmelose*	62.2 ± 12.13 [c]	9.0 ± 0.17 [d]
Diabetic+*A. marmelose*+Pyridoxine	73.3 ± 10.29 [d]	9.0 ± 0.21 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p < 0.05$, [b] $p<0.001$ when compared to control; [c] $p<0.01$, [d] $p<0.001$ when compared to diabetic group.

Figure-7

Scatchard analysis of [^{3}H] Ketanserin receptors binding aganist ketanserin in the Cerebral cortex of Control and Experimental rats

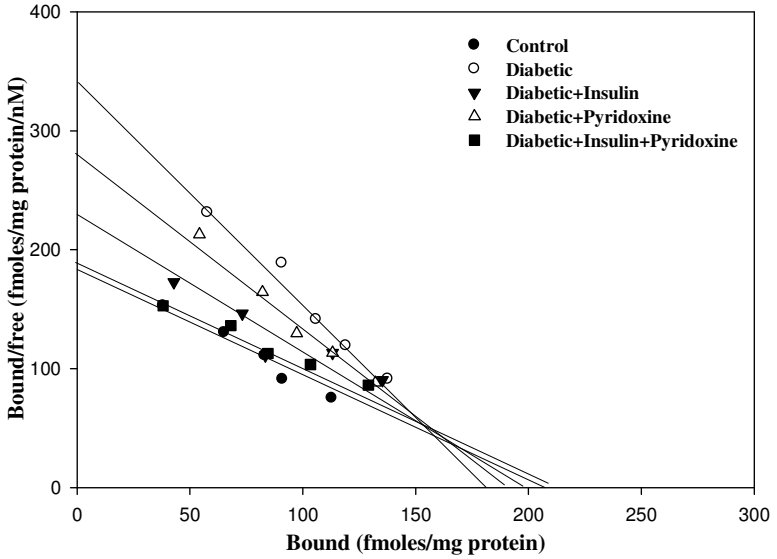


Table-16

[^{3}H] Ketanserin binding parameters in the cerebral cortex of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	208.7 ± 3.11	1.15 ± 0.10
Diabetic	185.5 ± 6.22	0.53 ± 0.07 [a]
Diabetic+Insulin	197.7 ± 1.96	0.92 ± 0.06 [c]
Diabetic+Pyridoxine	189.3 ± 6.08	0.67 ± 0.28 [b,d]
Diabetic+Insulin+Pyridoxine	207.3 ± 3.00	1.06 ± 0.16 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared to control group; [c] $p<0.001$, [d] $p<0.05$ when compared to diabetic group.

Figure-8

Scatchard analysis of [^{3}H] Ketanserin receptors binding aganist ketanserin in the Cerebral cortex of Control and Experimental rats

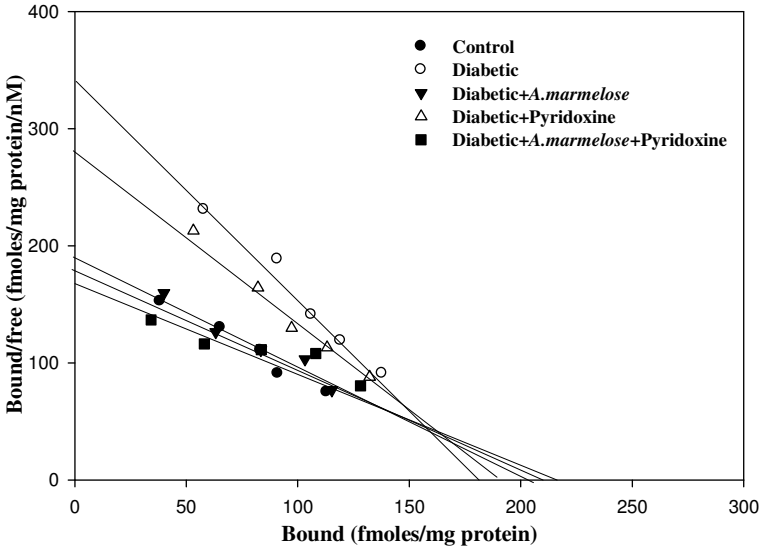


Table-17

[^{3}H] Ketanserin binding parameters in the cerebral cortex of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	208.7 ± 3.11	1.15 ± 0.10
Diabetic	185.5 ± 6.22	0.53 ± 0.07 [a]
Diabetic+Pyridoxine	189.3 ± 6.08	0.67 ± 0.28 [b,d]
Diabetic+*A. marmelose*	203.1 ± 2.16	1.06 ± 0.06 [c]
Diabetic+*A. marmelose*+Pyridoxine	214.3 ± 3.18	1.26 ± 0.11 [c]

Values are mean ± S.E.M of 6-8 separate experiments.Each group consists of 6-8 rats.[a] $p<0.001$, [b] $p<0.01$ when compared to control group; [c] $p<0.001$, [d] $p<0.05$ when compared to diabetic group.

Figure-9

Scatchard analysis of [^{3}H] Glutamate against glutamate in the cerebral cortex of control and experimental rats

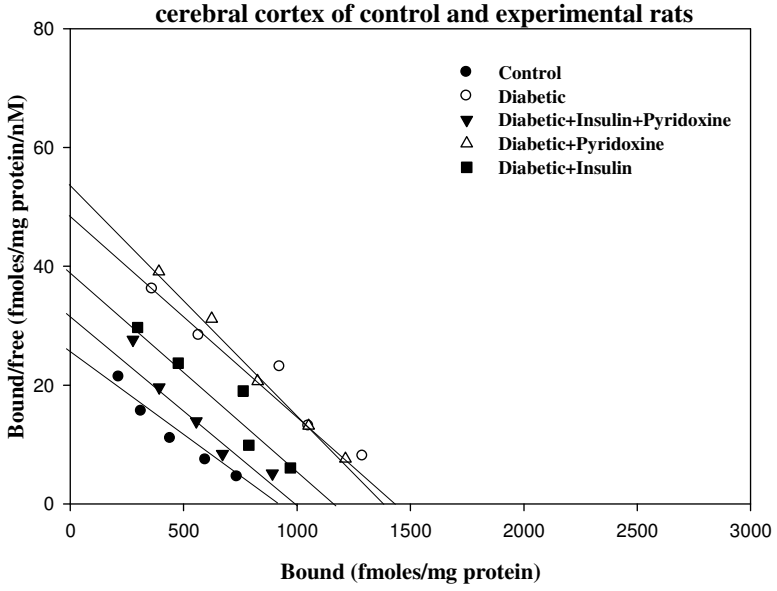


Table-18

[^{3}H] Glutamate binding parameters in the cerebral cortex of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	908.4 ± 0.23	35.0 ± 0.04
Diabetic	1426.8 ± 0.39 [c]	28.0 ± 0.17 [c]
Diabetic+Insulin	1164.6 ± 0.12 [c,d]	34.0 ± 0.19 [d]
Diabetic+Pyridoxine	1364.4 ± 0.09 [c]	25.2 ± 0.07 [c]
Diabetic+Insulin+Pyridoxine	989.4 ± 0.17 [a, d]	32.0 ± 0.13 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats.[a] p <0.05, [b] p<0.01, [c] p<0.001 when compared to control; [d] p<0.001 when compared to diabetic group

Figure-10

Scatchard analysis of [^{3}H] Glutamate against glutamate in the cerebral cortex control and experimental rats

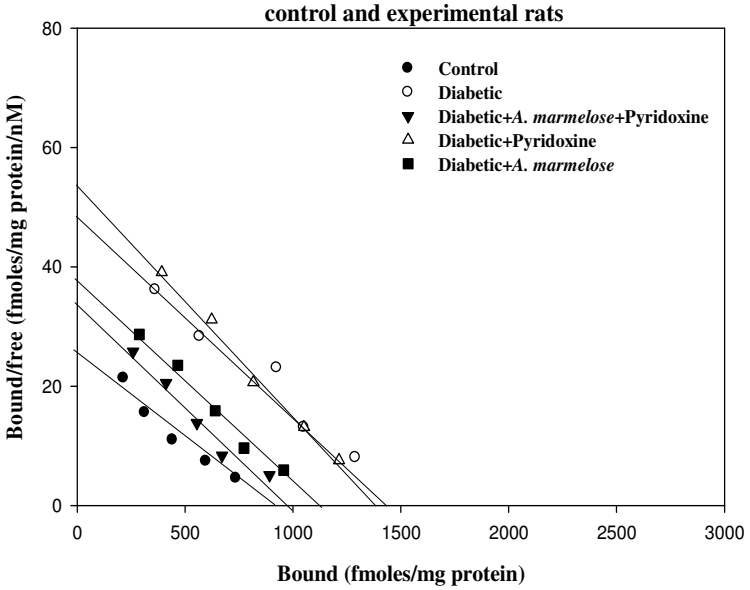


Table-19

[^{3}H] Glutamate binding parameters in the cerebral cortex of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	908.4 ± 0.23	35.0 ± 0.04
Diabetic	1426.8 ± 0.39 [c]	28.0 ± 0.17 [c]
Diabetic+Pyridoxine	1364.4 ± 0.09 [c]	25.2 ± 0.07 [c]
Diabetic +*A. marmelose*	1120.8 ± 0.29 [c,d]	32.0 ± 0.19 [d]
Diabetic +*A. marmelose*+Pyridoxine	976.0 ± 0.31 [b,d]	32.5 ± 0.14 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] p <0.05, [b] p<0.01, [c] p<0.001 when compared to control; [d] p<0.001 when compared to diabetic group.

Figure-11

Real Time PCR amplification of 5-HT_{2A} receptor mRNA from the cerebral cortex of control and experimental rats.

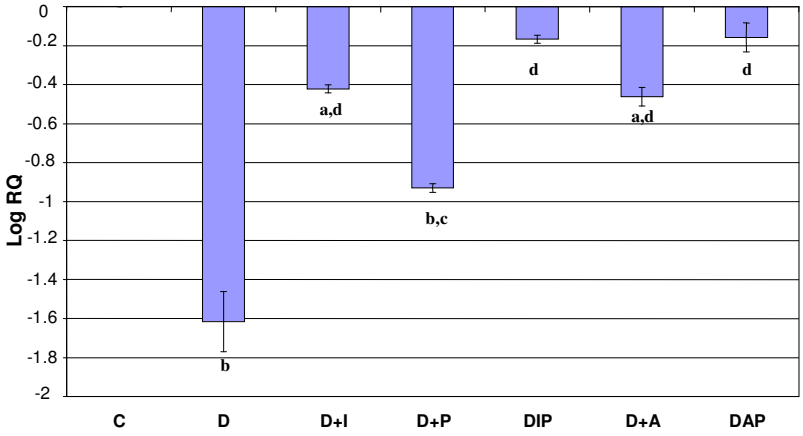


Table-20

Real Time PCR amplification of 5-HT_{2A} receptor mRNA from the cerebral cortex of control and experimental rats.

Experimental Group	Log RQ value
Control	0
Diabetic	-1.615 ± 0.154 [b]
Diabetic+Insulin	-0.421 ± 0.019 [a,d]
Diabetic+Pyridoxine	-0.929 ± 0.021 [b,c]
Diabetic+Insulin+Pyridoxine	-0.166 ± 0.020 [d]
Diabetic+*A. marmelose*	-0.461 ± 0.047 [a,d]
Diabetic+*A. marmelose*+Pyridoxine	-0.157 ± 0.074 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.05$, [b] $p<0.001$ when compared to control group; [c] $p<0.05$, [d] $p<0.001$ when compared to diabetic group.

Figure-12

Real Time PCR amplification of 5-HTT mRNA from the cerebral cortex of control and experimental rats

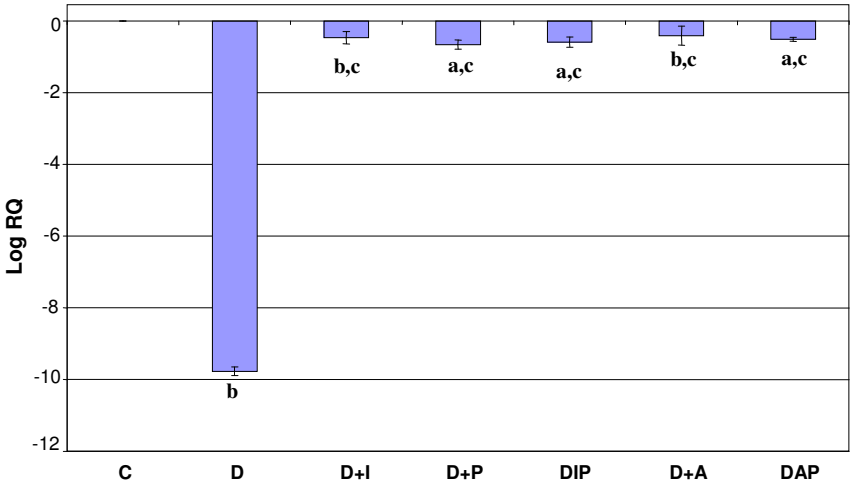


Table-21

Real Time PCR amplification of 5-HTT mRNA from the cerebral cortex of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-9.770 ± 0.080 [b]
Diabetic+Insulin	-0.490 ± 0.094 [b,c]
Diabetic+Pyridoxine	-0.660 ± 0.090 [a,c]
Diabetic+Insulin+Pyridoxine	-0.588 ± 0.458 [a,c]
Diabetic+*A. marmelose*	-0.408 ± 0.13 [b,c]
Diabetic+*A. marmelose*+Pyridoxine	-0.516 ± 0.09 [a,c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.05$, [b] $p<0.001$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-13

Real Time PCR amplification of mGluR5 mRNA from the cerebral cortex of control and experimental rats

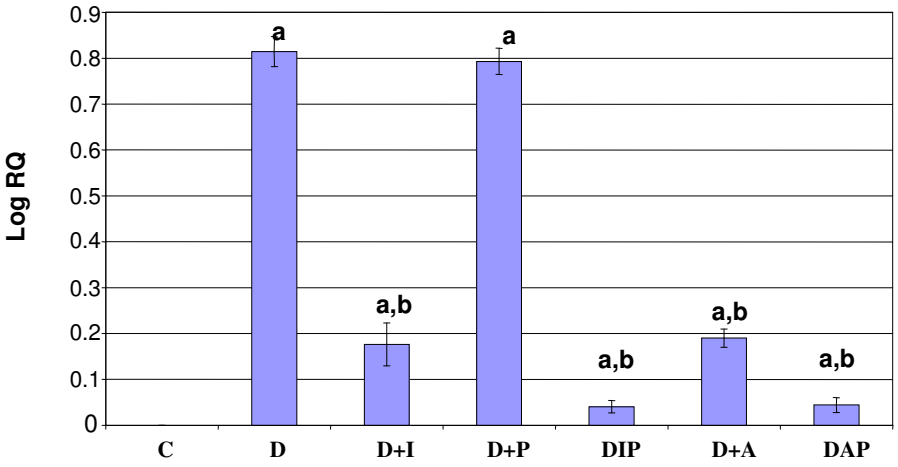


Table-22

Real Time PCR amplification of mGluR5 mRNA from the cerebral cortex of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	0.814 ± 0.032 [a]
Diabetic+Insulin	0.176 ± 0.046 [a,b]
Diabetic+Pyridoxine	0.793 ± 0.028 [a]
Diabetic+Insulin+Pyridoxine	0.040 ± 0.013 [a,b]
Diabetic+*A. marmelose*	0.190 ± 0.020 [a,b]
Diabetic+ *A. marmelose*+Pyridoxine	0.044 ± 0.016 [a,b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-14

Real Time PCR amplification of GLAST glutamate transporter mRNA from the cerebral cortex of control and experimental rats

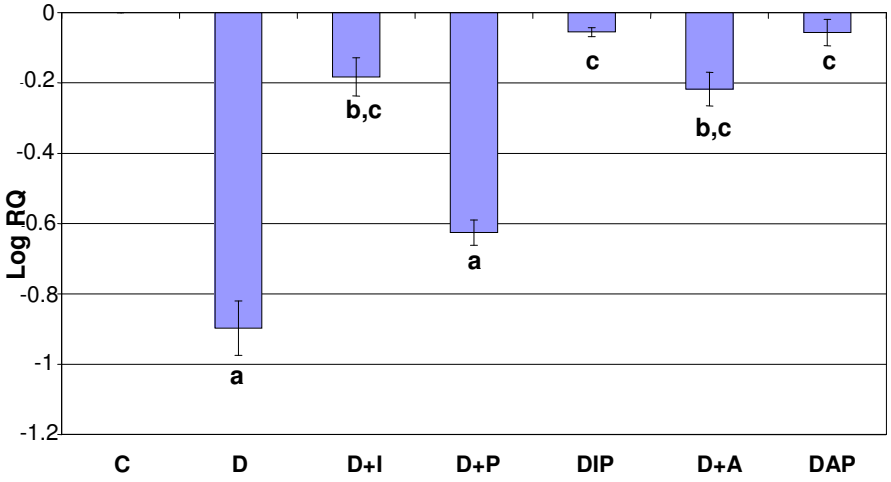


Table-23

Real Time PCR amplification of GLAST glutamate transporter mRNA from the cerebral cortex of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.897 ± 0.077 [a]
Diabetic+Insulin	-0.182 ± 0.054 [b,c]
Diabetic+Pyridoxine	-0.625± 0.035 [a]
Diabetic+Insulin+Pyridoxine	-0.055 ± 0.013 [c]
Diabetic+*A. marmelose*	-0.217 ± 0.047 [b,c]
Diabetic+*A. marmelose*+Pyridoxine	-0.056 ± 0.036 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.05$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-15

Real Time PCR amplification of Insulin Receptor mRNA from the cerebral cortex of control and experimental rats

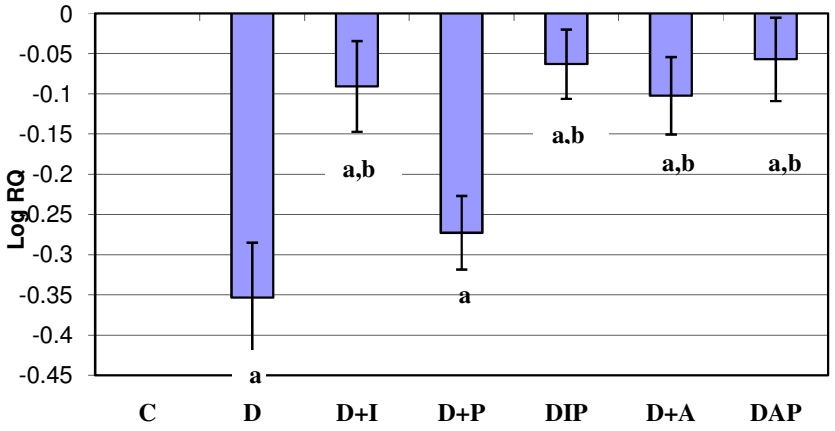


Table-24

Real Time PCR amplification of Insulin Receptor mRNA from the cerebral cortex of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.353 ± 0.036 [a]
Diabetic+Insulin	-0.190 ± 0.024 [a,b]
Diabetic+Pyridoxine	-0.325 ± 0.015 [a]
Diabetic+Insulin+Pyridoxine	-0.074 ± 0.018 [a,b]
Diabetic+*A. marmelose*	-0.227 ± 0.017 [a,b]
Diabetic+ *A. marmelose*+Pyridoxine	-0.086 ± 0.011 [a,b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-16

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

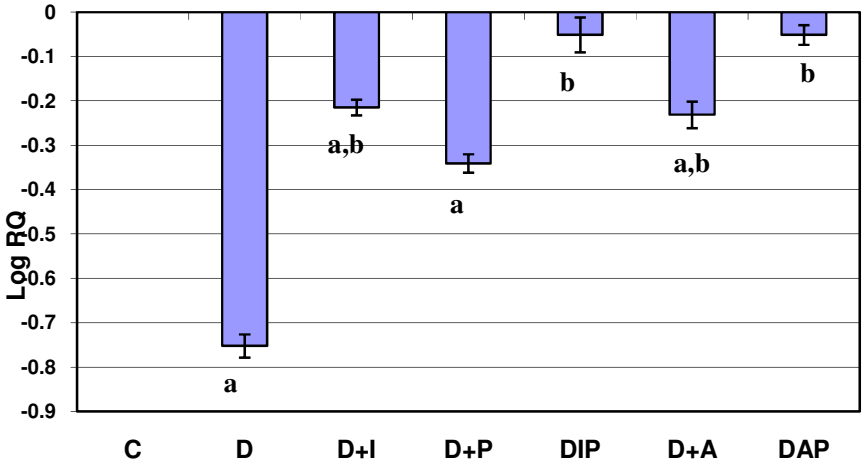


Table-25

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

Experimental Group	Log RQ value
Control	0
Diabetic	-0.752 ± 0.026 [a]
Diabetic+Insulin	-0.214 ± 0.017 [a,b]
Diabetic+Pyridoxine	-0.340 ± 0.020 [a]
Diabetic+Insulin+Pyridoxine	-0.051 ± 0.049 [b]
Diabetic+*A. marmelose*	-0.231 ± 0.040 [a,b]
Diabetic+*A. marmelose*+Pyridoxine	-0.055 ± 0.022 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-17

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

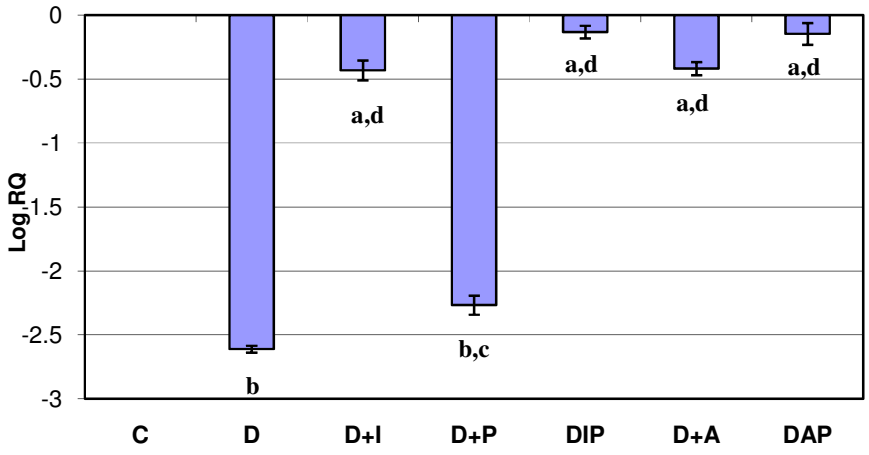


Table-26

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

Experimental Group	Log RQ value
Control	0
Diabetic	-2.61 ± 0.02 [a]
Diabetic+Insulin	-0.43 ± 0.06 [a,b]
Diabetic+Pyridoxine	-2.26 ± 0.07 [a]
Diabetic+Insulin+Pyridoxine	-0.13 ± 0.03 [b]
Diabetic+*A. marmelose*	-0.41 ± 0.05 [a,b]
Diabetic+*A. marmelose*+Pyridoxine	-0.14 ± 0.04 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-18

Scatchard analysis of 5-HT receptors using [^{3}H] 5-HT binding aganist 5-HT in brainstem of Control and Experimental Rats

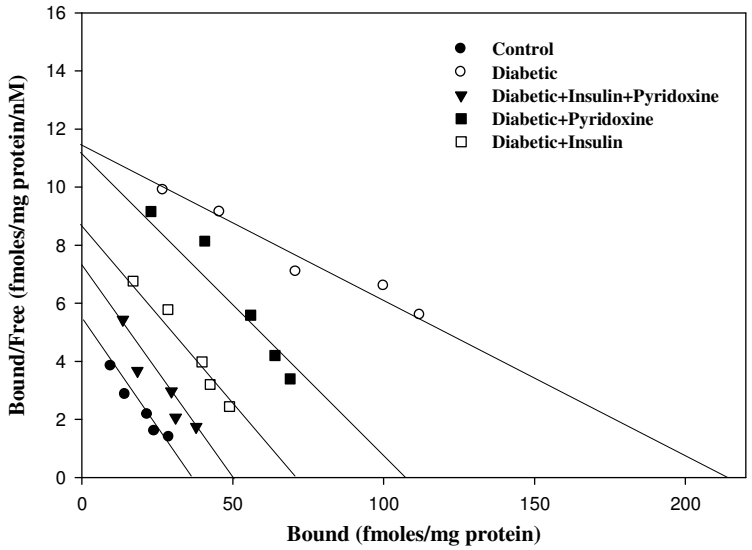


Table-27

[^{3}H] 5-Hydroxytryptamine binding parameters in the brain stem of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	36.2 ± 0.92	5.0 ± 0.10
Diabetic	214.0 ± 0 .41 [b]	20.0 ± 0.33 [b]
Diabetic+Insulin	70.0 ± 0.24 [b,d]	10.0 ± 0.18 [a,d]
Diabetic+Pyridoxine	106.0 ± 1.46 [b,c]	10.0 ± 0.76 [a,d]
Diabetic+Insulin+Pyridoxine	50.0 ± 3.16 [d]	7.2 ± 0.45 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.05$, [b] $p<0.001$ when compared to control group; [c] $p<0.01$, [d] $p<0.001$ when compared to diabetic group.

Figure-19

Scatchard analysis of 5-HT receptors using [^{3}H] 5-HT binding aganist 5-HT in brainstem of Control and Experimental Rats

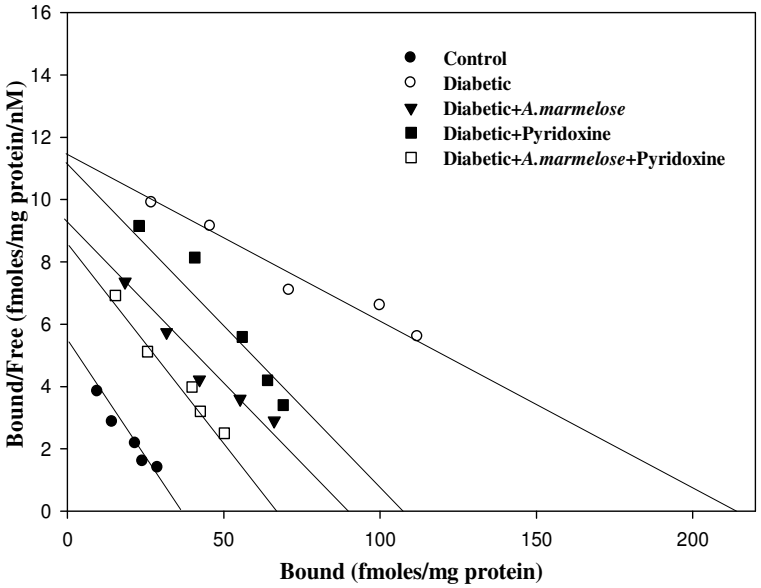


Table-28

[^{3}H] 5-Hydroxytryptamine binding parameters in the brain stem of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	36.2 ± 0.92	5.0 ± 0.10
Diabetic	214.0 ± 0 .41 [b]	20.0 ± 0.33 [b]
Diabetic+Pyridoxine	106.0 ± 1.46 [b,c]	10.0 ± 0.76 [a,d]
Diabetic+*A. marmelose*	82.3 ± 0.23 [b,d]	9.6 ± 0.18 [a,d]
Diabetic+*A. marmelose*+Pyridoxine	62.5 ± 0.31 [d]	7.2 ± 0.23 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.05$, [b] $p<0.001$ when compared to control group; [c] $p<0.01$, [d] $p<0.001$ when compared to diabetic group.

Figure-20

Scatchard analysis of [^{3}H] Ketanserin binding aganist ketanserin in the brainstem of control and experimental rats

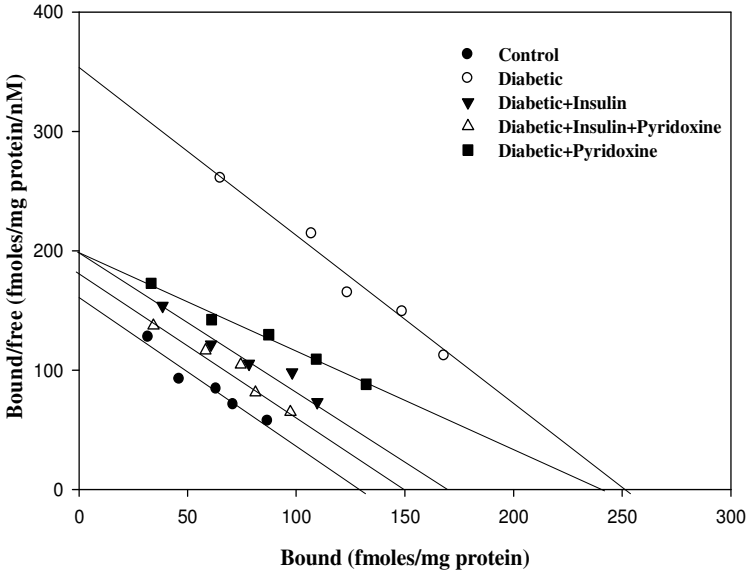


Table-29

[^{3}H] Ketanserin binding parameters in the brainstem of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	128.7 ± 2.21	0.6 ± 0.10
Diabetic	252.2 ± 1.32 [a]	1.0 ± 0.17
Diabetic+Insulin	168.7 ± 5.17 [b,d]	1.0 ± 0.28
Diabetic+Pyridoxine	240.0 ± 2.16 [a]	1.0 ± 0.06
Diabetic +Insulin+Pyridoxine	148.4 ± 3.98 [d]	0.7 ± 0.19

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to control group; [d] $p<0.001$ when compared to diabetic group.

Figure-21

Scatchard analysis of [^{3}H] Ketanserin binding aganist ketanserin in the brainstem of control and experimental rats

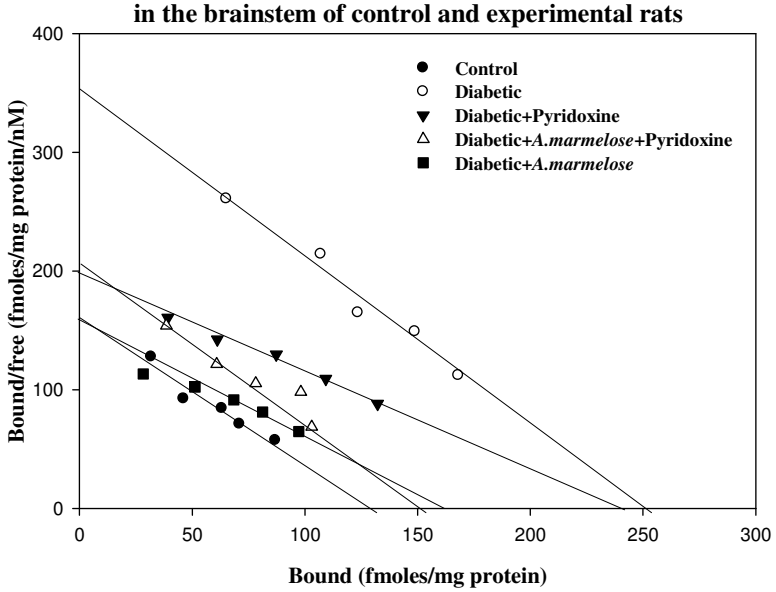


Table-30

[^{3}H] Ketanserin binding parameters in the brainstem of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	128.7 ± 2.21	0.6 ± 0.10
Diabetic	252.2 ± 1.32 [a]	1.0 ± 0.17
Diabetic+Pyridoxine	240.0 ± 2.16 [a]	1.0 ± 0.06
Diabetic+*A. marmelose*	162.6 ± 2.19 [c,d]	0.7 ± 0.19
Diabetic+*A. marmelose*+Pyridoxine	150.8 ± 3.14 [d]	0.7 ± 0.14

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats .[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to control group; [d] $p<0.001$ when compared to diabetic group.

Figure-22

Scatchard analysis of [^{3}H] Glutamate against glutamate in the brain stem of Control and Experimental rats

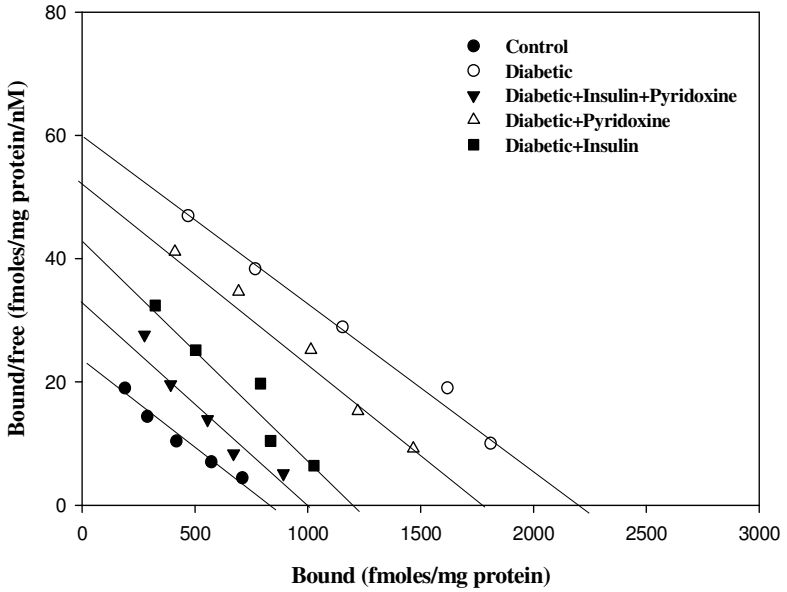


Table-31

[^{3}H] Glutamate binding parameters in the brainstem of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	837.0 ± 0.12	34.5 ± 0.04
Diabetic	2245.0 ± 0.09 [a]	35.0 ± 0.17
Diabetic+Insulin	1208.4 ± 0.26 [b]	32.0 ± 0.08
Diabetic+Pyridoxine	1800.0 ± 0.31 [a,c]	34.5 ± 0.19
Diabetic+Insulin+Pyridoxine	996.0 ± 0.11 [b]	34.0 ± 0.31

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control; [b] $p<0.001$, [c] $p<0.01$ when compared to diabetic group.

Figure-23

Scatchard analysis of [^{3}H] Glutamate aganist glutamate in the brain stem of control and experimental rats

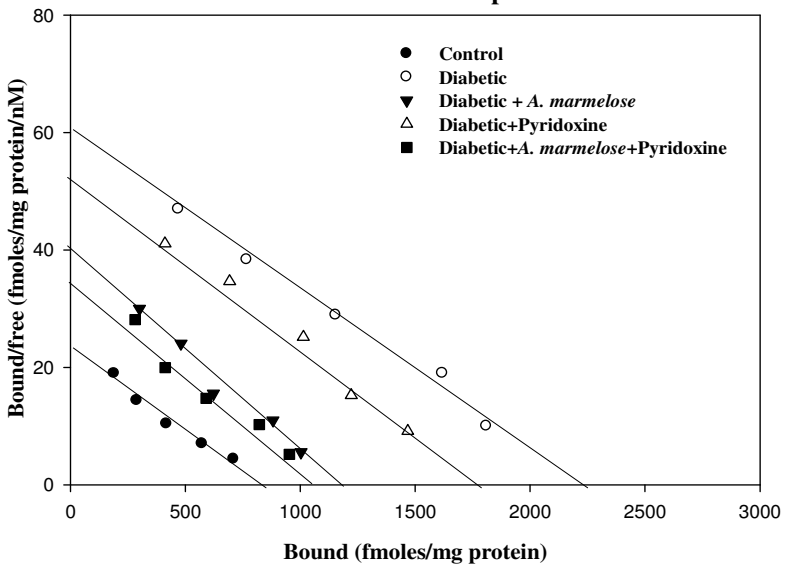


Table-32

[^{3}H] Glutamate binding parameters in the brainstem of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	837.0 ± 0.12	34.5 ± 0.04
Diabetic	2245.0 ± 0.09 [a]	35.0 ± 0.17
Diabetic+Pyridoxine	1800.0 ± 0.31 [a,c]	34.5 ± 0.19
Diabetic+*A. marmelose*	1180.0 ± 0.42 [b]	34.0 ± 0.24
Diabetic +*A. marmelose*+ Pyridoxine	1030.0 ± 0.15 [b]	34.0 ± 0.16

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control; [b] $p<0.001$, [c] $p<0.01$ when compared to diabetic group.

Figure-24

Real Time PCR amplification of 5-HT_{2A} receptor mRNA from the brain stem of control and experimental rats.

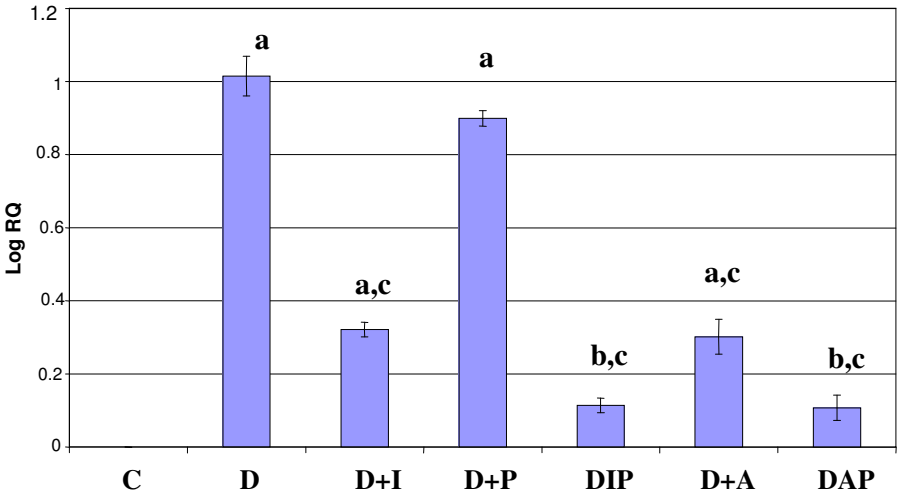


Table-33

Real Time PCR amplification of 5-HT_{2A} receptor mRNA from the brain stem of control and experimental rats.

Experimental Group	Log RQ value
Control	0
Diabetic	1.015 ± 0.05 [a]
Diabetic+Insulin	0.321 ± 0.01 [a,c]
Diabetic+Pyridoxine	0.899 ± 0.02 [a]
Diabetic+Insulin+Pyridoxine	0.113 ± 0.02 [b,c]
Diabetic +*A. marmelose*	0.301 ± 0.04 [a,c]
Diabetic +*A. marmelose*+Pyridoxine	0.107 ± 0.03 [b,c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-25

Real Time PCR amplification of 5-HTT mRNA from the brain stem of control and experimental rats.

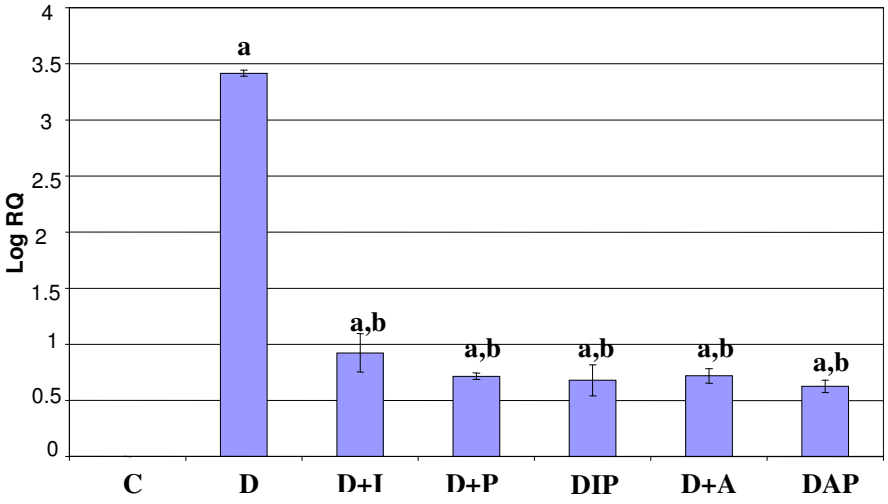


Table-34

Real Time PCR amplification of 5-HTT mRNA from the brain stem of control and experimental rats.

Experimental Group	Log RQ value
Control	0
Diabetic	3.417 ± 0.02 [a]
Diabetic+Insulin	0.924 ± 0.04 [a,b]
Diabetic+Pyridoxine	0.715 ± 0.10 [a,b]
Diabetic+Insulin+Pyridoxine	0.678 ± 0.10 [a,b]
Diabetic+*A. marmelose*	0.718 ± 0.13 [a,b]
Diabetic+*A. marmelose*+Pyridoxine	0.626 ± 0.09 [a,b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats.[a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-26

Real Time PCR amplification of mGluR5 mRNA from the brain stem of control and experimental rats

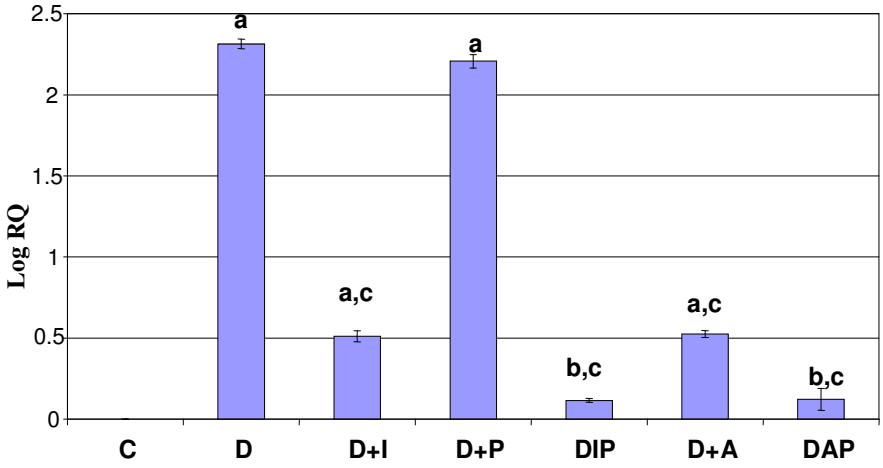


Table-35

Real Time PCR amplification of mGluR5 mRNA from the brain stem of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	2.314 ± 0.029 [a]
Diabetic+Insulin	0.510 ± 0.033 [a,c]
Diabetic+Pyridoxine	2.207± 0.041 [a]
Diabetic+Insulin+Pyridoxine	0.116 ± 0.012 [b,c]
Diabetic+*A. marmelose*	0.525 ± 0.020 [a,c]
Diabetic+*A. marmelose*+Pyridoxine	0.122 ± 0.067 [b,c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-27

Real Time PCR amplification of GLAST glutamate transporter mRNA from the brain stem of control and experimental rats.

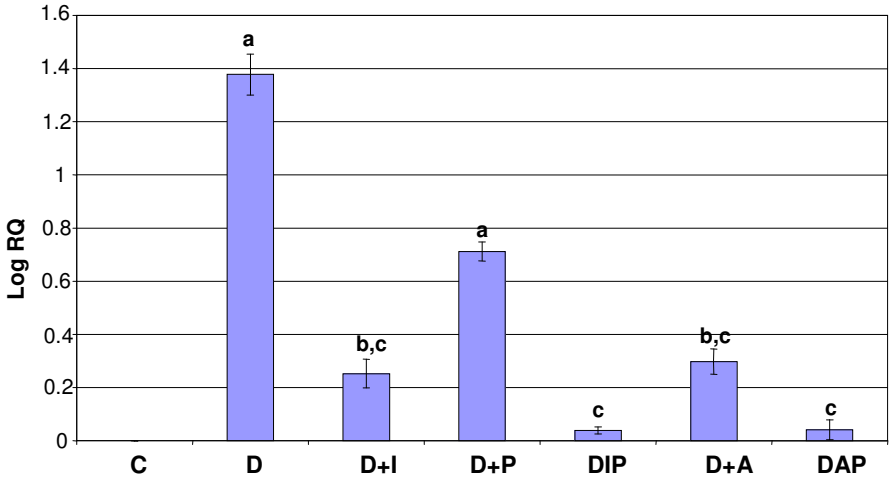


Table-36

Real Time PCR amplification of GLAST glutamate transporter mRNA from the brain stem of control and experimental rats.

Experimental Group	Log RQ value
Control	0
Diabetic	1.377 ± 0.077 [a]
Diabetic+Insulin	0.252 ± 0.054 [b,c]
Diabetic+Pyridoxine	0.711 ± 0.035 [a]
Diabetic+Insulin+Pyridoxine	0.038 ± 0.013 [c]
Diabetic+*A. marmelose*	0.297 ± 0.037 [b,c]
Diabetic+*A. marmelose* +Pyridoxine	0.041 ± 0.046 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-28

Real Time PCR amplification of Insulin Receptor mRNA from the brainstem of control and experimental rats

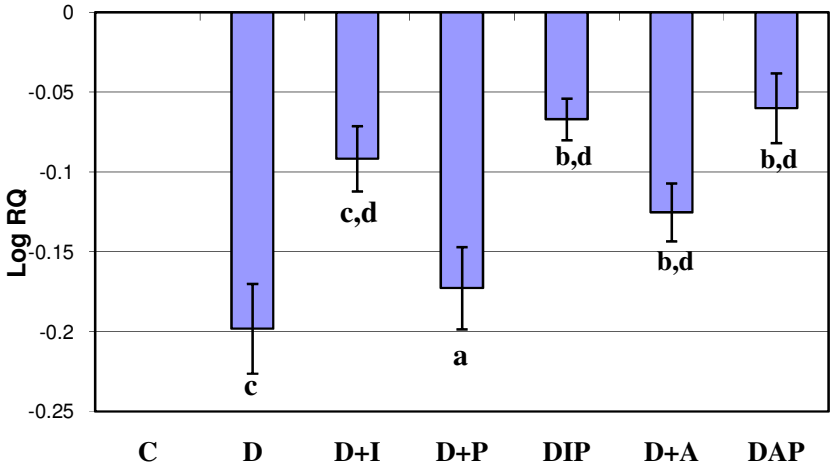


Table-37

Real Time PCR amplification of Insulin Receptor mRNA from the brainstem of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.198 ± 0.19 [c]
Diabetic+Insulin	-0.091 ± 0.11 [c,d]
Diabetic+Pyridoxine	-0.180 ± 0.10 [a]
Diabetic+Insulin+Pyridoxine	-0.172 ± 0.09 [b,d]
Diabetic+*A. marmelose*	-0.125 ± 0.04 [b,d]
Diabetic+ *A. marmelose*+Pyridoxine	-0.059 ± 0.06 [b,d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to control group; [d] $p<0.001$ when compared to diabetic group.

Figure-29

Real Time PCR amplification of SOD mRNA from the brainstem of control and experimental rats

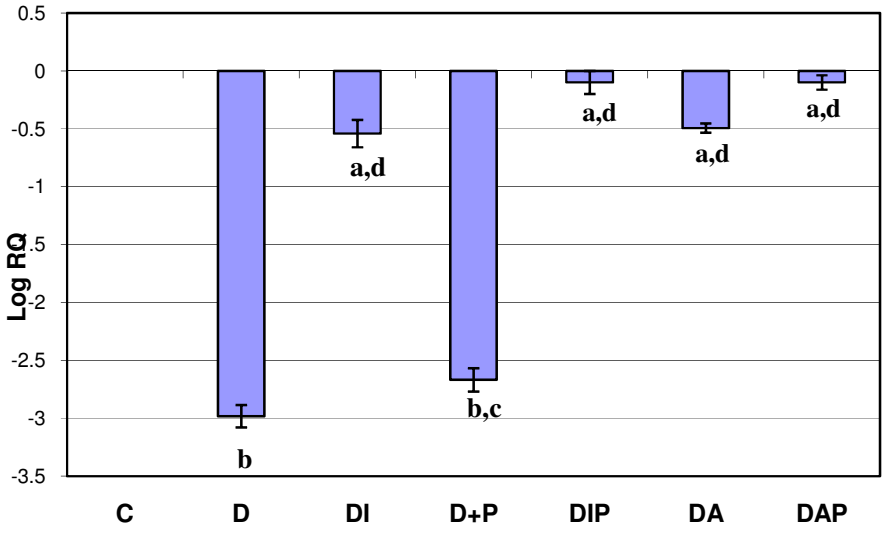


Table-38

Real Time PCR amplification of SOD mRNA from the brainstem of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-2.981 ± 0.09 [a]
Diabetic+Insulin	-0.540 ± 0.11 [a,b]
Diabetic+Pyridoxine	-2.661 ± 0.10 [a]
Diabetic+Insulin+Pyridoxine	-0.098 ± 0.09 [b]
Diabetic+*A. marmelose*	-0.493 ± 0.04 [a,b]
Diabetic+ *A. marmelose*+Pyridoxine	-0.099 ± 0.06 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-30

Real Time PCR amplification of GPx mRNA from the brainstem of control and experimental rats

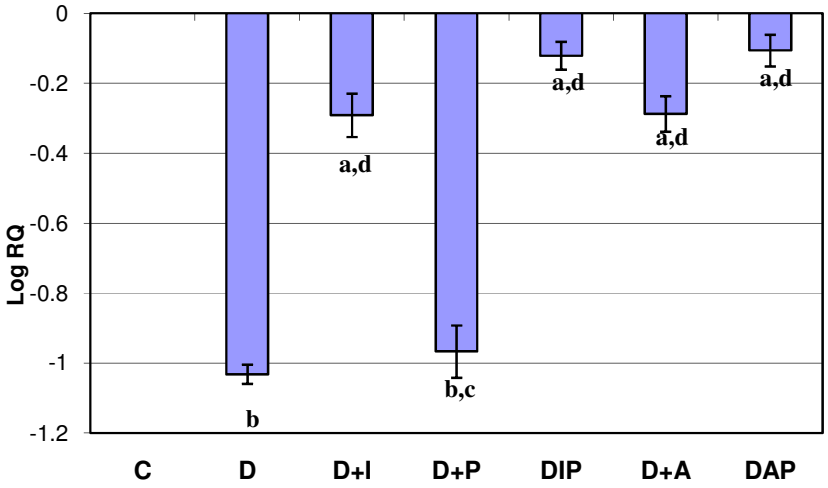


Table-39

Real Time PCR amplification of GPx mRNA from the brainstem of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-1.03 ± 0.02 [b]
Diabetic+Insulin	-0.29 ± 0.06 [a,b]
Diabetic+Pyridoxine	-0.96 ± 0.07 [a]
Diabetic+Insulin+Pyridoxine	-0.12 ± 0.03 [b]
Diabetic+*A. marmelose*	-0.28 ± 0.05 [a,b]
Diabetic+*A. marmelose*+Pyridoxine	-0.10 ± 0.04 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats .[a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-31

Scatchard analysis of [^{3}H] 5-HT binding aganist 5-HT in the cerebellum of Control and Experimental rats

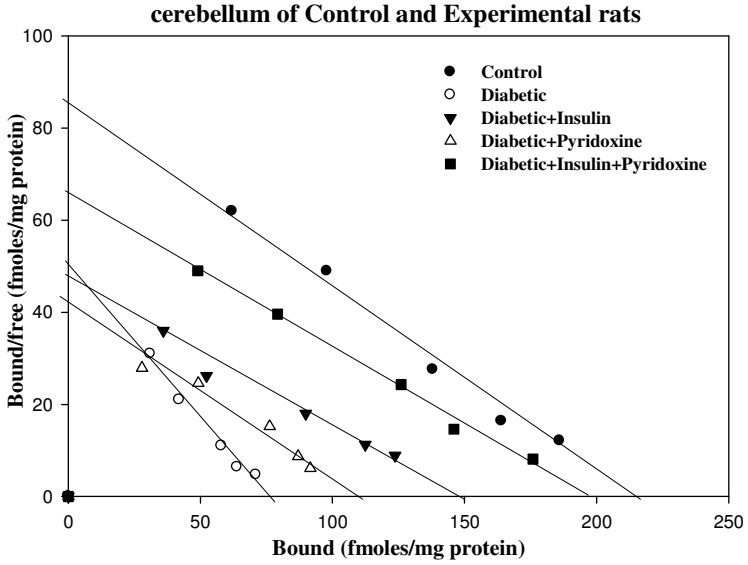


Table-40

[^{3}H] 5-Hydroxytryptamine binding parameters in the cerebellum of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	214.0 ± 6.21	2.60 ± 0.60
Diabetic	76.5 ± 2.34 [a]	1.85 ± 0.43 [a]
Diabetic+Insulin	149.8 ± 5.66 [a,b]	2.80 ± 0.18 [b]
Diabetic+Pyridoxine	109.2 ± 1.03 [a,b]	2.82 ± 0.52 [b]
Diabetic+Insulin+Pyridoxine	197.6 ± 8.20 [b]	2.80 ± 0.15 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-32

Scatchard analysis of [^{3}H] 5-HT binding aganist 5-HT in the cerebellum of Control and Experimental rats

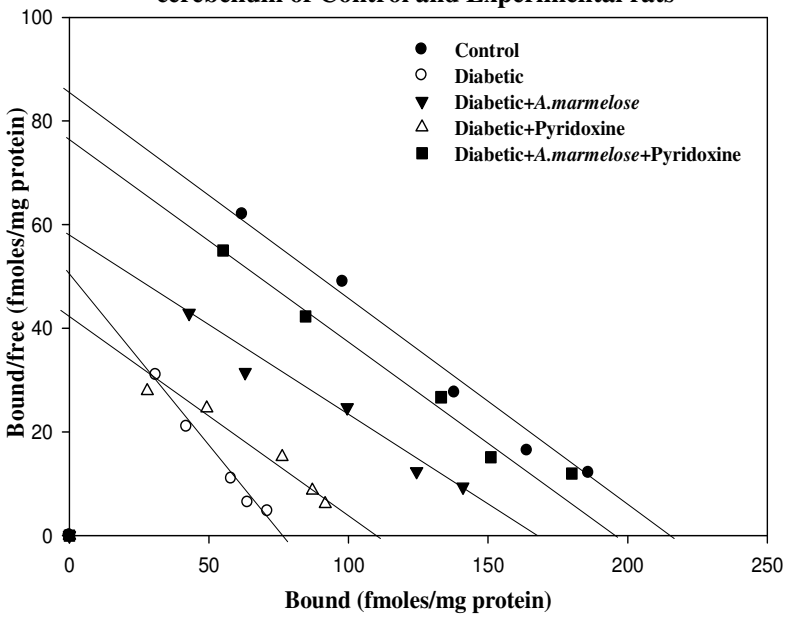


Table-41

[^{3}H] 5-Hydroxytryptamine binding parameters in the cerebellum of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	214.0 ± 6.21	2.60 ± 0.60
Diabetic	76.5 ± 2.34 [a]	1.85 ± 0.43 [a]
Diabetic +Pyridoxine	109.2 ± 1.03 [a,b]	2.82 ± 0.52 [b]
Diabetic+*A. marmelose*	166.0 ± 4.33 [a,b]	2.80 ± 0.42 [b]
Diabetic+*A. marmelose*+Pyridoxine	194.8 ± 3.42 [b]	2.65 ± 0.21 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-33

Scatchard analysis of [^{3}H] Ketanserin binding aganist ketanserin in the cerebellum of control and experimental rats

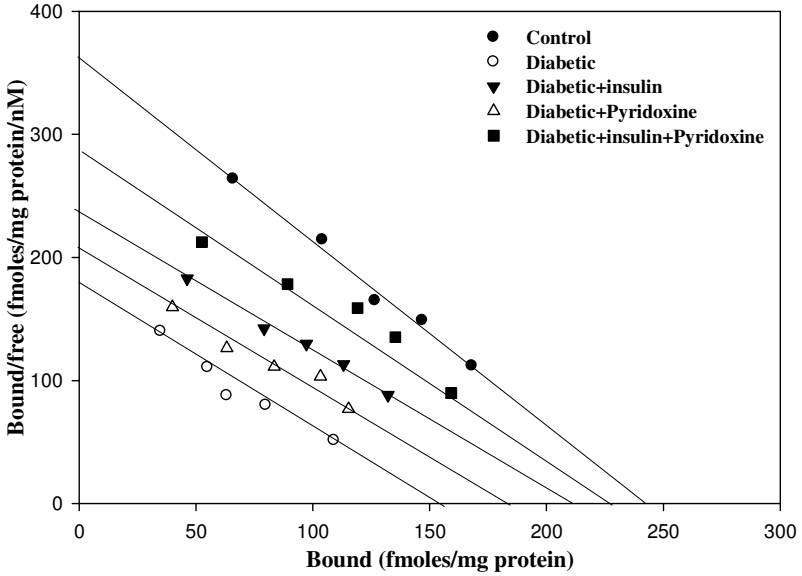


Table-42

[^{3}H] Ketanserin binding parameters in the cerebellum of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	245.0 ± 0.92 [b]	0.80 ± 0.10
Diabetic	154.3 ± 0 .41 [a]	0.86 ± 0.33
Diabetic + Insulin	210.0 ± 0.24 [a,b]	0.82 ± 0.18
Diabetic + Pyridoxine	182.5 ± 1.46 [a,b]	0.85 ± 0.76
Diabetic + Insulin+ Pyridoxine	228.2 ± 3.16 [b]	0.81 ± 0.45

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-34

Scatchard analysis of [^{3}H] Ketanserin binding aganist ketanserin in the cerebellum of control and experimental rats

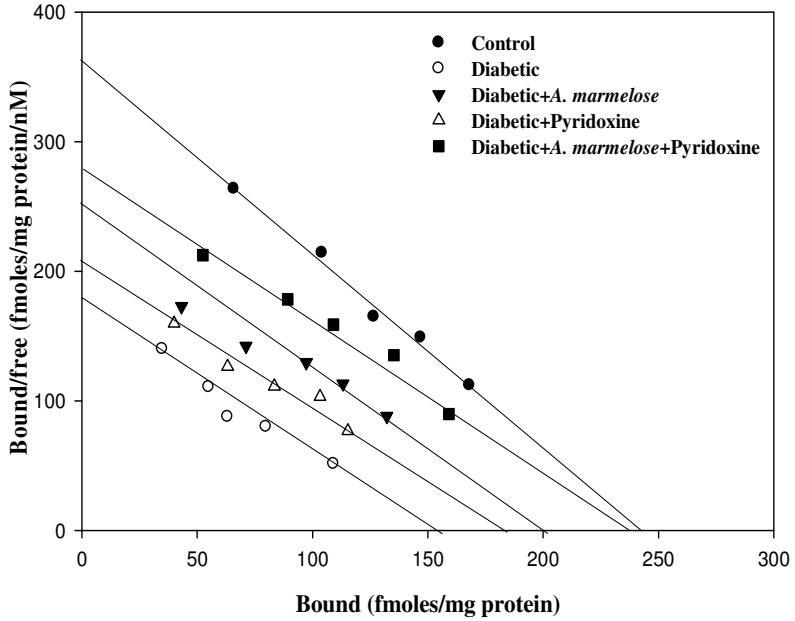


Table-43

[^{3}H] Ketanserin binding parameters in the cerebellum of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	245.0 ± 0.92 [b]	0.80 ± 0.10
Diabetic	154.3 ± 0 .41 [a]	0.86 ± 0.33
Diabetic+*A. marmelose*	197.4 ± 0.24 [a,b]	0.82 ± 0.18
Diabetic+Pyridoxine	182.5 ± 1.46 [a,b]	0.85 ± 0.76
Diabetic+*A. marmelose*+Pyridoxine	232.9 ± 3.16 [b]	0.81 ± 0.45

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-35

Scatchard analysis of [^{3}H] Glutamate against glutamate in the cerebellum of control and experimental rats

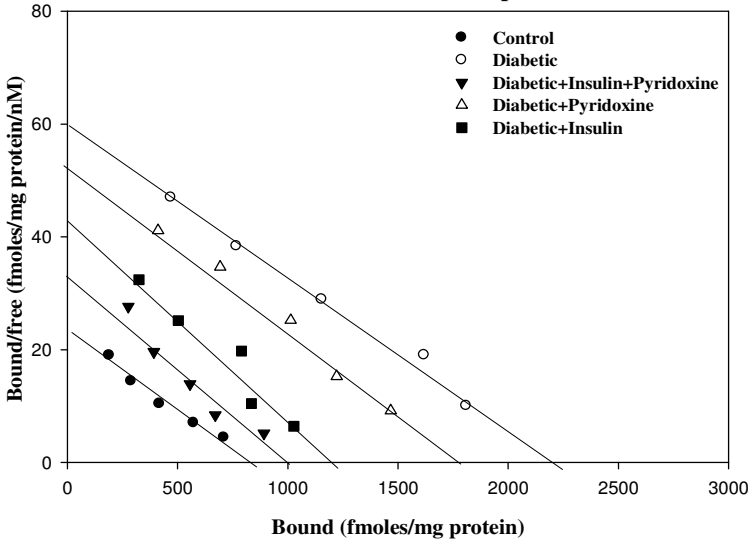


Table-44

[^{3}H] Glutamate binding parameters in the cerebellum of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	937.0 ± 0.20	34.5 ± 0.12
Diabetic	2345.0 ± 0.16 [a]	35.0 ± 0.25
Diabetic+Insulin	1112.0 ± 0.11 [b]	32.0 ± 0.18
Diabetic+Pyridoxine	1418.5 ± 0.24 [a,c]	34.5 ± 0.22
Diabetic+Insulin+Pyridoxine	976.0 ± 0.31 [b]	34.0 ± 0.19

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control; [b] $p<0.001$, [c] $p<0.01$ when compared to diabetic group.

Figure-36

Scatchard analysis of [^{3}H] Glutamate aganist glutamate in the cerebellum control and experimental rats

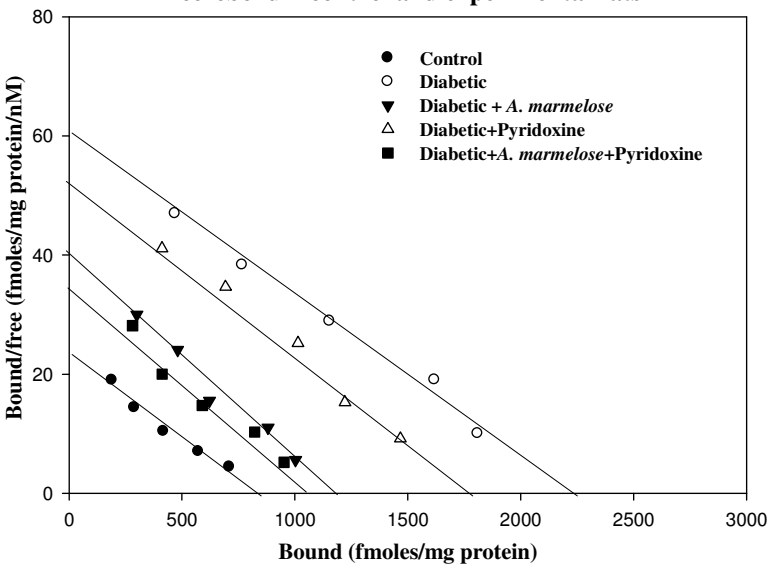


Table-45

[^{3}H] Glutamate binding parameters in the cerebellum of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	937.0 ± 0.20	34.5 ± 0.12
Diabetic	2345.0 ± 0.16 [a]	35.0 ± 0.25
Diabetic+Pyridoxine	1112.0 ± 0.11 [a,c]	32.0 ± 0.18
Diabetic+*Aegle marmelose*	1098.2 ± 0.34 [b]	34.5 ± 0.22
Diabetic+*Aegle marmelose* + Pyridoxine	996.7 ± 0.21 [b]	34.0 ± 0.19

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control; [b] $p<0.001$, [c] $p<0.01$ when compared to diabetic group.

Figure-37

Real Time PCR amplification of 5-HT_{2A} receptor mRNA from the cerebellum of control and experimental rats

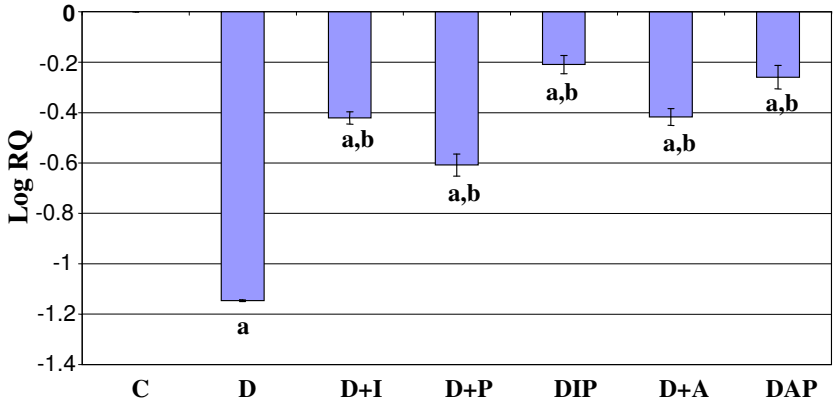


Table-46

Real Time PCR amplification of 5-HT_{2A} receptor mRNA from the cerebellum of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-1.146 ± 0.096 [a]
Diabetic+Insulin	-0.421 ± 0.094 [a,b]
Diabetic+Pyridoxine	-0.608 ± 0.060 [a,b]
Diabetic+Insulin+Pyridoxine	-0.209 ± 0.458 [a,b]
Diabetic+*A. marmelose*	-0.417 ± 0.033 [a,b]
Diabetic *A. marmelose*+Pyridoxine	-0.259 ± 0.048 [a,b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-38

Real Time PCR amplification of 5-HTT mRNA from the cerebellum of control and experimental rats

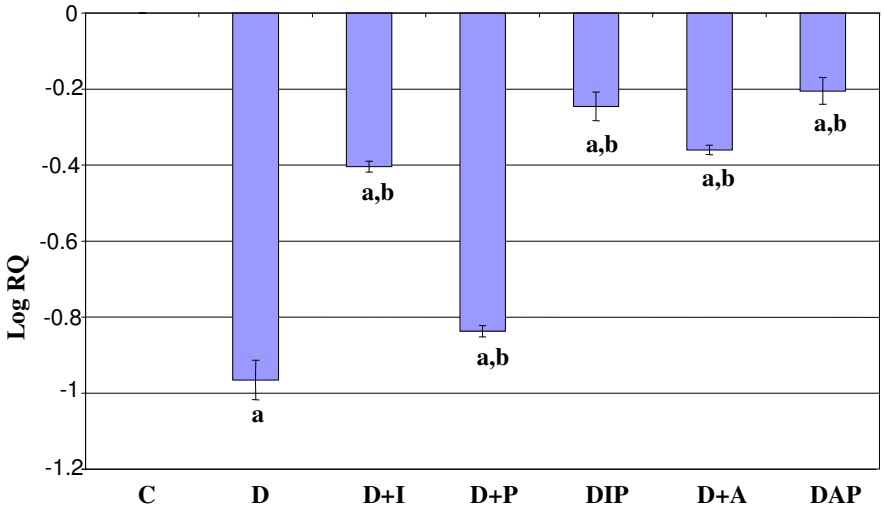


Table-47

Real Time PCR amplification of 5-HTT mRNA from the cerebellum of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.965 ± 0.05 [a]
Diabetic+Insulin	-0.403 ± 0.14 [a,b]
Diabetic +Pyridoxine	-0.837 ± 0.11 [a,b]
Diabetic+Insulin+Pyridoxine	-0.245 ± 0.03 [a,b]
Diabetic+*A. marmelose*	-0.360 ± 0.01 [a,b]
Diabetic+ *A. marmelose* +Pyridoxine	-0.204 ± 0.07 [a,b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-39

Real Time PCR amplification of mGluR5 mRNA from the cerebellum of Control and Experimental rats

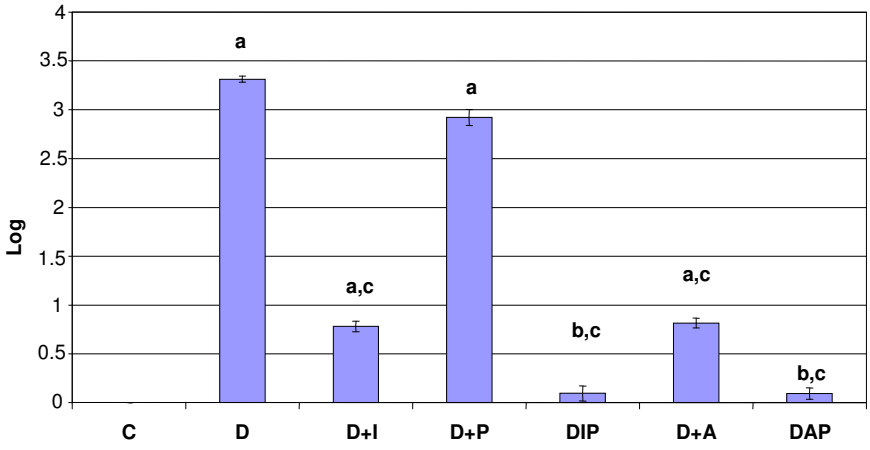


Table-48

Real Time PCR amplification of mGluR5 mRNA from the cerebellum of Control and Experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	3.211 ± 0.029 [a]
Diabetic+Insulin	0.780 ± 0.033 [a,c]
Diabetic +Pyridoxine	2.921 ± 0.041 [a]
Diabetic+Insulin+Pyridoxine	0.094 ± 0.012 [b,c]
Diabetic+*A. marmelose*	0.815 ± 0.020 [a,c]
Diabetic+ *A. marmelose* +Pyridoxine	0.092 ± 0.067 [b,c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-40

Real Time PCR amplification of GLAST glutamate transporter mRNA from the cerebellum of Control and Experimental rats

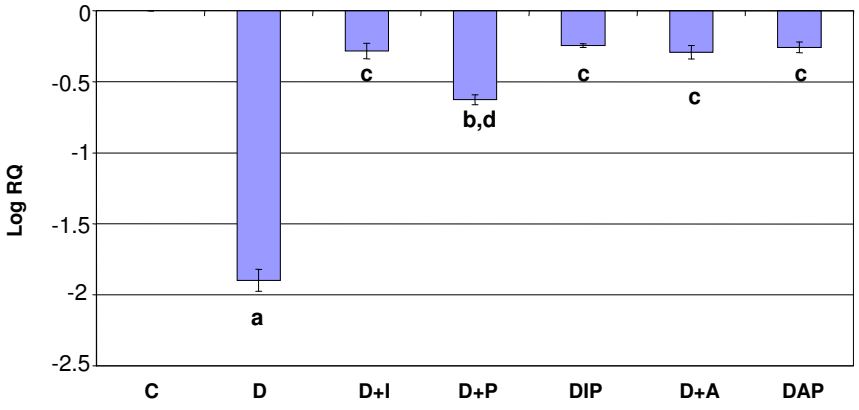


Table-49

Real Time PCR amplification of GLAST glutamate transporter mRNA from the cerebellum of Control and Experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-1.897 ± 0.077 [a]
Diabetic+Insulin	-0.282 ± 0.054 [c]
Diabetic +Pyridoxine	-0.625 ± 0.035 [b,d]
Diabetic+Insulin+Pyridoxine	-0.244 ± 0.013 [c]
Diabetic+*A. marmelose*	-0.291 ± 0.047 [c]
Diabetic+ *A. marmelose* +Pyridoxine	-0.257 ± 0.036 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.05$ when compared to control group; [c] $p<0.01$, [d] $p<0.05$ when compared to diabetic group.

Figure-41

Real Time PCR amplification of Insulin receptor mRNA from the cerebellum of Control and Experimental rats

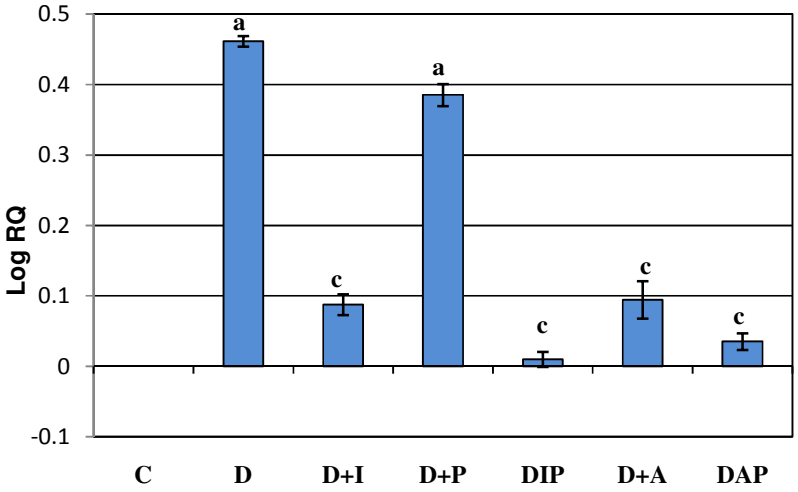


Table-50

Real Time PCR amplification of Insulin receptor mRNA from the cerebellum of Control and Experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	0.461 ± 0.077 [a]
Diabetic+Insulin	0.087 ± 0.054 [c]
Diabetic +Pyridoxine	0.385 ± 0.035 [a]
Diabetic+Insulin+Pyridoxine	0.010 ± 0.013 [c]
Diabetic+*A. marmelose*	0.094 ± 0.047 [c]
Diabetic+ *A. marmelose* +Pyridoxine	0.035 ± 0.036 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-42

Real Time PCR amplification of SOD mRNA from the cerebellum of Control and Experimental rats

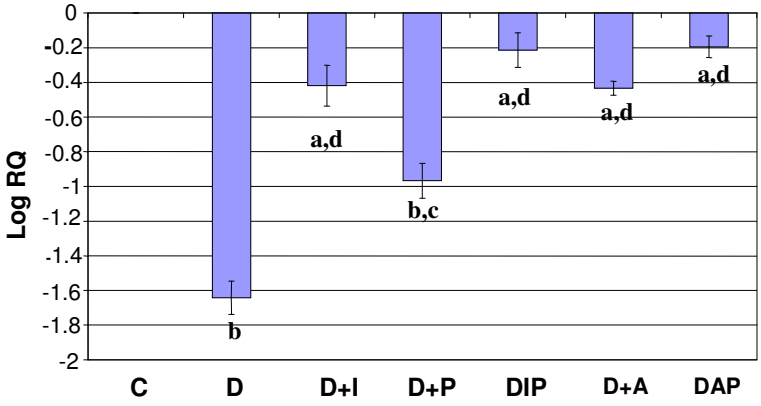


Table-51

Real Time PCR amplification of SOD mRNA from the cerebellum of Control and Experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-1.641 ± 0.09 [b]
Diabetic+Insulin	-0.418 ± 0.14 [a,d]
Diabetic+Pyridoxine	-0.966 ± 0.10 [b,c]
Diabetic+Insulin+Pyridoxine	-0.213 ± 0.07 [a,d]
Diabetic+*A. marmelose*	-0.433 ± 0.04 [a,d]
Diabetic+*A. marmelose*+Pyridoxine	-0.194 ± 0.06 [a,d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.05$, [b] $p<0.001$ when compared to control group; [c] $p<0.01$, [d] $p<0.001$ when compared to diabetic group.

Figure-43

Real Time PCR amplification of GPx mRNA from the cerebellum of Control and Experimental rats

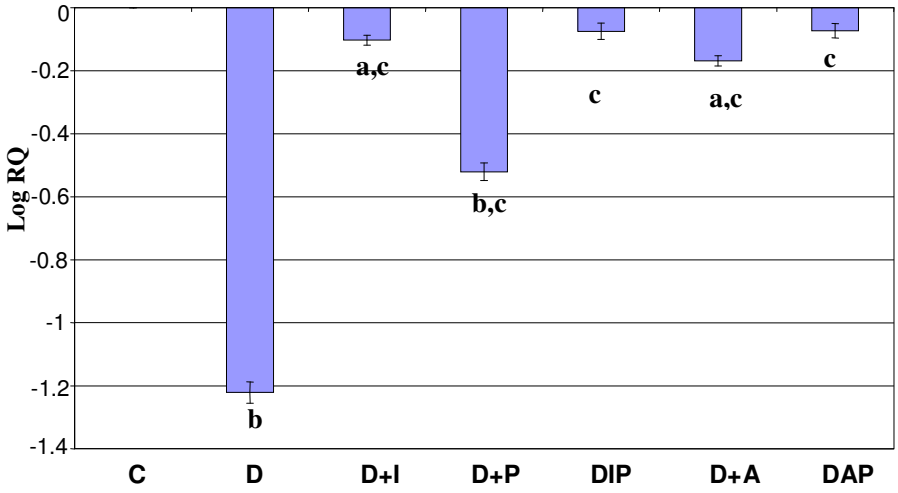


Table-52

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

Experimental Group	Log RQ value
Control	0
Diabetic	-1.221 ± 0.09 [b]
Diabetic+Insulin	-0.102 ± 0.14 [a,c]
Diabetic+Pyridoxine	-0.520 ± 0.10 [b,c]
Diabetic+Insulin+Pyridoxine	-0.107 ± 0.07 [c]
Diabetic+*A. marmelose*	-0.118 ± 0.04 [a,c]
Diabetic+*A. marmelose*+Pyridoxine	-0.102 ± 0.06 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.05$, [b] $p<0.001$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-44

Scatchard analysis of [^{3}H] 5-HT binding aganist 5-HT in the hippocampus of Control and Experimental rats

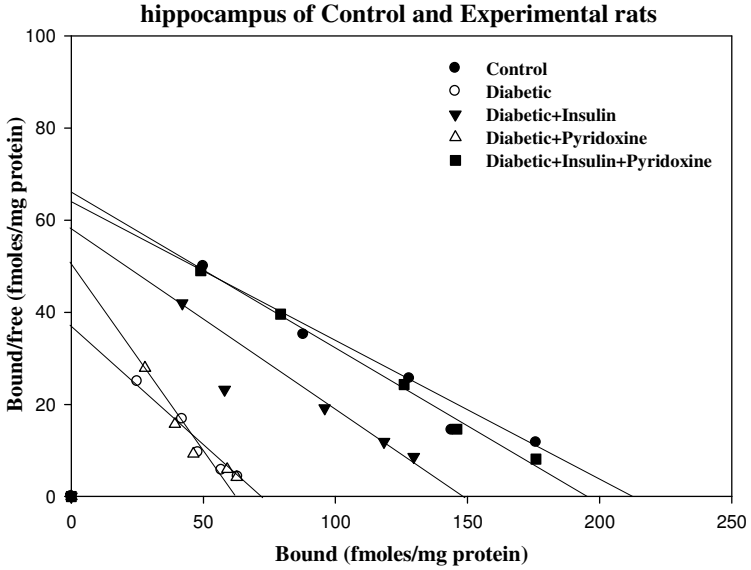


Table-53

[^{3}H] 5-Hydroxytryptamine binding parameters in the hippocampus of control and experimental rats.

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	212.5 ± 2.11	3.22 ± 0.54
Diabetic	72.4 ± 3.21 [a]	1.94 ± 0.41 [a]
Diabetic+Pyridoxine	62.8 ± 2.06 [a]	1.40 ± 0.29 [a]
Diabetic+Insulin	148.4 ± 2.33 [a,c]	2.60 ± 0.49 [b]
Diabetic+Insulin+Pyridoxine	196.0 ± 1.43 [c]	3.20 ± 0.17 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.05$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-45

Scatchard analysis of [^{3}H] 5-HT binding aganist 5-HT in the hippocampus of Control and Experimental rats

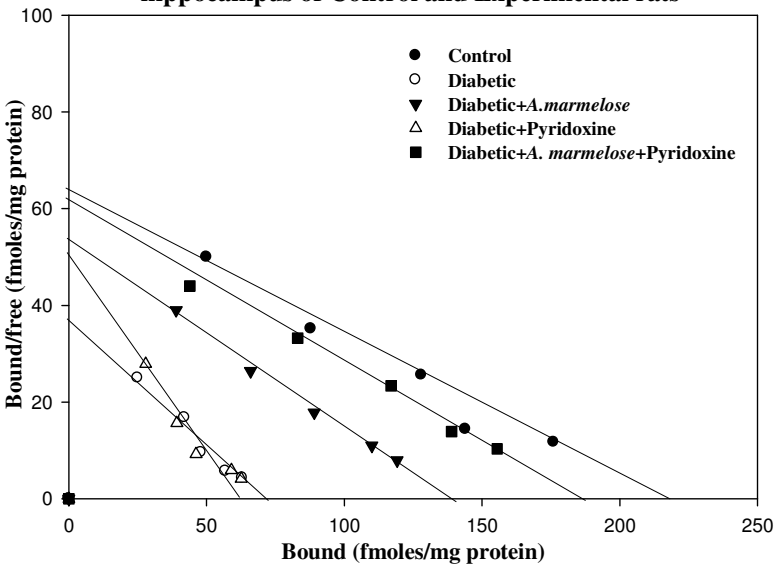


Table-54

[^{3}H] 5-Hydroxytryptamine binding parameters in the hippocampus of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	212.5 ± 6.21	3.20 ± 1.12
Diabetic	72.4 ± 2.34 [a]	1.94 ± 1.03 [a]
Diabetic+Pyridoxine	62.8 ± 1.03 [a]	1.40 ± 0.96 [a]
Diabetic+*A. marmelose*	140.1 ± 4.33 [a,c]	2.57 ± 1.42 [b]
Diabetic+*A. marmelose*+Pyridoxine	186.4 ± 2.42 [c]	3.05 ± 1.31 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.05$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-46

Scatchard analysis of [^{3}H] Ketanserin binding aganist ketanserin in the hippocampus of control and experimental rats

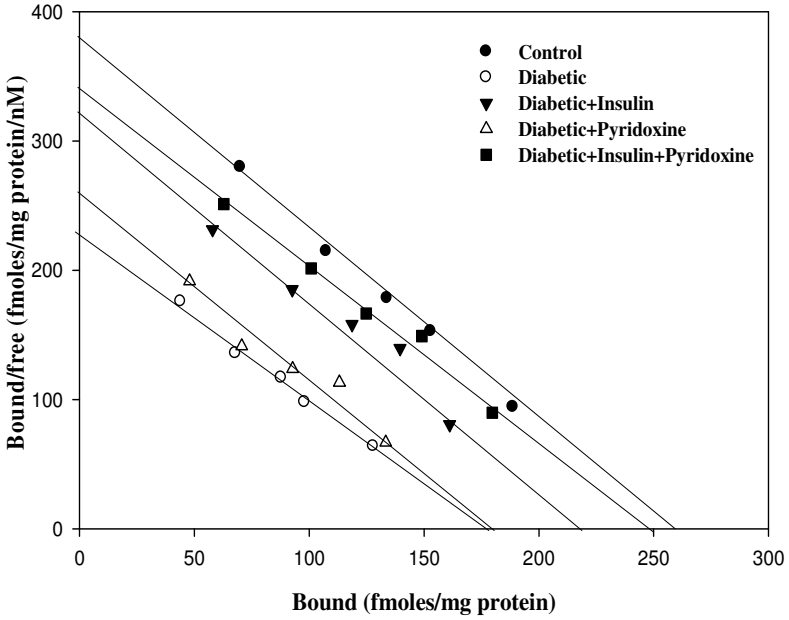


Table-55

[^{3}H] Ketanserin binding parameters in the hippocampus of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	260.5 ± 0.35 [e]	0.68 ± 0.08
Diabetic	176.2 ± 0.19 [b]	0.77 ± 0.17 [a]
Diabetic+Insulin	218.1 ± 0.32 [e]	0.67 ± 0.09 [c]
Diabetic+Pyridoxine	180.6 ± 0.27 [b]	0.70 ± 0.06 [c]
Diabetic+Insulin+Pyridoxine	244.0 ± 0.26 [e]	0.68 ± 0.11 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] p <0.05, [b] p<0.001 when compared to control; [c] p <0.05, [d] p <0.01, [e] p<0.001 when compared to diabetic group.

Figure-47

Scatchard analysis of [^{3}H] Ketanserin binding aganist ketanserin in the hippocampus of control and experimental rats

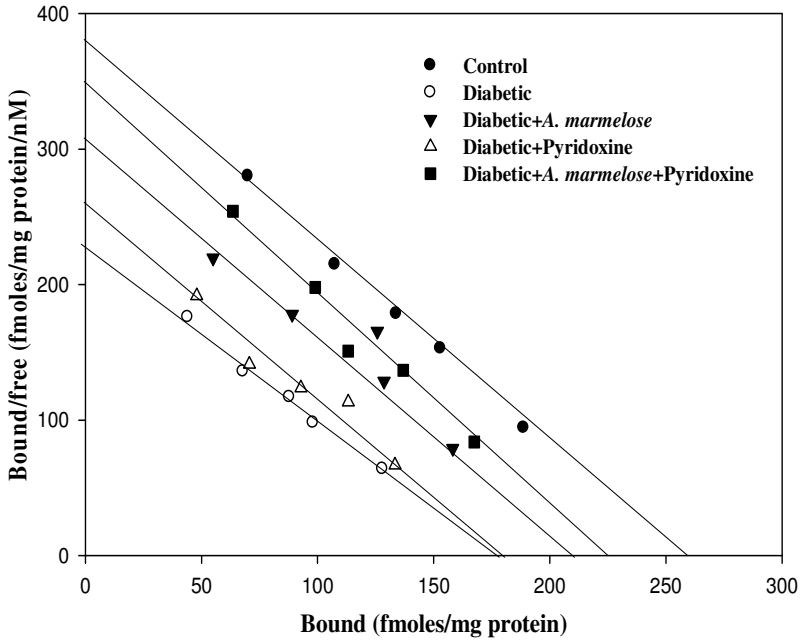


Table-56

[^{3}H] Ketanserin binding parameters in the hippocampus of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	260.5 ± 0.35 [d]	0.68 ± 0.08 [c]
Diabetic	176.2 ± 0.19 [b]	0.77 ± 0.17 [a]
Diabetic+*A. marmelose*	209.3 ± 0.22 [d]	0.68 ± 0.11 [c]
Diabetic+Pyridoxine	180.6 ± 0.27 [b]	0.70 ± 0.06 [c]
Diabetic+*A. marmelose*+Pyridoxine	228.2 ± 0.29 [d]	0.67 ± 0.07 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] p <0.05, [b] p<0.001 when compared to control; [c] p <0.05, [d] p<0.001 when compared to diabetic group.

Figure-48

Scatchard analysis of [^{3}H] Glutamate against glutamate in the hippocampus of control and experimental rats

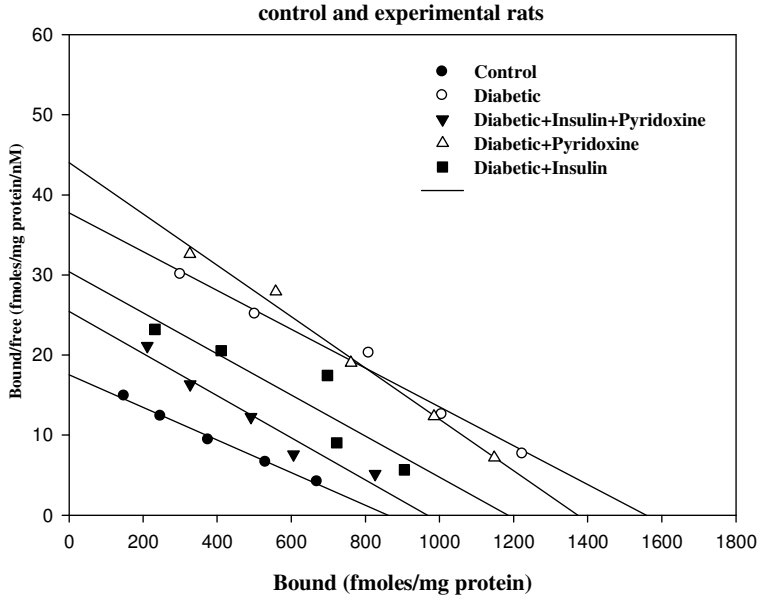


Table-57

[^{3}H] Glutamate binding parameters in the hippocampus of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	845.3 ± 0.16	42.5 ± 0.12
Diabetic	1551.2 ± 0.20 [a]	45.0 ± 0.25
Diabetic+Insulin	1188.4± 0.11 [b,c]	42.0 ± 0.18
Diabetic+Pyridoxine	1371.3 ± 0.31 [a]	42.5 ± 0.22
Diabetic+Insulin+Pyridoxine	952.1 ± 0.24 [c]	42.0 ± 0.19

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared to control; [c] $p<0.001$ when compared to diabetic group.

Figure-49

Scatchard analysis of [^{3}H] Glutamate against glutamate in the hippocampus of control and experimental rats

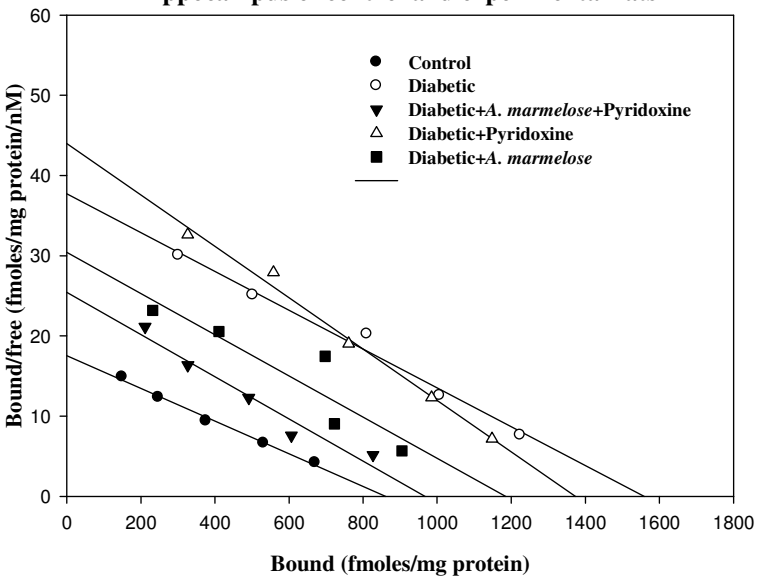


Table-58

[^{3}H] Glutamate binding parameters in the hippocampus of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	845.3 ± 0.16	42.5 ± 0.12
Diabetic	1554.2 ± 0.20 [a]	45.0 ± 0.25
Diabetic+Pyridoxine	1188.4± 0.11 [a]	42.0 ± 0.18
Diabetic+*A. marmelose*	1099.1 ± 0.31 [b,c]	42.5 ± 0.22
Diabetic+*A. marmelose*+Pyridoxine	982.0 ± 0.24 [c]	42.0 ± 0.19

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared to control; [c] $p<0.001$ when compared to diabetic group.

Figure-50

Real Time PCR amplification of 5-HT_{2A} mRNA from the hippocampus of Control and Experimental rats

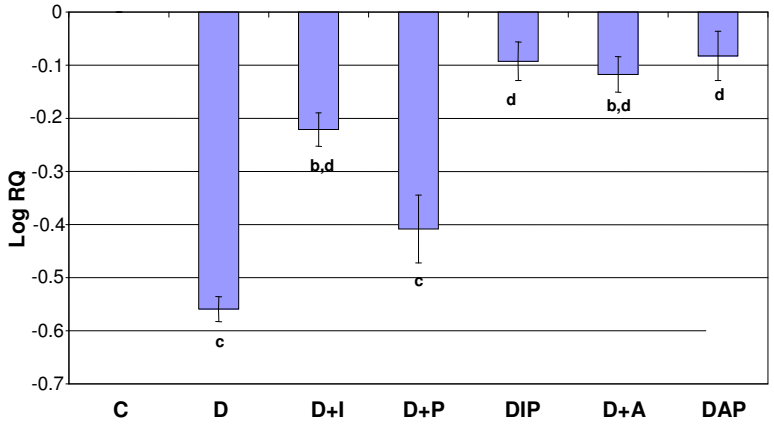


Table-59

Real Time PCR amplification of 5-HT_{2A} mRNA from the hippocampus of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.559 ± 0.023 [c]
Diabetic+Insulin	-0.221 ± 0.031 [b,d]
Diabetic+Pyridoxine	-0.408 ± 0.063 [c]
Diabetic+Insulin+Pyridoxine	-0.092 ± 0.036 [d]
Diabetic+*A. marmelose*	-0.117 ± 0.031 [a,d]
Diabetic+*A. marmelose*+Pyridoxine	-0.082 ± 0.046 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ when compared to control group; [d] $p<0.001$ when compared to diabetic group.

Figure-51

Real Time PCR amplification of 5-HTT mRNA from the hippocampus of control and experimental rats

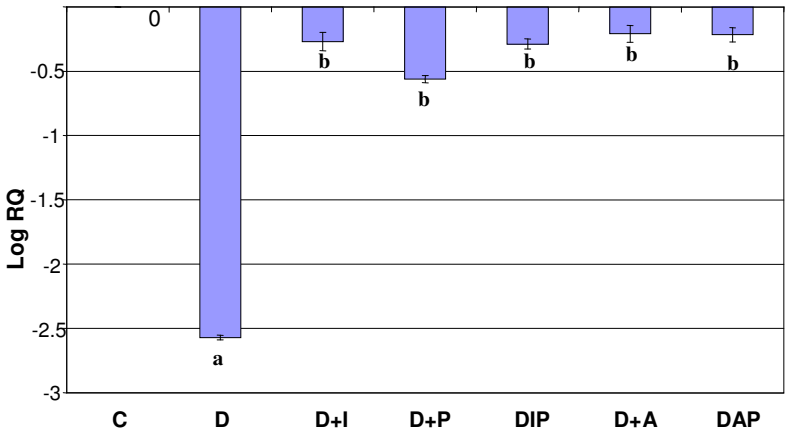


Table-60

Real Time PCR amplification of 5-HTT mRNA from the hippocampus of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-2.570 ± 0.017 [a]
Diabetic+Insulin	-0.269 ± 0.071 [b]
Diabetic+Pyridoxine	-0.560 ± 0.028 [b]
Diabetic+Insulin+Pyridoxine	-0.288 ± 0.039 [b]
Diabetic+*A. marmelose*	-0.207 ± 0.064 [b]
Diabetic+*A. marmelose*+Pyridoxine	-0.211± 0.054 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.01$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-52

Real Time PCR amplification of mGluR5 mRNA from the hippocampus of Control and Experimental rats

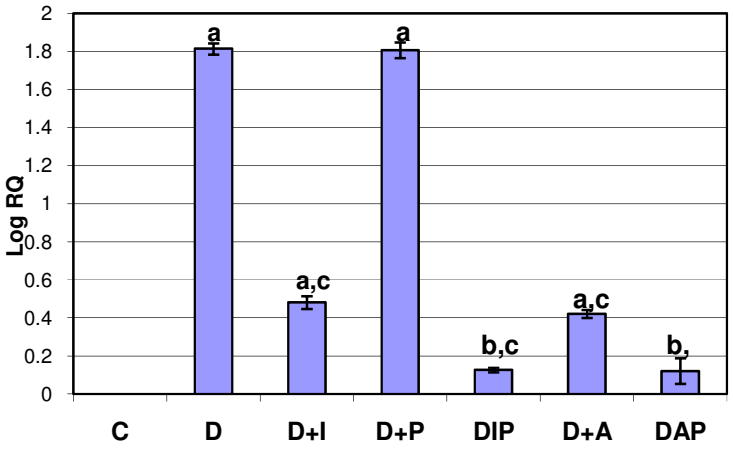


Table-61

Real Time PCR amplification of mGluR5 mRNA from the hippocampus of Control and Experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	1.814 ± 0.029 [a]
Diabetic+Insulin	0.480 ± 0.038 [a,c]
Diabetic+Pyridoxine	1.807± 0.031 [a]
Diabetic+Insulin+Pyridoxine	0.126 ± 0.011 [b,c]
Diabetic+*A. marmelose*	0.421 ± 0.019 [a,c]
Diabetic+*A. marmelose*+Pyridoxine	0.120 ± 0.054 [b,c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-53

Real Time PCR amplification of GLAST glutamate transporter mRNA from the hippocampus of control and experimental rats

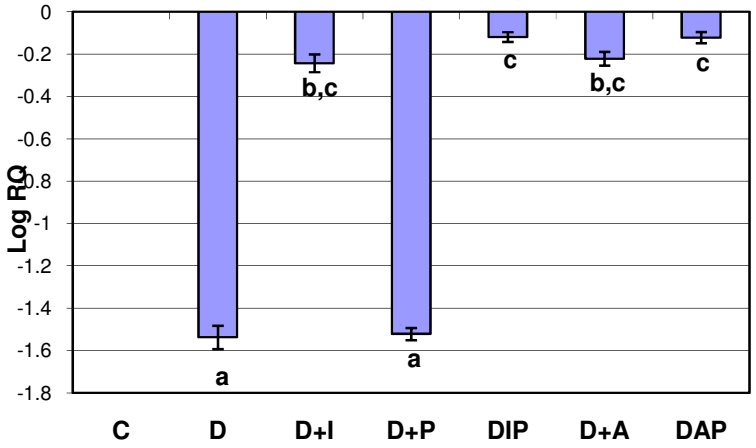


Table-62

Real Time PCR amplification of GLAST glutamate transporter mRNA from the hippocampus of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-1.537 ± 0.055 [a]
Diabetic+Insulin	-0.242 ± 0.042 [c]
Diabetic+Pyridoxine	-1.521 ± 0.028 [b,d]
Diabetic+Insulin+Pyridoxine	-0.118 ± 0.023 [c]
Diabetic+*A. marmelose*	-0.221 ± 0.032 [c]
Diabetic+ *A. marmelose*+Pyridoxine	-0.121 ± 0.026 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.05$ when compared to control group; [c] $p<0.01$, [d] $p<0.05$ when compared to diabetic group.

Figure-54

Real Time PCR amplification of Insulin Receptor mRNA from the hippocampus of Control and Experimental rats

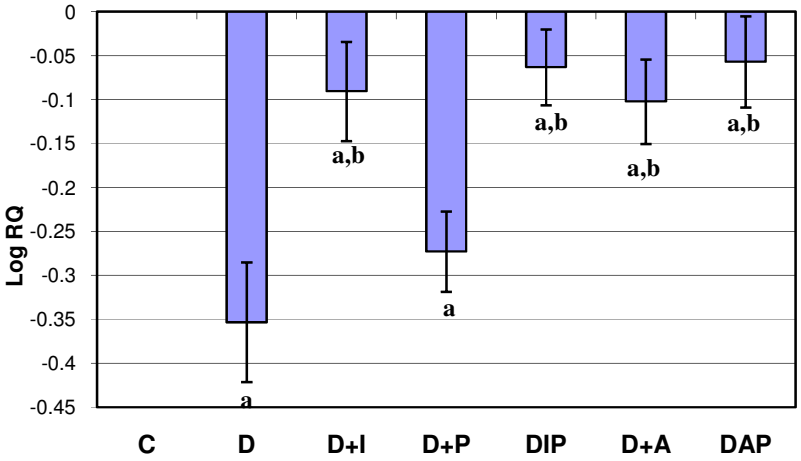


Table-63

Real Time PCR amplification of Insulin Receptor mRNA from the hippocampus of Control and Experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.283 ± 0.30 [a]
Diabetic+Insulin	-0.190 ± 0.25 [a,b]
Diabetic+Pyridoxine	-0.290 ± 0.22 [a]
Diabetic+Insulin+Pyridoxine	-0.064 ± 0.20 [a,b]
Diabetic+*A. marmelose*	-0.197 ± 0.21 [a,b]
Diabetic+*A. marmelose*+Pyridoxine	-0.078 ± 0.27 [a,b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-55

Real Time PCR amplification of SOD mRNA from the hippocampus of Control and Experimental rats

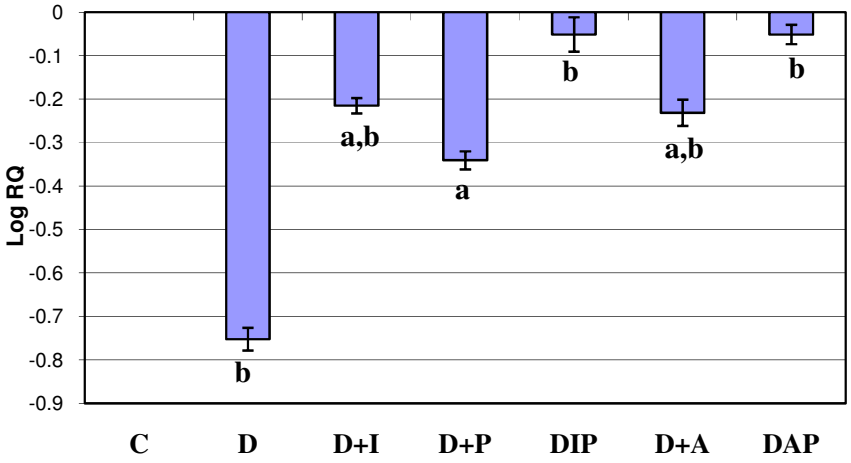


Table-64

Real Time PCR amplification of SOD mRNA from the hippocampus of Control and Experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.781 ± 0.023
Diabetic+Insulin	-0.241 ± 0.018 [a,b]
Diabetic+Pyridoxine	-0.350 ± 0.021 [a]
Diabetic+Insulin+Pyridoxine	-0.093 ± 0.045 [b]
Diabetic+*A. marmelose*	-0.243 ± 0.039 [a,b]
Diabetic+*A. marmelose*+Pyridoxine	-0.091 ± 0.019 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-56

Real Time PCR amplification of GPx mRNA from the hippocampus of Control and Experimental rats

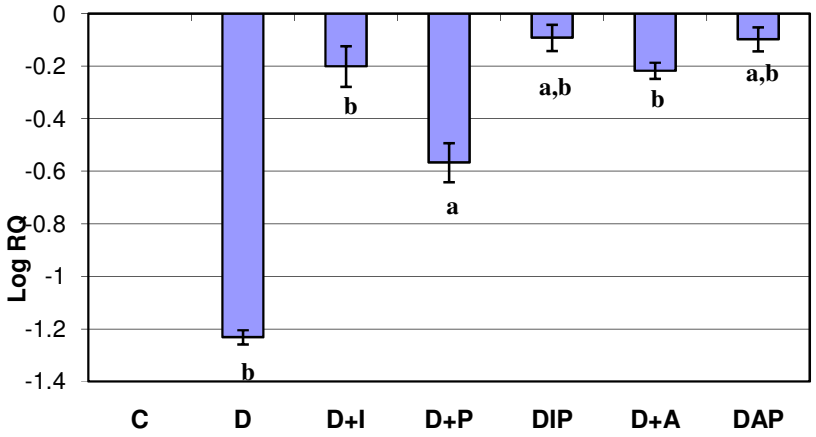


Table-65

Real Time PCR amplification of GPx mRNA from the hippocampus of Control and Experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.675 ± 0.017 [b]
Diabetic+Insulin	-0.108 ± 0.023 [b]
Diabetic +Pyridoxine	-0.466 ± 0.026 [a]
Diabetic+Insulin+Pyridoxine	-0.032 ± 0.019 [a,b]
Diabetic+*A. marmelose*	-0.117 ± 0.021 [b]
Diabetic+*A. marmelose*+Pyridoxine	-0.030 ± 0.029 [a,b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-57

Scatchard analysis of [^{3}H] 5-HT binding aganist 5-HT in the pancreas of Control and Experimental rats

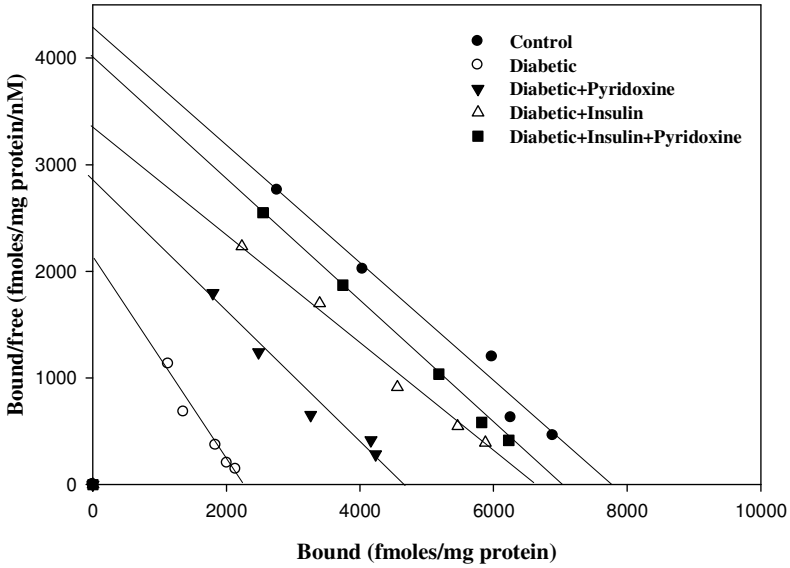


Table-66

[^{3}H] 5-Hydroxytryptamine binding parameters in the pancreas of Control and Experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	7787.0 ± 2.11	1.81 ± 0.14
Diabetic	2219.0 ± 3.21 [a]	1.04 ± 0.23 [a]
Diabetic+Pyridoxine	4653.0 ± 2.06 [a,b]	1.60 ± 0.19 [b]
Diabetic+Insulin	6559.4 ± 2.33 [b]	1.83 ± 0.25 [b]
Diabetic+Insulin+Pyridoxine	7024.0 ± 1.43 [b]	1.82 ± 0.37 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-58

Scatchard analysis of [^{3}H] 5-HT binding aganist 5-HT in the pancreas of Control and Experimental rats

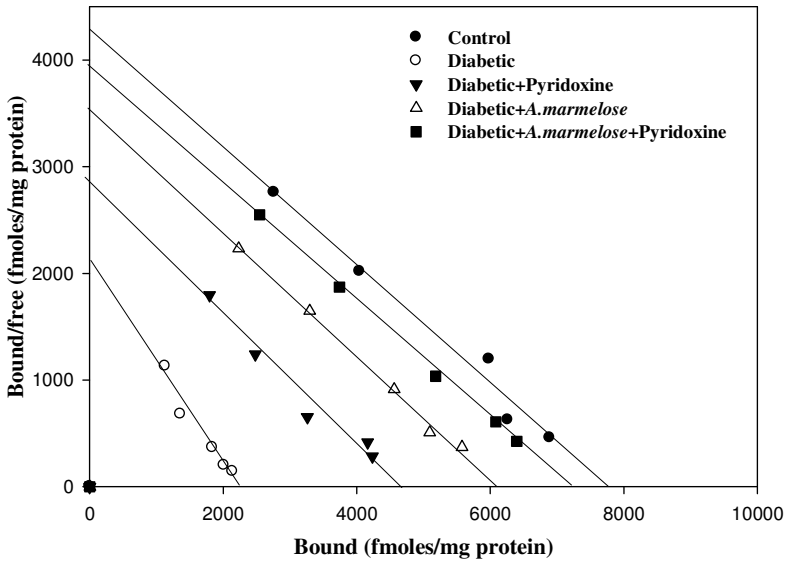


Table-67

[^{3}H] 5-Hydroxytryptamine binding parameters in the pancreas of Control and Experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	7787.0 ± 2.11	1.81 ± 0.14
Diabetic	2219.0 ± 3.21 [a]	1.04 ± 0.23 [a]
Diabetic+Pyridoxine	4653.0 ± 2.06 [a,b]	1.60 ± 0.19 [b]
Diabetic+*A. marmelose*	6059.1 ± 2.45 [b]	1.73 ± 0.25 [b]
Diabetic+*A. marmelose*+Pyridoxine	7258.6 ± 2.32 [b]	1.82 ± 0.37 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-59

Scatchard analysis of [^{3}H] Ketanserin binding aganist ketanserin in the pancreas of control and experimental rats

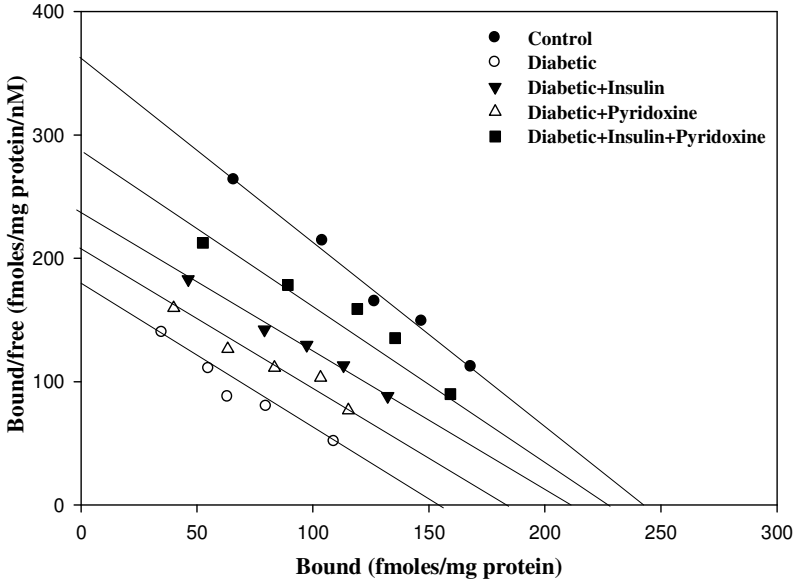


Table-68

[^{3}H] Ketanserin binding parameters in the pancreas of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	235.0 ± 0.92	0.80 ± 2.30
Diabetic	161.3 ± 0 .41 [a]	0.86 ± 3.33
Diabetic+Insulin	217.0 ± 0.24 [a,b]	0.82 ± 2.43
Diabetic+Pyridoxine	171.2 ± 1.46 [a,b]	0.85 ± 2.76
Diabetic+Insulin+Pyridoxine	229.0 ± 3.16 [b]	0.81 ± 3.38

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group;

[b] $p<0.001$ when compared to diabetic group.

Figure-60

Scatchard analysis of [^{3}H] Ketanserin receptors binding aganist ketanserin in the pancreas of control and experimental rats

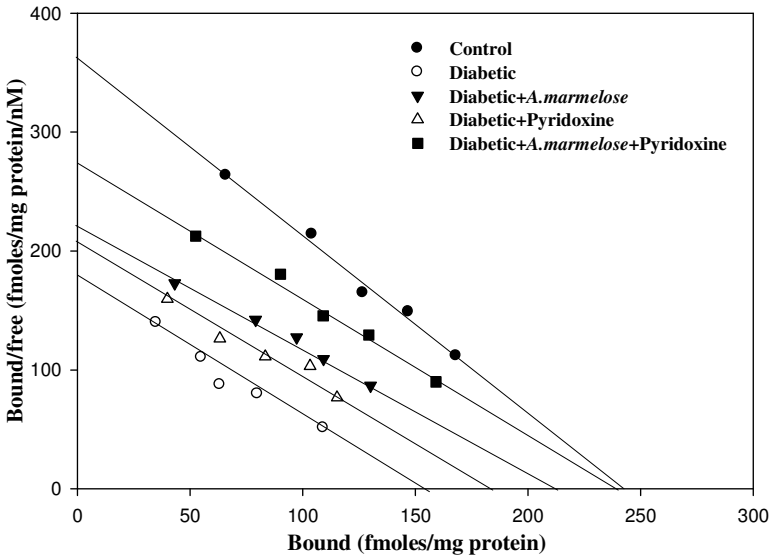


Table-69

[^{3}H] Ketanserin binding parameters in the pancreas of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	235.0 ± 0.92	0.80 ± 2.30
Diabetic	161.3 ± 0 .41 [a]	0.86 ± 3.33
Diabetic+*A. marmelose*	212.0 ± 0.24 [a,b]	0.86 ± 2.98
Diabetic+Pyridoxine	171.2 ± 1.46 [a,b]	0.85 ± 2.76
Diabetic+*A. marmelose*+Pyridoxine	234.0 ± 3.06 [b]	0.86 ± 2.75

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-61

Scatchard analysis of [^{3}H] Glutamate against glutamate in the pancreas of control and experimental rats

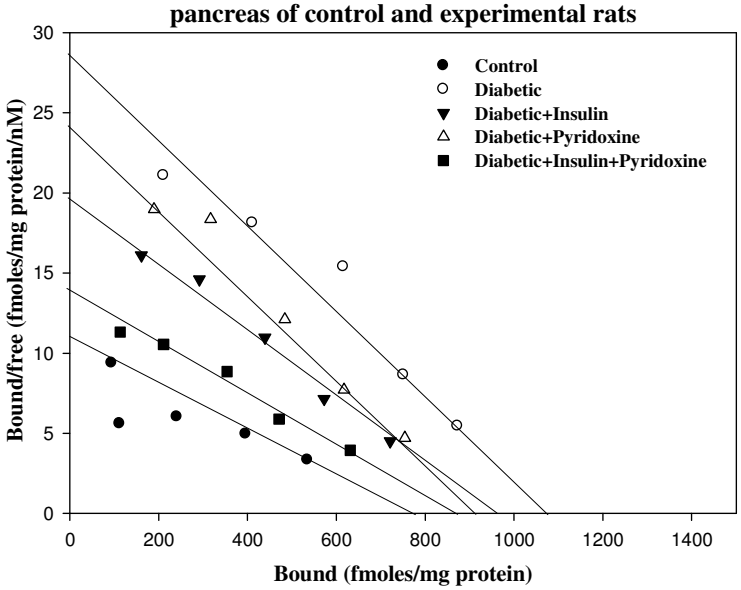


Table-70

[^{3}H] Glutamate binding parameters in the pancreas of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	767.8 ± 0.31	68.0 ± 0.19
Diabetic	1079.6 ± 0.20 [a]	37.5 ± 0.25 [a]
Diabetic+Insulin	968.1 ± 0.24 [b,e]	50.5 ± 0.18 [c,e]
Diabetic+Pyridoxine	910.2 ± 0.11 [c,d]	38.7 ± 0.18 [a]
Diabetic+Insulin+Pyridoxine	863.2 ± 0.16 [d]	62.6 ± 0.12 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 groups. [a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to control; [d] $p<0.001$, [e] $p<0.01$ when compared to diabetic group.

Figure-62

Scatchard analysis of [^{3}H] Glutamate against glutamate in the pancreas of control and experimental rats

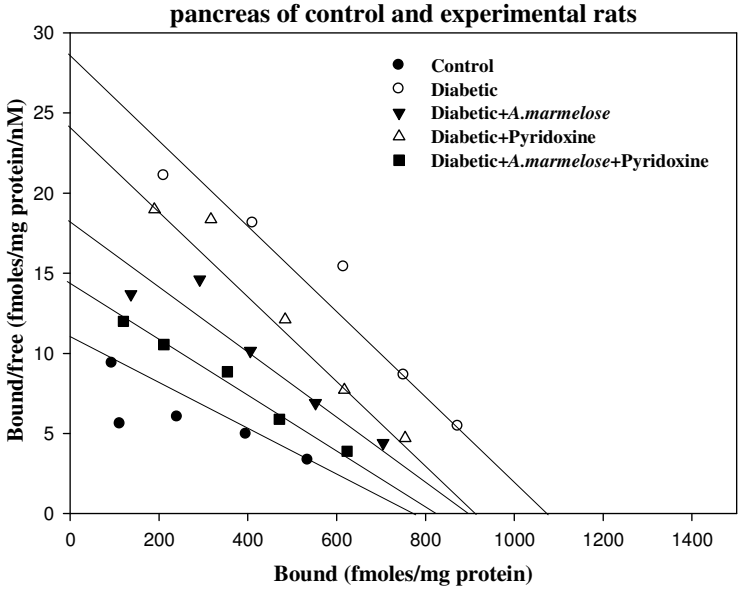


Table-71

[^{3}H] Glutamate binding parameters in the pancreas of control and experimental rats

Experimental Groups	B_{max} (fmoles/ mg protein)	K_d (nM)
Control	767.8 ± 0.31	68.0 ± 0.19
Diabetic	1079.6 ± 0.20 [a]	37.5 ± 0.25 [a]
Diabetic+Pyridoxine	910.2 ± 0.11 [c,d]	38.7 ± 0.18 [a]
Diabetic+*A. marmelose*	888.5 ± 0.16 [a,d]	50.5 ± 0.21 [c,e]
Diabetic+*A. marmelose*+Pyridoxine	818.7 ± 0.13 [d]	59.5 ± 0.17 [d]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 groups. [a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to control; [d] $p<0.001$, [e] $p<0.01$ when compared to diabetic group.

Figure-63

Real Time PCR amplification of 5-HT_{2A} receptor mRNA from the pancreas of control and experimental rats

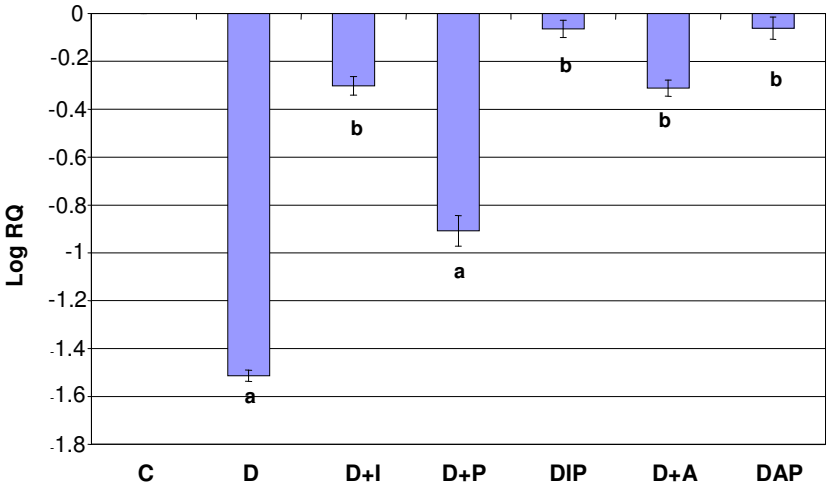


Table-72

Real Time PCR amplification of 5-HT_{2A} receptor mRNA from the pancreas of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-1.513 ± 0.023 [a]
Diabetic+Insulin	-0.302 ± 0.031 [b]
Diabetic+Pyridoxine	-0.908 ± 0.063 [a]
Diabetic+Insulin+Pyridoxine	-0.064 ± 0.036 [b]
Diabetic+*A. marmelose*	-0.312 ± 0.031 [b]
Diabetic+*A. marmelose*+Pyridoxine	-0.061 ± 0.046 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-64

Real Time PCR amplification of 5-HTT mRNA from the pancreas of control and experimental rats

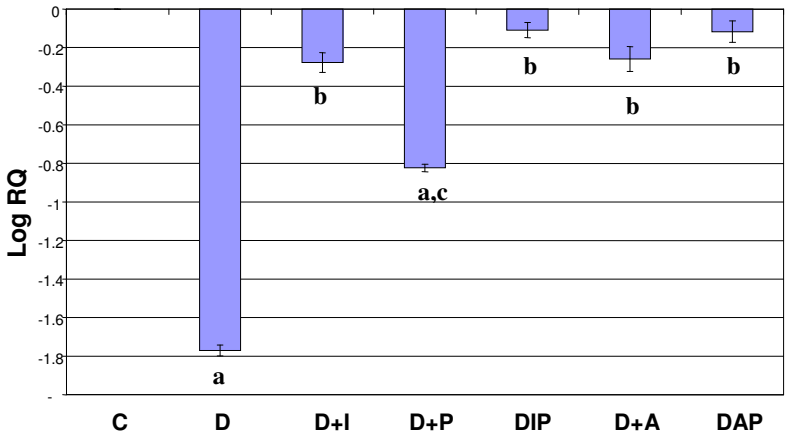


Table-73

Real Time PCR amplification of 5-HTT mRNA from the pancreas of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-1.770 ± 0.027 [a]
Diabetic+Insulin	-0.276 ± 0.050 [b]
Diabetic+Pyridoxine	-0.823 ± 0.019 [a,c]
Diabetic+Insulin+Pyridoxine	-0.108 ± 0.039 [b]
Diabetic+*A. marmelose*	-0.258 ± 0.064 [b]
Diabetic+*A. marmelose*+Pyridoxine	-0.116 ± 0.054 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group;
[b] $p<0.001$, [c] $p<0.01$ when compared to diabetic group

Figure-65

Real Time PCR amplification of mGluR5 mRNA from the pancreas of control and experimental rats

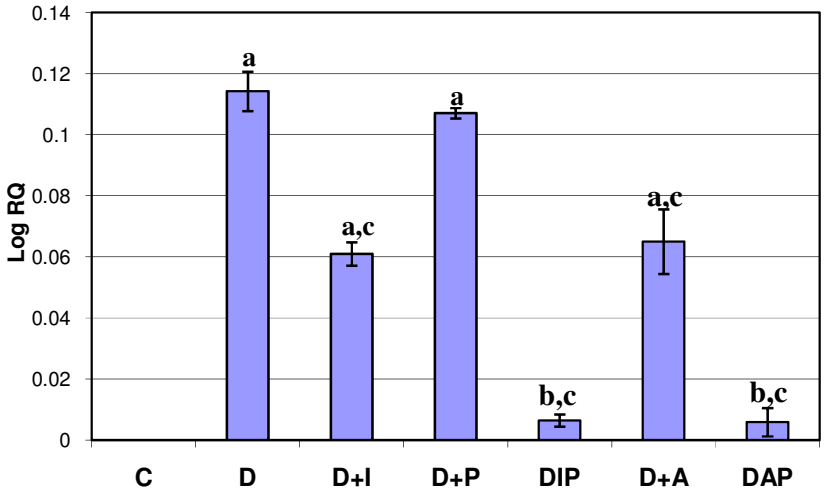


Table-74

Real Time PCR amplification of mGluR5 mRNA from the pancreas of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	0.115 ± 0.006 [a]
Diabetic+Insulin	0.060 ± 0.003 [a,c]
Diabetic +Pyridoxine	0.107± 0.001 [a]
Diabetic+Insulin+Pyridoxine	0.006 ± 0.002 [b,c]
Diabetic+*A. marmelose*	0.065 ± 0.010 [a,c]
Diabetic+ *A. marmelose*+Pyridoxine	0.005 ± 0.04 [b,c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.01$ when compared to control group; [c] $p<0.001$ when compared to diabetic group.

Figure-66

Real Time PCR amplification of GLAST glutamate transporter mRNA from the pancreas of control and experimental rats

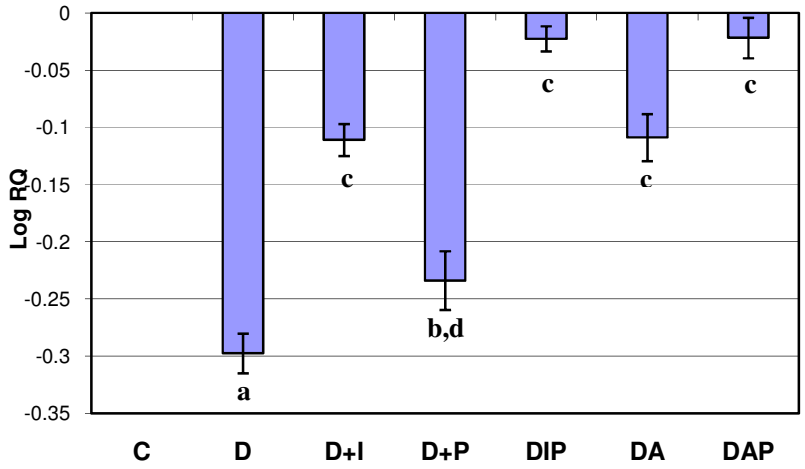


Table-75

Real Time PCR amplification of GLAST glutamate transporter mRNA from the pancreas of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.297 ± 0.017 [a]
Diabetic+Insulin	-0.110 ± 0.014 [c]
Diabetic+Pyridoxine	-0.233 ± 0.025 [b,d]
Diabetic+Insulin+Pyridoxine	-0.022 ± 0.011 [c]
Diabetic+*A. marmelose*	-0.108 ± 0.020 [c]
Diabetic+*A. marmelose*+Pyridoxine	-0.021 ± 0.017 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.05$ when compared to control group; [c] $p<0.01$, [d] $p<0.05$ when compared to diabetic group.

Figure-67

Real Time PCR amplification of Insulin receptor mRNA from the pancreas of control and experimental rats

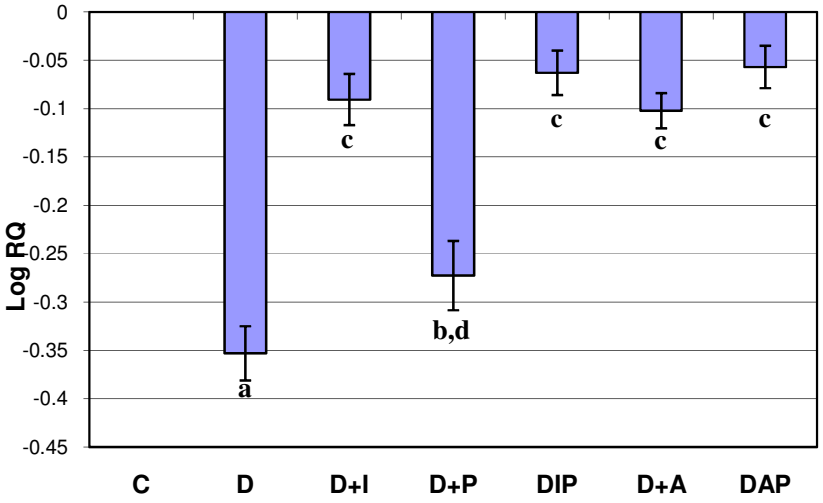


Table-76

Real Time PCR amplification of Insulin receptor mRNA from the pancreas of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	- 0.385 ± 0.020 [a]
Diabetic+Insulin	-0. 090 ± 0.018 [c]
Diabetic+Pyridoxine	-0.272 ± 0.015 [b,d]
Diabetic+Insulin+Pyridoxine	-0.063 ± 0.021 [c]
Diabetic+*A. marmelose*	-0.102 ± 0.017 [c]
Diabetic+*A. marmelose*+Pyridoxine	-0.056 ± 0.012 [c]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [b] $p<0.05$ when compared to control group; [c] $p<0.01$, [d] $p<0.05$ when compared to diabetic group.

Figure-68

Real Time PCR amplification of SOD mRNA from the pancreas of control and experimental rats

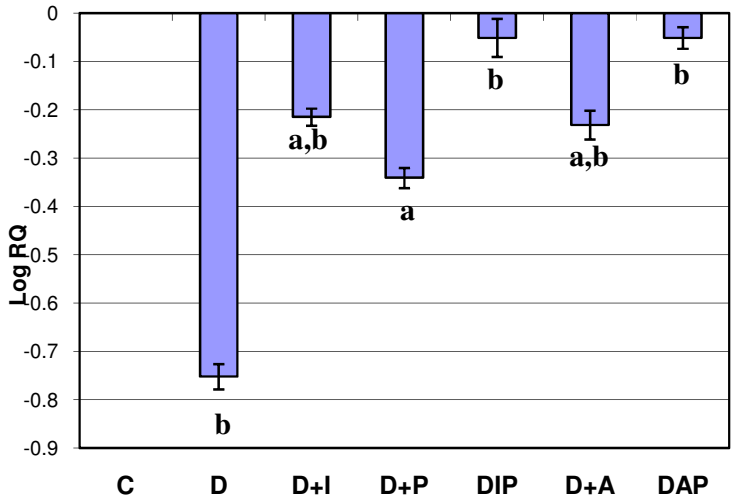


Table-77

Real Time PCR amplification of SOD mRNA from the pancreas of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-0.752 ± 0.027 [a]
Diabetic+Insulin	-0.214 ± 0.014 [a,b]
Diabetic+Pyridoxine	-0.340 ± 0.021 [a]
Diabetic+Insulin+Pyridoxine	-0.051 ± 0.039 [b]
Diabetic+*A. marmelose*	-0.231 ± 0.030 [a,b]
Diabetic+*A. marmelose*+Pyridoxine	-0.050 ± 0.022 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Figure-69

Real Time PCR amplification of GPx mRNA from the pancreas of control and experimental rats

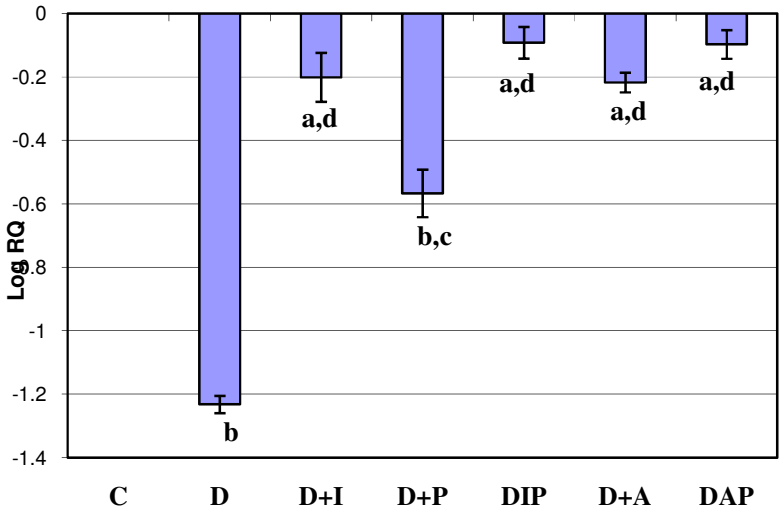


Table-78

Real Time PCR amplification of GPx mRNA from the pancreas of control and experimental rats

Experimental Group	Log RQ value
Control	0
Diabetic	-1.231 ± 0.027 [a]
Diabetic+Insulin	-0.201 ± 0.077 [a,b]
Diabetic+Pyridoxine	-0.566 ± 0.074 [a]
Diabetic+Insulin+Pyridoxine	-0.092 ± 0.049 [b]
Diabetic+*A. marmelose*	-0.217 ± 0.030 [a,b]
Diabetic+*A. marmelose*+Pyridoxine	-0.097 ± 0.045 [b]

Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.001$ when compared to diabetic group.

Table-79

Rotarod performance of control and experimental rats after 15 days treatment

Experimental groups	Retention time on the rod (in seconds)		
	10 rpm	**25 rpm**	**50 rpm**
C	182.00 ± 4.61	169.33 ± 6.30	143.33 ± 3.38
D	79.33 ± 2.40 [b]	41.33 ± 2.18 [b]	26.33 ± 4.97 [b]
D+I	158.00 ± 0.57 [a,d]	114.16 ± 4.70 [b,d]	55.00 ± 6.42 [b,d]
D+P	113.00 ± 5.00 [a,b]	66.66 ± 3.48	29.33 ± 5.45
DIP	175.66 ± 0.88 [d]	152.66 ± 1.43 [d]	135.33 ± 3.75 [d]
D+A	153.22 ± 0.57 [a,d]	118.26 ± 4.70 [b,d]	53.00 ± 6.42 [b,d]
D+AP	179.56 ± 0.68 [d]	147.66 ± 1.89 [d]	145.33 ± 3.25 [d]

Values are Mean ± SEM of 4-6 separate experiments (n = 5-6 rats per group) ANOVA followed by Students-Newman-Keul Test.

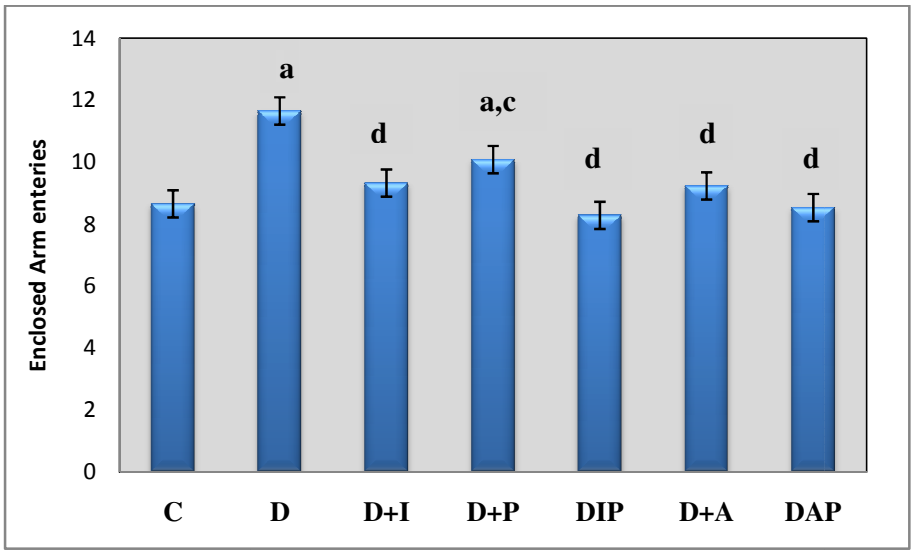
[a] $p<0.05$, [b] $p<0.001$ when compared to control group;

[c] $p<0.05$, [d] $p<0.001$ when compared to diabetic group.

Figure-70

Elevated plus-maze exploration by rats

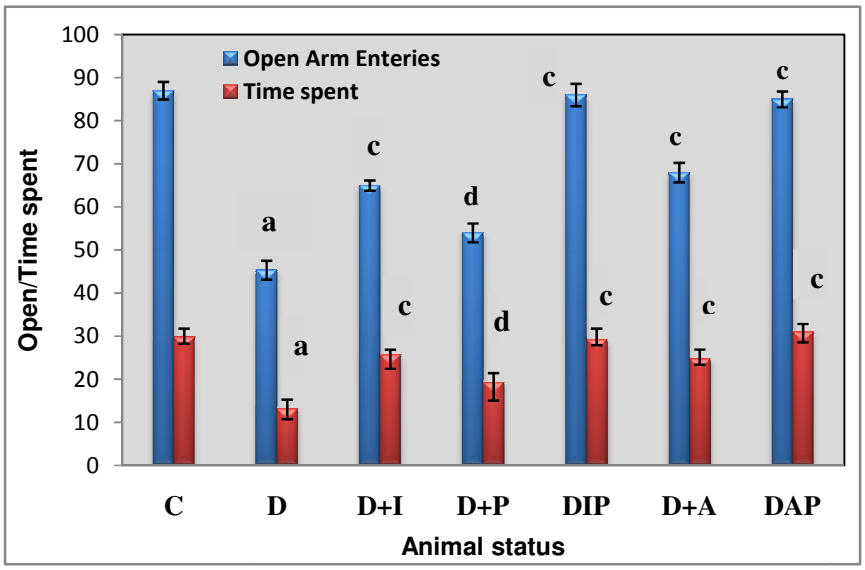
Closed Arm Entry attempts (Counts/5 minutes) by Control and Experimental Rats



Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats.[a] $p<0.001$ when compared to control group; [c] $p<0.01$, [d] $p<0.001$ when compared to diabetic group.

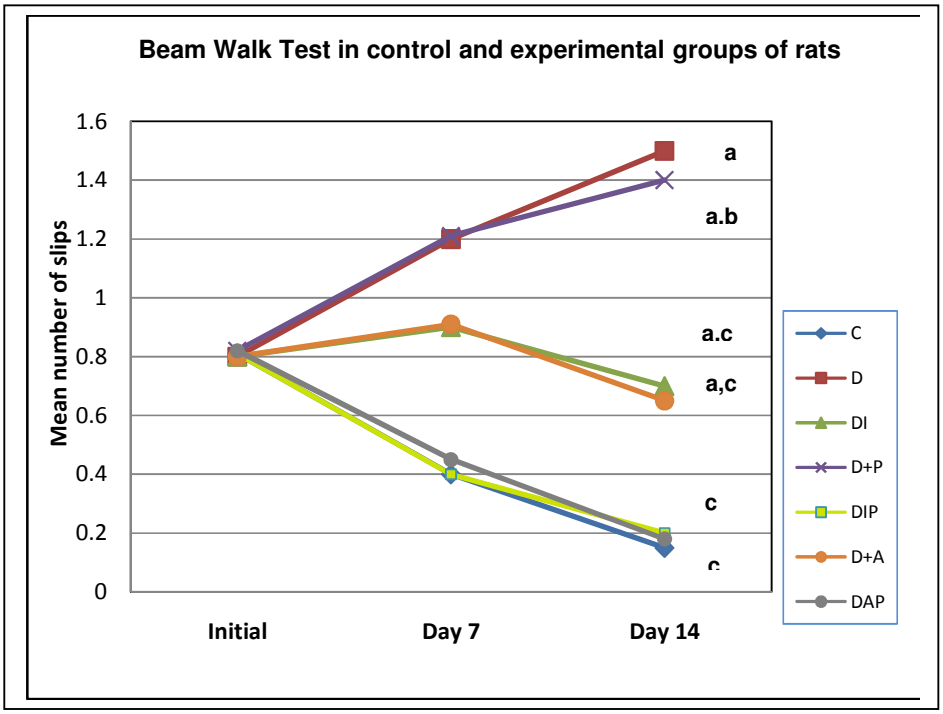
Figure-71

Time Spent in Open Arm Entry attempts (Counts/5 minutes) by control and experimental rats



Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats.[a] $p<0.001$ when compared to control group;
[c] $p<0.01$, [d] $p<0.001$ when compared to diabetic group.

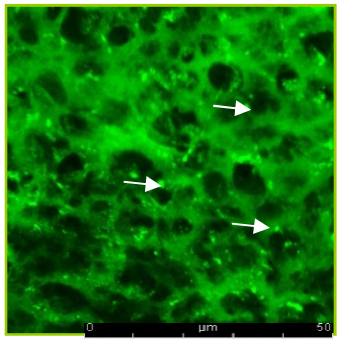
Figure-72



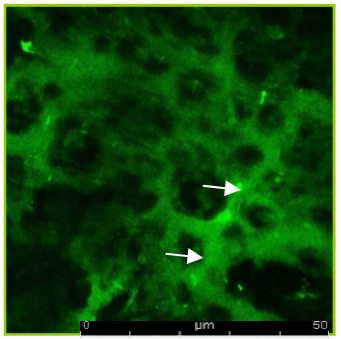
Values are mean ± S.E.M of 6-8 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control group; [b] $p<0.01$, [c] $p<0.001$ when compared to diabetic group.

Figure-73

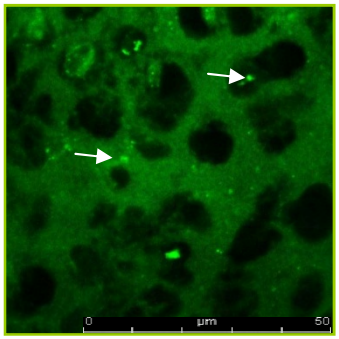
5-HT_{2A} Receptor antibody staining in Cerebral cortex of control and experimental groups of rats



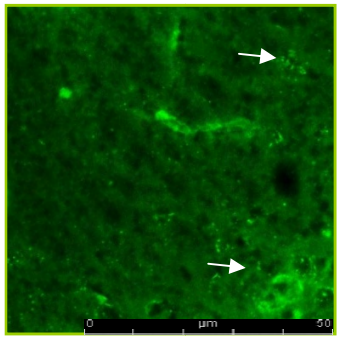
Control



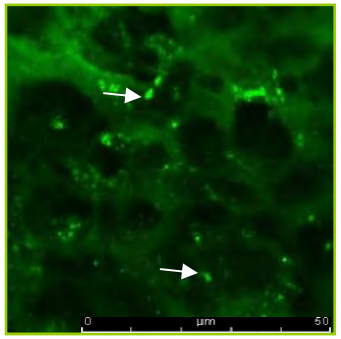
Diabetic



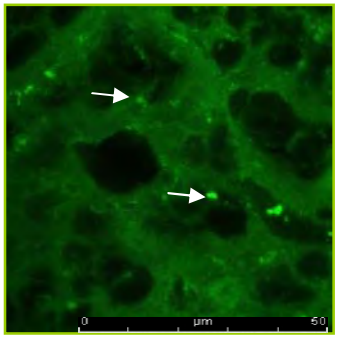
Diabetic+Insulin



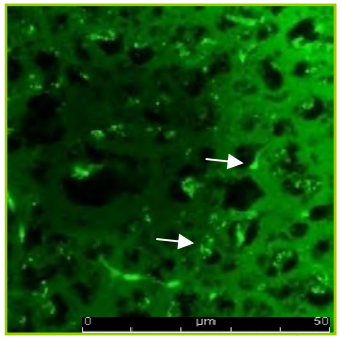
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose*+Pyridoxine

Table-80

5-HT_{2A} antibody staining in cerebral cortex control and experimental groups of rats

Experimental Group	Pixel intensity
Control	423625 ± 1878
Diabetic	223339 ± 1552 [a]
Diabetic+Insulin	386451 ± 2098 [b]
Diabetic +Pyridoxine	209787 ± 1426 [a]
Diabetic+Insulin+Pyridoxine	422101 ± 2123 [b]
Diabetic+*A. marmelose*	375684 ± 1534 [b]
Diabetic+ *A. marmelose* +Pyridoxine	421878 ± 1986 [b]

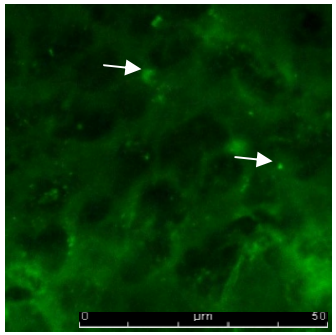
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

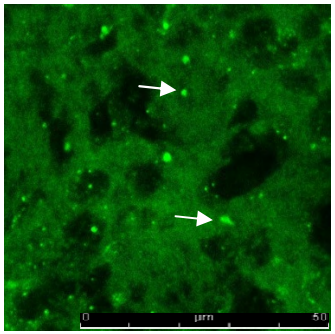
[b] $p<0.001$ when compared to diabetic group.

Figure-74

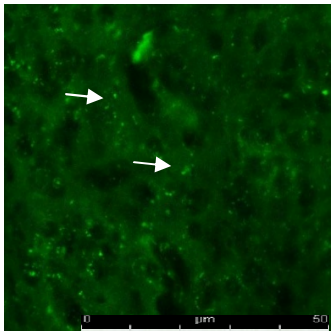
5-HT transporter antibody staining in Cerebral cortex of control and experimental groups of rats



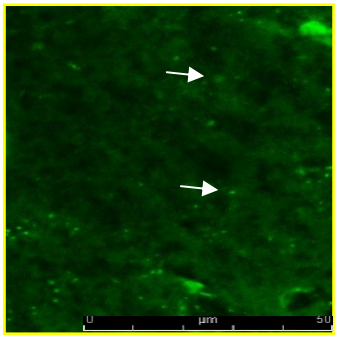
Control



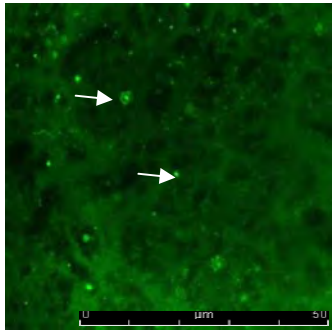
Diabetic



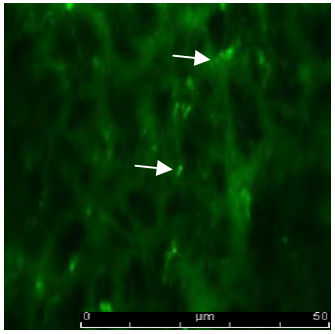
Diabetic+Insulin



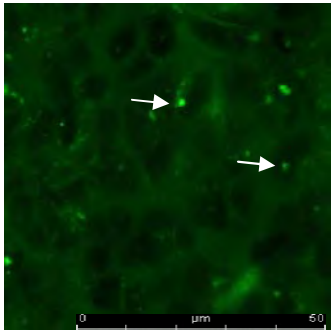
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose* +Pyridoxine

Table-81

5-HT transporter antibody staining in cerebral cortex control and experimental groups of rats

Experimental Group	Pixel intensity
Control	223625 ± 3878
Diabetic	423339 ± 4552 [a]
Diabetic+Insulin	286451 ± 4098 [b]
Diabetic+Pyridoxine	409787 ± 3426 [a]
Diabetic+Insulin+Pyridoxine	222101 ± 4123 [b]
Diabetic+*A. marmelose*	275684 ± 4534 [b]
Diabetic+ *A. marmelose*+Pyridoxine	229878 ± 3986 [b]

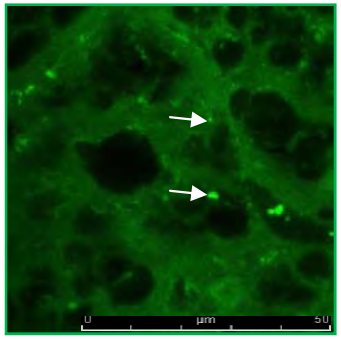
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

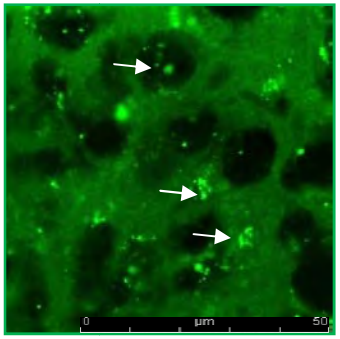
[b] $p<0.001$ when compared to diabetic group.

Figure-75

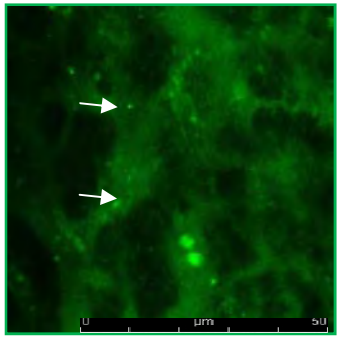
mGluR5 antibody staining in Cerebral cortex of control and experimental groups of rats



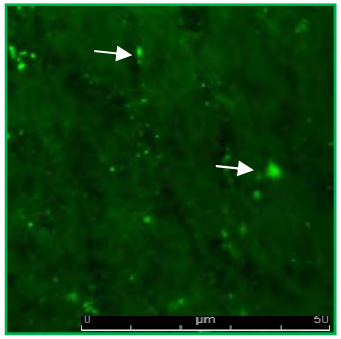
Control



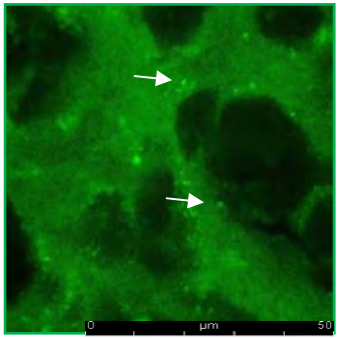
Diabetic



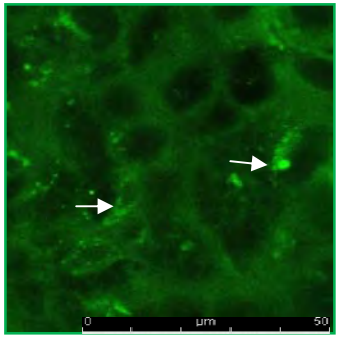
Diabetic+Insulin



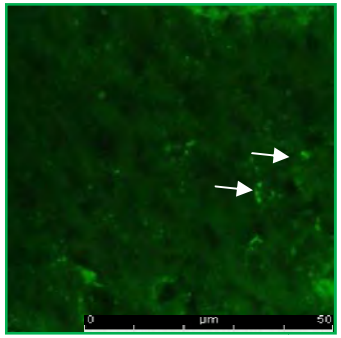
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose*+Pyridoxine

Table-82

mGluR5 antibody staining in cerebral cortex control and experimental groups of rats

Experimental Group	Pixel intensity
Control	223625 ± 3878
Diabetic	423339 ± 4552 [a]
Diabetic+Insulin	286451 ± 4098 [b]
Diabetic +Pyridoxine	409787 ± 3426 [a]
Diabetic+Insulin+Pyridoxine	222101 ± 4123 [b]
Diabetic+*A. marmelose*	275684 ± 4534 [b]
Diabetic+ *A. marmelose* +Pyridoxine	229878 ± 3986 [b]

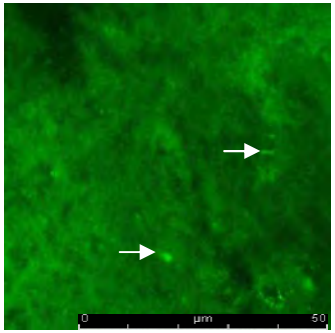
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

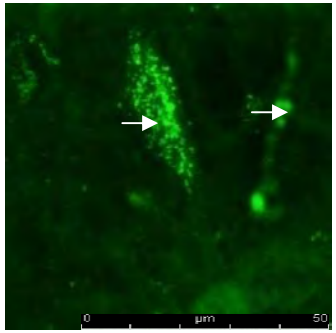
[b] $p<0.001$ when compared to diabetic group.

Figure-76

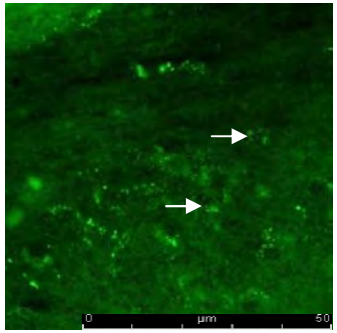
5-HT_{2A} Receptor antibody staining in brain stem Control and Experimental groups of rats



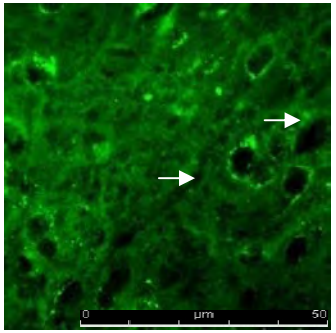
Control



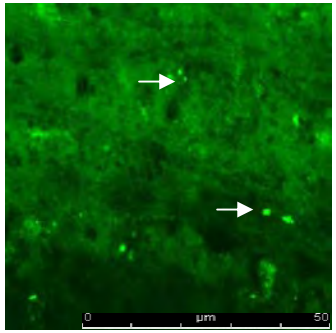
Diabetic



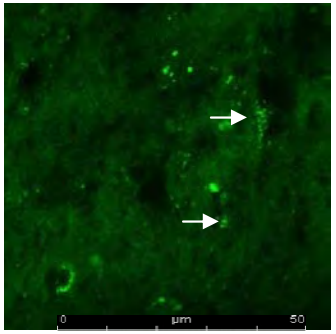
Diabetic+Insulin



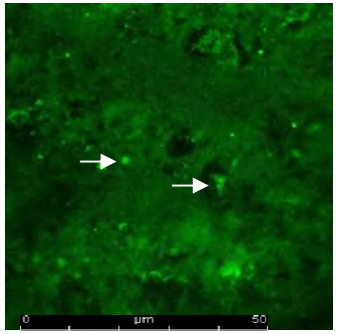
Diabetic + Pyridoxine



Diabetic + Insulin+ Pyridoxine



Diabetic+*A. marmelose*



Diabetic+ *A. marmelose* +Pyridoxine

Table-83

5-HT_{2A} Receptor antibody staining in brain stem of control and experimental groups of rats

Experimental Group	Pixel intensity
Control	226731 ± 2566
Diabetic	975469 ± 6422 [b]
Diabetic+Insulin	386451 ± 3524 [b,c]
Diabetic +Pyridoxine	787239 ± 4411 [b,c]
Diabetic+Insulin+Pyridoxine	278098 ± 5433 [c]
Diabetic+*A. marmelose*	389874 ± 3230 [a,c]
Diabetic+ *A. marmelose* +Pyridoxine	231387 ± 5766 [c]

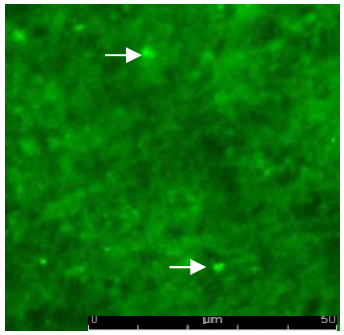
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.01$, [b] $p<0.001$ when compared to control group;

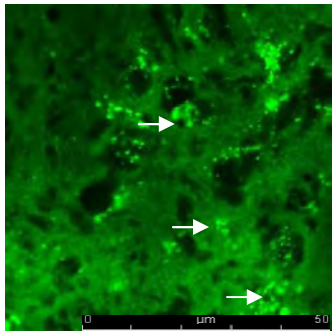
[c] $p<0.001$ when compared to diabetic group

Figure-77

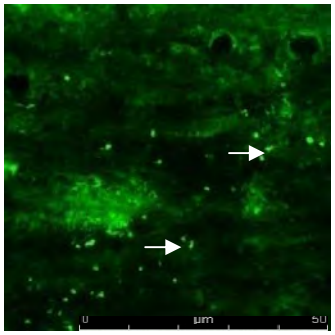
5-HTT Receptor antibody staining in brain stem of control and experimental groups of rats



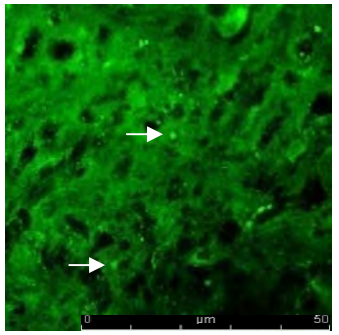
Control



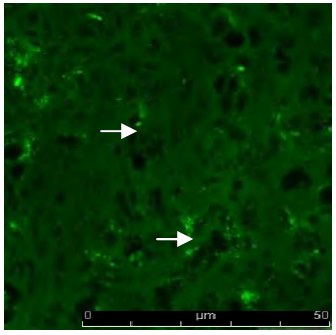
Diabetic



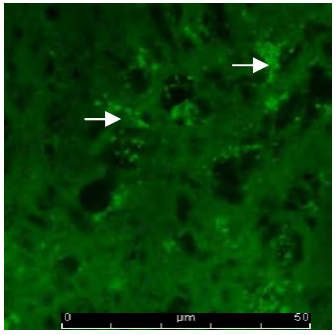
Diabetic+Insulin



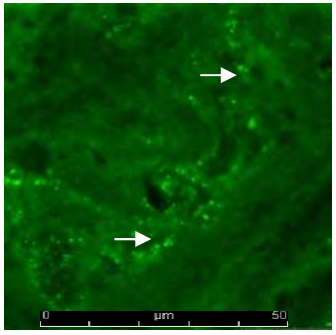
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose*+Pyridoxine

Table-84

5-HTT Receptor Gene expression in the brain stem of control and experimental rats

Experimental Group	Pixel intensity
Control	298825 ± 4218
Diabetic	872132 ± 4742 [a]
Diabetic+Insulin	686451 ± 3898 [a,b]
Diabetic +Pyridoxine	797887 ± 4410 [a]
Diabetic+Insulin+Pyridoxine	222101 ± 3543 [b]
Diabetic+*A. marmelose*	696848 ± 2834 [a,b]
Diabetic+ *A. marmelose* +Pyridoxine	224317 ± 3419 [b]

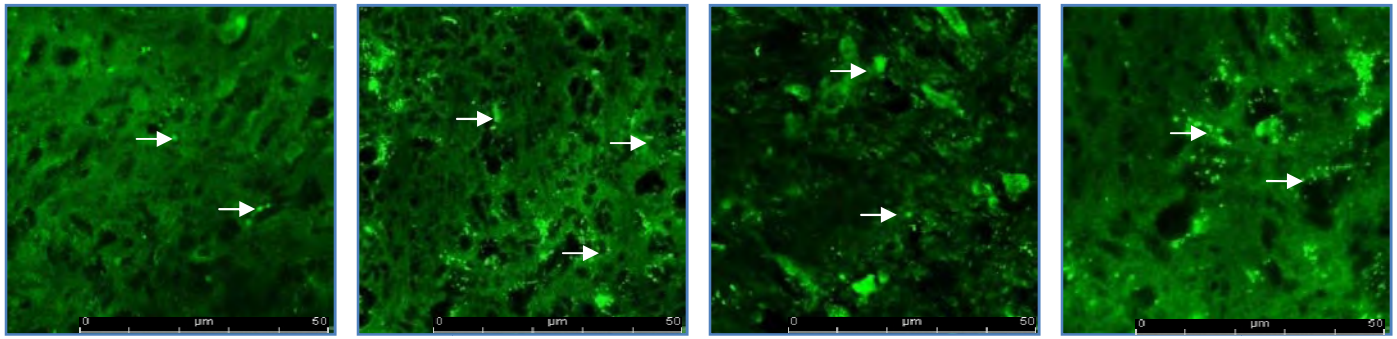
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

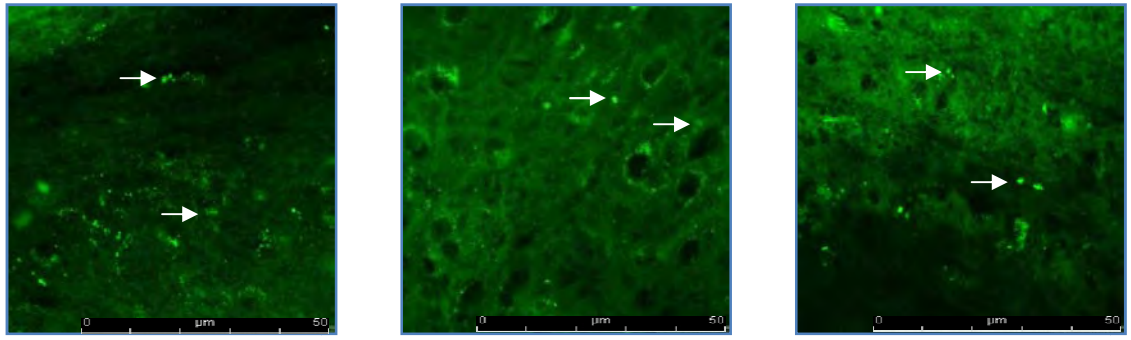
[b] $p<0.001$ when compared to diabetic group.

Figure-78

mGluR5 antibody staining in brain stem of control and experimental groups of rats



Control **Diabetic** **Diabetic+Insulin** **Diabetic+Pyridoxine**



Diabetic+Insulin+Pyridoxine **Diabetic+*A. marmelose*** **Diabetic+*A. marmelose*+Pyridoxine**

Table-85

mGluR5 Gene expression in the brain stem of control and experimental rats

Experimental Group	Pixel intensity
Control	223625 ± 3878
Diabetic	323339 ± 4552 [a]
Diabetic+Insulin	286451 ± 4098 [a,b]
Diabetic +Pyridoxine	309787 ± 3426 [a]
Diabetic+Insulin+Pyridoxine	222101 ± 4123 [b]
Diabetic+*A. marmelose*	275684 ± 4534 [a,b]
Diabetic+ *A. marmelose* +Pyridoxine	229878 ± 3986 [b]

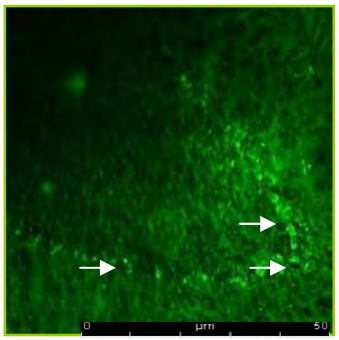
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

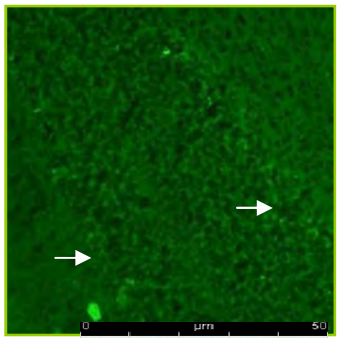
[b] $p<0.001$ when compared to diabetic group.

Figure-79

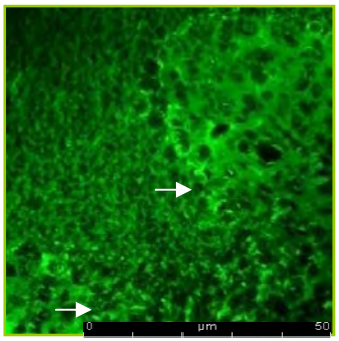
5-HT_{2A} Receptor antibody staining in Cerebellum control and experimental groups of rats



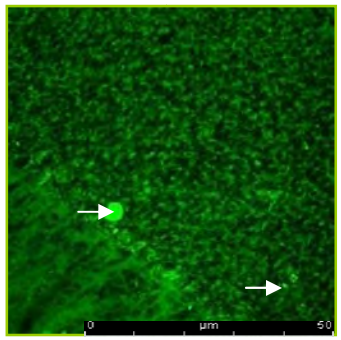
Control



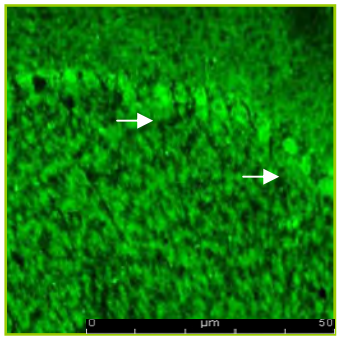
Diabetic



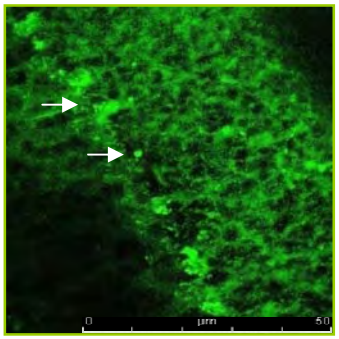
Diabetic+Insulin



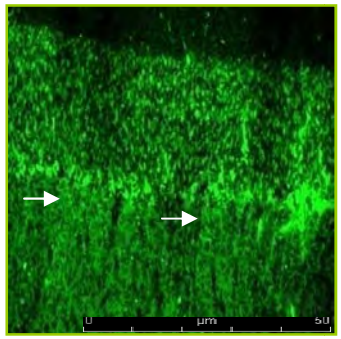
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose*+Pyridoxine

Table-86

5-HT_{2A} Receptor antibody staining in control and experimental groups of rats

Experimental Group	Pixel intensity
Control	63841280 ± 5505
Diabetic	22334083 ± 1770 [a]
Diabetic+Insulin	41191355 ± 5064 [a,c]
Diabetic +Pyridoxine	29510250 ± 5760 [a,b]
Diabetic+Insulin+Pyridoxine	68987716 ± 6305 [c]
Diabetic+*A. marmelose*	40029173 ± 6009 [a,c]
Diabetic+ *A. marmelose* +Pyridoxine	66849887 ± 6542 [c]

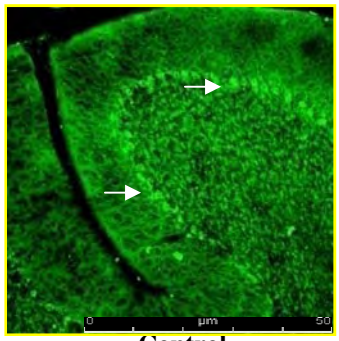
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

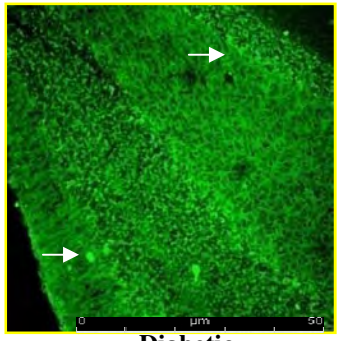
[b] $p<0.05$, [c] $p<0.001$ when compared to diabetic group.

Figure-80

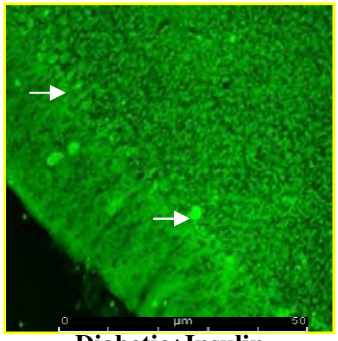
5-HTT Receptor antibody staining in Cerebellum control and experimental groups of rats



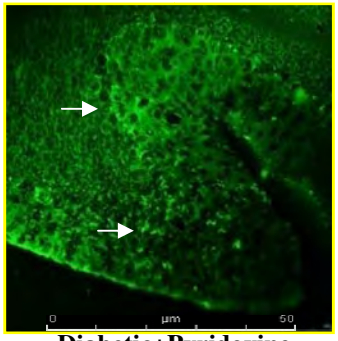
Control



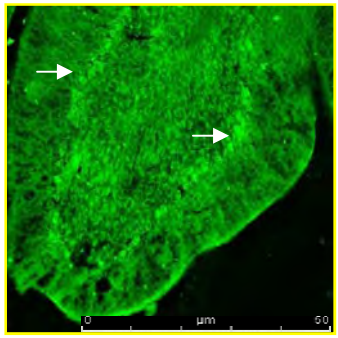
Diabetic



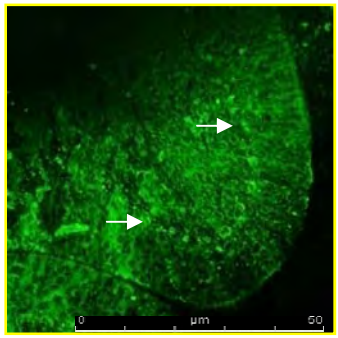
Diabetic+Insulin



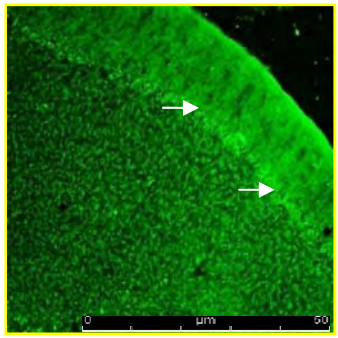
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+ *A. marmelose*+Pyridoxine

Table-87

5-HTT Receptor antibody staining in cerebellum of control and experimental groups of rats

Experimental Group	Pixel intensity
Control	42356535 ± 1960
Diabetic	28334083 ± 1978 [b]
Diabetic+Insulin	39646688 ± 1670 [b,c]
Diabetic +Pyridoxine	28975878 ± 3426 [b]
Diabetic+Insulin+Pyridoxine	41210173 ± 2723 [a,c]
Diabetic+*A. marmelose*	38668444 ± 1534 [b,c]
Diabetic+ *A. marmelose* +Pyridoxine	40987895 ± 2086 [a,c]

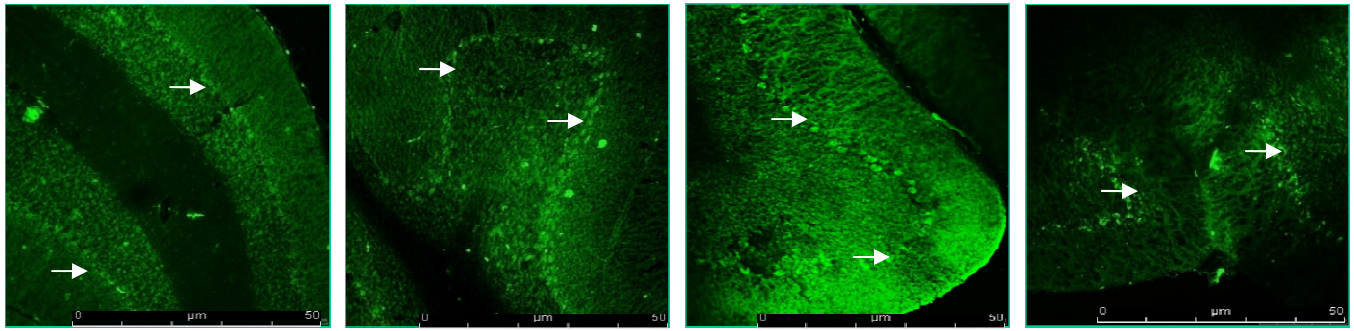
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.05$, [b] $p<0.001$ when compared to control group;

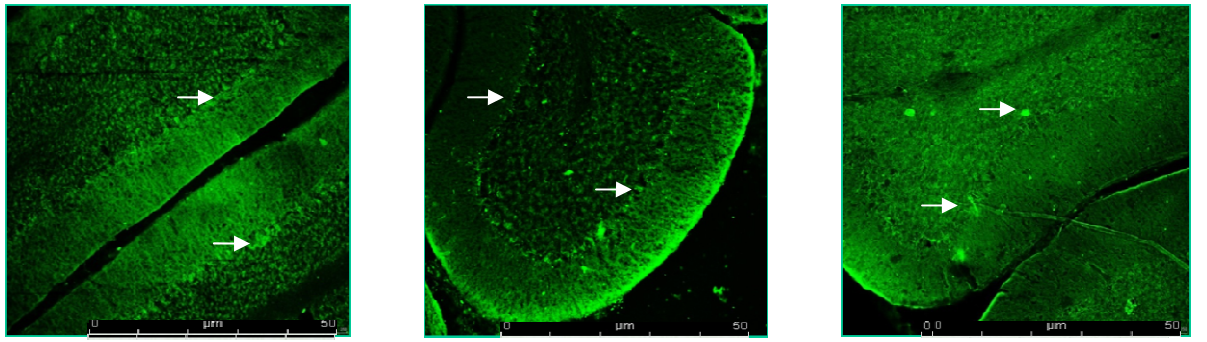
[c] $p<0.001$ when compared to diabetic group.

Figure-81

mGluR5 antibody staining in Cerebellum of control and experimental groups of rat



Control **Diabetic** **Diabetic+Insulin** **Diabetic+Pyridoxine**



Diabetic + Insulin+ Pyridoxine **Diabetic+*A. marmelose*** **Diabetic+ *A. marmelose* +Pyridoxine**

Table-88

mGluR5 antibody staining in cerebellum of control and experimental groups of rats

Experimental Group	Pixel intensity
Control	228625 ± 3878
Diabetic	623339 ± 4552 [b]
Diabetic+Insulin	306451 ± 4098 [b,c]
Diabetic +Pyridoxine	609787 ± 3426 [b]
Diabetic+Insulin+Pyridoxine	222101 ± 4123 [a,c]
Diabetic+*A. marmelose*	315684 ± 4534 [b,c]
Diabetic+ *A. marmelose* +Pyridoxine	224878 ± 3986 [a,c]

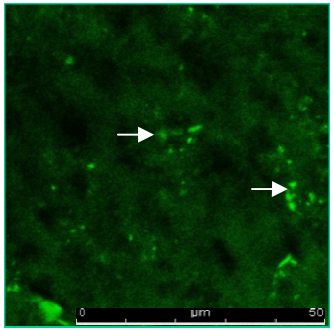
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.05$, [b] $p<0.001$ when compared to control group;

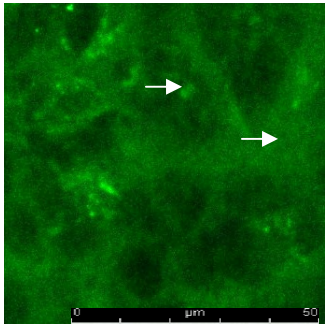
[c] $p<0.001$ when compared to diabetic group.

Figure-82

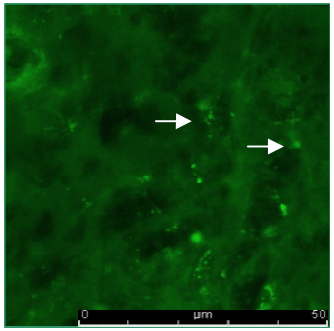
5-HT_{2A} Receptor antibody staining in the hippocampus of control and experiment



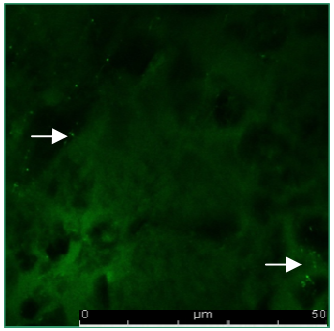
Control



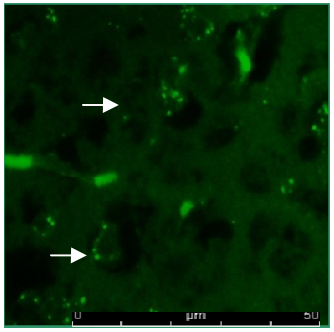
Diabetic



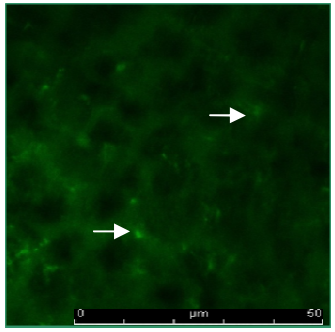
Diabetic+Insulin



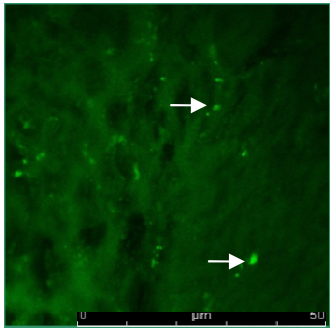
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+ *A. marmelose*+Pyridoxine

Table-89

5-HT_{2A} Receptor antibody staining in hippocampus of control and experimental groups of rats

Experimental Group	Pixel intensity
Control	384132 ± 1454
Diabetic	133475 ± 1431 [a]
Diabetic+Insulin	229123 ± 1453 [a,c]
Diabetic +Pyridoxine	151012 ± 2662 [a,b]
Diabetic+Insulin+Pyridoxine	398791 ± 2105 [c]
Diabetic+*A. marmelose*	222921 ± 1097 [a,c]
Diabetic+ *A. marmelose* +Pyridoxine	314997 ± 1084 [c]

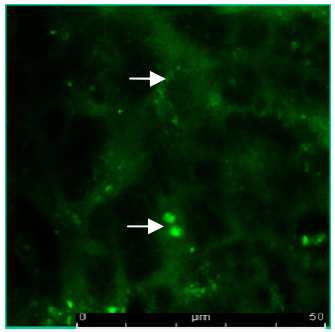
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

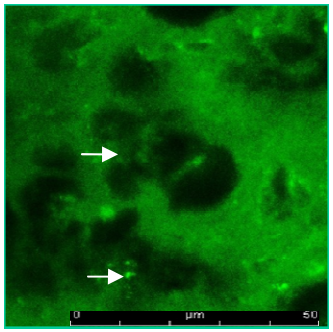
[b] $p<0.05$, [c] $p<0.001$ when compared to diabetic group.

Figure-83

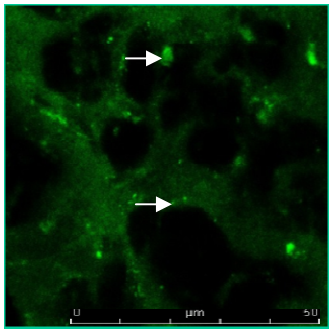
5-HT transporter antibody staining in the hippocampus of control and experimental rats



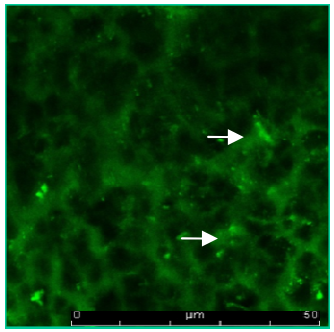
Control



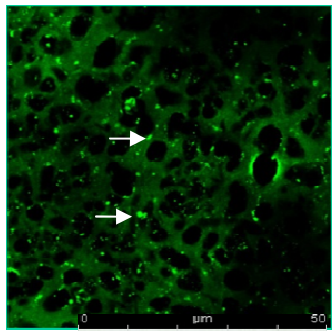
Diabetic



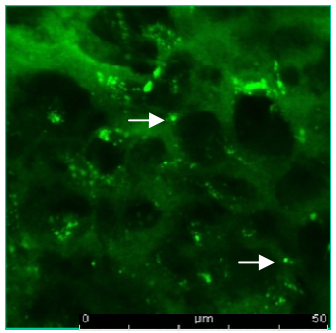
Diabetic+Insulin



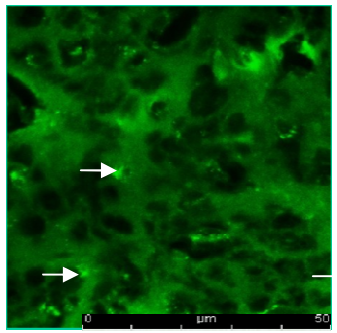
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose*+Pyridoxine

Table-90

5-HT transporter antibody in the hippocampus of control and experimental rats

Experimental Group	Pixel intensity
Control	4235653 ± 1960
Diabetic	2833408 ± 1978[a]
Diabetic+Insulin	3964668 ± 1670[a,b]
Diabetic +Pyridoxine	2897587 ± 3426[a]
Diabetic+Insulin+Pyridoxine	4121017 ± 2723[b]
Diabetic+*A. marmelose*	3866844 ± 1534[a,b]
Diabetic+ *A. marmelose* +Pyridoxine	4098789 ± 2086[b]

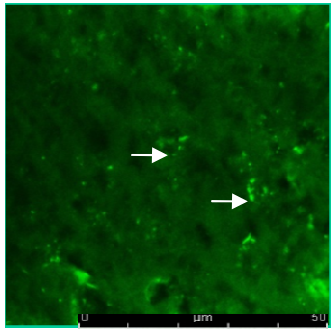
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

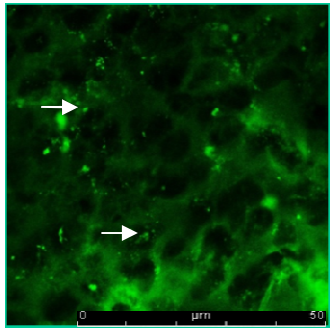
[b] $p<0.001$ when compared to diabetic group.

Figure-84

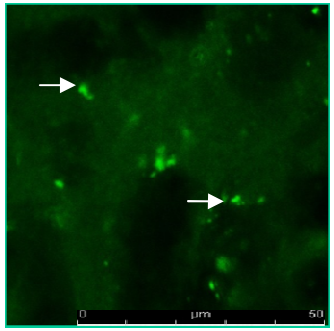
mGluR5 antibody staining in hippocampus of control and experimental groups of rats



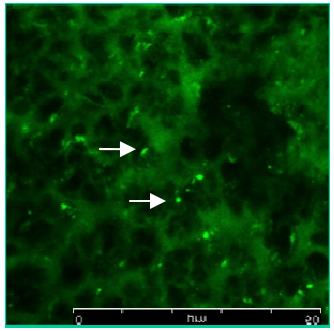
Control



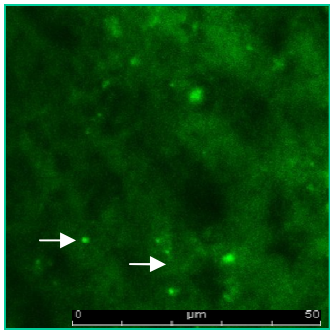
Diabetic



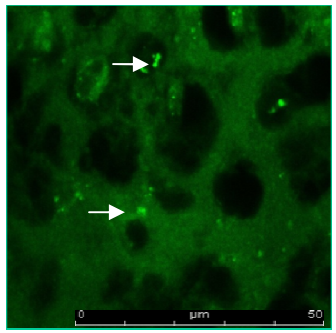
Diabetic+Insulin



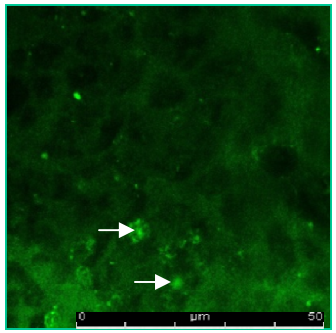
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose*+Pyridoxine

Table-91

mGluR5 antibody staining in hippocampus of control and experimental groups of rats

Experimental Group	Pixel intensity
Control	123625 ± 3218
Diabetic	245339 ± 3512 [a]
Diabetic+Insulin	186451 ± 4898 [a,b]
Diabetic +Pyridoxine	209787 ± 4426 [a]
Diabetic+Insulin+Pyridoxine	129101 ± 5123 [b]
Diabetic+*A. marmelose*	185684 ± 5534 [a,b]
Diabetic+ *A. marmelose* +Pyridoxine	129318 ± 5986 [b]

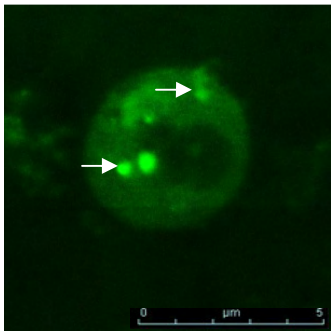
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

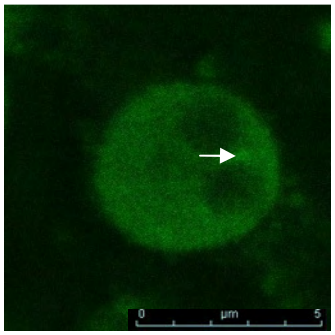
[b] $p<0.001$ when compared to diabetic group

Figure-85

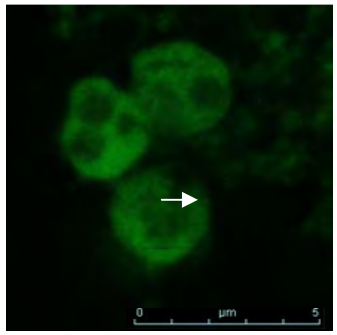
5-HT_{2A} Receptor antibody staining in the pancreas of control and experimental rats



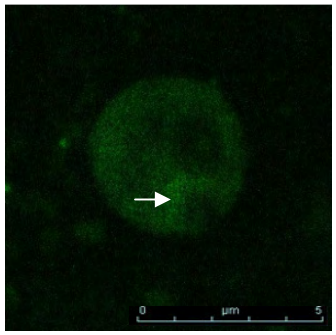
Control



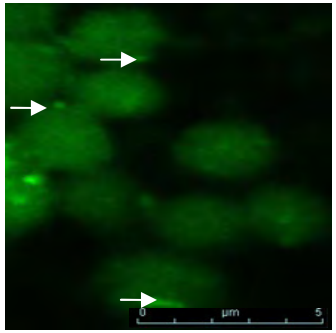
Diabetic



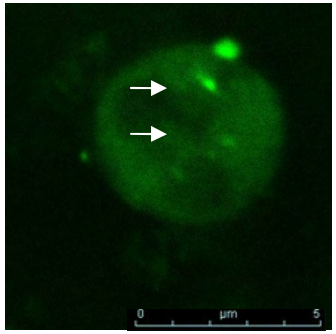
Diabetic+Insulin



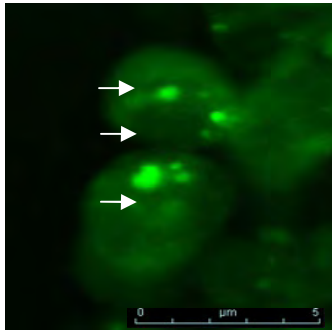
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose*+Pyridoxine

Table-92

5-HT_{2A} Receptor antibody staining in the pancreas of control and experimental rats

Experimental Group	Pixel intensity
Control	1,26,830 ± 1505
Diabetic	80,232 ± 1770 [a]
Diabetic+Insulin	1,17,591 ± 2064 [a,c]
Diabetic +Pyridoxine	99,341 ± 1760 [a,b]
Diabetic+Insulin+Pyridoxine	1,33,716 ± 2305 [d]
Diabetic+*A. marmelose*	1,03,931 ± 1309 [a,b]
Diabetic+ *A. marmelose* +Pyridoxine	1,39,432 ± 2542 [d]

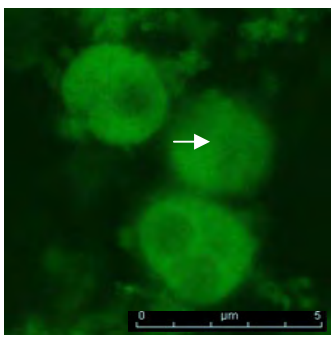
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

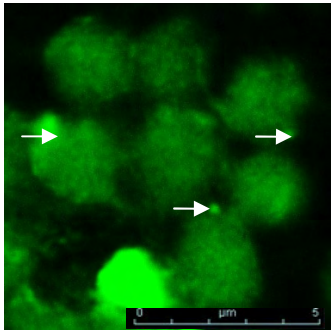
[b] $p<0.05$, [c] $p<0.01$, [d] $p<0.001$ when compared to diabetic group.

Figure-86

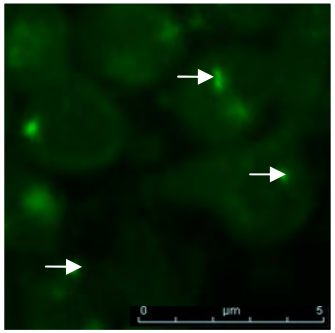
mGluR5 antibody staining in the pancreas of control and experimental rats



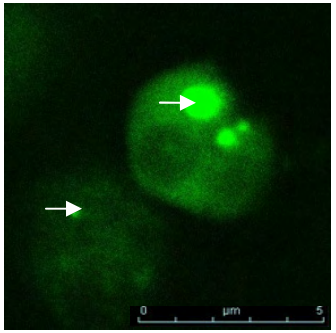
Control



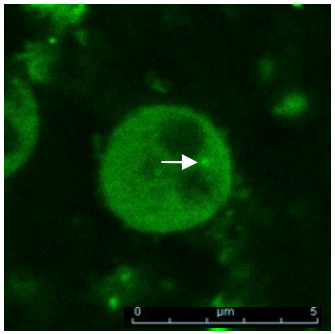
Diabetic



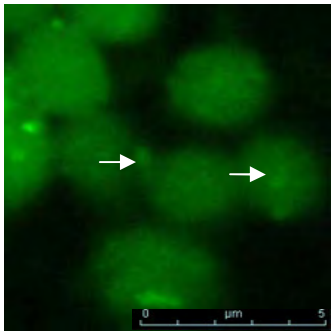
Diabetic+Insulin



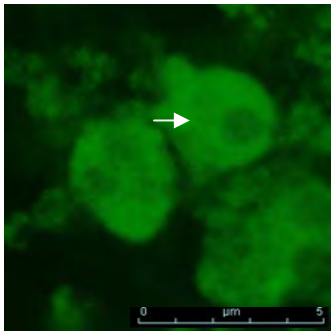
Diabetic + Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose*+Pyridoxine

Table-93

mGluR5 antibody staining in the pancreas of control and experimental rats

Experimental Group	Pixel intensity
Control	181435 ± 1571
Diabetic	319867 ± 1535 [a]
Diabetic+Insulin	217211 ± 1649 [a,c]
Diabetic +Pyridoxine	322341 ± 1769 [a]
Diabetic+Insulin+Pyridoxine	183423 ± 1655 [d]
Diabetic+*A. marmelose*	203931 ± 1913 [a,b]
Diabetic+ *A. marmelose* +Pyridoxine	189112 ± 1428 [d]

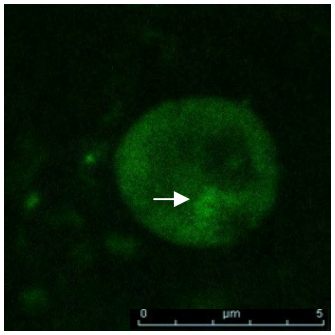
Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

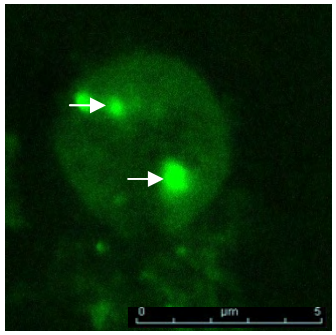
[b] $p<0.05$, [c] $p<0.01$, [d] $p<0.001$ when compared to diabetic group.

Figure-87

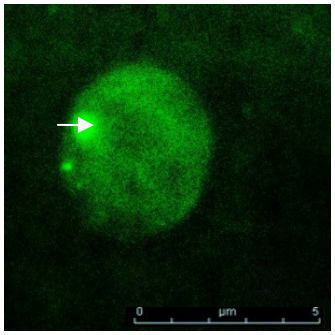
Intracellular Calcium imaging in pancreatic islets of Control and Experimental rats



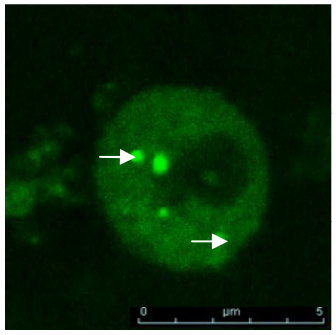
Control



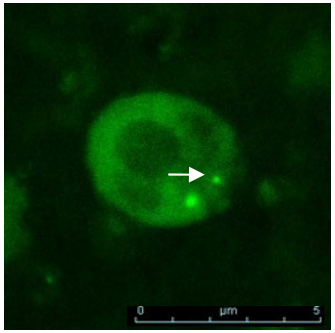
Diabetic



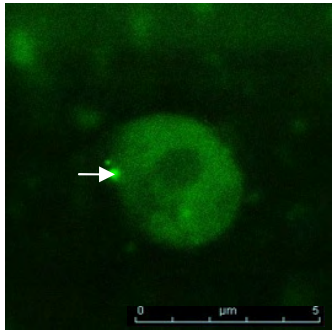
Diabetic+Insulin



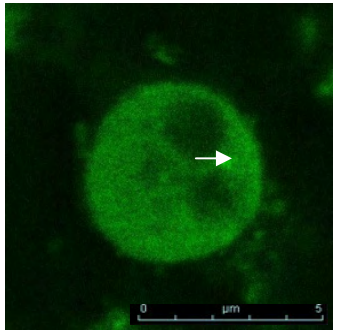
Diabetic+Pyridoxine



Diabetic+Insulin+Pyridoxine



Diabetic+*A. marmelose*



Diabetic+*A. marmelose*+Pyridoxine

Table-94

Intracellular Calcium imaging in pancreatic islets of Control and Experimental rats

Experimental Group	Pixel intensity
Control	181435 ± 1571
Diabetic	319867 ± 1535 [a]
Diabetic+Insulin	217211 ± 1649 [a,b]
Diabetic +Pyridoxine	322341 ± 1769 [a]
Diabetic+Insulin+Pyridoxine	183423 ± 1655 [c]
Diabetic+*A. marmelose*	203931 ± 1913 [a,b]
Diabetic+ *A. marmelose* +Pyridoxine	189112± 1428 [c]

Values are mean ± S.E.M of 4-6 rats in each group. Each group consists of 6-8 rats.

[a] $p<0.001$ when compared to control group;

[b] $p<0.05$, [c] $p<0.001$ when compared to diabetic group.

Figure Legends

Figure 73

Confocal image of 5-HT_{2A} receptors in the cerebral cortex of control and experimental rats using immunofluorescent 5-HT_{2A} receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of 5-HT_{2A} receptors in the cerebral cortex of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment.⟶ in white shows 5-HT_{2A} receptors expression.

Figure 74

Confocal image of 5-HTT receptors in the cerebral cortex of control and experimental rats using immunofluorescent 5-HTT receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of 5-HTT receptors in the cerebral cortex of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment.⟶ in white shows 5-HTT receptors expression.

Figure 75

Confocal image of mGluR5 receptors in the cerebral cortex of control and experimental rats using immunofluorescent mGluR5 receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of mGluR5 receptors in the cerebral cortex of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows mGluR5 receptors expression.

Figure 76

Confocal image of 5-HT_{2A} receptors in the brain stem of control and experimental rats using immunofluorescent 5-HT_{2A} receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of 5-HT_{2A} receptors in the brain stem of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows 5-HT_{2A} receptors expression.

Figure 77

Confocal image of 5-HTT receptors in the brain stem of control and experimental rats using immunofluorescent 5-HTT receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of 5-HTT receptors in the brain stem of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows 5-HTT receptors expression.

Figure 78

Confocal image of mGluR5 receptors in the brain stem of control and experimental rats using immunofluorescent mGluR5 receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of mGluR5 receptors in the brain stem of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows mGluR5 receptors expression.

Figure 79

Confocal image of 5-HT_{2A} receptors in the cerebellum of control and experimental rats using immunofluorescent 5-HT_{2A} receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of 5-HT_{2A} receptors in the cerebellum of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows 5-HT_{2A} receptors expression.

Figure 80

Confocal image of 5-HTT receptors in the cerebellum of control and experimental rats using immunofluorescent 5-HTT receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of 5-HTT receptors in the cerebellum of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows 5-HTT receptors expression.

Figure 81

Confocal image of mGluR5 receptors in the cerebellum of control and experimental rats using immunofluorescent mGluR5 receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of mGluR5 receptors in the cerebellum of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows mGluR5 receptors expression.

Figure 82

Confocal image of 5-HT_{2A} receptors in the hippocampus of control and experimental rats using immunofluorescent 5-HT_{2A} receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of 5-HT_{2A} receptors in the hippocampus of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows 5-HT_{2A} receptors expression.

Figure 83

Confocal image of 5-HTT receptors in the hippocampus of control and experimental rats using immunofluorescent 5-HTT receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of 5-HTT receptors in the hippocampus of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows 5-HTT receptors expression.

Figure 84

Confocal image of mGluR5 receptors in the hippocampus of control and experimental rats using immunofluorescent mGluR5 receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of mGluR5 receptors in the hippocampus of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows mGluR5 receptors expression.

Figure 85

Confocal image of 5-HT_{2A} receptors in the pancreas of control and experimental rats using immunofluorescent 5-HT_{2A} receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of 5-HT_{2A} receptors in the pancreas of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows 5-HT_{2A} receptors expression.

Figure 86

Confocal image of mGluR5 receptors in the pancreas of control and experimental rats using immunofluorescent mGluR5 receptor specific primary antibody and FITC as secondary antibody. There was a significant decrease of mGluR5 receptors in the pancreas of diabetic rats which was reversed to control with *Aegle marmelose* and insulin treated alone and in combination with pyridoxine treatment. ⟶ in white shows mGluR5 receptors expression.

Figure 87

Confocal image of calcium release from the pancreas using Fluo-4 significantly increased the calcium release from pancreatic islets of diabetic rats which was reversed to near control after treatment with insulin and *Aegle marmelose* alone and in combination therapy with pyridoxine ⟶ in white shows calcium release.

LIST OF PUBLICATIONS:

1. Amee Krishnakumar, **Pretty Mary Abraham**, Jes Paul and C. S. Paulose. **(2009).** Downregulation of cerebellar 5-HT_{2C} receptors in pilocarpine-induced epilepsy in rats: Therapeutic role of *Bacopa monnieri* extract. Journal of Neurological Sciences. 284, 124–128.

2. C. S. Paulose, P. S. John, Sreekanth R, Mathew Philip, Padmarag Mohan C, Jobin Mathew, Peeyush Kumar T, Jes Paul, **Pretty Mary Abraham**, Sherin Antony, Binoy Joseph, Anu Joseph, Amee Krishnakumar, Anju T R, Reas Khan S, Santhosh Thomas K and Nandhu M S. **(2009).** Spinal Cord Regeneration and Functional Recovery: Neurotransmitter's Combination and Bone Marrow Cells Supplementation. *Current Science*.Q869.

ABSTRACTS PRESENTED

1. **Pretty Mary Abraham,** Jayanarayanan S, Smijin Soman and C. S. Paulose. Oxidative stress affects Glutamate receptor functional regulation in cerebral cortex of Streptozotocin induced Diabetic Rats: Neuroprotective role of pyridoxine and *Aegle marmelose***.** International Conference on Neuroscience Updates, Annual meeting of Society for Neurochemistry organised by Centre for Neuroscience, Department of Biotechnology, CUSAT, Cochin. December 7-14, 2009.

2. Anju T. R, **Pretty Mary Abraham** and C. S. Paulose. Enhanced $5HT_{2A}$ Receptors in the Cerebral Cortex of Hypoxia Induced Neonatal Rats: Effect of Glucose and Oxygen Supplementation. 36^{th} ACBICON 2009 National Conference of Association of Clinical Biochemists of India conducted by Amrita Institute of Medical Sciences, Cochin.

3. Anitha Malat, **Pretty Mary Abraham** and C.S.Paulose. Serotonergic receptor down regulation in brain stem of Streptozotocin induced Diabetic Rats: Antagonism by pyridoxine and insulin. 78th Annual Meeting of the Society of Biological Chemists (India), held at NCCS, Pune. October, 2009.

4. **Pretty Mary Abraham**, Amee Krishnakumar and C.S.Paulose. Effect of pyridoxine and *Aegle marmelose* leaf extract and serotonergic receptor functional regulation in Streptozotocin induced Diabetic Rats**.** 77th Annual Meeting of the Society of Biological Chemists (India), held at IIT, Madras. December, 2008.

5. **Pretty Mary Abraham**, Korah P Kuruvilla, & C.S.Paulose**.** Altered $5HT_{2A}$ Receptor Gene Expression In The Brain Stem Of Diabetic Rats: Supplementation of Pyridoxine And Aegle Marmelose Leaf Extract. International Conference on Advances in Neurosciences. XXVI Annual meeting in Indian Academy of Neurosciences, held at CUSAT, Cochin. (December, 2008).

6. Amee Krishnakumar, **Pretty Mary Abraham** and C. S. Paulose. "Enhanced 5-HT_{2C} receptor gene expression in the cerebral cortex of pilocarpine induced epileptic rats: Neuroprotective role of Bacopa monnieri." International symposium on Regenerative Neuroscience, National Institute of Mental Health & Neuro Sciences (NIMHANS) Bangalore. (January, 2008).

7. **Pretty Mary Abraham,** Savitha Balakrishnan & C.S.Paulose. Kinetic Parameters of Glutamate dehydrogenase in the Kidney of Streptozotocin induced and Insulin Treated Diabetic Rats as a Function of Age. 76th Annual Meeting of the Society of Biological Chemists (India), held at Sri Venketeswara University , Tirupati, 25-26th November, 2007.