Ph. D. Thesis

PEEYUSH KUMAR T

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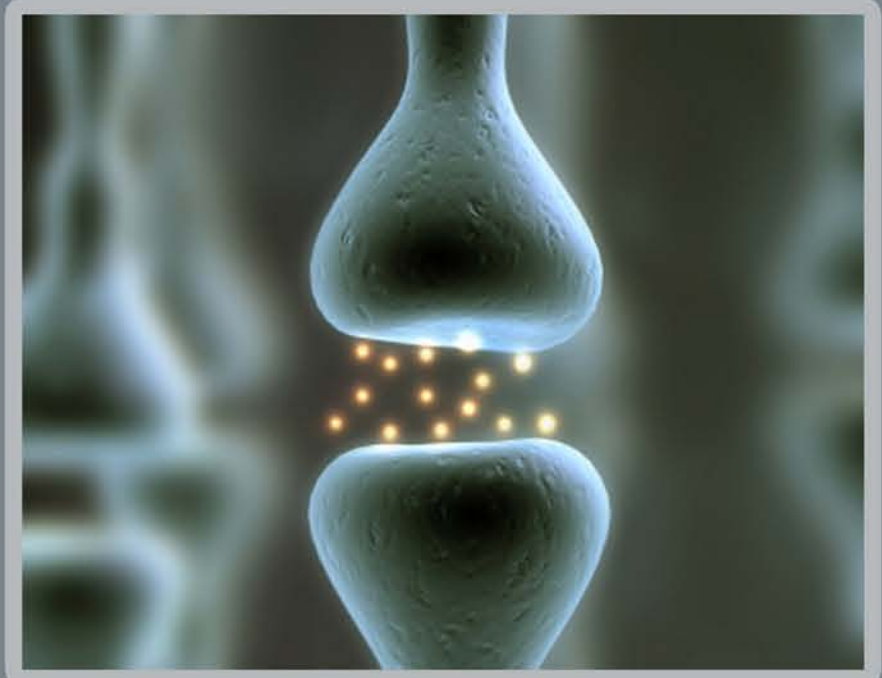
Brain Cholinergic and Dopaminergic Functions in Streptozotocin Induced Diabetic Rats: Effects of Curcumin and Vitamin D_3 Supplementation



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

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BRAIN CHOLINERGIC AND DOPAMINERGIC FUNCTIONS IN STREPTOZOTOCIN INDUCED DIABETIC RATS: EFFECTS OF CURCUMIN AND VITAMIN D_3 SUPPLEMENTATION

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BY

PEEYUSH KUMAR T

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

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DECLARATION

I hereby declare that the thesis entitled **"Brain Cholinergic and Dopaminergic Functions in Streptozotocin Induced Diabetic Rats: Effects of Curcumin and Vitamin D_3 Supplementation"** is the authentic record of 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 and that no part thereof has previously formed the basis for the award of any degree or diploma, associateship or other similar titles or recognition.

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Peeyush Kumar T

Reg. No. 3403

Department of Biotechnology

Cochin University of Science and Technology

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"Real life isn't always going to be perfect or go our way, but the recurring acknowledgement of what is working in our lives can help us not only to survive but surmount our difficulties."

The recognition of ***The Almighty God*** *as the ruling and leading power in the universe and the grateful acknowledgment of His favours and blessings are necessary which I need forever.*

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Dedicated to my Beloved

Parents, Brother, Friends and Dear Ones...

ABBREVIATIONS

1,25(OH)2D3	1α,25-dihydroxyvitamin D_3
5-HIAA	5-hydroxy indole - 3 acetic acid
5-HT	5-Hydroxy tryptamine
ACh	Acetylcholine
AChE	Acetylcholine esterase
AD	Alzheimers disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
B_{max}	Maximal binding
BSA	Bovine serum albumin
cAMP	Cylic adenosine monophosphate
ChAT	Choline acetyltransferase
CNS	Central Nervous System
Ct	Crossing threshold
DA	Dopamine
DAMP	Deoxy acetyl methyl piperidine
DEPC	Di ethyl pyro carbonate
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
EPI	Epinephrine

EPSCs	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
FITC	Florescent isothiocyanate
GABA	Gamma amino butyric acid
GLUT2	Glucose transporter type 2
GLUT3	Glucose transporter type 3
GLUT4	Glucose transporter type 4
GPCR	G protein-coupled receptor
HBSS	Hang's balanced salt solution
IGF	Insulin-like growth factor
INS	Insulin
K_d	Dissociation constant
L-DOPA	L-3,4 Dihydroxy phenyl alanine
LTD	Long term depression
mRNA	Messenger ribonucleic acid
NE	Norepinephrine
NMDA	N-methyl-D-aspartate
P	Level of significance
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline Triton X- 100
PCR	Polymerase Chain Reaction

PFC	Prefrontal cortex
PLC	Phospholipase C
QNB	Quinuclidinylbenzilate
RNA	Ribonucleic acid
SEM	Standard error of mean
Ser	Serine
SOD	Superoxide dismutase
STZ	Streptozotocin
T3	Triiodothyronine
T4	Thyroxine
Thr	Threonine
Tyr	Tyrosine
VDR	Vitamin D receptor
VICC	Voltage - insensitive calcium channels

CONTENTS

Introduction

Diabetes mellitus is a common metabolic disorder characterised by hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Feldman, 1997). Diabetes mellitus is known to be associated with neurological complications in both the peripheral nervous system (PNS) and the central nervous system (CNS) (Greene, 1999). 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 makes an important contribution to the overall regulation of pancreatic beta cell function. Controlling blood sugar is essential for avoiding long-term complications of diabetes like learning and memory deficit. Greater understanding of CNS involvement could lead to new strategies to prevent or reverse the damage caused by diabetes mellitus. Acetylcholine, a major neurotransmitter from the autonomic nervous system, regulates the cholinergic stimulation of insulin secretion, through interactions with muscarinic receptors (Satin & Kinard, 1998; Ahren, 2000; Gilon & Henquin, 2001). Dopamine in the CNS is involved in the control of both motor and emotional behaviour (Vallone *et al.,* 2000) and peripherally modulates insulin secretion in the pancreatic islets (Nogueira *et al.,* 1994)

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. These effects are mediated by acetylcholine, which is released from nerve terminals and acts upon muscarinic cholinergic receptors in the β-cell plasma membrane (Sharp *et al*., 1974; Berthoud *et al*., 1980; Mathias *et al*., 1985; Ahren, 2000). Cholinergic agonist carbachol increases insulin secretion from isolated rat islets (Zawalich, 1989b). Carbachol stimulated insulin secretion is inhibited by atropine, a general muscarinic antagonist, confirming the role of

muscarinic receptors in cholinergic induced insulin secretion. Reverse transcription analysis of rat pancreatic islets indicated that muscarinic M1 and M3 are predominant receptors in the islets (Lismaa *et al*., 2000). Muscarinic M1 and M3 receptors function differentially regulate glucose induced insulin secretion (Renuka *et al*., 2006). Increased activity of muscarinic M1 and M3 receptor subtypes stimulate insulin secretion and islet cell proliferation during the regeneration of pancreas (Renuka *et al*., 2005) The muscarinic receptor stimulation by acetylcholine leads to activation of phospholipase C (PLC), which, in turn, hydrolyses phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce Inositol triphosphate (IP3) and diacylglycerol (DAG) (Best & Malaisse, 1983; Zawalich *et al*., 1989). In pancreatic β-cells, IP3 mobilises 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).

Dopamine plays an important role both centrally and peripherally. It also plays a major role in the regulation of appetite and growth hormone. Dopamine is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised. Dopamine interacts with specific membrane receptors to produce its effects. These effects are terminated by reuptake of dopamine into the presynaptic neuron by a dopamine transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-O-methyltransferase (COMT). The recent identification of five dopamine receptor subtypes provides a basis for understanding dopamine's central and peripheral actions. Dopamine receptors are classified into two major groups: dopamine D_1 like and dopamine D_2 like. Dopamine D_1 like receptors consists of dopamine D_1 and dopamine D_5 receptors. Dopamine D_2 like receptors consists of dopamine D_2, dopamine D_3 and dopamine D_4 receptors. Stimulation of the dopamine D_1 receptor give rise to increased production of cAMP. Dopamine D_2 receptors inhibit cAMP production, but

activate the inositol phosphate second messenger system (Seeman, 1980). An imbalance between dopaminergic neurotransmission and dopamine receptors is known to be associated with the symptomatology of numerous neuropsychiatric disorders like schizophrenia, psychosis, mania and depression as well as neuropathological disorders like Parkinson's disease and Huntington's disease (Carlsson 1988, 1993; Bermanzohn & Siris 1992, Brown & Gershon 1993, Jakel & Maragos 2000, Kostrzewa & Segura-Aguilar 2003). Hyperglycaemia during diabetes is reported to damage dopaminergic functions. The progression of diabetes is associated with an impaired ability of the neurons in the CNS to release neurotransmitters resulting in behavioural changes (Broderick & Jacoby, 1989). The dopaminergic cells in particular are highly sensitive to excitotoxicity and oxidative stress when the energy metabolism is impaired (Callahan *et al*., 1998).

cAMP responsive element binding protein (CREB) is a protein that is a transcription factor. It binds to certain DNA sequences called cAMP response elements and thereby increases or decreases the transcription of the downstream genes (Lauren, 2005). In neuronal tissue, CREB regulation by nerve growth factor and insulin-like growth factor-1 is essential for neuronal plasticity, full axonal development, memory consolidation and neuroprotection (Spaulding, 1993; Shimomura, *et al.,* 1998). The PLC activity decline in the brain is expected to affect DAG which is the principal molecular species of phosphoinositides in the nervous tissue (Whiting *et al*., 1979). Alterations in glucose utilisation are known to occur in the important regions of brain connected with learning and memory (Auer & Siesjo, 1993). The brain glucose uptake is ultimately dependent on facilitative glucose transporters. GLUT3 is the main neuronal glucose transporter (Kamal *et al*., 2000) abundant in the brain. Insulin receptor in peripheral tissues participates mainly in glucose metabolism; however its role in the CNS appears not to be related to glucose metabolism but to other neuronal activities such as memory (Zhao *et al*., 1999). Recently, much evidence has been presented

regarding the role of brain insulin or insulin receptors in memory formation (Frolich *et al*., 1998).

Nutritional therapy is a major key in controlling diabetes. Antioxidant agents from diet have a significant therapeutic influence on various neurodegenerative disorders associated with diabetes and oxidative stress. Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and exhibits powerful anti-oxidant, anti-diabetic, anti-inflammatory and anti-cancer properties (Commandeur & Vermeulen, 1996; Miller, 2001; Surh *et al.,* 2001). A number of experimental studies have demonstrated curcumin's antioxidant and neuroprotective potential (Bala *et al.,* 2006; Kuhad & Chopra, 2007). Also, curcumin modulates the expression of various molecular targets, such as transcription factors, enzymes, cytokines, cell cycle proteins, receptors and adhesion molecules (Shishodia *et al.,* 2005). Curcumin antagonise the deficit of glucose energy metabolism or oxidative stress related to cognitive impairment associated with diabetes.

Vitamin D_3 is either synthesised in the epidermis from 7-dehydrocholesterol by the absorption of ultraviolet light, or obtained from the diet in a limited number of foods such as eggs, fish oils and fortified milk . The biological actions of Vitamin D_3 are mediated through binding to the vitamin D receptor (VDR), a member of the nuclear steroid hormone receptor family. An increased prevalence of diabetes has been described in vitamin D-deficient individuals (Chiu *et al.,* 2004). Insulin synthesis and secretion has been shown to be impaired in β cells in vitamin D-deficient animals. Immunohistochemistry showed the presence of VDR in human pituitary gland (Perez-Fernandez *et al.,* 1997), suggesting a possible role of Vitamin D in regulation of the brain endocrine system. It is of particular importance that VDR and catalytic enzymes are colocalised in the brain, supporting an autocrine/paracrine function for Vitamin D. These findings support a functional role for Vitamin D in the human brain (McGrath *et al.,* 2001).

Approaches to the control and prevention of hyperglycemia are central to the management of diabetes mellitus (Herman & Crofford, 1997). The development of new dietary adjuncts and novel antidiabetic agents, which reinstate a normal metabolic environment and thereby reducing the long term complications associated with diabetes, is required. Such agents would both ideally stimulate the secretion and improve the action of insulin (Bailey & Flatt, 1995). Diabetes mellitus is associated with cognitive deficits and neurophysiological and structural changes in the brain (Brands *et al.,* 2003; Mijnhout *et al.,* 2006). However, the action mechanisms of this remain obscure. Factors that contribute to cognitive deficits as well as the protective factors that reduce the impact of diabetes on brain functions are still an enigma. The present study was designed to investigate the beneficial effect of curcumin and Vitamin D_3 on impairment in the functional role of cholinergic, dopaminergic, insulin, Vitamin D receptor, GLUT3, PLC and CREB expression in the brain regions and pancreas of streptozotocin (STZ)-induced diabetic rats. Also, interaction of curcumin and Vitamin D_3 with pancreatic muscarinic receptors and vesicular acetylcholine transporters were studied thereby, evaluating the therapeutic role of curcumin and Vitamin D_3 in regulating insulin synthesis and release. Behavioural studies were conducted to evaluate the motor function and cognitive deficit in control and experimental rats. Our present study on curcumin and Vitamin D_3 dependent regulation of cholinergic, dopaminergic, insulin and VDR in CNS and pancreas will certainly enlighten novel therapeutic possibilities for diabetes treatment.

OBJECTIVES OF THE PRESENT STUDY

1. To study the anti-hyperglycemic activity of curcumin and Vitamin D_3 in STZ-induced diabetic animal model.

2. To measure the circulating insulin and T3 concentration of control, diabetic, insulin, curcumin and Vitamin D_3 treated diabetic rats.

3. To study the behavioural changes in control and experimental rats using Y-maze, rotarod test, grid walk and beam walk test.

4. To study the total muscarinic, muscarinic M1 and muscarinic M3 receptor subtypes binding parameters in cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and pancreas of control, diabetic, insulin, curcumin and Vitamin D_3 treated diabetic rats.

5. To study the total dopamine binding parameters in cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus of control, diabetic, insulin, curcumin and Vitamin D_3 treated diabetic rats.

6. To study the expression of acetylcholine esterase, choline acetyltransferase, muscarinic M1, muscarinic M3, α7 nicotinic acetylcholine, dopamine D1, dopamine D2, insulin and VDR gene expression in the cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus, hypothalamus and pancreas of control, diabetic, insulin, curcumin and Vitamin D_3 treated diabetic rats using Real Time PCR.

7. To study the gene expression status of GLUT2/GLUT3, PLC, CREB and superoxide dismutase in the cerebral cortex, cerebellum, brain stem,

corpus striatum, hippocampus, hypothalamus and pancreas of control, diabetic, insulin, curcumin and Vitamin D_3 treated diabetic rats using Real Time PCR.

8. To study the localisation and expression status of muscarinic M1, muscarinic M3, α7 nicotinic acetylcholine receptor (α7 nAchR), in the brain slices of cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus of control, diabetic, insulin, curcumin and Vitamin D_3 treated diabetic rats using specific antibodies in confocal microscope.

9. To study the localisation and expression status of acetylcholine esterase, muscarinic M1, muscarinic M3, vesicular acetylcholine transporter in the pancreas of control, diabetic, insulin, curcumin and Vitamin D_3 treated diabetic rats using using specific antibodies in confocal microscope.

Literature Review

Diabetes mellitus is a chronic disease characterized by relative or absolute deficiency of insulin, resulting in glucose intolerance. Diabetes mellitus is a major global health problem that affects more than 185 million people around the world (Zimmet *et al*., 2001). The classic symptoms of diabetes mellitus results from abnormal glucose metabolism. The lack of insulin activity results in failure of transfer of glucose from the plasma into the cells. This situation so called "starvation in the midst of plenty". The body responds as if it were in the fasting state, with stimulation of glucogenolysis, gluconeogenesis and lipolysis producing ketone bodies. The disease is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycemia (Dunne *et al*., 2004; Kumar *et al*., 2002). The number of diabetic patients is expected to reach 300 million by the year 2025. 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 acetylcholine plays a prominent role.

The pancreas

The pancreas is a mixed gland, with a large exocrine and a much smaller endocrine gland. The endocrine cells are arranged into small islands of cells called the islets of Langerhans. The interactive function of both the exocrine and the endocrine parts are particularly important for the normal functioning of the body. The endocrine cells produce indispensable hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide, which are crucial to the optimum functioning of body metabolism. The pancreas is well innervated by autonomic nerves rich in different

types of neuropeptides including vasoactive intestinal polypeptide and neuropeptide Y; galanin, Calcitonin-gene-related-peptide, cholecystokinin and leucine-enkephaline (Adeghate *et al.,* 2001). In addition to the presence of neuropeptides, neurotransmitters such as serotonin, GABA or neurotransmitter-regulating enzymes such as tyrosine hydroxylase and dopamine hydroxylase have been identified in the pancreas. (Adeghate & Donáth 1991; Adeghate & Ponery 2001; Adeghate & Ponery 2002).

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 and also responds to other substances such as glucagon and acetylcholine. Insulin responses 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 secretions. 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).

The endocrine pancreas is richly innervated, but the abundance and organisation of these innervations are highly variable between species (Kobayashi & Fujita, 1969). Most of the nerve fibers enter the pancreas along the arteries (Miller, 1981; Woods & Porte, 1974). 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, 1986a; Radke & Stach, 1986b).

The autonomic innervations of the endocrine pancreas have several origins. Classically, the autonomic nervous system uses two interconnected neurons to control effectors 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 (Verchere *et al*., 1996; Sheikh *et al*., 1988, 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 (Berthoud *et al*., 1990; Berthoud & Powley, 1991; Chen *et al*., 1996) and possibly also in the nucleus ambiguus (Luiten *et al*., 1986) which are both under the control of the hypothalamus. They are organized in well separated branches traveling 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 (Berthoud & Powley, 1990) Preganglionic vagal fibers release acetylcholine that binds to nicotinic receptors on intraganglionic neurons. Postganglionic vagal fibers release several neurotransmitters: acetylcholine, Vasoactive Intestinal Peptide (VIP), gastrin-releasing peptide (GRP), nitric oxide (NO), and pituitary adenylate cyclase-activating polypeptide (PACAP) (Havel *et al*., 1997, Love & Szebeni , 1999; Wang *et al*., 1999; Ahrén *et al*., 1999; Ahrén, 2000; Myojin *et al*., 2000). Cholinergic terminals are found in the neighborhood of all islet cell types at the periphery and within the islet (Van der Zee *et al*., 1992; Love & Szebeni, 1999). The importance of the cholinergic innervation of the endocrine pancreas is attested by the presence of a 10-fold higher activity of choline acetyltransferase and acetylcholine esterase (the enzymes involved in the synthesis and the degradation of acetylcholine respectively) in the islets than in the surrounding exocrine tissue (Godfrey & Matschinsky, 1975). Cholinergic synapses with endocrine cells have been observed in some species (Golding & Pow, 1990).

Understanding the organisation of the pancreatic innervations permits correct interpretation of some experiments using different cholinergic antagonists. The stimulation of insulin release occurring upon electrical stimulation of vagal nerves in the dog is abolished by both nicotinic and muscarinic antagonists (Ahrén & Taborsky Jr, 1986). In the perfused rat pancreas, nicotine produces an increase of insulin secretion that is blocked by atropine (Miller, 1981). These observations can be explained by the presence of nicotinic receptors on pancreatic ganglia and nerves (Stagner & Samols, 1986; Karlsson & Ahrén, 1998; Kirchgessner & Liu, 1998) and muscarinic receptors on ß-cells.

The overall effect of a parasympathetic stimulation is an increase of insulin secretion because postganglionic fibers contain various neurotransmitters in addition to the classic neurotransmitter acetylcholine. It is important to keep in mind that parasympathetic neurotransmission is the sum of various biological effects. VIP and PACAP stimulate insulin secretion by increasing cAMP levels (Ahrén, 2000). They act on the same family of receptors (Jian *et al.*, 1999) and exert their action by two mechanisms, directly by stimulating ß-cells through the PLC-PKC pathway (Ahrén, 2000) and indirectly by activating intrapancreatic postganglionic nerves that stimulate insulin secretion (Karlsson *&* Ahrén, 1998).

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 (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*., 1998). The preganglionic fibers release acetylcholine that acts on nicotinic receptors on intraganglionic neurons, whereas the postganglionic fibers release several neurotransmitters: norepinephrine, galanin, (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 (Radke & Stach, 1986c).

Impact of Diabetes on Central nervous system

The brain has not traditionally been considered a target for diabetic complications, but new research has shown that the disease does have particular effects on the CNS. These include impaired learning and memory, neurodegeneration and loss of synaptic plasticity. Most drug discovery efforts aimed at diabetes target insulin action in peripheral tissues. There is evidence that there is substantial overlap between the CNS circuits that regulate energy balance and those that regulate glucose levels, suggesting that their dysregulation could link obesity and diabetes. Some of the abnormalities demonstrated in experimental diabetic neuropathy include a decreased axonal transport, a reduced nerve conduction velocity, increase in resistance to ischemic conduction failure and impaired axon regeneration (Calcutt, 1994; Biro, 1997; Longo, 1986). Diabetes also leads to clinically relevant end-organ damage in the CNS as a result of both acute and chronic metabolic and vascular disturbances (McCall, 1992; Biessels *et al.,* 1994). The consequences of acute metabolic and vascular insults to the brain, such as hypoglycaemia and stroke, are well recognized and have been reviewed extensively. Moreover, recent epidemiological studies demonstrate an association between diabetes and vascular dementia as well as AD (Stewart. *et al.,* 1999; Ott *et al.,* 1999).

Brain neurotransmitter changes during diabetes

Neurotransmitters have been reported to show significant alterations during hyperglycemia 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 (Tassava *et al*., 1992; Chen & Yang, 1991), 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). STZ- 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 epinephrine and dopamine activity in specific hypothalamic nuclei. This lead to the development of secondary neuroendocrine abnormalities known to occur in the diabetes. The dopamine 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 dopamine 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).

Acetylcholine

Cholinergic system plays an important role in physiological and behavioural functions. Acetylcholine acts by binding to specific membrane receptors and is divided into muscarinic and nicotinic receptors. Cholinergic stimulation of pancreatic β-cells increases insulin secretion (Kaneto *et al*., 1967). These are mediated by muscarinic cholinergic, rather than nicotinic receptors (Stubbe & Steffens, 1993; Ahren *et al*., 1990) and is dependent on extracellular glucose concentration (Henquin *et al*., 1988). Acetylcholine stimulated insulin secretion coupling is mediated by complex mechanisms of signal transduction. It has been proposed that acetylcholine activates phospholipid turnover and thereby increases the intracellular calcium level. Normal β-cells' voltage-dependent sodium channels are important for membrane depolarisation. acetylcholine increases sodium influx into the cells (Henquin *et al*., 1988). Acetylcholine hyperpolarises the cell by increasing potassium permeability. Quist (1982) reported that carbachol causes Ca^{2+}-dependent stimulation of phosphate incorporation into phosphatidyl inositol phosphates in the canine heart. Cholinergic stimulation of phosphatidyl inositol phosphates synthesis is blocked by muscarinic antagonist atropine (Brown & Brown, 1983).

Muscarinic receptors

Muscarinic receptors are a family of G protein-coupled receptors that have a primary role in central cholinergic neurotransmission. Specific agonists, which activate postsynaptic muscarinic receptors, stimulate cholinergic signaling (Valentin *et al*., 2006). The muscarinic acetylcholine receptors are widely distributed throughout the body and subserve numerous vital functions in both the brain and autonomic nervous system (Hassal *et al*., 1993). Activation of muscarinic receptors in the periphery causes decrease in heart rate, relaxation of blood vessels, constriction in the

airways of the lung, increase in the secretions and motility of the various organs of the gastrointestinal tract, increase in the secretions of the lacrimal and sweat glands, and constriction in the iris sphincter and ciliary muscles of the eye (Wess, 1993). In the brain, muscarinic receptors participate in many important functions such as learning, memory and the control of posture.

Muscarinic receptors are members of a large family of plasma membrane receptors that transduce the intracellular signals *via* coupling to guanine nucleotide binding regulatory proteins (G proteins) (Nathanson, 1987; Bonner, 1989; Hulme *et al*., 1990). Molecular cloning studies have revealed the existence of five molecularly distinct mammalian muscarinic receptor proteins (Bonner, 1989; Hulme *et al*., 1990).

All mammalian muscarinic receptor genes share one common feature with several other members of G-protein receptor gene family *i.e.,* their open reading frame contained within a single exon (Bonner *et al*., 1987). Like all other G protein coupled receptors, the muscarinic receptors are predicted to conform to a generic protein fold consisting of seven hydrophobic transmembrane helices joined by alternating intracellular and extracellular amino-terminal domain and a cytoplasmic carboxy-terminal domain. The five mammalian muscarinic receptors display a high degree of sequence identity sharing about 145 amino acids. Characteristically all muscarinic receptors contain a very large third cytoplasmic loop, which, except for the proximal portions, displays virtually no sequence identity among the different subtypes (Bonner, 1989). Agonist binding to muscarinic receptors is thought to trigger conformational changes within the helical bundle, which are then transmitted to the cytoplasmic face where the interaction with specific G proteins are known to occur. Site directed mutagenesis and receptor-modeling studies suggest that almost all G protein coupled receptors plays a pivotal role in mediating the conformational changes associated with receptor activation (Wess, 1993).

The ligand binding to muscarinic receptors is predicted to occur in a pocket formed by the ring like arrangement of the seven transmembrane domains (Wess *et al*., 1991; Hulme *et al*., 1990). Ligand binding appears to be initiated by ion-ion interaction between positively charged amino head present in virtually all muscarinic receptor ligands and a conserved Asp residue located in TM III. In addition a previous mutagenesis study has shown that replacement of the conserved TM III Asp residue in the rat muscarinic M1 receptor with Asn results in a receptor unable to bind to [^{3}H] QNB.

Sequence analysis shows that the hydrophobic core of all muscarinic receptors contains a series of conserved Ser, Thr and Tyr residues, most of which do not occur in other G protein coupled receptors. Pharmacological analysis of mutant M3 muscarinic receptors showed that two Thr residues (Thr231 and Thr234) and four Tyr residues (Tyr148, Tyr506, Tyr529 and Tyr533) are important for high affinity acetylcholine binding (Wess *et al*., 1991). It has been shown that a Pro 201 to Ala mutant M3 muscarinic receptor exhibits affinities for both muscarinic agonists and antagonists 80-450 times less than those of the wild type (Wess *et al*., 1993).

In the periphery, among other effects, muscarinic receptors mediate smooth muscle contraction, glandular secretion and modulation of cardiac rate and force. In the CNS there is evidence that muscarinic receptors are involved in motor control, temperature regulation, cardiovascular regulation and memory. Interest in the classification of muscarinic receptors involved in functions at different locations has been heightened by the potential therapeutic application of selective agents in areas such as AD, Parkinson's disease, asthma, analgesia, and disorders of intestinal motility, cardiac and urinary bladder function (Caulfield & Birdsall, 1998).

Classification

Muscarinic receptors are widely distributed throughout the central and peripheral nervous system. They have critical functions in learning and memory, attention and motor activity (Bonner, 1989; Weiner *et al*., 1990; Levey, 1993). The five muscarinic receptor subtypes are designated as M1 - M5. The odd-numbered receptors (M1, M3, and M5) couple to Gq/11, and thus activate PLC, which initiates the phosphatidyl inositol trisphosphate cascade. This leads to the dissociation of phosphatidyl 4, 5- bisphosphates (PIP2) into two components, i.e., IP_3 and DAG. IP_3 mediates Ca^{2+} release from the intracellular pool (endoplasmic reticulum), whereas DAG is responsible for activation of protein kinase C. On the other hand, PIP2 is required for the activation of several membrane protein, such as the "M current" channel and Na^{+}/Ca^{2+} exchanger, and muscarinic receptor- dependent depletion of PIP2 inhibits the function of these proteins (Bonner *et al*., 1987; Caulfield & Birdsall, 1998; Bonner *et al*., 1988; Fuster *et al*., 2004; Suh & Hille, 2005; Winks *et al*., 2005; Meyer *et al*., 2001). The M1, M2 and M4 subtypes of macetylcholineRs are the predominant receptors in the CNS. These receptors activate a multitude of signaling pathways important for modulating neuronal excitability, synaptic plasticity and feedback regulation of acetylcholine release (Volpivelli *et al*., 2004).

Muscarinic M1 receptor

M1 receptors are predominantly expressed in the forebrain, including the cerebral cortex, hippocampus and corpus striatum, where this sub-type contributes by 50-60% to the total of the muscarinic receptors (Hamilton *et al*., 1997; Gerber *et al*., 2001; Miyakawa *et al*., 2001). The M1 receptor subtype, which is also expressed in peripheral tissues, has been implicated in stress adaptive cardiovascular reflexes and central blood pressure control. Studies have shown that central administration of the

M1 specific antagonist pirenzepine lowered the blood pressure (Brezenoff & Xiao, 1986; Buccafusco, 1996). A putative overexpression of the M1 subtype in selected brain areas of spontaneously hypertensive rats has been reported (Scheucher *et al*., 1991). Muscarinic agonist depolarisation of rat isolated superior cervical ganglion is mediated through M1 receptors (Brown *et al*., 1980). M1 is one of the predominant muscarinic receptor subtypes expressed in pancreatic islets (Gilon & Henquin, 2001). Studies in pancreatic islets revealed that activation of muscarinic receptors is pertusis toxin insensitive and Gq mediated. Muscarinic M1 receptor number decreased in the brainstem at time of pancreatic regeneration without any change in the affinity (Renuka *et al*., 2006).

Muscarinic M2 receptor

Muscarinic receptor activation in guinea pig heart produces a reduction in force of contraction and a decrease in the rate of beating. These effects are probably the consequence of inhibition of voltage-gated Ca^{2+} channels and activation of inwardly rectifying K^{+} channels, respectively. Extensive studies with many antagonists have defined this response as being mediated by the M2 receptor (Caulfield, 1993). Muscarinic M2 receptors mediate both negative and positive ionotropic responses in the left atrium of the reserpinized rat, latter effect being insensitive to pertusis toxin (Kenakin & Boselli, 1990). Central cholinergic transmission is activated by inhibition of the presynaptic M2 acetylcholine autoreceptor using selective antagonists. The presynaptic M2 autoreceptor negatively influences the release of acetylcholine in several brain regions, including the striatum, hippocampus, and cerebral cortex (Billard *et al*., 1995; Kitaichi *et al*., 1999; Zhank *et al*., 2002). A direct consequence of brain M2 autoreceptor inhibition is an elevation of acetylcholine release in the synaptic cleft. Methoctramine and other M2 receptor

antagonists have been shown to enhance the release of acetylcholine in different brain structures (Stillman *et al*., 1993; Stillman *et al.*, 1996).

Muscarinic M3 receptor

M3 muscarinic receptors are broadly expressed in the brain, although the expression level is not high, compared to those of the M1 and M2 receptors (Levey, 1993). Muscarinic M3 receptor is widely distributed in the peripheral autonomic organs with the highest expression found in the exocrine glands (Candell *et al*., 1990; Pedder *et al*., 1991; Kashihara *et al*., 1992; Matsui *et al*., 2000). Expression of the M3 receptor in the rat pancreatic islets and insulin secreting cell lines has been established (Lismaa, 2000). M3 receptor also triggers direct contractions of smooth muscle, however, it only represents a minor fraction of total muscarinic receptor population in smooth muscle. It is expressed in relatively low density throughout the brain. Studies using knock out mice for M3 receptors gave evidences for the primary importance of these receptors in the peripheral cholinergic system. In urinary bladder, pupillary muscles and intestinal smooth muscles the cholinergic contractions are mediated predominately through M3 receptors (Matsui *et al*., 2000).

Muscarinic M4 receptor

Muscarinic M4 receptor is known to be abundantly expressed in the striatum (Levey, 1993). Muscarinic M4 receptors act as inhibitory muscarinic autoreceptors in the mouse (Zhang *et al*., 2002). The neuroblastoma-glioma hybrid cell line NG108–15 expresses M4 mRNA and M4 receptors can be detected readily in radioligand binding assays (Lazareno *et al*., 1990). Inhibition of adenylyl cyclase activity by muscarinic agonists in rat corpus striatum is mediated by M4 receptors (Caulfield, 1993; Olianas *et al*., 1996).

Muscarinic M5 receptor

The M5 receptor was the last muscarinic acetylcholine receptor cloned. Localisation studies have revealed that the M5R is abundantly expressed in dopamine-containing neurons of the substantia nigra par compacta, an area of the midbrain providing dopaminergic innervation to the striatum. Concordantly, oxotremorine-mediated dopamine release in the striatum was markedly decreased in M5R-deficient mice. More intriguingly, in M5R-deficient mice, acetylcholine induced dilation of cerebral arteries and arterioles was greatly attenuated (Yamada *et al*., 2001), suggesting that the M5 receptor is suitable target for the treatment of cerebrovascular ischemia. Muscarinic M5 receptor subtype is expressed at low levels in the brain (Hulme *et al*., 1990; Hosey, 1992).

Studies of the M5 receptor have been hampered both by the lack of selective ligands and of tissues or cell lines that endogenously express the native receptor protein. Immunoprecipitation and RT-PCR studies have shown that the M5 receptor is expressed at very low densities in the mammalian brain. However, in situ hybridisation studies have demonstrated that M5 transcripts are highly concentrated in the basal ganglia and are the only muscarinic receptor transcripts expressed on dopaminergic neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) (Reever *et al*., 1997). Another potentially useful system is the eosinophilic leukemia cell line (EoL-1) where M5 receptors are induced on differentiation with interferon-γ (Mita *et al*., 1996).

Signal transduction by muscarinic activation

Gq-protein-coupled receptors (GqPCRs) are widely distributed in the CNS and play fundamental roles in a variety of neuronal processes. Their activation results in phosphatidyl inositol 4,5-bisphosphate (PIP2) hydrolysis and Ca^{2+} release from

intracellular stores *via* the PLC-inositol 1,4,5-trisphosphate (IP_3) signaling pathway. Because early GqPCR signaling events occur at the plasma membrane of neurons, they are influenced by changes in membrane potential (Billups *et al*., 2006). Muscarinic receptors, which are G protein coupled, stimulate signaling by first binding to G protein complex ($\alpha\beta\gamma$) which provides specificity for coupling to an appropriate effector. The α subunit interacts with an effector protein or ion channel to stimulate or inhibit release of intracellular second messengers. Mutation analysis showed that the G protein is primarily but not exclusively acts through interaction with the third cytoplasmic loop. It is suggested that the short sequences, N terminal 16-21 and C terminal 19 amino acids of the loop play a key role in determining the specificity (Wess *et al*., 1989).

Cyclic adenosine monophosphate

Adenylate cyclase is either positively or negatively regulated by G protein coupled receptors resulting in an increase or decrease in the generation of the second messenger, Cyclic adenosine monophosphate (cAMP). The stimulation of muscarinic M2 and M4 receptors endogenously expressed in cell lines, results in the inhibition of adenylate cyclase. G protein reconstitution experiments have shown that M2 receptors inhibit adenylate cyclase through Gi and possibly through the pertusis toxin insensitive Gz. In neuroblastoma SK-N-SH cells which express endogenous muscarinic M3 receptors stimulate adenylate cyclase activity (Baumgold & Fishman, 1988). The muscarinic M1 receptor which ectopically expressed at physiological levels in A9L cells, was shown to stimulate adenylate cyclase through an IP_3 and Ca^{2+} dependent mechanism (Felder *et al*., 1989). In contrast, M1 receptors stimulate adenylate cyclase in CHO cells predominantly through an IP_3 and Ca^{2+} independent

mechanism that also contained a small Ca^{2+} dependent component (Gurwitz *et al*., 1994).

Phospholipase C

The family of PLC enzymes has been grouped into three classes, β, γ and δ (Rhee & Choi, 1992). PLC serves as the primary effector for the muscarinic M1 receptor that is coupled through Gq α subunits (Berstein *et al*., 1992). Muscarinic M1, M3 and M5 receptors stimulate the production of IP_3, independent of direct PLCβ and G protein interaction (Gusovsky, 1993). This alternate route for the generation of IP_3 involves the tyrosine kinase dependent phosphorylation of PLCγ, a mechanism normally stimulated by growth factors and their receptors (Meisenhelder *et al.*,, 1989). Expression studies revealed that the cloned muscarinic M2 receptor stimulates PLC through a pertusis toxin-sensitive G protein although with lower efficiency than M1 or M3 receptors (Ashkenazi *et al*., 1987). Inhibition of PLC by an endogenously expressed M2 receptor has been reported in FRTL5 cells suggesting that negative regulation occur in some cells (Bizzarri *et al*., 1990).

Phospholipase A2

Phospholipase A2 catalyzes the hydrolysis of membrane phospholipids to generate free arachidonic acid and the corresponding lysophospholipid. Muscarinic receptors have been shown to stimulate the release of arachidonic acid and its eicosanoid metabolites in a variety of tissues including heart, brain and muscle (Abdel-Latif, 1986). Ectopic transfection experiments indicate that the muscarinic M1, M3 or M5 receptors, but not M2 or M4 receptors are linked to phospholipase A2 activation (Felder *et al*., 1990; Liao *et al*., 1990). Muscarinic receptor stimulated release of arachidonic acid occurs predominantly through the activation of

phospholipase A2 and phosphatidylcholine serves as the primary substrate. Studies suggested that calcium influx, through voltage independent calcium channel activation and diacylglycerol, through PLC activation were essential for phospholipase A2 activation (Brooks *et al*., 1989; Felder *et al*., 1990). In ileal smooth muscle cells, carbachol stimulated phospholipase A2 itself caused calcium influx, implicating an amplification mechanism in phospholipase A2 regulation (Wang *et al*., 1993).

Phospholipase D

Muscarinic receptor stimulated phospholipase D has been reported in a number of cell types including canine synaptosomes (Qian & Drewes, 1989), rat astrocytoma cells (Martinson, 1990), human neuroblastoma cells (Sandmann & Wurtman, 1991) and rat parotid cells (Guillemain & Rossignol, 1992). Association of muscarinic subtypes with phospholipase D has been shown in human embryonic kidney cells transfected with the muscarinic M1-M4 receptors. In most cells studied, phospholipase C and phospholipase D are usually stimulated simultaneously following receptor activation (Liscovitch, 1991).

Calcium influx and release from intracellular stores

Muscarinic receptors typically stimulate biphasic increases in intracellular calcium in most cells. The transient phase represents the release of calcium from IP_3 sensitive intracellular Ca^{2+} stores. Ca^{2+} influx through Ca^{2+} channels play a central role in the regulation of multiple signaling pathways activated by muscarinic receptors. In excitable cells such as neurons and muscle cells, Ca^{2+} passes predominantly through voltage sensitive Ca^{2+} channels. In non-excitable cells, such as fibroblasts and epithelial cells, Ca^{2+} passes through a family of poorly characterised voltage - insensitive Ca^{2+} channels (Fasolato *et al*., 1994). Voltage-independent Ca^{2+}

channels open in response to receptor activation and have been classified into (1) receptor operated Ca^{2+} channels which are second messenger independent (2) second messenger - operated Ca^{2+} channels and (3) depletion operated Ca^{2+} channels which open following IP_3 mediated depletion of intracellular stores and provide a source of Ca^{2+} for refilling the stores.

α7 nicotinic acetylcholine receptor

The nicotinic acetylcholine receptor (nAChR), a key player in neuronal communication, converts neurotransmitter binding into membrane electrical depolarization. This protein combines binding sites for the neurotransmitter acetylcholine and a cationic transmembrane ion channel. The nAChR also binds the addictive drug nicotine. It mediates synaptic transmission at the junction between nerve and muscle cells and various types of nAChR are expressed in the brain. It is involved in several neurological pathologies. Several genes have been identified in rat and chick neural or sensory tissue that encode for neuronal nAChR subunits that are distinct from those in the muscle nAChR, providing for a multitude of potential subtypes of neuronal nAChRs. The wide distribution of the some of these transcripts in mammalian brain indicates that neuronal nAChRs represent a major neurotransmitter receptor superfamily related to other ligand gated ion channels including serotonin ($5HT_3$), $GABA_A$, N-methyl-D-aspartate, and glycine. However, in contrast to these other ligand gated ion channels where established pharmacology rapidly segued into the molecular biology, the pharmacology of neuronal nAChRs has only started to emerge as a result of the rapid advances in the molecular biology of the nAChR family (Changeux *et al*., 1998).

The α7 nicotinic receptor, also known as the α7 receptor, is a type of nicotinic acetylcholine receptor, consisting entirely of α7 subunits (Rang *et al.,* 2003). As with

other nicotinic acetylcholine receptors, functional α7 receptors are pentameric (i.e., (α7)5 stoichiometry). It is located in the brain, where activation yields post- and presynaptic excitation (Rang *et al.,* 2003), mainly by increased Ca^{2+} permeability. Neuronal nicotinic cholinergic receptors are crucial to acetylcholine neurotransmission in both the CNS and autonomic nervous system. However, in the CNS, these receptors are more often associated with modulation of release of several neurotransmitters including dopamine, norepinephrine, GABA and glutamate (Wonnacott, 1997; Girod & Role, 2001). In the CNS, nicotinic acetylcholine receptors mediate the release of glutamate (Reno *et al*., 2004; De Filippi *et al*., 2001; Rossi *et al*., 2003) and norepinephrine (O Leary & Leslie, 2003). Thus, these receptors significantly influence the activity within the CNS circuitry and deregulation of this activity could contribute to diabetes mellitus associated disorders involving the CNS. Abnormalities of nicotinic acetylcholine receptor function in the hippocampus lead to cognitive and memory impairments (Green *et al*., 2005; Levin *et al*., 2002) and sensory gating deficits (Adler *et al*., 1998).

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 (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.

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). 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).

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 Ca^{2+} (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).

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 Ca^{2+} current through Ca^{2+} 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 and 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).

Role of neurotransmitters in insulin regulation & secretion

Acetylcholine

Acetylcholine is one of the principal neurotransmitters of the parasympathetic system. Acetylcholine, through vagal muscarinic and non-vagal muscarinic pathways

(Greenberg & Pokol, 1994) increases insulin secretion (Tassava *et al*., 1992). They function through muscarinic receptors present on pancreatic islet cells (Ostenson *et al*., 1993). Acetylcholine agonist, carbachol, at low concentration (10^{-7} M) stimulated insulin secretion at 4 mM and 20 mM concentrations of glucose (Renuka *et al*., 2006).

Dopamine

Dopamine is reported to inhibit glucose stimulated insulin secretion from pancreatic islets (Tabeuchi *et al*., 1990). Eswar *et al*., (2006) reported that dopamine significantly stimulated insulin secretion at a concentration of 10^{-8} M in the presence of high glucose (20mM). Reports show that experimental diabetes and insulin deficiency result in the rapid onset of detectable alterations in dopaminergic activity in specific hypothalamic nuclei. The uptake affinity and velocity of dopamine in synaptosomes decreased significantly during diabetes. The dopamine content was increased in the cerebral cortex and hypothalamus of diabetic rats (Shiimzu, 1991; Tassava *et al*., 1992; Ohtani *et al*., 1997). The altered turnover ratio in the limbic forebrain is reported to cause enhanced spontaneous locomotor activity in diabetic rats (Kamei *et al*., 1994).

High concentrations of dopamine in pancreatic islets decrease glucose stimulated insulin secretion (Tabeuchi *et al*., 1990). L-DOPA, the precursor of dopamine had similar effect to that of dopamine (Lindstrom & Sehlin, 1983). Dopamine D3 receptors are implicated in the control of blood glucose levels (Alster & Hillegaart, 1996). Dopamine D1 receptors have also been reported to be present on pancreatic β-cells (Tabeuchi *et al*., 1990). These clearly indicate the role of dopamine in the regulation of pancreatic function.

Gamma-Aminobutyric acid

Gamma aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the CNS. GABA is reported to present in the endocrine pancreas at concentrations comparable with those found in CNS. 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). GABA through its receptors has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic α–cells and δ-cells respectively (Gaskins, 1995). It is present in the cytoplasm and in synaptic-like microvesicles (Reetz, 1991) and is co-released with insulin from β-cells in response to glucose. The released GABA inhibits islet α -and β -cell hormonal secretion in a paracrine manner. During diabetes the destruction of β -cells will lead to decrease in GABA release resulting in the enhancement of glucagon secretion from α-cells leading to hyperglycemia. The brain GABAergic mechanisms also play an important role in glucose homeostasis. Inhibition of central $GABA_A$ receptors increases plasma glucose concentration (Lang, 1995). $GABA_A$ receptors in brainstem have a regulatory role in pancreatic regeneration (Kaimal *et al*., 2007) Thus, any impairment in the GABAergic mechanism in the CNS and/or in the pancreatic islets is important in the pathogenesis of diabetes.

Serotonin

Serotonin 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 (Sumiyoshi *et al*., 1997; Sandrini *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 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).

Epinephrine and Norepinephrine

These are secreted by the adrenal medulla. Norepinephrine (NE) is a principal neurotransmitter of sympathetic nervous system. These hormones inhibit insulin secretion, both *in vivo* and *in vitro* (Porte, 1967; Renstrom *et al*., 1996). Epinephrine exerts opposite effects on peripheral glucose disposal and glucose stimulated insulin secretion (Avogaro *et al*., 1996). 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 hypoglycemia, a 15 to 40 fold increase of epinephrine plays a pivotal role in increasing glucose production independently of glucagon (Gauthier *et al*., 1980). It is already known that, when used in high doses *in vivo* or *in vitro*, epinephrine reduces the insulin response to stimulators (Malaisse, 1972). *In vitro* studies with yohimbine – α_2-adrenergic receptor antagonist, showed that the insulin secretion from the pancreatic islets increased significantly suggesting that when the alpha 2-adrenergic receptors are blocked, it enhances islet cell proliferation and insulin secretion (Ani *et al*., 2006). EPI and NE have an antagonistic effect on insulin secretion and glucose uptake (Porte *et al*., 1966). 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 phospho-fructokinase. In humans, adrenaline stimulates lipolysis, ketogenesis, thermogenesis and glycolysis and raises plasma glucose concentrations by stimulating both glycogenolysis and gluconeogenesis. Adrenaline is, however, known to play a secondary role in the physiology of glucose counter-regulation. Indeed, it has been shown to play a critical role in one pathophysiological state, the altered glucose counter-regulation in patients with established insulin-dependent diabetes mellitus (Cryer, 1993). The inhibitory effect of EPI upon insulin secretion induced by glucose was reported by Coore and Randle, (1964), who incubated pancreatic tissue from the rabbit. As judged by Malaisse *et al*., (1967) the inhibitory effect of EPI on glucose-induced insulin secretion is mediated through the activation of α-adrenoreceptors.

Central muscarinic regulation of glucose homeostasis

The acetylcholine esterase inhibitor, soman induced marked and sustained hypertension in rats (Letienne *et al.*, 1999). Stimulation of muscarinic receptors in the nucleus tractus solitarius (NTS) of the rat decreases arterial blood pressure and heart rate. Atropine injected into the NTS of rats produced a dose-dependent inhibition of cardiovascular response elicited by injection of acetylcholine into the same site. It is suggested that cholinergic mechanisms in the NTS are not involved in the tonic regulation of cardiovascular function or the baroreceptor reflex (Tsukamoto *et al*., 1994).

When carbachol, muscarine, bethanechol, methacholine, or neostigmine was injected into the third cerebral ventricle, it caused a dose-dependent increase in the hepatic venous plasma glucose concentration. However, in the case of 1, 1-dimethylphenyl-4-piperazinium iodide (DMPP) or nicotine, the level of hepatic venous glucose did not differ from that of the saline-treated control rats. The increase in glucose level caused by neostigmine was dose-dependently suppressed by co-administration of atropine. These facts suggest that cholinergic activation of muscarinic receptors in the CNS plays a role in increasing hepatic glucose output. Injection of neostigmine, an inhibitor of cholinesterase, into the ventricle resulted in the increase of not only glucose, but also glucagon, epinephrine, and norepinephrine in the hepatic venous plasma. Neostigmine-induced increments in glucose did not occur in adrenalectomized rats. This suggests that the secreted epinephrine acts directly on the liver to increase hepatic glucose output (Iguchi *et al*., 1986).

The injection of adrenaline and carbachol into the third cerebral ventricle resulted in a marked hyperglycemia associated with increased immunoreactive glucagon. Adrenaline-induced hyperglycemia was not affected by bilateral adrenalectomy, while carbachol-induced hyperglycemia was completely inhibited by

adrenalectomy. The injection of somatostatin with adrenaline into the third cerebral ventricle did not influence adrenaline-induced hyperglycemia, while carbachol-induced hyperglycemia was inhibited by co-administration with somatostatin (Iguchi *et al*., 1985).

Atropine injected into the third cerebral ventricle suppressed epinephrine secretion and dose-dependently inhibited hepatic venous hyperglycemia induced by neostigmine in intact rats. The neostigmine-induced glucagon secretion which occurs in adrenalectomised rats was suppressed by atropine. Atropine also prevented the neostigmine-induced hyperglycemia in adrenalectomised rats receiving constant somatostatin infusion through femoral vein. Phentolamine, propranolol and hexamethonium showed no significant inhibitory effect on neostigmine-induced hyperglycemia, epinephrine and glucagon secretion in intact rats, glucagon secretion in adrenalectomised rats, or hyperglycemia in adrenalectomised rats. These results suggest that neostigmine-induced epinephrine and glucagon secretion and increased hepatic glucose output stimulated by direct neural innervation to liver is mediated by central muscarinic receptor in fed rats (Iguchi *et al*., 1990)

Studies by Iguchi *et al*., (1992) suggest that the glucoregulatory hippocampal activity evoked by the acetylcholine esterase inhibitor, neostigmine transmitted to peripheral organs *via* the ventromedial hypothalamus. The ventromedial hypothalamus, lateral hypothalamus, paraventricular hypothalamus and median site of the lateral-preoptic area were involved in increasing the plasma levels of glucose and epinephrine by cholinergic stimulation (Honmura *et al*., 1992).

Atropine in a dose-dependent manner suppressed the hyperglycemia induced by hippocampal administration of neostigmine, whereas hexamethonium had no significant effect. These observations suggest that the pathway for this experimental hyperglycemia involves, at least in part, the muscarinic cholinergic neurons in the

ventromedial hypothalamus (Iguchi *et al*., 1991). Takahashi *et al*., (1993) reported that neostigmine induced hyperglycemia affects not only the cholinergic system but also the noradrenergic and dopaminergic systems in the hypothalamus (Takahashi *et al*., 1993). Muscarinic cholinergic system is reported to participate in the $HgCl_2$-induced central hyperglycemic effect through the function of the adrenal medulla. Norepinephrine and dopamine content were found to be decreased suggesting that their neurons have hypothalamic glycoregulation (Takahashi *et al*., 1994).

Microinjections of carbachol or neostigmine into the ventromedial nucleus of the hypothalamus of fed, conscious rats produced marked increases in plasma glucose and lactate, which were suppressed or markedly reduced by previous adrenodemedullation. The reports suggest that cholinergic synapses in the ventromedial hypoythalamus participate in a central glucoregulatory system that increases hepatic glucose production mainly through a stimulation of adrenal medulla epinephrine secretion (Brito *et al*., 1993).

Neostigmine caused significant increases in serum glucose concentrations, hypothalamic noradrenergic and dopaminergic neuronal activities, and significantly suppressed hypothalamic serotonergic neuronal activity. All these responses to neostigmine were completely inhibited by the co-administration of atropine. These observations emphasize the important role of the interactions between cholinergic (muscarinic) and monoaminergic neurons in the brain (Gotoh & Smythe, 1992). In the ventromedial hypothalamic nucleus, lateral hypothalamus and paraventricular nucleus the cholinergic activity is increased after 2-D glucose administration (Takahashi *et al*., 1994 & 1996).

Central cholinergic-muscarinic activation with neostigmine stimulates sympathetic nervous activity in the liver, heart, pancreas and interscapular brown adipose tissue (Gotoh & Smythe, 1992). Histamine induction of CNS-mediated

hyperglycemia involves neuronal transmission not only *via* H1 receptors but also, at least in part, by muscarinic cholinergic neurons (Nonogaki *et al*., 1993). The action of acetylcholine within the hypothalamus on the pancreatic hormone secretions is mediated to a large part through sympatho-adrenomedullary activity. However, a part of the decreased insulin response to glucose is mediated by direct innervation of the pancreas (Ishikawa *et al*., 1982).

Intravenous 2-D glucose induced a marked increase in plasma glucose that was not affected by intracerebroventricular administration. However, the hyperglycemia induced by intracerebroventricular 2-D glucose was significantly reduced by previous intracerebroventricular injection of atropine. Central cholinergic neurons participate in the complex neural events responsible for the hyperglycemic response to neurocytoglucopenia and to stressful situations (Brito *et al*., 2001). Intravenous administration of 2-D glucose caused neuroglycopenia and marked hyperglycemia. The cholinergic activity was increased after 2-D glucose administration (Takahashi *et al*., 1996).

Dopamine, a neurotransmitter in the CNS

Dopamine is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake and endocrine regulation. This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function and gastrointestinal motility (Missale *et al*., 1998).

Dopamine containing neurons arise mainly from dopamine cell bodies in the substantia nigra and ventral tegmental area in mid-brain region (Carlsson, 1993; Tarazi *et al*., 1997 a, b; 1998 a, b, 2001). Dopaminergic system is organized into four

major subsystems (i) the *nigrostriatal* system involving neurons projecting from the substantia nigra, pars compacta to the caudate-putamen of the basal ganglia. This is the major dopamine system in the brain as it accounts for about 70% of the total dopamine in the brain, and its degeneration makes a major contribution to the patho-physiology of Parkinson's disease; (ii) *the mesolimbic system* that originates in the midbrain tegmentum and projects to the nucleus accumbens septi and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus and the entorhinal cortex, all of which are considered components of the limbic system and so are of particular interest for the patho-physiology of idiopathic psychiatric disorders; (iii) the *mesocortical* system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions; (iv) the *tuberinfundibular* pathway, which is a neuroendocrinological pathway arising from the arcuate and other nuclei of the hypothalamus and ending in the median eminence of the inferior hypothalamus. Dopamine released in this system exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin. Dopamine is involved in the control of both motor and emotional behaviour. Despite the large number of crucial functions it performs, this chemical messenger is found in a relatively small number of brain cells. In fact, while there are a total of 10 billion cells in the cerebral cortex alone, there are only one million dopaminergic cells in the entire brain (Missale *et al*., 1998).

Biosynthesis of dopamine

Dopamine is synthesized from the amino acid L-tyrosine**.** L-tyrosine is hydroxylated by the enzyme tyrosine hydroxylase (TH) to give L-3, 4-dihydroxyphenylalanine (L-DOPA) which is the rate limiting step**.** L-DOPA is subsequently decarboxylated to dopamine by the enzyme aromatic L-amino acid

decarboxylase. Therefore, it is not possible to enhance the levels of dopamine by providing L-tyrosine. The activity of tyrosine hydroxylase is regulated by several endogenous mechanisms. For example, the enzyme is activated by increased neuronal impulse flow, but is inactivated either by dopamine itself as an end-product inhibitor, or by activation of presynaptic dopamine receptors. On the other hand, the enzyme aromatic L-amino acid decarboxylase converts L-DOPA to dopamine instantaneously. Therefore, providing L-DOPA creates a possibility to enhance the formation of dopamine.

Dopamine receptors

Dopamine mediates its actions via membrane receptor proteins. Dopamine receptors are found on postsynaptic neurons in brain regions that are dopamine-enriched. In addition, they reside presynaptically on dopamine neuronal cell bodies and dendrites in the midbrain as well as on their terminals in the forebrain. Dopamine receptors belong to a family of large peptides that are coupled to G-proteins which are modified by attached carbohydrate, lipid-ester or phosphate groups. The topologies of the five dopamine receptors are predicted to be the same as all the other G-protein-coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions. The third intracytoplasmic loop is functionally critical and interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects (Carlsson, 1993; Tarazi *et al*., 1997 a, b, 1998 a, b). In their putative transmembrane domains, the dopamine D_1 and D_5 receptors are 79% identical to each other, while they are only 40–45% identical to the dopamine D_2, D_3, and D_4 receptors. Conversely, the dopamine D_2, D_3, and D_4 receptors are between 75% and 51% identical to each other. They contain seven putative membrane-spanning helices which would form a narrow dihedral

hydrophobic cleft surrounded by three extracellular and three intracellular loops. The receptor polypeptides are probably further anchored to the membranes through palmitoylation of a conserved Cys residue found in their carboxy tails, 347 in dopamine D_1, the C-terminus in dopamine D_2 like receptors. The dopamine receptors are glycosylated in their N-terminal domains. Dopamine D_1 like subtypes has potential glycosylation sites in their first extra cytoplasmic loop.

Dopamine receptors are divided into two families on the presence or absence of ability of dopamine to stimulate adenylyl cyclase and produce the second-messenger molecule cyclic-AMP (Kebabian & Calne, 1979; Schwartz *et al*., 1992; Civelli *et al.*, 1993; O'Dowd, 1993; Jackson & Westlind, 1994; Ogawa, 1995; Strange, 1996). This classification is based on similarities in structure, pharmacology, function and distribution. Dopamine D1 like receptors are characterized initially as mediating the stimulation of cAMP production. Dopamine D2 like receptors inhibits the production of cAMP. This pharmacological characterisation is based on the ability of some dopamine agents to block adenylyl cyclase activity to inhibit the release of prolactin *in vivo* and *in vitro* in a cAMP-independent fashion (Seeman, 1980). Applications of recent technical advances in molecular genetics have greatly facilitated the isolation and characterisation of novel dopamine receptors**,** dopamine D3, D4 and D5 with different anatomical localisation from traditional dopamine D1 or dopamine D2 receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families**:** the dopamine D1 like family which includes dopamine D1 and D5 receptors. The dopamine D2 like family includes dopamine D2, D3 and D4 receptors (Schwartz *et al*., 1992; Grandy *et al*., 1993; Sibley *et al*., 1993). The genomic organisations of the dopamine receptors demonstrate that they are derived from the divergence of two gene families that mainly differ in the absence or the presence of

introns in their coding sequences. Dopamine D1 like receptors genes do not contain introns in their coding regions, a characteristic shared with most G protein-coupled receptors. The genes encoding the dopamine D2 like receptors are interrupted by introns (Gingrich & Marc, 1993). Furthermore, most of the introns in the dopamine D2-like receptor genes are located in similar positions.

Dopamine D1-like family

The dopamine D1 receptor is the most abundant dopamine receptor in the CNS. The dopamine D1 like receptors are characterized by a short third loop as in many receptors coupled to Gs protein (Civelli *et al*., 1993; Gingrich & Canon *et al*., 1993; O'Dowd, 1993). The dopamine D1 like receptors have short third intracellular loops and long carboxy terminal tails. The dopamine D_1 like receptors are classified into dopamine D1 and D5. In the dopamine D1 and D5 receptor third intracellular loop and the carboxy terminus are similar in size but divergent in their sequence. In contrast, the small cytoplasmic loops 1 and 2 are highly conserved so that any difference in the biology of these receptors is probably related to the third cytoplasmic loop and the carboxy terminal tail (Civelli *et al*., 1993, Gingrich & Canon *et al*., 1993; O'Dowd, 1993). The external loop between transmembrane domain (TM) TM4 and TM5 is considerably different in the two receptor subtypes, being shorter (27 amino acids) in the D1 receptor than in the D5 receptor (41 amino acids). The amino acid sequence of this loop is divergent in the dopamine D5 receptor (Marc *et al*., 1998).

Dopamine D1 receptor

Dopamine D1 receptors are found at high levels in the typical dopamine regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercles. Dopamine D1 receptor seems to mediate important actions of

dopamine to control movement, cognitive function and cardiovascular function. The dopamine D1 receptor gene, which lacks introns, encodes a protein that extends for 446 amino acids (Dohlman *et al*., 1991). In humans dopamine D_1 receptor gene has been localized to chromosome 5 (Sunahara *et al.,* 1990). The dopamine D1 receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5-trisphosphate (IP_3) and diacylglycerol *via* the activation of PLC (Monsma *et al.,* 1990; Sibley *et al.,* 1990)**.** Dopamine D1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. Dopamine D_1 receptors messenger ribonucleic acid (mRNA) is colocalized in striatal neurons of the basal ganglia with mRNA for dopamine receptor phosphor protein (DARPP-32; KD) which is a dopamine and cAMP-regulated phosphoprotein. Dopamine Receptor Phosphor Protein contributes to the actions of dopamine D_1 receptor (Hemmings & Greengard, 1986; Greengard, *et al*., 1987). The dopamine D_1 receptors in the brain are linked to episodic memory, emotion, and cognition.

Dopamine D5 receptors

The dopamine D5 receptor gene is intronless and encodes a protein that extends for 47 amino acids (George *et al.,* 1991). This protein has an overall 50% homology with dopamine D1 receptor and 80% if only the seven transmembrane segments are considered. The gene encoding the human dopamine D5 protein is located at the short arm of chromosome 4, the same region where the Huntington disease gene has been located. Two dopamine D5 receptor pseudogenes having 154 amino acids have been identified with 90% homology (Gusella, 1989). These pseudogenes, however, contain stop codons in their coding regions that prevent them from expressing functional receptors. The functions of these pseudogenes, which appear so far to be specific to humans, are not yet known.

Dopamine D5 receptor mRNA expression is unique and limited to the hippocampus and parafascicular nucleus of the thalamus (Civelli *et al*., 1992). It is involved in the thalamic processing of painful stimuli (Giesler *et al*., 1979). Dopamine D5 receptors appear to interact with G-proteins and can stimulate adenylyl cyclase, with relatively high affinity for dopamine and dopamine D1-selective agonists (George *et al*., 1991).

Dopamine D_2 like family

Dopamine D2 like receptors belong to the G-protein coupled receptors and has 400 amino acid residues. Dopamine D2-like receptors are characterized by a long extracellular amino terminus which has several glycosylation sites and a shorter carboxy terminal tail with putative phosphorylation sites. The function of sugar moieties is unclear (Marie *et al*., 1996; Sibley, 1999). It is generally believed that the membrane enclosed part of the amino-acid chain of G-protein coupled receptors is folded into seven α-helices. The transmembrane helices consist primarily of hydrophobic amino-acid residues. The unique feature of dopamine D_2 like receptors family is that they posses a bigger third cytoplasmic (intracellular) loop in common, which is thought to be the site where the G-protein couples (Marie *et al*., 1996). Between the different dopamine receptors, the third loop also displays the greatest variability in amino-acid sequence. This has consequences for their respective second messenger systems. The dopamine D2-like receptors are coupled to Gi-protein and inhibit the formation of cAMP. The dopamine D2 receptors tertiary structure is stabilized by two cysteine disulphide bridges.

Dopamine D2 receptors

The dopamine D2 receptor gene encodes a protein that extends for 415 amino acids. Similar to other G-protein coupled receptors, the dopamine D2 receptor has seven transmembrane segments, but in contrast to dopamine D_1-like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. Unlike the dopamine D1-like receptor genes, the dopamine D2 receptor gene contains seven introns that are spliced out during mRNA transcription (Fischer *et al*., 1989). The gene encoding this receptor was found to reside on q22-q23 of human chromosome 11 (Makam *et al.,* 1989). The dopamine D2 receptor was the first receptor to be cloned (Bunzow *et al*., 1988). The dopamine D2 receptors are involved in several signal transduction cascades, including inhibition of cAMP production (Vallar & Meldolesi, 1989), inhibition of phosphoinositide turnover (Epelbaum J *et al.,* 1986), activation of potassium channels and potentiation of arachidonic acid release (Axelrod, 1991). The dopamine D2 receptors are highly expressed in basal ganglia, nucleus accumbens septi and ventral tegmental area (Schwartz *et al.,* 1998).

The dopamine D2 receptor exists as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop and are designated as dopamine D2S and dopamine D2L (Seeburg *et al*.,1989; Marc *et al*., 1998). Because this loop seems to play a central role in receptor coupling, the existence of a splicing mechanism at this level could imply functional diversity. However, in spite of the efforts of several groups, no obvious differences have emerged so far between the two dopamine D2 receptor isoforms. The two isoforms derived from the same gene by alternative RNA splicing which occurs during the maturation of the dopamine D2 receptor pre-mRNA (Schwartz *et al*., 1989a). Dopamine D2 receptor isoforms (dopamine D2L and dopamine D2S) vary within each species by the presence or absence of a 29-amino acid sequence in the third

cytoplasmic domain of the dopamine D2 receptor peptide chain. Both variants share the same distribution pattern; with the shorter form less abundantly transcribed in addition they appear to differ in their mode of regulation (Marc *et al*., 1998). Pharmacologically, both isoforms exhibit nearly similar profiles in terms of their affinities to different dopamine D2 selective agents and inhibit adenylyl cyclase activity. However, these isoforms display an opposite regulatory effect (Sibley *et al*., 1993). These isoforms have the same pharmacological profile, even though a marginal difference in the affinity of some substituted response to dopamine treatment is reported: Dopamine induces the up regulation of dopamine D2L isoform of dopamine D2 receptors (Castro & Strange, 1993). When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc *et al*., 1998; Sibley, 1999). However, the dopamine D2S receptor isoform displayed higher affinity than the dopamine D2L in this effect (Seeburg *et al*., 1993). The isoforms of dopamine D2 mediate a phosphatidylinositol-linked mobilisation of intracellular Ca^{2+} in mouse Ltk fibroblasts. Protein kinase C, however, differentially modulates dopamine D2S and D2L activated transmembrane signalling in this system with a selective inhibitory effect on the dopamine D_{2S}-mediated response.

Dopamine D3 receptors

Dopamine D3 receptor gene contains five introns and encodes a 446 amino acid protein (Schwartz *et al*., 1992). The gene encoding this receptor resides on chromosome 3 (Giros *et al*., 1990). The dopamine D3 receptors bear close structural and pharmacological similarities to the dopamine D2 receptors. Dopamine D3 mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz *et al*., 1992). Distribution of dopamine D3 receptor mRNA are distributed and expressed mainly in subcortical limbic regions including islands of Calleja, nucleus accumbens

septi and olfactory tubercle, with low levels of expression in the basal ganglia. D3 receptor mRNA has also been found in neurons of the cerebellum, which regulate eye-movements (Levesque *et al*., 1992). The status of the dopamine D3 molecular entity as a functional receptor remains uncertain since it neither couples to G-proteins nor consistently transduces an effector mechanism. However, the structural similarity with dopamine D2 receptor raises the possibility that dopamine D3 receptor also inhibit adenylyl cyclase activity in its normal cellular setting. More recent studies reported that dopamine D3 receptors mediate positive regulatory influences of dopamine on production of the peptide neurotensin (Sokoloff *et al*., 1990; Schwartz *et al*., 1992).

Dopamine D4 receptors

Dopamine D4 receptor gene contains four introns and encodes a 387 amino acid protein (Van Tol *et al*., 1991). The overall homology of the dopamine D4 receptor to the dopamine D2 and D3 receptors is about 41% and 39% respectively, but this homology increases to 56% for both receptors when only the transmembrane spanning segments are considered. The gene encoding the human dopamine D4 protein is located at the tip of the short arm of chromosome 11 (Civelli & Bunzow, 1993; Missale *et al*., 1998). Dopamine D4 receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes. The stimulation of dopamine D4 receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Misalle *et al*., 1998). In humans, dopamine D4 receptor occurs in several genomic polymorphic variants that contain two to eleven repeats of a 48 base pair segment that is expressed in the third cytoplasmic domain (Van Tol *et al*., 1992; Misalle *et al*., 1998). These are called the dopamine D4 alleles

which are represented as dopamine D4.2, D4.4 and D4.7. This contributes to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind, 1994).

Dopamine and its receptor alterations during diabetes

Dopamine is implicated in diabetes. Hyperglycaemia in rats is reported to decrease dopaminergic activity in the striata suggesting the up regulation of dopamine receptors possibly due to the decreased dopamine metabolism (Hio *et al*., 1994). In experimental diabetes and insulin deficiency there is a rapid onset of detectable alterations in hypothalamic dopamine activity leading to secondary neuroendocrine abnormalities. Lim *et al*. (1994) have described an increase in the striatal dopamine and decrease in its metabolites dihydroxyphenylacetic acid and HVA. Tyrosine hydroxylase is reported to be depleted in nigrostriatal neurons in the genetically diabetic rat causing marked reduction in mesolimbic dopamine system. Insulin treatment could not restore the decreased dopamine to controlled conditions, impairing the dopamine biosynthesis (Kamei & Saitoh, 1994). Dopamine uptake affinity and velocity in synaptosomes is decreased significantly during diabetes. The dopamine content was increased in cerebral cortex and hypothalamus of diabetic rats (Chen & Yang, 1991; Tassava *et al*., 1992; Ohtani *et al.,* 1997). Diabetes is reported to cause increased dopamine release with altered turnover ratio of dopamine metabolites from the mesolimbic systems. This resulted in the enhanced spontaneous locomotor activity which is suggested to be due to the up regulation of δ-opioid receptor-mediated functions (Kamei *et al*., 1994). The decrease in striatal dopamine transporter mRNA in experimental diabetes is suggested to be a possible cause for the disturbance in dopamine metabolism (Figlewicz *et al*., 1996). The dopamine turnover ratio in the limbic forebrain and midbrain in diabetic mice were significantly greater than those in non-diabetic mice (Kamei & Saitoh, 1996). Yawning behaviour in STZ

induced diabetes was significantly lowered when compared with their age-matched normal controls as a result of altered dopamine metabolism and decreased turnover to its metabolites (Heaton & Varrin, 1993).

Dopamine receptors are reported to be increased in diabetes causing significant alterations in central dopaminergic system (Lozovsky *et al*., 1981). Dopamine D_2 receptor density has been reported to be increased in the striatum of diabetic rats (Lozovsky *et al*., 1981; Trulson & Hummel, 1983). Intracerebroventricular application of alloxan and STZ in rat striatum is reported to have caused an alteration in dopamine receptors and increased dopamine content which had a similar effect to peripheral, diabetogenic administration of these drugs (Salkovic *et al*., 1992). The affinity of striatal dopamine D_1 receptors was significantly increased without changes in the number of binding sites, while the binding of dopamine D_2 receptors was significantly increased without affecting its affinity in the diabetic rats (Hio *et al*., 1994). Dopamine D_1 receptors are reported to decrease in hyporesponsiveness (Kamei *et al*., 1994). The increase in the central dopaminergic postsynaptic receptors has been related to decrease the locomotor and ambulatory activity in STZ-induced diabetic rats (Kobayashi *et al*., 1990; Shimomura *et al*., 1990). Studies from our laboratory reported dopamine D_2 receptor alterations in the brain and pancreas of STZ- induced diabetic rats (Eswar *et al*., 2007).

Diabetes mellitus causes a condition called as neurocytoglucopenia where the increased glucose results in an increased sympathetic outflow into the liver, pancreas, adrenal medulla, adipose tissue and the circulation. This causes an increased hepatic glucose production, inhibition of insulin secretion and free fatty acid mobilisation from the adipose tissue (Oliveira *et al*., 1998). Participation of dopaminergic tone in the control of insulin secretion and hyperglycaemia has been given little focus. Studies have shown that dopamine agonists play an important role in lowering the

elevated shift in the sympathetic tone as a result of increased glucose levels and stimulate the parasympathetic tone which increases the insulin response (Oliveira *et al*., 1998).

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 utilisation and glucose production, both processes being tightly coordinated (Nevado *et al*., 2006). 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, GLUT4 is the main isoform of glucose transporters (Burant *et al*., 1991). In adipose tissue the concentrations of GLUT4 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 GLUT4 protein and mRNA are marginally altered (Garvey *et al*., 1989; Bourey *et al*., 1990). In liver, GLUT2 is the main isoform of glucose transporters (Thorens *et al*., 1988). Much less information is available concerning the expression of GLUT2 in liver of diabetic rats and the results are somewhat contradictory.

Insulin and the brain

Two decades ago both insulin and its receptor were discovered in the brain (Havrankova *et al.,* 1978). Moreover, contrary to old assumptions, it is now known that insulin is actively transported across the blood–brain barrier and it is produced locally in the brain (Schwartz *et al.,* 1998). Concentrations of insulin receptors in the brain are particularly high in neurons, with abundant insulin receptor protein in both cell bodies and synapses (Zhao *et al*., 1999).

These findings have raised questions about the physiological role of insulin in the brain. Some suggest that, as in peripheral tissues, insulin mainly acts by mediating cerebral glucose uptake (Hoyer, 1998), but this opinion is not shared by others. Insulin and insulin receptors appear to play a modulatory role in certain behaviours, such as feeding behaviour and learning and memory (Wickelgren, 1998; Kumagai , 1999). For example, after training in a water maze, insulin receptor mRNA levels were increased in the hippocampus of rats, in parallel with accumulation of insulin receptor protein. Moreover, intracerebroventricular administration of insulin facilitated retention of a passive-avoidance task in rats (Park *et al*., 2000).

The complexity of the mechanisms underlying these behavioural findings is only now starting to be appreciated (Fernandes *et al*., 1999). When applied to brain slices, insulin inhibits the spontaneous firing rate of hippocampal pyramidal neurones and the frequency of AMPA-receptor mediated miniature EPSCs of cerebellar Purkinje neurones. In addition, insulin attenuates the amplitude of AMPA-receptor-mediated currents in cerebellar Purkinje neurons (Palovcik *et al*., 1984), through the stimulation of clathrin-dependent receptor internalisation, a phenomenon that appears to have links with cerebellar LTD (Wang *et al*., 2000). These same authors have reported no effect of insulin on NMDA-receptor-mediated currents in cerebellar Purkinje neurons. Conversely, in hippocampal slices insulin has been shown to

increase NMDA-receptor mediated EPSPs (Liu *et al*., 1995). These different findings are possibly due to variations in insulin signalling in different brain regions. Insulin thus appears to play a modulatory role in synaptic transmission in the brain. However, studies of its involvement in behaviour and synaptic transmission have so far mainly examined its effects after local (for example, intracerebroventricular) administration or *ex vivo*. The challenge for future studies will be to determine whether systemic insulin also has neuromodulatory effects under physiological conditions and to dissociate these effects from the associated effects of insulin on peripheral and central glucose homeostasis.

The cAMP responsive element binding protein (CREB)

The cAMP responsive element binding protein (CREB) is a nuclear protein that modulates the transcription of genes with cAMP responsive elements in their promoters. Increases in the concentration of either Ca^{2+} or cAMP can trigger the phosphorylation and activation of CREB. This transcription factor is a component of intracellular signaling events that regulate a wide range of biological functions, from spermatogenesis to circadian rhythms and memory. Evidence from *Aplysia*, *Drosophila*, mice and rats shows that CREB-dependent transcription is required for the cellular events underlying long-term but not short-term memory (Byrne, 1993). While the work in *Aplysia* and *Drosophila* only involved CREB function in very simple forms of conditioning, genetic and pharmacological studies in mice and rats demonstrate that CREB is required for a variety of complex forms of memory, including spatial and social learning, thus indicating that CREB may be a universal modulator of processes required for memory formation (Silva, 1998).

Curcumin

India has a rich history of using plants for medicinal purposes. Turmeric (*Curcuma longa* L.) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases (Ammon & Wahl, 1991; Eigner & Scholz, 1999) *C. longa* L., botanically related to ginger (Zingiberaceae family), is a perennial plant having a short stem with large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in colour. Turmeric is used as a food additive (spice), preservative and colouring agent in Asian countries, including China and South East Asia. It is also considered as auspicious and is a part of religious rituals. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury1. In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis (Ammon *et al.,* 1992). The colouring principle of turmeric is the main component of this plant and is responsible for the antiinflammatory property. Turmeric was described as *C. longa* by Linnaeus and its taxonomic position is as follows:

Class	Liliopsida
Subclass	Commelinids
Order	Zingiberales
Family	Zingiberaceae
Genus	*Curcuma*
Species	*Curcuma longa*

Medicinal Properties:

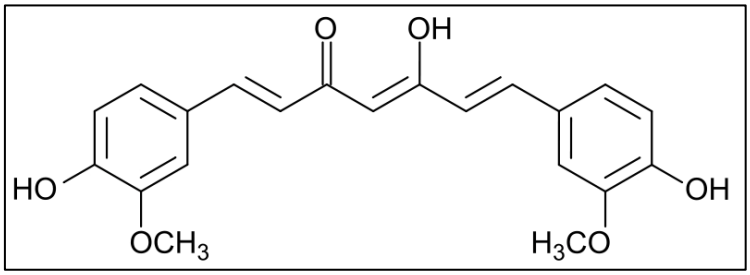
- anti-oxidant;
- arthritis: anti-inflammatory effects, possibly inhibits something in the pathway of Cox-2 but not Cox-2 itself; not only does it not cause ulcers but is

currently being used experimentally as a treatment for ulcers in western countries.

- anti-Alzheimer's: inhibits formation of, and breaks down, Amyloid-beta oligomers (fibres) and aggregates in rodents;
- anti-platelet;
- anti-cancer effects: causes apoptosis in various cancer cell types including skin, colon, forestomach, duodenum and ovary in the laboratory; we await clinical trials in humans;
- anti: -viral, -fungal, -bacterial effects (inhibits Helicobacter Pylori);
- inhibits NFkappaB, 5-lipoxygenase, glutathione S-transferase and cytochrome P-450;
- Anti- diabetic effects in rodents; we await clinical trials in humans.

Curcumin and Alzheimer's Disease (AD)

Recently curcumin has been proposed as a potential remedy against brain ageing and neurodegenerative disorders (Cole *et al*., 2007), and it has been evaluated in a pilot clinical trial in AD patients, with encouraging preliminary results (Baum L. *et al*., 2008). Curcumin is highly lipophilic and cross the blood-brain barrier. Although its bioavailability is very low, since the drug is rapidly metabolized by conjugation, curcumin reach brain in a sufficient concentration to activate signal transduction events and to decrease Amyloid β aggregation (Yang *et al*., 2005). Epidemiological studies suggested that curcumin, one of the most prevalent nutritional and medical compounds used by the Indian population, is responsible for the significantly reduced (4.4-fold) prevalence of AD in India compared to United States (Chandra *et al*., 2001). Furthermore elderly Singaporeans who eat curry with turmeric had higher Mini-Mental State Examination scores than those who did not.



- **Chemical Formula: $C_{21}H_{20}O_6$**
- **Molecular Weight: 368.38**

Vitamin D_3

Several forms of Vitamin D are distinguished, namely Vitamin D_1, D_2, D_3 and D_4. Of these, the most important for humans is D_3. The action of UV-B rays with a wavelength of 290–315 nm1 on the skin results in photochemical synthesis of previtamin D_3 from the provitamin 7-dehydrocholesterol, which in turn is formed in the liver from cholesterol. Over a period of two to three days, a thermally induced change in molecular structure (isomerisation) then results in the conversion of previtamin D_3 to vitamin D_3 (cholecalciferol) in a process that does not require sunlight. Vitamin D_3 then undergoes enzymatic conversion in the liver and the kidneys to the active substance 1,25-dihydroxycholecalciferol, also known as 1,25-dihydroxyvitamin D_3 (1,25(OH)2D_3). 1,25-dihydroxyvitamin D_3 is the active form of the Vitamin in human beings (Holick, 1987; Haussler *et al*., 1998).

Vitamin D receptor

Certain VDR gene polymorphisms are associated with type 1 diabetes (Mathieu *et al*., 1994; Ambrosio *et al*., 1998). Vitamin D receptors are activated when certain mediator substances, or ligands, dock at them. This ligand function can be

exerted not only by vitamin D compounds, but also by steroid hormones, thyroid hormones and Vitamin A1 acid. By binding to the receptor, these ligands regulate the metabolism of Ca^{2+} and phosphate, and thus also of bone and control cell replication and differentiation. This occurs *via* an influence on the synthesis of certain regulatory proteins. When a VDR is activated by binding of a ligand, it exerts its action as a transcription factor. This means that it binds to specific sites on DNA (deoxyribonucleic acid), the molecule in the cell nucleus that bears genetic information and thereby initiates the synthesis of certain regulatory proteins.

Vitamin D and diabetes

The discovery of receptors for 1,25(OH)2D3, the activated form of vitamin D, in tissues with no direct role in calcium and bone metabolism (e.g. pancreatic beta cells and cells of the immune system) has broadened our view of the physiological role of this molecule (Holick, 1987; Haussler *et al*., 1998). An increased prevalence of type 2 diabetes has been described in Vitamin D-deficient individuals (Boucher *et al.,* 1995; Chiu *et al.,* 2004) and insulin synthesis and secretion have been shown to be impaired in beta cells from Vitamin D-deficient animals. Glucose tolerance is restored when vitamin D levels return to normal. The identification of receptors for 1,25(OH)2D3 in cells of the immune system led to experiments in animal models of type 1 diabetes in which the administration of high doses of 1,25(OH)2D3 was shown to prevent type 1 diabetes (Mathieu *et a*l., 1994; Boucher *et al*., 1995), mainly through immune regulation. It has been demonstrated that 1, 25(OH) 2D3 is one of the most powerful blockers of dendritic cell differentiation and that it directly blocks IL-12 secretion (Ambrosio *et al*., 1998). Lymphocyte proliferation is inhibited and regulator cell development is enhanced (Halteren *et al*., 2002). This review provides an overview of the data available on the role of Vitamin D in type 1 and type 2 diabetes

and discusses possible applications of the molecule or its synthetic analogues (Bouillon *et al*., 2003) in clinical disease. The terminology used in many papers to describe vitamin D and its metabolites is confusing, with misnomers leading to misunderstanding and over-interpretation of data. In this review the term vitamin D refers to the product that is in food (vitamins D_2 and D_3) and is synthesised in the skin under the influence of UVB radiation (vitamin D_3), whereas the metabolically active molecule is referred to as 1,25 (OH)2D3.

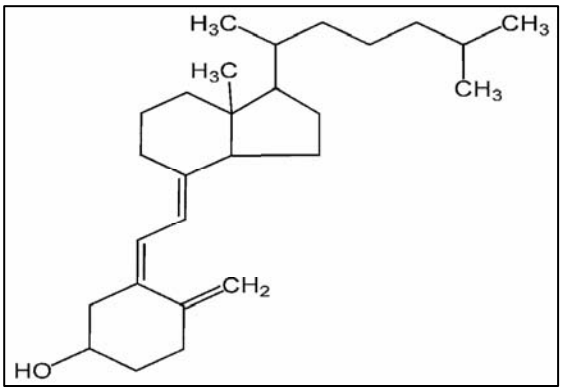
Vitamin D_3 and Central nervous system

Regulatory effect of vitamin D on NGF and GDNF suggests that it is a potent neuroprotective agent (Kalueff & Tuohimaa, 2007). The active form of vitamin D, 1,25(OH)2D would appear to provide some protection against excitatory neurotransmitters such as glutamate (Ibi *et al.,* 2001). Vitamin D also protect the brain against reactive oxygen species *via* up regulation of antioxidant molecules, such as glutathione, in non-neuronal cells (Garcion *et al.,* 1999). Vitamin D suppress macrophage activity in the brain after lipopolysaccharide- induced brain inflammation (Garcion *et al.,* 1998). Inflammatory mechanisms induced by experimental autoimmune encephalitis (EAE) are also diminished by this vitamin (Nataf *et al.,* 1996). It has been shown *in vitro* that activated microglia metabolise 25(OH) D and produce the biologically active 1, 25(OH) 2D (Neveu *et al.,* 1994). Thus, non-neuronal cells in the brain mediate anti-inflammatory effects of vitamin D *via* its local synthesis. Vitamin D has also been shown to preserve dopamine and serotonin content in the brains of animals repeatedly administered with neurotoxic doses of methamphetamine (Cass *et al.,* 2006).

Similar to the benefits of traditional antioxidant nutrients, Vitamin D_3 inhibits inducible nitric oxide synthase (Garcion *et al.,* 1997), an enzyme that is up regulated

during ischemic events and in patients with Alzheimer's and Parkinson's disease. Vitamin D_3 also enhances innate antioxidant pathways. The hormone up regulates gamma glutamyl transpeptidase (Baas *et al.,* 2000) and subsequently increases glutathione. Glutathione is an innate antioxidant which protects oligodendrocytes and the integrity of the nerve conduction pathway critical to mental processing.

Vitamin D has been detected in the cerebrospinal fluid and this hormone has been shown to cross the blood- brain barrier (Balabanova *et al.,* 1984; Gascon-Barre & Huet., 1983; Pardridge, Sakiyama, & Coty, 1985) The presence of VDR in the limbic system, cortex, cerebellum of rodents and humans (Eyles *et al.,* 2005; Langub *et al.,* 2001; Musiol *et al.,* 1992; Walbert, Jirikowski, & Prufer, 2001) support a functional role for Vitamin D in the regulation of behaviour and cognitive functions. It is also consistent with the distribution of other neurosteroids (Prufer & Jirikowski., 1997). VDR is found in the olfactory, visual and auditory sensory systems (Glaser *et al.,* 1999; Prufer *et al.,* 1999; Zou *et al.,* 2008), suggesting that the somatosensory system is also a target of 1,25(OH)2D VDR like immunoreactivity was found in the nucleus vestibularis, which extends its efferents to cerebellar Purkinje cells and the thalamic part of the vestibular system, nucleus ventrolateralis, suggesting that the vestibular system is also a target of VD (Prufer *et al.,* 1999). Expression of VDR in motor neurons (Prufer et al., 1999) suggests its role in regulation of motor functions. A putative receptor for Vitamin D has been detected in chick brain (Jia & Nemere, 1999), allowing speculation that Vitamin D could act like other neuroactive hormones in modulating neuronal activity and neurotransmitter receptors (Zakon, 1998; Rupprecht & Holsboer, 1999). It is of particular importance that VDR and catalytic enzymes are colocalized in the brain (Baulieu, 1998; Melcangi & Panzica, 2001), supporting an autocrine/paracrine function for Vitamin D. These findings support a functional role for Vitamin D_3 in the human brain (McGrath *et al.,* 2001).



Vitamin D_3

Molecular formula $C_{27}H_{44}O$

Molar mass 384.64 g/mol

Materials and Methods

CHEMICALS USED IN THE STUDY AND THEIR SOURCES

Biochemicals

Curcumin, Cholecalciferol (Vitamin D_3), dopamine, pirenzepine, atropine, 4-DAMP mustard (4-deoxy acetyl methyl piperidine mustard), ethylene diamine tetra acetic acid - EDTA, HEPES - [n′ (2-hydroxy ethyl)] piperazine-n′-[2-ethanesulfonic acid], Streptozotocin, citric acid, Tris HCl, foetal calf serum (heat inactivated), D-glucose, calcium chloride, collagenase type XI, bovine serum albumin fraction V and RPMI-1640 medium were purchased from Sigma Chemical Co., St. Louis, MO. USA). All other reagents were of analytical grade purchased locally.

Radiochemicals

Quinuclidinylbenzilate, L-[Benzilic-4,4'-^{3}H]-[4-^{3}H] (Sp. Activity 42 Ci/mmol), [^{3}H] Dopamine (Sp. activity- 45.1Ci/mmol) and 4-DAMP, [N-methyl-^{3}H] (Sp. Activity 83 Ci/mmol) was purchased from NEN life sciences products Inc., Boston, U.S.A.

Radioimmunoassay kit for insulin was purchased from Baba Atomic Research Centre (BARC), Mumbai, India.

Molecular Biology Chemicals

Tri-reagent kit was purchased from Sigma chemicals Co., St. Louis, MI, 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.

Confocal Dyes

Rat primary antibody for muscarinic M1 (Cat. No. 087k1395), M3 (Cat. No. 126k1205), α7 nicotinic acetylcholine receptor (Cat. No. 018k4811), acetylcholine esterase (Cat. No. 097k1431) and vesicular acetylcholine transporter (Cat. No. 077k4838) and FITC coated secondary antibody (Cat. No. No-AP307R) were purchased from Sigma Aldrich and Chemicon, USA.

ANIMALS

Adult male Wistar rats of 180-240g body weight purchased from Amrita Institue of Medical Sciences, Cochin and Kerala Agriculture Unviersity, Mannuthy were used for all experiments. They were housed in separate cages under 12 hour light and 12 hour dark periods and were maintained on standard food pellets and water *ad libitum*.

DIABETES INDUCTION

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 anaesthesia (Junod *et al.,*1969). Streptozotocin was given at a dose of 55mg/Kg body weight (Hohenegger & Rudas, 1971; Arison *et al.,* 1967).

DETERMINATION OF BLOOD GLUCOSE

The diabetic state of animals was assessed by measuring blood glucose concentrations 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 CURCUMIN AND VITAMIN D_3

Animals used in this study were randomly divided into the following groups. Each group consisted of 6-8 animals.

a) Group 1: Control (given citrate buffer injection)
b) Group 2: Diabetic
c) Group 3: Diabetic rats treated with insulin
d) Group 4: Diabetic rats treated with Curcumin
e) Group 5: Diabetic rats treated with Vitamin D_3

The insulin treated diabetic group (Group 3) received subcutaneous injections (1Unit/kg body weight) of insulin daily during the entire period of the experiment. A mixture of both Lente and Plain insulin (Abbott India) were given for the better control of glucose (Sasaki & Bunag, 1983). The last injection was given 24 hr before sacrificing the diabetic rats.

Curcumin was given orally to the 4th group of diabetic rats in the dosage of 60mg/Kg body weight suspension of curcumin orally at 24 hour intervals. Curcumin was suspended in 0.5% w/v sodium carboxymethylcellulose immediately before administration in constant volume of 5ml/kg body weight (Sharma *et al.,* 2006). Cholecalciferol was given orally to the 5th group of diabetic rats in the dosage of 12 µg/Kg body weight dissolved in 0.3 ml of coconut oil (Rosanne *et al.,* 2005). Blood samples were collected from the tail vein at 0 hours (Before the start of the experiment), 3rd, 6th, 10th and 14th day and the glucose levels were estimated. Blood samples were collected 3hrs after the administration of morning dose. Changes in the body weight of animals were monitored on 1st Day (before the start of the experiment), 7th and 15th day.

SACRIFICE AND TISSUE PREPARATION

The animals were then sacrificed on 15th day by decapitation. The cerebral cortex, cerebellum, brain stem, corpus striatum, and hypothalamus were dissected out quickly over ice according to the procedure of Glowinski & Iversen, (1966) and the pancreas was dissected quickly over ice. Hippocampus was dissected 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 -80° C until assay. All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines.

ESTIMATION OF BLOOD GLUCOSE

Blood glucose was estimated using Glucose estimation kit (Merck). The spectrophotometric method using glucose oxidase-peroxidase reactions is as follows:

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

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

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

The hydrogen peroxide formed in this reaction reacts 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 accelerates the reactions. The amount of dye formed is proportional to the glucose concentration. The absorbance was read at 510nm 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 is 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 200 µU/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 [^{125}I] insulin (50µl) was added and incubated at room temperature for 3 hours. The second antibody was added (50µl) 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.

A standard curve was plotted with %B/Bo on the Y-axis and insulin concentration/ml on the X-axis of a log-logit graph. %B/Bo 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).

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 specifically 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 1500xg for 20 minutes. The supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

A standard curve was plotted with $\%B/B_o$ on the Y-axis and T3 concentration (ng /ml) on the X-axis of a log-logit graph. $\%B/B_o$ was calculated as:

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

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

BEHAVIOURAL STUDIES

Y-Maze Test

The Y-maze was made of grey wood, covered with black paper and consisted of three arms with an angle of 120 degrees between each of the arms. Each arm was 8 cm width ×30 cm length ×15 cm height. The three identical arms were randomly designated: Start arm, in which the rat started to explore (always open); Novel arm, which was blocked at the 1st trial, but open at the 2nd trial; and the other arm (always open). The maze was placed in a separate room with enough light. The floor of the maze was covered with sawdust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Visual cues were placed on the walls of the maze.

The Y-maze test consisted of two trials separated by an inter-trial interval (ITI). The first trial (training) was of 10 minutes duration and allowed the rat to explore only two arms (start arm and the other arm) of the maze, with the third arm (novel arm) blocked. After a 1 hour ITI (Ma *et al*., 2007), the second trial (retention) was conducted, during which all three arms were accessible and novelty *vs* familiarity was analyzed through comparing behavior in all three arms. For the second trial, the rat was placed back in the maze in the same starting arm, with free access to all three arms for 5 minutes. The time spent in each arm was analyzed. Data was expressed as percentage of performance in all three arms during the five minutes of test (Akwa *et al*., 2001, Jobin, *et al*., 2010).

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 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 in such a manner that it allowed 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, 15 and 25 rpm after 15 days of treatment in all groups of rats.

Grid Walk Test

Deficits in descending motor control were examined by assessing the ability to navigate across a 1 m long runway with irregularly assigned gaps (0.5–5 cm) between round metal bars. Crossing this runway requires that animals accurately place their limbs on the bars. In baseline training and postoperative testing, every animal had to cross the grid for at least three times. The number of footfalls (errors) was counted in each crossing for 3 minute and a mean error rate was calculated (Z'Graggen *.et al.*, 1998).

Narrow Beam Test

The narrow beam test was performed according to the descriptions of Haydn and Jasmine (1975). A rectangular 1.2-cm wide beam, 1.05m long and elevated 30 cm from the ground was used for the study. After training, normal rats were able to traverse the horizontal beams with less than three footfalls. When occasionally their feet slipped off the beam, they were retrieved and repositioned precisely. The time the rats could remain balanced on the beam was counted.

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

Binding studies in the Brain regions

Total muscarinic, muscarinic M1 and M3 receptor binding studies

[^{3}H] QNB and [^{3}H] DAMP binding assay in cerebral cortex, cerebellum, brain stem, hippocampus, corpus striatum and pancreas were done according to the modified procedure of Yamamura & Snyder (1981). Brain tissues were homogenised in a polytron homogeniser with 20 volumes of cold 50mM Tris-HCl buffer, containing 1mM EDTA (pH.7.4). The supernatant was then centrifuged at 30,000xg for 30 minutes and the pellets were resuspended in appropriate volume of Tris-HCl-EDTA buffer.

Total muscarinic and muscarinic M1 receptor binding parameter assays were done using [^{3}H] QNB (0.1-2.5nM) and M3 receptor using [^{3}H] DAMP (0.01-5nM) in the incubation buffer, pH 7.4 in a total incubation volume of 250µl containing appropriate protein concentrations (200-250µg). The non-specific binding was determined using 100µM atropine for total muscarinic, pirenzepine for muscarinic M1 and 4-DAMP mustard for muscarinic M3 receptor. Total incubation volume of 250 µl contains 200-250µg protein concentrations. Tubes were incubated at 22°C for 60 minutes and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris-HCl buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. The non-specific binding determined showed 10% in all our experiments.

Total Dopamine receptor binding studies.

Dopamine DA receptor assay was done using [^{3}H] DA as per Madras *et al.*, (1988) and Hamblin & Creese, (1982). Brain tissues were homogenised in a polytron homogeniser with 20 volumes of cold 50mM Tris-HCl buffer, along with 1mM EDTA, 0.01% ascorbic acid, 4mM $MgCl_2$, 1.5 mM $CaCl_2$, pH.7.4 and centrifuged at 38,000xg for 30min. at 4^{o}C. The pellet was washed twice by homogenization and centrifuged twice at 38,000 g for 30min. at 4^{o}C. This was resuspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.25nM-1.5nM of [^{3}H] DA in 50mM Tris-HCl buffer, along with 1mM EDTA, 0.01% ascorbic acid, 1mM $MgCl_2$, 2 mM $CaCl_2$, 120mM NaCl, 5mM KCl, pH.7.4 in a total incubation volume of 250µl containing 200-300 µg of proteins. Specific binding was determined using 100µM unlabelled dopamine. Tubes were incubated at 25^{0}C for 60 min. and filtered rapidly through GF/Bfilters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Protein determination

Protein was measured by the method of Lowry *et al*., (1951) using bovine serum albumin as standard. The intensity of the purple blue colour formed was proportional to the amount of protein which was read in a spectrophotometer 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 IN DIFFERENT BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS

Isolation of RNA

RNA was isolated from the brain regions and pancreas of control and experimental rats using the Tri reagent from Sigma Chemicals Co., St. Louis, MO, U.S.A). 25-50 mg tissue homogenates were made in 0.5 ml Tri Reagent. The homogenate was kept in the room temperature for 5 minutes. 100 µl of chloroform was added to the homogenate, mixed vigorously for 15 seconds kept in the RT for 10-15 minutes and was centrifuged at 12,000xg for 15 minutes at 4°C. The upper aqueous phase was transferred to a fresh tube and 250µl of isopropanol was added and the tubes were allowed to stand at room temperature for 10 minutes. The tubes were centrifuged at 12,000xg for 10 minutes at 4°C. RNA precipitated as a pellet on the sides and bottom of the tube. The supernatant was removed and the RNA pellet was washed with 500µl of 75% ethanol, vortexed and centrifuged at 12,000xg 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 260nm and 280nm. For pure RNA preparation the ratio of absorbance at 260/280 was ≥ 1.7. The concentration of RNA was calculated as one absorbance $_{260} = 42\mu g$.

REAL-TIME POLYMERASE CHAIN REACTION

cDNA synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA arhive kit in 0.2ml microfuge tubes. The reaction mixture of 20 µl contained 0.2µg total RNA, 10 X RT buffer, 25 X dNTP mixture, 10 X 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 assays

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 muscarinic M1, M3, α7 nicotinic acetylcholine, dopamine D1, dopamine D2, insulin, Vitamin D reecptors, acetylcholine esterase, choline acetyl transferase, GLUT3, GLUT2, super oxide dismutase, phospholipase C, PDX1 and CREB. 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 ($2^{-\Delta\Delta}C\ T$).

IMMUNOHISTOCHEMISTRY OF MUSCARINIC M1, M3 AND α7 NICOTINIC ACETYLCHOLINE RECEPTOR IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS USING CONFOCAL MICROSCOPE

Control and experimental rats were deeply anesthetized with ether. The rat were 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. 10 µm sections were cut using Cryostat (Leica, CM1510 S). The sections were treated with PBST (PBS in 0.01% Triton X-100) for 20 min. Brain slices were incubated overnight at 4°C with either rat primary antibody for muscarinic M1, M3 and α7 nicotinic acetylcholine receptor, 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 FITC. The sections were observed and photographed using confocal imaging system (Leica SP 5). The specificity of the immunohistochemical procedure is validated by negative controls to ensure that the labelling method accurately, identified the antibody bound to the specific muscarinic M1, M3 and α7 nicotinic acetylcholine receptor in the brain regions. Expressions of muscarinic M1, M3 and α7 nicotinic acetylcholine receptor were analysed using pixel intensity method. The given mean pixel value is the net value which is deducted from the negative control pixel value (Peeyush *et al.,* 2010).

IMMUNOCYTOCHEMISTRY OF MUSCARINIC M1, M3 RECEPTORS, ACETYLCHOLINE ESTERASE AND VESICULAR ACETYLCHOLINE TRANSPORTER EXPRESSION IN THE PANCREAS OF CONTROL AND EXPERIMENTAL RATS USING CONFOCAL MICROSCOPE

Pancreatic islets were isolated from control and experimental 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: 137mM Choline chloride, 5.4mM KCl, 1.8mM $CaCl_2$, 0.8mM $MgSO_4$, 1mM KH_2PO_4, 14.3mM $KHCO_3$ and 10mM HEPES**.** The pancreas was aseptically transferred to a sterile glass vial containing 2.0ml 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 (300rpm/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% was assessed by Trypan Blue. The islets were seeded in culture wells and allowed to adhere to the plate. The islets were rinsed with PBS and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH- 7.0., for 30 minutes on ice. After fixation, the islets were washed thrice with blocking buffer containing 0.1 M phosphate buffer, pH- 7.0., 0.1% Triton X and 10% BSA. Then the islets were incubated with primary antibody for muscarinic M1, M3 receptors, acetylcholine esterase and vesicular acetylcholine transporter, diluted in PBST at 1: 1000 dilution), prepared in blocking buffer with 1% serum and incubated overnight at 4°C. After the incubation, the islets were washed thrice with blocking buffer. Then the islets were incubated with secondary antibody tagged with FITC (No: AB7130F, Chemicon, diluted in PBST at 1: 1000 dilution) diluted in blocking buffer with 1% serum and incubated at room temperature in dark for two hours. After incubation the islets were rinsed with blocking buffer and were observed and

photographed using confocal imaging system (Leica SP 5). The specificity of the immunocytochemical procedure is validated by negative controls to ensure that the labelling method accurately identifies the antibody bound to the specific muscarinic M1, M3 receptors, acetylcholine esterase and vesicular acetylcholine transporter in the pancreatic islets. Expressions of muscarinic M1, M3 receptors, acetylcholine esterase and vesicular acetylcholine transporter were analysed using pixel intensity method. The given mean pixel value is the net value which is deducted from the negative control pixel value (Peeyush *et al.,* 2010).

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

The body weight was significantly decreased ($p<0.001$) in the diabetic rats when compared to control group. After insulin treatment, curcumin and Vitamin D_3 supplementation for 14 days, the body weight reversed to near the initial body weight (Table-1, Figure-1).

BLOOD GLUCOSE LEVEL

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 group. Insulin curcumin and Vitamin D_3 treatments were able to significantly reverse ($p<0.001$) the increased blood glucose level to near the control level when compared to diabetic group (Table-2, Figure-2).

CIRCULATING INSULIN LEVEL

There was a significant decrease in the serum insulin level of the diabetic group when compared to control ($p<0.001$). Insulin curcumin and Vitamin D_3 treatment for 14 days significantly increased ($p<0.001$) the serum insulin level to near control level when compared to diabetic group (Table-3, Figure-3).

CIRCULATING TRIIDOTHYRONINE (T3) CONTENT LEVEL

There was a significant decrease in the serum T3 level of the diabetic group when compared to control group ($p<0.001$). Insulin curcumin and Vitamin D_3

treatment for 14 days significantly increased ($p<0.001$) the serum T3 level to near control level when compared to diabetic group (Table-4, Figure-4).

BEHAVIOURAL STUDIES

Behavioural response of control and experimental rats on Y-Maze performance

Number of visits and time spent in the novel arm decreased significantly ($p<0.001$) in the diabetic group compared to control. Lower percentage of arm visits between the novel arm and the start arm and decreased time spent in the novel arm compared to the other two arms within the diabetic rats showed their decreased exploratory behaviour. Time spent in the novel arm and number of visit to the novel arm reversed to near control in the diabetic rats treated with insulin, curcumin and Vitamin D_3 (Table-5, Figure-5).

Rotarod performance of control and experimental groups of rats

Rotarod experiment showed a significant decrease in the retention time on the rotating rod in the diabetic rats at 10 ($p<0.01$), 15 ($p<0.001$) and 25 ($p<0.001$) revolutions per minute (rpm) when compared to control. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats significantly reversed the retention time near to control at 10 ($p<0.001$), 15 ($p<0.001$) and 25 ($p<0.001$) rpm (Table -6, Figure-6).

Behavioural response of control and experimental rats on grid walk test

There was significant increase ($p<0.001$) in the foot falls in diabetic rats compared to control. Foot falls significantly reversed to near control in diabetic rats administered with insulin ($p<0.001$), curcumin ($p<0.001$) and Vitamin D_3 ($p<0.001$) (Table-7, Figure-7).

Behavioural response of control and experimental rats on narrow beam test

There was significant decrease in the retention of balance on the narrow beam ($p<0.001$) in diabetic rats compared to control. Balance on the narrow beam significantly reversed to near control in diabetic rats treated with insulin ($p<0.001$), curcumin ($p<0.001$) and Vitamin D_3 ($p<0.001$) (Table-8, Figure-8).

NEUROTRANSMITTERS, VITAMIN D, INSULIN RECEPTORS, GLUT3, PHOSPHOLIPASE C, CREB AND SUPEROXIDE DISMUTASE EXPRESSION IN THE BRAIN REGIONS AND PANCREAS OF EXPERIMENTAL RATS

CEREBRAL CORTEX

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebral cortex of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, [^{3}H] QNB and muscarinic general antagonist atropine. The Scatchard analysis showed that the B_{max} ($p<0.001$) and K_d ($p<0.05$) decreased significantly in diabetic rats compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} ($p<0.001$) and K_d ($p<0.01$) significantly reversed to near control value when compared to diabetic group (Table-9, 10 & Fig-9, 10).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebral cortex of control and experimental rats.

Binding analysis of muscarinic M1 receptor was done using [^{3}H] QNB and M1 subtype specific antagonist pirenzepine. The B_{max} and K_d decreased significantly ($p<0.001$) in diabetic group when compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} ($p<0.001$) and K_d ($p<0.001$) significantly reversed to near control value when compared to diabetic group (Table-11, 12 & Fig-11, 12).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor, antagonist, 4-DAMP mustard in the cerebral cortex of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using [^{3}H] DAMP and M3 subtype specific antagonist 4-DAMP mustard. The B_{max} and K_d was increased significantly ($p<0.001$) in diabetic group when compared to control. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} ($p<0.001$) and K_d ($p<0.001$) significantly reversed to near control value when compared to diabetic group (Table-13, 14 & Fig-13, 14).

Dopamine receptor analysis

Scatchard analysis of [^{3}H] dopamine binding against dopamine in the cerebral cortex of control and experimental rats.

Binding analysis of dopamine receptors was done using [^{3}H] dopamine and unlabelled dopamine. The B_{max} and K_d was increased significantly ($p<0.001$) in diabetic group when compared to control. The K_d also increased significantly when compared to control group ($p<0.001$). In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} ($p<0.001$) and K_d ($p<0.001$) significantly reversed to near control value when compared to diabetic group (Table-15, 16 & Fig-15, 16).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation ($p<0.001$) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the altered expression to near control (Table-17, Figure-17).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control (Table-18, Figure-18).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression was decreased significantly ($p<0.001$) in diabetic rats and it was reversed significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-19, Figure-19).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression was increased significantly ($p<0.001$) in diabetic rats and it was reversed significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-20, Figure-20).

Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression was increased significantly ($p<0.001$) in diabetic rats and it was reversed significantly to near control in curcumin and Vitamin D_3 treated diabetic rats. Insulin treatment did not show any significant change in α7 nicotinic acetylcholine receptor gene expression when compared to diabetes (Table-21, Figure-21).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression was increased significantly ($p<0.001$) in diabetic rats and it was reversed

significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-22, Figure-22).

Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression was increased significantly ($p<0.001$) in diabetic rats and it was reversed significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-23, Figure-23).

Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats

Real Time-PCR analysis showed that the Vitamin D receptor gene expression was decreased significantly ($p<0.001$) in diabetic rats and it was reversed significantly ($p<0.001$) to near control in insulin and Vitamin D_3 treated diabetic rats. Curcumin treatment did not show any significant change in Vitamin D receptor gene expression when compared to diabetes (Table-24, Figure-24).

Real Time-PCR analysis of insulin receptor in the control and experimental rats

Real Time-PCR analysis showed that the insulin receptor gene expression was decreased significantly ($p<0.001$) in diabetic rats and it was reversed ($p<0.001$) significantly to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-25, Figure-25).

Real Time-PCR analysis of GLUT3 in the control and experimental rats

Gene expression of GLUT3 mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control (Table-26, Figure-26).

Real Time-PCR analysis of phospholipase C in the control and experimental rats

Gene expression of phospholipase C mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control (Table-27, Figure-27).

Real Time-PCR analysis of CREB in the control and experimental rats

Gene expression of CREB mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic rats compared to control. Treatment using curcumin ($p<0.001$) and Vitamin D_3 ($p<0.001$) significantly reversed these changes to near control. Insulin treatment did not show any significant change in CREB mRNA expression when compared to diabetes (Table-28, Figure-28).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control (Table-29, Figure-29).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the cerebral cortex of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the cerebral cortex showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats significantly reversed ($p<0.001$) the muscarinic M1 receptor expression in the cerebral cortex to near control (Table-30, Figure-30).

Muscarinic M3 receptor antibody staining in the cerebral cortex of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the cerebral cortex showed a significant increase ($p<0.001$) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats significantly reversed ($p<0.001$) the muscarinic M3 receptor expression in the cerebral cortex to near control (Table-31, Figure-31).

α7 nicotinic acetylcholine receptor antibody staining in the cerebral cortex of control and experimental rats

α7 nicotinic acetylcholine receptor subunit antibody staining in the cerebral cortex showed a significant increase ($p<0.001$) in the mean pixel value in diabetic rats compared to control. Curcumin and Vitamin D_3 treated diabetic rats significantly reversed ($p<0.001$) the muscarinic M3 receptor expression in the cerebral cortex to near control. Insulin treatment did not show any significant reversal when compared to diabetic rats (Table-32, Figure-32).

CEREBELLUM

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebellum of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, [^{3}H] QNB and muscarinic general antagonist atropine. The Scatchard analysis showed that the B_{max} ($p<0.001$) and K_d ($p<0.01$) increased significantly in diabetic rats compared to control group. In insulin treated diabetic rats B_{max} and K_d significantly ($p<0.001$) reversed to near control when compared to diabetic group. Curcumin and Vitamin D_3 treatment significantlyly reversed the B_{max} ($p<0.01$) to near control value with out any change in K_d when compared to diabetic group (Table-33, 34 & Fig-33, 34).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebellum of control and experimental rats

Binding analysis of muscarinic M1 receptors was done using [^{3}H] QNB and M1 subtype specific antagonist pirenzepine. The B_{max} and K_d increased significantly ($p<0.001$) in diabetic group when compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats B_{max} ($p<0.001$) and K_d ($p<0.01$) significantly reversed to near control value when compared to diabetic group (Table-35, 36 & Fig-35, 36).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebellum of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using [^{3}H] DAMP and M3 subtype specific antagonist 4-DAMP mustard. The B_{max} and K_d was increased significantly ($p<0.001$) in diabetic group when compared to control group. In insulin and curcumin treated diabetic rats, B_{max} ($p<0.001$) and K_d ($p<0.01$) significantly reversed to near control when compared to diabetic group. Vitamin D_3 treatment significantly reverse the B_{max} ($p<0.001$) and K_d ($p<0.001$) to near control value when compared to diabetic group (Table-38 & Fig-38).

Dopamine receptor analysis

Scatchard analysis of [^{3}H] dopamine binding against dopamine in the cerebellum of control and experimental rats.

Binding analysis of total dopamine receptors was done using [^{3}H] dopamine and unlabelled dopamine. The B_{max} ($p<0.001$) and K_d ($p<0.01$) decreased significantly in diabetic group when compared to control. In insulin, curcumin and Vitamin D_3 treated diabetic rats B_{max} ($p<0.001$) and K_d ($p<0.01$) significantly reversed to near control value when compared to diabetic group (Table-39, 40 & Fig-39, 40).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation ($p<0.001$) in the cerebellum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the altered expression to near control (Table-41, Figure-41).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic rats compared to control. Curcumin and Vitamin D_3 ($p<0.001$), insulin ($p<0.01$) treatment significantly reversed the changes to near control (Table-42, Figure-42).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it reversed significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-43, Figure-43).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression was increased significantly ($p<0.001$) in diabetic condition and it reversed

significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-44, Figure-44).

Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it reversed to near control value in curcumin and Vitamin D_3 ($p<0.001$) treated diabetic rats. Insulin treatment did not show any significant change in α7 nicotinic acetylcholine receptor gene expression when compared to diabetes (Table-45, Figure-45).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it reversed significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-46, Figure-46).

Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it reversed significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-47, Figure-47).

Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats

Real Time-PCR analysis showed that the Vitamin D receptor gene expression significantly ($p<0.001$) increased in diabetic condition and insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed to near control (Table-48, Figure-48).

Real Time-PCR analysis of insulin receptor in the control and experimental rats

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly ($p<0.001$) in diabetic rats and treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed to near control. (Table-49, Figure-49).

Real Time-PCR analysis of GLUT3 in the control and experimental rats

Gene expression of GLUT3 mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control (Table-50, Figure-50).

Real Time-PCR analysis of phospholipase C in the control and experimental rats

Gene expression of phospholipase C mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic rats compared to control. Treatment using curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control. Insulin treatment did not show any significant change when compared to diabetic (Table-51, Figure-51).

Real Time-PCR analysis of CREB in the control and experimental rats

Gene expression of CREB mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.01$) reversed the changes to near control (Table-52, Figure-52).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control (Table-53, Figure-53).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the cerebellum of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the cerebellum showed a significant increase ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treated diabetic rats significantly ($p<0.001$) reversed the muscarinic M1 receptor expression in the cerebellum to near control level (Table-54, Figure-54).

Muscarinic M3 receptor antibody staining in the cerebellum of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the cerebellum showed a significant increase ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treated diabetic rats significantly ($p<0.001$) reversed the muscarinic M3 receptor expression in the cerebellum to near control level (Table-55, Figure-55).

α7 nicotinic acetylcholine receptor antibody staining in the cerebellum of control and experimental rats

α7 nicotinic acetylcholine receptor subunit antibody staining in the cerebellum showed a significant increase ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Curcumin and Vitamin D_3 treated diabetic rats significantly reversed ($p<0.001$) the muscarinic M3 receptor expression in the cerebellum to near control level. Insulin treatment did not show any significant reversal when compared to diabetic (Table-56, Figure-56).

BRAIN STEM

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the brain stem of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, [^{3}H] QNB and muscarinic general antagonist, atropine. The Scatchard analysis showed that the B_{max} increased significantly ($p<0.001$) in diabetic rats with out any significant change in the K_d when compared to control. Treatment with insulin,

curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the B_{max} to near control when compared to diabetic group. K_d did not show any significant change when compared to diabetic (Table-57, 58 & Figure- 57, 58).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the brain stem of control and experimental rats.

Binding analysis of muscarinic M1 receptors was done using [^{3}H] QNB and M1 subtype specific antagonist pirenzepine. The B_{max} and K_d decreased significantly ($p<0.001$) in diabetic rats when compared to control. In insulin, curcumin and Vitamin D_3 treated diabetic rats B_{max} ($p<0.001$) and K_d ($p<0.01$) significantly reversed to near control value when compared to diabetic group (Table-59, 60 & Fig-59, 60).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the brain stem of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using [^{3}H] DAMP and M3 subtype specific antagonist 4-DAMP mustard. The B_{max} ($p<0.001$) and K_d ($p<0.01$) increased significantly in diabetic group when compared to control group. B_{max} of insulin, Vitamin D_3 ($p<0.01$) and curcumin ($p<0.001$) treated diabetic rats significantly reversed to near control when compared to diabetic. In insulin, curcumin ($p<0.05$) and Vitamin D_3 ($p<0.001$), K_d significantly reversed to near control when compared to diabetic (Table-61, 62 & Fig-61, 62).

Dopamine receptor analysis

Scatchard analysis of [^{3}H] dopamine binding against dopamine in the brain stem of control and experimental rats.

Binding analysis of total dopamine receptors was done using [^{3}H] dopamine and unlabelled dopamine. The B_{max} and K_d increased significantly ($p<0.001$) in diabetic group when compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} and K_d significantly ($p<0.001$) reversed to near control when compared to diabetic group (Table-63, 64 & Fig-63, 64).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation ($p<0.001$) in the brain stem of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the expression to near control (Table-65, Figure-65).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant up regulation ($p<0.001$) in the brain stem of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the expression to near control (Table-66, Figure-66).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression was decreased significantly ($p<0.001$) in diabetic rats and it was significantly ($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 ($p<0.001$) treated diabetic rats (Table-67, Figure-67).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it was significantly ($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 ($p<0.001$) treated diabetic rats (Table-68, Figure-68).

Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it was significantly reversed ($p<0.001$) to near control in curcumin and Vitamin D_3 treated diabetic rats. Insulin treatment did not show any significant change in α7 nicotinic acetylcholine receptor gene expression when compared to diabetes (Table-69, Figure-69).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it was significantly

($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-70, Figure-70).

Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression decreased significantly ($p<0.001$) in diabetic rats and it was significantly ($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-71, Figure-72).

Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats

Real Time-PCR analysis showed that the Vitamin D receptor gene expression increased significantly ($p<0.001$) in diabetic rats and insulin ($p<0.01$), curcumin and Vitamin D_3 ($p<0.001$) treated diabetic rats, it was reversed significantly to near control (Table-72, Figure-72).

Real Time-PCR analysis of insulin receptor in the control and experimental rats

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly ($p<0.001$) in diabetic condition and it was significantly ($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-73, Figure-73).

Real Time-PCR analysis of GLUT3 in the control and experimental rats

Gene expression of GLUT3 mRNA showed significant up regulation ($p<0.001$) in the brain stem of diabetic rats compared to control. Treatment using

insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control (Table-74, Figure-74).

Real Time-PCR analysis of phospholipase C in the control and experimental rats

Gene expression of phospholipase C mRNA showed significant down regulation ($p<0.001$) in the brain stem of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control (Table-75, Figure-75).

Real Time-PCR analysis of CREB in the control and experimental rats

Gene expression of CREB mRNA showed significant down regulation ($p<0.001$) in the brain stem of diabetic rats compared to control. Treatment using curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control. Insulin treatment did not show any significant change in CREB mRNA expression when compared to diabetes (Table-76, Figure-76).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant up regulation ($p<0.001$) in the brain stem of diabetic rats compared to control. Treatment using insulin ($p<0.01$), curcumin and Vitamin D_3 ($p<0.001$) significantly reversed these changes to near control when compared to diabetic. (Table-77, Figure-77).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the brain stem of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the brainstem showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats significantly reversed ($p<0.001$) the muscarinic M1 receptor expression in the brain stem to near control (Table-78, Figure-78).

Muscarinic M3 receptor antibody staining in the brain stem of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the brainstem showed a significant increase ($p<0.001$) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats significantly reversed ($p<0.001$) the muscarinic M3 receptor expression in the brainstem to near control (Table-79, Figure-79).

α7 nicotinic acetylcholine receptor antibody staining in the brain stem of control and experimental rats

α7 nicotinic acetylcholine receptor antibody staining in the brainstem showed a significant increase ($p<0.001$) in the mean pixel value in diabetic rats compared to control. Curcumin and Vitamin D_3 treated diabetic rats significantly ($p<0.001$) reversed the muscarinic M3 receptor expression in the brain stem to near control level. Insulin treatment did not show any significant reversal when compared to diabetic (Table-80, Figure-80).

CORPUS STRIATUM

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the corpus striatum of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, [^{3}H] QNB and muscarinic general antagonist, atropine. The Scatchard analysis showed that the B_{max} decreased significantly ($p<0.001$) in diabetic rats with out any significant change in the K_d when compared to control group. Treatment with insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the B_{max} to near control when compared to diabetic group. K_d did not show any significant change when compared to diabetic (Table-81, 82 & Figure- 81, 82).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the corpus striatum of control and experimental rats

Binding analysis of muscarinic M1 receptors was done using [^{3}H] QNB and M1 subtype specific antagonist pirenzepine. The B_{max} increased and K_d decreased significantly ($p<0.001$) in diabetic group when compared to control group. In insulin ($p<0.001$), curcumin and Vitamin D_3 ($p<0.01$) treated diabetic rats B_{max} significantly reversed to near control when compared to diabetic group. K_d in insulin, curcumin ($p<0.001$) and Vitamin D_3 ($p<0.01$) treated diabetic rats significantly reversed to near control when compared to diabetic group (Table-83, 84 & Fig-83, 84).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the corpus striatum of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using [^{3}H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard. The B_{max} decreased significantly ($p<0.001$) in diabetic group without any change in K_d when compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} was significantly ($p<0.001$) reversed back to near control when compared to diabetic group. K_d did not show any significant change when compared to diabetic (Table-85, 86 & Fig-85, 86).

Dopamine receptor analysis

Scatchard analysis of [^{3}H] dopamine binding against dopamine in the corpus striatum of control and experimental rats.

Binding analysis of total dopamine receptors was done using [^{3}H] dopamine and unlabelled dopamine. The B_{max} and K_d decreased significantly ($p<0.001$) in diabetic group when compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} significantly ($p<0.001$) reversed back to near control. K_d of curcumin and Vitamin D_3 treated diabetic rats significantly reversed the changes when compared to diabetic group whereas K_d of insulin treated diabetic rats did not show any significant change when compared to diabetic group (Table-87, 88 & Fig-87, 88).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control when compared to diabetic (Table-89, Figure-89).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control when compared to diabetic (Table-90, Figure-90).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression was increased significantly ($p<0.001$) in diabetic rats and it was reversed significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-91, Figure-91).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression decreased significantly ($p<0.001$) in diabetic rats. Treatment using insulin, curcumin ($p<0.01$) and Vitamin D_3 ($p<0.001$) significantly reversed these changes to near control when compared to diabetic (Table-92, Figure-92).

Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression was increased significantly ($p<0.001$) in diabetic rats. Treatment using insulin, curcumin ($p<0.01$) and Vitamin D_3 ($p<0.001$) significantly reversed these changes to near control when compared to diabetic (Table-93, Figure-93).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression was decreased significantly ($p<0.001$) in diabetic rats and it was reversed significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-94, Figure-94).

Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression increased significantly ($p<0.001$) in diabetic rats. Treatment using insulin,

curcumin ($p<0.001$) and Vitamin D_3 ($p<0.01$) significantly reversed these changes to near control when compared to diabetic (Table-95, Figure-95).

Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats

Real Time-PCR analysis showed that the Vitamin D receptor gene expression decreased significantly ($p<0.001$) in diabetic rats. Treatment using insulin, curcumin ($p<0.01$) and Vitamin D_3 ($p<0.001$) significantly reversed these changes to near control when compared to diabetic (Table-96, Figure-96).

Real Time-PCR analysis of insulin receptor in the control and experimental rats

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it was reversed significantly ($p<0.001$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-97, Figure-97).

Real Time-PCR analysis of GLUT3 in the control and experimental rats

Gene expression of GLUT3 mRNA showed significant up regulation ($p<0.001$) in the corpus striatum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control (Table-98, Figure-98).

Real Time-PCR analysis of phospholipase C in the control and experimental rats

Gene expression of phospholipase C mRNA showed significant up regulation ($p<0.001$) in the corpus striatum of diabetic rats compared to control. Treatment using

insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control (Table-99, Figure-99).

Real Time-PCR analysis of CREB in the control and experimental rats

Gene expression of CREB mRNA showed significant up regulation ($p<0.001$) in the corpus striatum of diabetic rats compared to control. Treatment using curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control. Insulin treatment did not show any significant change in CREB mRNA expression when compared to diabetic rats (Table-100, Figure-100).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed these changes to near control (Table-101, Figure-101).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the corpus striatum of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the corpus striatum showed a significant increase ($p<0.001$) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats

significantly reversed ($p<0.001$) the muscarinic M1 receptor expression in the corpus striatum to near control (Table-102, Figure-102).

Muscarinic M3 receptor antibody staining in the corpus striatum of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the corpus striatum showed a significant decrease ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats significantly reversed ($p<0.001$) the muscarinic M3 receptor expression in the corpus striatum to near control (Table-103, Figure-103).

α7 nicotinic acetylcholine receptor antibody staining in the corpus striatum of control and experimental rats

α7 nicotinic acetylcholine receptor subunit antibody staining in the corpus striatum showed a significant increase ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Curcumin and Vitamin D_3 treated diabetic rats significantly reversed ($p<0.001$) the muscarinic M3 receptor expression in the corpus striatum to near control level. Insulin treatment did not show any significant reversal when compared to diabetic (Table-104, Figure-104).

HIPPOCAMPUS

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the hippocampus of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, [^{3}H] QNB and muscarinic general antagonist, atropine. The Scatchard analysis showed that the B_{max} and K_d decreased significantly ($p<0.001$) in diabetic rats. Treatment with insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the B_{max} and K_d to near control (Table-105, 106 & Figure-105, 106).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the hippocampus of control and experimental rats

Binding analysis of muscarinic M1 receptors was done using [^{3}H] QNB and M1 subtype specific antagonist pirenzepine. The B_{max} decreased significantly ($p<0.001$) in diabetic group with out any change in K_d when compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} significantly ($p<0.001$) reversed to near control when compared to diabetic. K_d did not show any significant change when compared to diabetic (Table-107, 108 & Figure- 107, 108).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the hippocampus of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using [^{3}H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard. The B_{max} and K_d increased significantly ($p<0.001$) in diabetic group when compared to control group. B_{max} in insulin ($p<0.001$), curcumin and Vitamin D_3 ($p<0.01$) treated diabetic rats significantly reversed back to near control value when compared to diabetic group. K_d did not show any significance change when compared to diabetic group (Table-109, 110 & Figure-109, 110).

Dopamine receptor analysis

Scatchard analysis of [^{3}H] dopamine binding against dopamine in the hippocampus of control and experimental rats.

Binding analysis of total dopamine receptors was done using [^{3}H] dopamine and unlabelled dopamine. The B_{max} increased significantly ($p<0.001$) in diabetic group with out any change in K_d when compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} significantly ($p<0.001$) reversed to near control when compared to diabetic group. K_d did not show any significance change in treatment groups when compared to diabetic (Table-111, 112 & Figure-111, 112).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation ($p<0.001$) in the hippocampus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control when compared to diabetic (Table-113 & Figure-113).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control when compared to diabetic (Table-114, Figure-114).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression decreased significantly ($p<0.001$) in diabetic condition and it was significantly reversed to near control in insulin and curcumin ($p<0.001$), Vitamin D_3 ($p<0.01$), when compared to diabetic rats (Table-115, Figure-115).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression increased significantly ($p<0.001$) in diabetic condition and it was reversed to near control in insulin, curcumin ($p<0.01$) and Vitamin D_3 ($p<0.001$) treated diabetic rats (Table-116, Figure-116).

Real Time-PCR analysis of α7 nicotinic acetylcholine receptor in the control and experimental rats

Real Time-PCR analysis showed that α7 nicotinic acetylcholine receptor gene expression decreased significantly ($p<0.001$) in diabetic condition and it was significantly reversed to near control value in curcumin ($p<0.01$) and Vitamin D_3 ($p<0.001$) treated diabetic rats. Insulin treatment did not show any significant change in α7 nicotinic acetylcholine receptor gene expression when compared to diabetic rats (Table-117, Figure-117).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it was reversed significantly ($p<0.001$) to near control in insulin and curcumin treated diabetic rats. Vitamin D_3 did not show any significantly change in dopamine D1 receptor gene expression when compared to diabetic rats (Table-118, Figure-118).

Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression increased significantly ($p<0.001$) in diabetic condition and it was reversed significantly ($p<0.001$) to near control in insulin and curcumin treated diabetic rats. Vitamin D_3 did not show any significant change in dopamine D2 receptor gene expression when compared to diabetic rats (Table-119, Figure-119).

Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats

Real Time-PCR analysis showed that the Vitamin D receptor gene expression decreased significantly ($p<0.001$) in diabetic condition and it was significantly ($p<0.01$) reversed to near control in insulin, curcumin and Vitamin D_3 treatment (Table-120, Figure-120).

Real Time-PCR analysis of insulin receptor in the control and experimental rats

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly ($p<0.001$) in diabetic condition and it was significantly ($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-121, Figure-121).

Real Time-PCR analysis of GLUT3 in the control and experimental rats

Gene expression of GLUT3 mRNA showed significant up regulation ($p<0.001$) in the hippocampus of diabetic rats compared to control. Treatment using insulin ($p<0.001$), curcumin and Vitamin D_3 ($p<0.01$), significantly reversed these changes to near control. (Table-122, Figure-122).

Real Time-PCR analysis of phospholipase C in the control and experimental rats

Gene expression of phospholipase C mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control when compared to diabetic rats (Table-123, Figure-123).

Real Time-PCR analysis of CREB in the control and experimental rats

Gene expression of CREB mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic rats compared to control. Treatment using curcumin and Vitamin D_3 significantly ($p<0.01$) reversed the changes to near control. Insulin treatment did not show any significant change in CREB mRNA expression when compared to diabetes (Table-124, Figure-124).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D3 significantly ($p<0.001$) reversed the changes to near control when compared to diabetic (Table-125, Figure-125).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the hippocampus of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the hippocampus showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats significantly reversed ($p<0.001$) the muscarinic M1 receptor expression in the hippocampus to near control (Table-126, Figure-126).

Muscarinic M3 receptor antibody staining in the hippocampus of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the hippocampus showed a significant increase ($p<0.001$) in the mean pixel value in diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats significantly reversed ($p<0.001$) the muscarinic M3 receptor expression in the hippocampus to near control (Table-127, Figure-127).

α7 nicotinic acetylcholine receptor antibody staining in the hippocampus of control and experimental rats

α7 nicotinic acetylcholine receptor subunit antibody staining in the hippocampus showed a significant decrease ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Curcumin and Vitamin D_3 treated diabetic rats significantly reversed ($p<0.001$) the α7 nicotinic acetylcholine receptor expression in the hippocampus to near control level. Insulin treatment did not show any significant reversal when compared to diabetic (Table-128, Figure-128).

HYPOTHALAMUS

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation ($p<0.001$) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control when compared to diabetic (Table-129 & Figure-129).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation ($p<0.001$) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control when compared to diabetic (Table-130, Figure-130).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression decreased significantly ($p<0.001$) in diabetic condition and it significantly reversed to near control in insulin, curcumin ($p<0.001$) and Vitamin D_3 ($p<0.01$) treated diabetic rats (Table-131, Figure-131).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it was significantly ($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-132, Figure-132).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression decreased significantly ($p<0.001$) in diabetic rats. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control when compared to diabetic rats (Table-133, Figure-133).

Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression decreased significantly ($p<0.001$) in diabetic rats. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control when compared to diabetic rats (Table-134, Figure-134).

Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats

Real Time-PCR analysis showed that the Vitamin D receptor gene expression increased significantly ($p<0.001$) in diabetic condition and it was significantly

reversed to near control value in insulin, Vitamin D_3 ($p<0.001$) and curcumin ($p<0.01$) treated diabetic rats (Table-135, Figure-135).

Real Time-PCR analysis of insulin receptor in the control and experimental rats

Real Time-PCR analysis showed that the insulin receptor gene expression increased significantly ($p<0.001$) in diabetic condition and it reversed to near control value in insulin ($p<0.001$), curcumin ($p<0.001$) and Vitamin D_3 ($p<0.001$) treated diabetic rats (Table-136, Figure-136).

Real Time-PCR analysis of GLUT3 in the control and experimental rats

Gene expression of GLUT3 mRNA showed significant up regulation ($p<0.001$) in the hypothalamus of diabetic rats compared to control. Treatment using insulin ($p<0.01$), curcumin and Vitamin D_3 ($p<0.001$) treatment significantly reversed the changes to near control. (Table-137 & Figure-137).

Real Time-PCR analysis of phospholipase C in the control and experimental rats

Gene expression of phospholipase C mRNA showed significant down regulation ($p<0.001$) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control (Table-138, Figure-138).

Real Time-PCR analysis of CREB in the control and experimental rats

Gene expression of CREB mRNA showed significant down regulation ($p<0.001$) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin ($p<0.01$) Vitamin D_3 ($p<0.001$) treatment significantly reversed the changes to near control (Table-139, Figure-139).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant up regulation ($p<0.001$) in the hypothalamus of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control (Table-140, Figure-140).

PANCREAS

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the pancreas of control and experimental rats

The total muscarinic receptor status was assayed using the specific ligand, [^{3}H] QNB and muscarinic general antagonist, atropine. The Scatchard analysis showed that the B_{max} and K_d decreased significantly ($p<0.001$) in diabetic rats when compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats B_{max} ($p<0.001$) and K_d ($p<0.01$) significantly reversed to near control value when compared to diabetic group (Table-141, 142 & Fig-141, 142).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the pancreas of control and experimental rats

Binding analysis of Muscarinic M1 receptors was done using [^{3}H] QNB and M1 subtype specific antagonist, pirenzepine. The B_{max} decreased significantly ($p<0.001$) in diabetic rat when compared to control. The K_d did not show any

significant change when compared to control. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} significantly ($p<0.001$) reversed back to near control when compared to diabetic group. K_d did not show any significant change in the treatment group. (Table-143, 144 & Fig-143, 144).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist 4-DAMP mustard in the pancreas of control and experimental rats.

Binding analysis of muscarinic M3 receptors was done using [^{3}H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard. The B_{max} decreased significantly ($p<0.001$) in diabetic group when compared to control group. The K_d did not show any significant change when compared to control group. In insulin, curcumin and Vitamin D_3 treated diabetic rats, B_{max} was significantly ($p<0.001$) reversed back to near control when compared to diabetic group. K_d did not show any significant change when compared to diabetic group. (Table-145, 146 & Fig-145, 146).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of acetylcholine esterase in the control and experimental rats

Gene expression of acetylcholine esterase mRNA showed significant up regulation ($p<0.001$) in the pancreas of diabetic rats compared to control. Treatment using insulin, Vitamin D_3 ($p<0.001$) and curcumin ($p<0.01$) significantly reversed the altered expression to near control (Table-147, Figure-147).

Real Time-PCR analysis of choline acetyltransferase in the control and experimental rats

Gene expression of choline acetyltransferase mRNA showed significant down regulation ($p<0.001$) in the pancreas of diabetic rats compared to control. Treatment using curcumin, Vitamin D_3 ($p<0.001$) and insulin ($p<0.01$) significantly reversed the altered expression to near control (Table-148, Figure-148).

Real Time-PCR analysis of muscarinic M1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M1 receptor gene expression decreased significantly ($p<0.001$) in diabetic rats. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the altered expression to near control (Table-149, Figure-149).

Real Time-PCR analysis of muscarinic M3 receptor in the control and experimental rats

Real Time-PCR analysis showed that the muscarinic M3 receptor gene expression decreased significantly ($p<0.001$) in diabetic rats and it was significantly ($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-150, Figure-150).

Real Time-PCR analysis of dopamine D1 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D1 receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it was significantly

($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-151, Figure-151).

Real Time-PCR analysis of dopamine D2 receptor in the control and experimental rats

Real Time-PCR analysis showed that the dopamine D2 receptor gene expression increased significantly ($p<0.001$) in diabetic rats and it was significantly ($p<0.001$) reversed to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-152, Figure-152).

Real Time-PCR analysis of Vitamin D receptor in the control and experimental rats

Real Time-PCR analysis showed that the Vitamin D receptor gene expression decreased significantly ($p<0.001$) in diabetic rats and it was significantly reversed to near control in insulin, Vitamin D_3 ($p<0.001$) and curcumin ($p<0.01$) treated diabetic rats (Table-153, Figure-153).

Real Time-PCR analysis of insulin receptor in the control and experimental rats

Real Time-PCR analysis showed that the insulin receptor gene expression decreased significantly ($p<0.001$) in diabetic rats and it was reversed significantly ($p<0.01$) to near control in insulin, curcumin and Vitamin D_3 treated diabetic rats (Table-154, Figure-154).

Real Time-PCR analysis of GLUT2 in the control and experimental rats

Gene expression of GLUT2 mRNA showed significant down regulation ($p<0.001$) in the pancreas of diabetic rats compared to control. Treatment using

insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control (Table-155, Figure-155).

Real Time-PCR analysis of phospholipase C in the control and experimental rats

Gene expression of phospholipase C mRNA showed significant down regulation ($p<0.001$) in the pancreas of diabetic rats compared to control. Treatment using insulin ($p<0.001$), curcumin ($p<0.01$) and Vitamin D_3 ($p<0.01$)significantly reversed the changes to near control (Table-156, Figure-156).

Real Time-PCR analysis of superoxide dismutase in the control and experimental rats

Gene expression of superoxide dismutase mRNA showed significant down regulation ($p<0.001$) in the pancreas of diabetic rats compared to control. Treatment using insulin, curcumin and Vitamin D_3 significantly ($p<0.001$) reversed the changes to near control (Table-157, Figure-157).

CONFOCAL STUDIES

Acetylcholine esterase antibody staining in the pancreas of control and experimental rats

Acetylcholine esterase antibody staining in the pancreas showed a significant decrease ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treated diabetic rats significantly ($p<0.001$) reversed the acetylcholine esterase expression in the pancreas to near control (Table-158, Figure-158).

Muscarinic M1 receptor antibody staining in the pancreas of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the pancreas showed a significant decrease ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treated diabetic rats showed a significant reversal ($p<0.001$) of muscarinic M1 receptor expression in the pancreas to near control level (Table-159, Figure-159).

Muscarinic M3 receptor antibody staining in the pancreas of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the pancreas showed a significant decrease ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treated diabetic rats showed a significant reversal ($p<0.001$) of muscarinic M3 receptor expression in the pancreas to near control level (Table-160, Figure-160).

Vesicular acetylcholine transporter antibody staining in the pancreas of control and experimental rats

Vesicular acetylcholine transporter antibody staining in the pancreas showed a significant decrease ($p<0.001$) in the mean pixel value of diabetic rats compared to control. Insulin, Curcumin and Vitamin D_3 treated diabetic rats showed a significant reversal ($p<0.001$) of vesicular acetylcholine transporter expression in the pancreas to near control level (Table-161, Figure-161).

Discussion

Diabetes mellitus is a major global health problem currently affecting more than 180 million people worldwide. The disease is one of the most severe metabolic disorders in humans and it is characterised by hyperglycaemia as a result of a relative or an absolute lack of insulin or the action of insulin on its target tissue or both. The neurological consequences of diabetes mellitus in the CNS are now receiving greater attention. Prolonged exposure to chronic hyperglycaemia in diabetes can lead to various complications, affecting the neurological, cardiovascular, renal and visual systems (Brownlee, 2001). The utilisation of glucose for cell's energy is critical in the functioning of the organs. Nutritional therapy is a challenging but necessary dimension in the management of diabetes and neurodegenerative changes associated with it.

BLOOD GLUCOSE & BODY WEIGHT

The STZ diabetic rat serves as an excellent model to study the molecular, cellular and morphological changes in brain induced by stress during diabetes (Aragno, *et al*., 2000). In the present study, STZ-induced rats were used as an experimental model for diabetes, since they provide a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycaemia (Low *et al*., 1997). The facts' that increased blood glucose level and decreased body weight, observed during diabetes, are similar with previous reports as a result of the marked destruction of insulin secreting pancreatic β-cells by STZ (Junod *et al*., 1969). Hyperglycemia 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 through acetyl-CoA.

Previous reports showed that curcumin has the potential to protect pancreatic islet cells against STZ-induced death dysfunction (Meghana *et al*., 2007) and increase plasma insulin level in diabetic mice (Seo *et al*., 2008). Previous studies showed that pancreatic insulin secretion is inhibited by Vitamin D deficiency (Norman *et al*., 1980). An increased prevalence of diabetes has been associated with Vitamin D-deficient individuals (Chiu *et al*., 2004). The results of this study have demonstrated that curcumin, Vitamin D_3 and insulin treatment to STZ-induced diabetic rats have beneficial effects in reducing blood glucose levels to near control. The results suggest that the mode of action of curcumin and Vitamin D_3 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 insulin, curcumin and Vitamin D_3 improved body weight significantly which indicate prevention of muscle tissue damage due to hyperglycemic condition. The central complications of hyperglycaemia also include potentiating of neuronal damage observed following hypoxic/ischemic events, as well as stroke. Glucose utilization is decreased in the brain during diabetes (McCall, 1992), providing a potential mechanism for increased vulnerability to acute pathological events.

CIRCULATING INSULIN LEVEL

There was a significant decrease in the circulating insulin level of diabetic rats when compared to control group. The increase in insulin levels in curcumin and Vitamin D_3 treated diabetic rats attribute to the stimulation of the surviving beta cells by the treatment, which in turn exerts an antihyperglycaemic action. Thus, it is suggested that the curcumin and Vitamin D_3 treatment induced insulin release from pancreas, thereby potentiating its effect. A possible mechanism of action is that the

curcumin and Vitamin D_3 stimulated the residual pancreatic β-cell function or produced the antihyperglycaemia through an extra-pancreatic mechanism, probably increasing peripheral utilization of glucose. This data confirmed the anti hyperglycemic activity of curcumin and Vitamin D_3.

CIRCULATING TRIIODOTHYRONINE (T3) LEVEL

Thyroid hormone is essential for maintaining normal neurological functions both during development and in adult life. Type III-iodothyronine deiodinase (D3) degrades thyroid hormones by converting thyroxine and 3, 3′, 5-triiodo-L-thyroinine (T3) to inactive metabolites. A regional expression of D3 activity has been observed in the human CNS and a critical role for D3 has been suggested in the regulation of local T3 content in concert with other enzymes. 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). T3 content in the serum was increased significantly in diabetic groups compared to control. Long term thyrotoxicosis has been shown to cause β cell dysfunction resulting in reduced pancreatic insulin content, poor insulin response to glucose and decreased rate of insulin secretion (Bech *et al*., 1996). Insulin, curcumin and Vitamin D_3 treatment significantly reversed the increased T3 content near to control. A reduced secretion of thyroid hormones with age has been documented in humans and animals with no substantial increase in TSH secretion, which is indicative of an age related impairment of the pituitary sensitivity to the negative control exerted by thyroid hormones (Schlienger *et al*., 1982).

BEHAVIOURAL DEFICITS IN DIABETIC RATS

Several studies have described the effects of diabetes in the central nervous system (CNS) as a series of neurochemical, neurophysiological and structural abnormalities, a condition referred to as diabetic encephalopathy (Biessels *et al*., 2002a; Sima *et al*., 2004). In addition to these abnormalities, impairments in cognitive function have been observed in diabetic patients and also in animal models of diabetes (Strachan *et al*., 2003; Brands *et al*., 2007). These impairments have been characterized mainly by moderate deficits in learning and memory, psychomotor slowing and reduced mental flexibility (Cukierman *et al*., 2005; Brands *et al*., 2007). Furthermore, diabetic patients also seem to double the probability of developing Alzheimer's disease and other dementias (Arvanitakis *et al*., 2004; Biessels *et al*., *2006*).

We evaluated the behavioural response of diabetic rats in Y-maze test and memory enhancing property of curcumin and Vitamin D_3. Y-maze is used to evaluate the spatial learning in different rat models (Murugesan, 2005). Also, motor performance of control and experimental rats on rotarod, beam walk and grid walk test were studied.

The Y-maze test is a classic model behavioral test, with a strong aversive component, utilized for evaluating learning and memory in rats and mice (Katz & Chudler, 1980; Woo et al., 2008). Y-maze performance showed that intensity of derangement in diabetic rats increased. These results are in agreement with other studies that have also verified cognitive impairment in STZ-induced diabetes mellitus (Kamal *et al*., 2000) which is associated with intensification of pathological processes within the cortical and other brain regions engaged in these processes (Artola *et al*., 2005). Furthermore, spatial memory and exploratory activity have an influence on behavioral tests including Y-maze performance. In this regard, the number of novel

arm entries and time spent was significantly lower in STZ-diabetic rats. There are also reports on the involvement of the cholinergic system abnormality in the impaired acquisition and/or retention of passive avoidance learning. In this respect, it has been postulated that the observed behavioral abnormalities consequent on an impairment of cerebral glucose metabolism suggestive of cholinergic dysfunction (Jackson *et al*., 2000). However, when the diabetic rats were treated with insulin, curcumin and Vitamin D_3, the time spent and number of novel arm entry in the Y-maze was similar to that found for rats from the control group. These findings indicate that curcumin and Vitamin D_3 were able to normalize the cholinergic receptor dysfunction which assists in lowering their time for spatial recognition and thus improving the cognitive functions.

Diabetes mellitus has been reported to be accompanied by a number of behavioural and hormonal abnormalities, including reduced locomotor activity (Marshall *et al*., 1976). Rotarod test has been used to examine the Motor in-coordination (Cendelin *et al*., 2008). The rotarod, beam walk and grid walk test experiment demonstrated the impairment of the motor function and coordination in the diabetic rats. Diabetic rats showed lower fall off time from the rotating rod when compared to control and increased number of foot slips in beam and grid walk test and decreased time spent in narrow beam test compared to control, suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture. At the same time, they adjusted their limb movements on the metallic rod which is indicative of cerebellar dysfunction. The insulin, curcumin and Vitamin D_3 treated diabetic rats showed an improved motor performance in rotarod, beam and grid walk test compared to STZ- induced diabetic rats. Our findings indicate that curcumin and Vitamin D_3 normalizes their alleviated stress level which assists in

lowering their time for spatial recognition and thus helps to maintain their posture during movement on the rod.

CHOLINERGIC ENZYME ALTERATIONS IN BRAIN AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS.

Choline acetyltransferase (ChAT) is the rate-limiting enzyme of generating acetylcholine (Ach), which is synthesized in cholinergic neuronal cell bodies and is often used in the studies of tissue localization and functional activity. The reduction of ChAT is correlated with the severity of dementia and pathologic changes (Rodrigo *et al*., 2004). The elevated activity of insulin could improve the expression of ChAT (Rivera *et al*., 2005). Acetylcholine is the primary neurotransmitter of the cholinergic system and its activity is regulated by acetylcholine esterase (AChE). The termination of nerve impulse transmission is accomplished through the degradation of acetylcholine into choline and acetyl CoA by AChE (Weihua Xie *et al*., 2000). Acetylcholine esterase activity has been used as a marker for cholinergic activity (Ellman *et al*., 1961). It has been well established that there is a marked change in the acetylcholine esterase activity in diabetic condition. Akmayev *et al*., (1978) showed that there is difference in distribution of the enzyme in the neurons of the central vagal nuclei and medulla oblongata in normal and diabetic adult male rats. It is suggested that the changes in the plasma glucose or insulin levels is influenced by the activity of cholinergic neurons. Cholinergic neurons may be regulated by insulin signaling, and require this signaling for repair and survival. Impairment of insulin signaling in cholinergic neurons results in a disorder of energy metabolism and impairs repair and cell survival, thus evoking a series of pathologic changes and corresponding clinical manifestations (Hongjuan *et al*., 2009). Thus central cholinergic activity is implicated in the insulin secretion.

Central cholinergic activity was studied in experimental rats after using ChAT and AChE as marker. Our results showed an increase expression of AChE in cerebral cortex, cerebellum, brainstem, hippocampus and hypothalamus of diabetic rats when compared to control group. In corpus striatum there was a decrease in the expression of AChE in diabetic group when compared to control rats. ChAT shows a decreased expression in cerebral cortex, cerebellum, corpus striatum, hippocampus and hypothalamus. In brain stem ChAT expression was increased. These results are in accordance with Kuhad *et al* (2007) where a significant elevation in AChE activity was observed in cerebral cortex from STZ-induced diabetic rats. AChE activation leads to a fast ACh degradation and a subsequent down regulation of ACh receptors causing undesirable effects on cognitive functions (Appleyard *et al*., 1990). In this context, it is suggested that the increase in AChE activity caused by experimental diabetes leads to a reduction in the efficiency of cholinergic neurotransmission due to a decrease in acetylcholine levels in the synaptic cleft, thus contributing to the progressive cognitive impairment and other neurological dysfunctions seen in diabetic patients (Biessels *et al*., 1994). STZ causes reduced cerebral energy metabolism leading to cognitive dysfunction by inhibiting the synthesis of adenosine triphosphate (ATP) and acetyl CoA which results in cholinergic deficiency supported by reduced ChAT activity in hippocampus (Prickaerts *et al*., 1999) and increased AChE activity in rat brain (Sonkusare *et al*., 2005). The enhancement of cholinergic activity by inhibition of AChE enzyme is the main stay of symptomatic treatment of dementia (Siddiqui & Levey, 1999).

In insulin, curcumin and Vitamin D_3 treated diabetic rats AChE and ChAT expression were reversed to near control. Our result showed that diabetic state influenced the expression of AChE and ChAT enzyme and the reversal of altered expression to near control found in the insulin, curcumin and Vitamin D_3 treated diabetic rat brain regions is a compensatory mechanism to maintain the normoglycemic level. The improvement of cognitive impairment by curcumin is suggestive of diverse mechanisms including increasing cholinergic activity by inhibiting acetylcholine esterase activity. Curcumin has been shown to lower the acetylcholine esterase level in the cerebral cortex and hippocampus of the rat brain (Sharma *et al*., 2009).

Pancreas

The pancreatic islets are richly innervated by parasympathetic, sympathetic and sensory nerves. Several different neurotransmitters are stored within the terminals of these nerves, acetylcholine, noradrenaline and several neuropeptides. Stimulation of the autonomic nerves and treatment with neurotransmitters affect islet hormone secretion. Insulin secretion is stimulated by parasympathetic nerves and inhibited by sympathetic nerves (Ahren, 2000). Acetylcholine mediates insulin release through vagal stimulation. Acetylcholine acts through the activation of Gq-phospholipase C. Expression of muscarinic receptors in rat islets, RINm5F cells and INS-1 cells was established by reverse transcriptase-polymerase chain reaction and quantified by RNase protection. Both methods indicated that M1 and M3 receptors were expressed approximately equally in the various cellular preparations (Lismaa *et al*., 2000). ACh is released from cholinergic synapses on β-cells during the cephalic phase of digestion causing a transient increase in insulin secretion. It has been proposed that ACh activates phospholipid turnover and thereby increases the

intracellular calcium levels. IP_3 mediates Ca^{2+} mobilization from intracellular Ca^{2+} stores and plays an important role in insulin secretion from pancreatic β-cells (Laychock, 1990). Our results showed an increased expression of AchE and decreased expression of ChAT in the pancreas of diabetic rats when compared to control. Treatment with insulin, curcumin and Vitamin D_3 reversed these altered expression to near control. Confocal studies using AchE specific antibodies in isolated pancreatic islets confirmed the results of gene expression studies. Our findings results emphasize the involvement of cholinergic enzyme dysfunction in the pancreas of diabetic animals and point towards the potential of curcumin and Vitamin D_3 as a therapy for treatment of diabetes.

CENTRAL MUSCARINIC RECEPTOR ALTERATIONS

Over the past decade, the role of muscarinic receptors in health was given much importance. Central muscarinic receptors, particularly M1 are involved in higher cognitive processes of learning and memory. Central muscarinic M1 antagonism lead to cognitive dysfunction and other CNS-related adverse events. Muscarinic M1 and M2 knockout mice, both demonstrate cognitive defects (Tzavara *et al*., 2003). The potential therapeutic value of various cholinergic agonists and antagonists has received increasing attention (Zwieten & Doods, 1995; Zwieten *et al*., 1995). Muscarinic receptors are a family of G protein-coupled receptors that have a primary role in central cholinergic neurotransmission. Specific agonists, which activate postsynaptic muscarinic receptors, stimulate cholinergic signaling (Valentin *et al*., 2006). It is known that different parts of the brain, particularly the hypothalamus and the brainstem are important centers involved in the monitoring of glucose status The effect of the cholinergic agonist blocked by the muscarinic antagonist atropine shows the involvement of muscarinic receptors in the central

cholinergic glucose homeostasis. The muscarinic M1 receptor is one of five known muscarinic subtypes in the cholinergic nervous system (Bonner *et al*., 1987; Hulme *et al*., 1990; van Zwieten & Doods, 1995). The muscarinic M1, M2 and M4 subtypes of mAChRs are the predominant receptors in the CNS. These receptors activate a multitude of signaling pathways important for modulating neuronal excitability, synaptic plasticity and feedback regulation of ACh release (Volpivelli *et al*., 2004)

Cerebral cortex

The RT-PCR and HPLC studies revealed that the M1 receptor was present in a relatively high density in the cerebral cortex (Jian *et al*., 1994; Oki *et al*., 2005). It is hypothesized that the cerebral cortex participates in the memory, attention, perceptual awareness, thought, language and consciousness which are necessary for the normal life style. The muscarinic M1, M3 and M5 receptors are located predominantly on postsynaptic nerve terminals and are thought to be responsible for the role of the muscarinic cholinergic system in cognition and long term potentiation in the hippocampus and cortex (Bartus, 2000). Immunoprecipitation and immunofluorescence studies indicate that muscarinic M1 and M3 receptors are expressed in cortex (Levey, 1993).

Binding studies using [^{3}H] QNB and muscarinic general antagonist, atropine revealed that total muscarinic receptors are decreased in the cerebral cortex during diabetic condition. In insulin, curcumin and Vitamin D_3 treated diabetic rats; binding parameters were reversed to near control. In these groups, treatment groups maintained glucose and circulating insulin levels to near control. Central cholinergic neurons participate in the complex neural events responsible for the hyperglycemic response to neurocytoglucopenia and to stressful situations. The hyperglycemia induced by intracerebroventricular 2-deoxyglucose (2-DG) was significantly reduced

by previous intracerebroventricular injection of atropine (Brito *et al*., 2001). Atropine injected into the third cerebral ventricle suppressed epinephrine secretion and dose-dependently inhibited hepatic venous hyperglycemia induced by neostigmine in intact rats (Iguchi *et al*., 1990). The down regulation of muscarinic receptors during diabetes is a compensatory mechanism to facilitate insulin secretion and maintenance of normoglycemia in diabetic rats.

Muscarinic M1 receptor changes during diabetes were studied using subtype specific antagonist, pirenzepine and [^{3}H] QNB. Muscarinic M1 receptors were decreased in diabetic rats, with a decrease in K_d indicating an increase in the affinity of receptors during diabetic state. Also, in STZ- induced diabetes, the mRNA level and binding parameter of muscarinic M3 receptors showed an increase in the cerebral cortex when compared to control. In insulin, curcumin and Vitamin D_3 treated diabetic rats, binding parameters were reversed to near control values. Down regulation of the muscarinic M1 receptor in the central nervous system helps to regulate the NE and EPI secretion which are inhibitory to insulin secretion (Apparsundaram *et al*., 1998). Real Time-PCR analysis also revealed a down regulation of the muscarinic M1 receptor mRNA level during diabetic condition. This is concordant with our receptor binding studies. Immunohistochemistry study using confocal microscope confirmed a similar expression pattern in localization of muscarinic M1 receptor in the cerebral cortex of control and experimental rats. Thus curcumin and Vitamin D_3 treatment contributes to amelioration of progressive cognitive impairment and other neurological dysfunctions associated with cortex seen in diabetes. Earlier reports showed significant alterations in neurotransmitters during hyperglycemia causing degenerative changes in neurons of the CNS (Bhardwaj *et al*., 1990; Garris, 1990). Curcumin and Vitamin D_3 treatment was able to significantly reverse these altered parameters to near the control value. Previous reports showed

that activation of muscarinic M1 cholinergic receptors produced an increase of glucose utilization (Hosey, 1992). Thus, we speculated that curcumin and Vitamin D_3 have ability to modulate muscarinic receptors, thereby ameliorating the impaired cognitive performance shown by STZ- induced diabetes.

Cerebellum

Cerebellum is a region of the brain that plays an important role in the integration of sensory perception, memory consolidation, coordination and motor control. In order to coordinate motor control, there are many neural pathways linking the cerebellum with the cerebral motor cortex and the spinocerebellar tract (Roberta & Peter, 2003). There is currently enough anatomical, physiological and theoretical evidence to support the hypothesis that cerebellum is the region of the brain for learning, basal ganglia for reinforcement learning and cerebral cortex for unsupervised learning (Doya, 1999). The cellular basis of motor learning has been mostly attributed to long term depression (LTD) at excitatory parallel fiber - purkinje cell synapses. LTD is induced when parallel fibers are activated in conjunction with a climbing fiber, the other excitatory input to Purkinje cells. Recently, by using whole-cell patch-clamp recording from Purkinje cells in cerebellar slices, a new form of synaptic plasticity was discovered.

Gene expression studies showed that the mRNA level of muscarinic M1 and M3 receptors in the cerebellum of diabetic rats substantially increased when compared to control. Binding parameters B_{max} of total muscarinic, muscarinic M1 and M3 receptors were increased in diabetic rats compared to control. Earlier reports showed significant alterations in neurotransmitters during hyperglycaemia and causes degenerative changes in neurons of the central nervous system (Garris, 1990; Lackovic *et al*., 1990; Bhardwaj *et al.,* 1999). Cerebellum participates in the learning

and coordination of anticipatory operations which are necessary for the effective and timely directing of cognitive and non-cognitive resources (Allen *et al*., 1997). The current study revealed the modulatory function of insulin, curcumin and Vitamin D_3 on total muscarinic, muscarinic M1 and M3 receptors by normalising the altered receptor gene expression and binding parameters to near control. Immunohistochemical analysis confirmed the result of mRNA expression and binding parameters. The cerebellum has generally been suggested to be involved in the control and integration of motor processes, as well as cognitive functions. In the current study, we observed the neuroprotective effect of curcumin and Vitamin D_3 on muscarinic receptors and muscarinic M1 and M3 receptor subtypes in cerebellum, which is responsible for the coordination of voluntary motor movement, balance and equilibrium and declarative memory.

Brain stem

The Brain Stem is a part of the brain located beneath the cerebrum and in front of the cerebellum. It connects the spinal cord to the rest of the brain. The brain stem controls involuntary muscles such as the stomach and the heart. The brain stem also acts as a relay station between the brain and the rest of the body. Brain stem reticular formation has been considered to play an important role in generating behavioural states as well as in the modulation of pain sensation (Paré & Steriade 1993, Steriade, 1996). These reticular functions originate from interacting neuronal groups in the brain stem, including cholinergic, adrenergic and serotoninergic neurons (Steriade, 1996). Brain stem along with hypothalamus serves as the key centre of the central nervous system regulating the body homeostasis. Stimulation of the peripheral vagus

nerve leads to an increase in circulating insulin levels. Anatomical studies suggest that the origin of these vagal efferent fibres is nucleus ambiguus and dorsal motor nucleus directly innervating pancreas (Bereiter *et al*., 1981).

The total muscarinic receptors of the brainstem are found to be increased during diabetic condition. Muscarinic M1 receptors are decreased and muscarinic M3 receptors are increased during diabetic state. In insulin treated, curcumin and Vitamin D_3 treated diabetic rats, binding parameters were reversed back to near control values.

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. Nucleus ambiguus stimulation reported to increase plasma insulin levels in rats (Bereiter *et al*., 1981). RT-PCR analysis also revealed a down regulation of the muscarinic M1 receptor mRNA level during diabetic condition. This is in accordance with our receptor binding studies. Also confocal studies using specific antibodies of muscarinic M1 and M3 brainstem confirmed the Real time PCR and Scatchard analysis. The brain stem provides the main motor and sensory innervation *via* the cranial nerves. Muscarinic alterations in brainstem during diabetes result in memory problems, difficulty concentrating, difficulty staying focused and physical defects including the inability to walk, remain balanced, and a loss of strength. Our results showed that curcumin and Vitamin D_3 restored the altered muscarinic functions associated with brainstem.

Corpus striatum

Densities of muscarinic M1 receptor subtype were highest in the corpus striatum (Oki *et al*., 2005). The corpus striatum is the largest component of the basal ganglia. Cholinergic terminals within the striatum contain presynaptic muscarinic

receptors that inhibit neurotransmitter release (Chesselet, 1984). Various anatomical, electrophysiological and pathological observations provide evidence that ACh plays a major role in the control of striatal function and in the regulation of motor control (Jabbari *et al*., 1989). Striatal ACh is released from a population of large cholinergic interneurons that establish complex synaptic contacts with dopamine terminals, originating from the substantia nigra and with several striatal neuronal populations (Lehmann & Langer, 1982, 1983; Wainer *et al*., 1984; Phelps *et al*., 1985; Izzo & Bolam, 1988; Vuillet *et al*., 1992). Corpus striatum regulates endocrine functions indirectly through the secretion of other hormones like thyroxine. Scatchard analysis of total muscarinic receptors revealed a decreased B_{max} in corpus striatum during diabetic condition. In insulin, curcumin and Vitamin D_3 treated diabetic rats, binding parameters were reversed to near control values. Muscarinic M1 receptors were increased and muscarinic M3 receptors decreased during diabetic state. Supplementation of insulin, curcumin and Vitamin D_3 to diabetic rats reversed the binding parameters to near control . mRNA level revealed an up regulation of the muscarinic M1 receptor and down regulation of M3 receptor during diabetic condition. The results of confocal studies confirmed the alterations of muscarinic M1 and M3 receptor at protein level. CNS mAChRs regulate a large number of important central functions including cognitive, behavioural, sensory, motor and autonomic processes (Wess, 1996; Felder *et al*., 2000; Eglen, 2005). The present study suggests that drugs that can selectively activate muscarinic receptors are of significant therapeutic benefit in the diabetes management. Thus our results revealed the significance of central muscarinic receptor changes during diabetes and the regulatory role of curcumin and Vitamin D_3 on muscarinic receptors in corpus striatum.

Hippocampus

Uncontrolled diabetes mellitus leads to severe complications of the peripheral and central nervous system. In addition to the well-known peripheral neuropathy, data from epidemiologic studies confirm that diabetes is a risk factor for brain aging, stroke, cerebrovascular diseases and Alzheimer's disease (Gispen & Biessels, 2000; Biessels *et al*., 2002). Deterioration of cognitive functions is also present in humans with type I diabetes (Gold *et al*., 1994). More than 20 neurodegenerative diseases are associated with diabetes mellitus in humans. These associations reflect direct effect of hyperglycemia on the brain, or of the diabetes-associated comorbidities of hypertension, dyslipidemia, or hyperinsulinemia (Makimattila *et al*., 2004). Pronounced pathological changes also characterize the brain of diabetic animals, particularly the hippocampus. There is damage to presynaptic and postsynaptic structures, dysregulation of Ca^{2+} homeostasis, neuronal loss, dendritic atrophy in CA3 neurons, reduced expression of insulin growth factors and their receptors and decreased neurogenesis (Jackson-Guilford *et al*., 2000; Saravia *et al*., 2004). In the hippocampus of diabetic rats, our results showed that total muscarinic, muscarinic M1 receptors binding parameters, B_{max} was decreased and muscarinic M3 receptors were increased in diabetic rats compared to control.

mRNA expression showed down regulation of M1 receptor and up regulation of M3 receptor in the hippocampus of diabetic rats. This suggests an impaired muscarinic receptor function in the hippocampus leading to deficits in cognitive performance and long term memory formation in diabetic rats. In correspondence with the hippocampal neuropathology, diabetic animals showed reduced learning and memory deficits (Gispen & Biessels, 2000). A recent report has pointed out an association between memory alterations of diabetic rodents and the decrease of

neuronal proliferation in the dentate gyrus (Jackson-Guilford *et al*., 2000). In dentate gyrus, as well as the subventricular zone (SVZ), neurogenesis continues throughout adulthood (Taupin & Gage, 2002).

Insulin, curcumin and Vitamin D_3 supplementation reversed the altered parameters to near control. Immunohistochemistry studies using confocal microscope confirmed the results of binding parameters and gene expression. In line with this, we suggest that regulation of muscarinic receptor function by curcumin and Vitamin D_3 contribute consequently to improve the cognitive functions, such as learning and memory.

Hypothalamus

Hypothalamus is the centre involved in the neuroendocrine regulation. It is the region of the central nervous system where the autonomic and endocrine systems are integrated. Hypothalamic paraventricular nucleus serves as the major neuroendocrine and autonomic output centre. Specialized subgroups of hypothalamic neurons exhibit specific excitatory or inhibitory electrical responses to changes in extracellular levels of glucose (Burdakov *et al*., 2005). Hypothalamic centers involved in the regulation of energy balance and endogenous glucose production constantly sense fuel availability by receiving and integrating inputs from circulating nutrients and hormones such as insulin and leptin. In response to these peripheral signals, the hypothalamus sends out efferent impulses that restrain food intake and endogenous glucose production. This promotes energy homeostasis and keeps blood glucose levels in the normal range. Disruption of this intricate neural control is likely to occur in type 2 diabetes and obesity which contribute to defects of glucose homeostasis and insulin resistance common to both diseases (Demuro & Obici, 2006).

The cholinergic glucoregulatory hippocampal activity transmitted to peripheral organs *via* the ventromedial hypothalamus (Iguchi *et al*., 1992). The ventromedial hypothalamus (VMH), lateral hypothalamus, paraventricular hypothalamus and median site of the lateral preoptic area are involved in increasing the plasma glucose and epinephrine levels (Honmura *et al*., 1992). The muscarinic antagonist atropine suppressed the hyperglycemia induced by administration of neostigmine in a dose-dependent manner, suggesting the involvement of muscarinic receptors of the VMH in the glucoregulation (Iguchi *et al*., 1991).

Gene expression of muscarinic M1 receptor was down regulated and muscarinic M3 receptor was up regulated in diabetic rats compared to control. Previous studies demonstrated that the distribution of mRNA of muscarinic receptor generally parallels with the distribution of their protein. These alterations in muscarinic transmission suggested impairing neuroendocrine function which includes disturbed secretion of pituitary hormones, notably growth hormone and cortisol, which, by impairing tissue sensitivity to insulin, contribute to poor metabolic control in diabetes. In insulin, curcumin and Vitamin D_3 treated diabetic rats the altered mRNA levels of muscarinic M1 and M3 receptors were reversed to near control. The ventromedial hypothalamus, lateral hypothalamus, paraventricular hypothalamus, and median site of the lateral-preoptic are involved in increasing the plasma levels of glucose and epinephrine by cholinergic stimulation (Honmura *et al*., 1992). These results unravelled the therapeutic effect of curcumin and Vitamin D3 supplementation on regulating hypothalamus mediated metabolic processes, secretion of neurohormones, secretion of pituitary hormones, control of body temperature, hunger, thirst fatigue and circadian cycles and other activities of the autonomic nervous system.

MUSCARINIC RECEPTORS AND VESICULAR ACETYLCHOLINE TRANSPORTER ALTERATIONS IN THE PANCREAS

The autonomic nervous system plays an important role in the insulin release. Physiological insulin secretion is initiated by glucose and augmented by nervous and humoral systems (Ahren *et al*., 1986). The pancreatic islets are richly innervated by parasympathetic, sympathetic and sensory nerves. Neurotransmitters are stored within the terminals of these nerves, both acetylcholine and noradrenalin and several neuropeptides. Expression of muscarinic receptors in rat islets was established by reverse transcriptase-polymerase chain reaction and quantified by RNase protection. Both methods indicated that muscarinic M1 and M3 receptors were expressed approximately equally in the various cellular preparations (Lismaa *et al*., 2000).

Stimulation of the autonomic nerves and treatment with neurotransmitters affect islet hormone secretion. Insulin secretion is stimulated by parasympathetic nerves and inhibited by sympathetic nerves (Ahren, 2000). Acetylcholine mediates insulin release through vagal stimulation. Acetylcholine acts through the activation of Gq-phospholipase C. It stimulates Ca^{2+} influx through the voltage dependent L-type Ca^{2+} channel that is primarily activated by glucose. Studies showed that muscarinic M1 and M3 are the major muscarinic receptors present in the pancreas (Lismaa *et al*., 2000). During diabetic condition, total muscarinic, muscarinic M1 and M3 receptor binding parameters decreased when compared to control. Gene expression studies also showed the down regulation of muscarinic receptors in diabetic rats. Insulin, curcumin and Vitamin D_3 treatment reversed the binding parameters to near control.

Localization of muscarinic M1, M3 receptors and vesicular acetylcholine transporter using confocal laser scanning microscopy showed a decreased mean pixel value in pancreatic islets of diabetic rats when compared to control. Administration of

choline to rats elevates serum insulin. Pretreatment with a peripheral muscarinic acetylcholine receptor antagonist, atropine methylnitrate blocked the choline-induced increase in blood insulin. The increase in serum insulin elicited by choline was prevented by pretreatment with the M1 antagonist, pirenzepine, or the muscarinic M1 and M3 antagonist, 4-DAMP. Pretreatment with an antagonist of ganglionic nicotinic acetylcholine receptors, hexamethonium, prevented the choline-induced increase in serum insulin. Choline increased the acetylcholine content of the pancreas and enhanced acetylcholine release from minced pancreas, which suggests that choline stimulates insulin secretion indirectly by enhancing acetylcholine synthesis and release (Ilcol *et al*., 2003).

Muscarinic M3 receptors appears to be the predominant subtype expressed by pancreatic β-cells (Gilon & Henquin, 2001; Lismaa *et al*., 2000). Earlier study demonstrated that muscarinic stimulation of pancreatic insulin and glucagon release is mediated by the M3 muscarinic receptor subtype (Duttaroy *et al*., 2004). Immunocytochemistry analysis in pancreas showed an increased expression of muscarinic M3 receptor in insulin, curcumin and Vitamin D_3 treated diabetic rats. An improvement in insulin secretion and response to an intravenous glucose tolerance test has also been seen with Vitamin D_3 replacement in Vitamin D deficient rabbits (Nyomba *et al*., 1984). In individuals with diabetes mellitus, Vitamin D treatment increased insulin secretion and improved glucose tolerance (Rudnicki & Molsted-Pedersen, 1997). Our result showed that Vitamin D_3 supplementation plays a pivotal role in regulating muscarinic M3 receptor expression through the VDR present in the pancreas and thereby enhancing the insulin synthesis and secretion. Thus our results demonstrate a possible mechanism of reducing the neuronal disorders in diabetes with Vitamin D_3 supplementation thereby mediating potential therapeutic effect through muscarinic M3 receptors in pancreas (Peeyush *et al*., 2010). Previous reports showed

that curcumin has the potential to protect pancreatic islets cells against streptozotocin-induced death dysfunction (Meghana *et al*., 2007) and increase plasma insulin level in diabetic mice (Seo *et al*., 2008). Thus it is suggested that curcumin supplementation ameliorated the decreased muscarinic receptor function and acetylcholine transport in pancreatic islets of diabetic rats. In insulin, curcumin and Vitamin D_3 treated diabetic rats, muscarinic M1, M3 receptor function and acetylcholine transport reversed to near control. The present findings showed the potential anti-diabetic effect of curcumin and Vitamin D_3.

α7 nicotinic receptor gene expression in control and experimental rats

The search for potential targets for a treatment of neurodegenerative diseases associated with cholinergic deficits has led to an increasing interest in nicotinic acetylcholine receptors (nAChR). The number of the different nAChR subunits and their possible combinations forming different receptor subtypes explain the individual anatomical distribution, the electrophysiological and pharmacological diversity as well as the variable effects of nAChR agonists and antagonists. Several lines of evidence suggest that the α7 nAChR is an important pharmacological target for the treatment of cognitive deficits. (Levin *et al*., 2006). Furthermore, the α7 nAChR agonist GTS-21 improves attentional function in patients with schizophrenia (Olincy *et al*., 2006; Freedman *et al*., 2008) and α7 nAChR agonists have been shown to improve performance on a variety of cognitive tests related to working, short-term and long- term memory function in animal models (Bitner *et al*., 2007; Boess *et al*., 2007; Hashimoto *et al*., 2008). However, the involvement of the α7 nAChR in the prevention of cognitive deficits in diabetes has not been addressed. The major aim of the study was to further explore whether diabetes is related to α7 nAChR modulation

in brain regions also to learn the neuroprotective role of curcumin and Vitamin D_3 treatment in restoring the changes.

In the brain, nicotinic receptors include several subtypes with differing properties and functions. The abundant presence of α7 nAChR's in the hippocampus, neocortex and basal ganglia (Clarke *et al.,* 1985), in conjunction with the memory-enhancing activity of selective α7 nicotinic agonists such as DMXB (Meyer *et al.,* 1997), suggests a significant role for α7 nAChR's in learning and memory. In addition, the protective action of nicotine is mediated, at least partially, through α7 nACh receptors. Our results showed an increased expression of α7 nAChR in cerebral cortex, cerebellum, brain stem, corpus striatum and decreased expression in hippocampus of diabetic rats compared to control. Confocal studies using specific antibody for α7 nAChR confirmed the mRNA expression in cerebral cortex, cerebellum, brainstem corpus striatum and hippocampus in diabetic rats. The α7 nAChR subunit has been linked to inhibit neuronal function in the hippocampus by several lines of investigation (Frazier *et al.,* 1998; Freedman *et al.,* 1999). In addition to this role in inhibitory neuronal function, the α7 nAChR has also been proposed to have a developmental role. The receptor is expressed by hippocampal neurons as soon as they have formed from the neuroepithelium (Adams *et al*., 1999). Because the receptor admits Ca^{2+} into the neuron (Vijayaraghavan *et al.,* 1992), it has the ability to affect neuronal migration (Komuro & Rakic, 1996) as well as other developmental functions such as apoptosis (Sastry & Rao, 2000). In line with this, α7 nAChR functional difference in diabetes is suggested to be one of the major factor causing memory and behavioral deficit.

Curcumin and Vitamin D_3 treatment significantly reversed the altered changes in the brain regions of diabetic rats to near control while insulin treatment did not show any significant reversal other than in corpus striatum. Neuronal nicotinic

acetylcholine receptors are crucial to acetylcholine neurotransmission in both the CNS and autonomic nervous system. However, in the CNS, these receptors are more often associated with modulation of release of several neurotransmitters including dopamine, norepinephrine, GABA and glutamate (Wonnacott, 1997; Girod and Role, 2001). Thus, these receptors significantly influence the activity within the CNS and deregulation of this activity contribute to diabetes mellitus associated disorders involving the CNS. Abnormalities of nAChR function in the hippocampus lead to cognitive and memory impairments (Levin *et al*., 2002; Green *et al*., 2005) and sensory gating deficits (Adler *et al*., 1998). Curcumin and Vitamin D_3 supplementation was found to be more effective in reversing the altered gene expression to near control stage.

CENTRAL DOPAMINERGIC RECEPTOR ALTERATIONS

Cerebral cortex

Diabetes is considered to be one of the most psychologically demanding chronic medical illnesses and is often associated with several psychiatric disorders (deGroot *et al*., 2001). Although the mechanism responsible for cognitive deficits in stress-related neuropsychiatric disorders has been obscure, prefrontal cortical (PFC) dopaminergic dysfunction is thought to be involved. In animals, the mesoprefrontal dopaminergic system is particularly vulnerable to stress. Prefrontal cortex is a cortical area involved in selecting and retaining information to produce complex behaviours (Arianna *et al*., 2007). Our results showed an up regulation of total dopamine receptors accompanied with decrease in its affinity in the cerebral cortex of diabetic rats. The dopamine neurons projecting to the prefrontal cortex are thought to be involved in various motor and behavioural functions (Tam & Roth, 1997). This

increased number of dopamine receptors could account for the behavioural supersensitivity to dopamine agonist as a result of damage in the dopamine functions (Cresse *et al*., 1977).

Dopamine D_1 receptors are located postsynaptically on the cortical neurons (Tassin *et al*., 1978, 1982) and the decreased dopamine level in the PFC induced by electrolytic lesion up regulates the dopamine D_1 receptor density in the PFC (Tassin *et al*., 1982). The mesoprefrontal dopaminergic system is particularly vulnerable to stress (Abercrombie *et al*., 1989) and that an over stimulation of dopamine D_1 receptor in the PFC impairs the working memory (Zahrt *et al*., 1997). We observed an increase in dopamine D_1 receptors mRNA level in the cerebral cortex of diabetic rats when compared to control. Excessive dopamine D1 receptor stimulation is sufficient to produce marked PFC dysfunction. Stress impairs PFC cognitive function through a hyperdopaminergic mechanism. It is reported that chronic stress induced depressive state is caused by a dopamine D1 receptor mediated hypodopaminergic mechanism in the PFC (Mizoguchi *et al*., 2002). Thus excessive cortical dopamine D1 receptor density with decreased dopamine is suggested to be the cause for cortical dysfunction during diabetes.

However, the finding that DA D1 receptor stimulation alone is sufficient to induce PFC dysfunction does not rule out an additional role for DA D2 receptors. Cognitive deficits induced by either stress exposure or ketamine (Verma & Moghaddam, 1996) is blocked by selective DA D2 receptor antagonists. These findings suggest that both DA D1 and DA D2 receptor families contribute to the detrimental actions of dopamine in the PFC and that the two may synergize to take the PFC "off-line" during stress. We observed that DA D2 receptors also increased significantly in the cerebral cortex of diabetic rats.

Stimulation of DA D_1/D_2 receptors under DA depleted conditions cause a

subtle impairment in spatial working memory performance (Ellis *et al*., *2005*). Dopaminergic neurotransmission is critically involved in many aspects of complex behaviour and cognition beyond reward/reinforcement and motor function. Our results showed that treatment with insulin, curcumin and Vitamin D_3 reversed the increased dopamine receptor expression in diabetic rats to near control. Previous findings suggest the antidepressant-like effects of curcumin involve the central monoaminergic neurotransmitter systems.(Ying Xu *et al*., 2005) Vitamin D help to protect against cognitive deterioration and dementia, specifically, vascular dementia and Alzheimer's disease, through vasculoprotection (Lind *et al*., 1987; Pfeifer *et al*., 2001; Wang *et al*., 2001; Zittermann *et al*., 2003; Wang *et al*., 2008a,b), preservation of neurons (Sutherland *et al*., 1992; Landfield & Cadwallader-Neal, 1998; Brewer *et al*., 2001) and protection against risk factors for cognitive dysfunction (Lind *et al*., 1987; Zittermann *et al*., 2003; Bischoff-Ferrari *et al*., 2004). Thus, curcumin and Vitamin D_3 treatment exerted antidepressant-like effect and reduce stress by normalising the increased expression of dopamine receptors in cerebral cortex.

Cerebellum

Dopamine is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake and endocrine regulation. This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function and gastrointestinal motility (Missale *et al*., 1998). Dopamine receptors are reported to be increased in diabetes causing significant alterations in central dopaminergic system (Lozovsky *et al*., 1981). Our results showed that total dopaminergic receptor binding parameters were decreased in the

cerebellum, which is responsible for the coordination of voluntary motor movement, balance, equilibrium and declarative memory. The decreased dopamine receptor density in the cerebellum of diabetic rats when compared to control indicates an imbalance in dopaminergic neural transmission. Furthermore, many behavioral studies have shown evidence that the dopamine system plays an important role in regulating exploratory and locomotor behavior (Fink & Smith, 1979; Funada *et al*., 1994). The current data reveal a significant reversal of this altered binding parameter to near control in insulin, curcumin and Vitamin D_3 treatment. Diabetes mellitus has been reported to be accompanied by a number of behavioural and hormonal abnormalities, including reduced locomotor activity (Marshall *et al*., 1976). The present experiments further revealed the effect of curcumin and Vitamin D_3 to modulate the dopaminergic receptors in the cerebellum by standardising the altered expression near to a normal level.

Dopamine D_1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. The gene expression studies of DA D1 receptors showed a decrease in the cerebellum of diabetic rats which confirm and extend our observations of total dopamine receptors. DA D1 receptor seems to mediate important actions of dopamine to control movement, cognitive function and cardiovascular function. The DA D_1 receptors in the brain are linked to episodic memory, emotion, and cognition. Diabetes mellitus has been reported to cause degenerative changes in neurons of the CNS (Bhattacharya & Saraswathi, 1991; Garris, 1990., Lackovic *et al*., 1990). Haloperidol and SCH23390, a selective dopamine D1 receptor antagonist, significantly reduced spontaneous locomotor activity in diabetic mice, but not in nondiabetic mice (Kamei *et al*., 1994). Our study showed that diabetes regulate the expression of DA D1 receptor which reduce the cerebellar function. In our study, insulin, curcumin and Vitamin D_3 increased the

dopamine D1 receptor expression levels in the cerebellum, which suggests that the curcumin supplementation modulated the functional regulation of these receptors to maintain normal dopaminergic function and this is involved as a mechanism for preventing cerebellar dysfunctions. Such interference with the dopaminergic system could explain, at least in part, the ameliorative effect of curcumin and Vitamin D_3 on CNS.

The interest in learning DA D2 receptor expression begins with the hypothesis that DA D2 receptors are involved in the pathophysiology of schizophrenia and in the mechanism of antipsychotic drug action (de Paulis, 2003). Thus, our findings bring attention to the cerebellum as a possible site of dysfunction in diseases like diabetes mellitus. To examine whether DA D2 receptors are altered in diabetes, we examined the expression of DA D2 in the cerebellum, to which dopaminergic neurons project, and are related to memory, attention, perceptual awareness, thought, language, consciousness and motor function. The present study showed that DA D2 receptors expression of cerebellum in diabetic rats were up regulated when compared to control. These results indicate an alteration of the dopaminergic function in diabetes, because it is known that dopamine is a principal modulator of higher functions including attention, working memory [Castellano *et al*., 1999] and motor control (Zhou & Palmiter, 1995). The increase in the central dopaminergic postsynaptic receptors has been related to decrease the locomotor and ambulatory activity in STZ-induced diabetic rats (Kobayashi & Shigeta, 1990; Shimomura *et al*., 1990). It was reported that injection of DA D2 agonist into lobules 9 and 10 of the cerebellum, induced balance and motor coordination disturbances in the rotarod test (Kolasiewicz & Maj 2001). It was observed that insulin, curcumin and vitamin D_3 reversed the adverse effects of diabetes on DA D2 receptors in the cerebellum to near control level.

Brainstem

Brainstem is an important part of the brain in monitoring the glucose status and the regulation of feeding (Guilford *et al*., 2000). When glucose levels were lowered to 2.8 mmol/l, brain function was impaired in nondiabetic rats as well. Our results showed an increased binding of total dopamine receptors with decreased affinity in the brainstem of diabetic rats compared to control. Our previous studies demonstrated adrenergic, serotonergic and DA D_2 receptor function alterations in the brainstem of diabetic rats (Abraham & Paulose, 1999; Padayatti & Paulose, 1999; Paulose *et al*., 1999; Eswar *et al*., 2007). In diabetic condition, DA D_1 receptors gene expression was up regulated in the brain stem. Gene expression studies using Real-Time PCR showed that DA D_2 receptors significantly down regulated in the brainstem of diabetic rats. Treatment with insulin, curcumin and Vitamin D_3 reversed the increased binding parameters of dopamine and altered gene expression of DA D1 and DA D2 in the brain stem. From our data we suggest that there is increased activation of sympathetic stimulation during diabetes as a result of increased NE and EPI (Tassava, *et al.,* 1992; Jackson, *et al*., 1997; Jackson & Paulose,; 1999) is because of decreased dopamine content in the brainstem with an up regulation of DA D_1 receptors and down regulation of DA D_2 receptors. In the brainstem there was a decrease in the expression of DA D_2 receptor mRNA as a result of diabetes. It has been reported that damages in the brain cause alterations in the expression of the DA D_{2L} isoform which is expressed in the *in vivo* condition (Neve *et al*., 1991; Snyder *et al*., 1991).

Modest reductions in plasma glucose to 3mM produce marked alterations in brainstem responses to auditory stimuli. Adverse effects of hyperglycemia on brain function are not limited to higher centers but also involve the brainstem (Jones *et al.*,

1990). We observed an up regulation of DA D_1 receptors and down regulation of DA D_2 receptors in the brainstem of diabetic rats. These results indicate that the dopaminergic activity in the brainstem altered in hyperglycaemic rats impairing dopamine related functions of brainstem. Earlier studies reported that brainstem is universally spared in hypoglycaemic brain damage (Auer, 2004). Our results showed a prominent dopaminergic functional improvement with curcumin and Vitamin D_3 supplementation in the brainstem of diabetic rats.

Corpus striatum

Striatal dopamine receptors were markedly decreased with increased affinity during diabetes with the depletion of dopamine in the striatum and an increased HVA metabolism. Striatal dopamine firing during diabetes is decreased affecting dopaminergic functions (Saller, 1984). The decreased dopamine receptor density during diabetes is related to the decreased locomotor activity in STZ-induced diabetic rats (Kobayashi *et al*., 1990; Shimomura *et al*., 1990). This finding correlates with our present data suggesting that the disturbances in the central dopaminergic receptors during STZ- induced diabetes affects dopamine related functions. The firing of dopamine neurons projecting from the substantia nigra to the striatum is reported to be rapidly suppressed by hyperglycaemia leading to the hypofunction of dopamine receptors (Saller, 1984). There are hypothesis that suggests activities related to the functional capacities of dopamine receptors like stereotypy, ambulation, behaviour are diminished due to hyperglycaemia (Lozovsky *et al*., 1981). Also, a decrease in dopamine receptors during diabetes results in hyporesponsiveness (Saitoh *et al*., 1998). In diabetic rats we observed a significant increase in striatal total dopamine receptors which is a compensatory response to decreased dopamine content. The

insulin, curcumin and Vitamin D_3 supplementation significantly modulates the altered binding parameters of dopamine receptors in the striatum to near control.

Real-Time PCR analysis showed a decreased expression of DA D_1 receptors in the striatum of diabetic rats. This correlates with previous reports that DA D_1 receptor density decreased in the striatum of alloxan induced diabetic rats (Salkovic & Lackovic, 1992). DA D_1 stimulated cAMP production was markedly increased in diabetic rats, whereas ability of DA D_2 receptor action to reduce cAMP formation was almost abolished during diabetes (Abbracchio *et al*., 1989). An imbalance between G_s -proteins and G_i/G_o protein mediated efficacy of G_s activity as a result of the loss of G_i/G_o inhibitory functions has been found in the striatum and other tissues of diabetic animals (Salkovic & Lackovic, 1992). Dopamine through its DA D_1 receptor stimulates adenylyl cyclase and inhibits adenylyl cyclase activity through its DA D_2 receptors. Decreased DA D_1 receptors expression during diabetes that we observed in the striatum is a major cause in affecting dopamine related functions. It has been suggested that curcumin and Vitamin D_3 reversed the effects of diabetes on DA D1 receptors in the brainstem and this is involved as a mechanism of preventing dopamine related functions in brainstem.

Gene expression studies showed that DA D2 receptors up regulated in diabetic rats compared to control. Insulin, curcumin and Vitamin D_3 treatment reversed the increased expression to near control. Previously [^{3}H] spiroperidol binding to DA D_2 receptors have been reported to be increased during diabetes (Trulson & Himmel, 1983). Striatal DA D_2 receptor primarly represents a population of dopamine D_2 sites (Marzella *et al*., 1997). During diabetes it has been documented that the sensitization of these receptors and their increased number results in a decreased locomotory and ambulatory activity (Kobayashi & Shigeta, 1990; Shimomura *et al*., 1990). DA D_2 receptor gene expression increased in the striatum during diabetes as a

result of the decreased transmission of dopamine. Hyperglycaemia depressed the dopaminergic function. Therefore a decreased dopaminergic activity is suggested to increase the DA D_2 receptors. A lesion in the striatum is reported to increase the expression of DA D_{2L} receptor gene (Zhang *et al*., 1994). *In vivo* release of dopamine from mesolimbic and neostriatal dopamine neurons appears to be modulated by DA D_2 but not by DA D_1 receptors, whereas both receptor types modulate dopamine metabolism (Boyar & Altar, 1987). DA D_2 receptors are reported to regulate the release of dopamine from dopaminergic neurons originating in the ventral tegmental area as well as in the substantia nigra (Plantje *et al*., 1987). The two dopamine receptor subtypes interact in a synergistic way to adapt to the alterations in glucose metabolism. The insulin, curcumin and Vitamin D_3 treatment regularise the imbalanced DA receptor functions in the corpus striatum.

Hippocampus

Previous reports suggest that, in both insulin-deficient rats and insulin-resistant mice, diabetes impairs hippocampus-dependent memory and learning, perforant path synaptic plasticity and adult neurogenesis (Alexis *et al*., 2008). The hippocampal formation receives a dopamine input from different midbrain groups and a more prominent dopamine input into the temporal pole of hippocampus (Hortnagl *et al*., 1991). The hippocampus has long been known to be important for memory function. It is reported that profound hypoglycaemia selectively damages CA1 and the dentate gyrus of the hippocampus (Tasker *et al*., 1992). The dopaminergic system is a strong candidate for mediating novelty acquisition and synaptic plasticity in CA1. We observed a significant up regulation of dopamine receptors in the hippocampus of diabetic rats. Our data suggest that the impairment in glucose metabolism caused up regulation of hippocampal dopamine receptors. Treatment using insulin, curcumin and

Vitamin D_3 reversed the increased binding of total dopamine receptor in the hippocampus of diabetic rats to near control.

The characterizations of neuronal populations expressing dopamine receptor subtypes in the hippocampus have shown a prominent labeling of DA D_1 receptors in dentate gyrus and subicular complex (Fremeau *et al*., 1991). Yokoyama (1995) demonstrated widespread distribution of DA D_2 like receptor in the hippocampus. DA D_2 receptors in the ventral hippocampus were shown to have important influences on spatial working memory (Wilkerson & Levin, 1999). DA D_2 receptor plays a role in hippocampal memory function (Hiroshige *et al*., 2005). An intact mesocortical dopaminergic input to the PFC has been reported to be necessary for long-term potentiation to occur at hippocampal-prefrontal cortex synapses. Earlier studies suggest that DA D_1 but not DA D_2 receptors are crucial for the dopamine control of the NMDA receptor-mediated synaptic response on a specific excitatory input to the PFC. The interactions of these receptors play a crucial role in the storage and transfer of hippocampal information in the PFC. Real-Time PCR analysis showed an increased expression of DA D_1 and DA D_2 receptors in the hippocampus of diabetic rats compared to control. The increase in dopamine receptor sensitivity is a compensatory response to diminished firing of dopamine. Insulin curcumin and vitamin D_3 treatment reversed the increased expression to near control. These findings suggest that the neuroprotective effects of curcumin in hippocampus involve the central monoaminergic neurotransmitter systems (Xu *et al*., 2005b). Recently it was shown that the VDR was distributed throughout rat hippocampus (Langub *et al*., 2001). Earlier reports have shown that Vitamin D_3 acts as a potent differentiation agent in rat hippocampal cultures as assessed by a reduction in mitosis and increased neurite outgrowth. In addition, vitamin D_3 induces NGF, a neurotrophin.

Hyperglycemia markedly affects hippocampally dependent spatial working memory task (McNay *et al*., 2006). DA D_1 and D_2 receptors are generally considered to exert opposite effects at the cellular level, but many behavioural studies find an apparent cooperative effect of DA D_1 and DA D_2 receptors in the nucleus accumbens. Opposing influences of DA D_1 and DA D_2 receptor activation on cAMP-dependent signaling have been reported in many studies (Kebabian & Calne, 1979; Missale *et al*., 1998), with DA D_1 receptors acting through the stimulatory G_s-like G_{olf}, and D_2 receptors acting through the inhibitory $G_{i/o}$ proteins. Hopf *et al*., (2003) reported that cooperative action of DA D_1 and DA D_2 receptors in the brain mediate dopamine-dependent behaviours. Recent studies explains that stimulation of DA D_1 and DA D_2 dopamine receptors has the potential to give rise to different intracellular signals depending on whether DA D_1 or DA D_2 receptors are activated alone or together (Pollack, 2004). Thus our results suggest that the co activation of DA D_1 and DA D_2 receptors with dopamine depletion have particular relevance in the impairment of glucose metabolism and dopamine related functions. Also, co-activation of DA D_1 and DA D_2 receptors is reported to enhance glutamate mediated cellular excitation (Hopf *et al*., 2003). The hippocampal cell populations in particular are important for learning and memory and impairment of cognitive abilities and neuronal damage in diabetes is ameliorated by curcumin and Vitamin D_3 treatment.

Hypothalamus

Dopaminergic action is important in the regulation of the hypothalamic-pituitary hormone release. Also, DA and its receptors are implicated in the satiety, hunger and body weight maintenance. The central vagal connection with dopaminergic innervation is reported to reach the pancreatic islets through the parahypothalamic ventricular (PHV) nucleus while aderenergic and serotonergic

innervations reach the pancreas through the brain stem (Smith & Davis, 1983). Altered DA is reported to affect the feeding pattern, as food intake is accompanied by DA release which differs significantly in the hypothalamus of obese and lean Zucker rats. The reduction in DA, NE and EPI levels in the hypothalamus suggests a low metabolism of monoamines (Bellush & Henley, 1990). They are responsible for the development of thermoregulatory deficits when exposed to cold environment (Leu, *et al*., 1986).

Our studies in the hypothalamus suggest that DA D1 receptor expression decreased during diabetes. An alteration in the sensitivity of the receptors during diabetes has been previously reported causing a difference in the modulation of innervating DA systems. Dopamine D1-like but not DA D2-like receptor antagonism in the LH attenuated taste avoidance learning (Fenu *et al*., 2001).The nucleus paraventricularis of the hypothalamus is regarded as an important region of the brain, operating as a neuronal interface between various brain structures and hormonal systems (Hoebel *et al*., 1989; Armstrong, 1995). There are several anatomical and functional evidences that DA and its receptors in the PVN might constitute an important afferent system controlling the activity of PVN neurons and subsequent release of hormones. Specifically, the PVN receives dopaminergic innervation from two major sources: the dorsal periventricular nucleus (group A14) and the zona incerta (group A13) (Cheung *et al*., 1998; Wagner *et al*., 1995). There are also evidences indicating that agonists of dopaminergic receptors might modulate the activity of PVN neurons and subsequent release of hormones such as TSH (Andersson, 1989). DA D1 receptors are localized in PVN neurons, which are activated tonically by neurotransmitters operating *via* receptors that elevate intracellular concentration of cAMP and CA 21 (Gonzalez, 1989), Colocalization of DA D1 receptor protein with pCREB may also, at the anatomical level, suggest that

dopamine, *via* the DA D1 receptors, may control not only the release of PVN hormones, but also the transcriptional activity of their genes. Diabetes associated impaired hypothalamic functions such as neuroendocrine regulation and memory processing through CREB is due to the altered expression of dopaminergic receptors is suggested. Insulin, curcumin and Vitamin D_3 treatment normalized the decreased expression to near control values thus proposing a potential nutritional value in managing diabetes.

Gene expression studies showed that DA D2 receptor down regulated in diabetic rats compared to control. The regional difference in the receptor status is relevant to the role which DA plays during various physiological and behavioural activites. In the intra lateral hypothalamic area (Intra-LHA) blockade of DA D2 receptors by specific antagonist in tumor bearing (TB) and non tumor bearing (NTB) rats increased food intake indicating the involvement of DA D2 receptors in feeding mechanisms (Zhang, *et al*., 2001). Thus during diabetes the decrease in DA D2 receptor expression could disturb hypothalamic functions. Impairment of DA D2 receptor is an important factor that leads to hyperphagic and polydypsic condition as DA participates in regulating meal size (Yang, *et al*., 1997). Dopamine–acetylcholine (DA-ACh) interaction within the lateral hypothalamus (LH) is involved in the regulation of locomotion, feeding behaviour and reinforcement (Baptista, *et al*., 1990; Hoebel, *et al*., 2000). The cholinergic stimulation of these activities is regulated by DA through D2 receptors in the hypothalamus. Thus DA in the hypothalamus is related to sensory input, feeding reflexes, food reward or memory processes (Hernandez & Hoebel, 1988). In the hypothalamus co-administration of dopamine D1 and DA D2 agonists inhibit the feeding effect mediated by the action on neuropeptide Y (NPY) (Kuo, 2002). This is effective in the reduction of food intake in diabetic rats, revealing the efficiency of DA D1/ D2 agonist in the improvement of

hyperphagia in diabetic animals. Decreased DA D2 receptor mRNA expression in diabetes is reversed to near control in insulin, curcumin and Vitamin D_3 treatment. We report an increased expression of DA D2 receptor mRNA during insulin treatment in diabetic rats. Modulated expression during treatment in the hypothalamus normalize the decreased number to control levels suggesting the therapeutic value of curcumin and Vitamin D_3.

PANCREATIC DOPAMINERGIC RECEPTOR EXPRESSION IN CONTROL AND EXPERIMENTAL RATS

DA is a neurotransmitter that plays a critical role in neurological and psychiatric disorders, such as schizophrenia, Parkinson disease, and drug addiction (Callier *et al*., 2003). Increasing evidence also shows implication of dopamine in various physiological functions such as cell proliferation (Hoglinger *et al*., 2004), gastrointestinal protection (Mezey *et al*., 1996) and inhibition of prolactin secretion (Freeman *et al*., 2000). Effects of DA on insulin secretion in general and on pancreatic beta cell function in particular have been poorly studied. Treatment with dopamine precursor L-dopa in humans suffering from Parkinson disease reduces insulin secretion upon oral glucose tolerance test (Rosati *et al*., 1976). In rodents, a single injection with L-dopa results in the accumulation of dopamine in beta cells and inhibition of the insulin secretory responses (Ericson *et al*., 1977; Zern *et al*., 1980). In isolated islets, analogues of DA inhibit glucose-stimulated insulin release (Arneric *et al*., 1984), whereas one study reports potentiation of insulin secretion upon acute DA accumulation (Ahren & Lundquist, 1985). Taken as a whole, previous studies suggest that beta cells are directly responsive to DA. Here, we investigated the molecular mechanisms implicated in beta cell responses to DA receptors action in diabetes and insulin, curcumin and Vitamin D_3 treated diabetic rats.

In particular, the present data demonstrate the up regulation of DA D1 receptors in the pancreas of diabetic rats when compared to control. Greengard *et al.*, (1942) reported that exogenous DA stimulated the pancreatic secretion of water and bicarbonate in anesthetized dogs. This has been confirmed in the isolated, perfused pancreas (Hashimoto *et al*., 1971; Furuta *et al*., 1974; Bastie *et al*., 1977). Receptor binding studies with [^{3}H] DA also demonstrated the presence of specific postsynaptic receptors for DA in the exocrine pancreas of the dog (Vayssette *et al.,* 1986). Previous studies reported that DA-stimulated pancreatic secretion is mediated by DA D1 receptors on the basis of the antagonism by SCH23390, a selective DA D1 receptor antagonist (Horiuchi *et al*., 1989). DA-induced pancreatic exocrine secretion is mediated by activation of DA D1 receptors of the pancreas in dogs (Horiuchi *et al*., 1989). Insulin curcumin and Vitamin D_3 treatment reversed the distorted DA D1 receptor in the pancreas of diabetic rats to near control. Thus our results showed the functional difference in DA D1 receptor in pancreas of diabetic rats contributing to the dysfunction of pancreatic islets. Curcumin and Vitamin D_3 proved a novel therapeutic role in modulating DA D1 receptor in the pancreas of diabetic rats.

Moreover, the inhibitory effects of dopamine are predominantly ascribed to activation of the DA D2-like receptor family members. DA receptors are present in INS-1E beta cells as well as rat, mouse and human islets. Dopamine inhibited glucose-stimulated insulin secretion, an effect reproduced by activation of DA D2-like receptors using the DA D2/D3 receptor agonist quinpirole (Blanca *et al*., 2005). DA D2 receptor expression was confirmed by immunodetection revealing localization on insulin secretory granules of INS-1E and primary rodent and human beta cells. DA (10M) and the D2-like receptor agonist quinpirole (5 M) inhibited glucose stimulated insulin secretion tested in several models, i.e. INS-1E beta cells, fluorescence-activated cell-sorted primary rat beta cells, and pancreatic islets of rat, mouse, and

human origin (Blanca *et al*., 2005). Our data showed an up regulation of DA D2 receptors in the pancreas of diabetic rats compared to control. Thus our findings proved that DA D2 like receptors are expressed in pancreatic beta cells and mediate inhibition of insulin secretion in diabetic rats. The role played by dopamine in glucose homeostasis involve dopamine receptors, expressed in pancreatic beta cells, modulating insulin release. Also, treatment with insulin, curcumin and Vitamin D_3 reversed the increased expression of DA D2 receptor to near control. Therefore, the potential of curcumin and Vitamin D_3 in modulating DA D2 receptor action on beta cells have relevant implications for the better management of diabetes.

INSULIN RECEPTOR ALTERATIONS IN BRAIN AND PANCREAS

Several studies have found high levels of insulin receptors in the CNS at specific locations. The highest concentrations of insulin receptors in the brain are in olfactory bulb, cerebral cortex, hippocampus, cerebellum and hypothalamus (Havrankova *et al*., 1978; Unger *et al*., 1989; 1991). Furthermore, areas with high levels of insulin receptors correspond to the areas with the highest level of extractable insulin (Baskin *et al*., 1983). Most insulin receptor immunoreactivity is on neurons, with very little seen on glial cells (Unger *et al*., 1991; Baskin *et al*., 1993). In the hippocampus, insulin binding is detected in the molecular layer of the dentate gyrus, and in the dendritic fields of CA1 pyramidal cells (stratum oriens and stratum radiatum) (Unger *et al*., 1991 Corp *et al*., 1986). Importantly, insulin binding in the hippocampus is associated with immunocytochemically detectable phosphotyrosine and IRS-1, one of the putative cellular intermediates in insulin action (Baskin *et al*., 1993; 1994).

Our results showed that insulin receptor expression down regulated in cerebral cortex and up regulated in cerebellum, brain stem, corpus striatum,

hippocampus and hypothalamus of diabetic rats when compared to control. An alteration in insulin signaling ability will have a major impact on cellular energy balance by affecting rate of uptake of glucose and other metabolic substrates and also directly by affecting the activity of enzymes involved in carbohydrate metabolism (e.g. glycolysis, glycogen synthesis, gluconeogenesis), lipid metabolism (lipolysis, fatty acid and triacylglycerol synthesis, and protein metabolism (protein synthesis and degradation) (Dimitriadis, 2000). Many or all of the enzymes involved in the mitochondrial tricarboxylic acid cycle, the final common catabolic sequence, appear to be modulated by insulin independently of insulin-stimulated glucose transport (Bessman & Mohan, 1997). Expression of the genes for many enzymes involved in metabolism also appears to be regulated by insulin (O'Brien, 1996). Thus an alteration of insulin signalling in brain regions have a profound effect on cellular energetics and is a contributing factor in the energetic deficit associated with the development of diabetes associated neurodegenerative diseases.

Our results suggest an altered insulin receptor expression in the brain regions of diabetic rats which could elicit cognitive deficits. Experiments have shown the ability of small doses of insulin (0.4–0.8 units/kg) to reverse the amnesia produced by a 2 mg/kg scopolamine injection (Messier & Destrade, 1994; Blanchard & Duncan, 1997) and intra-cerebro-ventricular injection of insulin facilitates memory (Park *et al*., 1968). The wide distribution of insulin and insulin receptors in the brain as well as the presence of insulin-dependent glucose transporters suggest that insulin in the brain participates in several cognitive functions, including learning and memory. An obvious problem that has impeded further research is that exogenous insulin injection can reduce blood glucose and lead to hypoglycaemia which is associated with impaired memory (Santucci *et al*., 1990; Kopf & Baratti, 1995; Kopf *et al*., 1998). Cognitive impairments associated with diabetes mellitus caused by inadequate

insulin/insulin receptor functions have also been documented. In this study, the altered expression of insulin receptor in the brain regions of diabetic rat brought back to near control level by the treatment with insulin, curcumin and Vitamin D_3. Animal model research indicates that insulin deficiency results in impairments in synaptic plasticity and cognitive processes while human studies suggest that insulin insensitivity also affect cognitive processing. These results provide a confirmatory evidence for prevention of insulin receptor dysfunction in brain with insulin, curcumin and Vitamin D_3 treatment and represent a novel possibility for the better management of diabetic mediated neurological complications.

Pancreas

Insulin regulates peripheral energy homeostasis by acting on multiple tissues to control carbohydrate, lipid and protein metabolism (Saltiel, 2001). It has also been demonstrated that insulin receptor and post-receptor signaling mechanisms are required for pancreatic beta cell function (Kulkarni, 2002). Recent studies has shown that the beta cell insulin receptor knock out mice failed to show the growth of islet cells while the control and IGF1knock out mice did exhibit this growth response. Mice with global deletion of insulin receptor substrate (IRS) 2 develop type 2 diabetes due to a combination of insulin resistance and beta cell failure (Withers *et al*., 1998; Kubota, *et al*., 2000). Furthermore, cell-specific gene targeting in mice using Cre/loxP-mediated recombination strategies has shown that beta cell deletion of the insulin receptor reduces first-phase insulin release and beta cell insulin content and causes a progressive deterioration in glucose tolerance (Kulkarni *et al*., 1999). Deletion of the insulin-like growth factor 1 receptor gene (Igf1r) likewise impairs insulin synthesis and secretion and combined deletion of the insulin receptor gene and Igf1r causes marked beta cell failure (Kulkarni *et al*., 2002; Ueki *et al*., 2006). Our

results showed a decreased expression of insulin receptor in the pancreatic islets of diabetic rats and treatment with insulin, curcumin and Vitamin D_3 reverse this decreased expression to near control. Our findings suggest that insulin receptor dependent mechanisms are required for normal growth and function of beta cell and suggest a novel role of curcumin and Vitamin D_3 for maintenance of a normal glucose homeostasis through modulating insulin receptors in pancreatic islets.

VITAMIN D RECEPTOR GENE EXPRESSION IN BRAIN AND PANCREAS

Vitamin D_3 regulate immune function (Deluca & Cantorna, 2001) and cell differentiation (Segaert & Bouillon, 1998). Vitamin D_3 acts *via* a member of the nuclear hormone receptor family to directly regulate gene transcription (Clancy *et al*., 2001). There is now accumulating evidence that Vitamin D_3 plays a role in the central nervous system (Garcion *et al*., 2002). The Vitamin D_3 receptor and key enzymes involved in the metabolism of Vitamin D_3 are expressed in the rat brain (Garcion *et al*., 2002). Both animal and clinical studies strongly support the notion that chronic VD deficiency is harmful to brain development and to adult neural functions. For example, in rodent models, VD deficiency leads to brain malformation and has effects on rodent behaviour. In humans, it is associated with mood disorders, multiple sclerosis, schizophrenia, and epilepsy. The functions of VD are mediated through the nuclear VD receptor (VDR), a member of the nuclear receptors (NR). VDR is widespread in both the developing and adult brain, as well as in the spinal cord, suggesting a potential role for VD and VDR in the brain (Eyles *et al*., 2005).

VDR is expressed in most brain areas. Vitamin D_3, has been detected in the cerebrospinal fluid, and this hormone has been shown to cross the blood- brain barrier (Gascon-Barre & Huet, 1983). The presence of VDR in the limbic system, cortex, cerebellum of rodents and humans (Musiol *et al*., 1992) support a functional role for

Vitamin D_3 in the regulation of behaviour and cognitive functions. The present study showed an increased expression of VDR in cerebellum, brain stem and hypothalamus and decreased expression in cerebral cortex, corpus striatum and hippocampus of diabetic rats compared to control. This varying expression of VDR in the brain of diabetic rats will confer to altered neuronal activity. Efferents to cerebellar Purkinje cells and the thalamic part of the vestibular system, nucleus ventrolateralis, suggesting that the vestibular system is also a target of VD (Prufer *et al*., 1999). Expression of VDR in motor neurons (Prufer *et al*., 1999) suggests its role in regulation of motor functions. A putative receptor for 1,25(OH)2D has been detected in chick brain (Jia and Nemere, 1999), allowing speculation that 1,25(OH)2D could act like other neuroactive hormones in modulating neuronal activity and neurotransmitter receptors (Zakon, 1998; Rupprecht & Holsboer, 1999).

Our results showed that insulin, curcumin and Vitamin D_3 reversed the altered expression of VDR in the brain regions of diabetic rats. VDR is found in the olfactory, visual and auditory sensory systems (Glaser *et al*., 1999; Prufer *et al*., 1999; Zou *et al*., 2008), suggesting that the somatosensory system is also a target of 1,25(OH)2D. Recent studies showed curcumin a nutritionally-derived ligand of VDR (Bartika *et al*., 2010). Studies have shown that Vitamin D confers regulatory benefits in neuronal Ca^{2+} homeostasis and protects neurons from excess calcium entry in the brain (Brewer *et al*., 2001). Regulation of brain calcium homeostasis occurs *via* down-regulation of the L-type voltage-sensitive Ca^{2+} channels (L-VSCCs) in hippocampal cultured neurons, thus contributing to protection from excitotoxic cell death (Brewer *et al*., 2001). Treatment with 1,25(OH)2D in aged rats restores aging neurons (Brewer *et al*., 2006). These beneficial changes protect neurons during ischemic events or excitotoxic insults. Neuroprotective effect of 1,25(OH)2D also happen through reduction of Ca^{2+} toxicity by stimulation of expression of Ca-binding proteins (de Viragh *et al*., 1989),

thus supporting the idea that Vitamin D regulates the changes in VDR expression in the brain regions of diabetic rats and prevent neuronal degeneration in diabetes.

Pancreas

In recent years, there have appeared several reports which suggest that the endocrine pancreas is also a target tissue for the hormonally active form of vitamin D3, 1,25-(OH)2-D3, along with the classical vitamin D target organs: the intestine, bone and kidney (Norman *et al*., 1982). These observations include: (a) the presence of a cytosol receptor protein for 1,25-(OH)2-D3 in the chick pancreas (Christakos & Norman, 1981; Pike *et al*., 1980; Pike, 1981). Previous studies have indicated that the pancreas has receptors specific for Vitamin D_3 and that Vitamin D_3 increases insulin secretion in vitamin D-deficient rats (Norman *et al*., 1980).

Our results showed a decreased expression of VDR mRNA in the pancreatic islets of diabetic rats Also, treatment with insulin curcumin and vitamin D_3 has reversed this expression to near control. Early *ex vivo* studies by Norman *et al*. (1980) have shown that insulin but not glucagon release after stimulation with glucose and arginine is reduced in the isolated perfused pancreas from vitamin D-deficient rats. Later on, the same group showed that glucose tolerance and insulin secretion are impaired in vitamin D-deficient rats *in vivo* (Cade & Norman, 1986) and that insulin secretion was improved within 3 h after a single administration of 1,25(OH)2D3 to vitamin D-deficient rats (Cade & Norman, 1987). In more recent studies, it was reported that *de novo* insulin synthesis is reduced in isolated islets from vitamin D-deficient rats and that insulin biosynthetic capacity is restored in vitro by addition of 1,25(OH)2D3 (Bourlon *et al*., 1999). It is proposed that curcumin act as an agonist for vitamin D receptors and there by modulating its expression in diabetic pancreas.

GLUT3 EXPRESSION IN BRAIN

Glucose transport into the brain is critical for the maintenance of brain metabolism. Although under basal conditions the rate of glucose transport is not the rate-limiting step for glycolysis in the central nervous system, hypoglycaemia or hyperglycaemia is known to change the glucose transport system in the brain (Devivo *et al*., 1991), suggesting that there is glucose-regulatable mechanisms associated with the transport of glucose.

The expression, regulation and activity of glucose transporters play an essential role in neuronal homeostasis, because glucose represents the primary energy source for the brain (Lund-Anderen, 1979; Pardridge, 1983). Although many isoforms of glucose transporters have been identified in the brain, GLUT-3, the neuron-specific glucose transporter, is solely responsible for the delivery of glucose into neurons in the central nervous system. GLUT-3 mRNA is widely expressed in the brain, including the pyramidal neurons of the hippocampus and the granule neurons of the dentate gyrus (Nagamatsu *et al*., 1992; Nagamatsu *et al*., 1993; McCall *et al*., 1995) and immunohistochemical analysis has demonstrated that GLUT-3 protein expression also exhibits a widespread distribution in the brain (Nagamatsu *et al*., 1993; McCall *et al*., 1994; Zeller *et al*., 1995). In the hippocampus, GLUT3 immunoreactivity has been identified in mossy fibers, the stratum radiatum and stratum oriens of Ammon's horn, and the molecular layer of the dentate gyrus (McCall *et al*., 1994; Gronlund *et al*., 1995). Our study investigated the effect of learning-induced neuronal activation on brain glucose utilization. Our data showed an up regulation of GLUT3 mRNA in the brain regions- cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and hypothalamus. Region-specific increased neuronal activity has been shown to be often associated with parallel increases in brain glucose uptake (Sokoloff *et al*., 1977; Sarter *et al*., 1989; Bontempi *et al*., 1996; Barrett *et al*., 2003). A number of studies

investigated the effect of learning-induced neuronal activation on local cerebral glucose utilization (LCGU). Learning and memory processing is usually found to produce increases of glucose metabolism in the hippocampus (Shimada *et al*., 1983; Friedman & Goldman-Rakic, 1988) and cortical brain regions (Matsunami *et al*., 1989; Friedman & Goldman-Rakic, 1994) that are functionally related to memory processing as well as to the sensorimotor task requirements (Matsunami *et al*., 1989; Friedman & Goldman-Rakic, 1994). Our results confirm the alterations in GLUT3 expression, a major glucose transporter in CNS with STZ-induced diabetes.

Also, insulin, curcumin and Vitamin D_3 treatment improved the glucose transport system in brain regions of diabetic rats by regulating the increased GLUT3 expression. Alterations in glucose utilization are known to occur in the important regions of brain connected with learning and memory (van der *et al*., 1992). Learning and memory processing is found to produce increases of glucose metabolism in the cortical brain regions that is functionally related to memory processing as well as to the sensorimotor task requirements (Friedman & Goldman-Rakic, 1994). Our findingS suggest a modulation of GLUT 3 expression in the brain with curcumin and Vitamin D_3 supplementation which consecutively normalise the glucose transport in CNS.

GLUT2 EXPRESSION IN PANCREAS

In previous morphological studies the changes in the pancreatic islets and the destruction of the beta cells during the development of diabetes have been documented (Like *et al*., 1974 a,b; Frankel *et al*., 1987). Together with glucokinase, the low-affinity plasma membrane GLUT2 glucose transporter in the pancreatic beta cell is responsible for recognition of glucose as the signal for glucose-induced insulin secretion (Lenzen, 1992; Matschinsky *et al*., 1993; Lenzen & Tiedge 1994). Rat pancreatic beta cells display a dense immunostaining for GLUT2 in the cell

membrane (Jetton & Magnuson, 1992). Loss of GLUT2 immunoreactivity is an early indicator of beta cell dysfunction and is an element of importance for the deterioration of glucose-induced insulin secretion in diabetic Chinese hamsters. The expression of GLUT2 in pancreatic beta cells has been suggested to be important for the normal glucose sensitivity of these cells (Unger, 1991; Thorens, 1992). Importantly, the expression of this transporter is reduced or suppressed in glucose-unresponsive beta cells from diabetic rats and mice, a phenomenon that participate in the beta cell dysfunctions associated with diabetes (Johnson *et al*., 1990b; Orci *et al*., 1990; Thorens *et al*., 1992). While in most situations decreased expression of GLUT2 correlates with a decrease in its mRNA levels, decreased GLUT2 expression in dexamethasone-treated rats has been reported to be controlled at the translational or posttranslational level (Ogawa *et al*., 1992). Our results showed a decreased mRNA expression of GLUT2 in the pancreatic islets of diabetic rats. Earlier studies reports that curcumin *in vitro* protects pancreatic islets against cytokine-induced death and dysfunction and *in vivo* prevents STZ-induced diabetes (Kanitkar *et al*., 2008). As glucose is absorbed, the process is reversed, Ca^{2+} absorption is down regulated as the apical membrane is repolarized and glucose absorption is down regulated by loss of apical GLUT2. The integration of glucose and Ca^{2+} absorption represents a complex nutrient sensing system, which allows both absorptive pathways to be regulated rapidly and precisely to match dietary intake (Emma *et al*., 2008). It is suggested that Vitamin D_3 through absorption of calcium through VDR regularise the decreased GLUT2 expression in diabetes to control. Our results showed a novel role of curcumin and Vitamin D_3 in reversing the altered expression of GLUT2 in pancreatic islets and thereby eliciting glucose induced insulin secretion.

PHOSPHOLIPASE C EXPRESSION IN BRAIN AND PANCREAS

There is now great interest in the identification of molecules involved in the regulation of both normal neuronal differentiation and its activity-dependent modification. While a variety of transmitter receptors have been implicated in neuronal plasticity, much less is known of the second messenger systems and intracellular signalling pathways that subsequently lead to changes in the structure and functional properties of brain cells. Phospholipase C mediates transduction of neurotransmitter signals across membranes via hydrolysis of phosphatidylinositol-4,5-bisphosphate, leading to generation of second messengers inositol- 1,4,5-trisphosphate and diacylglycerol. In the CNS, neurotransmitter receptor coupling to phospholipase C (PLC) has been extensively documented in [^{3}H] inositol-labeled tissue slices and synaptosomes obtained from animal brains (Fisher & Agranoff, 1987; Stephens & Logan, 1989; Chandler & Crews, 1990). In the present study, we observed diabetes-mediated alterations in phospholipase C expression in the brain regions- cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and hypothalamus. Further we extended the studies to phospholipase C regulation with insulin, curcumin and Vitamin D_3 treatment for potential therapeutic drugs which modulate signal transduction pathway thereby contributing the prevention of CNS dysfunction in diabetes. Our results showed a decreased expression of phospholipase C in the cerebral cortex, cerebellum, brain stem, hippocampus and hypothalamus of diabetic rats and an increased expression in corpus striatum when compared to control. The DA D1 receptors showed characteristic ability to stimulate adenylyl cyclase and generated inositol 1, 4, 5-trisphosphate (IP3) and diacylglycerol *via* the activation of phospholipase C (Monsma *et al*., 1990; Sibley *et al*., 1993). Muscarinic receptors M1–M3–M5 typically couple *via* α subunits of the Gq/11 family to activate phospholipase C (PLC), stimulating phosphoinositide (PI) hydrolysis (Caulfield & Birdsall, 1998). In

particular, reconstitution experiments with purified muscarinic m1 receptors, G protein subunits, and PLC suggested that the β1 subtype of PLC serves as the primary effector for the muscarinic m1 receptor (Felder, 1995). We considered that the down regulation of the Phospholipase C in rat cerebral cortex and cerebellum during diabetes contribute to the impaired signal transduction of G-protein coupled neurotransmitter receptors. Phospolipase C performs a catalytic mechanism, generating inositol triphosphate (IP3) and diacylglycerol (DAG). Altered phospholipase C expression fails to modulate the activity of downstream proteins important for cellular signaling. Defective expression of phospholipase C results in low levels of IP3 causing the impaired release of Ca^{2+} and bring down the level of intracellular calcium and thus failed to execute the normal neuronal function in cerebral cortex and cerebellum. Previous studies reports that phospholipase C-mediated signaling, initiated by growth factor receptor types, are involved in long-term memory formation, a process that requires gene expression (Paul *et al*., 1999). These evidences led us to propose that the enhancement of diabetes-mediated phospholipase C gene expression could impart damage to the central cognitive functions, which has been effectively protected by curcumin and Vitamin D_3 treatment.

Pancreas

In response to glucose stimulation, a variety of metabolic, ionic, and signal transduction events occur contemporaneously (Hedeskov, 1980; Henquin, 1985; Rasmussen *et al*., 1995; Zawalich, 1996) These events culminate in a rapid biphasic insulin secretory response from the perfused rat pancreas and from freshly isolated perifused rat pancreatic islets (Grodsky, 1972; Gerich *et al*., 1974; Grill *et al*., 1978; Bolaffi *et al*., 1986; Zawalich *et al*.,1989a; Zawalich, 1990). These events include

not only the cation Ca^{2+}, which gains access to the β-cell via the opening of voltage-regulated channels, but also cyclic adenosine monophosphate and phosphoinositide derived second-messenger molecules, generated as a consequence of PLC activation. The underlying explanation for impaired insulin secretion in diabetes resides, at least in part, in the inability of glucose to activate information flow in the phospholipase C/protein kinase C (PLC/PKC) signal transduction system to the same quantitative extent in mouse islets as it does in rat and, presumably, human islets as well. Our study showed a decreased expression of phospholipase C expression in the pancreatic islets of diabetic rats. Treatment using insulin, curcumin and Vitamin D_3 normalized these change in expression to near control. Stimulation of muscarinic M1 and M3 receptor activate PLC–phosphoinositide 3-kinase (PI3K) pathway to increase glucose uptake (Biddlecome *et al*., 1996; Elmendorf, 2002; Hutchinson & Bengtsson, 2005). Thus, we conclude that curcumin and Vitamin D_3 has a regulatory role on phospholipase C expression and thereby controlling insulin synthesis and release from the pancreas at the second messenger level.

CREB EXPRESSION IN BRAIN

The CREB plays a pivotal role in dopamine receptor-mediated nuclear signaling and neuroplasticity (Finkbeiner, 2000). Here we demonstrated the significance of CREB gene expression in the brain regions- cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and hypothalamus of STZ-induced diabetes rats. CREB-responsive transcription plays a central role in the formation of long-term memory in *Drosophila, Aplysia* and mice (Alcino *et al*., 1998). Agents that disrupt the activity of CREB specifically block the formation of long-term memory, whereas agents that increase the amount or activity of the transcription factor accelerate the process.

CREB plays a pivotal role in dopamine receptor-mediated nuclear signaling and neuroplasticity (Finkbeiner, 2000). Our findings showed a significant down regulation of CREB in cerebral cortex, cerebellum, brain stem, hippocampus and hypothalamus and up regulation in corpus striatum of diabetic rats, when compared to control. Electrophysiological studies with hippocampal slices suggest that cAMP-dependent transcription is required for the maintenance of LTP (Frey *et al*., 1990; 1993; Huang & Kandel 1994; Impey *et al*., 1996). CREB activation is detected in cultured hippocampal neurons using an antibody (Ginty *et al*., 1993) specific to phosphorylated CREB proteins (Ser133 of CREB) (Deisseroth *et al*., 1996). The study of the cholinergic and dopamine receptors expression in relation with CREB phosphorylation in diabetes is an important step toward elucidating the relationship between molecular adaptations and behavioural consequences. CREB proteins in neurons are thought to be involved in the formation of long-term memories; this has been shown in the marine snail Aplysia, the fruit fly *Drosophila melanogaster*, and in rats. CREB is necessary for the late stage of long-term potentiation. CREB also has an important role in the development of drug addiction (Mayr & Montminy, 2001**).** It is therefore important to identify the elements that elicit phosphorylation of CREB and thereby its expression in the nucleus.

Our results showed that curcumin and Vitamin D_3 treatment reversed the decreased expression of CREB in diabetes to near control. The curcumin and Vitamin D_3 supplementation significantly modulated the altered gene expression of CREB in the brain regions of diabetic rats to near control. Insulin treatment did not show any significant effect in the CREB expression of diabetic rats in cerebral cortex, brain stem and hippocampus whereas cerebellum, corpus striatum and hypothalamus showed a significant reversal. This study demonstrated that curcumin and Vitamin D_3 possess regulatory effect in the transcription factor CREB expression, which is crucial

in maintaining the normal neuronal function and better management in diabetes. The DA D1 signal transduction pathway, activation of the transcription factor CREB and dopamine-mediated gene expression are critically involved in memory processing, behavioural responses and drug addiction (Nestler, 2001). Interruption of this pathway interferes with important cognitive performance and behavioural aspects associated with CNS. The effect of curcumin and Vitamin D_3 in interacting with the cholinergic, dopaminergic receptor and CREB in STZ-induced diabetes proved its potential in managing CNS disorders in diabetes.

SUPEROXIDE DISMUTASE EXPRESSION IN BRAIN AND PANCREAS

Glucose utilization is decreased in the brain of diabetic patients (McCall, 1992) providing a potential mechanism for increased vulnerability to acute pathological events. Since glucose is the main brain energy supply for the maintenance of the nervous system, the deficiency of glucose in the cell trigger neuronal injury (Seo *et al*., 1999). Impaired energy metabolism in neurons induce production of increased amount of free radicals (Coyl & Puttfarcken, 1993) and initiate excitotoxic neuronal cell damage (Simon *et al*, 1984; Monyer *et al*., 1989). The increased oxidative stress in diabetes (Baynes 1991; Wolff, 1993; Travero, 1998) and immobilization of stress produces oxidative damage in many regions of rat brain including the hippocampus (Liu *et a.l*., 1996). Furthermore, oxidative damage in rat brain is increased by experimentally induced hyperglycemia (Aragno, 1997). Oxidative damage to various brain regions constitute into the long term complications, morphological abnormalities and memory impairments (Aksenov et al., 2001; Bunsey et al., 1996; Eichenbaum et al., 1992; Regan et al, 2001; Suzuki & Clayton, 2000). Protection of brain cells from degeneration should be an effective strategy to prevent

or to slow the progression of disease. Compounds that prevent oxidative damage increase the resistance of neuronal cells to degeneration.

It has been suggested that free radical species responsible for STZ toxicity is the hydroxyl radical. The destruction of superoxide radical or H_2O_2 by SOD or CAT would ameliorate STZ toxicity, as would substances able to scavenge the hydroxyl radical (Walling, 1975; Lubec, 1996). Vulnerability of brain to oxidative stress induced by oxygen free radicals seems to be due to the fact that, on one hand, the brain utilizes about one fifth of the total oxygen demand of the body and on the other, that it is not particularly enriched, when compared with other organs, in any of the antioxidant enzymes. Relatively low levels of these enzymes are responsible in part for the vulnerability of this tissue (Baynes & Thrope, 1999). Our results showed a decreased expression of SOD in diabetic rats compared to control in cerebral cortex, cerebellum and hippocampus and an increased expression in brain stem, corpus striatum and hypothalamus. The decreased SOD activity in organs suggests that the accumulation of superoxide anion radical is responsible for increased lipid peroxidation. The inactivity of the antioxidant enzymes, SOD in the diabetes-induced groups was attributed to peroxidative damage to the tissues caused by administering STZ (Kwag, 2001). The decreased activities of SOD is a response to increased production of H_2O_2 and O_2 by the autoxidation of glucose and non-enzymatic glycation (Aragno *et al*., 2000). This alteration of SOD represents one of the important factors for the vulnerability of the brain against oxygen free radicals or is relevant to the pathophysiology of diabetes in Wistar rats. Treatment with insulin, curcumin and Vitamin D_3 ameliorated the expression of enzyme and helps to control free radicals in brain regions. Curcumin has been shown to have a broad spectrum of biological activities such as anti-inflammatory, anti-neoplastic, antimutagenic and antioxidant (Naik *et al*., 2004). Research has shown curcumin to be a powerful

scavenger of the superoxide anion, the hydroxyl radical and nitrogen dioxide (Daniel *et al*., 2004). Previous studies report that Vitamin D_3 exhibit membrane anti oxidant property and an ability to inhibit iron-dependent lipid peroxidation in liposomes (Wiseman, 1993). Our data proved the anti oxidant property of curcumin and Vitamin D_3 in the brain regions, which could exert a beneficial action against numerous morphological and functional alterations during diabetes caused by the presence of free radicals in STZ diabetes.

Pancreas

In the past, numerous studies established a crucial role of reactive oxygen species in the pathogenesis of acute and chronic pancreatitis (Guyan *et al*, 1990; Schoen *et al*, 1992; Antosiewicz *et al.*, 1995). The damage of pancreatic acinar cells by oxidative stress leads to an uncontrolled release of digestive enzymes from the zymogen granula, which then causes the destruction of the surrounding tissue. Mechanisms have been detected resulting in an increase of oxygen radicals in pancreatic tissue (Uden *et al.*, 1988). Recently, Kishimoto et al. successfully detected superoxide production in rat pancreas using the well-established model ofceruleininduced pancreatitis (Kishimoto *et al.*, 1995; Ito *et al.*, 1996*).* SOD is implicated in the pathophysiology of various disease states including diabetes mellitus. Oxygen free radicals exert their cytotoxic effect by peroxidation of membrane phospholipids leading to change in permeability and loss of membrane integrity (Meerson *et al*., 1982). Pancreatic β-cell death underlies the pathogenesis of Type I (insulin-dependent) diabetes mellitus and liver is an important organ which offers an adequate site for various metabolic functions. Oxygen free radicals have been implicated in both β cell destruction as well as in liver injury (Roza *et al.*, 1985; Poli *et al*., 1989; Hunt *et al*., 1990; Robinovitch *et al*., 1992). Our results showed a

decreased expression of SOD in the pancreas of diabetic rats when compared to control.

The treatment using insulin, curcumin and vitamin D_3 reversed the pancreatic SOD expression in diabetes to near control. It is known that pancreatic β -cells contain very low levels of antioxidant enzymes which render them more susceptible to reactive oxygen species-induced toxicity as compared to other cell types (Tiedge *et al*., 1997). Hence, curcumin and Vitamin D_3 showed a prominent anti oxidant activity by normalizing the SOD expression to near control.

Thus our results showed that uncontrolled hyperglycaemia, deficiencies of central insulin, or both contributes to CNS disorders mediated through cholinergic, dopaminergic, insulin and Vitamin D receptor. Also, gene expression of cholinergic enzymes, glucose transporter GLUT3/2, transcription factor CREB, second messenger enzyme phospholipase C and anti oxidant enzyme, superoxide dismutase is found to be altered in the CNS of diabetic rats. Nutritional therapy using curcumin and Vitamin D_3 exhibited a potential effect in improving glucose homeostasis and reversing the altered functional regulation of receptors and enzymes of STZ induced diabetic rats to near normal. These results provide a confirmatory evidence for neuroprotective role of curcumin and Vitamin D_3 and represent a novel therapeutic possibility for the better management of diabetic mediated neurological complications.

Summary

1. Streptozotocin induced diabetic rats were used as model to study the alterations of cholinergic, dopaminergic, insulin, Vitamin D receptors, GLUT3, second messenger enzyme phospholipase C, CREB and anti oxidant enzyme super oxide dismutase and their regulation by curcumin and Vitamin D_3 in insulin secretion.

2. Antihyperglycemic activity of curcumin and Vitamin D_3 were evaluated by the blood glucose and circulating insulin level measurement of experimental rats. Diabetic rats showed increased blood glucose and decreased insulin level. Curcumin and Vitamin D_3 supplementation to diabetic rats reversed the blood glucose and circulating insulin level to control.

3. Serum T3 concentration was decreased in diabetic rats. Insulin, curcumin and Vitamin D_3 treatment reversed the T3 concentration to near control.

4. Behavioural studies: Y maze, rotarod, beam walk and grid walk test were conducted to assess the motor learning and memory in control and experimental rats. Diabetic rats showed a significant deficit in cognition, memory and motor learning. Insulin, curcumin and Vitamin D_3 treated diabetic rats reversed the behavioral response to near control when compared to diabetic rats.

5. Acetylcholine esterase expression level has been used as a marker for cholinergic activity. Acetylcholine esterase expression was analysed in the brain regions and pancreas. During diabetic stage the expression was increased in the cerebral cortex, cerebellum, brainstem, hippocampus and

hypothalamus while in corpus striatum it was decreased. Pancreas showed an up regulation in diabetic rats compared to control. In insulin treated, curcumin and Vitamin D_3 treated diabetic rats, the expression of the enzyme reversed to near control. Immunocytochemical studies using specific antibodies of acetylcholine esterase confirmed the mRNA expression at protein level in pancreas of control and experimental rats by reversing the changes in diabetic rats.

6. Choline acetyltransferase expression level has been used as a marker for acetylcholine synthesis. Choline acetyltransferase expression was analysed in the brain regions and pancreas. During diabetes, the expression was decreased in the cerebral cortex, cerebellum, corpus striatum, hippocampus and hypothalamus while in brain stem it was increased. Pancreas showed a down regulation in diabetic rats compared to control. In insulin, curcumin and Vitamin D_3 treated diabetic rats, the expression of the enzyme reversed to near control.

7. Total muscarinic receptor was analysed in the brain regions and pancreas of control and experimental rats. Total muscarinic receptor binding was decreased in cerebral cortex, corpus striatum and hippocampus while cerebellum and brainstem showed increased expression in diabetic rats. The Scatchard analysis and gene expression studies of muscarinic M1 receptor revealed a down regulation in cerebral cortex, brainstem, hippocampus and hypothalamus whereas in cerebellum and corpus striatum it was up regulated. Muscarinic M3 receptor binding and expression in cerebral cortex, cerebellum, brain stem, hippocampus and hypothalamus were increased and in corpus striatum there was a decrease in diabetic rats compared to control. In pancreas total muscarinic, muscarinic M1 and muscarinic M3 receptors were down regulated in

diabetic condition. Insulin, curcumin and Vitamin D_3 supplementation restored the binding and expression of total muscarinic, muscarinic M1 and muscarinic M3 receptors in brain regions and pancreas to near control. Immunohistochemistry studies using specific antibodies confirmed the Scatchard analysis and Real Time PCR analysis of muscarinic receptor expression at protein level in control and experimental rats.

8. α7 nicotinic acetylcholine receptor gene expression was studied in brain regions of experimental rats. In diabetic condition α7 nicotinic acetylcholine receptor was increased in cerebral cortex, cerebellum, brain stem and corpus striatum and decreased in hippocampus when compared to control. Treatment using curcumin and Vitamin D_3 in diabetic rats reversed the altered expression in the brain regions to near control whereas insulin treatment to diabetic rats did not significantly restore the altered α7 nicotinic acetylcholine receptor gene expression to control. Immunohistochemistry studies using specific antibodies confirmed the gene expression of α7 nicotinic acetylcholine receptor expression at protein level in control and experimental rats.

9. Total dopamine receptor binding was analysed in the brain regions of control and experimental rats. Total dopamine receptor binding was increased in cerebral cortex, brain stem and hippocampus while cerebellum and corpus striatum showed increased expression in diabetic rats. The gene expression studies of dopamine D1 receptor revealed an up regulation in cerebral cortex, brain stem and hippocampus whereas in cerebellum, corpus striatum and hypothalamus it was down regulated. Dopamine D2 receptor expression in cerebral cortex, cerebellum, corpus striatum and hippocampus was increased and in brain stem there was a

decrease in diabetic rats compared to control. In pancreas dopamine D1 and D2 receptor expression decreased in diabetic condition. Insulin, curcumin and Vitamin D_3 supplementation brought back the altered expression of total dopamine, DA D1 and DA D2 receptors to near control.

10. Vitamin D receptor status in the brain regions and pancreas of experimental rats were analysed using Real Time PCR. Cerebral cortex, corpus striatum, and hippocampus showed a decreased Vitamin D receptor mRNA level while an increased mRNA expression level in cerebellum, brain stem and hypothalamus of diabetic rats. There was decreased expression of Vitamin D receptor in pancreas of diabetic rats when compared to control. Restoration of disrupted Vitamin D receptor expression was seen with insulin, curcumin and Vitamin D_3 treatment to diabetic rats.

11. Insulin receptor mRNA level was studied in the brain regions and pancreas of experimental rats. A decreased expression of insulin receptor was observed in cerebral cortex whereas in cerebellum, brain stem, corpus striatum, hippocampus and hypothalamus, there was an increased expression in diabetic rats. Pancreas of diabetic rats showed decreased insulin receptor expression. Insulin, curcumin and Vitamin D_3 treatment to diabetic rats considerably ameliorated the altered insulin receptor expression to near control.

12. Gene expression studies showed insulin, curcumin and Vitamin D_3 treatment substantially reversed the increased expression of GLUT3 in brain regions- cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and hypothalamus of diabetic rats to near control. GLUT2

expression was studied in the pancreas and showed down regulation in diabetic rats when compared to control. The treatment groups reversed the decreased expression of GLUT2 to near control.

13. Second messenger enzyme - phospholipase C showed a decreased expression in diabetic brain regions - cerebral cortex, cerebellum, brain stem, hippocampus, hypothalamus and increased expression in corpus striatum. Diabetic pancreas also showed a decreased phospholipase C expression when compared to control. Insulin, curcumin and Vitamin D_3 administration to diabetic rats reversed the altered phospholipase C expression to near control.

14. Transcription factor, CREB expression in the brain regions - cerebral cortex, cerebellum, brain stem, hippocampus and hypothalamus showed decreased expression in diabetic rats. In corpus striatum, there was an increased CREB expression in diabetic rats compared to control. Diabetes induced altered CREB expression in brain regions was reversed with insulin, curcumin and Vitamin D_3 treatment to near control.

15. Antioxidant enzyme, superoxide dismutase expression was studied in experimental rats. Results showed that in diabetic rats, its mRNA level was down regulated in cerebral cortex, cerebellum, hippocampus and hypothalamus whereas in brain stem and corpus striatum, it was up regulated when compared to control. Pancreatic expression of superoxide dismutase in diabetic rats was decreased compared to control. Oxidative stress seen in diabetic brain regions and pancreas was considerable lowered by reversing the expression of superoxide dismutase to near control by treatment with insulin, curcumin and Vitamin D_3.

In summary, we conclude that brain and pancreatic cholinergic, dopaminergic, Vitamin D, insulin receptor, GLUT3/2, phospholipase C, CREB and superoxide dismutase functional balance has a major role in regulating the insulin secretion and modulating behavioural and cognitive process. The present study demonstrates the therapeutic role of nutritional agents, curcumin and Vitamin D_3 in ameliorating CNS dysfunctions and insulin synthesis and secretion from pancreas. Thus our results confirmed neuroprotective role of curcumin and Vitamin D_3 through cholinergic and dopaminergic functional regulation and glucose homeostasis which in turn lead to a novel therapeutic management of diabetes.

Conclusion

Diabetes mellitus, a chronic metabolic disorder and its long-term complications have devastating consequences like cognitive dysfunctions, neurophysiological and structural changes in the CNS. Nutritional therapy is a challenging but necessary dimension in the management of diabetes and neurodegenerative changes associated with it. Behavioural studies showed deficit in spatial learning, memory and motor control in diabetic rats. Our results showed that cholinergic and dopaminergic functional regulations were impaired in diabetes contributing to the neurological dysfunction which is suggested to cause behavioural deficits. Functional role of Vitamin D receptor in diabetes showed changes in brain regions and pancreas thereby contributing to behavioural and cognitive deficit, impaired insulin synthesis and release from pancreas. In diabetes, brain insulin receptor and glucose transporter GLUT3 expression showed alterations which are functionally related to cognitive deficit. Altered pancreatic insulin receptor and GLUT2 expression resulted in decreased insulin synthesis and release. Down regulation of phospholipase C, a second messenger enzyme in the brain regions of diabetic rats showed a defective signal transduction at second messenger level. Decreased CREB mRNA expression induced by diabetes showed impaired long term memory processing. Differential expression of anti oxidant enzyme, superoxide dismutase in diabetes imparts increased oxidative stress. Treatment of diabetic rats with insulin, curcumin and Vitamin D_3 reversed the altered cholinergic and dopaminergic neurotransmission, insulin, Vitamin D receptor, GLUT3/GLUT2, phospholipase C, CREB and superoxide dismutase expression in brain and pancreas. Thus our results showed that curcumin and Vitamin D_3 have neuroprotective role in diabetes by increasing insulin synthesis and release from pancreas, maintaining glucose homeostasis, which in turn lead to a novel therapeutic management of diabetes.

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1. **Peeyush Kumar T**, Gireesh G, Jobin Mathew Paulose CS**.** (2009). Neuroprotective Role of Curcumin in the Cerebellum of Streptozotocin Induced Diabetic Rats. Life Sciences, 85: 704-710.

2. **Peeyush Kumar T**, Savitha Balakrishnan, Sherin antony Anju TR, Jes paul and Paulose CS. (2010). Cholinergic, Dopaminergic and Insulin Receptors Gene Expression in the Cerebellum of Streptozotocin Induced Diabetic Rats: Functional Regulation with Vitamin D3 Supplementation. Pharmacology Biochemistry and behavior, 95: 216–222.

3. **Peeyush Kumar T**, Sherin Antony, Nandhu MS, Jayanarayanan S, Naijil George, Paulose CS. (2010)Vitamin D_3 Restores Altered Cholinergic and Insulin Receptor expression in the Cerebral Cortex and Muscarinic M3 receptor expression in Pancreatic Islets of Streptozotocin Induced Diabetic Rats. Journal of Nutritional Biochemistry**,** doi: 01.2045/j.nb.2010.03.0435.

4. Gireesh G, Balarama Kaimal S, **Peeyush Kumar** T, and Paulose CS. (2008). Decreased Muscarinic M1 Receptor Gene Expression in the Hypothalamus, Brainstem, and Pancreatic Islets of Streptozotocin-Induced Diabetic Rats. Journal of Neuroscience Research, 86:947–953

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6. Gireesh G, **Peeyush Kumar T**, Jobin Mathew Paulose CS. (2009). Enhanced muscarinic M1 receptor gene expression in the corpus striatum

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9. Savitha Balakrishnan, **Peeyush Kumar T**, Paulose CS. (2009). Glutamate (mGluR-5) gene expression in brain regions of streptozotocin induced diabetic rats as a function of age: role in regulation of calcium release from the pancreatic islets in vitro. Journal of biomedical science, 16: 99

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12. Anu Joseph, **Peeyush Kumar T**, Nandhu MS CS Paulose. (2010). Enhanced Nmdar1, Nmda2b and Mglur5 Receptors Gene Expression In The Cerebellum Of Insulin Induced Hypoglycaemic And Streptozotocin Induced Diabetic Rats. European Journal of pharmacology, doi:10.1016/j.ejphar.2009.12.024.

13. Jobin Mathew, **Peeyush Kumar T**, Reas Khan S Paulose CS. (2010). Decreased GABA Receptor in the Cerebellum of Epileptic Rats and Behavioural Deficit: Effect of Bacopa monnieri and Bacoside-A.Epilepsy and Behavior, doi:10.1016/j.yebeh.2010.01.012 (In Press).

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15. **Peeyush Kumar T.,** Sherin Antony., Gireesh G., Naijil George & Paulose C. S. Curcumin modulates dopaminergic receptor, CREB and phospholipase c gene expression in the cerebral cortex and cerebellum of streptozotocin induced diabetic rats. Journal of Biomedical Sciences (Accepted).

SEMINAR/CONFERENCE PRESENTATION

1. **Peeyush Kumar T**, Gireesh G and C. S. Paulose Increased Acetylcholine Esterase Activity In The Hypothalamus Of Diabetic Rats: Anti Diabetic Effect Of Aegle Marmelose And Costus Pictus Leaf Extracts. International Conference on Advances in Neuroscience & XXVI Annual Meeting of Indian Academy of Neurosciences held at Banaras Hindu University, Varanasi. (December 2007)

2. **Peeyush Kumar T**, Gireesh G and C. S. Paulose Altered Muscarinic M3 receptor gene expression in the cerebral cortex and Cerebellum of Diabetic rats: Supplementation of Vitamin D_3. Society for Biotechnologist Annual meeting held at Madras University Chennai National Conference On New frontiers and Current trends in Biotechnology & Platinum Jubilee celebrations of Department of Biochemistry. (October 2008).

3. **Peeyush Kumar T**, Gireesh G and C. S. Paulose. Altered Muscarinic M3 receptor gene expression in the cerebral cortex and Cerebellum of Diabetic rats: Supplementation of Vitamin D3. International Conference on Advances in Neuroscience & XXVI Annual Meeting of Indian Academy of Neurosciences. Department of Biotechnology. Cochin University of Science and Technology. (December 2008)

4. Sherin Antony, Anu Joseph, **Peeyush Kumar T**, C. S. Paulose. Enhanced Muscarinic M3 receptor gene expression in the cerebral cortex of Diabetic and Insulin Induced Hypoglycemic rats. 77th Annual Meeting of the Society of Biological Chemists (India) IIT Madras, Chennai. (December 2008)

5. Jobin Mathew, **Peeyush Kumar T**, C.S. Paulose GABA receptor functional regulation in the cerebral cortex of pilocarpine induced epileptic rats: neuroprotective role of Bacopa monnieri. 21st Kerala science congress, Kollam (January 2009)

6. Jayanarayanan S, **Peeyush Kumar T**, and C. S. Paulose. Enhanced Muscarinic M3 Receptor Gene Expression in the Cerebral Cortex of Diabetic rats: Supplementation of Curcumin. Society for Biotechnologist Annual meeting and National Conference on Biotechnology for Human Development held at Vellore institute of science and technology, Vellore. (November 2009)

7. **Peeyush Kumar T**., Sherin Antony and Paulose C. S. Vitamin D_3 restores altered cholinergic receptors in the cerebral cortex and Pancreas of diabetic rats. International conference on Neuroscience Updates. Department of Biotechnology. Cochin University of Science and Technology. (December 2009).

Figure-1

Body weight (gm) of Experimental rats

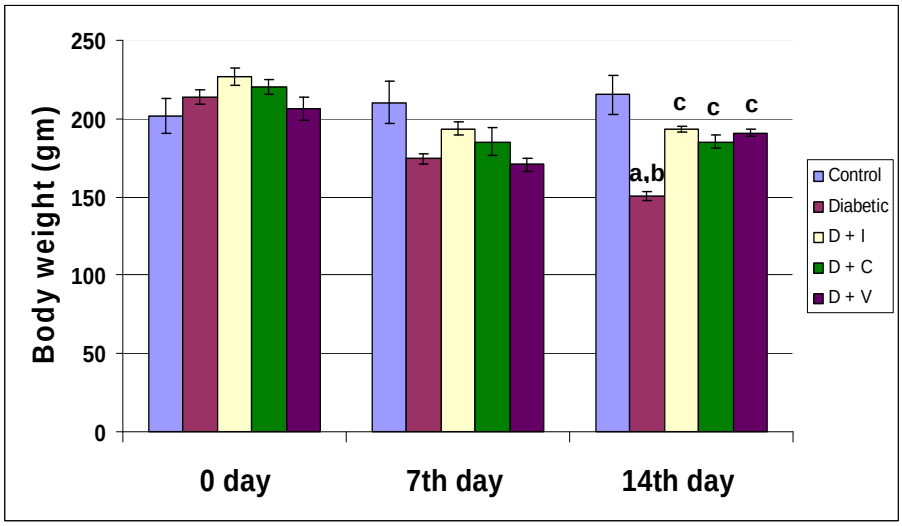


Table-1

Body weight (gm) of Experimental rats

Experimental groups	0 Day (Initial)	7th day	14th day
Control	202.0 ± 11.2	210.5 ± 13.3	215.3 ± 12.5
Diabetic	214.0 ± 4.7	174.3 ± 3.5	150.4 ± 2.6 [a,b]
D + I	226.6 ± 5.3	193.6 ± 4.1	193.2 ± 2.2 [c]
D + C	220.3 ± 4.7	185.3 ± 8.8	200.3 ± 4.5 [c]
D + V	206.3 ± 7.8	170.6 ± 4.3	190.7 ± 2.5 [c]

Values are mean ± S.E.M of 4-6 separate experiment. Each group consist of 6-8 rats. [a] $p<0.001$ when compared with control. [b] $p<0.001$ when compared with initial weight, [c] $p<0.001$ when compared with diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-2

Blood glucose (mg/dL) level in Experimental rats

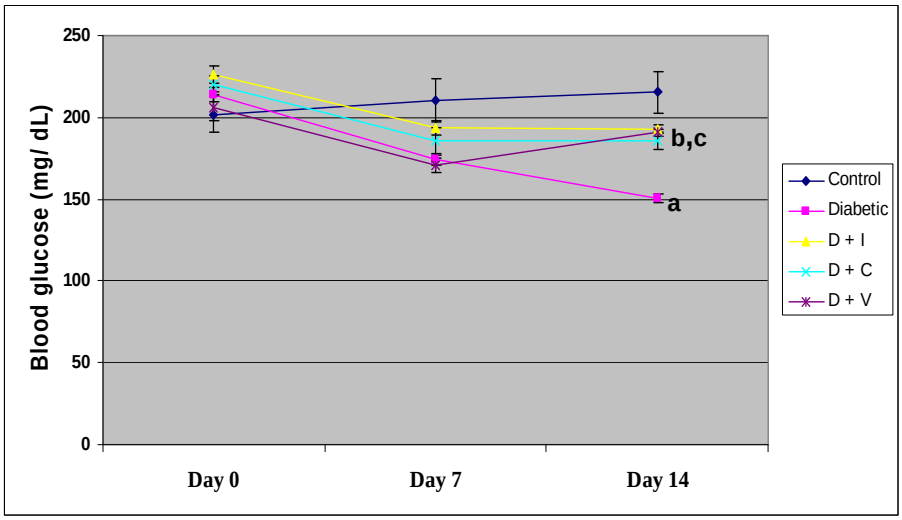


Table-2

Blood glucose (mg/dL) level in Experimental rats

Experimental groups	0 day (Before STZ injection)	3rd day (Initial)	6th day	10th day	14th day (Final)
Control	82.3 ± 1.6	86.5 ± 1.6	89.6 ± 1.2	92.3 ± 1.4	90.7 ± 1.2
Diabetic	80.3 ± 1.3	255.1 ± 0.8	317.3 ± 1.4	306.8 ± 0.7	313.3 ± 1.4 [a]
D + I	84.2 ± 0.8	256.8 ± 0.5	303.6 ± 0.7	190.9 ± 1.5	137.0 ± 1.3 [b,c]
D + C	84.2 ± 1.2	255.6 ± 1.1	310.0 ± 0.8	213 ± 1.5	170.2 ± 1.4 [b,c]
D + V	86.3 ± 1.5	257.4 ± 1.4	310.0 ± 0.8	195 ± 1.5	148.4 ± 2.5 [b,c]

Values are mean ± S.E.M of 4-6 separate experiment. Each group consist 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. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-3

Circulating insulin level in the plasma of control and experimental rats

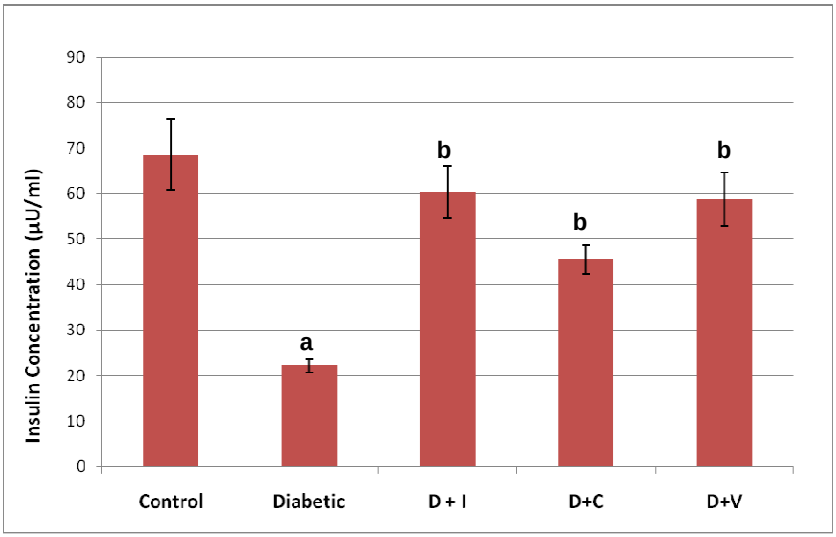


Table-3

Circulating insulin level in the plasma of control and experimental rats

Experimental groups	Insulin Concentration (µU/ml)
Control	68.6 ± 7.81
Diabetic	22.2 ± 1.45 [a]
D + I	60.4 ± 5.68 [b]
D + C	45.5 ± 3.09 [b]
D + V	58.8 ± 5.90 [b]

Values are mean ± S.E.M of 4-6 separate experiment. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-4

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

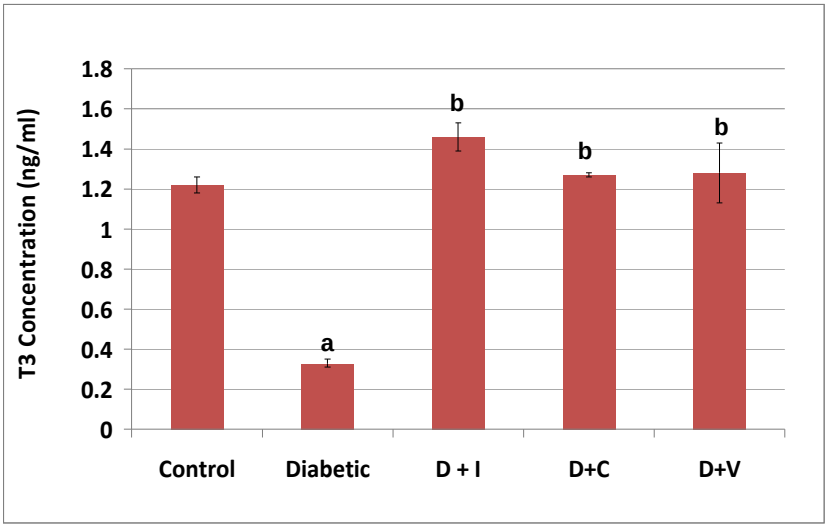


Table-4

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

Experimental groups	Concentration (ng/ml)
Control	1.22 ± 0.04
Diabetic	0.33 ± 0.02 [a]
D + I	1.46 ± 0.07 [b]
D + C	1.27 ± 0.01 [b]
D + V	1.28 ± 0.15 [b]

Values are mean ± S.E.M of 4-6 separate experiment. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-5

Behavioural response of control and experimental rats on Y maze

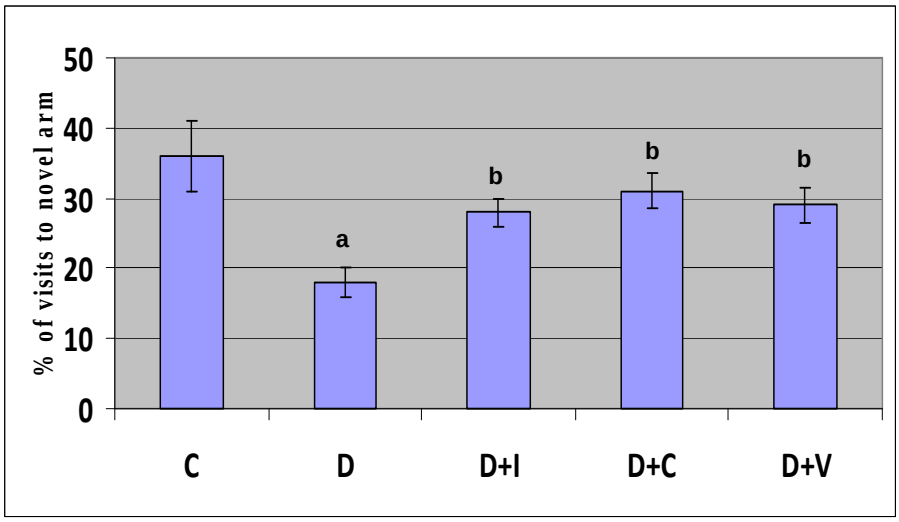


Table-5

Behavioural response of control and experimental rats on Y maze

Experimental groups	% of visits to novel arm
Control	36 ± 5.0
Diabetic	18 ± 2.2 [a]
D + I	28 ± 2.0 [b]
D + C	31 ± 2.5 [b]
D + V	29 ± 2.5 [b]

Values are mean ± S.E.M of 4-6 separate experiment. Each group consist of 5-6 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-6

Time spent on metallic rod of control and experimental rats in rotarod experiment

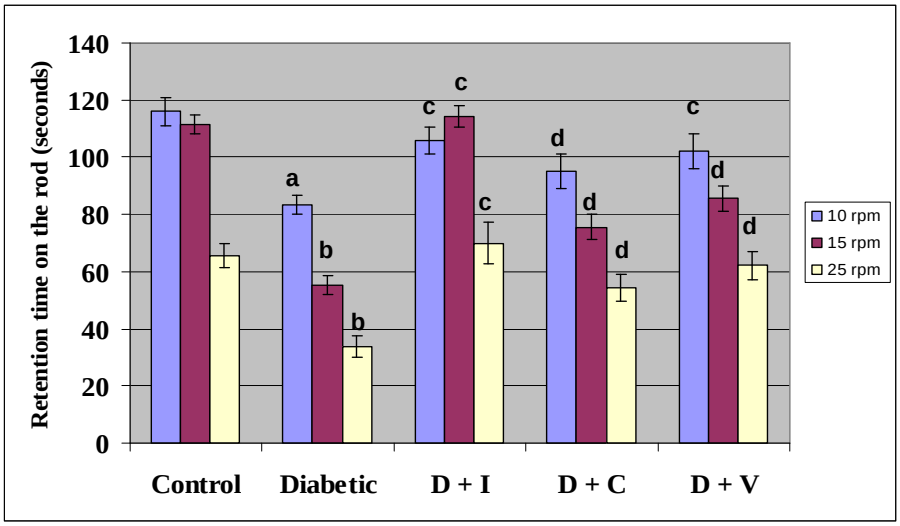


Table-6

Time spent on metallic rod of control and experimental rats in rotarod experiment

Experimental groups	Retention Time on the Rod (in seconds)		
	10 rpm	15 rpm	25 rpm
Control	115.00 ± 5.61	109.33 ± 4.30	68.34 ± 5.38
Diabetic	80.33 ± 2.40 [a]	57.33 ± 4.38 [b]	35.63 ± 4.65 [b]
D + I	106.00 ± 0.47 [c]	114.45 ± 3.70 [c]	70.00 ± 7.42 [c]
D + C	95.00 ± 6.12 [d]	75.45 ± 4.48 [d]	54.33 ± 4.95 [d]
D + V	102.00 ± 6.12 [c]	85.52 ± 4.48 [d]	62.23 ± 4.95 [d]

Values are mean ± S.E.M of 4-6 separate experiment. Each group consist of 5-6 rats. [a] $P<0.01$, [b] $P<0.001$ when compared to control group, [c] $P<0.001$, [d] $P<0.01$ when compared to diabetic group,. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-7

Behavioural response of control and experimental rats on grid walk test

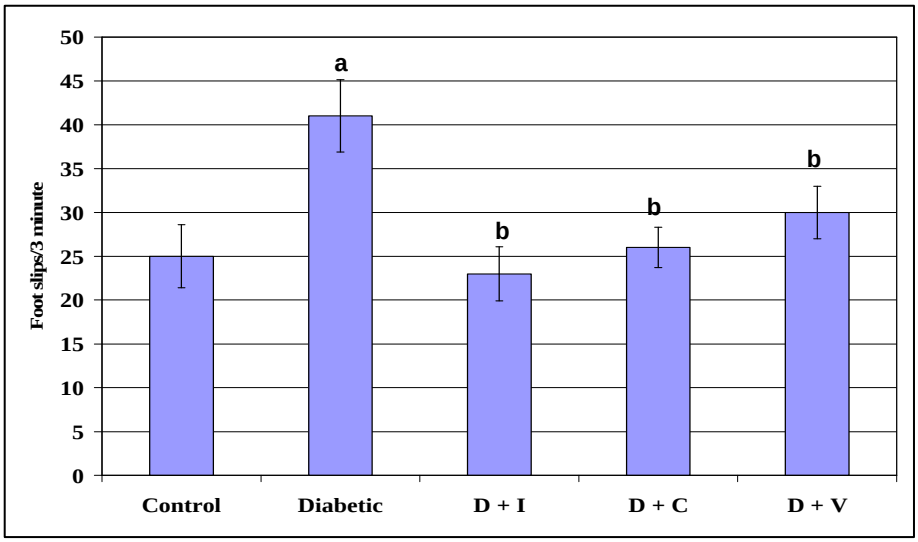


Table-7

Behavioural response of control and experimental rats on grid walk test

Experimental groups	Foot slips/3 minutes
Control	25 ± 3.8
Diabetic	41 ± 4.3 [a]
D + I	23 ± 3.1 [b]
D + C	26 ± 2.3 [b]
D + V	30 ± 3.0 [b]

Values are mean ± S.E.M of 4-6 separate experiment. Each group consist of 5-6 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-8

Behavioural response of control and experimental rats on narrow beam test

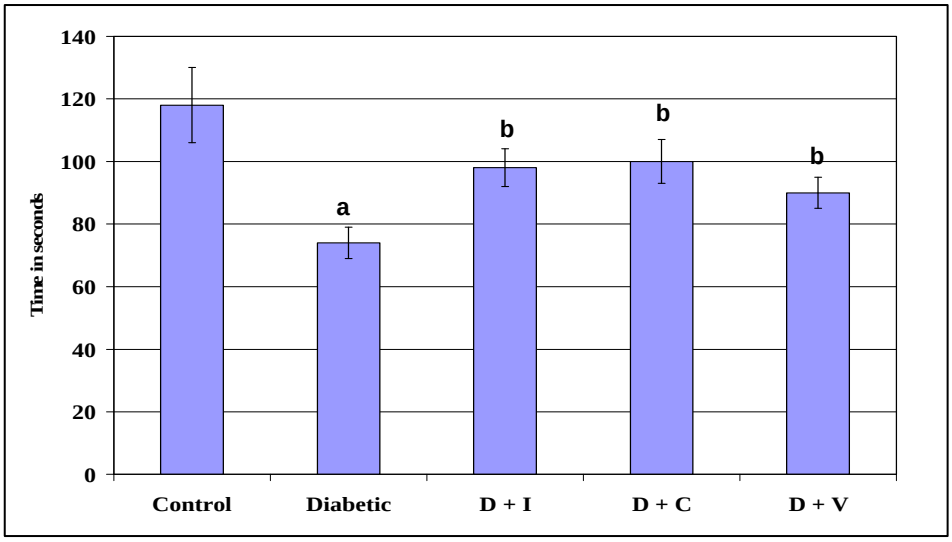


Table-8

Behavioural response of control and experimental rats on narrow beam test

Experimental groups	Time in seconds
Control	118 ± 9.4
Diabetic	74 ± 5.1 [a]
D + I	98 ± 6.2 [b]
D + C	100 ± 7.5 [b]
D + V	90 ± 5.3 [b]

Values are mean ± S.E.M of 4-6 separate experiment. Each group consist of 5-6 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-9

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebral cortex of control and experimental rats

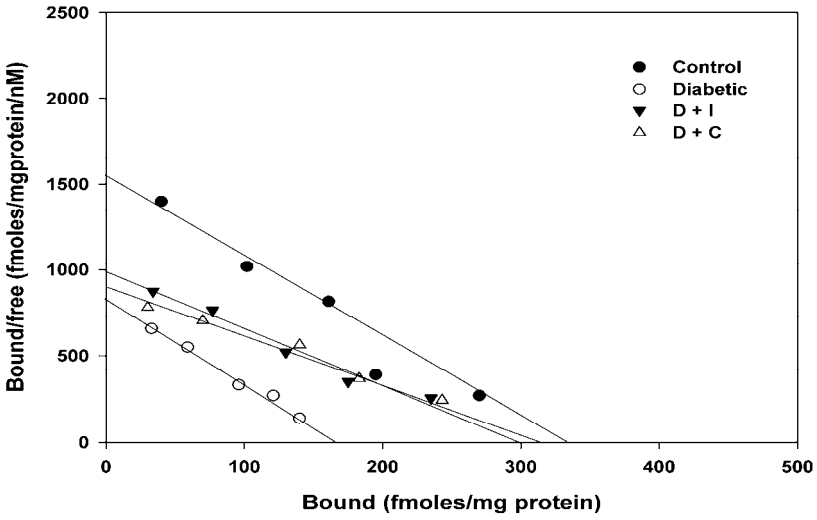


Table-9

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	316.2 ± 8..5	0.21 ± 0.05
Diabetic	160.5 ± 6.2 [a]	0.17 ± 0.04 [c]
D + I	300.3 ± 8.2 [b]	0.30 ± 0.01 [d]
D + C	308.6 ± 8.6 [b]	0.32 ± 0.03 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats [a] P<0.001, [c] P<0.05 when compared to control, [b] P<0.001, [d] P<0.01 when compared to diabetic group, when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- curcumin treated diabetic rats.

Figure-10

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebral cortex of control and experimental rats

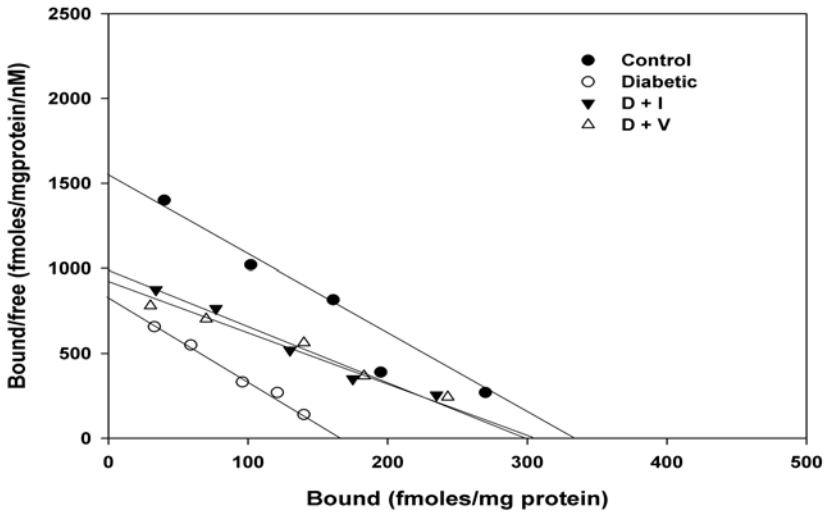


Table-10

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	316.2 ± 8.5	0.21 ± 0.02
Diabetic	160.5 ± 6.2 [a]	0.17 ± 0.02 [c]
D + I	300.3 ± 8.2 [b]	0.30 ± 0.01 [d]
D + V	301.0 ± 7.4 [b]	0.30 ± 0.08 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats [a] P<0.001, [c] P<0.05 when compared to control, [b] P<0.001, [d] P<0.01 when compared to diabetic group, when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-11

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebral cortex of control and experimental rats

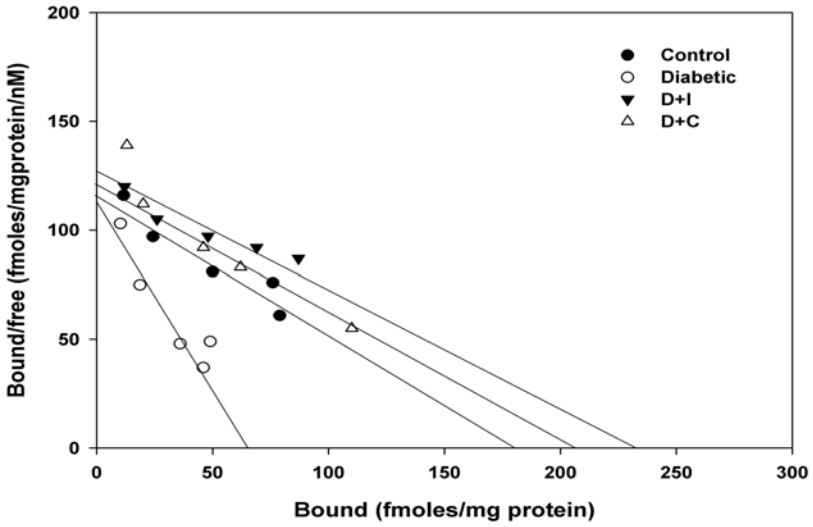


Table-11

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	180 ± 12.4	1.60 ± 0.02
Diabetic	65 ± 13.2 [a]	0.58 ± 0.02 [a]
D + I	225 ± 8.6 [b]	1.80 ± 0.01 [b]
D + C	205 ± 9.2 [b]	1.78 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- curcumin treated diabetic rats.

Figure-12

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebral cortex of control and experimental rats

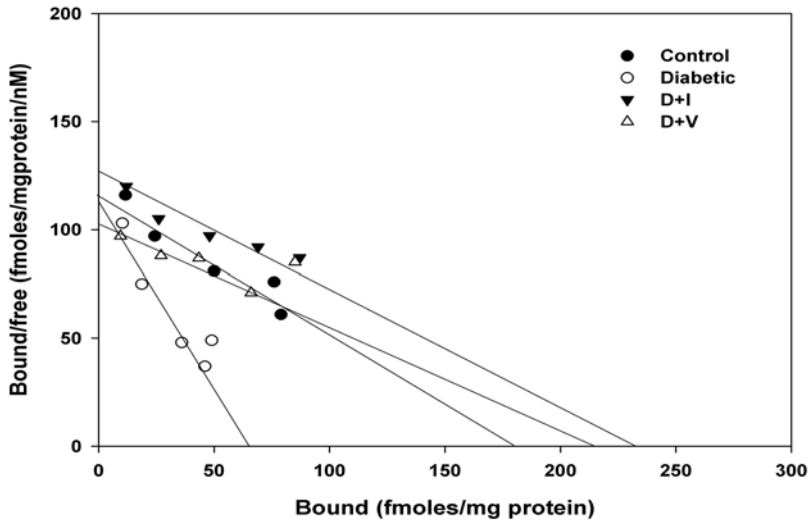


Table-12

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	180.4 ± 12.4	1.6 ± 0.20
Diabetic	65.3 ± 13.2 [a]	0.5 ± 0.02 [a]
D + I	225.5 ± 8.6 [b]	1.8 ± 0.01 [b]
D + V	215.6 ± 8.4 [b]	2.1 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-13

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebral cortex of control and experimental rats

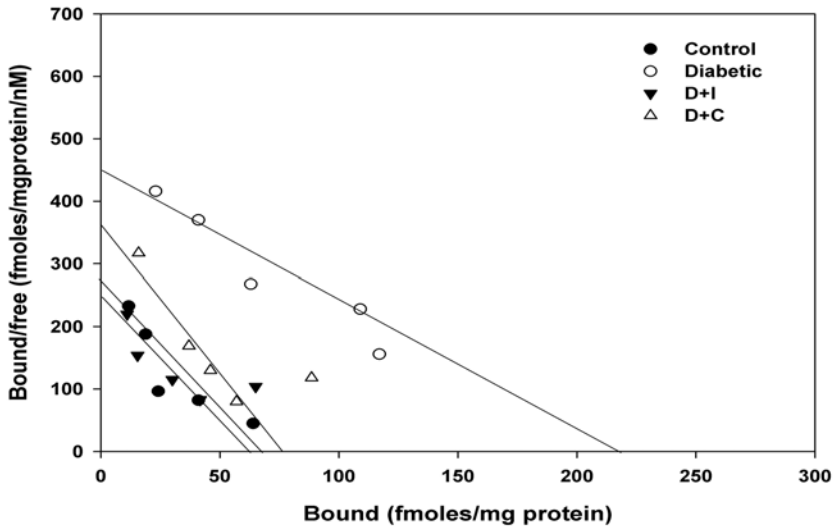


Table-13

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	56 ± 1.4	0.20 ± 0.02
Diabetic	202 ± 2.2 [a]	0.49 ± 0.02 [a]
D + I	52 ± 0.5 [b]	0.25 ± 0.01 [b]
D + C	75 ± 0.4 [b]	0.20 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- curcumin treated diabetic rats.

Figure-14

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebral cortex of control and experimental rats

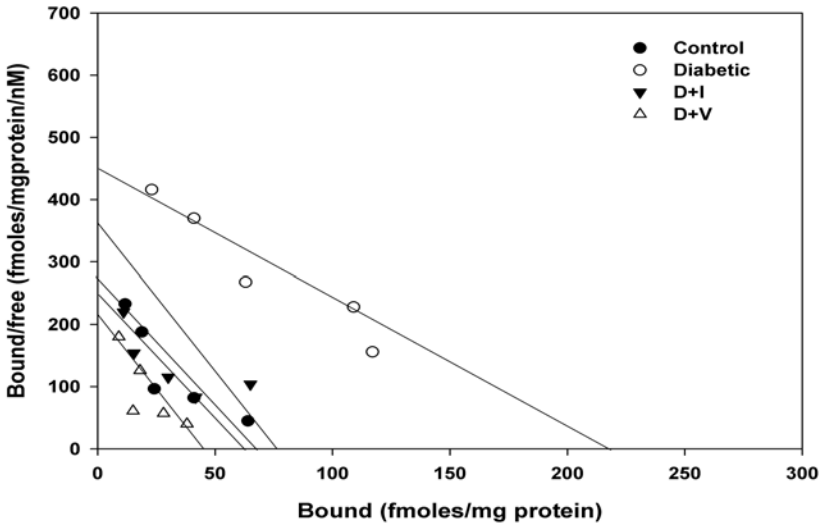


Table-14

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	56 ± 1.4	0.20 ± 0.02
Diabetic	202 ± 2.2 [a]	0.49 ± 0.02 [a]
D + I	52 ± 0.5 [b]	0.25 ± 0.01 [b]
D + V	75 ± 0.4 [b]	0.20 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-15

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the cerebral cortex of control and experimental rats

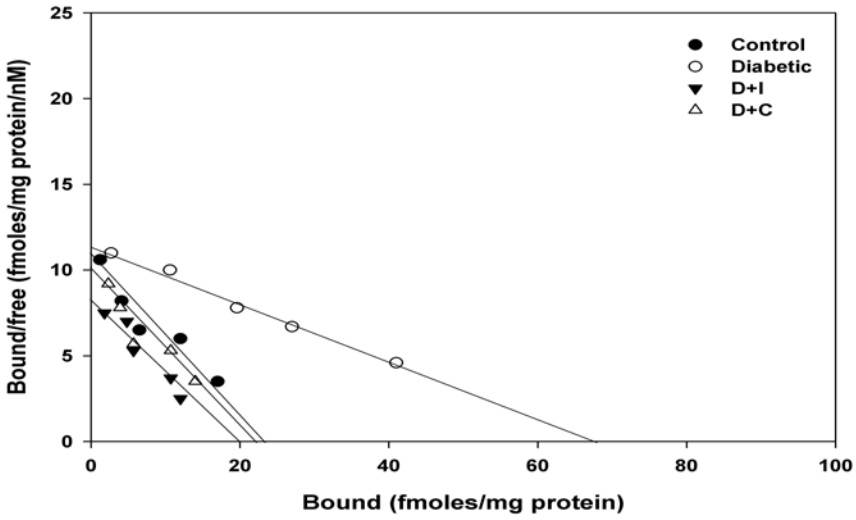


Table-15

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	23 ± 2.1	2.09 ± 0.09
Diabetic	67 ± 2.6 [a]	6.09 ± 0.13 [a]
D + I	19 ± 1.5 [b]	2.30 ± 0.08 [b]
D + C	21 ± 0.9 [b]	2.12 ± 0.08 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- curcumin treated diabetic rats.

Figure-16

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the cerebral cortex of control and experimental rats

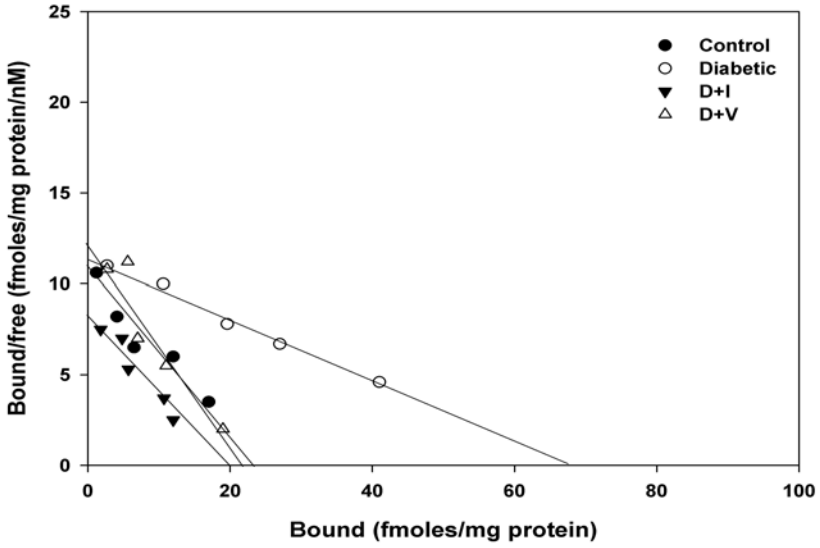


Table-16

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	23 ± 2.1	2.09 ± 0.02
Diabetic	67 ± 2.6 [a]	6.09 ± 0.02 [a]
D + I	19 ± 1.5 [b]	2.30 ± 0.01 [b]
D + V	21 ± 1.3 [b]	1.90 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-17

Real Time amplification of acetylcholine esterase mRNA from the cerebral cortex of control and experimental rats

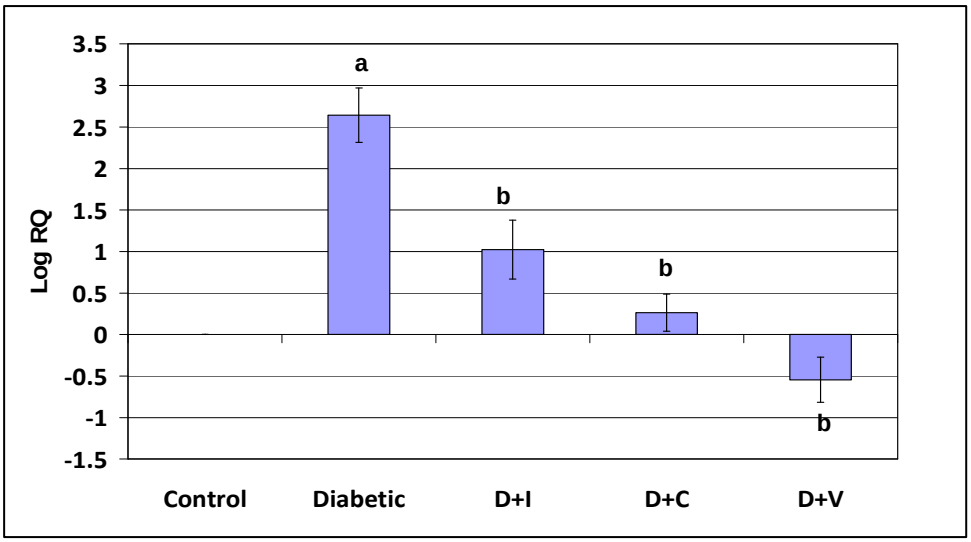


Table-17

Real Time amplification of acetylcholine esterase mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	2.53 ± 0.03^{a}
D + I	1.02 ± 0.02^{b}
D + C	0.25 ± 0.03^{b}
D + V	-0.52 ± 0.03^{b}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** $P<0.001$ when compared to control, **b** $P<0.001$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-18

Real Time amplification of choline acetyl transferase mRNA from the cerebral cortex of control and experimental rats

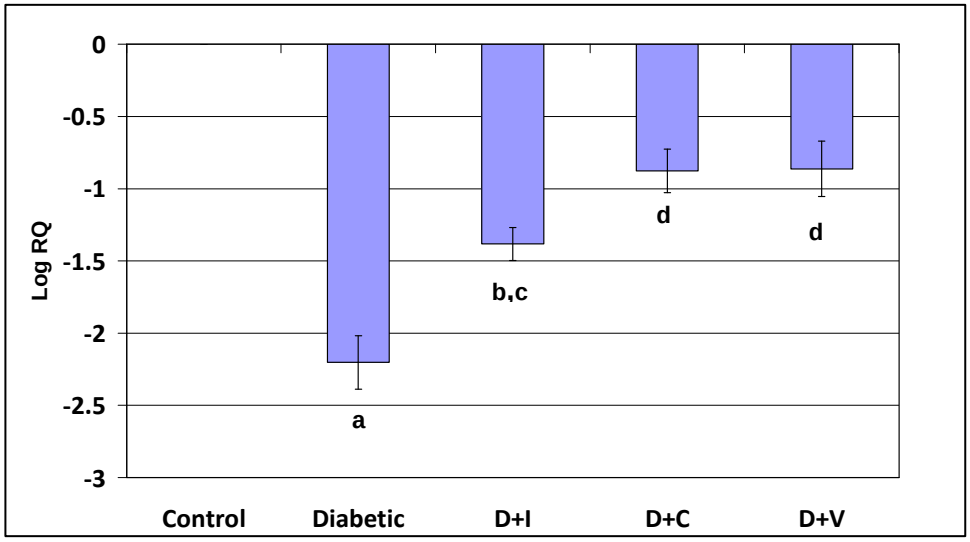


Table-18

Real Time amplification of choline acetyl transferase mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-2.20 ± 0.18 [a]
D + I	-1.38 ± 0.11 [b,c]
D + C	-0.87 ± 0.15 [d]
D + V	-0.86 ± 0.19 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01, **d** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-19

Real Time amplification of muscarinic M1 receptor mRNA from the cerebral cortex of control and experimental rats

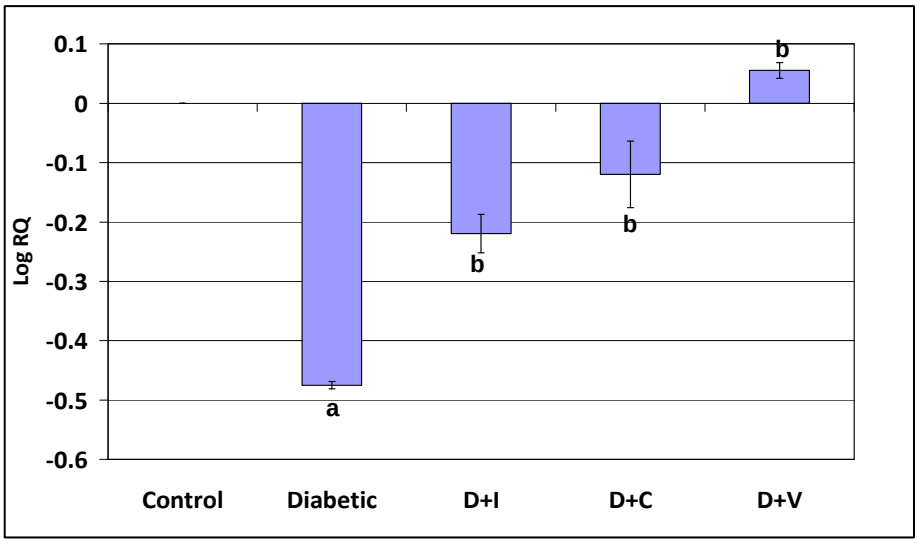


Table-19

Real Time amplification of muscarinic M1 receptor mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.47 ± 0.01 [a]
D + I	-0.31 ± 0.03 [b]
D + C	-0.11 ± 0.09 [b]
D + V	0.05 ± 0.01 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-20

Real Time amplification of muscarinic M3 receptor mRNA from the cerebral cortex of control and experimental rats

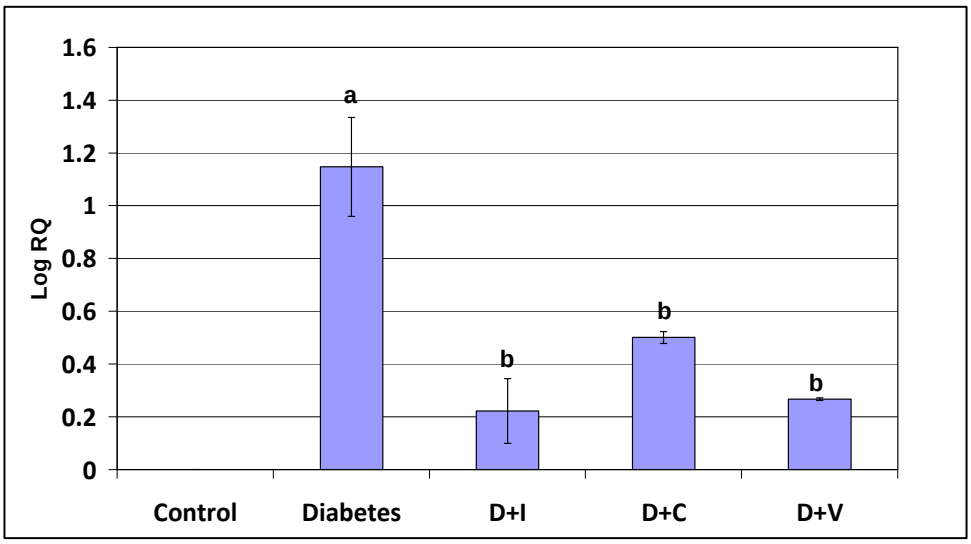


Table-20

Real Time amplification of muscarinic M3 receptor mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.15 ± 0.18 [a]
D + I	0.22 ± 0.03 [b]
D + C	0.25 ± 0.09 [b]
D + V	0.26 ± 0.01 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-21

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the cerebral cortex of control and experimental rats

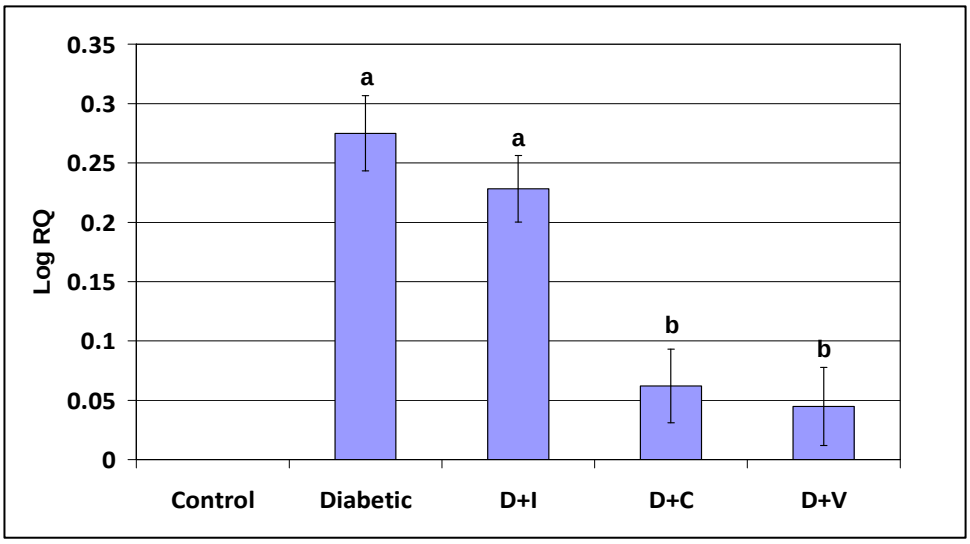


Table-21

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the cerebral cortex of control and experimental rat

Experimental groups	Log RQ
Control	0
Diabetic	0.27 ± 0.03 [a]
D + I	0.22 ± 0.02 [a]
D + C	0.06 ± 0.03 [b]
D + V	0.04 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-22

Real Time amplification of dopamine D1 receptor mRNA from the cerebral cortex of control and experimental rats

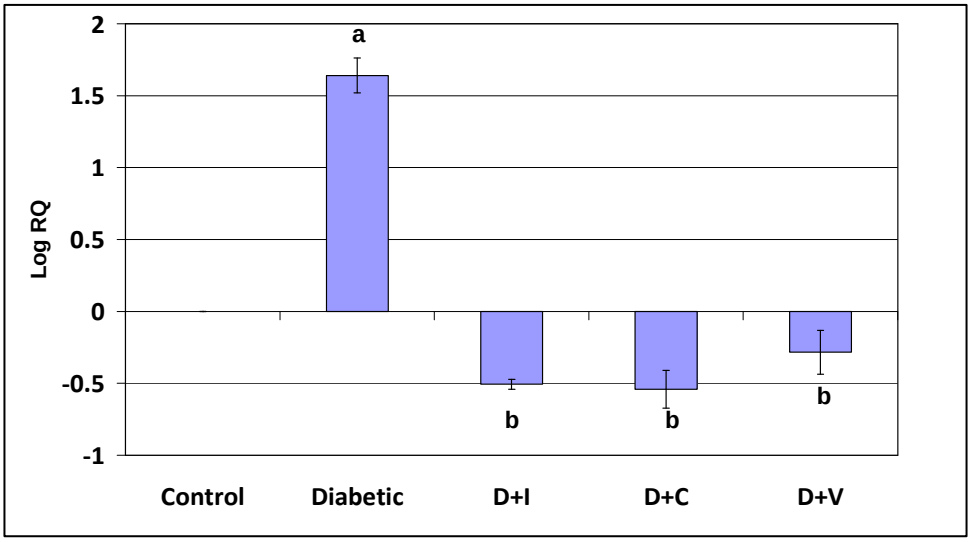


Table-22

Real Time amplification of dopamine D1 receptor mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.63 ± 0.12 [a]
D + I	-0.50 ± 0.03 [b]
D + C	-0.54 ± 0.13 [b]
D + V	-0.28 ± 0.15 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-23

Real Time amplification of dopamine D2 receptor mRNA from the cerebral cortex of control and experimental rats

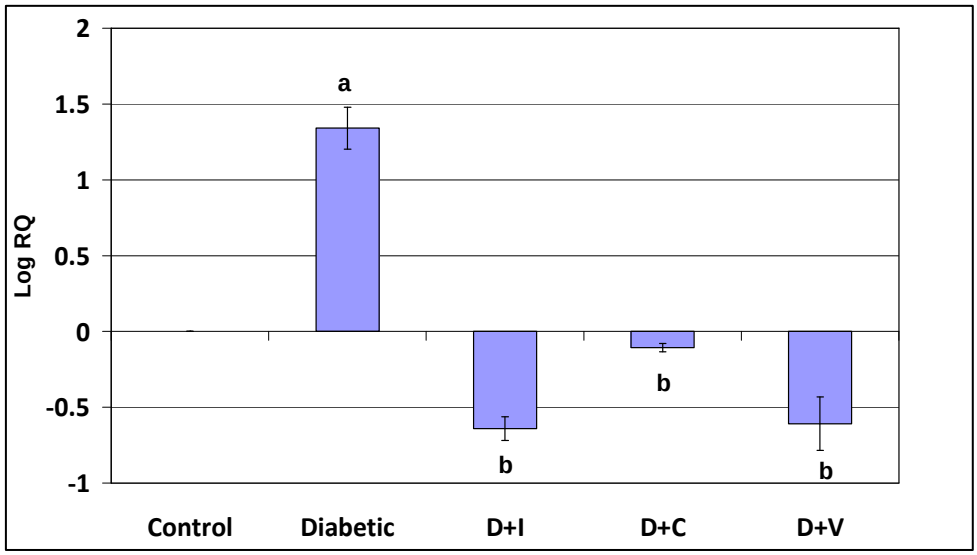


Table-23

Real Time amplification of dopamine D2 receptor mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.34 ± 0.13 [a]
D + I	-0.64 ± 0.07 [b]
D + C	-0.10 ± 0.02 [b]
D + V	-0.60 ± 0.17 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-24

Real Time amplification of Vitamin D receptor mRNA from the cerebral cortex of control and experimental rats

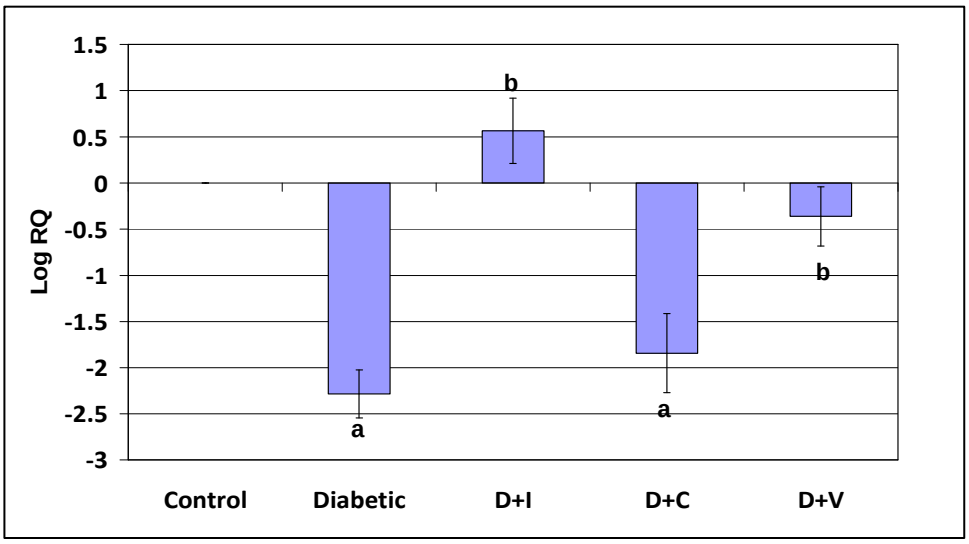


Table-24

Real Time amplification of Vitamin D receptor mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-2.28 ± 0.20 [a]
D + I	0.56 ± 0.15 [b]
D + C	-1.84 ± 0.22 [a]
D + V	-0.36 ± 0.10 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-25

Real Time amplification of insulin receptor mRNA from the cerebral cortex of control and experimental rats

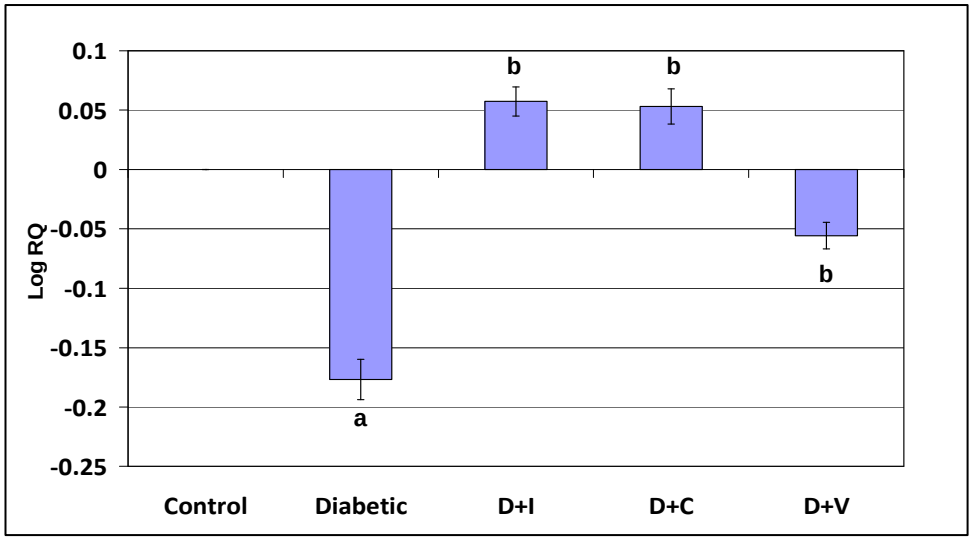


Table-25

Real Time amplification of insulin receptor mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.17 ± 0.02 [a]
D + I	0.05 ± 0.01 [b]
D + C	0.05 ± 0.01 [b]
D + V	-0.05 ± 0.01 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-26

Real Time amplification of GLUT3 mRNA from the cerebral cortex of control and experimental rats

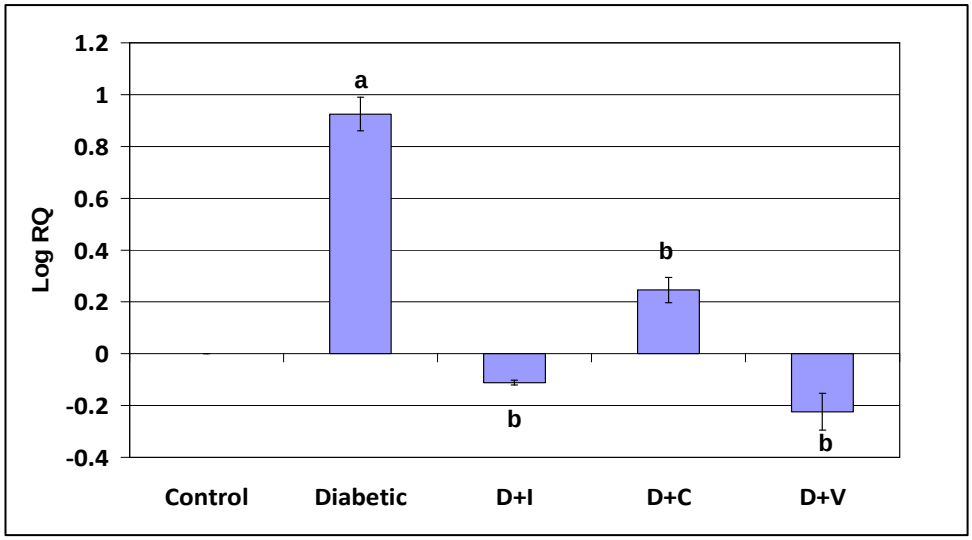


Table-26

Real Time amplification of GLUT3 mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.92 ± 0.06 [a]
D + I	-0.11 ± 0.01 [b]
D + C	0.24 ± 0.04 [b]
D + V	-0.22 ± 0.07 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-27

Real Time amplification of phospholipase C mRNA from the cerebral cortex of control and experimental rats

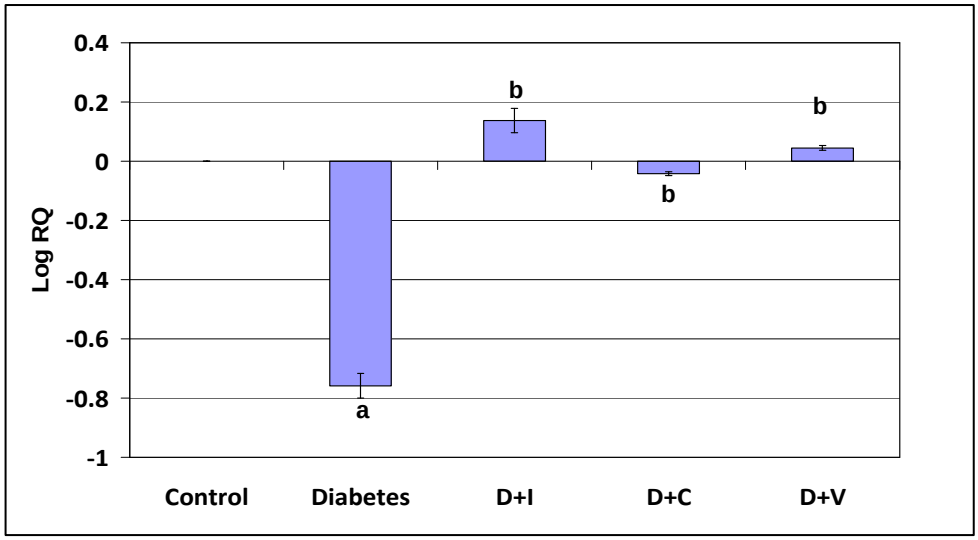


Table-27

Real Time amplification of phospholipase C mRNA from the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.78 ± 0.04 [a]
D + I	0.13 ± 0.04 [b]
D + C	-0.04 ± 0.01 [b]
D + V	0.04 ± 0.08 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-28

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

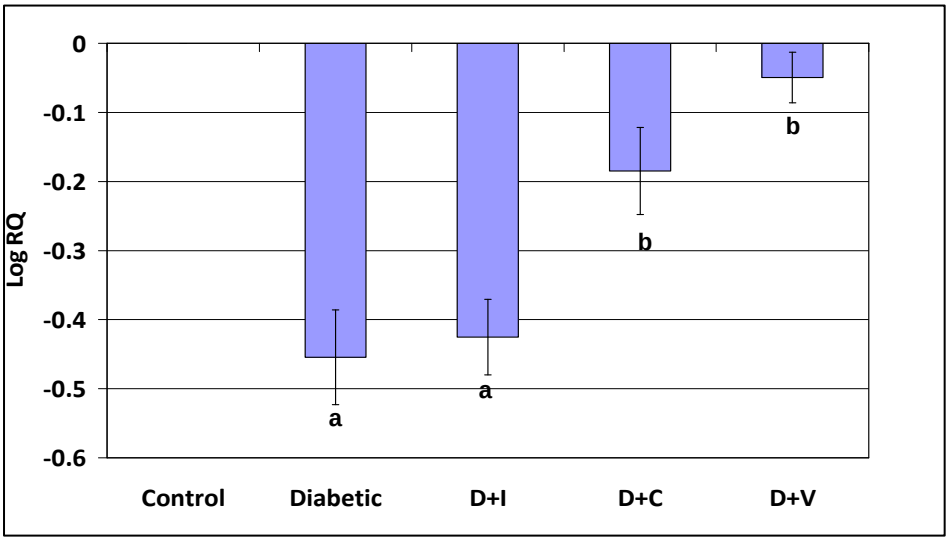


Table-28

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

Experimental groups	Log RQ
Control	0
Diabetic	-0.45 ± 0.06 [a]
D + I	-0.42 ± 0.05 [a]
D + C	-0.18 ± 0.06 [b]
D + V	0.04 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **[a]** P<0.001 when compared to control, **[b]** P<0.001 when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-29

Real Time amplification of superoxide dismutase mRNA from the cerebral cortex of control and experimental rats

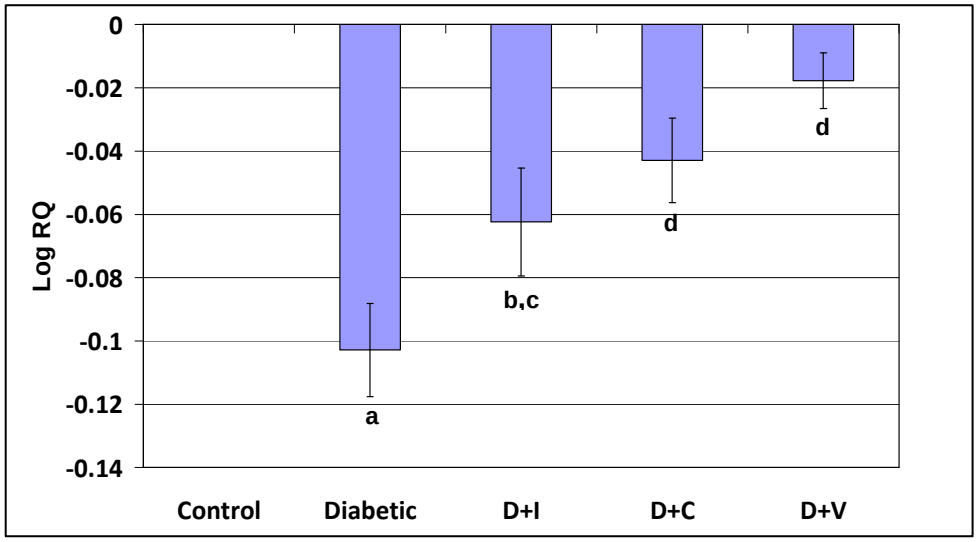


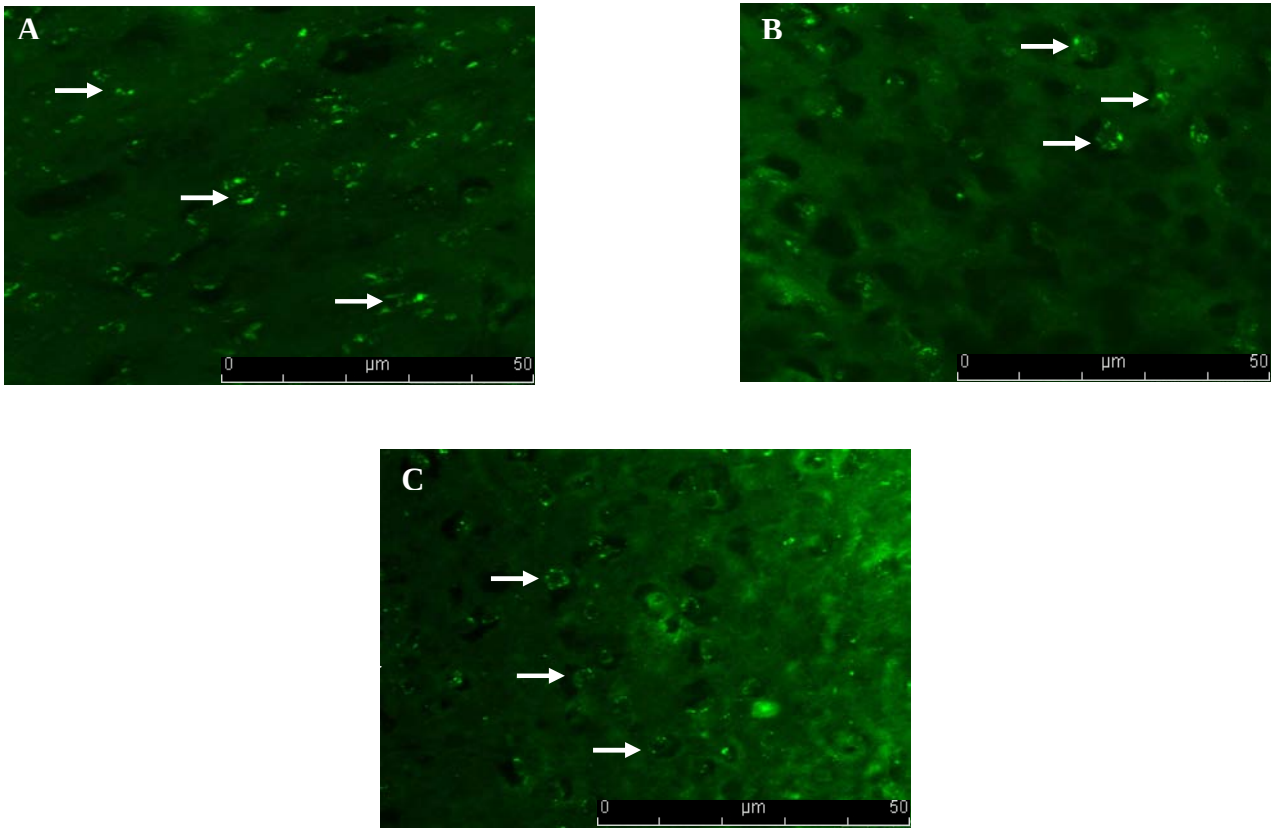
Table-29

Real Time amplification of superoxide dismutase mRNA from the cerebral cortex of control and experimental rats

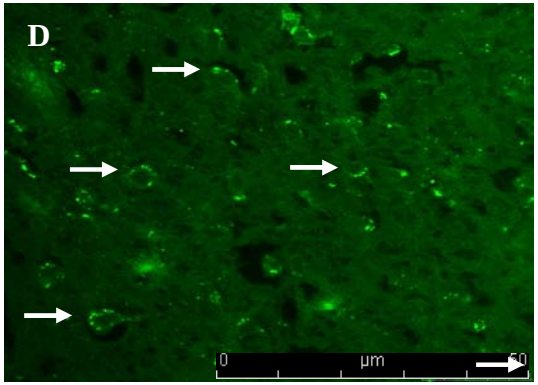
Experimental groups	Log RQ
Control	0
Diabetic	-0.10 ± 0.01 [a]
D + I	-0.06 ± 0.02 [b,c]
D + C	-0.04 ± 0.01 [d]
D + V	-0.01 ± 0.01 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01, **d** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-30
Muscarinic M1 receptor expression in the cerebral cortex of control and experimental rats



→ **Muscarinic M1 receptor**



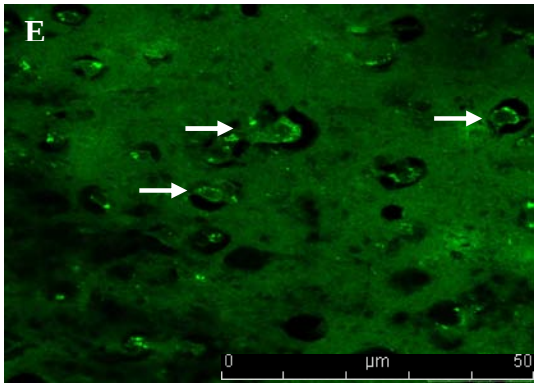


Table--30

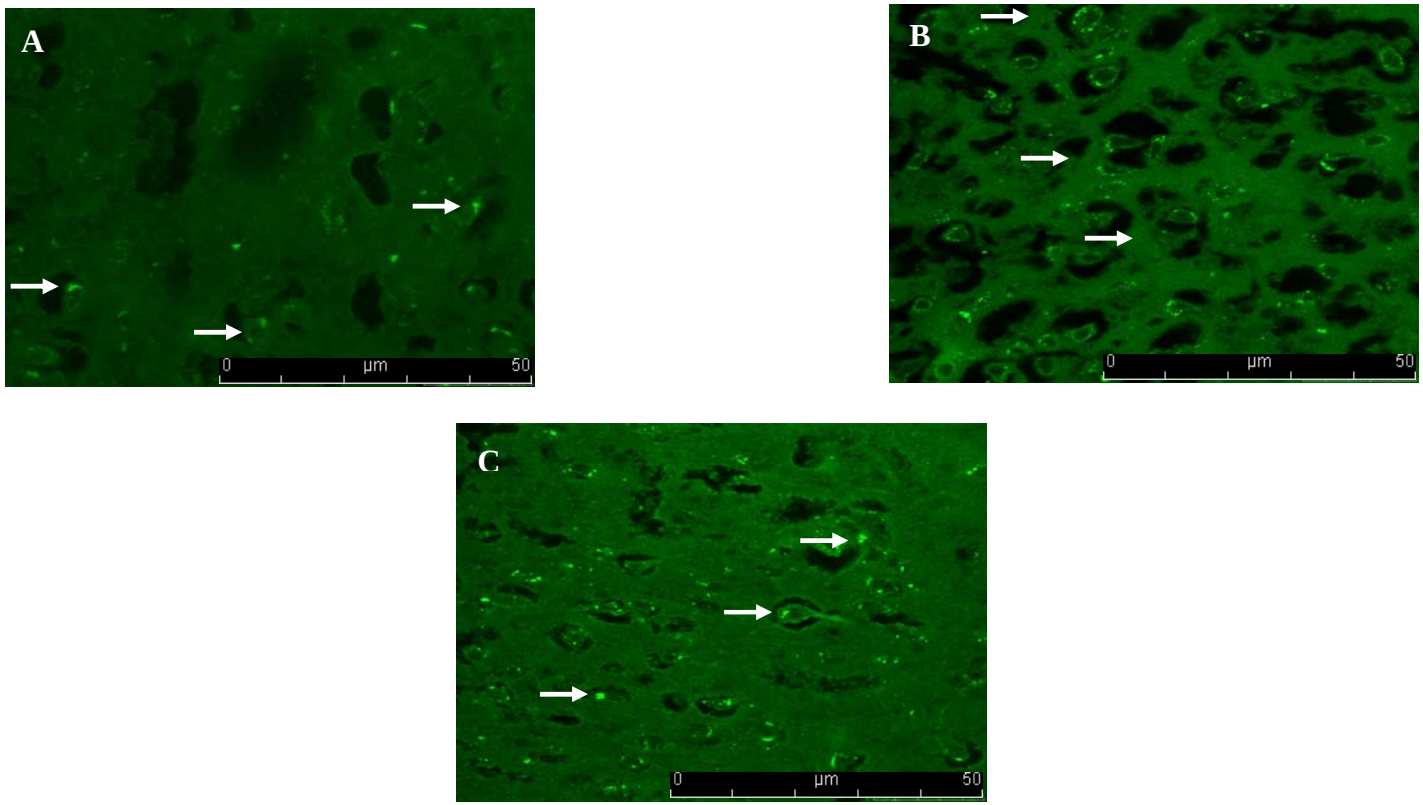
Muscarinic M1 receptor expression in the cerebral cortex of control and experimental rats

Condition	Mean Pixel Value
Control	64 ± 5.2
Diabetic	28 ± 3.4 [a]
D + I	58 ± 5.2 [b]
D + C	59 ± 4.2 [b]
D + V	52 ± 4.3 [b]

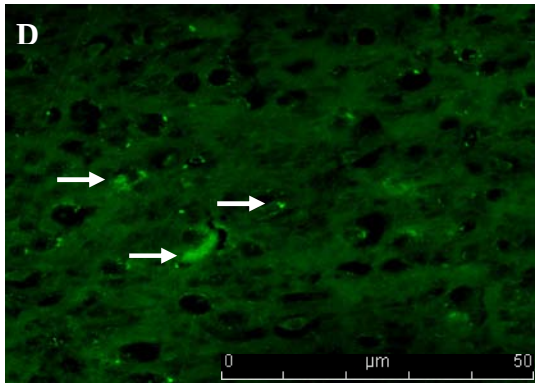
Confocal image of muscarinic M1 receptors in the cerebral cortex of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M1 receptors. [a] when compared to control, [b] when compared to diabetic. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-31

Muscarinic M3 receptor expression in the cerebral cortex of control and experimental rats



→ **Muscarinic M3 receptor**



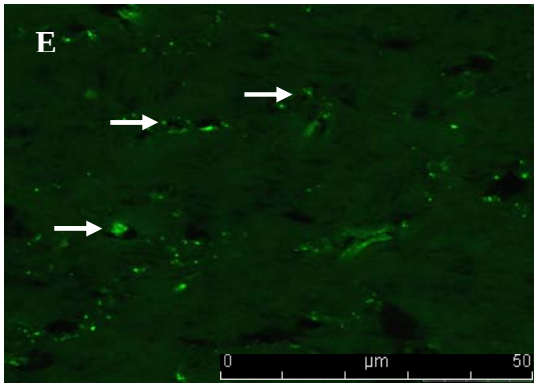


Table-31

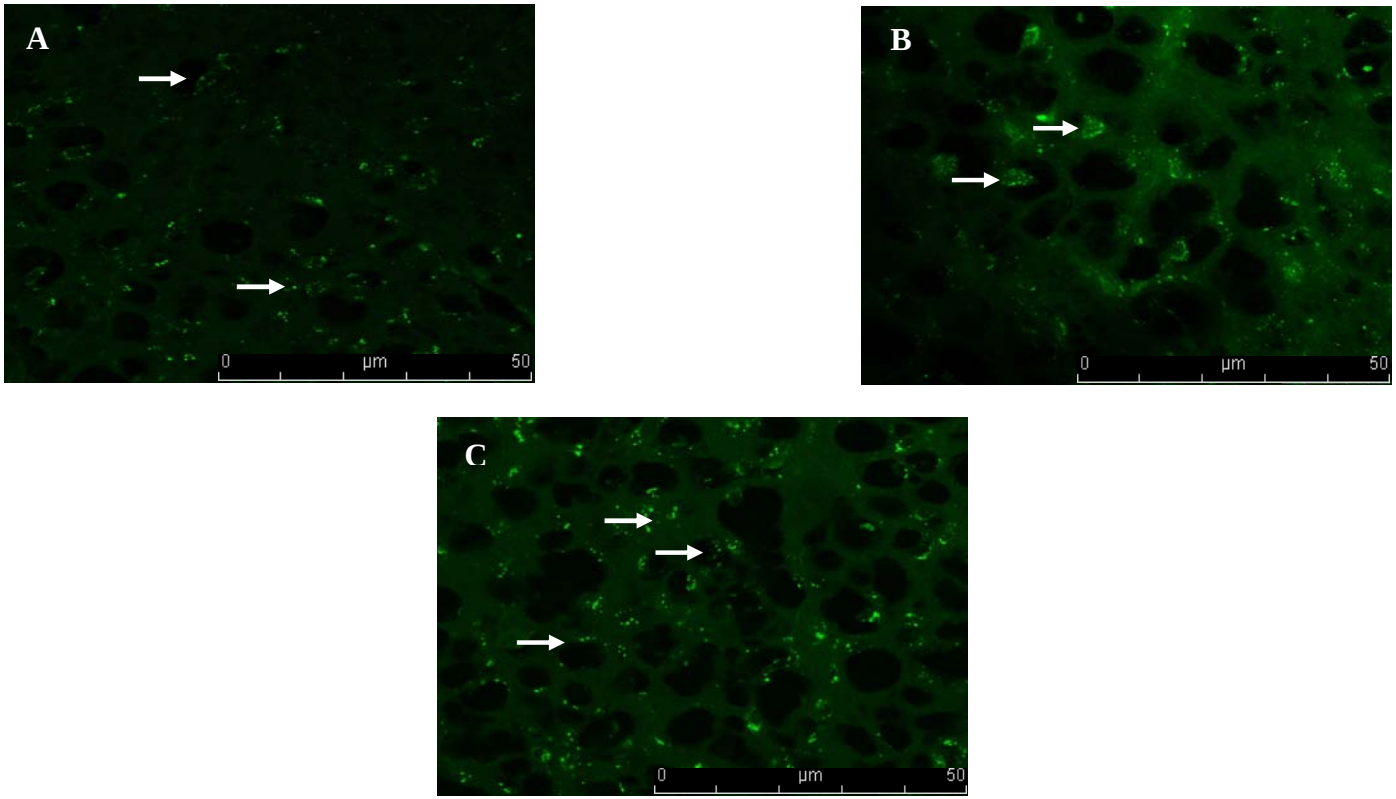
Muscarinic M3 receptor expression in the cerebral cortex of control and experimental rats

Condition	Mean pixel value
Control	31 ± 4.2
Diabetic	85 ± 3.1 [a]
D + I	55 ± 2.8 [b]
D + C	37 ± 3.4 [c]
D + V	36 ± 3.1 [c]

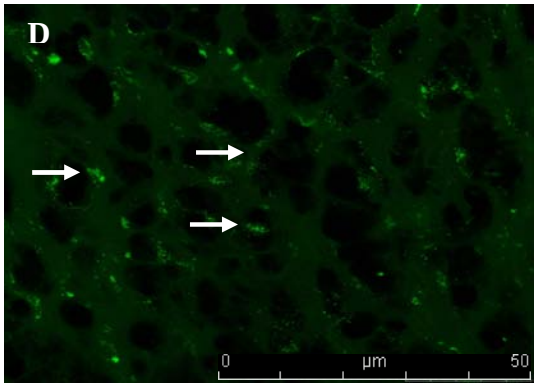
Confocal image of muscarinic M3 receptors in the cerebral cortex of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M3 receptor specific ρrimary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M3 receptors. [a] P<0.001 when compared to control, [b] P<0.01 when compared to diabetic group, [c] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-32

α7 nicotinic acetylcholine receptor expression in the cerebral cortex of control and experimental rats



→ **α7 nicotinic acetylcholine receptor**



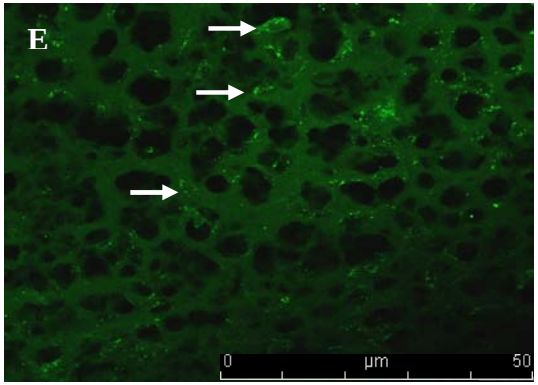


Table-32

α7 nicotinic acetylcholine receptor expression in the cerebral cortex of control and experimental rats

Condition	Mean pixel value
Control	46 ± 05
Diabetic	69 ± 03 [a]
D + I	58 ± 07 [a]
D + C	48 ± 06 [b]
D + V	58 ± 03 [b]

Confocal image of α7 nicotinic acetylcholine receptor in the cerebral cortex of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent α7 nicotinic acetylcholine receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows α7 nicotinic acetylcholine receptor. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-33

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebellum of control and experimental rats

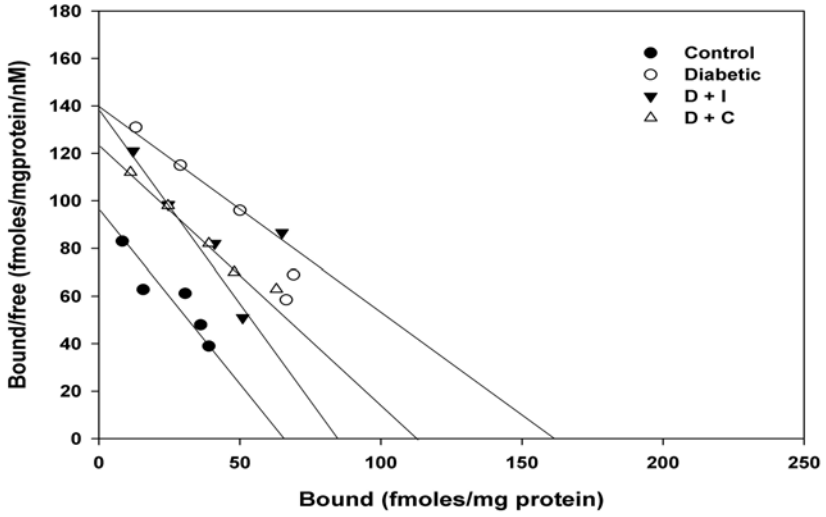


Table-33

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebellum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	65 ± 6.1	0.60 ± 0.02
Diabetic	160 ± 9.2 [a]	1.10 ± 0.02 [a]
D + I	84 ± 5.5 [b]	0.65 ± 0.01 [b]
D + C	111 ± 8.4 [c]	0.90 ± 0.03

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + C- curcumin treated diabetic rats.

Figure-34

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebellum of control and experimental rats

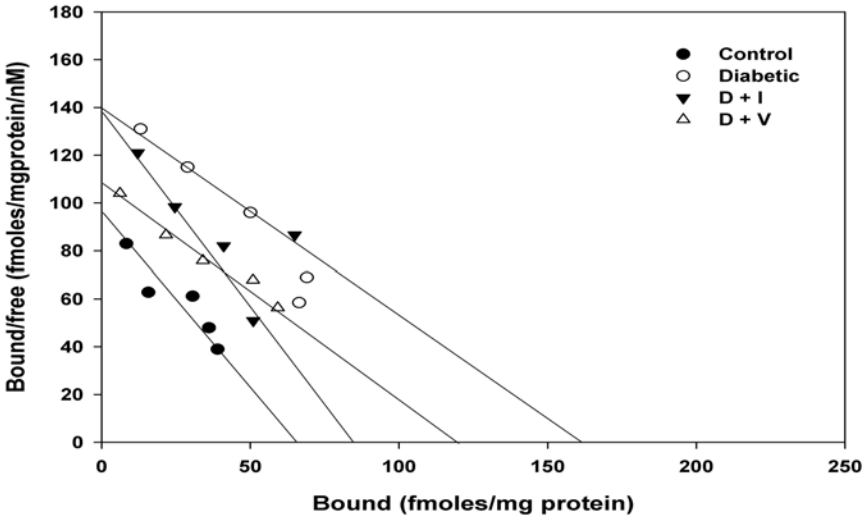


Table-34

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	65 ± 6.1	0.60 ± 0.02
Diabetic	160 ± 9.2 [a]	1.10 ± 0.02 [d]
D + I	84 ± 5.5 [b]	0.65 ± 0.01 [e]
D + V	115 ± 7.6 [c]	1.06 ± 0.04

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [d] P<0.05 when compared to control, [b] P<0.001, [c] P<0.01, [e] P<0. 05 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-35

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebellum of control and experimental rats

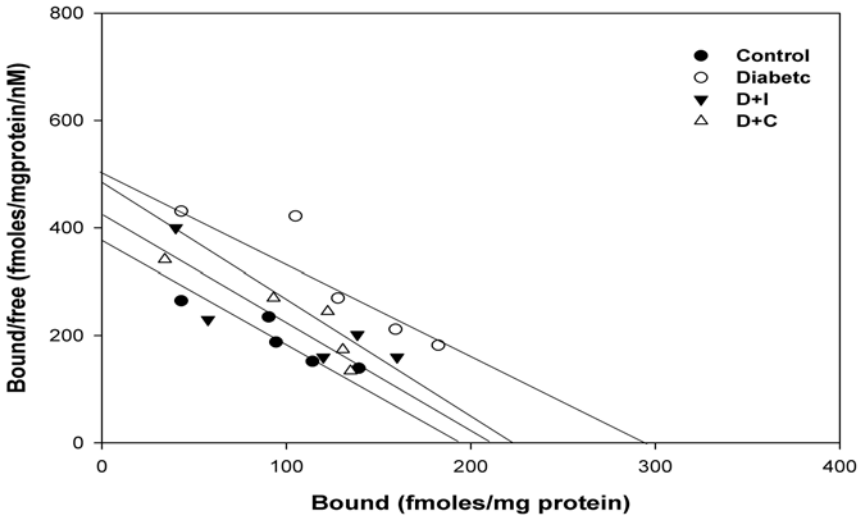


Table-35

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebellum of control and experimental rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	192 ± 12.4	0.55 ± 0.02
Diabetic	294 ± 13.2 [a]	0.98 ± 0.02 [a]
D + I	210 ± 8.6 [b]	0.50 ± 0.01 [b]
D + C	220 ± 8.4 [b]	0.45 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- curcumin treated diabetic rats.

Figure-36

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebellum of control and experimental rats

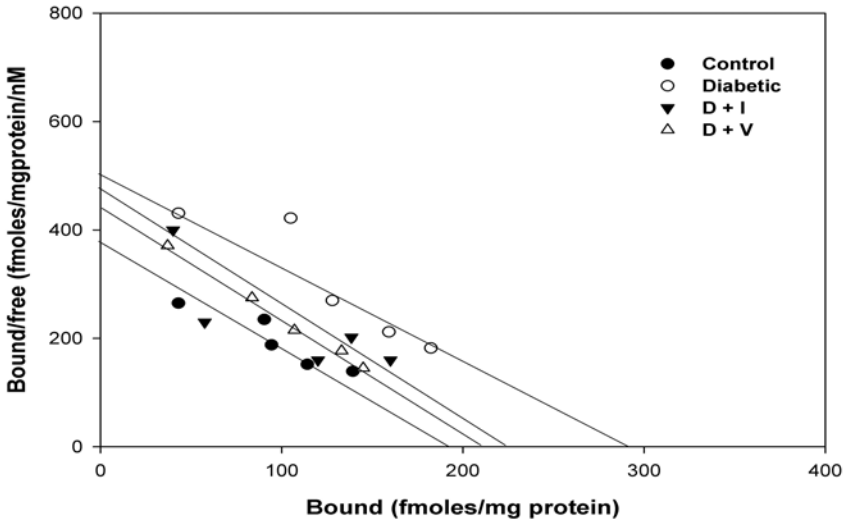


Table-36

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	192 ± 12.4	0.55 ± 0.02
Diabetic	294 ± 13.2 [a]	0.98 ± 0.02 [a]
D + I	210 ± 8.6 [b]	0.50 ± 0.01 [b]
D + V	210 ± 8.4 [b]	0.47 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-37

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebellum of control and experimental rats

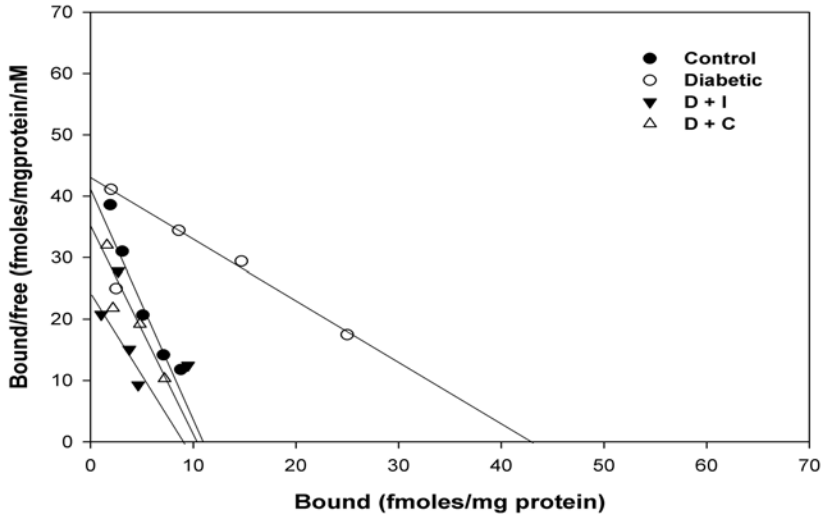


Table-37

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	11 ± 1.4	0.25 ± 0.02
Diabetic	43 ± 2.2 [a]	1.05 ± 0.02 [a]
D + I	9 ± 0.5 [b]	0.30 ± 0.01 [b]
D + C	10.5 ± 0.4 [b]	0.28 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- curcumin treated diabetic rats.

Figure-38

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebellum of control and experimental rats

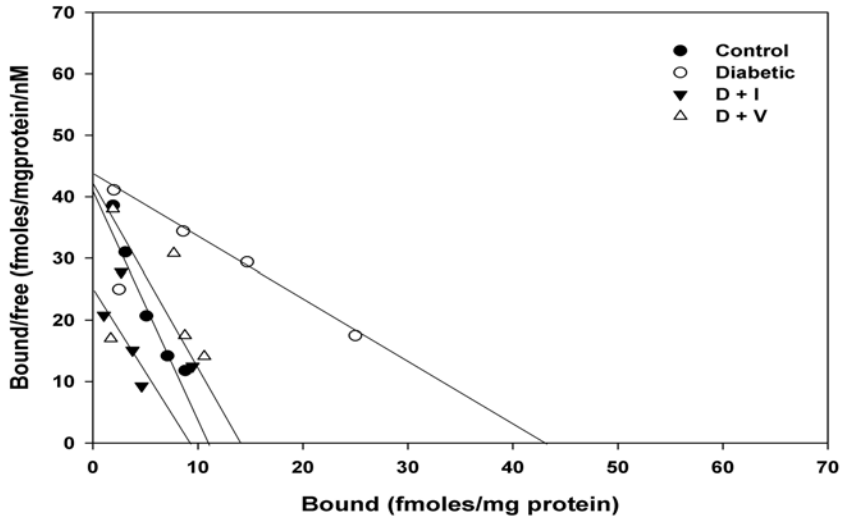


Table-38

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	11 ± 1.4	0.25 ± 0.02
Diabetic	43 ± 2.2 [a]	1.05 ± 0.02 [a]
D + I	9 ± 0.5 [b]	0.30 ± 0.01 [b]
D + V	14 ± 0.7 [b]	0.33 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-39

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the cerebellum of control and experimental rats

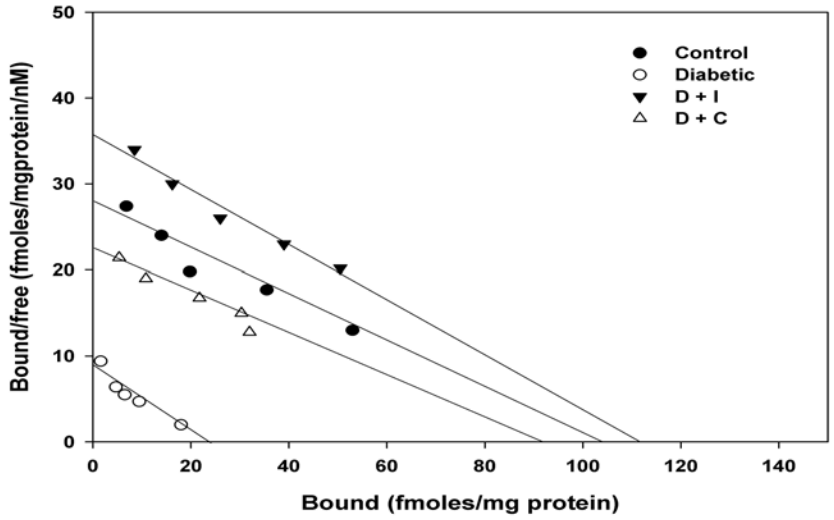


Table-39

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	112 ± 5.4	3.8 ± 0.14
Diabetic	22 ± 3.6 [a]	2.3 ± 0.05 [c]
D + I	116 ± 4.3 [b]	3.2 ± 0.13 [d]
D + C	91 ± 3.8 [b]	4.0 ± 0.03 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [c] P<0.01 when compared to control, [b] P<0.001, [d] P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-40

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the cerebellum of control and experimental rats

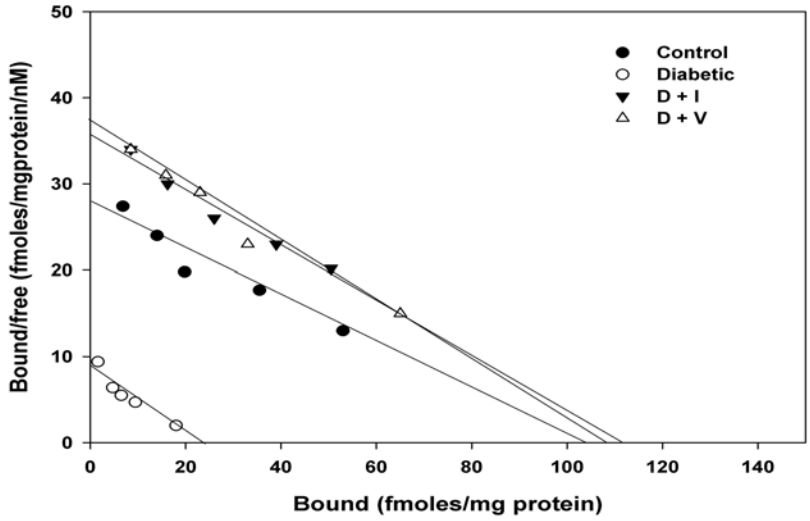


Table-40

Scatchard analysis of total dopamine receptor [^{3}H] dopamine binding against dopamine in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	112 ± 5.4	3.8 ± 0.14
Diabetic	22 ± 3.6 [a]	2.3 ± 0.05 [c]
D + I	116 ± 4.3 [b]	3.2 ± 0.13 [d]
D + V	114 ± 6.5 [b]	2.9 ± 0.09 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [c] P<0.01 when compared to control, [b] P<0.001, [d] P<0.01 when compared to diabetic group, when compared to control group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-41

Real Time amplification of acetylcholine esterase mRNA from the cerebellum of control and experimental rats

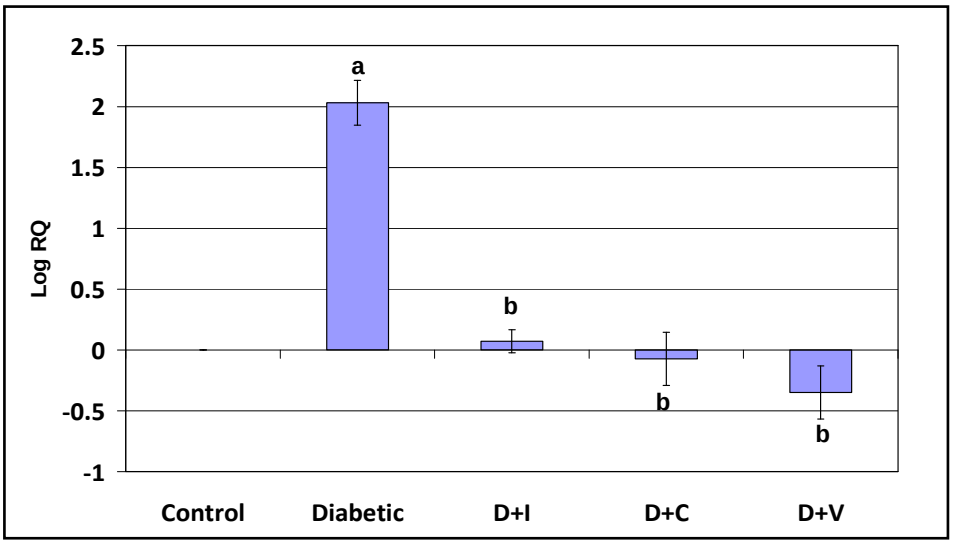


Table-41

Real Time amplification of acetylcholine esterase mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	2.03 ± 0.18 [a]
D + I	0.07 ± 0.09 [b]
D + C	-0.07 ± 0.21 [b]
D + V	-0.35 ± 0.21 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.01$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-42

Real Time amplification of choline acetyl transferase mRNA from the cerebellum of control and experimental rats

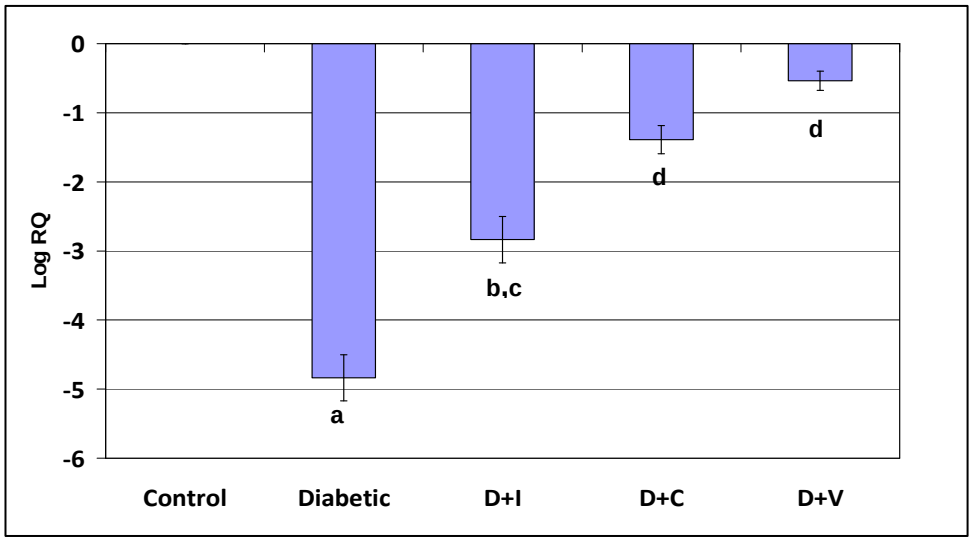


Table-42

Real Time amplification of choline acetyl transferase mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-4.83 ± 0.33 [a]
D + I	-2.83 ± 0.33 [b,c]
D + C	-1.39 ± 0.20 [d]
D + V	-0.53 ± 0.13 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01, **d** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-43

Real Time amplification of muscarinic M1 receptor mRNA from the cerebellum of control and experimental rats

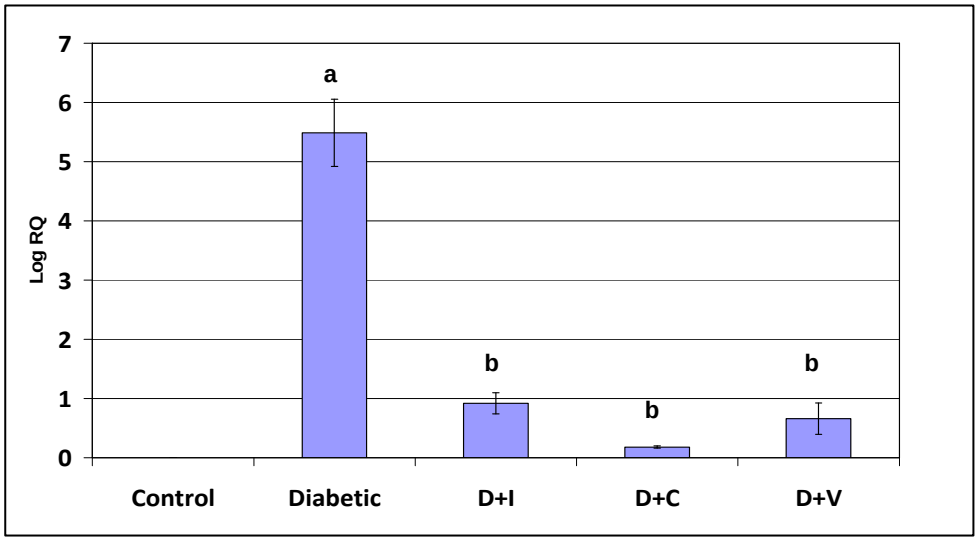


Table-43

Real Time amplification of muscarinic M1 receptor mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	5.48 ± 0.56 [a]
D + I	0.91 ± 0.47 [b]
D + C	0.17 ± 0.05 [b]
D + V	0.65 ± 0.263 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-44

Real Time amplification of muscarinic M3 receptor mRNA from the cerebellum of control and experimental rats

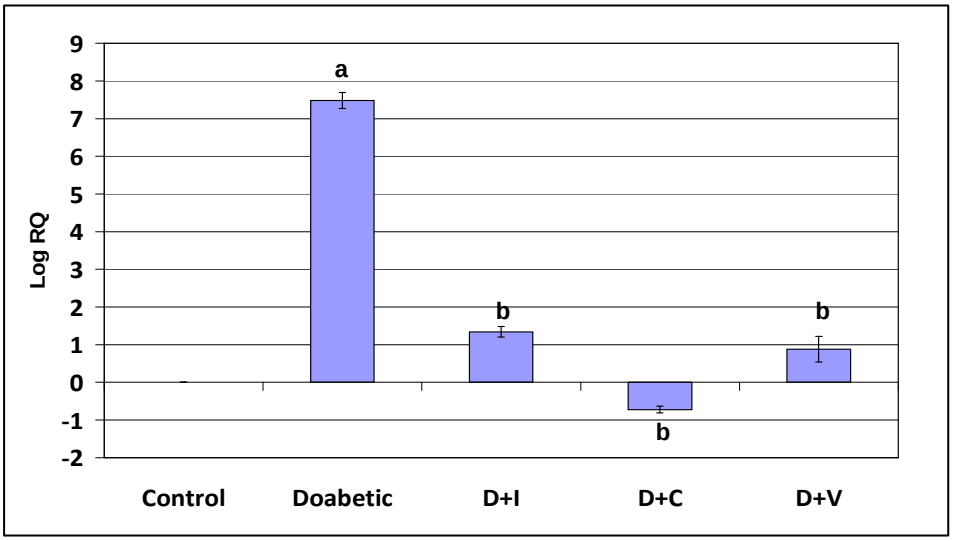


Table-44

Real Time amplification of muscarinic M3 receptor mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	7.48 ± 0.21 [a]
D + I	1.33 ± 0.13 [b]
D + C	-0.72 ± 0.09 [b]
D + V	0.87 ± 0.34 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-45

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the cerebellum of control and experimental rats

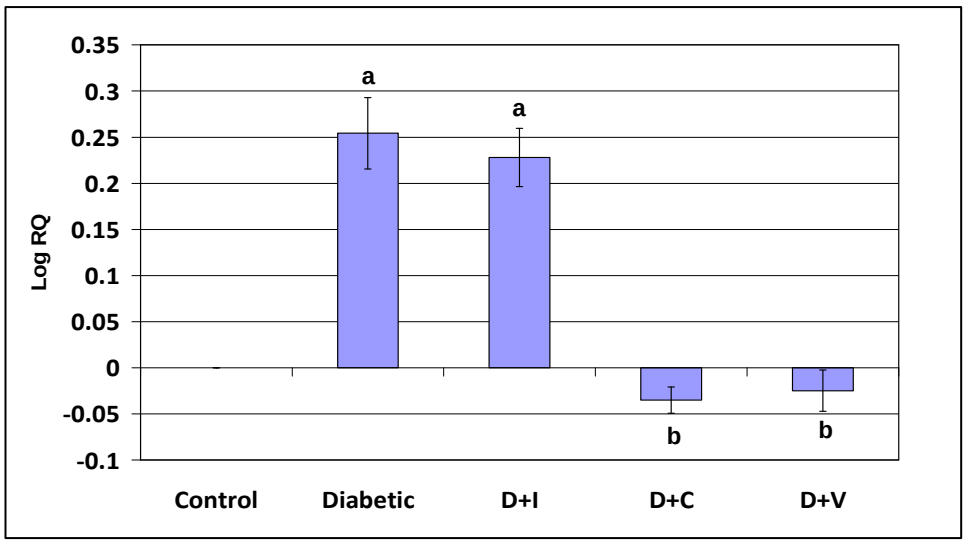


Table-45

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.25 ± 0.03 [a]
D + I	0.22 ± 0.03 [a]
D + C	-0.03 ± 0.01 [b]
D + V	-0.02 ± 0.02 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-46

Real Time amplification of dopamine D1 receptor mRNA from the cerebellum of control and experimental rats

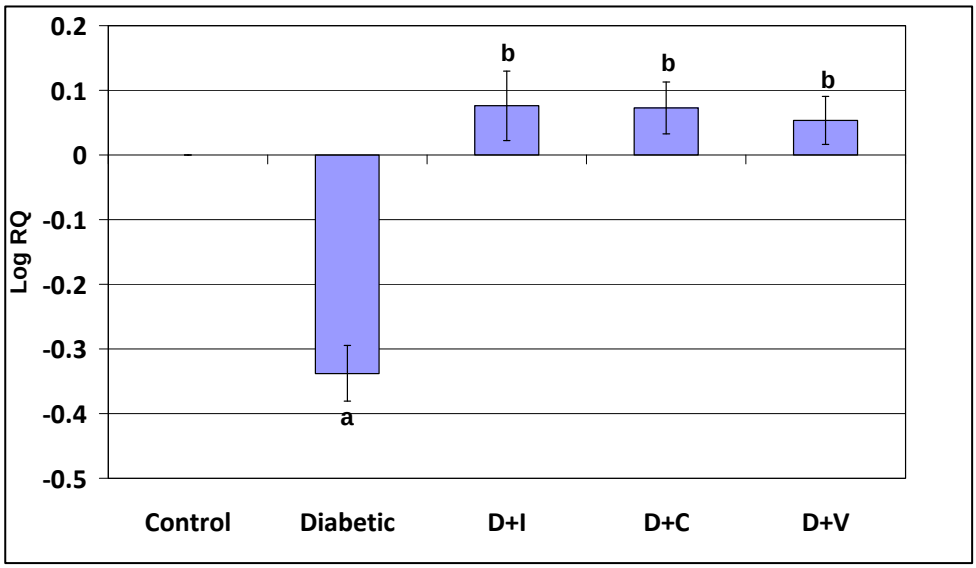


Table-46

Real Time amplification of dopamine D1 receptor mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.33 ± 0.04 [a]
D + I	0.07 ± 0.05 [b]
D + C	0.07 ± 0.04 [b]
D + V	0.05 ± 0.04 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-47

Real Time amplification of dopamine D2 receptor mRNA from the cerebellum of control and experimental rats

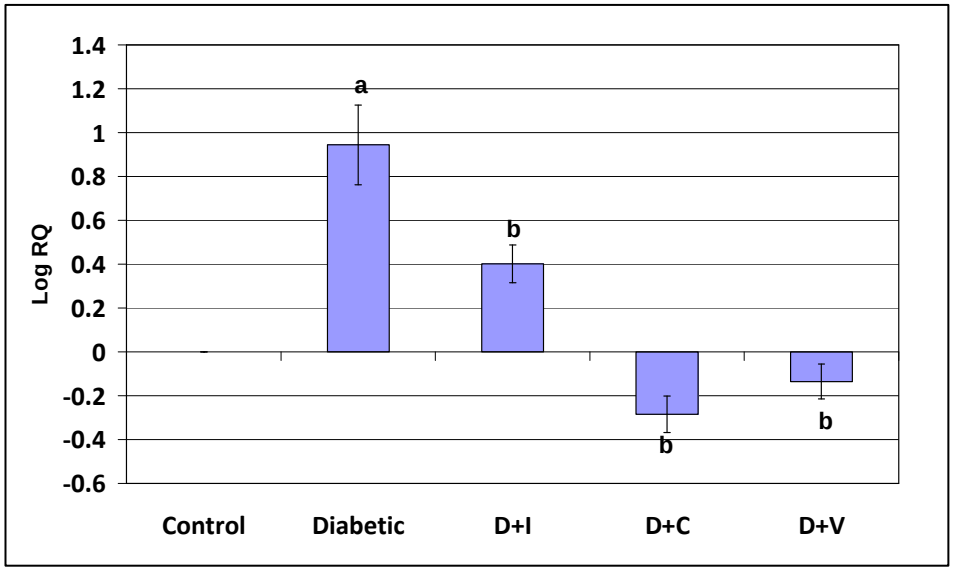


Table-47

Real Time amplification of dopamine D2 receptor mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.94 ± 0.18 [a]
D + I	0.40 ± 0.08 [b]
D + C	-0.28 ± 0.08 [b]
D + V	-0.13 ± 0.07 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-48

Real Time amplification of Vitamin D receptor mRNA from the cerebellum of control and experimental rats

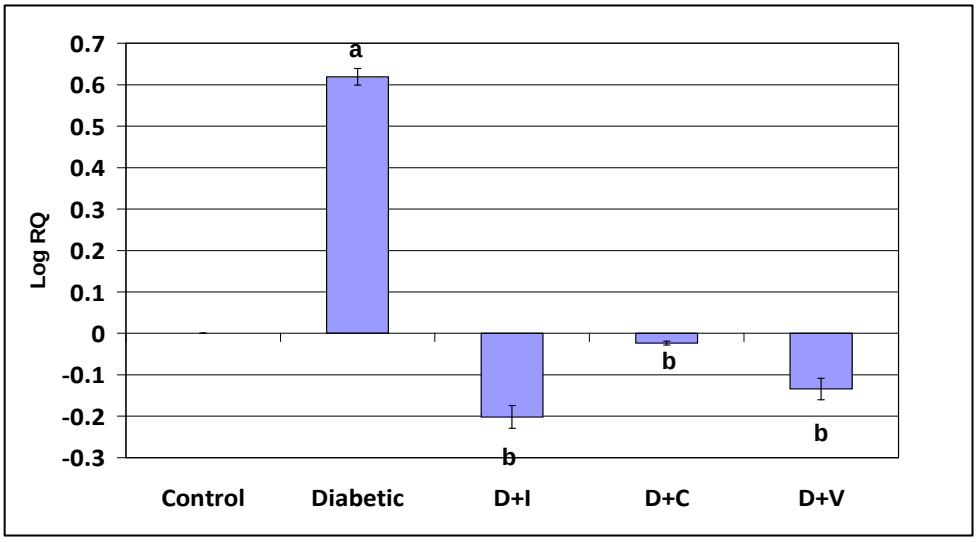


Table-48

Real Time amplification of Vitamin D receptor mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.61 ± 0.01 [a]
D + I	-0.20 ± 0.02 [b]
D + C	-0.02 ± 0.01 [b]
D + V	-0.13 ± 0.02 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-49

Real Time amplification of insulin receptor mRNA from the cerebellum of control and experimental rats

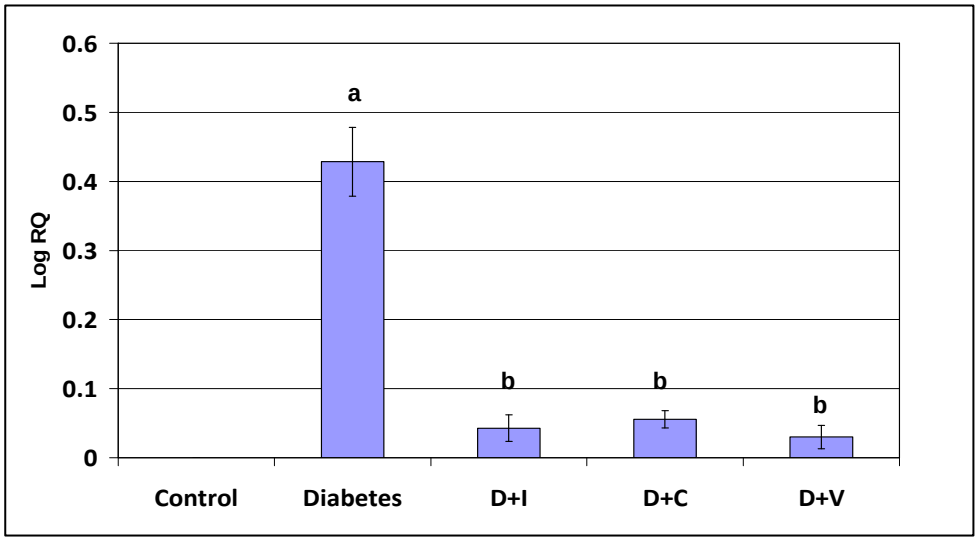


Table-49

Real Time amplification of insulin receptor mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.42 ± 0.04^{a}
D + I	0.04 ± 0.04^{b}
D + C	0.05 ± 0.03^{b}
D + V	0.02 ± 0.02^{b}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-50

Real Time amplification of GLUT3 mRNA from the cerebellum of control and experimental rats

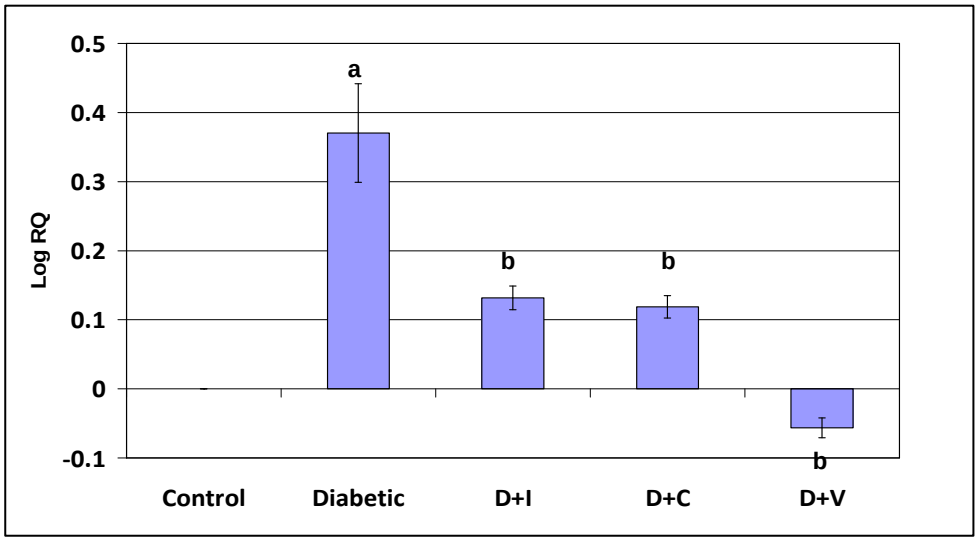


Table-50

Real Time amplification of GLUT3 mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.37 ± 0.07 [a]
D + I	0.13 ± 0.01 [b]
D + C	0.11 ± 0.01 [b]
D + V	-0.05 ± 0.01 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-51

Real Time amplification of phospholipase C mRNA from the cerebellum of control and experimental rats

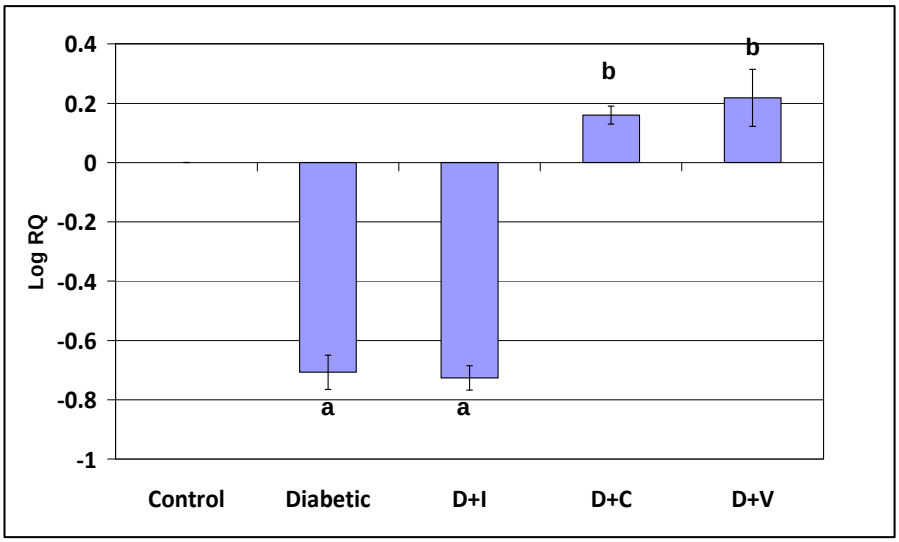


Table- 51

Real Time amplification of phospholipase C mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.70 ± 0.05 [a]
D + I	-0.72 ± 0.04 [a]
D + C	0.15 ± 0.03 [b]
D + V	0.21 ± 0.09 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-52

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

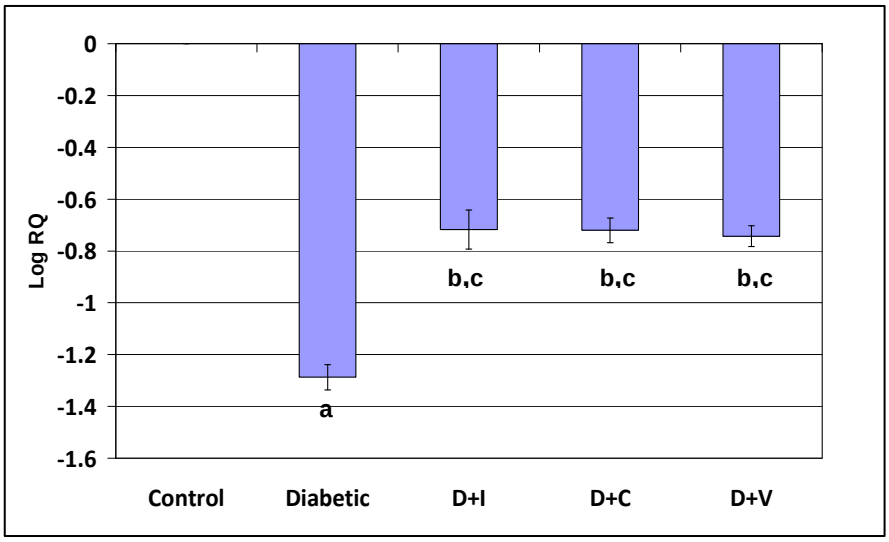


Table-52

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

Experimental groups	Log RQ
Control	0
Diabetic	-1.28 ± 0.04 [a]
D + I	-0.71 ± 0.07 [b,c]
D + C	-0.72 ± 0.04 [b,c]
D + V	-0.74 ± 0.04 [b,c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [c] P<0.01 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rat

Figure-53

Real Time amplification of superoxide dismutase mRNA from the cerebellum of control and experimental rats

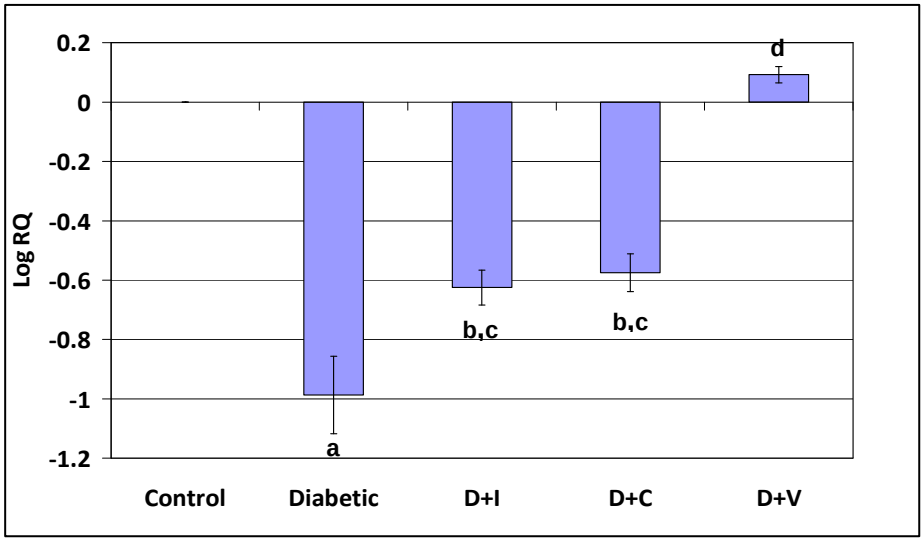


Table-53

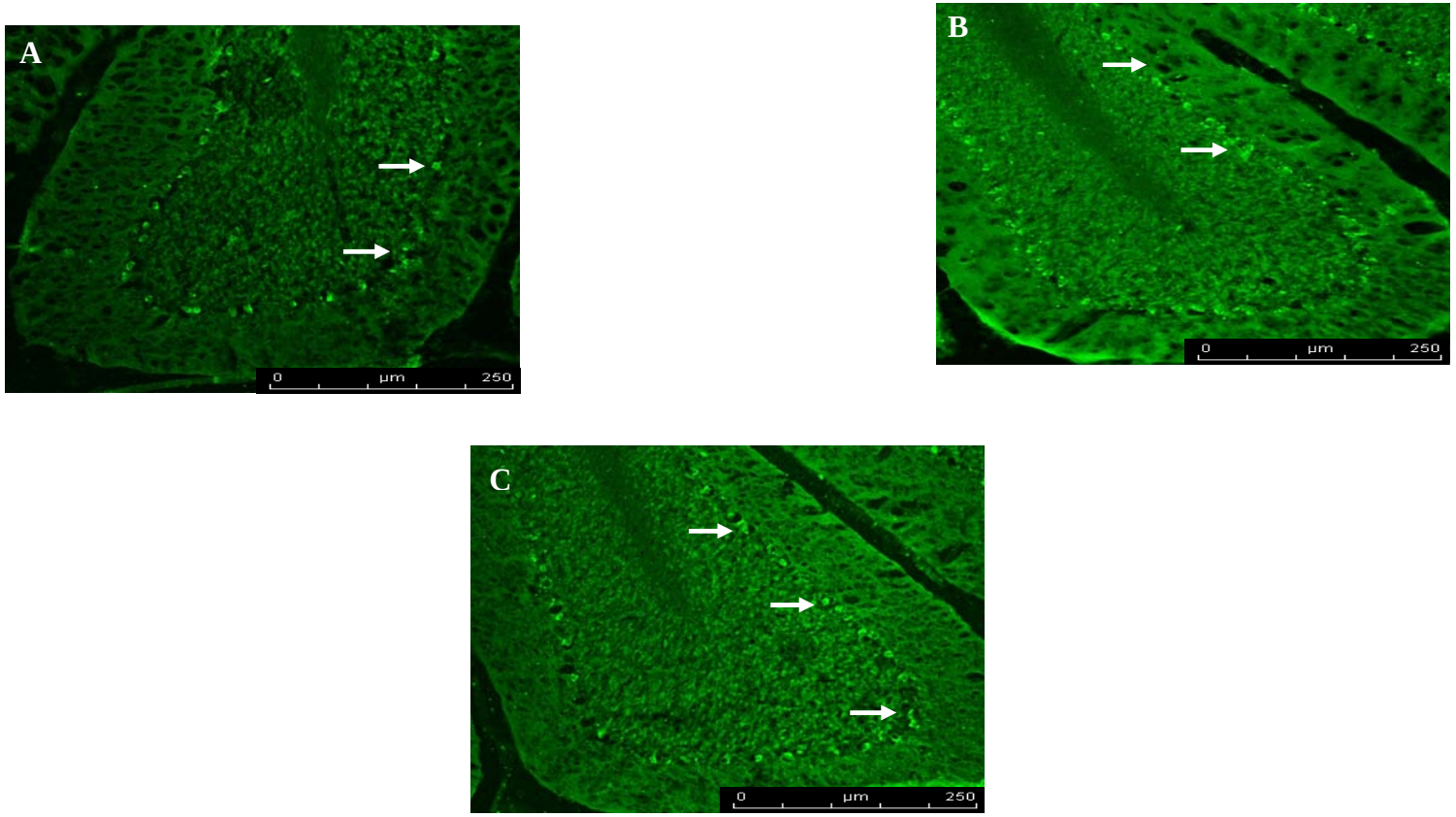
Real Time amplification of superoxide dismutase mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.98 ± 0.13 [a]
D + I	-0.62 ± 0.05 [b,c]
D + C	-0.57 ± 0.06 [b,c]
D + V	0.09 ± 0.27 [d]

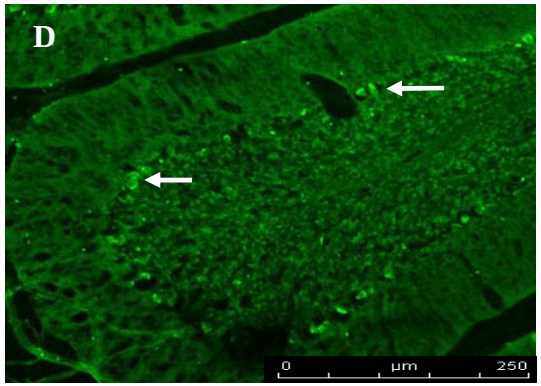
Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01, **d** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rat

Figure-54

Muscarinic M1 receptor expression in the cerebellum of control and experimental rats



→ **Muscarinic M1 receptor**



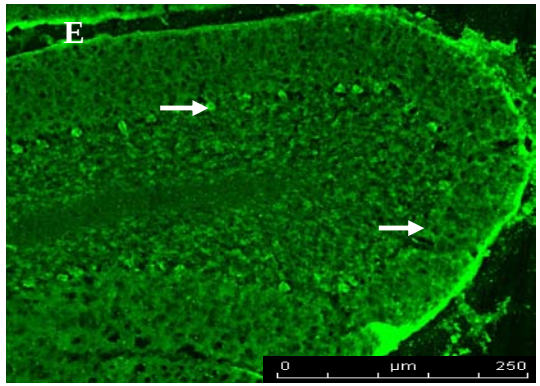


Table-54

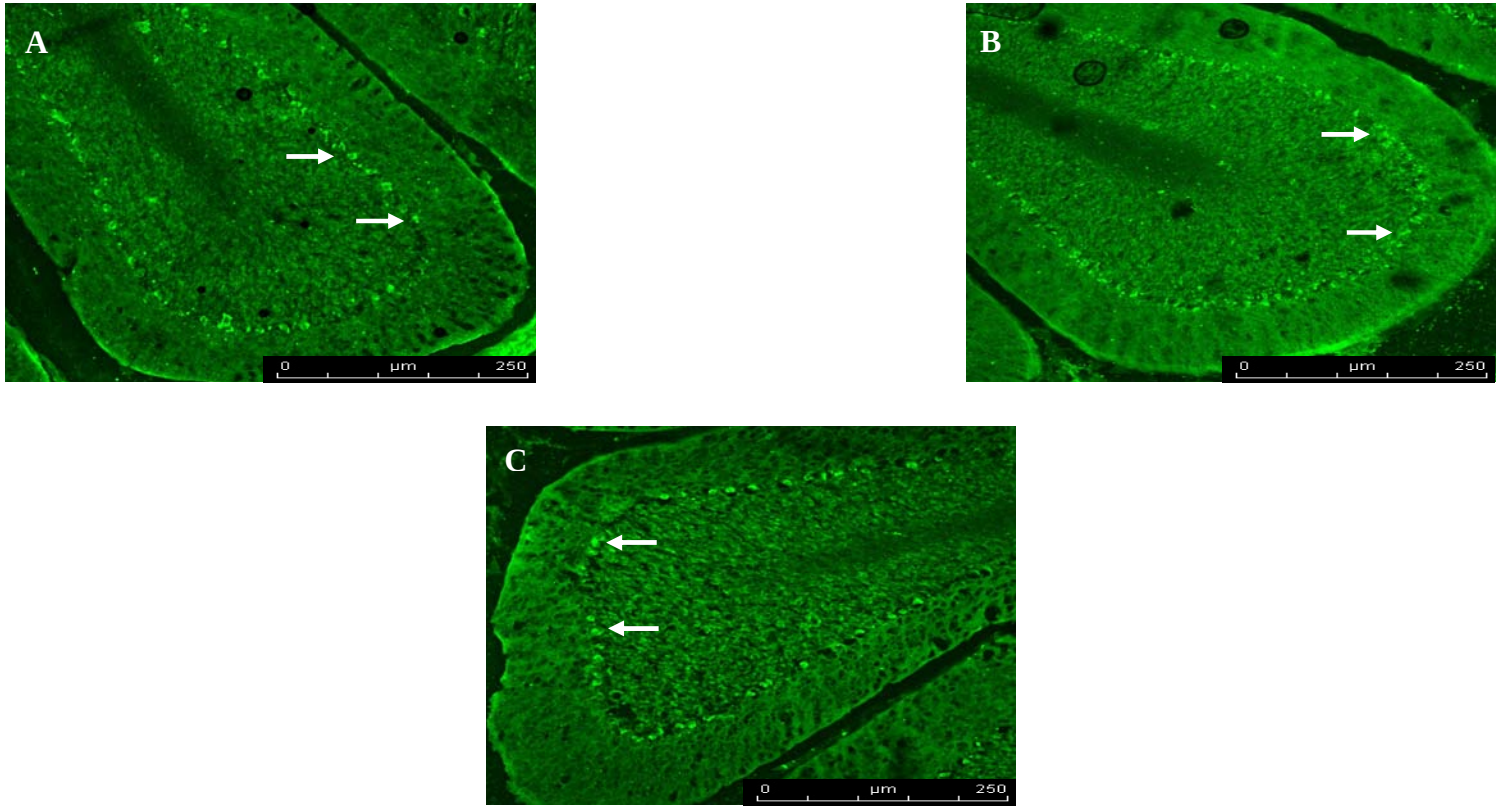
Muscarinic M1 receptor expression in the cerebellum of control and experimental rats

Condition	Mean pixel value
Control	28 ± 3.1
Diabetic	66 ± 4.4 [a]
D + I	31 ± 2.4 [b]
D + C	33 ± 3.3 [b]
D + V	35 ± 2.5 [b]

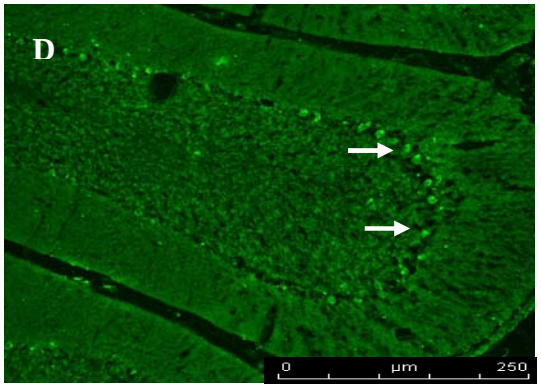
Confocal image of muscarinic M1 receptors in the cerebellum of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M1 receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 250 µm.

Figure-55

Muscarinic M3 receptor expression in the cerebellum of control and experimental rats



→ **Muscarinic M3 receptor**



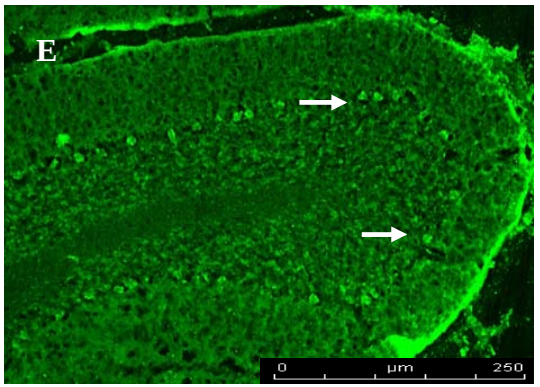


Table- 55

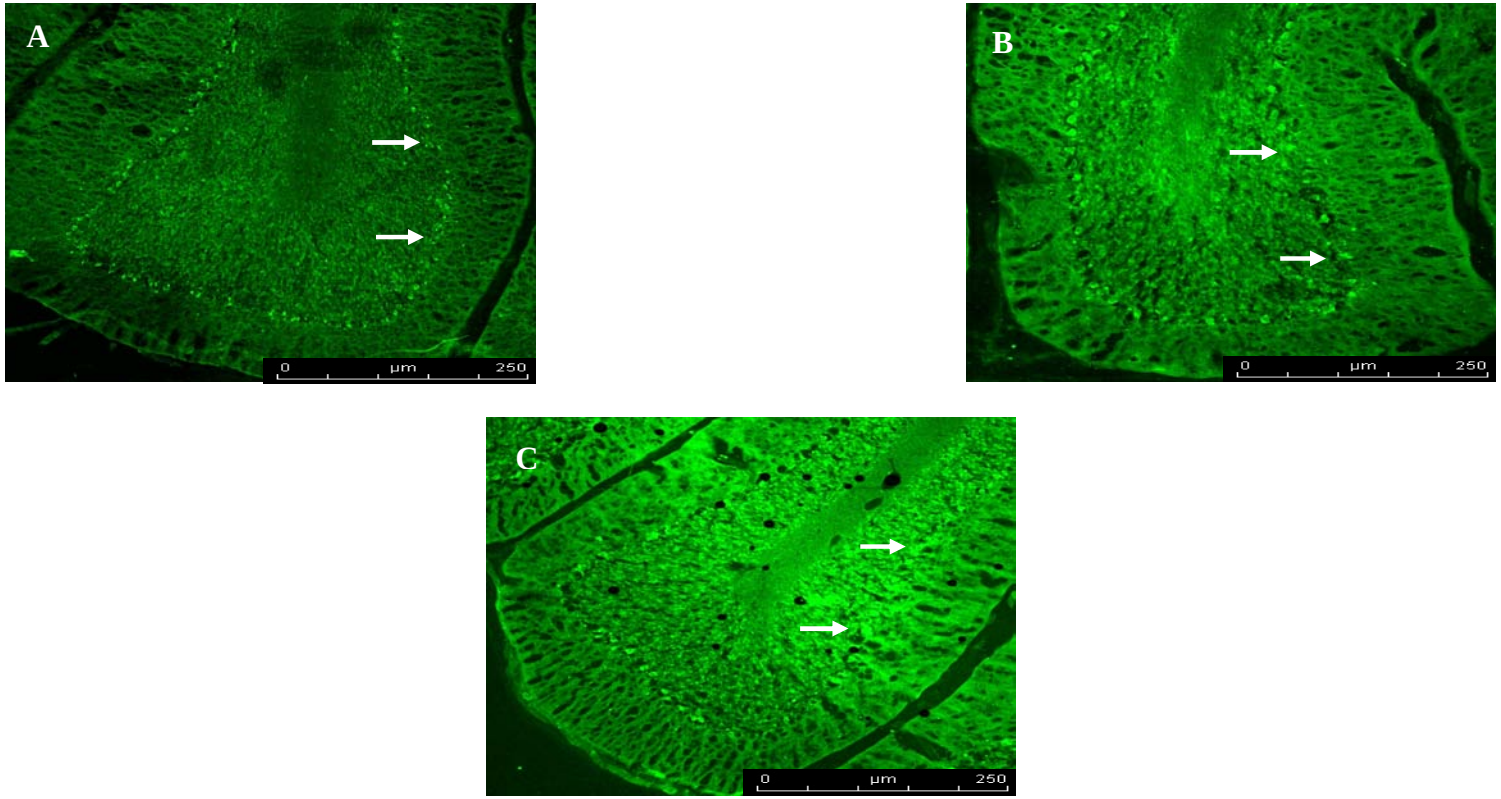
Muscarinic M3 receptor expression in the cerebellum of control and experimental rats

Condition	Mean pixel value
Control	36 ± 3.7
Diabetic	84 ± 4.3 [a]
D + I	26 ± 3.3 [b]
D + C	33 ± 4.1 [b]
D + V	31 ± 2.3 [b]

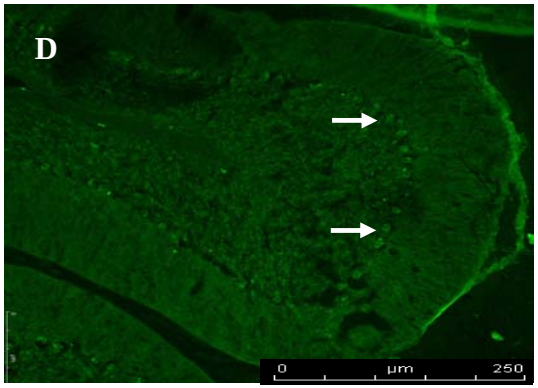
Confocal image of muscarinic M3 receptors in the cerebellum of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M3 receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 250 µm.

Figure-56

α7 nicotinic acetylcholine receptor expression in the cerebellum of control and experimental rats



→ **α7 nicotinic acetylcholine receptor**



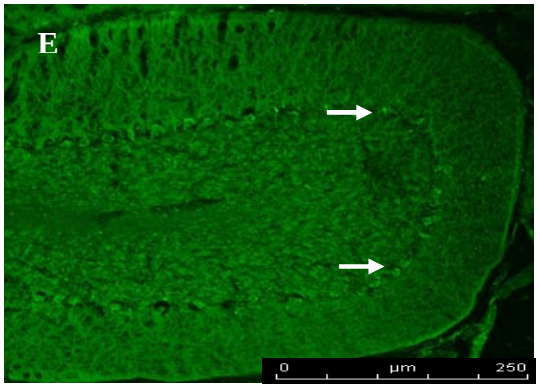


Table-56

α7 nicotinic acetylcholine receptor expression in the cerebellum of control and experimental rats

Condition	Mean pixel value
Control	35 ± 4.1
Diabetic	88 ± 5.4 [a]
D + I	77 ± 3.4 [a]
D + C	27 ± 3.2 [b]
D + V	25 ± 3.5 [b]

Confocal image of α7 nicotinic acetylcholine receptor in the cerebellum of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent α7 nicotinic acetylcholine receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows α7 nicotinic acetylcholine receptor. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 250 µm.

Figure-57

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the brain stem of control and experimental rats

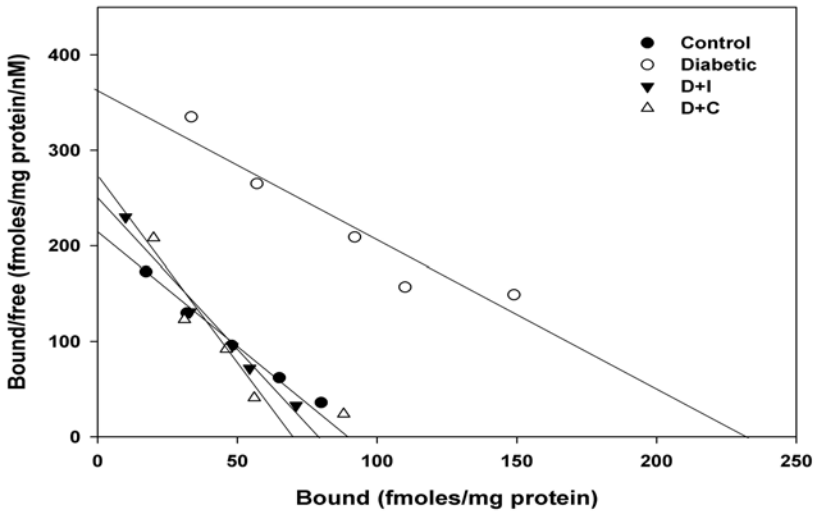


Table-57

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the brain stem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	87 ± 4.2	0.4 ± 0.04
Diabetic	230 ± 7.1 [a]	0.6 ± 0.07
D + I	77 ± 4.7 [b]	0.3 ± 0.04
D + C	68 ± 3.7 [b]	0.2 ± 0.03

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats.

Figure-58

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the brain stem of control and experimental rats

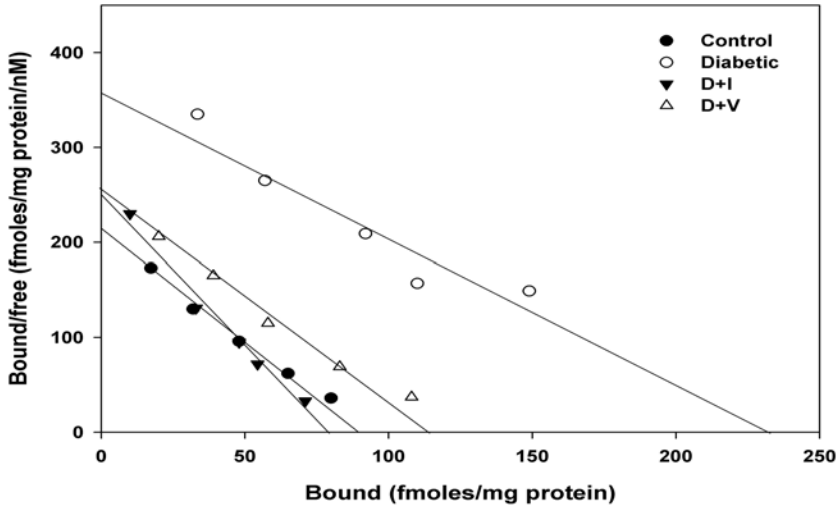


Table-58

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the brain stem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	87 ± 4.2	0.4 ± 0.04
Diabetic	230 ± 7.1 [a]	0.6 ± 0.07
D + I	77 ± 4.7 [b]	0.3 ± 0.04
D + V	110 ± 2.3 [b]	0.4 ± 0.03

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-59

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the brain stem of control and experimental rats

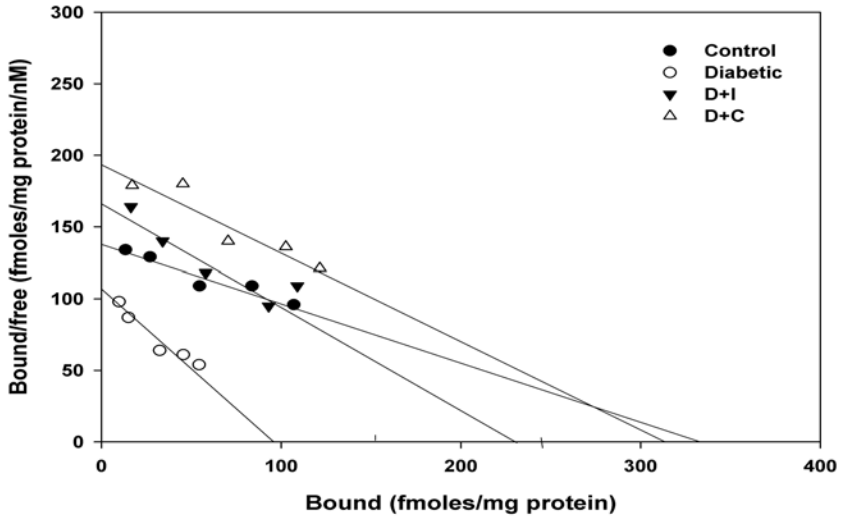


Table-59

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the brain stem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	331 ± 8.7	2.3 ± 0.54
Diabetic	94 ± 6.6 [a]	0.8 ± 0.15 [a]
D + I	228 ± 7.3 [b]	1.3 ± 0.43 [c]
D + C	311 ± 8.3 [b]	1.6 ± 0.29 [c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.01, [c] P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-60

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the brain stem of control and experimental rats

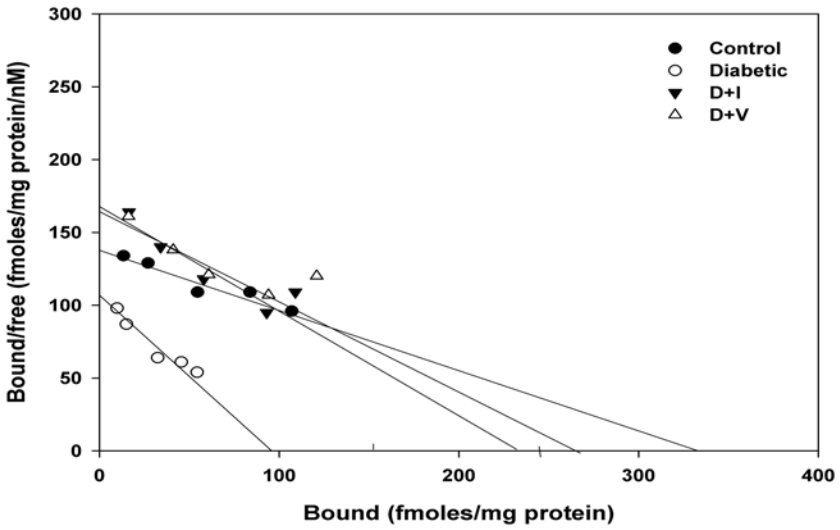


Table-60

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the brain stem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	331 ± 8.7	2.3 ± 0.54
Diabetic	94 ± 6.6 [a]	0.8 ± 0.15 [a]
D + I	228 ± 7.3 [b]	1.3 ± 0.43 [c]
D + V	263 ± 7.6 [b]	1.5 ± 0.29 [c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.01$, [c] $P<0.01$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-61

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the brain stem of control and experimental rats

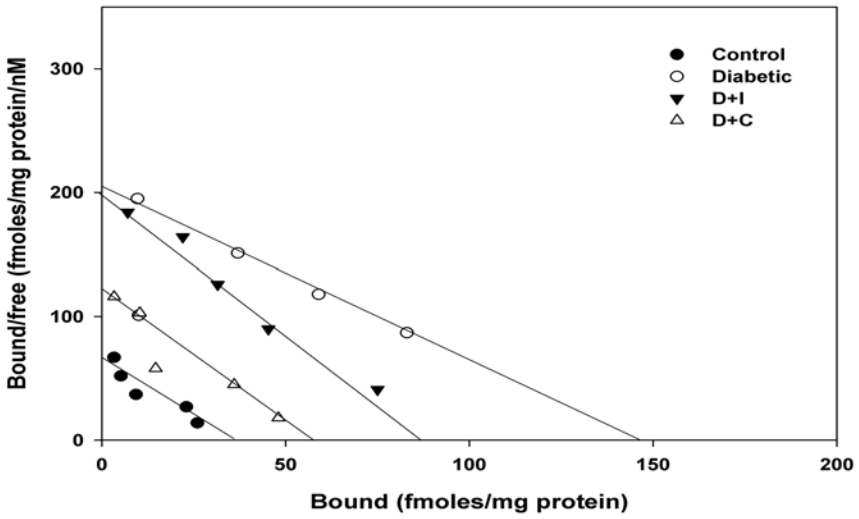


Table-61

Scatchard analysis of muscarinic M3 receptor using [^{3}H] DAMP binding against DAMP in the brain stem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	35 ± 4.2	0.4 ± 0.54
Diabetic	145 ± 7.1 [a]	0.7 ± 0.05 [d]
D + I	86 ± 5.6 [b]	0.4 ± 0.13 [e]
D + C	57 ± 5.2 [c]	0.5 ± 0.12 [e]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [d] P<0.01 when compared to control, [b] P<0.01, [c] P<0.001, [e] P<0.05 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats.

Figure-62

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the brain stem of control and experimental rats

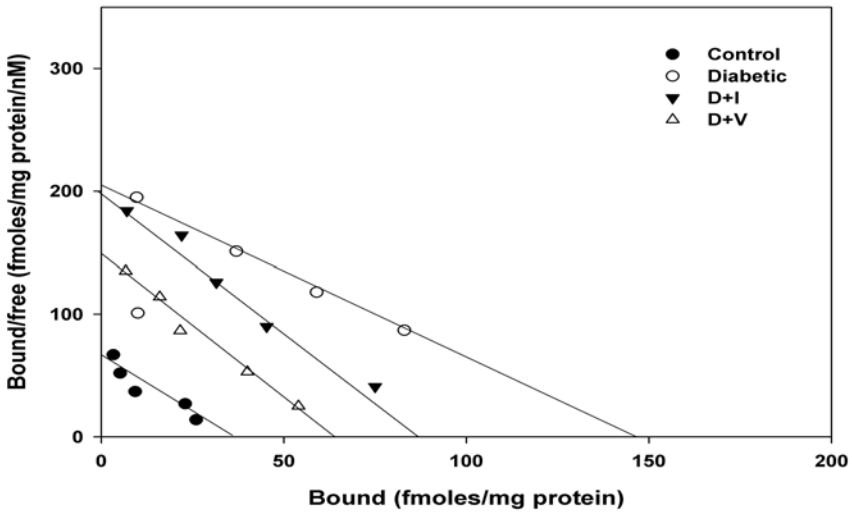


Table-62

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the brain stem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	35 ± 4.2	0.4 ± 0.05
Diabetic	145 ± 7.1 [a]	0.7 ± 0.06 [a]
D + I	86 ± 5.6 [b]	0.4 ± 0.13 [e]
D + V	63 ± 4.6 [b]	0.4 ± 0.09 [e]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [d] P<0.01 when compared to control, [b] P<0.01, [e] P<0.05 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats..

Figure-63

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the brain stem of control and experimental rats

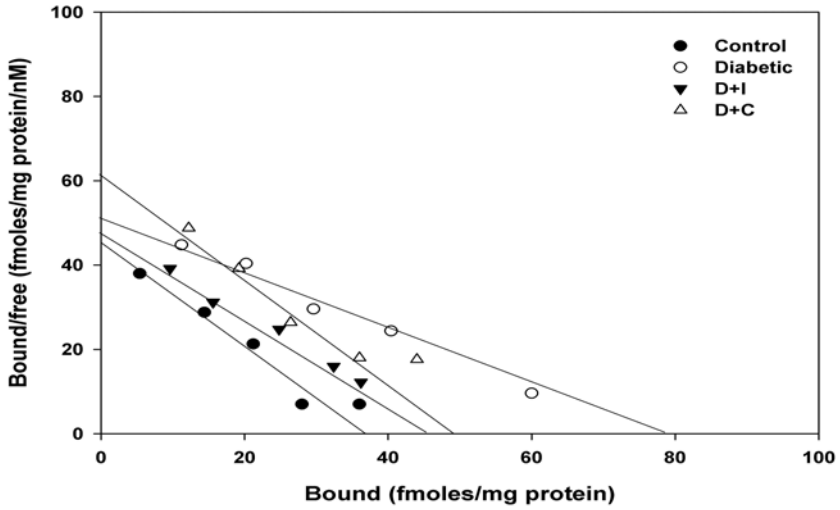


Table-63

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the brain stem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	36 ± 4.5	0.8 ± 0.04
Diabetic	78 ± 5.3 [a]	1.5 ± 0.07 [a]
D + I	44 ± 3.5 [b]	0.9 ± 0.04 [b]
D + C	48 ± 3.9 [b]	0.4 ± 0.06 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats.

Figure-64

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the brain stem of control and experimental rats

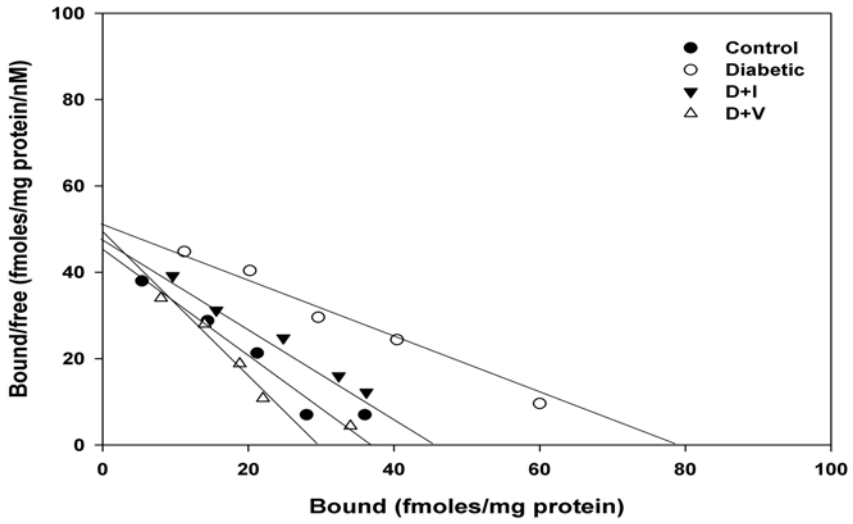


Table-64

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the brain stem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	36 ± 4.5	0.8 ± 0.04
Diabetic	78 ± 5.3 [a]	1.5 ± 0.04 [a]
D + I	44 ± 3.5 [b]	0.9 ± 0.02 [b]
D + V	29 ± 3.2 [b]	0.5 ± 0.04 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats..

Figure-65

Real Time amplification of acetylcholine esterase mRNA from the brain stem of control and experimental rats

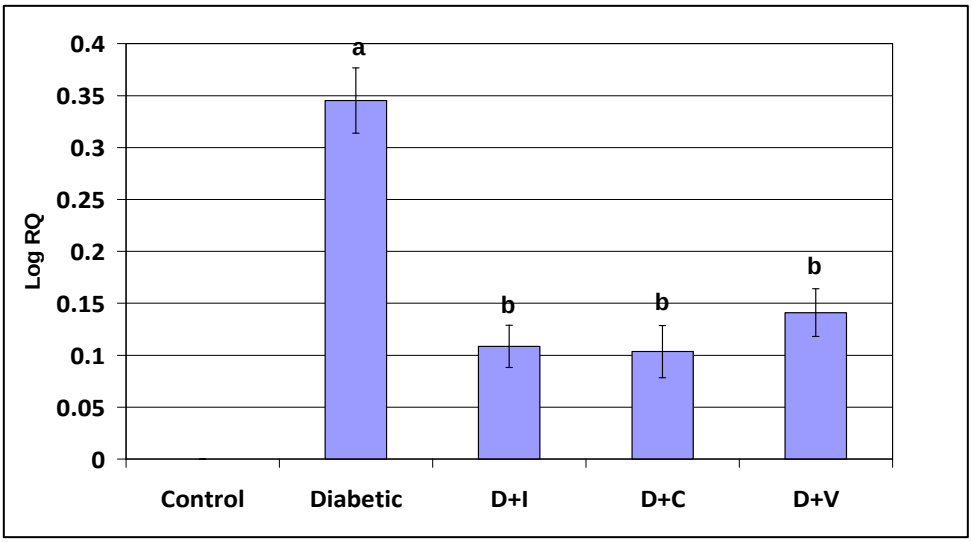


Table-65

Real Time amplification of acetylcholine esterase mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.34 ± 0.03^{a}
D + I	0.10 ± 0.02^{b}
D + C	0.10 ± 0.02^{b}
D + V	0.14 ± 0.02^{b}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001 when compared to control, **b** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-66

Real Time amplification of choline acetyl transferase mRNA from the brain stem of control and experimental rats

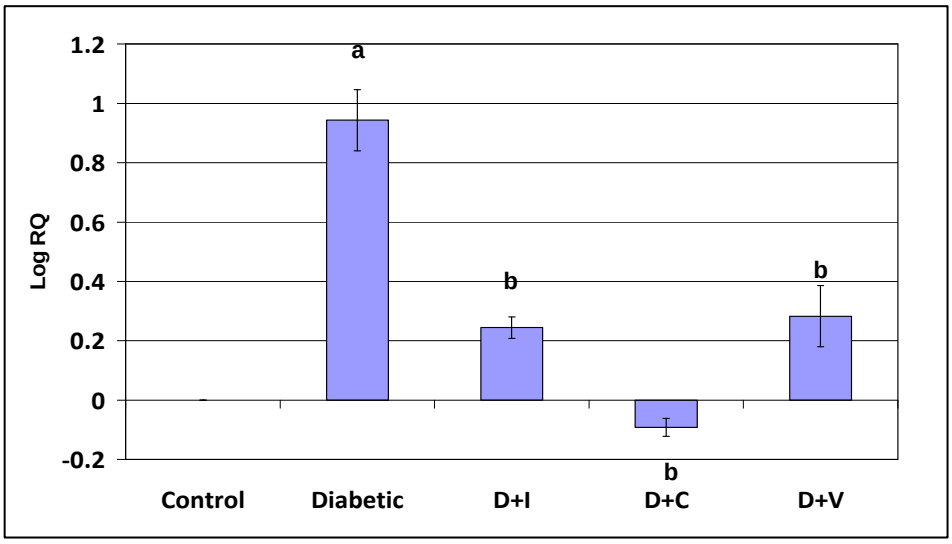


Table-66

Real Time amplification of choline acetyl transferase mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.94 ± 0.10^{a}
D + I	0.24 ± 0.03^{b}
D + C	-0.09 ± 0.03^{b}
D + V	0.28 ± 0.10^{b}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001 when compared to control, **b** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-67

Real Time amplification of muscarinic M1 receptor mRNA from the brain stem of control and experimental rats

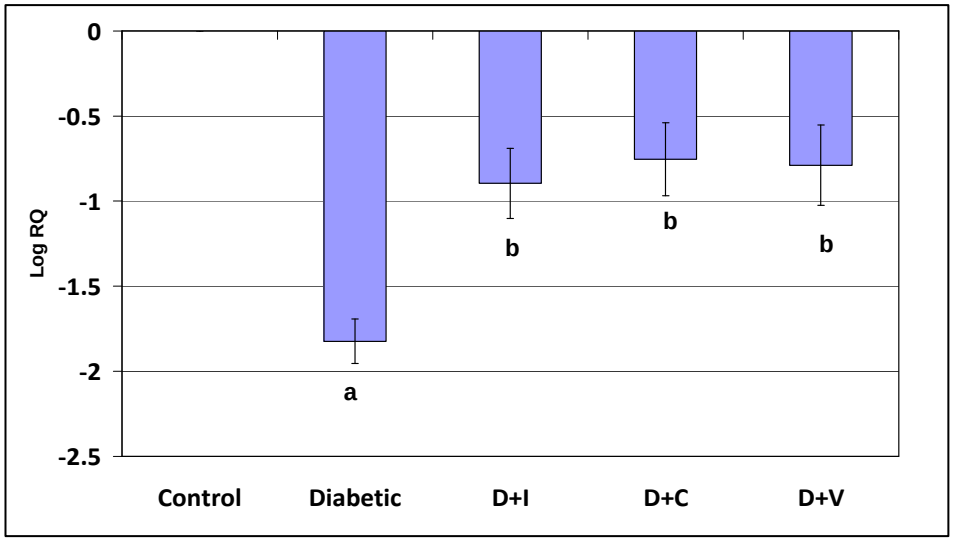


Table-67

Real Time amplification of muscarinic M1 receptor mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.82 ± 0.13 [a]
D + I	-0.89 ± 0.20 [b]
D + C	-0.75 ± 0.21 [b]
D + V	-0.78 ± 0.23 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.01$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-68

Real Time amplification of muscarinic M3 receptor mRNA from the brain stem of control and experimental rats

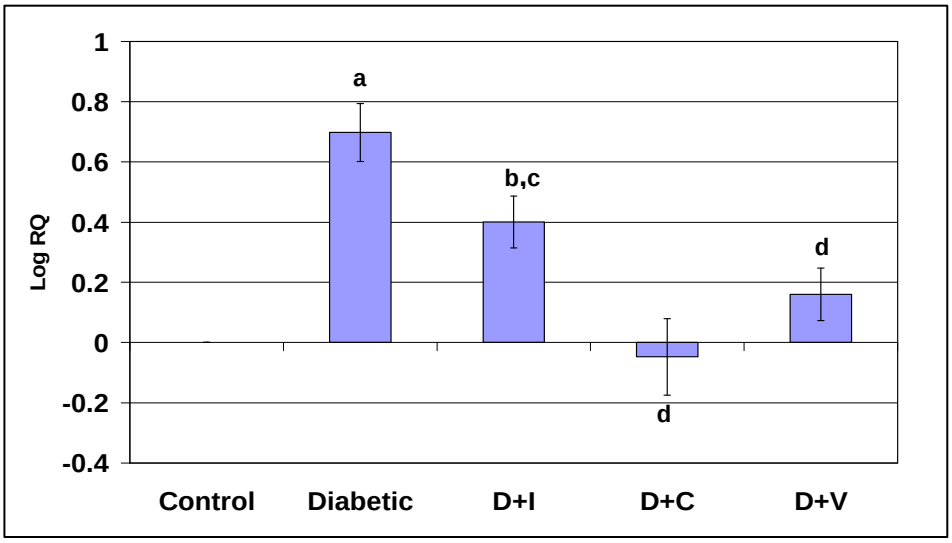


Table-68

Real Time amplification of muscarinic M3 receptor mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.69 ± 0.09^{a}
D + I	$0.40 \pm 0.08^{b,c}$
D + C	-0.04 ± 0.12^{d}
D + V	0.16 ± 0.08^{d}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01, **d** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-69

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the brain stem of control and experimental rats

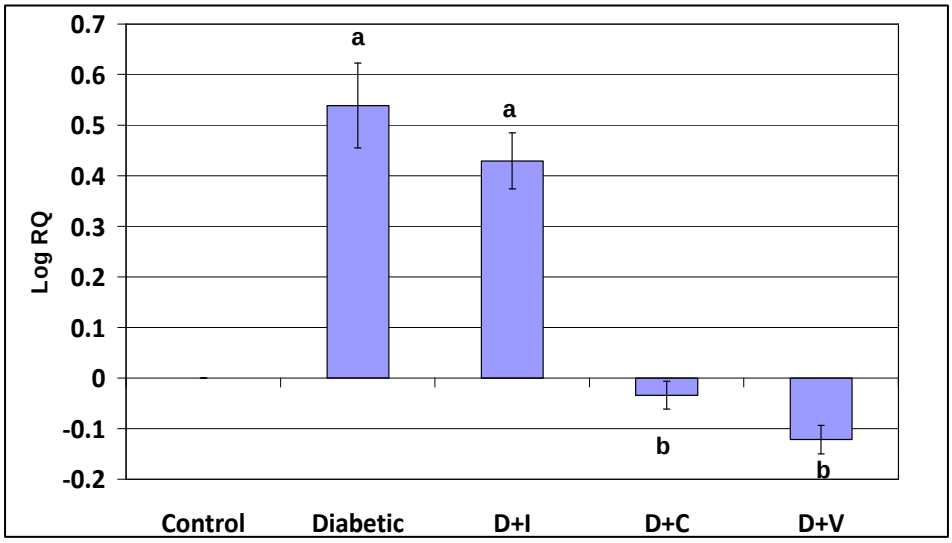


Table-69

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.53 ± 0.08 [a]
D + I	0.42 ± 0.05 [a]
D + C	0.03 ± 0.02 [b]
D + V	0.12 ± 0.02 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.01$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-70

Real Time amplification of dopamine D1 receptor mRNA from the brain stem of control and experimental rats

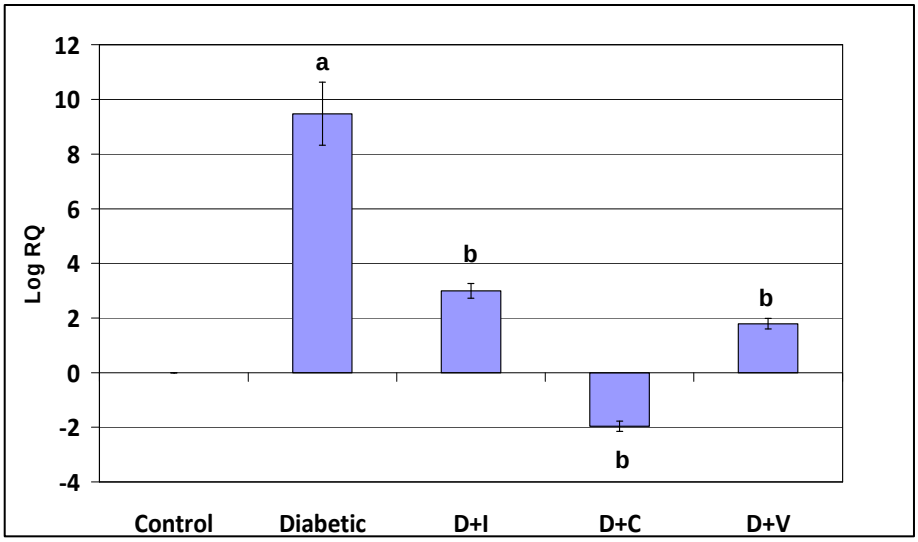


Table-70

Real Time amplification of dopamine D1 receptor mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	9.47 ± 1.16 [a]
D + I	2.99 ± 0.26 [b]
D + C	-1.96 ± 0.18 [b]
D + V	1.78 ± 0.19 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-71

Real Time amplification of dopamine D2 receptor mRNA from the brain stem of control and experimental rats

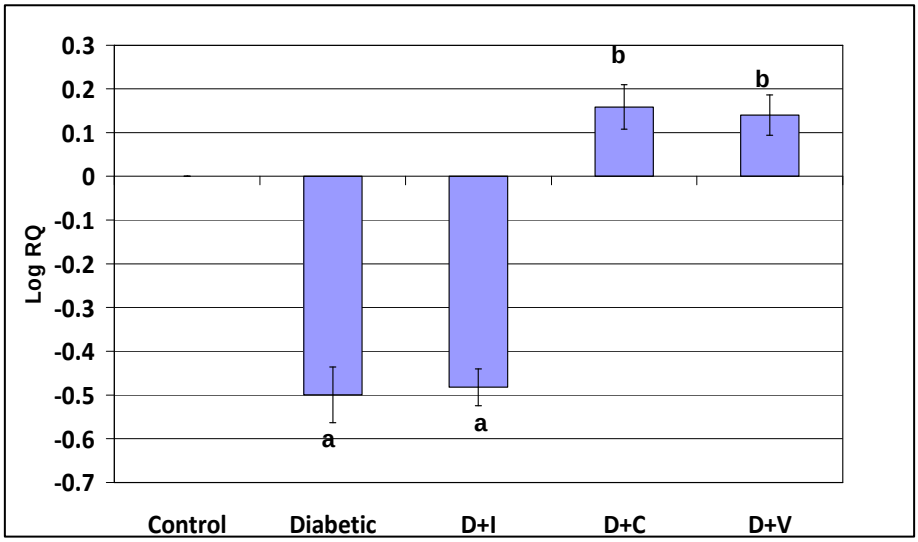


Table-71

Real Time amplification of dopamine D2 receptor mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.49 ± 0.06 [a]
D + I	-0.48 ± 0.04 [a]
D + C	0.15 ± 0.05 [b]
D + V	0.13 ± 0.04 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.01$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-72

Real Time amplification of Vitamin D receptor mRNA from the brain stem of control and experimental rats

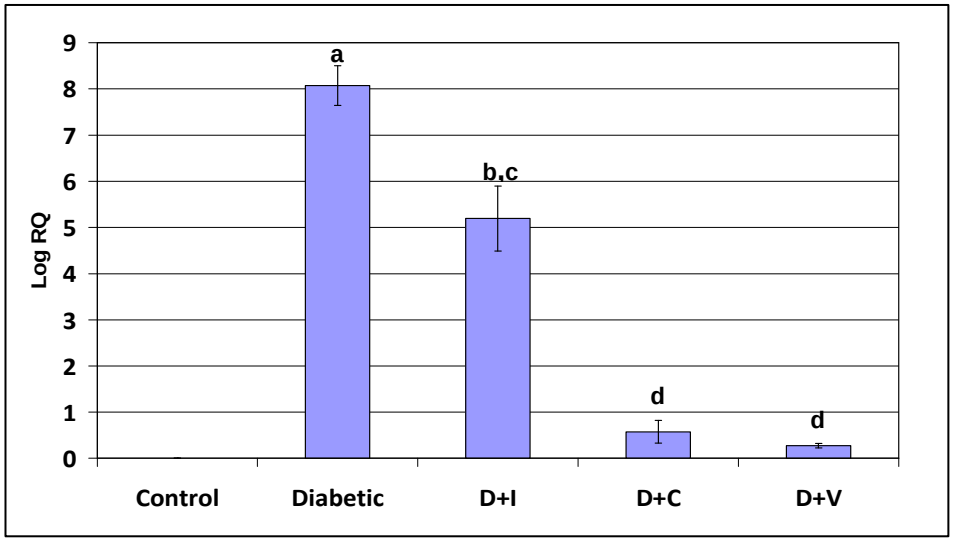


Table-72

Real Time amplification of Vitamin D receptor mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	8.07 ± 0.43^{a}
D + I	$5.19 \pm 0.70^{b,c}$
D + C	0.57 ± 0.24^{d}
D + V	0.27 ± 0.05^{d}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01, **d** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-73

Real Time amplification of insulin receptor mRNA from the brain stem of control and experimental rats

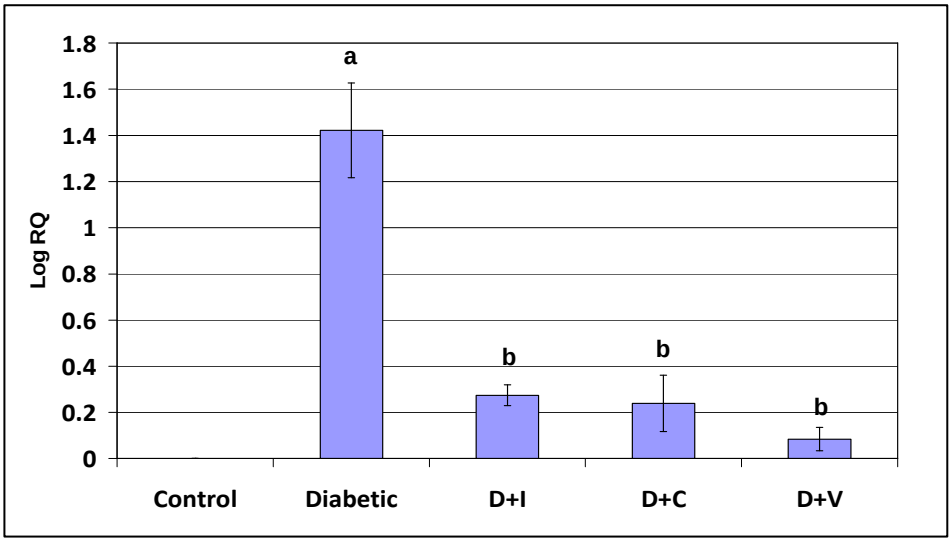


Table-73

Real Time amplification of insulin receptor mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.42 ± 0.20 [a]
D + I	0.27 ± 0.04 [b]
D + C	0.23 ± 0.12 [b]
D + V	-0.08 ± 0.05 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.01$ when compared to diabetic group, D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-74

Real Time amplification of GLUT3 mRNA from the brain stem of control and experimental rats

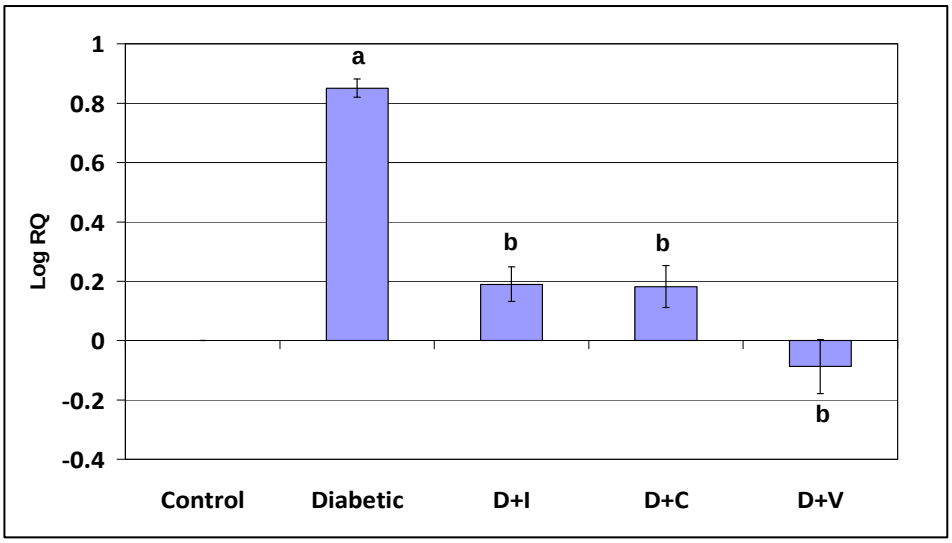


Table-74

Real Time amplification of GLUT3 mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.85 ± 0.03 [a]
D + I	0.18 ± 0.05 [b]
D + C	0.18 ± 0.07 [b]
D + V	-0.08 ± 0.09 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.01$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-75

Real Time amplification of phospholipase C mRNA from the brain stem of control and experimental rats

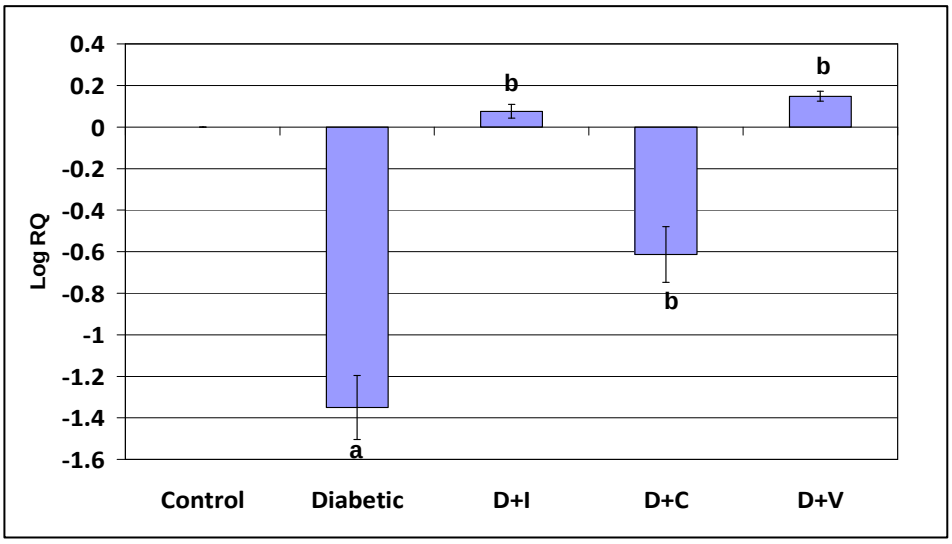


Table-75

Real Time amplification of phospholipase C mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.34 ± 0.15 [a]
D + I	0.07 ± 0.03 [b]
D + C	-0.06 ± 0.13 [b]
D + V	0.14 ± 0.02 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-76

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

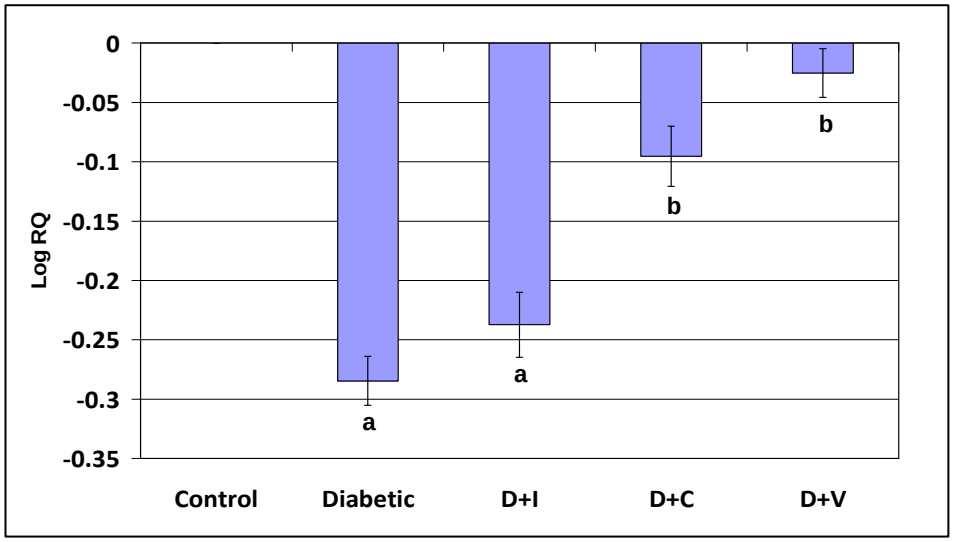


Table-76

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

Experimental groups	Log RQ
Control	0
Diabetic	-0.28 ± 0.02 [a]
D + I	-0.23 ± 0.02 [a]
D + C	-0.09 ± 0.02 [b]
D + V	-0.25 ± 0.020 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **[a]** $P<0.001$ when compared to control, **[b]** $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-77

Real Time amplification of superoxide dismutase mRNA from the brain stem of control and experimental rats

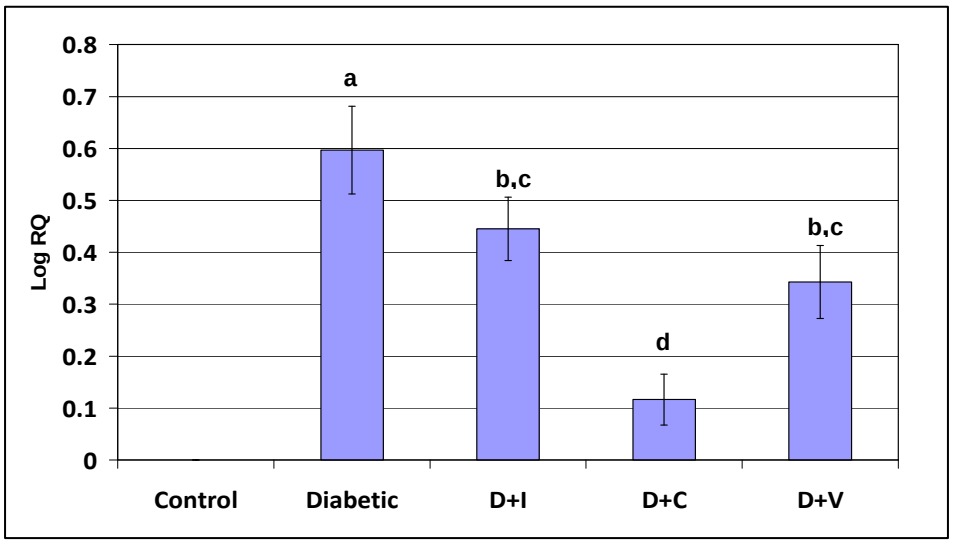


Table-77

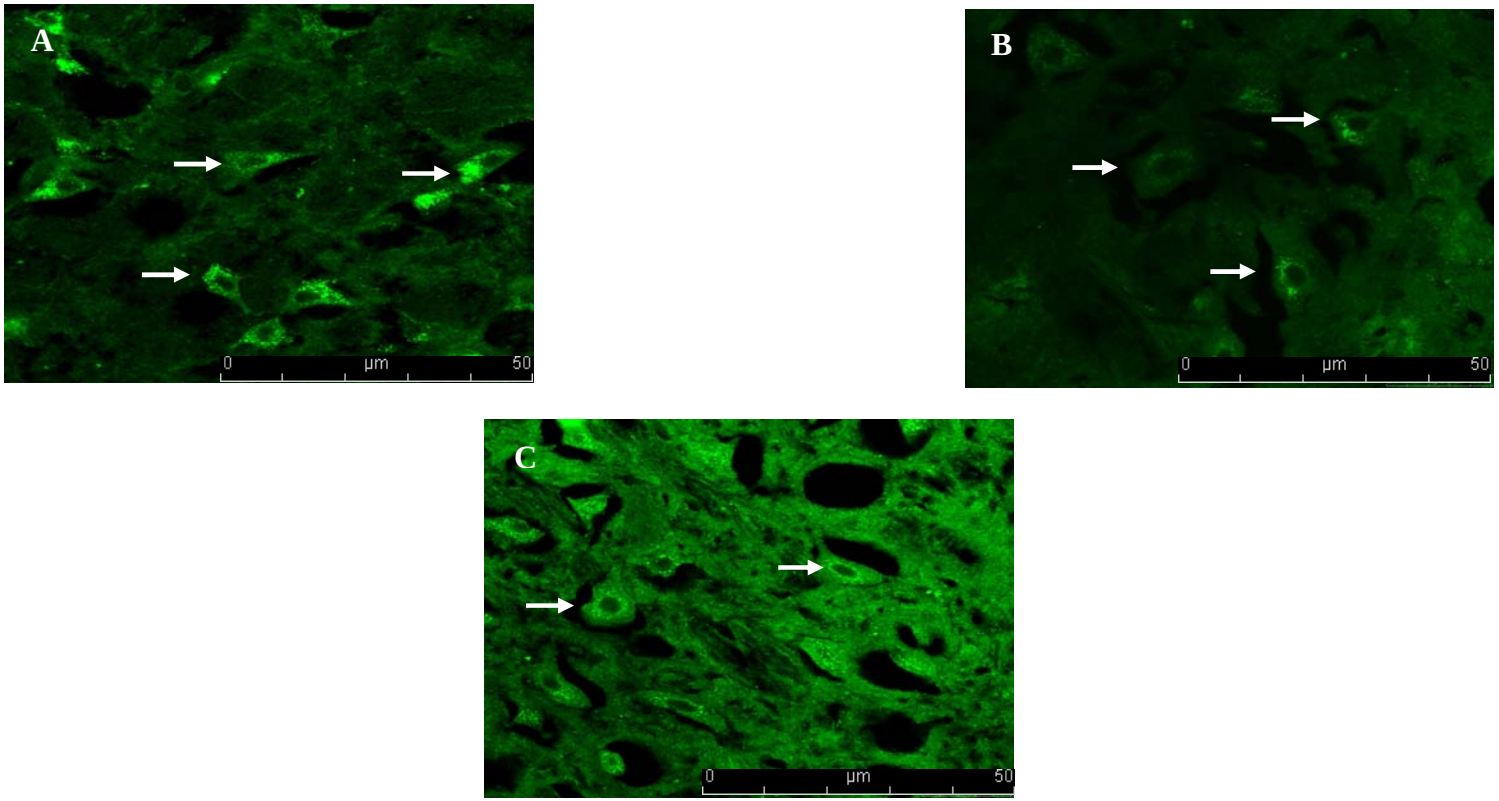
Real Time amplification of superoxide dismutase mRNA from the brain stem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.59 ± 0.08^{a}
D + I	$0.44 \pm 0.06^{b,c}$
D + C	0.11 ± 0.48^{d}
D + V	$0.34 \pm 0.07^{b,c}$

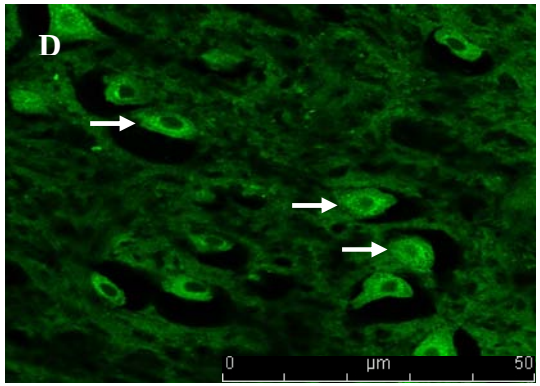
Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01, **d** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-78

Muscarinic M1 receptor expression in the brainstem of control and experimental rats



→ **Muscarinic M1 receptor**



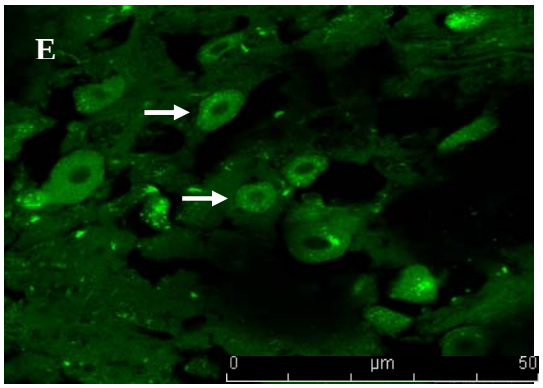


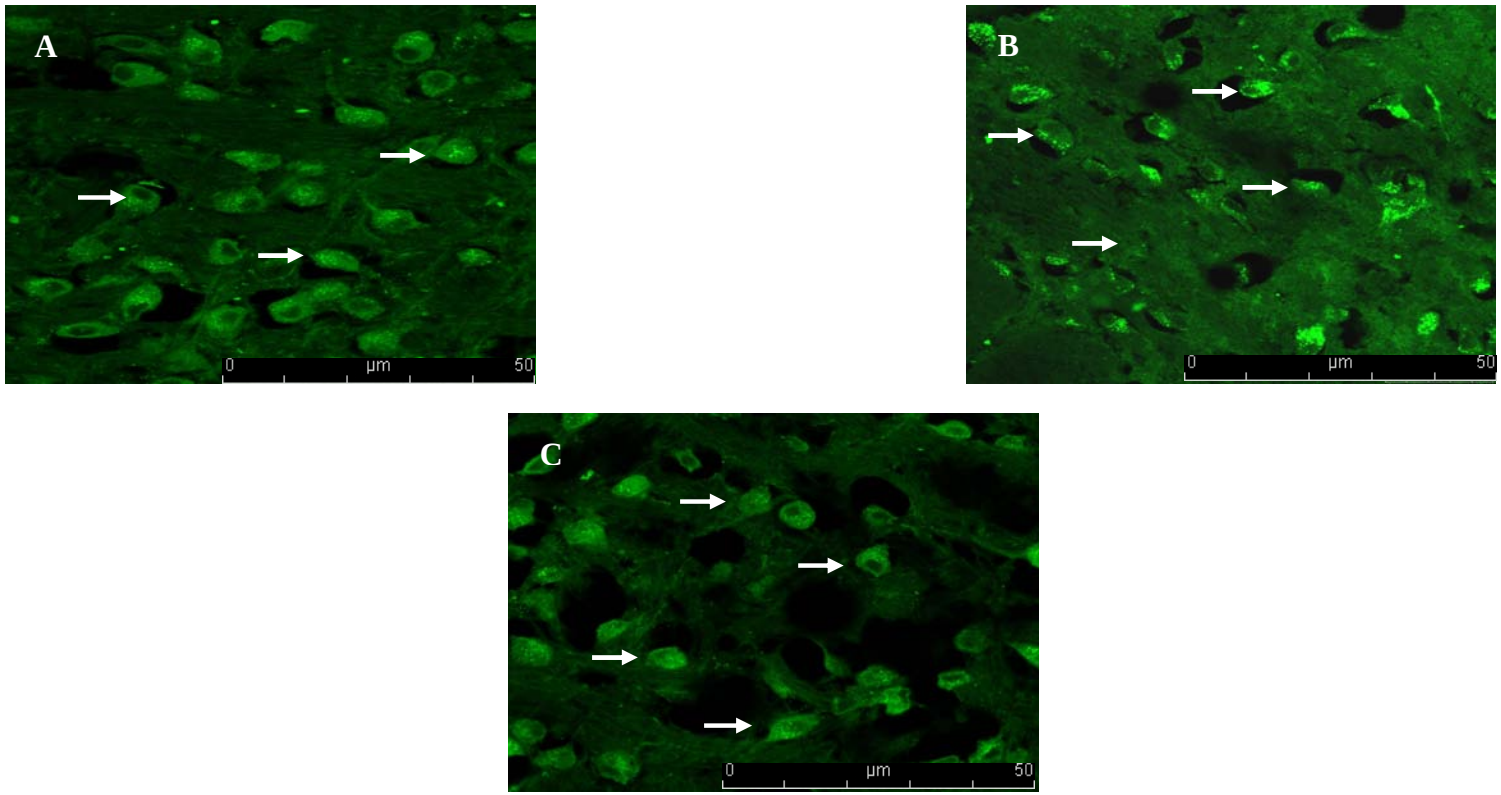
Table-78
Muscarinic M1 receptor expression in the brainstem of control and experimental rats

Condition	Mean pixel value
Control	77 ± 4.5
Diabetic	28 ± 2.6 [a]
D + I	54 ± 3.4 [b]
D + C	56 ± 3.4 [b]
D + V	45 ± 2.3 [b]

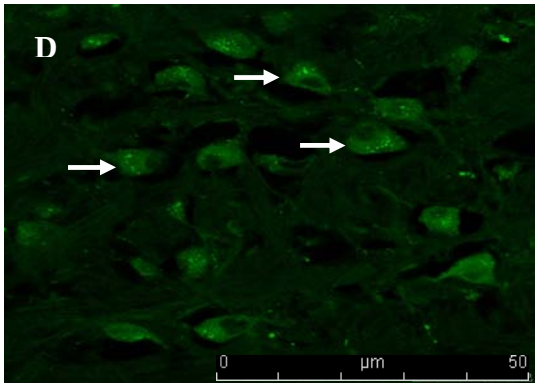
Confocal image of muscarinic M1 receptors in the cerebral cortex of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M1 receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-79

Muscarinic M3 Receptor Expression in the brainstem of Control and Experimental Rats



→ **Muscarinic M3 receptor**



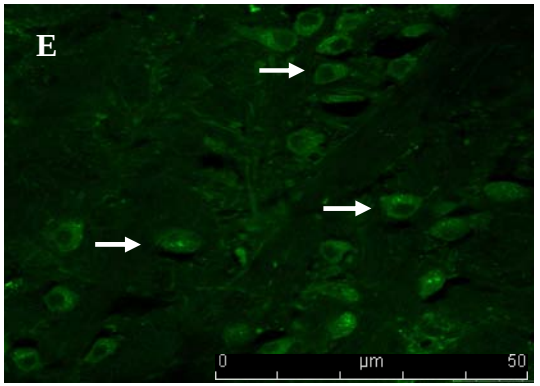


Table-79

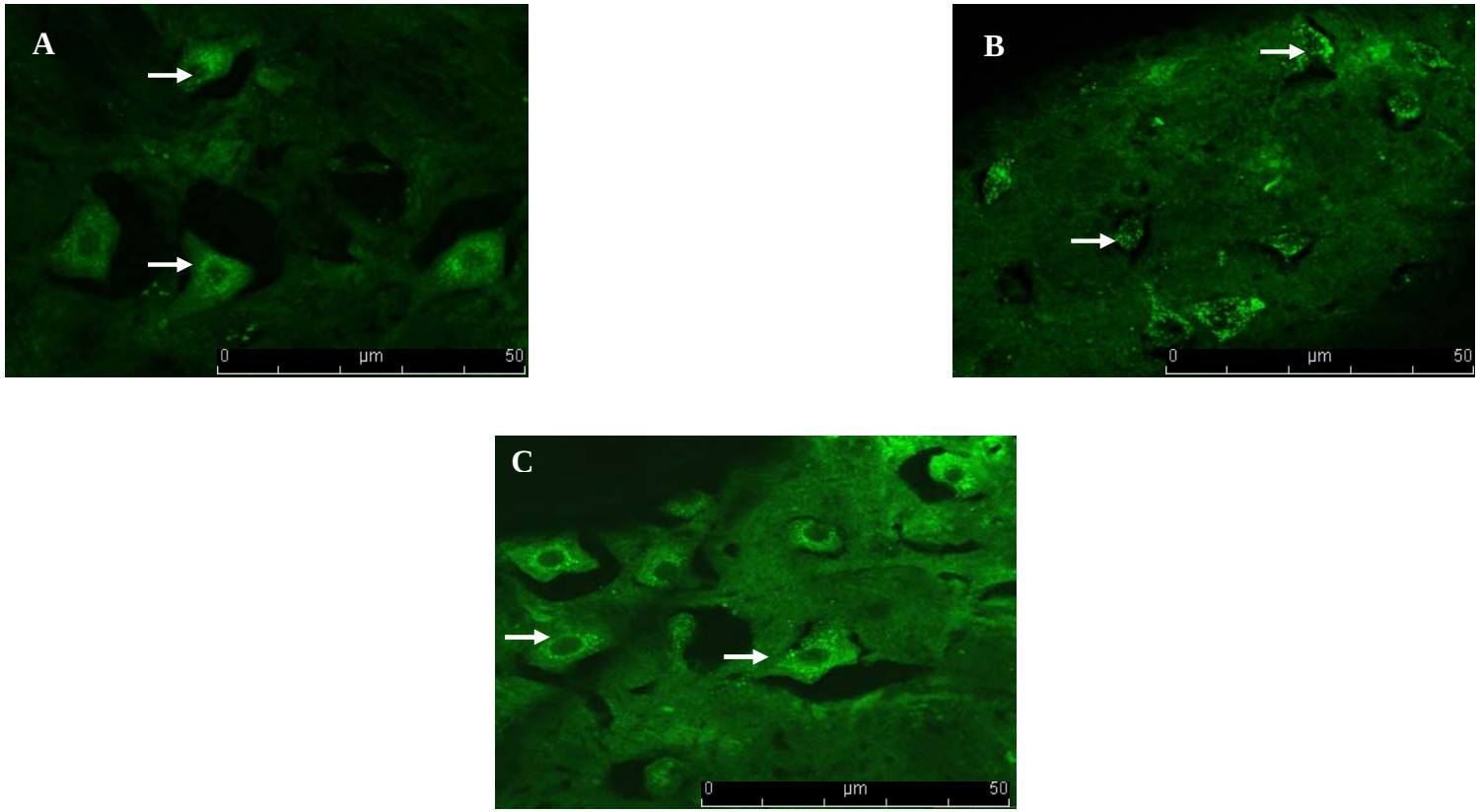
Muscarinic M3 receptor expression in the brainstem of control and experimental rats

Condition	Mean pixel value
Control	28 ± 2.3
Diabetic	62 ± 4.7 [a]
D + I	38 ± 3.2 [b]
D + C	27 ± 2.6 [b]
D + V	26 ± 2.5 [b]

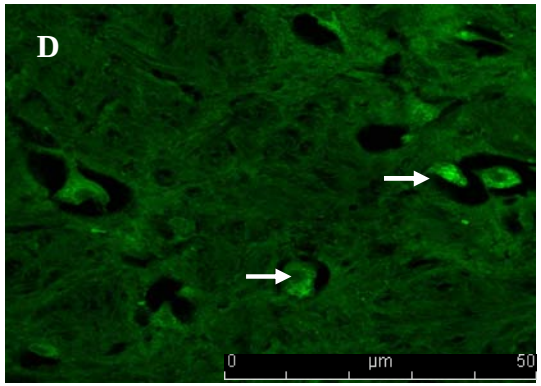
Confocal image of muscarinic M3 receptors expression in the brainstem of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M3 receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-80

α7nicotinic acetylcholine receptor expression in the brainstem of control and experimental rats



→ α7nicotinic acetylcholine receptor



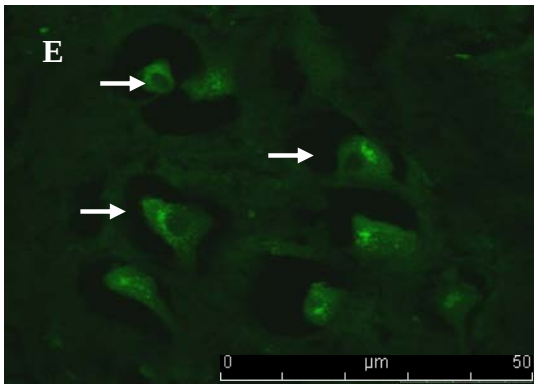


Table-80

α7nicotinic acetylcholine receptor expression in the brainstem of control and experimental rats

Condition	Mean pixel value
Control	30 ± 3.3
Diabetic	75 ± 5.5 [a]
D + I	35 ± 2.8 [b]
D + C	32 ± 4.2 [b]
D + V	39 ± 5.6 [b]

Confocal image of α7nicotinic acetylcholine receptor expression in the brainstem of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent α7nicotinic acetylcholine receptors specific primary antibody and FITC as secondary antibody (⟶) in white shows α7nicotinic acetylcholine receptor. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-81

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the corpus striatum of control and experimental rats

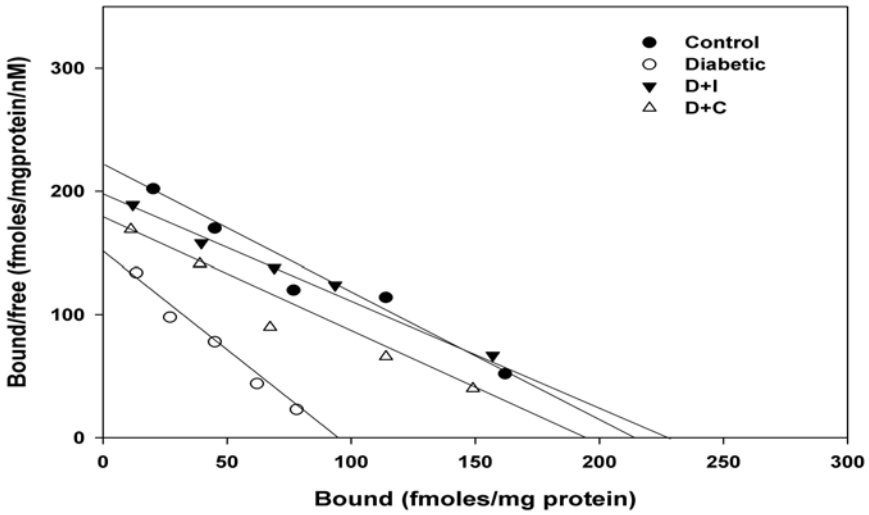


Table-81

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	212 ± 7.1	0.9 ± 0.06
Diabetic	93 ± 4.2 [a]	0.8 ± 0.04
D + I	227 ± 7.6 [b]	1.1 ± 0.05
D + C	197 ± 5.8 [b]	1.0 ± 0.07

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-82

Scatchard analysis of total muscarinic receptor using [^{3}H] QNB binding against atropine in the corpus striatum of control and experimental rats

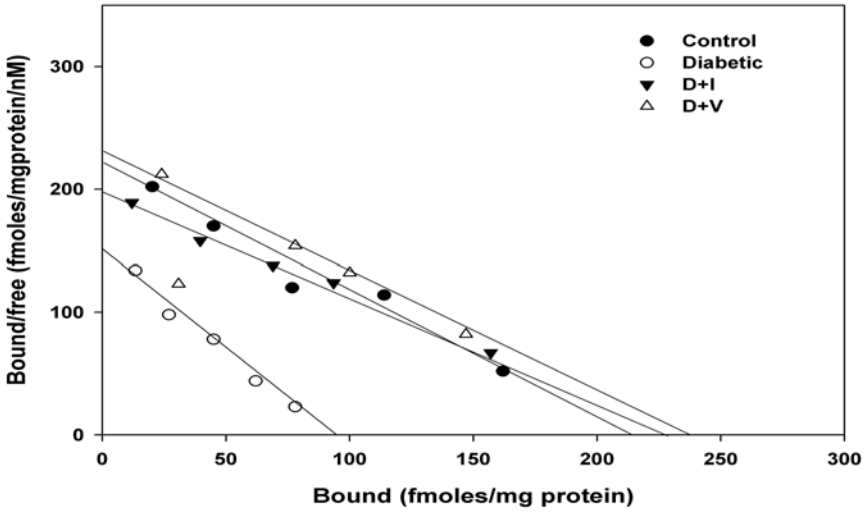


Table-82

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	212 ± 7.1	0.9 ± 0.06
Diabetic	93 ± 4.2 [a]	0.8 ± 0.04
D + I	227 ± 7.6 [b]	1.1 ± 0.05
D + V	245 ± 7.3 [c]	1.0 ± 0.07

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-83

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the corpus striatum of control and experimental rats

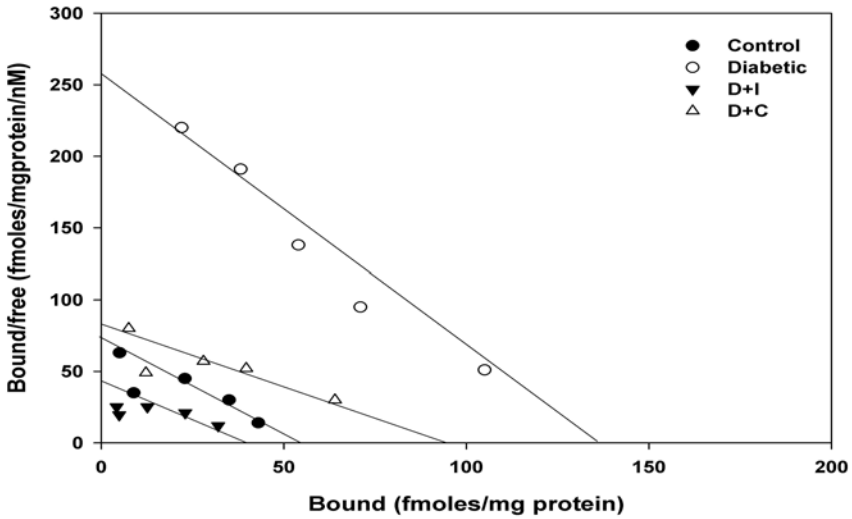


Table-83

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	54 ± 3.1	0.7 ± 0.05
Diabetic	135 ± 6.2 [a]	0.3 ± 0.03 [a]
D + I	38 ± 2.4 [b]	0.9 ± 0.04 [b]
D + C	93 ± 4.7 [c]	1.1 ± 0.07 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-84

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the corpus striatum of control and experimental rats

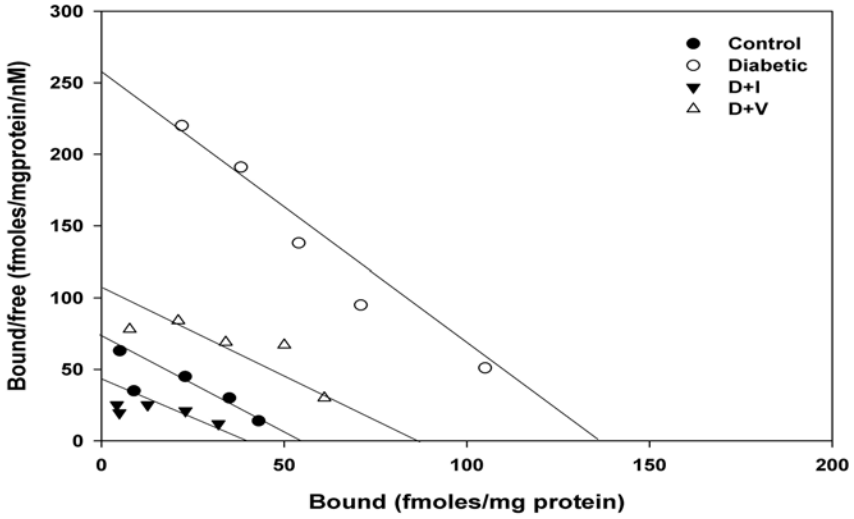


Table-84

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	54 ± 3.1	0.7 ± 0.05
Diabetic	135 ± 6.2 [a]	0.3 ± 0.03 [a]
D + I	38 ± 2.4 [b]	0.9 ± 0.04 [b]
D + V	85 ± 4.4 [c]	0.8 ± 0.05 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure- 85

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the corpus striatum of control and experimental rats

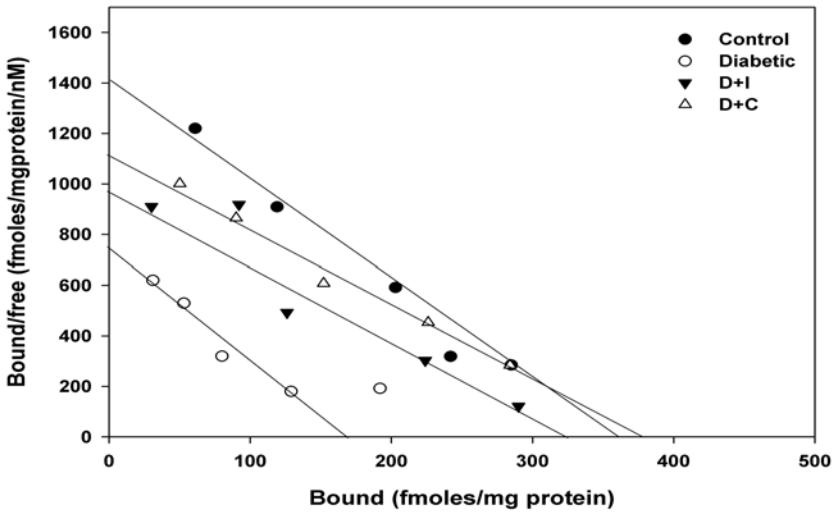


Table-85

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	358 ± 6.2	0.2 ± 0.06
Diabetic	163 ± 5.3[a]	0.2 ± 0.04
D + I	320 ± 7.4[b]	0.3 ± 0.04
D + C	374 ± 8.2[b]	0.3 ± 0.04

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001 when compared to control, **b** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-86

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the corpus striatum of control and experimental rats

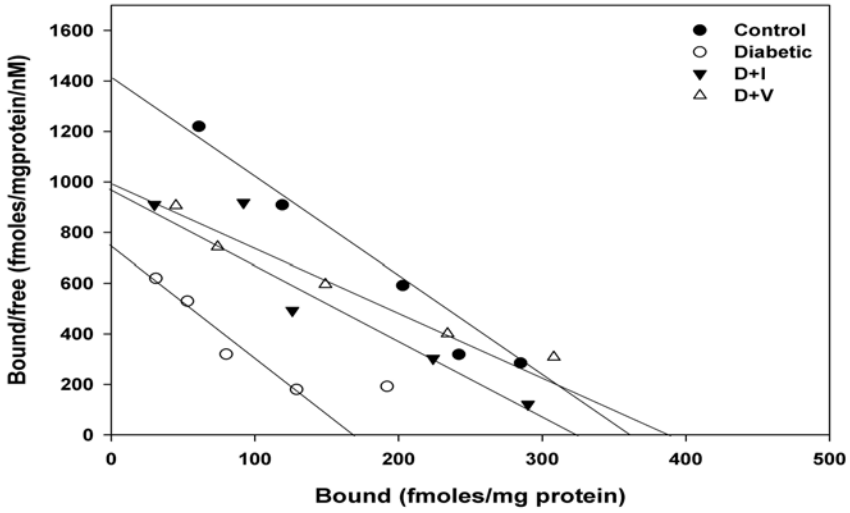


Table-86

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	358 ± 6.2	0.2 ± 0.05
Diabetic	163 ± 5.3 [a]	0.2 ± 0.03
D + I	320 ± 7.4 [b]	0.3 ± 0.04
D + V	384 ± 8.2 [b]	0.3 ± 0.05

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group, [c] $P<0.01$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-87

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the corpus striatum of control and experimental rats

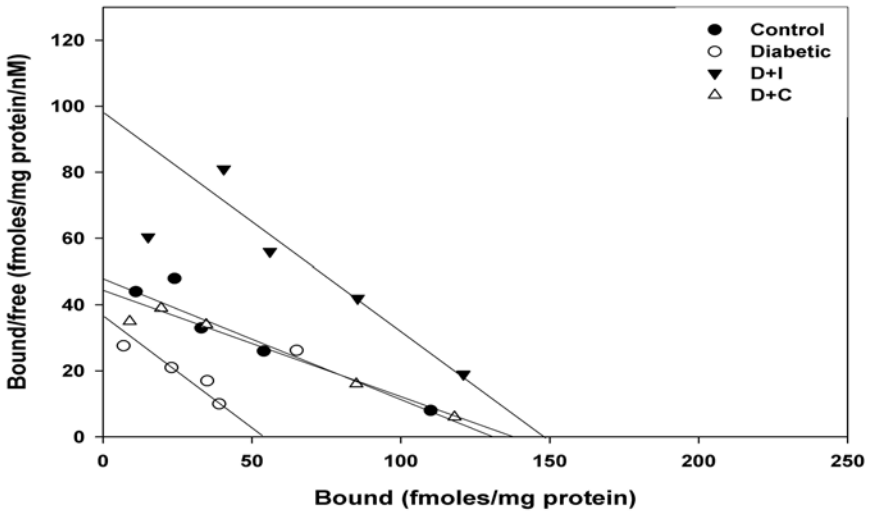


Table-87

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	130 ± 5.2	2.7 ± 0.15
Diabetic	51 ± 3.3 [a]	1.4 ± 0.10 [a]
D + I	149 ± 6.6 [b]	1.5 ± 0.12
D + C	133 ± 5.5 [b]	2.9 ± 0.17 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-88

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the corpus striatum of control and experimental rats

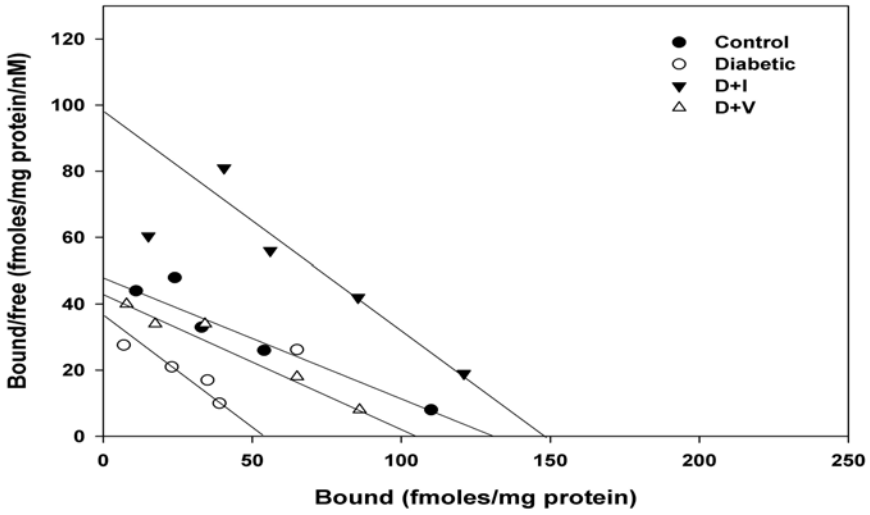


Table-88

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine binding against dopamine in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	130 ± 5.2	2.7 ± 0.15
Diabetic	51 ± 3.3 [a]	1.4 ± 0.10 [a]
D + I	149 ± 6.6 [b]	1.5 ± 0.12
D + V	102 ± 5.5 [b]	2.4 ± 0.16 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-89

Real Time amplification of acetylcholine esterase mRNA from the corpus striatum of control and experimental rats

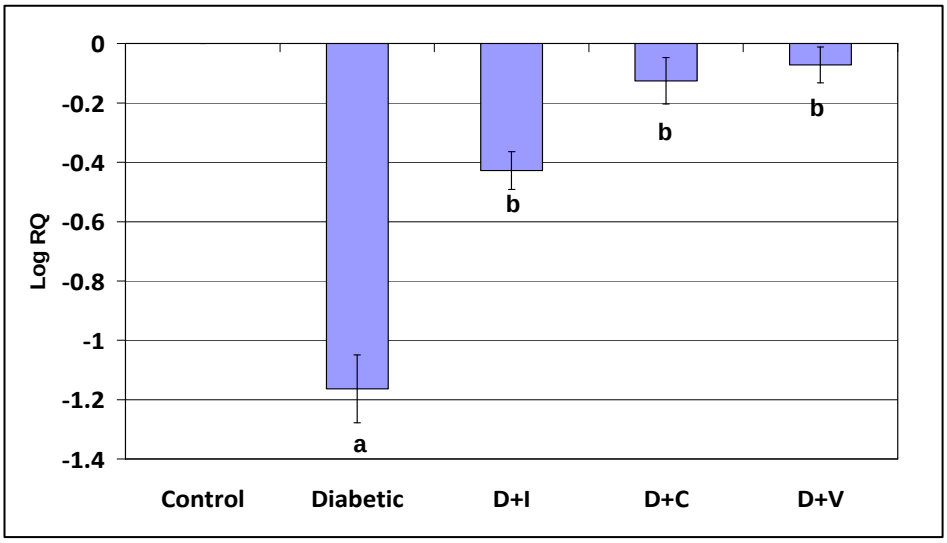


Table-89

Real Time amplification of acetylcholine esterase mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.16 ± 0.11 [a]
D + I	-0.42 ± 0.06 [b]
D + C	-0.12 ± 0.07 [b]
D + V	-0.07 ± 0.06 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-90

Real Time amplification of choline acetyl transferase mRNA from the corpus striatum of control and experimental rats

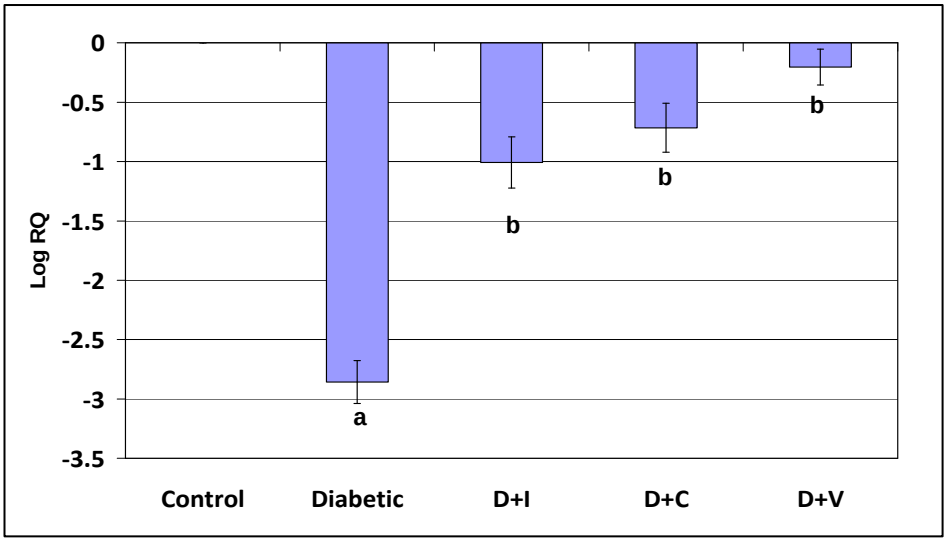


Table-90

Real Time amplification of choline acetyl transferase mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-2.85 ± 0.17 [a]
D + I	-1.00 ± 0.21 [b]
D + C	-0.71 ± 0.20 [b]
D + V	-0.20 ± 0.15 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-91

Real Time amplification of muscarinic M1 receptor mRNA from the corpus striatum of control and experimental rats

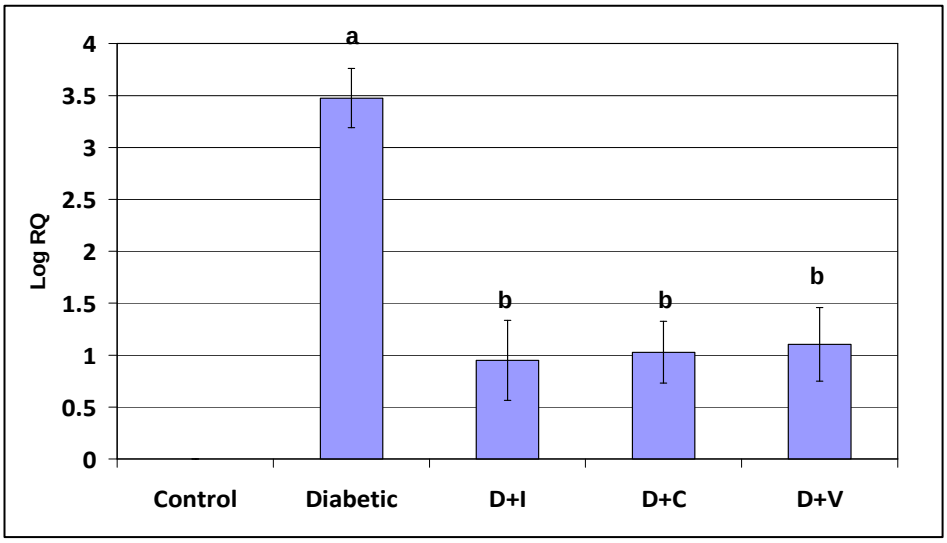


Table-91

Real Time amplification of muscarinic M1 receptor mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	3.47 ± 0.28 [a]
D + I	0.95 ± 0.38 [b]
D + C	1.02 ± 0.29 [b]
D + V	1.10 ± 0.35 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-92

Real Time amplification of muscarinic M3 receptor mRNA from the corpus striatum of control and experimental rats

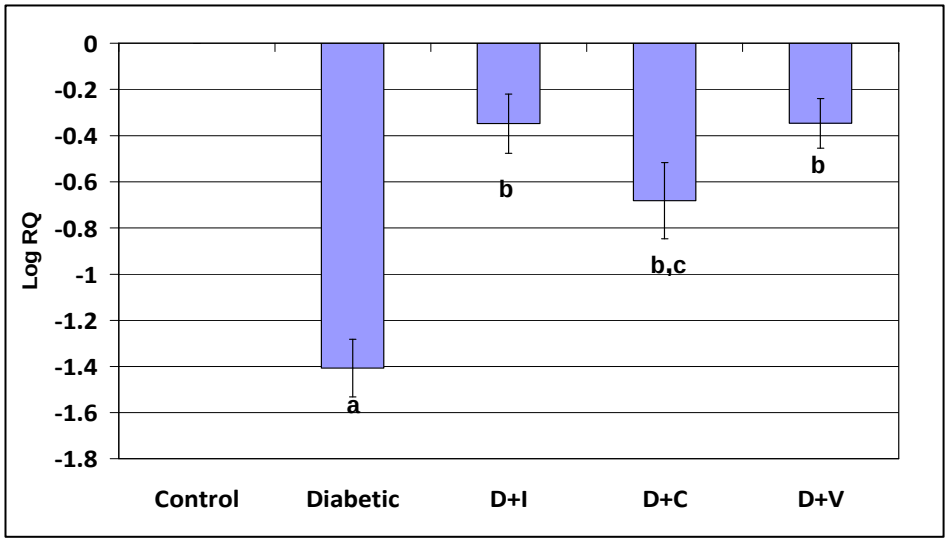


Table-92

Real Time amplification of muscarinic M3 receptor mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.40 ± 0.12 [a]
D + I	-0.34 ± 0.12 [b]
D + C	-0.68 ± 0.16 [b,c]
D + V	-0.34 ± 0.10 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$, [c] $P<0.05$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-93

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the corpus striatum of control and experimental rats

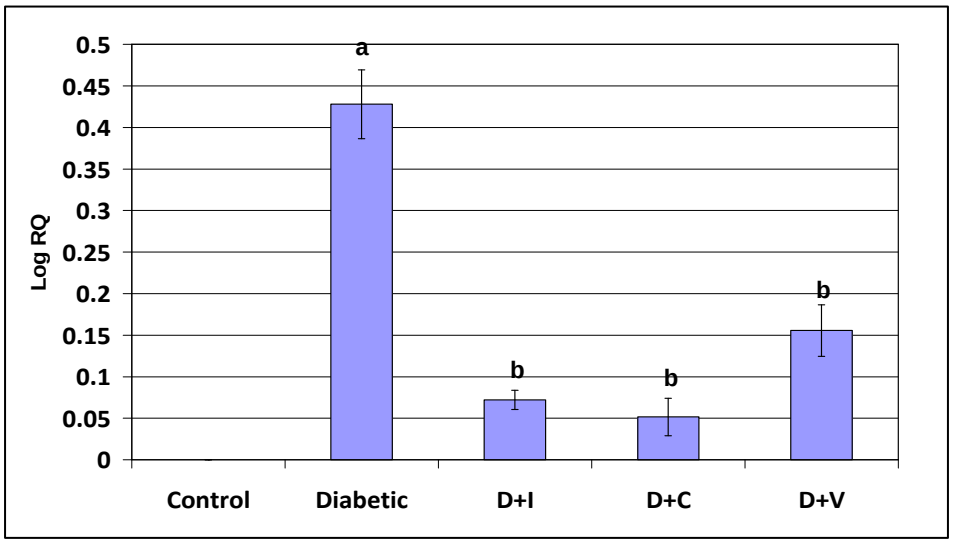


Table-93

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.42 ± 0.04 [a]
D + I	0.07 ± 0.01 [b]
D + C	0.05 ± 0.02 [b]
D + V	0.15 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-94

Real Time amplification of dopamine D1 receptor mRNA from the corpus striatum of control and experimental rats

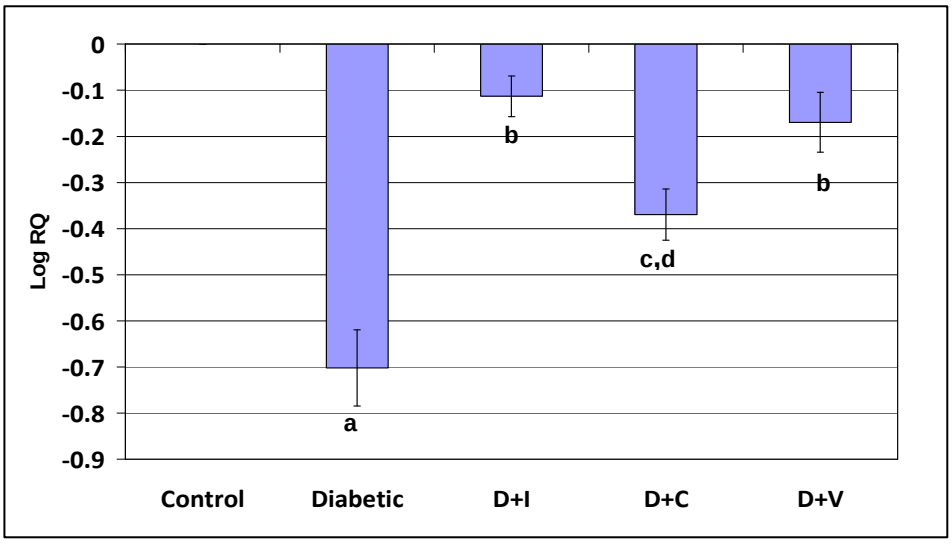


Table-94

Real Time amplification of dopamine D1 receptor mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.70 ± 0.08 [a]
D + I	-0.11 ± 0.04 [b]
D + C	-0.36 ± 0.05 [c,d]
D + V	-0.16 ± 0.06 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [c] P<0.05 when compared to control, [b] P<0.001, [d] P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-95

Real Time amplification of dopamine D2 receptor mRNA from the corpus striatum of control and experimental rats

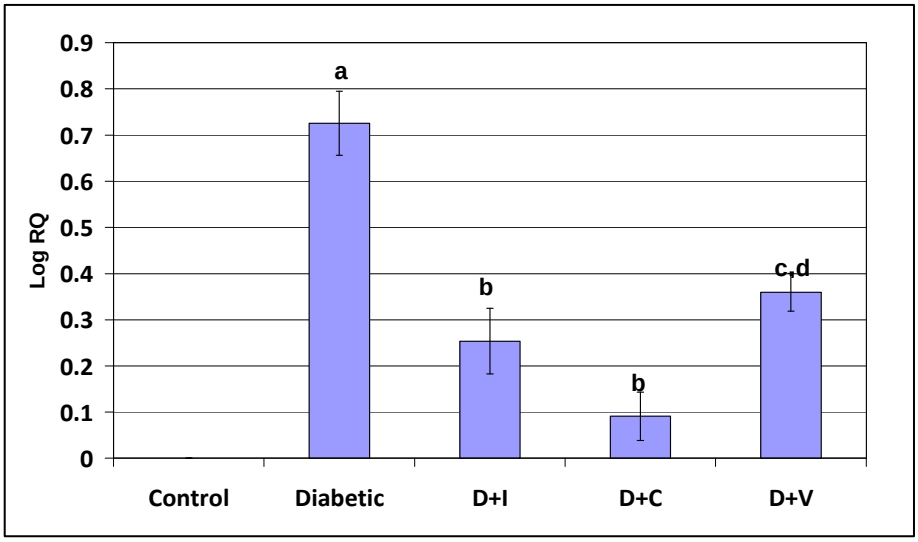


Table-95

Real Time amplification of dopamine D2 receptor mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.72 ± 0.06 [a]
D + I	0.25 ± 0.07 [b]
D + C	0.09 ± 0.05 [b]
D + V	0.35 ± 0.04 [c,d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [c] P<0.05 when compared to control, [b] P<0.001, [d] P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-96

Real Time amplification of Vitamin D receptor mRNA from the corpus striatum of control and experimental rats

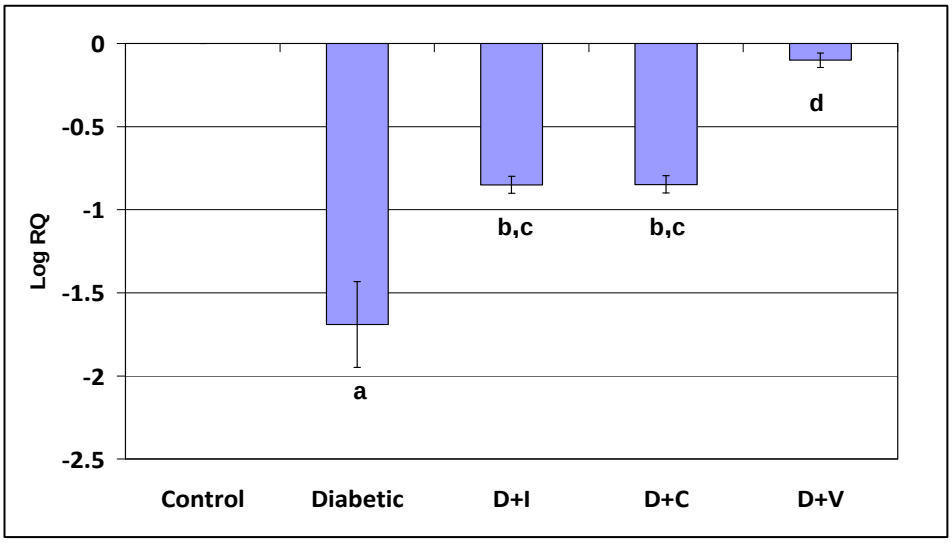


Table-96

Real Time amplification of Vitamin D receptor mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.69 ± 0.25 [a]
D + I	-0.85 ± 0.05 [b,c]
D + C	-0.84 ± 0.05 [b,c]
D + V	-0.10 ± 0.04 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [c] P<0.05 when compared to control, [b] P<0.01, [d] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-97

Real Time amplification of insulin receptor mRNA from the corpus striatum of control and experimental rats

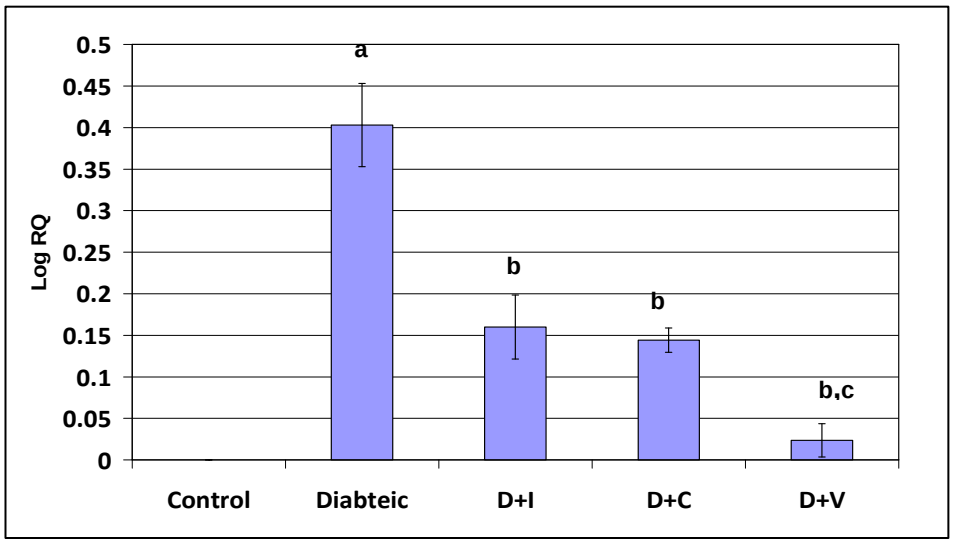


Table-97

Real Time amplification of insulin receptor mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.40 ± 0.04 [a]
D + I	0.15 ± 0.03 [b]
D + C	0.14 ± 0.01 [b]
D + V	0.02 ± 0.02 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-98

Real Time amplification of GLUT3 mRNA from the corpus striatum of control and experimental rats

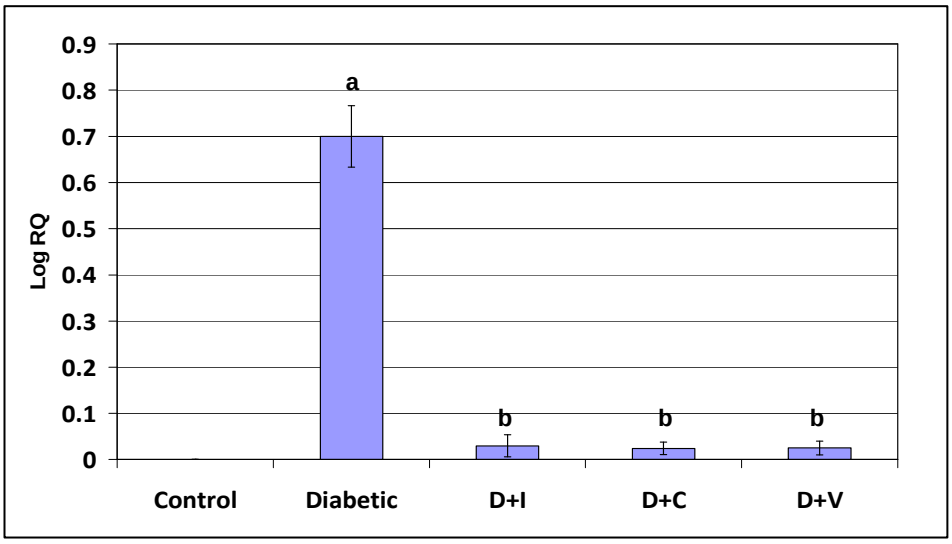


Table-98

Real Time amplification of GLUT3 mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.69 ± 0.06^{a}
D + I	0.03 ± 0.02^{b}
D + C	0.02 ± 0.01^{b}
D + V	0.02 ± 0.01^{b}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** $P<0.001$ when compared to control, **b** $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-99

Real Time amplification of phospholipase C mRNA from the corpus striatum of control and experimental rats

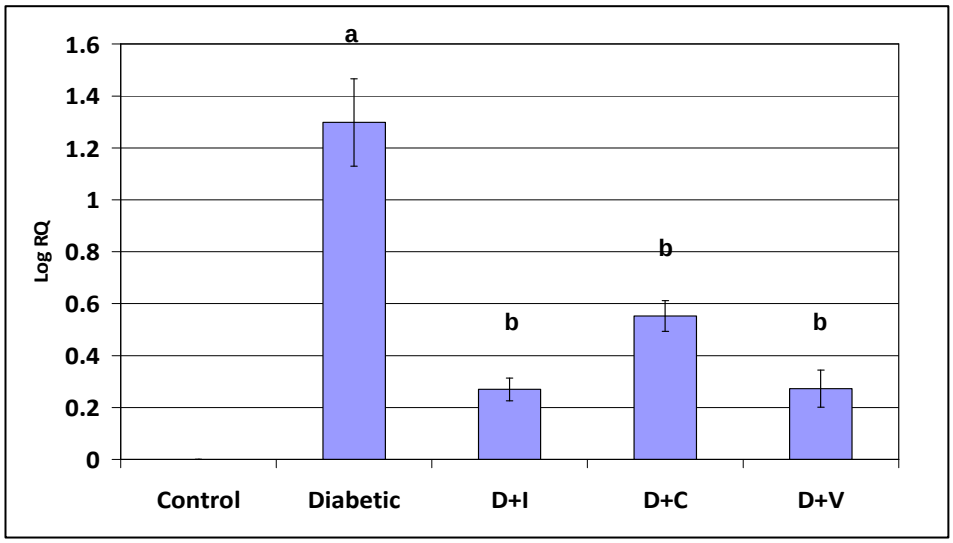


Table-99

Real Time amplification of phospholipase C mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.29 ± 0.16 [a]
D + I	0.29 ± 0.04 [b]
D + C	0.55 ± 0.05 [b]
D + V	0.27 ± 0.07 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-100

Real Time amplification of CREB mRNA from the corpus striatum of control and experimental rats

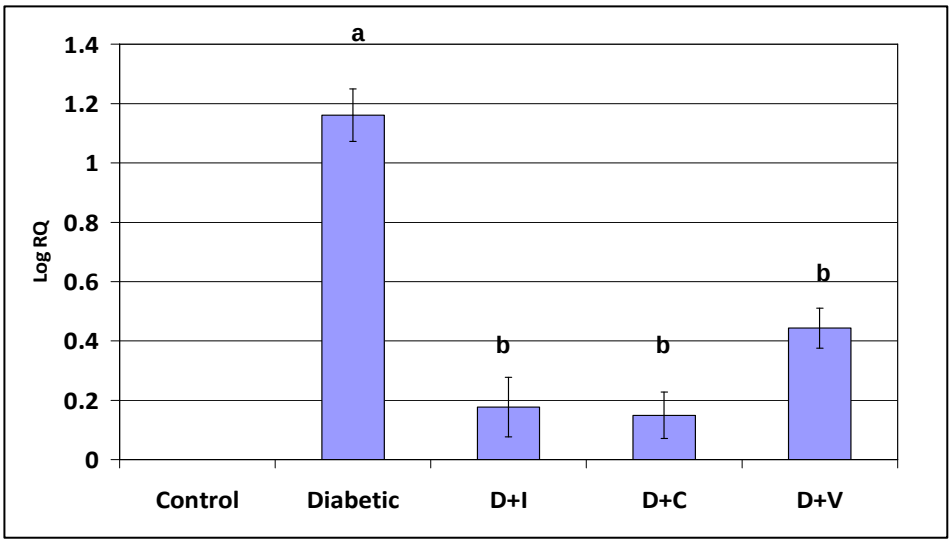


Table-100

Real Time amplification of CREB mRNA from the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.16 ± 0.08 [a]
D + I	0.17 ± 0.10 [b]
D + C	0.14 ± 0.07 [b]
D + V	0.44 ± 0.06 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-101

Real Time amplification of superoxide dismutase mRNA from the corpus striatum of control and experimental rats

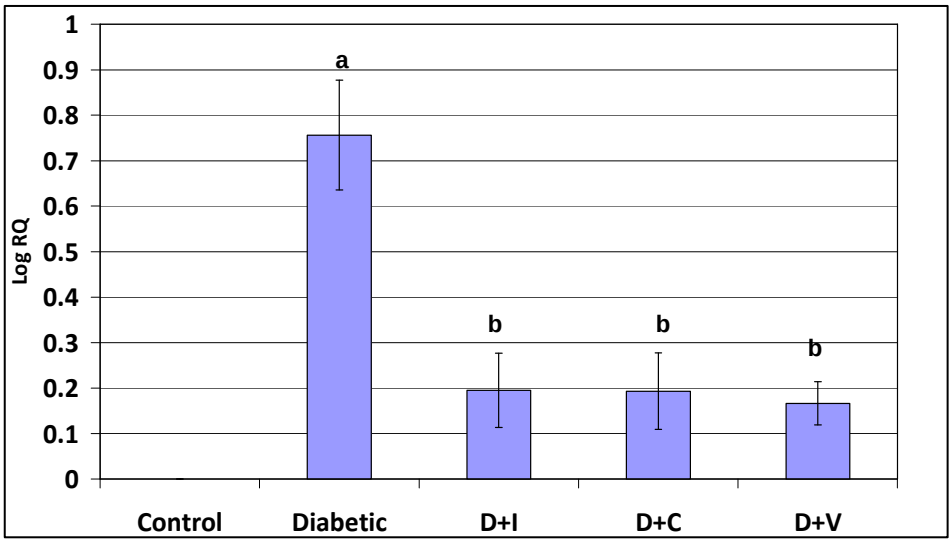


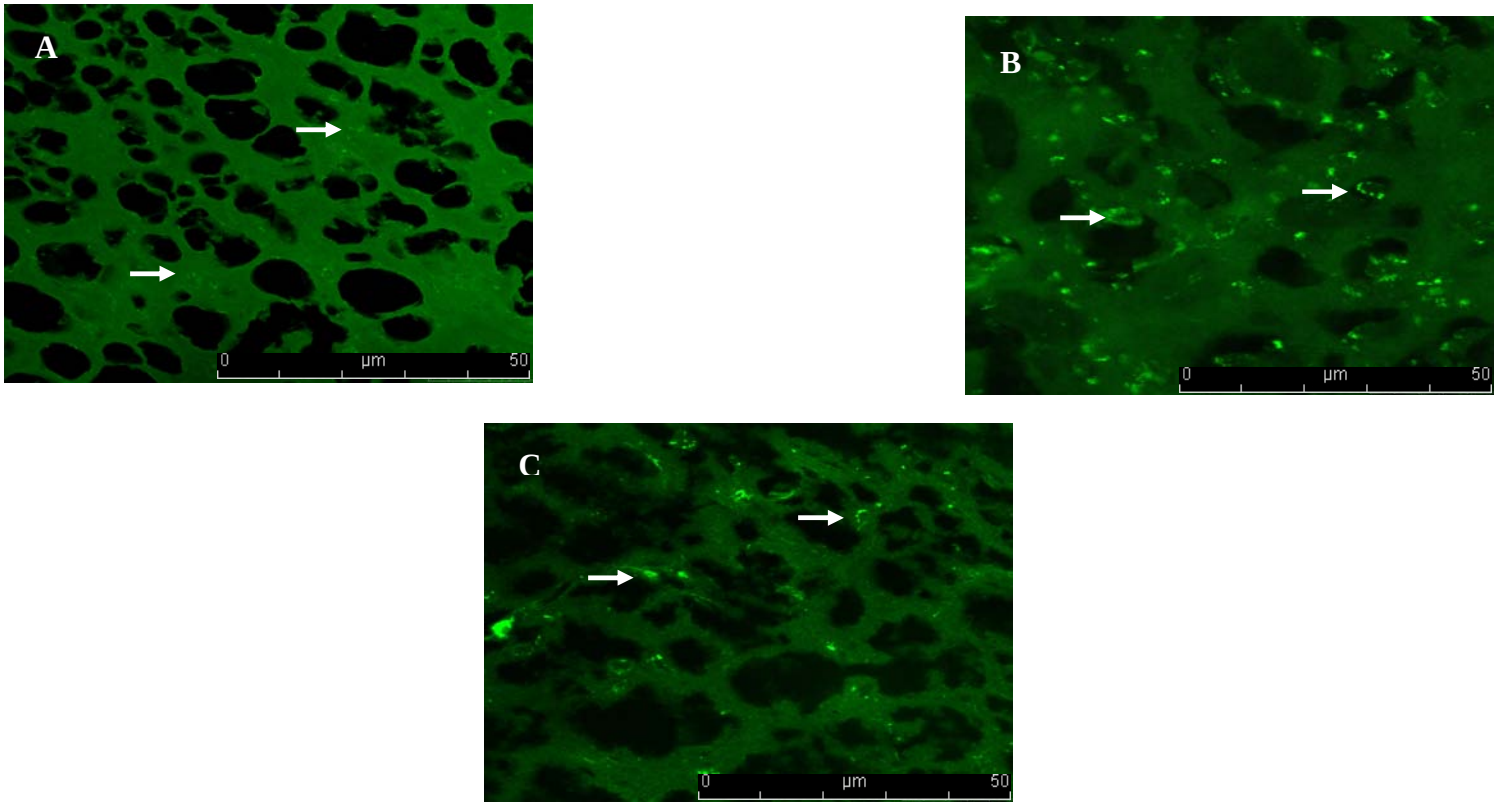
Table-101

Real Time amplification of superoxide dismutase mRNA from the corpus striatum of control and experimental rats

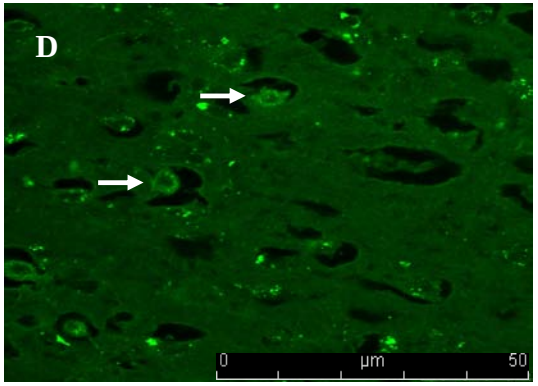
Experimental groups	Log RQ
Control	0
Diabetic	0.75 ± 0.12 [a]
D + I	0.19 ± 0.08 [b]
D + C	0.19 ± 0.09 [b]
D + V	0.16 ± 0.04 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-102
Muscarinic M1 receptor expression in the corpus striatum of control and experimental rats



→ **Muscarinic M1receptor**



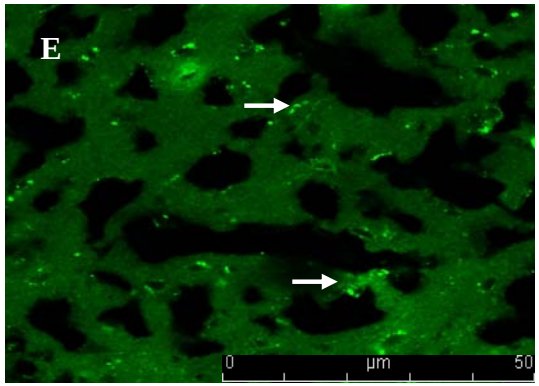
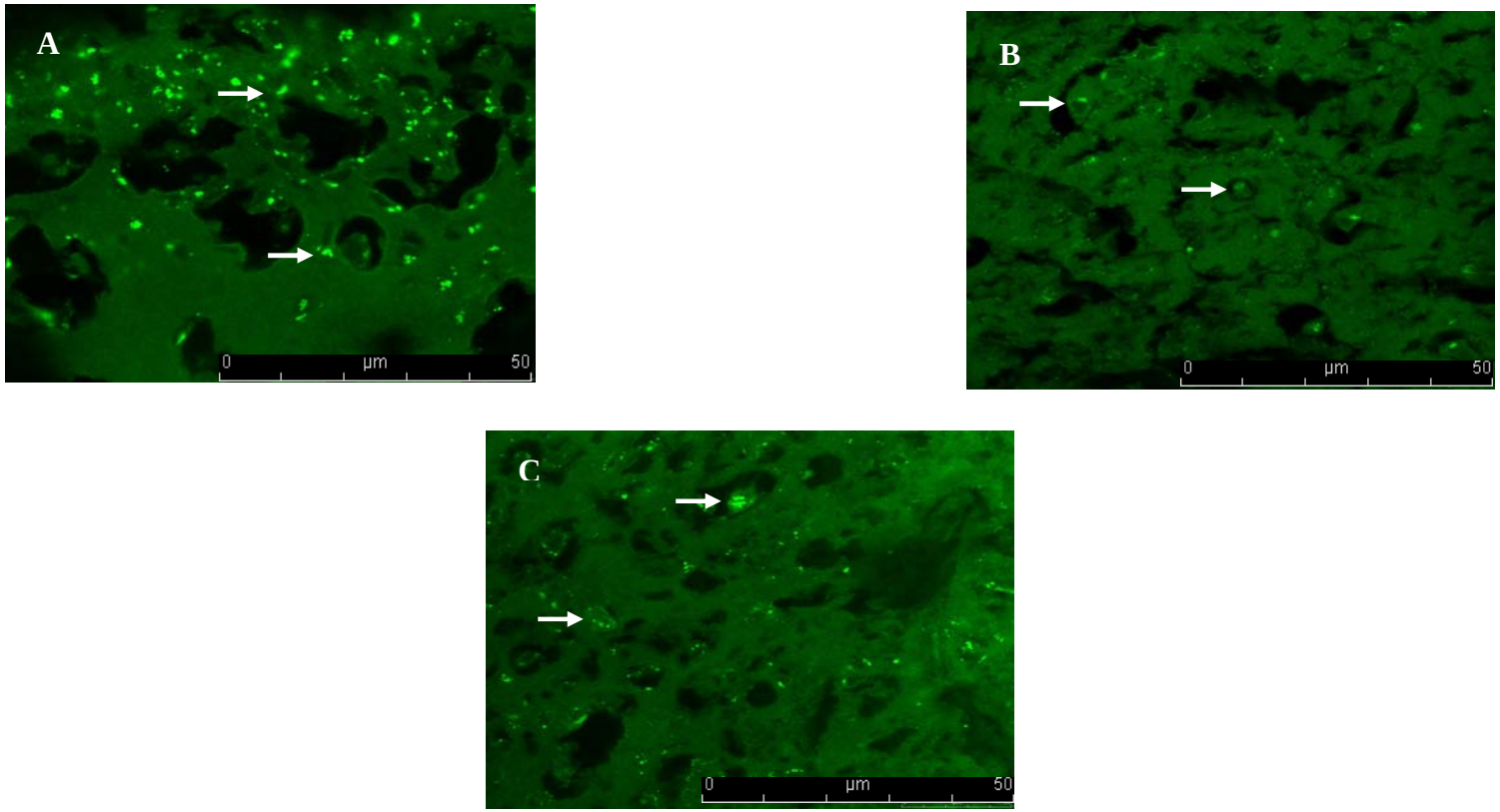


Table-102
Muscarinic M1 receptor expression in the corpus striatum of control and experimental rats

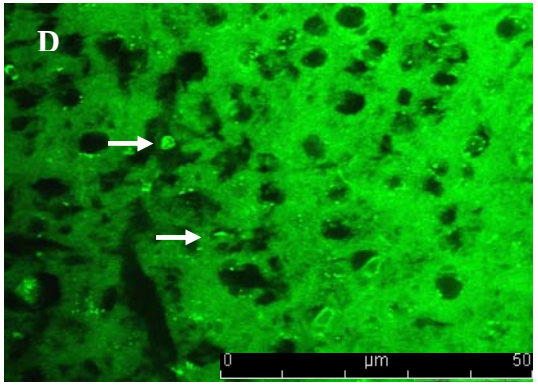
Condition	Mean pixel value
Control	27 ± 2.1
Diabetic	76 ± 5.3 [a]
D + I	35 ± 1.9 [b]
D + C	33 ± 2.3 [b]
D + V	35 ± 3.1 [b]

Confocal image of muscarinic M1 receptors in the corpus striatum of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M1 receptors. . [a] $P<0.001$ when compared to control, [b] $P<0.001$. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-103
Muscarinic M3 receptor expression in the corpus striatum of control and cxperimental Rats



→ **Muscarinic M3 receptor**



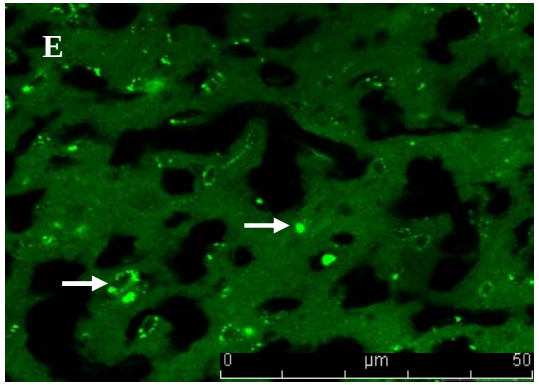


Table-103

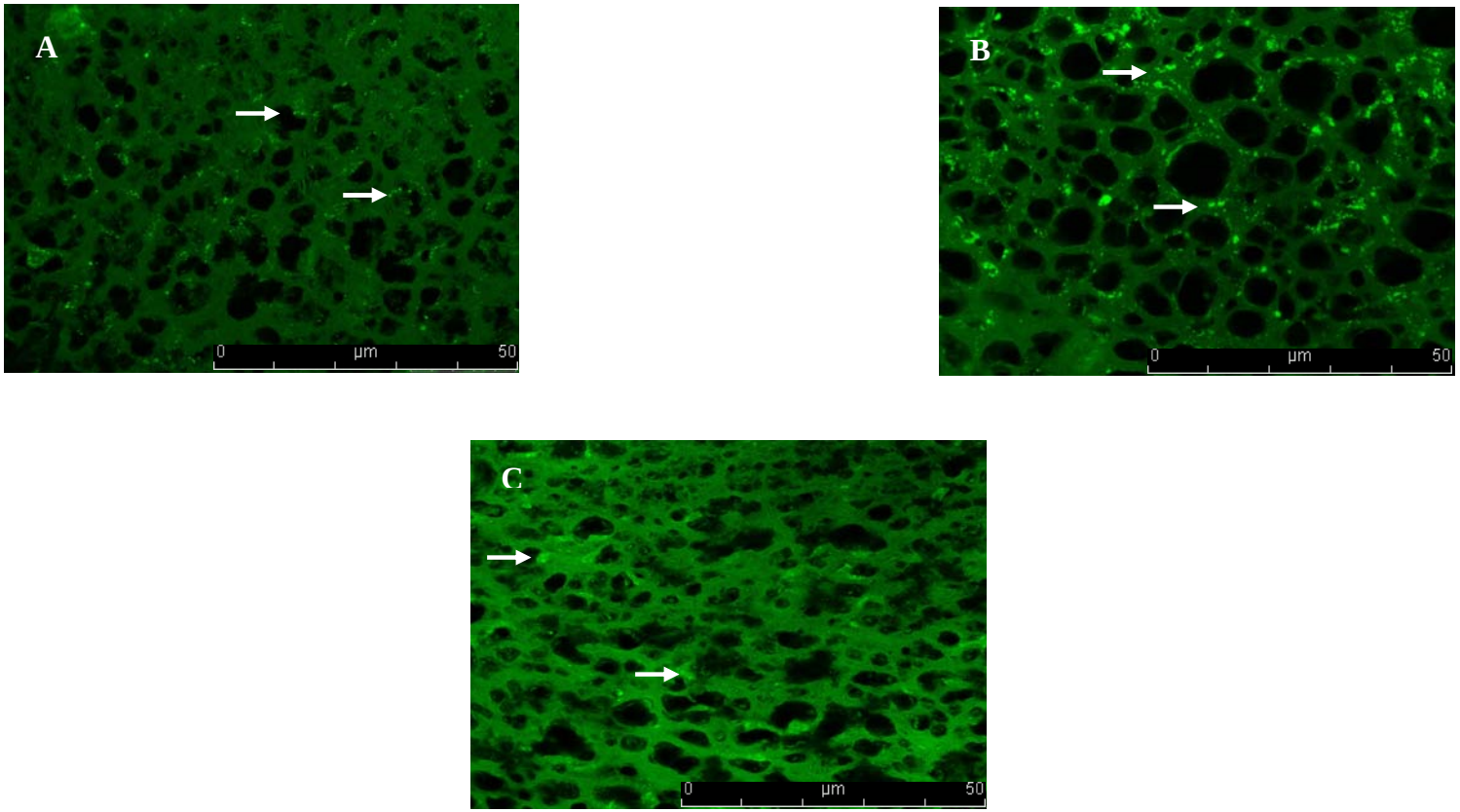
Muscarinic M3 receptor expression in the corpus striatum of control and experimental rats

Condition	Mean pixel value
Control	82 ± 5.4
Diabetic	26 ± 1.8[a]
D + I	65 ± 2.7[b]
D + C	64 ± 2.3[b]
D + V	67 ± 2.6[b]

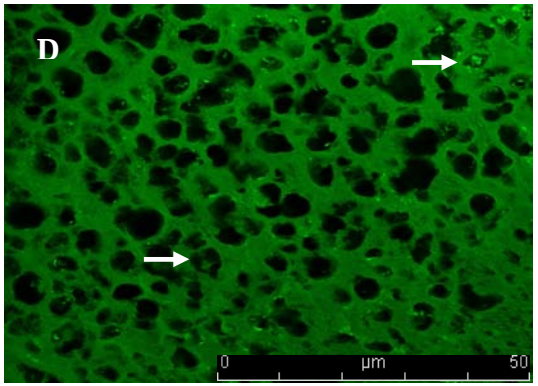
Confocal image of M3 receptor expression in the corpus striatum of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M3 receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-104

α7nicotinic acetylcholine receptor expression in the corpus striatum of control and experimental Rats



→ α7nicotinic acetylcholine receptor



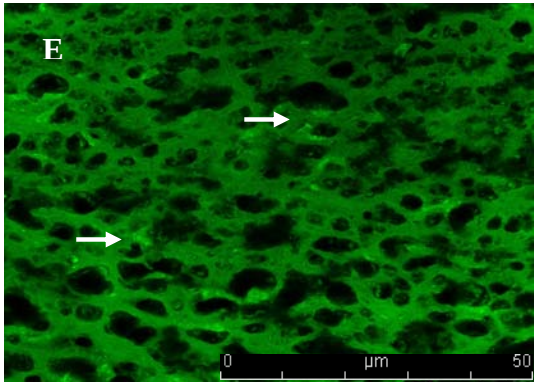


Table-104

α7nicotinic acetylcholine receptor expression in the corpus striatum of control and experimental rats

Condition	Mean pixel value
Control	32 ± 2.7
Diabetic	78 ± 3.8 [a]
D + I	55 ± 3.2 [b]
D + C	37 ± 2.3 [b]
D + V	38 ± 2.3 [b]

Confocal image of α7nicotinic acetylcholine receptors in the corpus striatum ex of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent α7nicotinic acetylcholine receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows α7 nicotinic acetylcholine receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-105

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the hippocampus of control and experimental rats

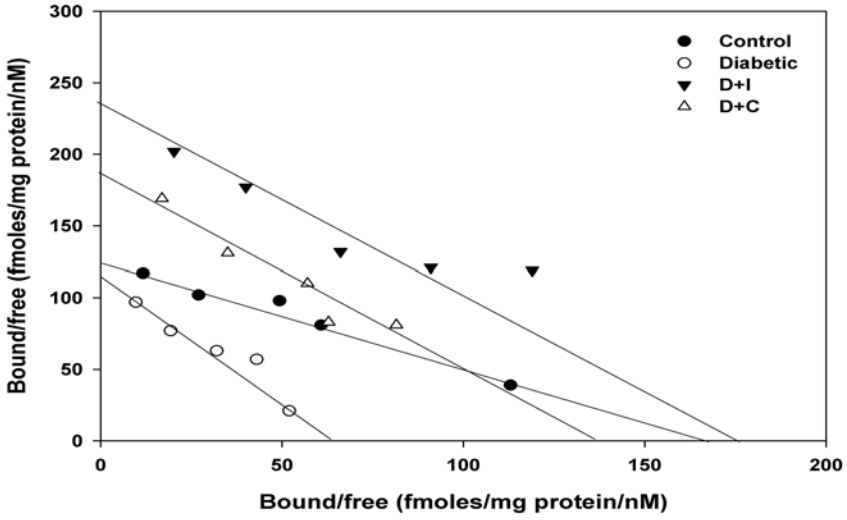


Table-105

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	165 ± 4.3	1.32 ± 0.09
Diabetic	70 ± 3.3 [a]	0.63 ± 0.03 [a]
D + I	182 ± 5.2 [b]	0.75 ± 0.06 [c]
D + C	140 ± 4.4 [b]	0.73 ± 0.04 [c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001, [c] P<0.05 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-106

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the hippocampus of control and experimental rats

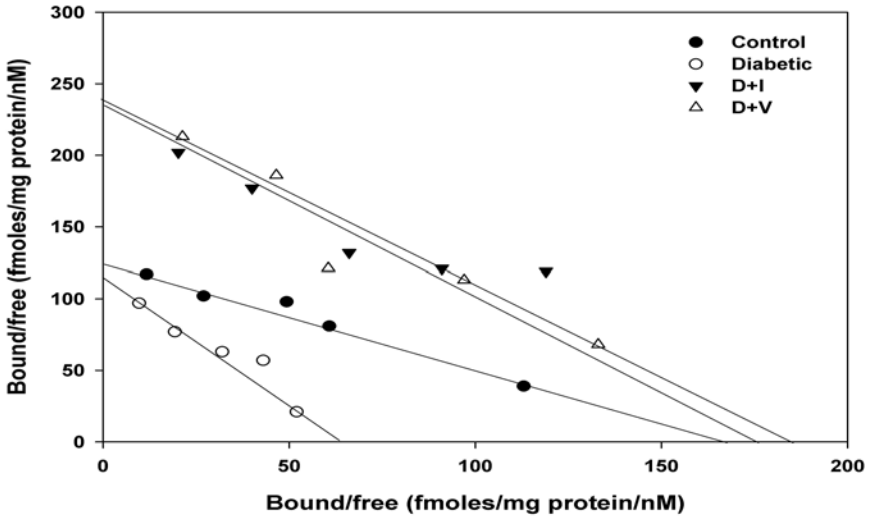


Table-106

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	170 ± 4.3	1.36 ± 0.05
Diabetic	60 ± 3.3 [a]	0.54 ± 0.03 [a]
D + I	175 ± 2.2 [b]	0.72 ± 0.04 [c]
D + V	180 ± 4.4 [b]	0.74 ± 0.05 [c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$, [c] $P<0.05$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-107

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the hippocampus of control and experimental rats

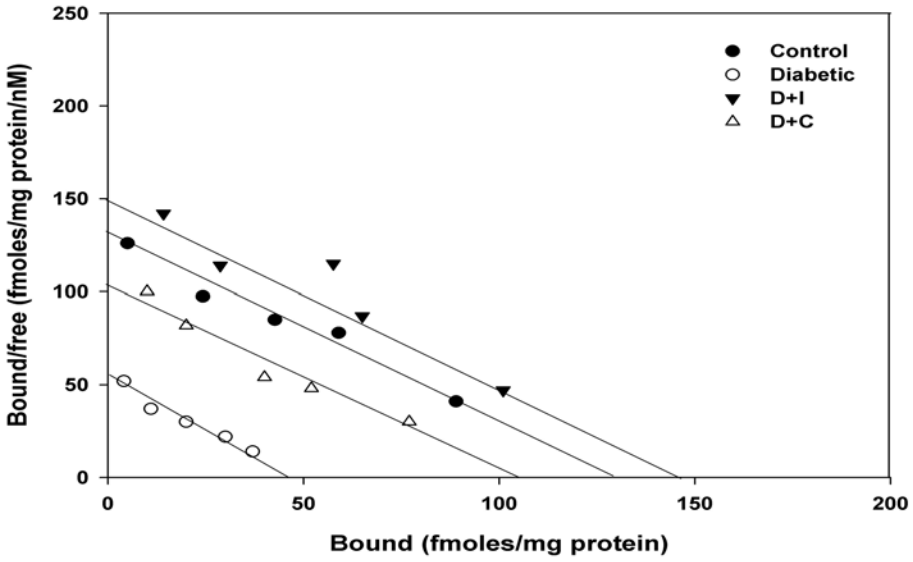


Table-107

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	129 ± 4.3	0.9 ± 0.06
Diabetic	49 ± 3.3[a]	0.9 ± 0.03
D + I	140 ± 2.2[b]	1.0 ± 0.05
D + C	104 ± 3.4[b]	1.0 ± 0.05

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-108

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the hippocampus of control and experimental rats

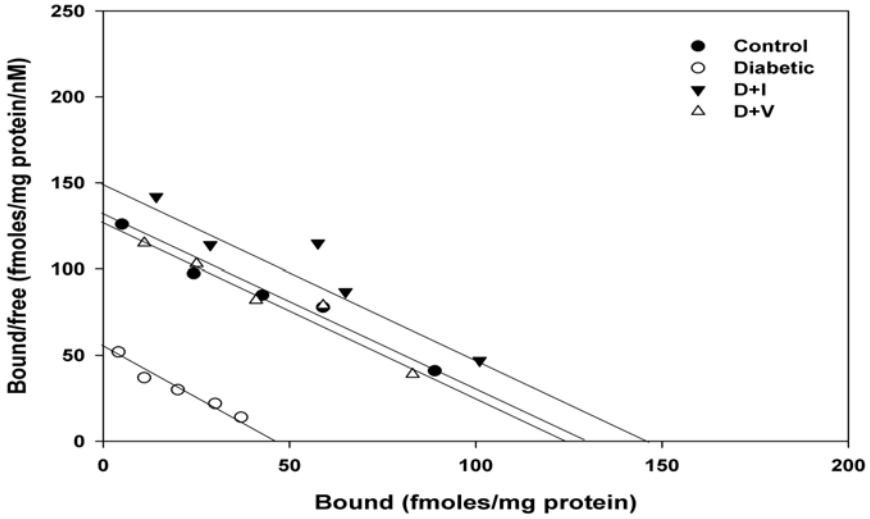


Table-108

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	129 ± 4.3	0.9 ± 0.05
Diabetic	49 ± 3.3 [a]	0.9 ± 0.03
D + I	144 ± 2.2 [b]	1.0 ± 0.04
D + V	125 ± 3.4 [b]	1.0 ± 0.05

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-109

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the hippocampus of control and experimental rats

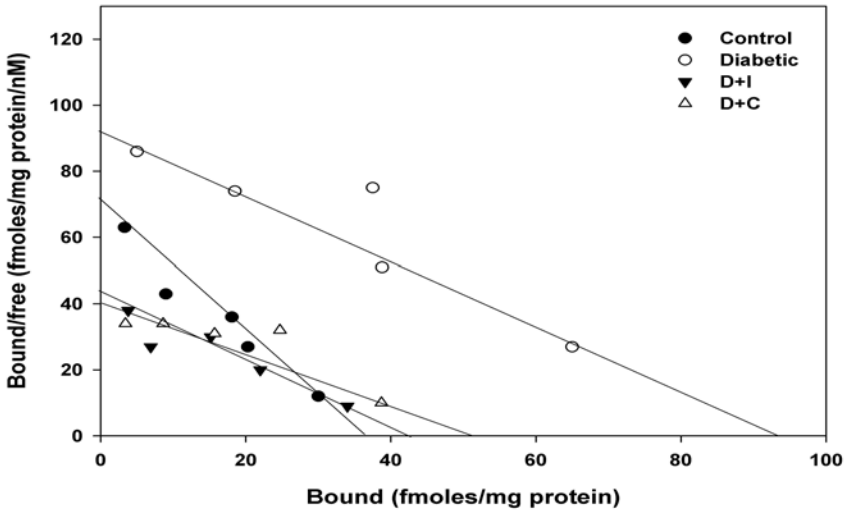


Table-109

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	38 ± 2.3	0.52 ± 0.04
Diabetic	92 ± 3.3 [a]	1.02 ± 0.07 [a]
D + I	42 ± 4.2 [b]	1.00 ± 0.08
D + C	50 ± 2.4 [c]	1.25 ± 0.09

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats

Figure-110

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the hippocampus of control and experimental rats

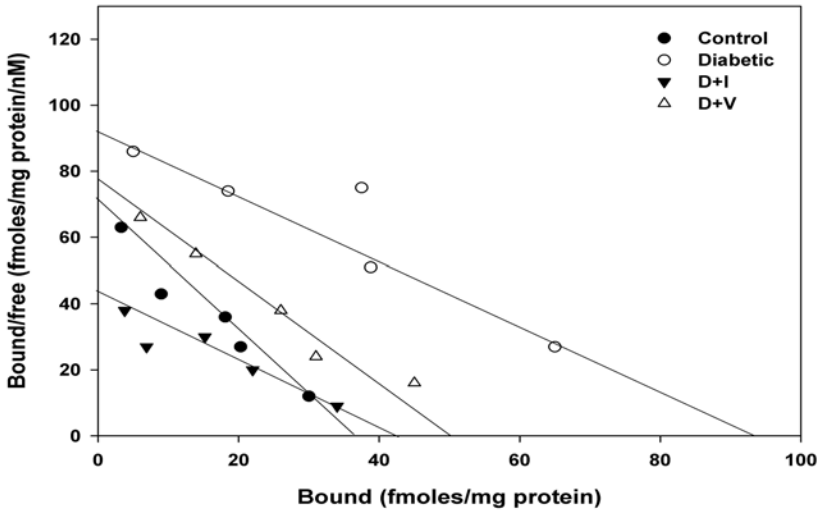


Table-110

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	38 ± 3.3	0.52 ± 0.04
Diabetic	92 ± 2.3 [a]	1.02 ± 0.07 [a]
D + I	42 ± 2.2 [b]	1.00 ± 0.08
D + V	50 ± 1.4 [c]	1.25 ± 0.07

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-111

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine against dopamine in the hippocampus of Control and experimental rats

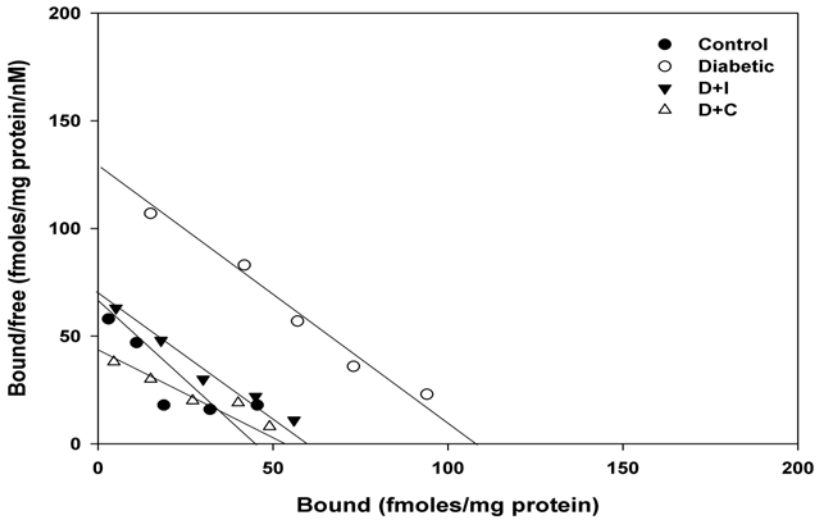


Table-111

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine against dopamine in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	48 ± 4.3	0.68 ± 0.09
Diabetic	110 ± 3.3 [a]	0.84 ± 0.07
D + I	60 ± 5.2 [b]	0.80 ± 0.10
D + C	52 ± 4.4 [b]	1.08 ± 0.08

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-112

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine against dopamine in the hippocampus of control and experimental rats

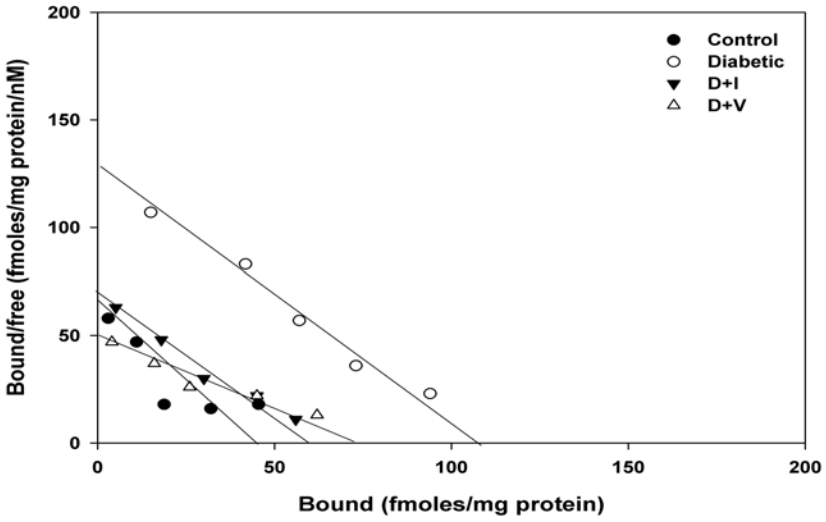


Table-112

Scatchard analysis of total dopamine receptor using [^{3}H] dopamine against dopamine in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	48 ± 2.3	0.68 ± 0.08
Diabetic	110 ± 3.3 [a]	0.84 ± 0.07
D + I	60 ± 2.2 [b]	0.80 ± 0.07
D + V	75 ± 2.4 [b]	1.50 ± 0.08

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-113

Real Time amplification of acetylcholine esterase mRNA from the hippocampus of control and experimental rats

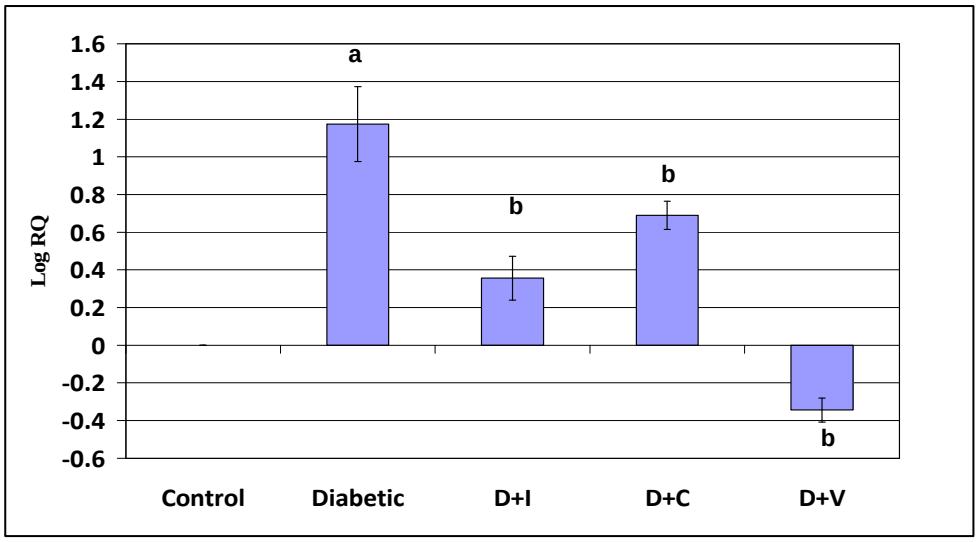


Table-113

Real Time amplification of acetylcholine esterase mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.17 ± 0.19 [a]
D + I	0.35 ± 0.11 [b]
D + C	0.68 ± 0.07 [b]
D + V	-0.34 ± 0.06 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **[a]** P<0.001 when compared to control, **[b]** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-114

Real Time amplification of choline acetyl transferase mRNA from the hippocampus of control and experimental rats

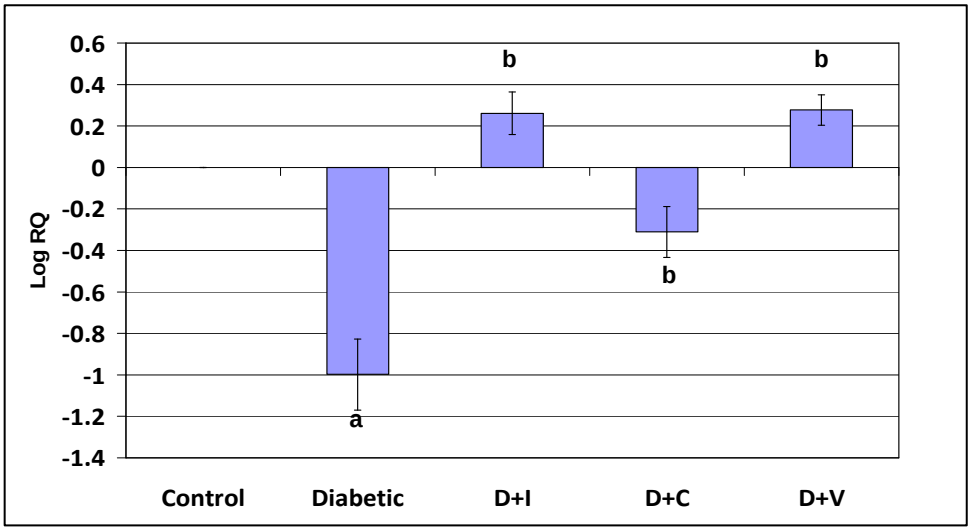


Table-114

Real Time amplification of choline acetyl transferase mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.17 ± 0.19 [a]
D + I	0.35 ± 0.11 [b]
D + C	-0.68 ± 0.07 [b]
D + V	0.34 ± 0.06 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-115

Real Time amplification of muscarinic M1 receptor mRNA from the hippocampus of control and experimental rats

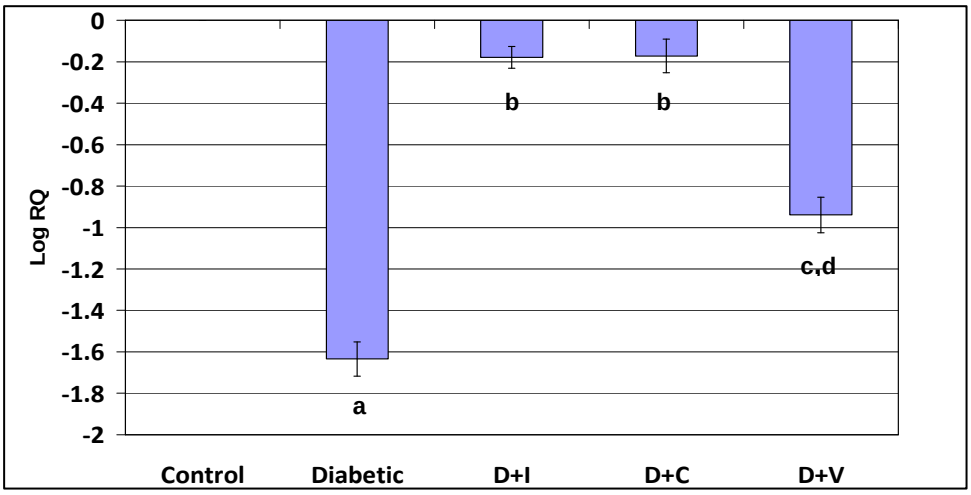


Table-115

Real Time amplification of muscarinic M1 receptor mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.63 ± 0.08 [a]
D + I	-0.17 ± 0.05 [b]
D + C	-0.17 ± 0.08 [b]
D + V	-0.93 ± 0.08 [c,d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **c** P<0.01 when compared to control, **b** P<0.001, **d** P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-116

Real Time amplification of muscarinic M3 receptor mRNA from the hippocampus of control and experimental rats

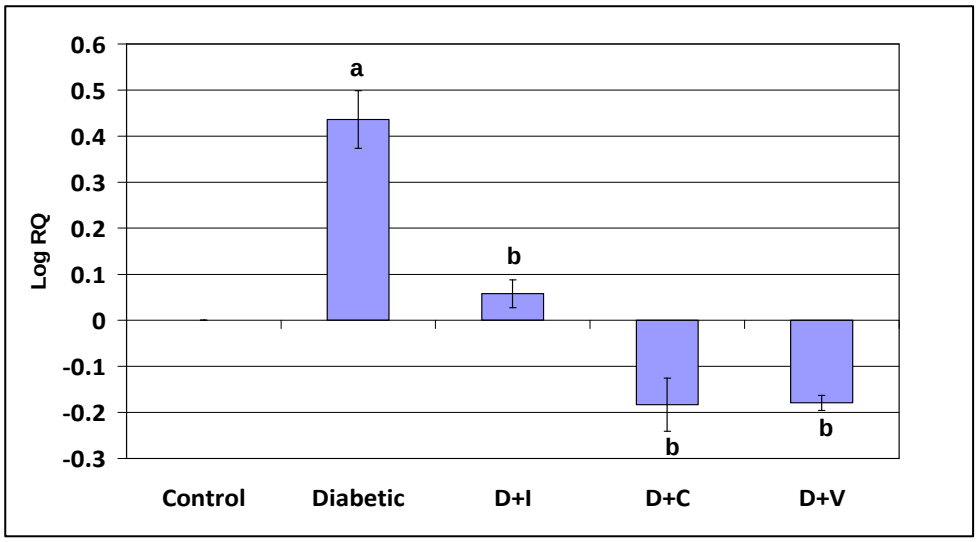


Table-116

Real Time amplification of muscarinic M3 receptor mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.43 ± 0.06 [a]
D + I	0.05 ± 0.03 [b]
D + C	-0.18 ± 0.05 [b]
D + V	-0.17 ± 0.01 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-117

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the hippocampus of control and experimental rats

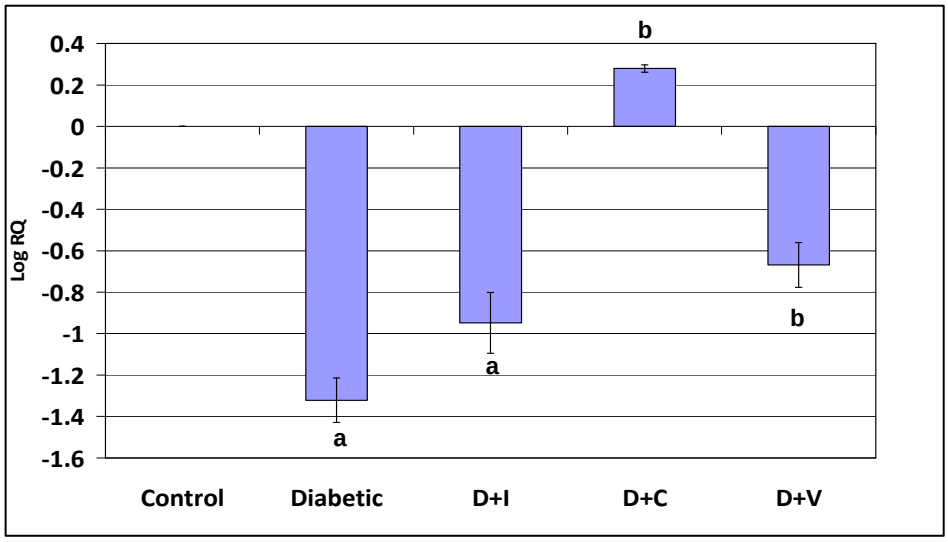


Table-117

Real Time amplification of α7 nicotinic acetylcholine receptor mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.13 ± 0.10 [a]
D + I	-0.94 ± 0.14 [a]
D + C	0.27 ± 0.01 [b]
D + V	-0.66 ± 0.10 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-118

Real Time amplification of dopamine D1 receptor mRNA from the hippocampus of control and experimental rats

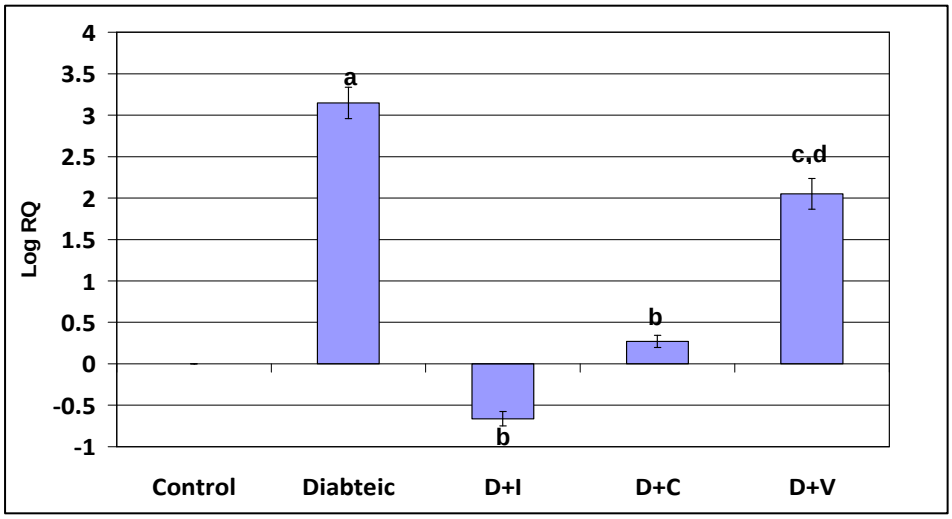


Table-118

Real Time amplification of dopamine D1 receptor mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	3.14 ± 0.17^{a}
D + I	-0.66 ± 0.03^{b}
D + C	0.26 ± 0.07^{b}
D + V	$2.05 \pm 0.13^{c,d}$

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **c** P<0.05 when compared to control, **b** P<0.001, **d** P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-119

Real Time amplification of dopamine D2 receptor mRNA from the hippocampus of control and experimental rats

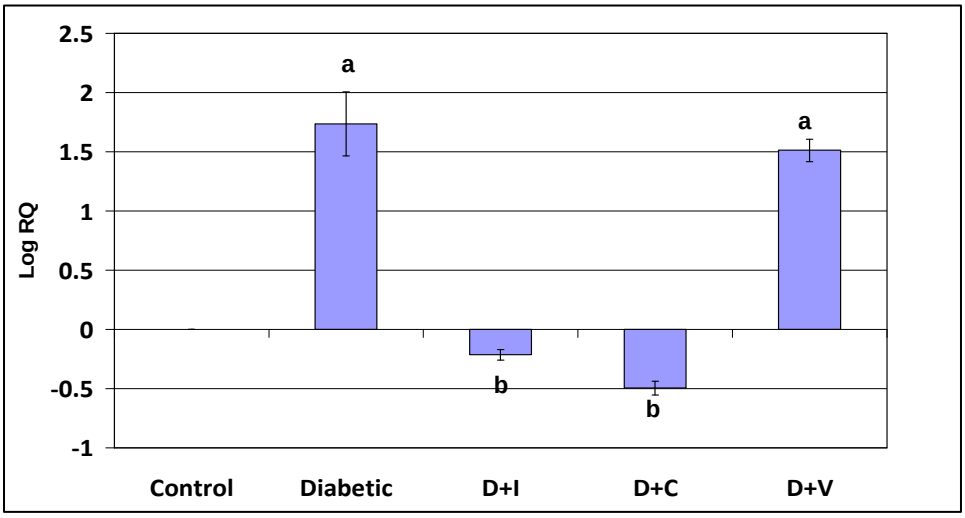


Table-119

Real Time amplification of dopamine D2 receptor mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.73 ± 0.17 [a]
D + I	-0.21 ± 0.04 [b]
D + C	-0.49 ± 0.05 [b]
D + V	1.50 ± 0.09 [a]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-120

Real Time amplification of Vitamin D receptor mRNA from the hippocampus of control and experimental rats

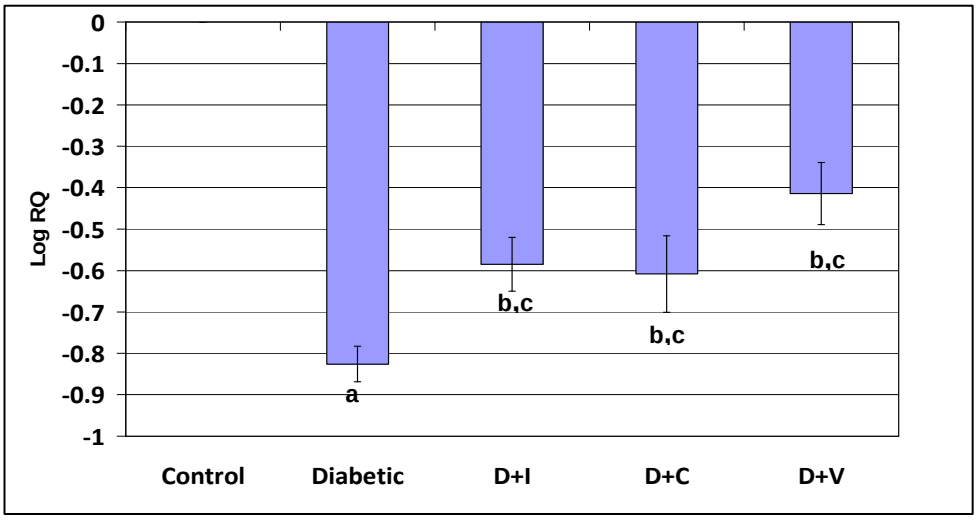


Table-120

Real Time amplification of Vitamin D receptor mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.82 ± 0.04 [a]
D + I	-0.58 ± 0.06 [b,c]
D + C	-0.60 ± 0.09 [b,c]
D + V	-0.41 ± 0.07 [b,c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [b] P<0.01 when compared to control, [c] P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-121

Real Time amplification of insulin receptor mRNA from the hippocampus of control and experimental rats

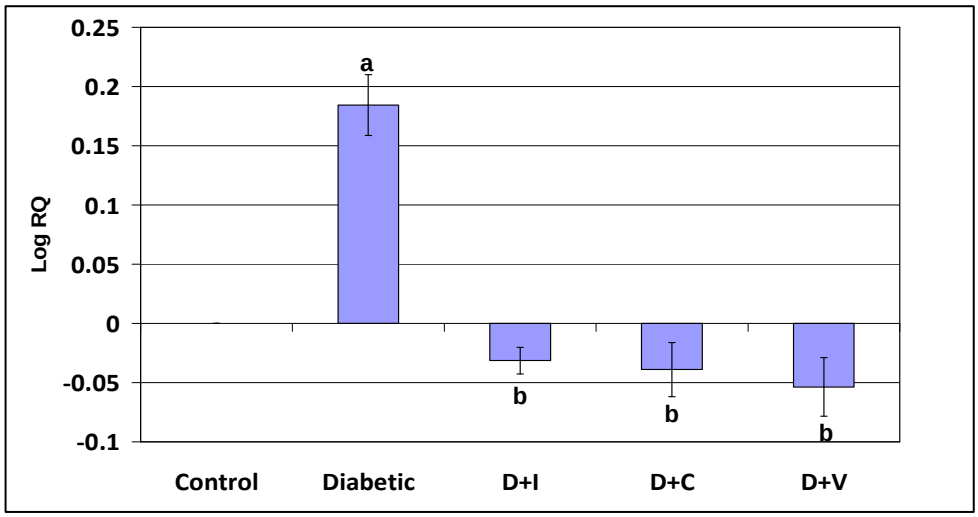


Table-121

Real Time amplification of insulin receptor mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.18 ± 0.02 [a]
D + I	-0.03 ± 0.01 [b]
D + C	-0.04 ± 0.02 [b]
D + V	-0.05 ± 0.02 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-122

Real Time amplification of GLUT3 mRNA from the hippocampus of control and experimental rats

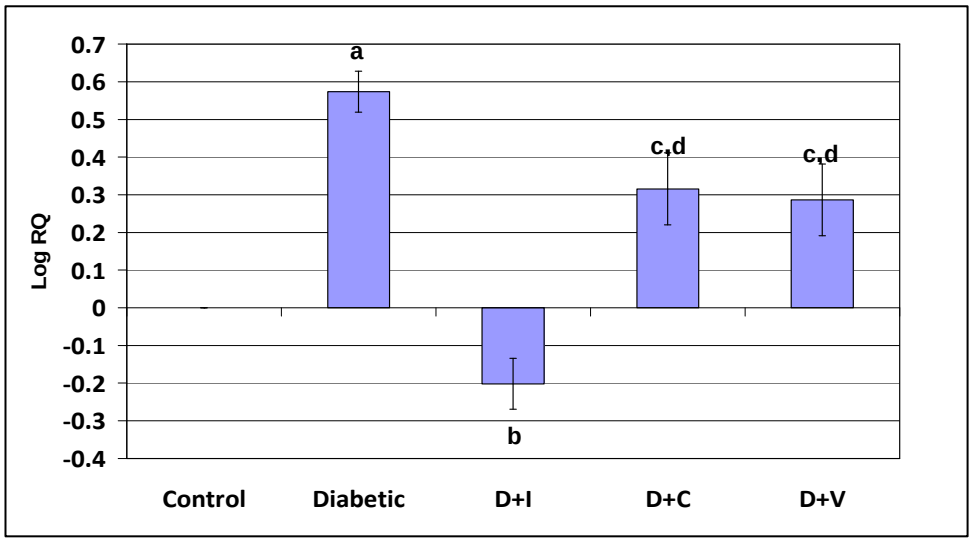


Table-122

Real Time amplification of GLUT3 mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.57 ± 0.05^{a}
D + I	-0.20 ± 0.06^{b}
D + C	$0.31 \pm 0.09^{c,d}$
D + V	$0.28 \pm 0.09^{c,d}$

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **c** P<0.01 when compared to control, **b** P<0.001, **d** P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-123

Real Time amplification of phospholipase C mRNA from the hippocampus of control and experimental rats

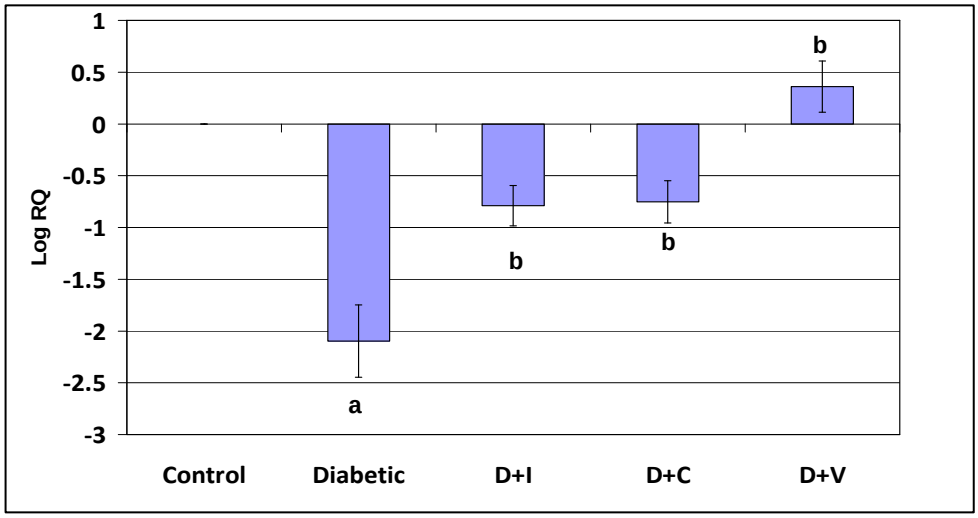


Table-123

Real Time amplification of phospholipase C mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-2.09 ± 0.34 [a]
D + I	-0.79 ± 0.19 [b]
D + C	-0.75 ± 0.20 [b]
D + V	0.36 ± 0.24 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-124

Real Time amplification of CREB mRNA from the hippocampus of control and experimental rats

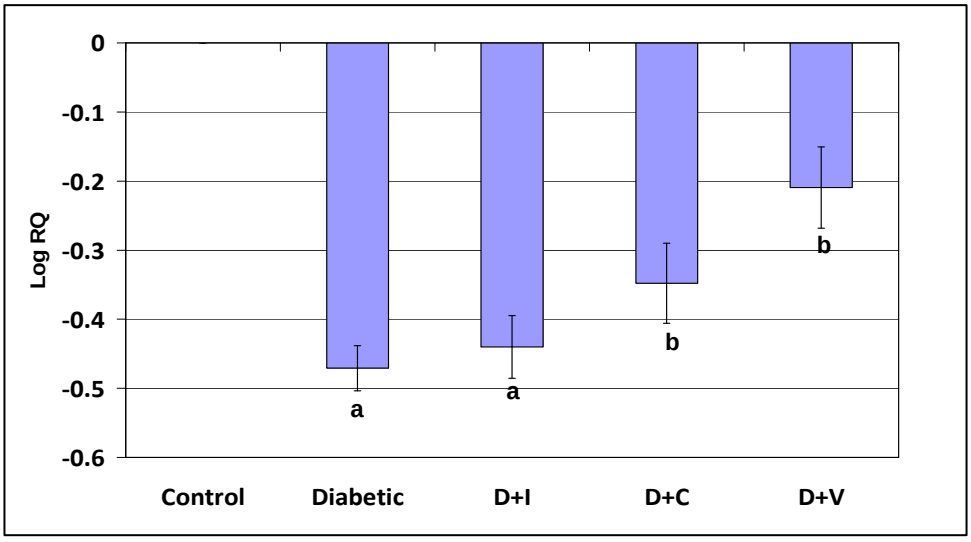


Table-124

Real Time amplification of CREB mRNA from the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.47 ± 0.03 [a]
D + I	-0.44 ± 0.04 [a]
D + C	-0.34 ± 0.05 [b]
D + V	-0.20 ± 0.05 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.01$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-125

Real Time amplification of superoxide dismutase mRNA from the hippocampus of control and experimental rats

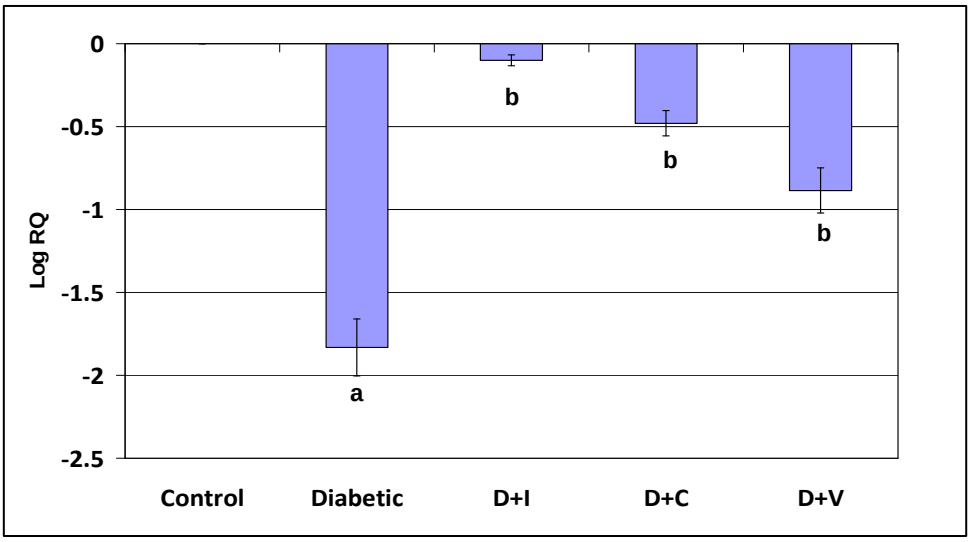


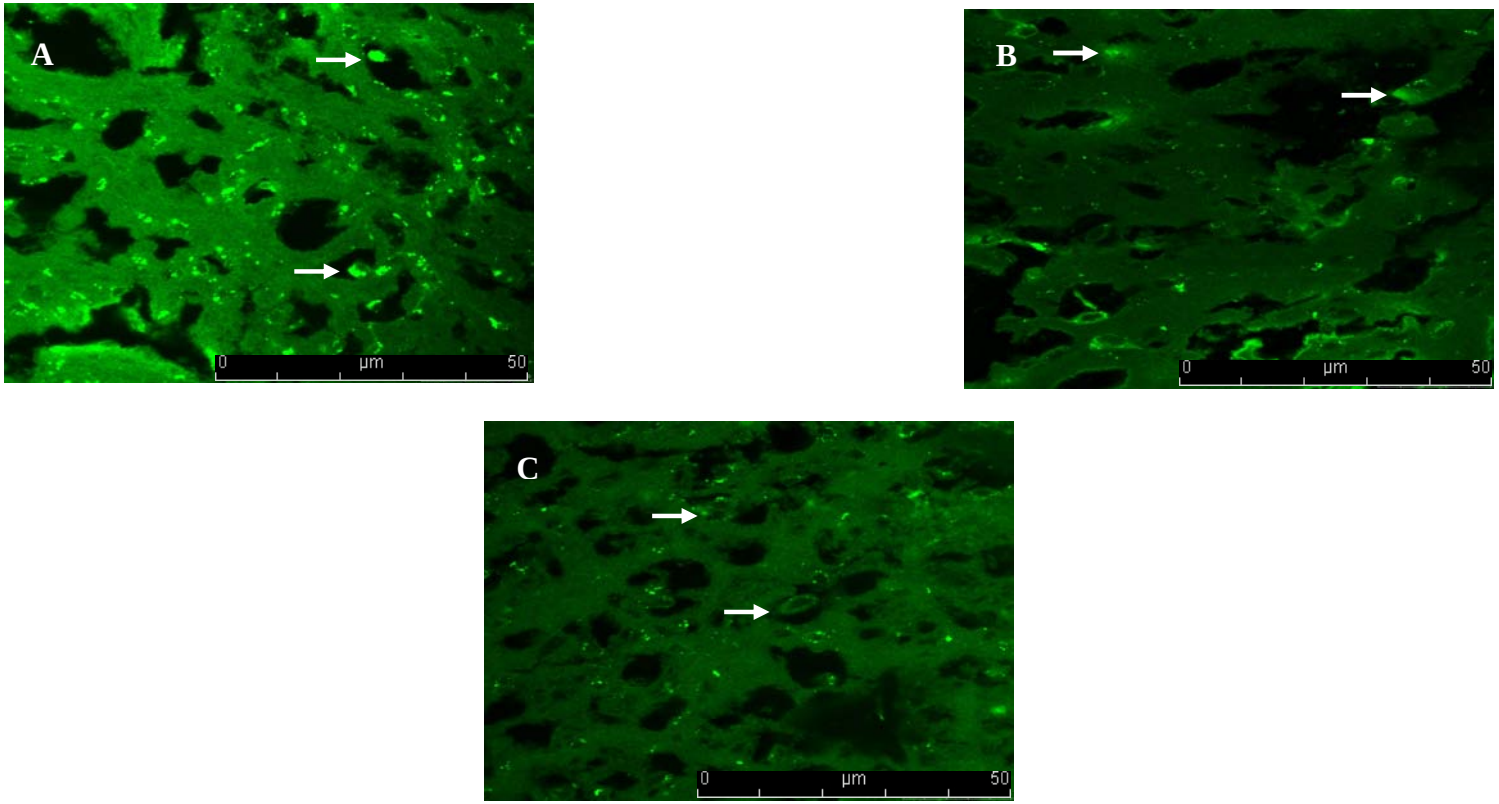
Table-125

Real Time amplification of superoxide dismutase mRNA from the hippocampus of control and experimental rats

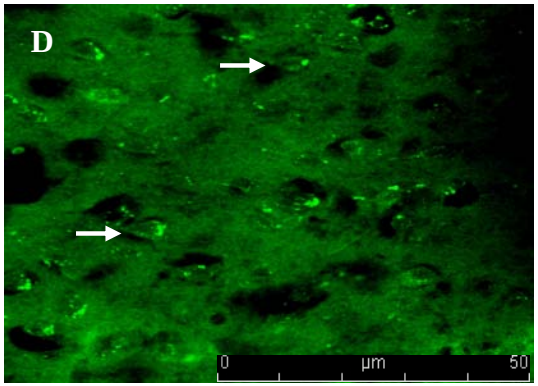
Experimental groups	Log RQ
Control	0
Diabetic	-1.83 ± 0.17 [a]
D + I	-0.09 ± 0.03 [b]
D + C	-0.47 ± 0.07 [b]
D + V	-0.88 ± 0.13 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-126
Muscarinic M1 receptor expression in the hippocampus of control and experimental rats



→ **Muscarinic M1 receptor**



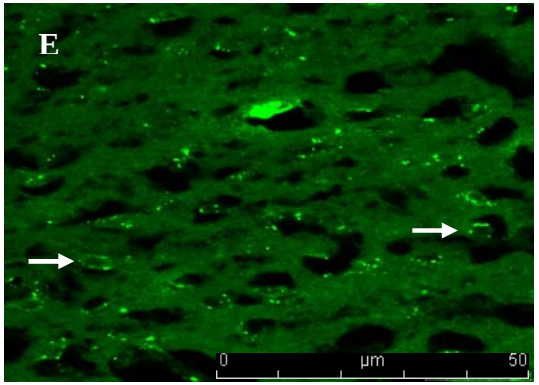
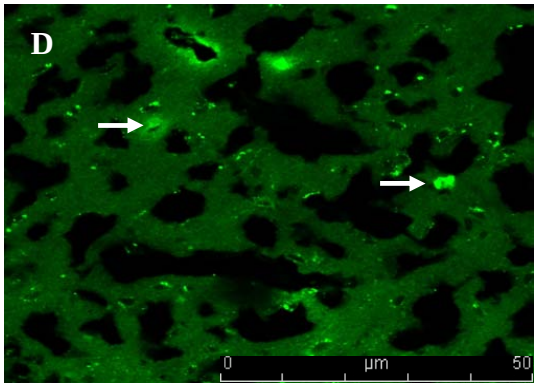


Table-126

Muscarinic M1 receptor expression in the hippocampus of control and experimental rats

Condition	Mean pixel value
Control	77 ± 5.3
Diabetic	28 ± 2.4 [a]
D + I	65 ± 4.7 [b]
D + C	69 ± 4.1 [b]
D + V	68 ± 3.9 [b]

Confocal image of muscarinic M1 receptor expression in the hippocampus of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M1 receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.



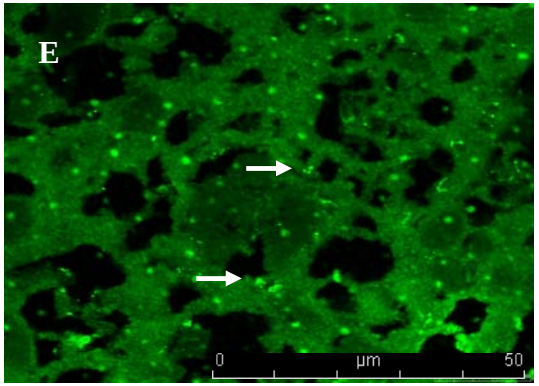
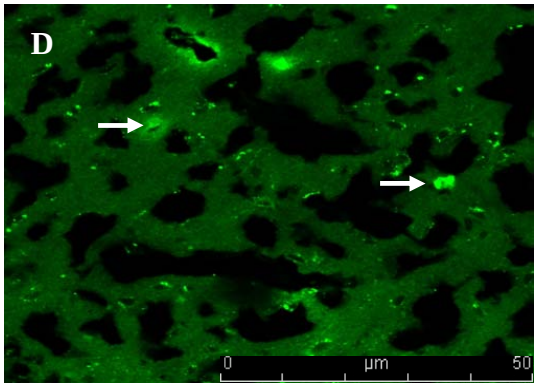


Table-127

Muscarinic M3 receptor expression in the hippocampus of control and experimental rats

Condition	Mean pixel value
Control	32 ± 1.7
Diabetic	77 ± 5.8 [a]
D + I	30 ± 1.9 [b]
D + C	36 ± 2.2 [b]
D + V	43 ± 3.2 [b]

Confocal image of muscarinic M3 Receptor Expression in the hippocampus of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M3 receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.



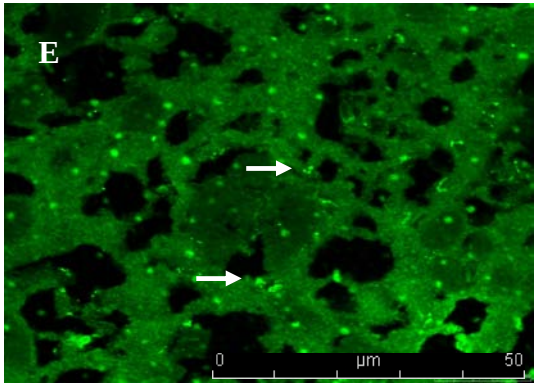


Table-127

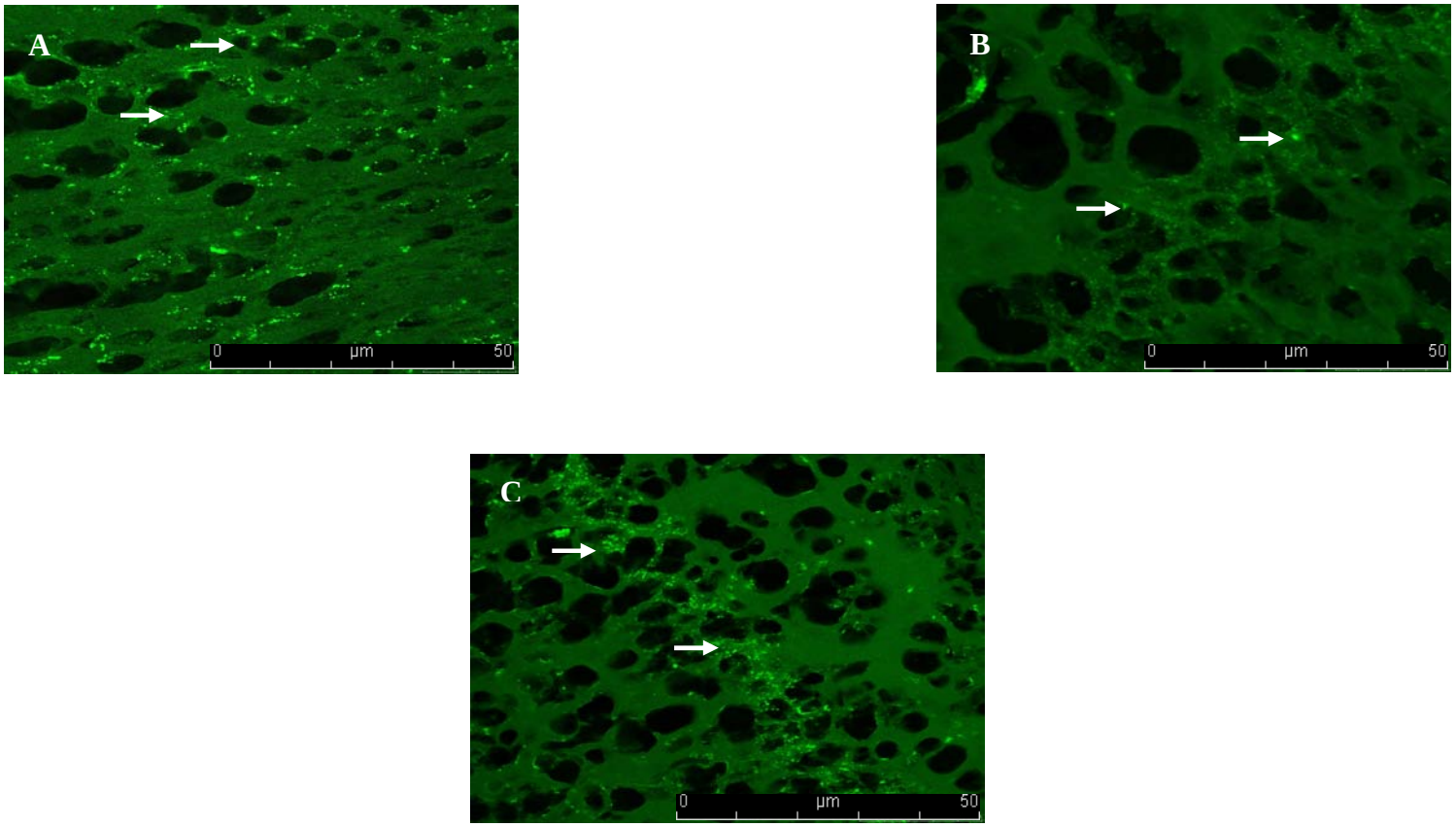
Muscarinic M3 receptor expression in the hippocampus of control and experimental rats

Condition	Mean pixel value
Control	32 ± 1.7
Diabetic	77 ± 5.8 [a]
D + I	30 ± 1.9 [b]
D + C	36 ± 2.2 [b]
D + V	43 ± 3.2 [b]

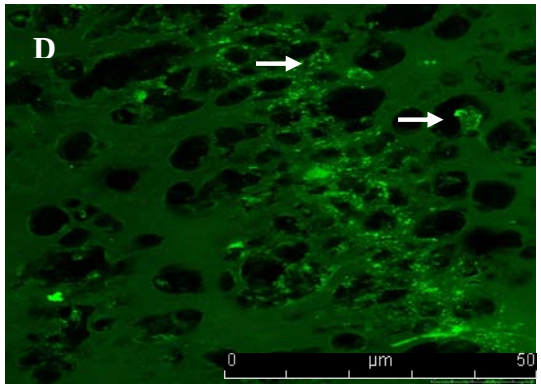
Confocal image of muscarinic M3 Receptor Expression in the hippocampus of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M3 receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-128

α7nicotinic acetylcholine receptor expression in the hippocampus of control and experimental rats



→ α7nicotinic acetylcholine receptor



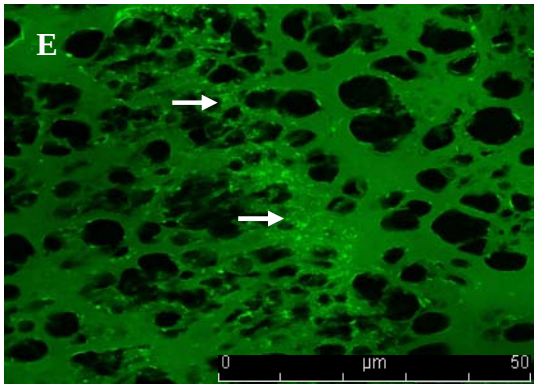


Table-128

α7nicotinic acetylcholine receptor expression in the hippocampus of control and experimental rats

Condition	Mean pixel value
Control	73 ± 2.7
Diabetic	28 ± 1.8 [a]
D + I	35 ± 192 [b]
D + C	68 ± 2.7 [b]
D + V	57 ± 2.9 [b]

Confocal image of α7 nicotinic acetylcholine receptor expression in the hippocampus of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent α7nicotinic acetylcholine receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows α7 nicotinic acetylcholine receptors. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group .D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 50 µm.

Figure-129

Real Time amplification of acetylcholine esterase mRNA from the hypothalamus of control and experimental rats

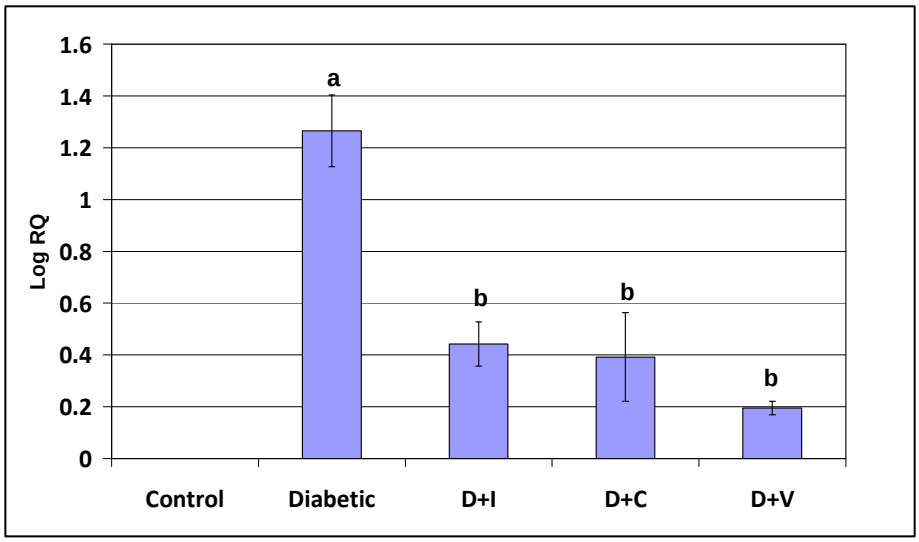


Table-129

Real Time amplification of acetylcholine esterase mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.26 ± 0.13 [a]
D + I	0.44 ± 0.08 [b]
D + C	0.39 ± 0.17 [b]
D + V	0.19 ± 0.02 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-130

Real Time amplification of choline acetyl transferase mRNA from the hypothalamus of control and experimental rats

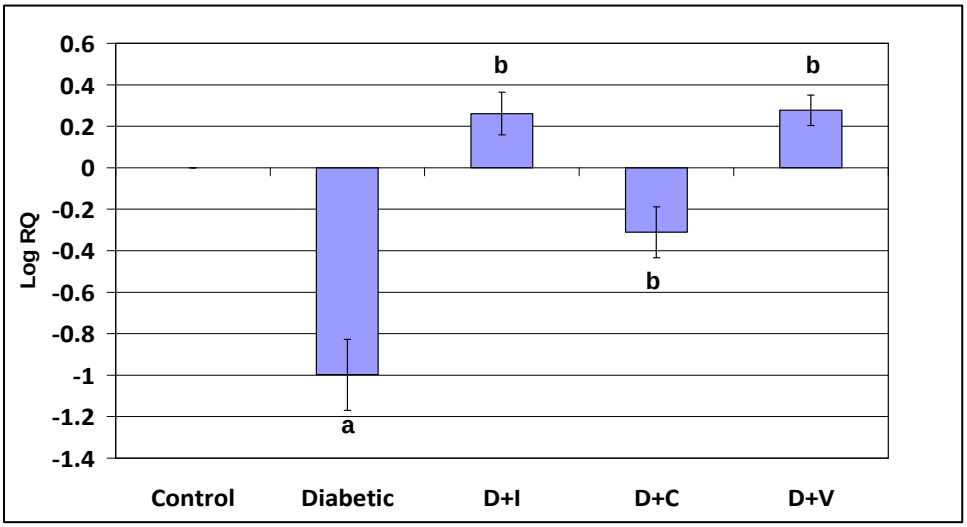


Table-130

Real Time amplification of choline acetyl transferase receptor mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.99 ± 0.17 [a]
D + I	0.26 ± 0.10 [b]
D + C	-0.33 ± 0.12 [b]
D + V	0.27 ± 0.07 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-131

Real Time amplification of muscarinic M1 receptor mRNA from the hypothalamus of control and experimental rats

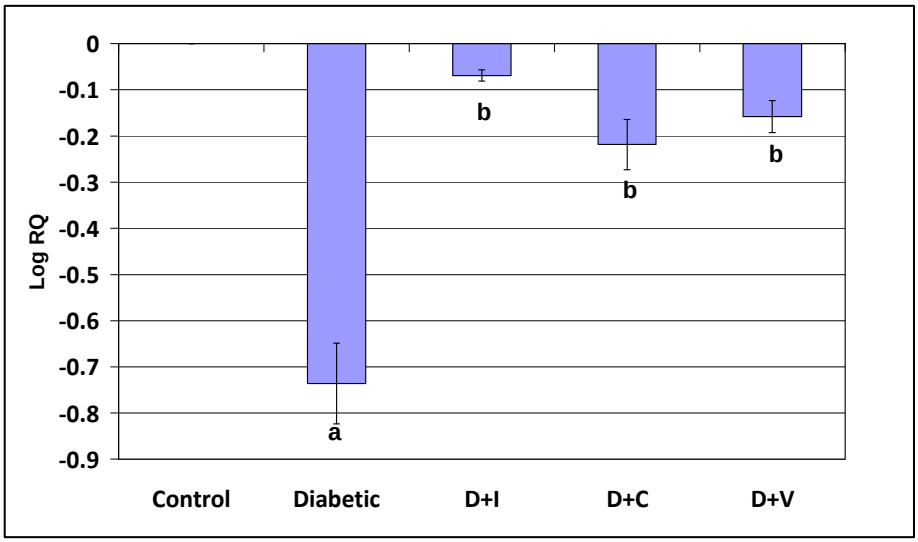


Table-131

Real Time amplification of muscarinic M1 receptor mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.73 ± 0.08 [a]
D + I	-0.06 ± 0.01 [b]
D + C	-0.21 ± 0.05 [b]
D + V	-0.15 ± 0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-132

Real Time amplification of muscarinic M3 receptor mRNA from the hypothalamus of control and experimental rats

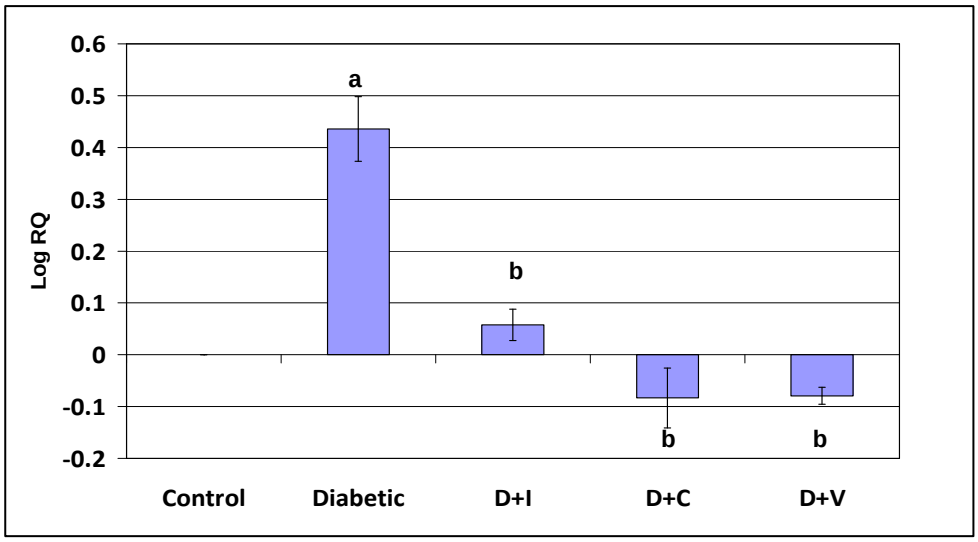


Table-132

Real Time amplification of muscarinic M3 receptor mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.43 ± 0.06^{a}
D + I	0.05 ± 0.03^{b}
D + C	-0.08 ± 0.05^{b}
D + V	-0.07 ± 0.01^{b}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** $P<0.001$ when compared to control, **b** $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-133

Real Time amplification of dopamine D1 receptor mRNA from the hypothalamus of control and experimental rats

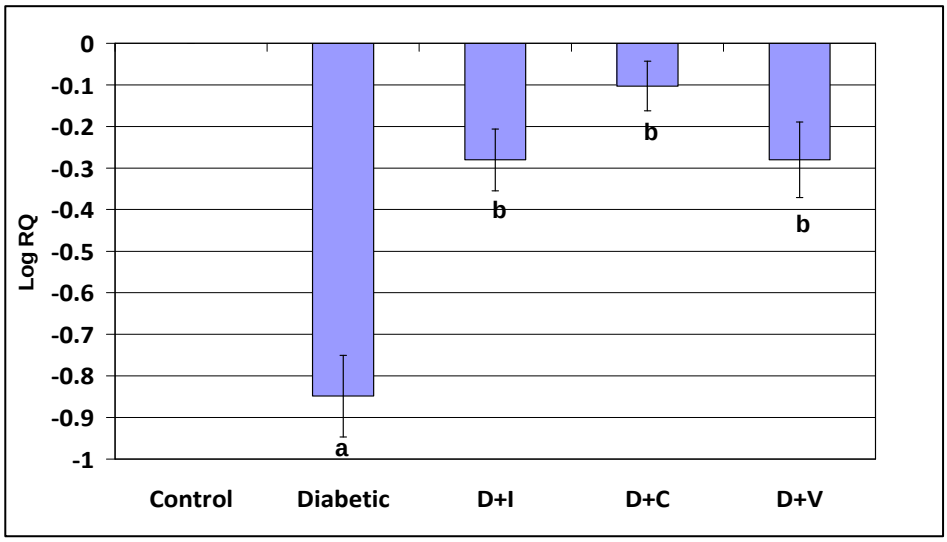


Table-133

Real Time amplification of dopamine D1 receptor mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.84 ± 0.09 [a]
D + I	-0.28 ± 0.07 [b]
D + C	-0.10 ± 0.05 [b]
D + V	-0.28 ± 0.09 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-134

Real Time amplification of dopamine D2 receptor mRNA from the hypothalamus of control and experimental rats

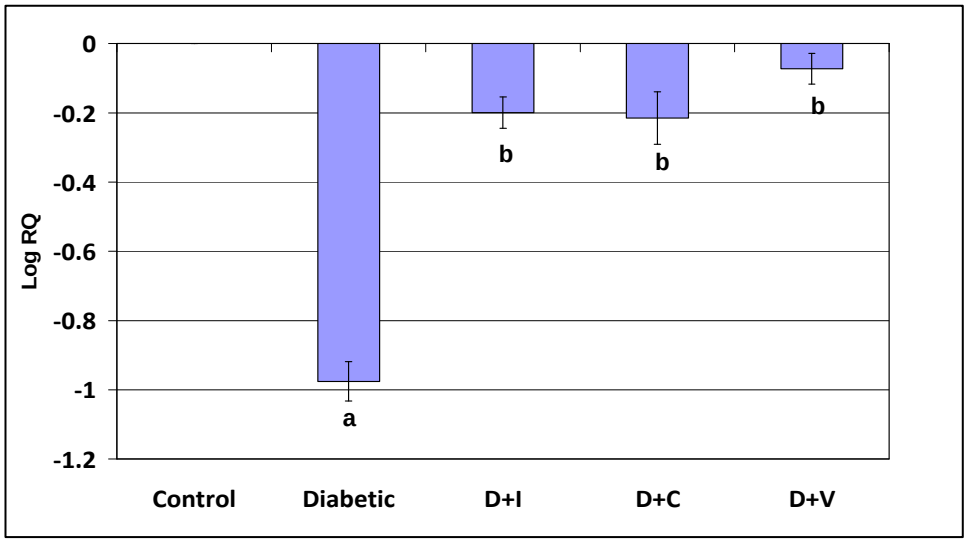


Table-134

Real Time amplification of dopamine D2 receptor mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.97 ± 0.05 [a]
D + I	-0.19 ± 0.04 [b]
D + C	-0.21 ± 0.07 [b]
D + V	-0.07 ± 0.04 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001 when compared to control, **b** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-135

Real Time amplification of Vitamin D receptor mRNA from the hypothalamus of control and experimental rats

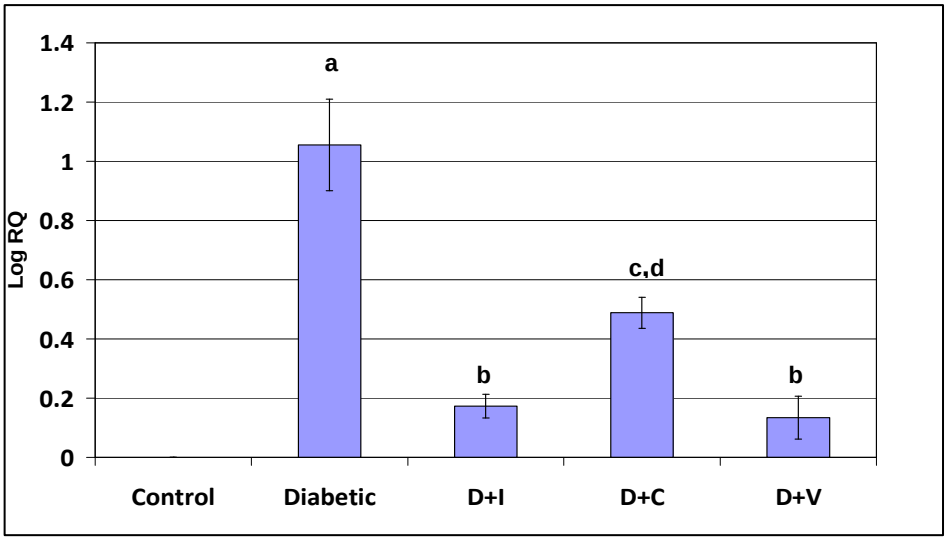


Table-135

Real Time amplification of Vitamin D receptor mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.05 ± 0.15^{a}
D + I	0.17 ± 0.04^{b}
D + C	$0.48 \pm 0.05^{c,d}$
D + V	0.13 ± 0.07^{b}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **c** P<0.01 when compared to control, **b** P<0.001, **d** P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-136

Real Time amplification of insulin receptor mRNA from the hypothalamus of control and experimental rats

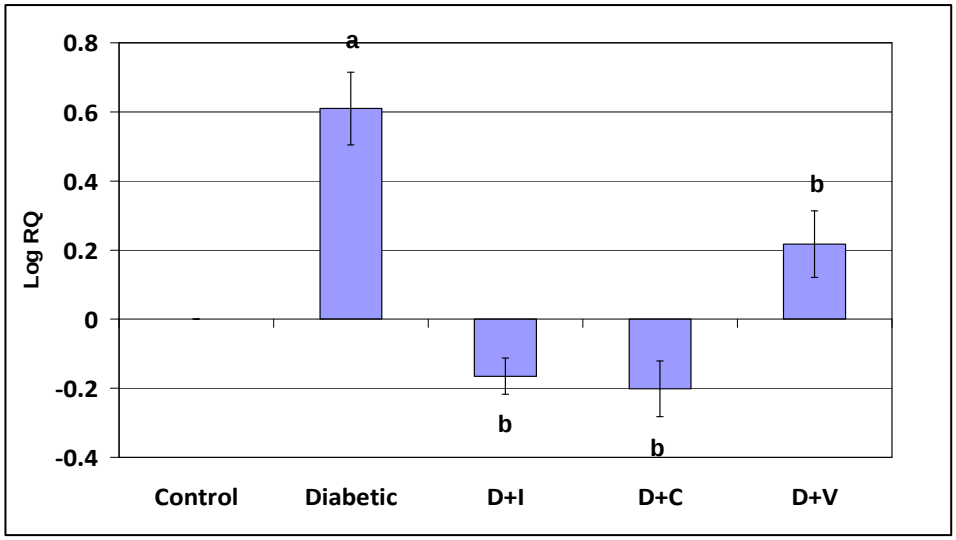


Table-136

Real Time amplification of insulin receptor mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.60 ± 0.10 [a]
D + I	-0.16 ± 0.05 [b]
D + C	-0.20 ± 0.08 [b]
D + V	0.21 ± 0.09 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-137

Real Time amplification of GLUT3 receptor mRNA from the hypothalamus of control and experimental rats

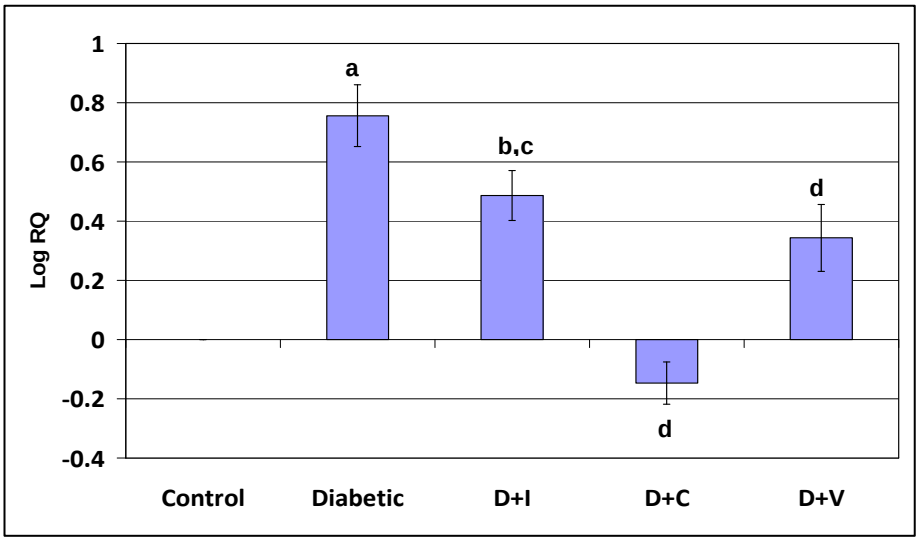


Table-137

Real Time amplification of GLUT3 receptor mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.75 ± 0.10^{a}
D + I	$0.48 \pm 0.08^{b,c}$
D + C	-0.14 ± 0.07^{d}
D + V	0.34 ± 0.11^{d}

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.05 when compared to control group, **c** P<0.01, **d** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-138

Real Time amplification of phospholipase C mRNA from the hypothalamus of control and experimental rats

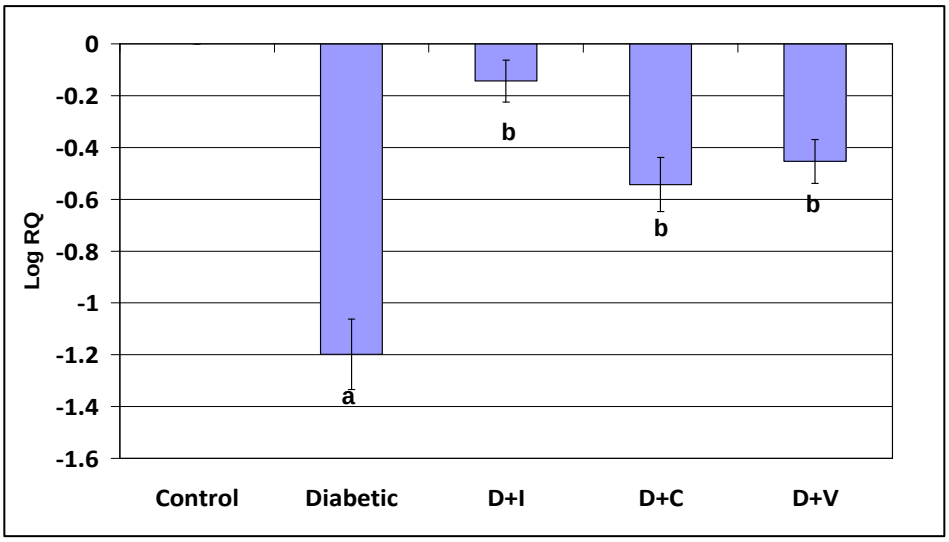


Table-138

Real Time amplification of phospholipase C mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.19 ± 0.13 [a]
D + I	-0.14 ± 0.08 [b]
D + C	-0.54 ± 0.10 [b]
D + V	-0.45 ± 0.08 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-139

Real Time amplification of CREB mRNA from the hypothalamus of control and experimental rats

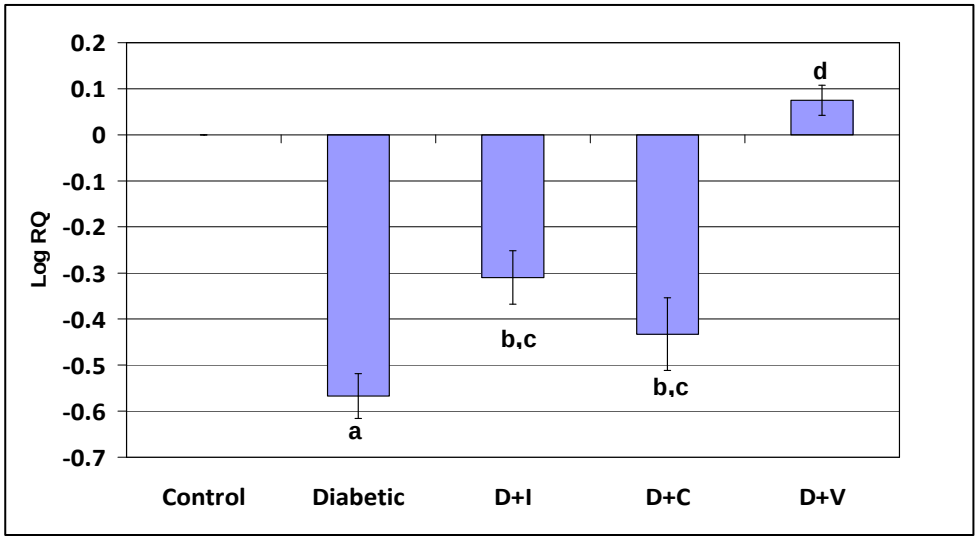


Table-139

Real Time amplification of CREB mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.56 ± 0.04 [a]
D + I	-0.30 ± 0.05 [b,c]
D + C	-0.43 ± 0.07 [b,c]
D + V	0.07 ± 0.03 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control group, **c** P<0.01, **d** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-140

Real Time amplification of superoxide dismutase mRNA from the hypothalamus of control and experimental rats

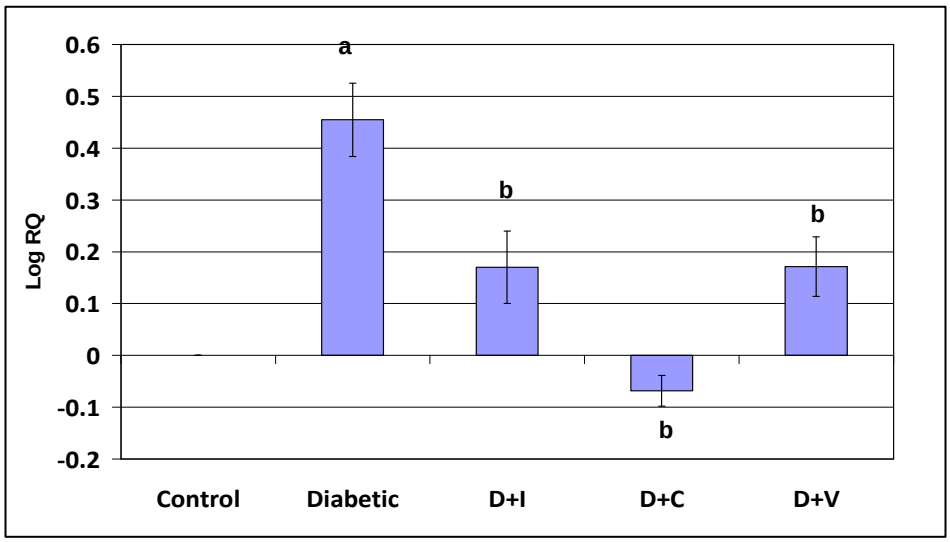


Table-140

Real Time amplification of superoxide dismutase mRNA from the hypothalamus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.45 ± 0.07 [a]
D + I	0.17 ± 0.06 [b]
D + C	-0.06 ± 0.02 [b]
D + V	-0.17 ± 0.05 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001 when compared to control, **b** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-141

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the pancreas of control and experimental rats

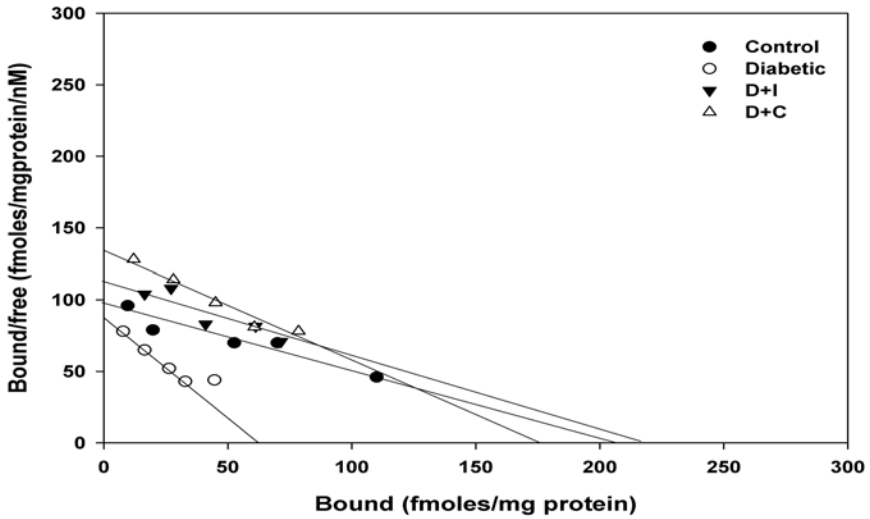


Table-141

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	205 ± 5.7	2.05 ± 0.11
Diabetic	60 ± 3.1 [a]	0.60 ± 0.07 [a]
D + I	213 ± 6.2 [b]	1.82 ± 0.09 [c]
D + C	175 ± 4.7 [b]	1.26 ± 0.12 [c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-142

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the pancreas of control and experimental rats

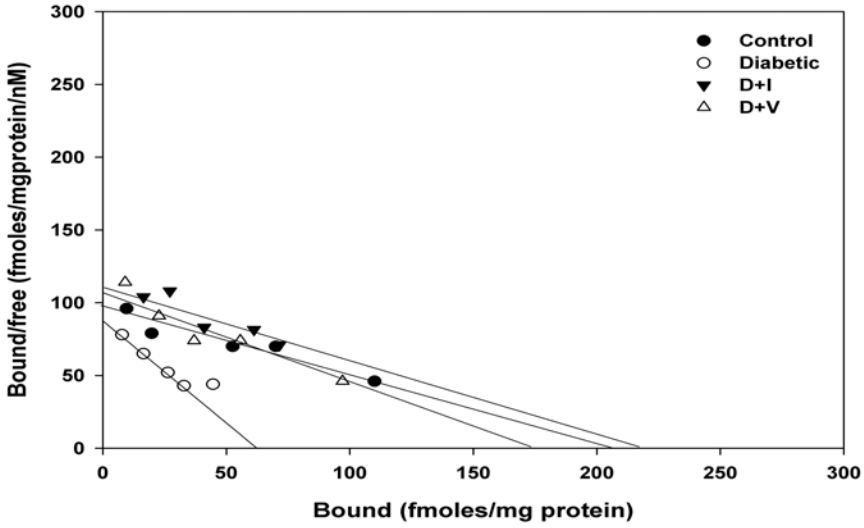


Table-142

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	205 ± 5.7	2.05 ± 0.11
Diabetic	60 ± 3.1 [a]	0.60 ± 0.07 [a]
D + I	213 ± 6.2 [b]	1.82 ± 0.09 [c]
D + V	172 ± 5.1 [b]	1.63 ± 0.12 [c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist 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. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats..

Figure-143

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the pancreas of control and experimental rats

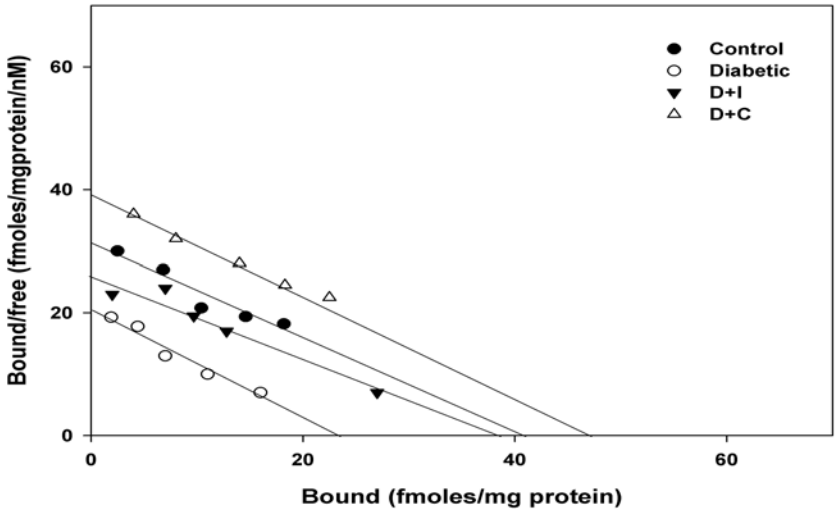


Table-143

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	41 ± 2.3	1.28 ± 0.08
Diabetic	22 ± 1.7 [a]	1.07 ± 0.07
D + I	38 ± 2.2 [b]	1.58 ± 0.09
D + C	46 ± 1.4 [b]	1.16 ± 0.09

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-144

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the pancreas of control and experimental rats

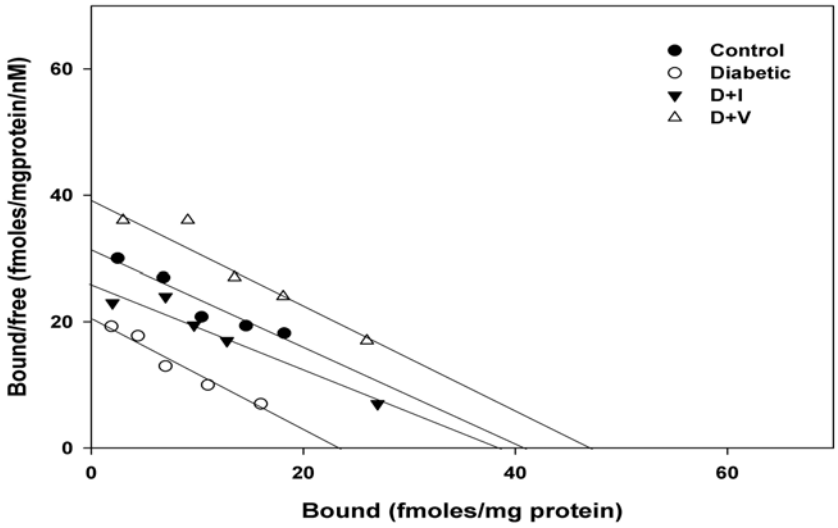


Table-144

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	41 ± 3.3	1.28 ± 0.08
Diabetic	22 ± 2.3 [a]	1.07 ± 0.07
D + I	38 ± 2.2 [b]	1.58 ± 0.09
D + V	46 ± 1.9 [b]	1.16 ± 0.10

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-145

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the pancreas of control and experimental rats

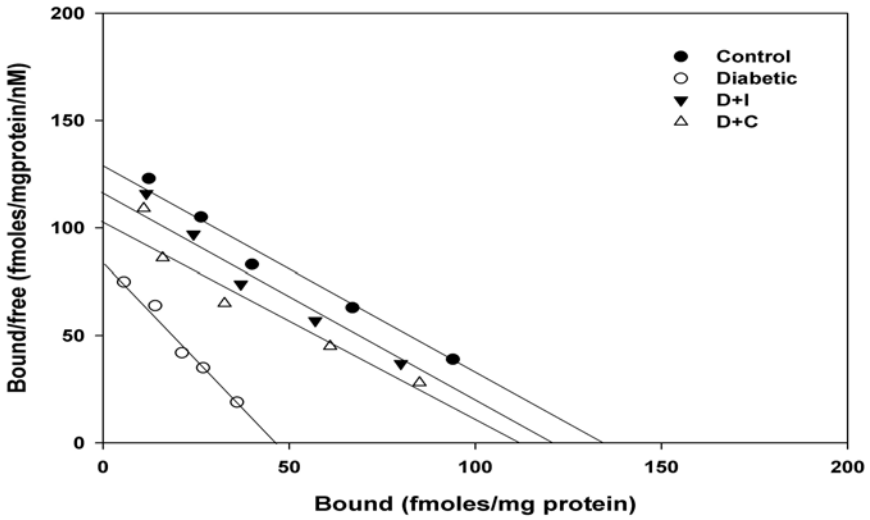


Table-145

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	127 ± 4.5	1.00 ± 0.08
Diabetic	49 ± 3.1 [a]	0.6 ± 0.15
D + I	124 ± 5.4 [b]	1.07 ± 0.09
D + C	110 ± 3.8 [b]	1.0 ± 0.08

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats and D + C- Curcumin treated diabetic rats.

Figure-146

Scatchard analysis of muscarinic M3 receptor using [^{3}H] DAMP against 4-DAMP mustard in the pancreas of control and experimental rats

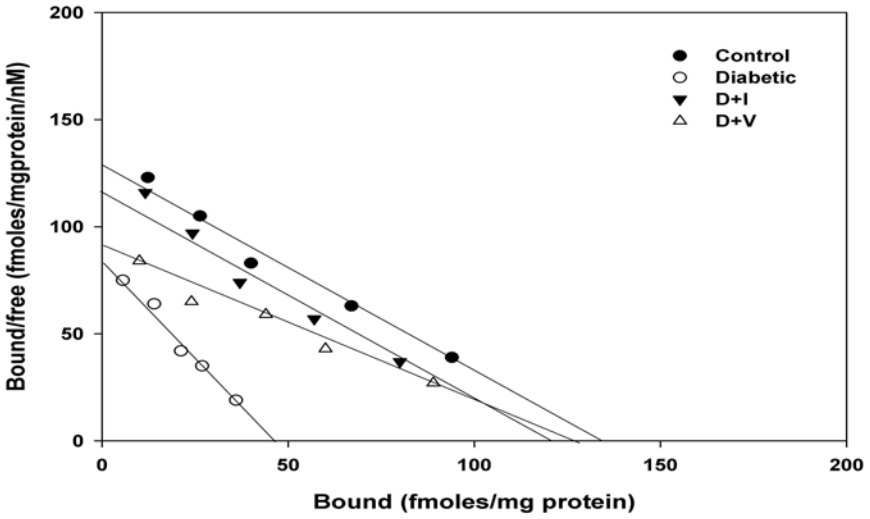


Table-146

Scatchard analysis of muscarinic M3 receptor using [^{3}H] DAMP against 4-DAMP mustard in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	127 ± 4.5	1.00 ± 0.08
Diabetic	49 ± 3.1 [a]	0.6 ± 0.15
D + I	124 ± 5.4 [b]	1.07 ± 0.09
D + V	125 ± 3.2 [b]	1.3 ± 0.10

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **[a]** P<0.001 when compared to control, **[b]** P<0.001 when compared to diabetic group. D + I- Insulin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-147

Real Time amplification of acetylcholine esterase mRNA from the pancreas of control and experimental rats

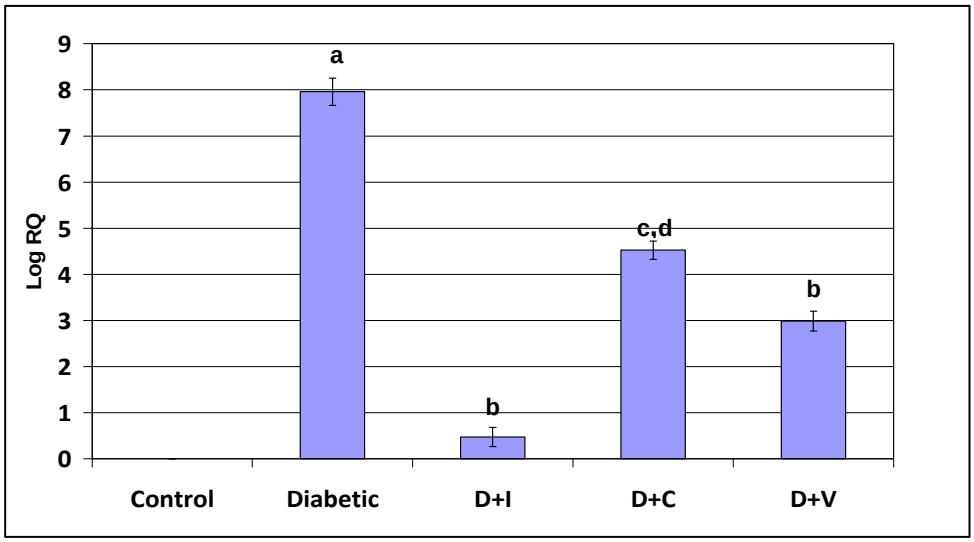


Table-147

Real Time amplification of acetylcholine esterase mRNA from the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	7.95 ± 0.29 [a]
D + I	0.47 ± 0.20 [b]
D + C	4.52 ± 0.19 [c,d]
D + V	2.98 ± 0.21 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001, [c] P<0.01 when compared to control, [b] P<0.001, [d] P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-148

Real Time amplification of choline acetyl transferase mRNA from the pancreas of control and experimental rats

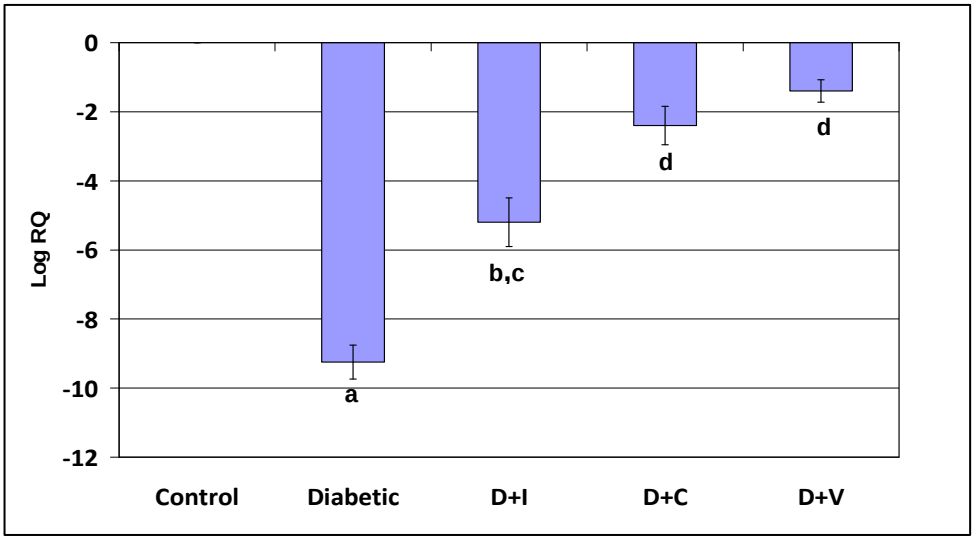


Table-148

Real Time amplification of choline acetyl transferase mRNA from the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-9.24 ± 0.49 [a]
D + I	-5.19 ± 0.70 [b,c]
D + C	-2.39 ± 0.55 [d]
D + V	-1.39 ± 0.32 [d]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **c** P<0.01 when compared to control, **b** P<0.001, **d** P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-149

Real Time amplification of muscarinic M1 receptor mRNA from the pancreas of control and experimental rats

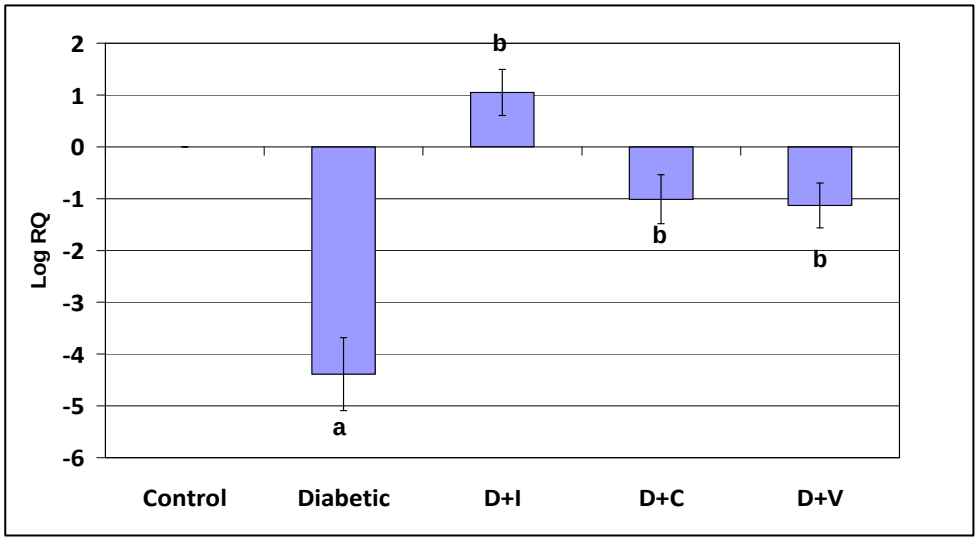


Table- 149

Real Time amplification of muscarinic M1 receptor mRNA from the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-4.38 ± 0.70 [a]
D + I	-1.05 ± 0.44 [b]
D + C	-1.01 ± 0.47 [b]
D + V	-1.13 ± 0.43 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-150

Real Time amplification of muscarinic M3 receptor mRNA from the pancreas of control and experimental rats

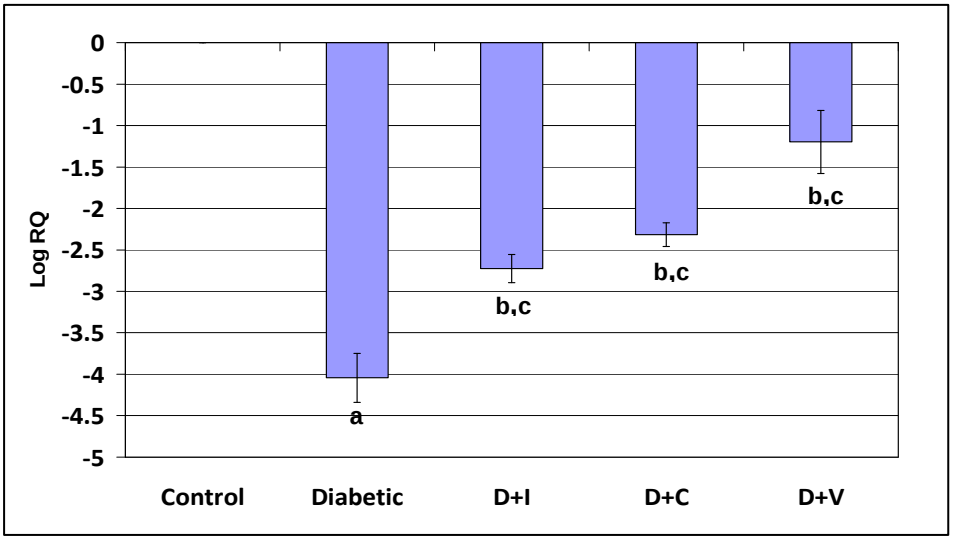


Table-150

Real Time amplification of muscarinic M3 receptor mRNA from the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-4.04 ± 0.29 [a]
D + I	-2.72 ± 0.16 [b,c]
D + C	-2.31 ± 0.14 [b,c]
D + V	-1.19 ± 0.38 [b,c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-151

Real Time amplification of dopamine D1 receptor mRNA from the pancreas of control and experimental rats

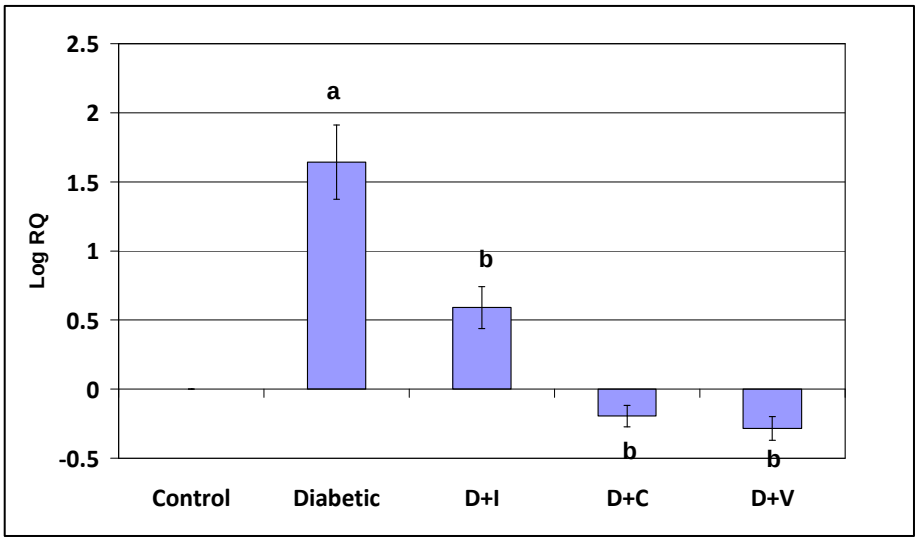


Table-151

Real Time amplification of dopamine D1 receptor mRNA from the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.6 ± 0.26 [a]
D + I	-0.59 ± 0.15 [b]
D + C	-0.19 ± 0.07 [b]
D + V	-0.28 ± 0.08 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group, . D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-152

Real Time amplification of dopamine D2 receptor mRNA from the pancreas of control and experimental rats

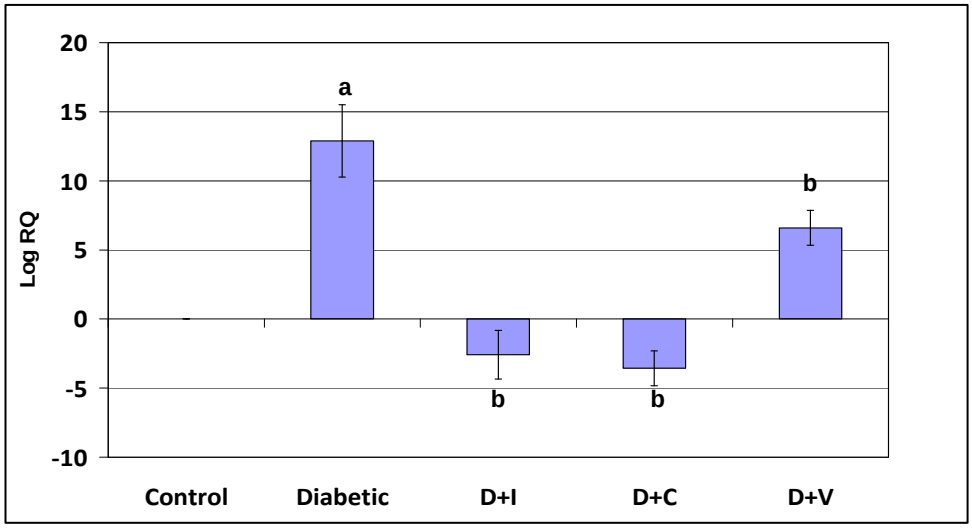


Table-152

Real Time amplification of dopamine D2 receptor mRNA from the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	12.8 ± 2.61 [a]
D + I	-2.5 ± 1.75 [b]
D + C	-3.5 ± 1.25 [b]
D + V	-6.5 ± 1.25 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] P<0.001 when compared to control, [b] P<0.001 when compared to diabetic group, . D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-153

Real Time amplification of Vitamin D receptor mRNA from the pancreas of control and experimental rats

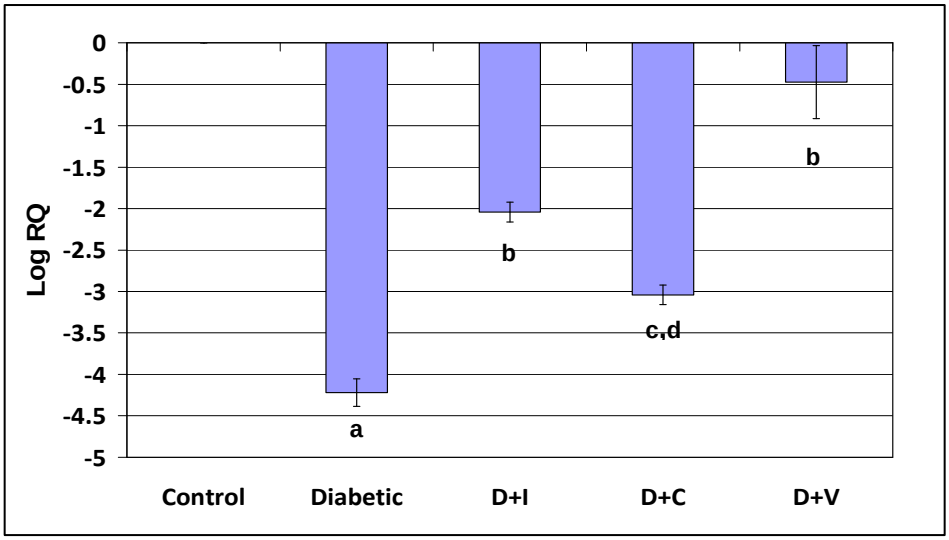


Table-153

Real Time amplification of Vitamin D receptor mRNA from the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-4.2 ± 0.16 [a]
D + I	-2.0 ± 0.11 [b]
D + C	-3.0 ± 0.11 [c,d]
D + V	-0.4 ± 0.44 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **c** P<0.01 when compared to control, **b** P<0.001, **d** P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats.

Figure-154

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

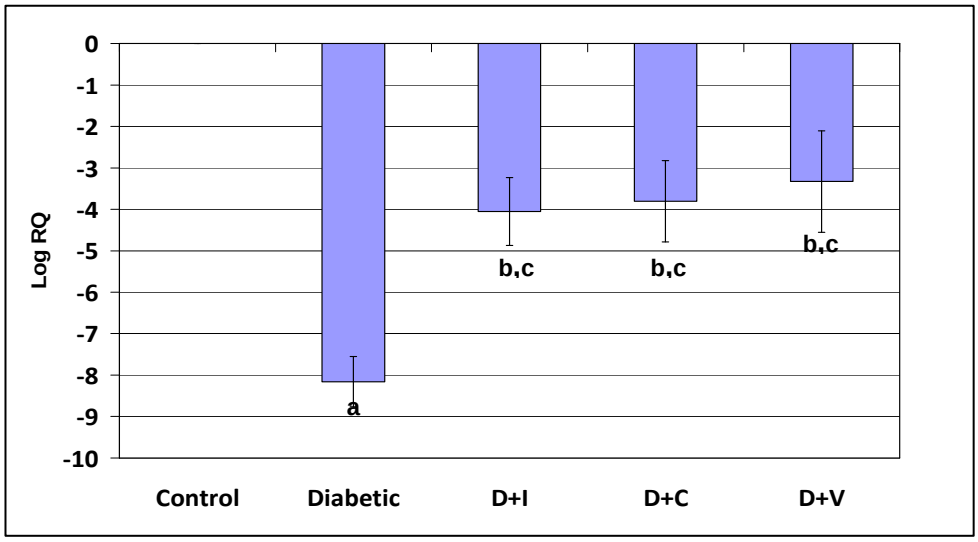


Table- 154

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

Experimental groups	Log RQ
Control	0
Diabetic	-8.04 ± 0.60 [a]
D + I	-4.72 ± 0.82 [b,c]
D + C	-3.31 ± 0.98 [b,c]
D + V	-3.19 ± 1.22 [b,c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01 when compared to diabetic group, . D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-155

Real Time amplification of GLUT2 mRNA from the pancreas of control and experimental rats

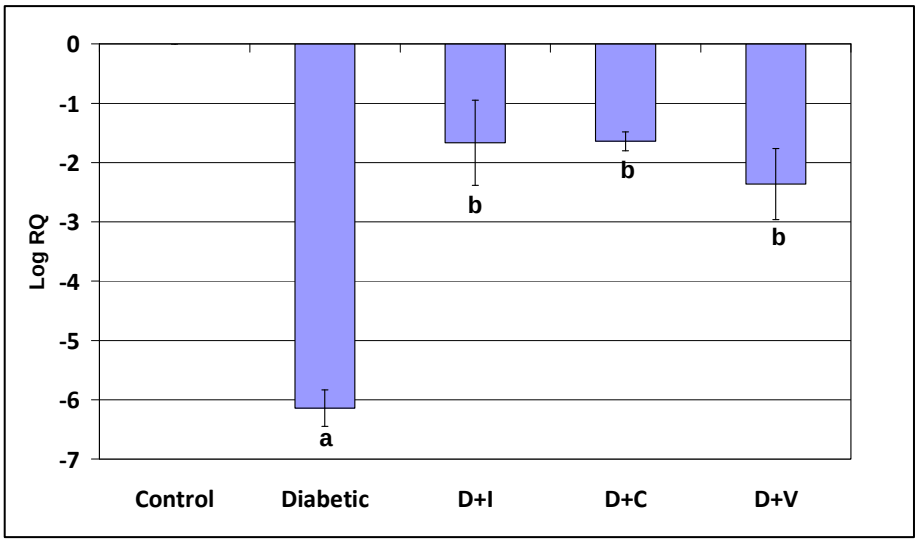


Table-155

Real Time amplification of GLUT2 mRNA from the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-6.1 ± 0.16 [a]
D + I	-1.6 ± 0.11 [b]
D + C	-1.6 ± 0.11 [b]
D + V	-2.3 ± 0.44 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **[a]** P<0.001 when compared to control, **[b]** P<0.001 when compared to diabetic group, . D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-156

Real Time amplification of phospholipase C mRNA from the pancreas of control and experimental rats

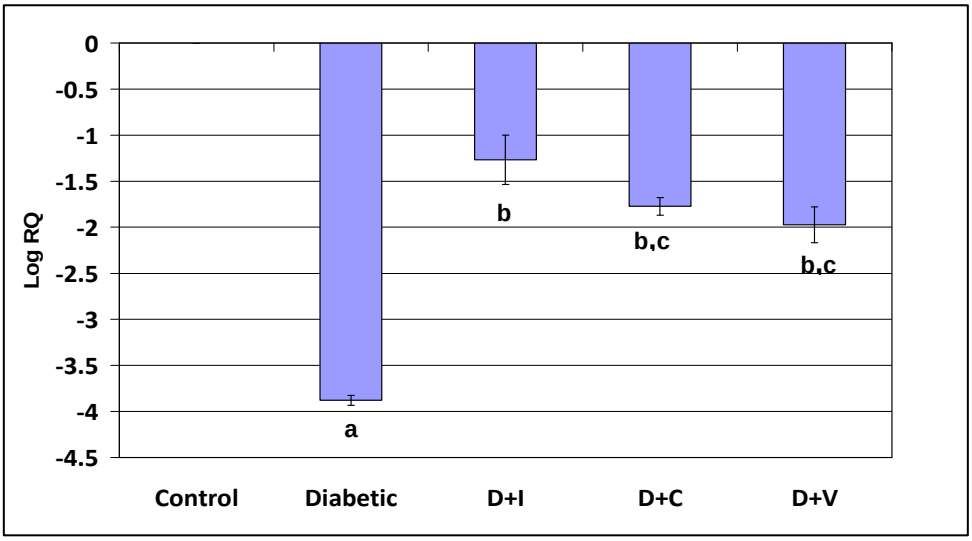


Table-156

Real Time amplification of phospholipase C mRNA from the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-3.8 ± 0.18 [a]
D + I	-1.2 ± 0.26 [b]
D + C	-1.7 ± 0.09 [b,c]
D + V	-1.9 ± 0.19 [b,c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. **a** P<0.001, **b** P<0.01 when compared to control, **c** P<0.01 when compared to diabetic group. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-157

Real Time amplification of superoxide dismutase mRNA from the pancreas of control and experimental rats

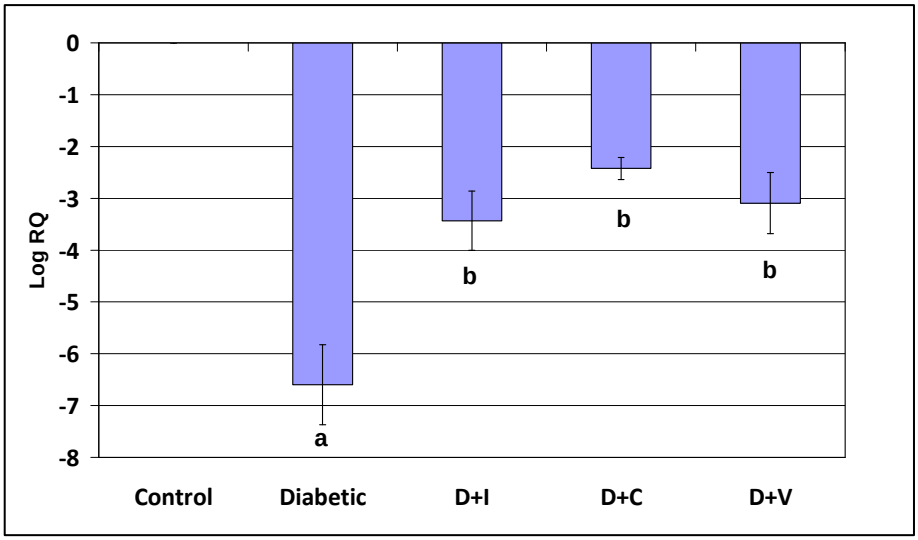


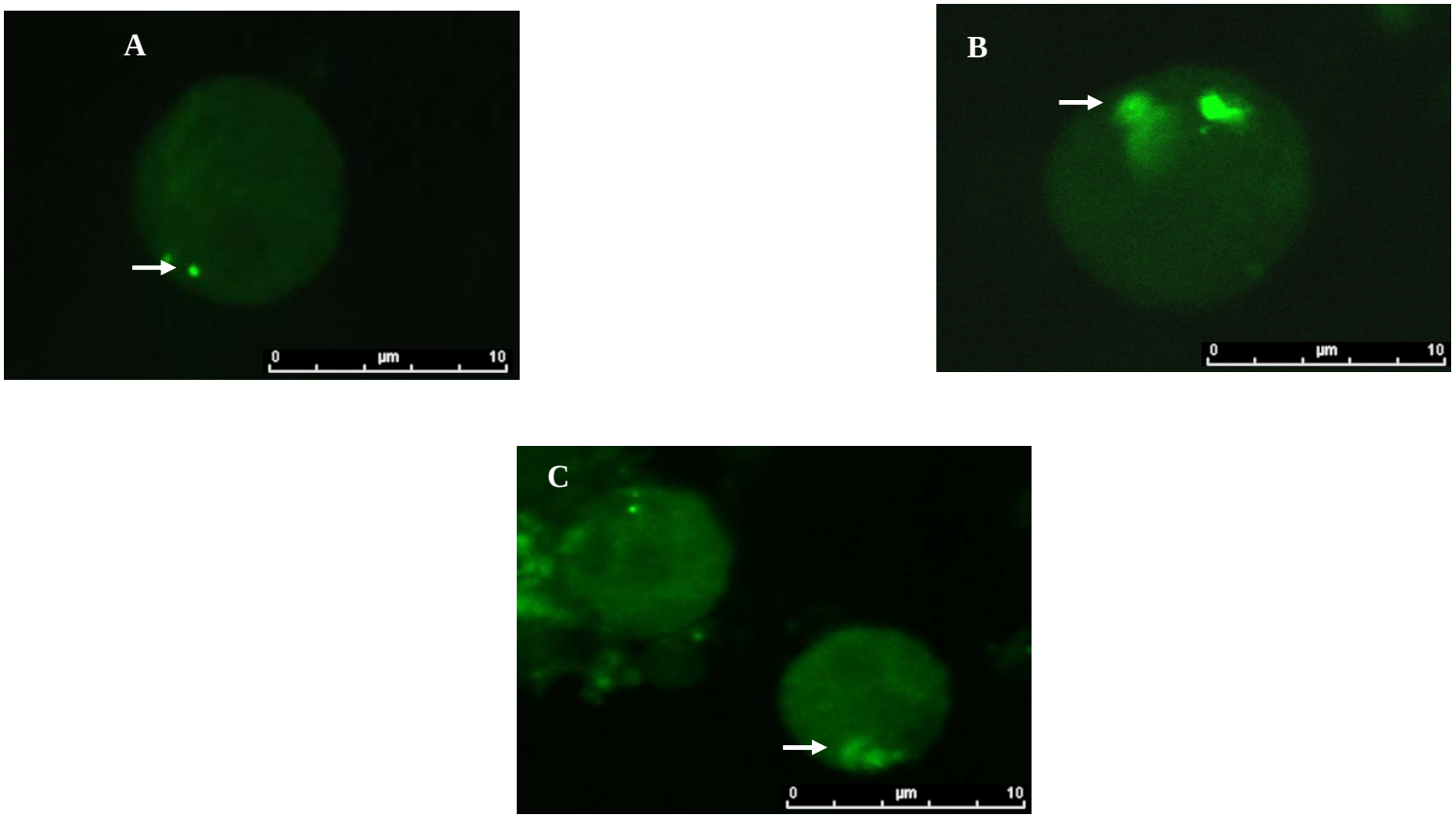
Table-157

Real Time amplification of superoxide dismutase mRNA from the pancreas of control and experimental rats

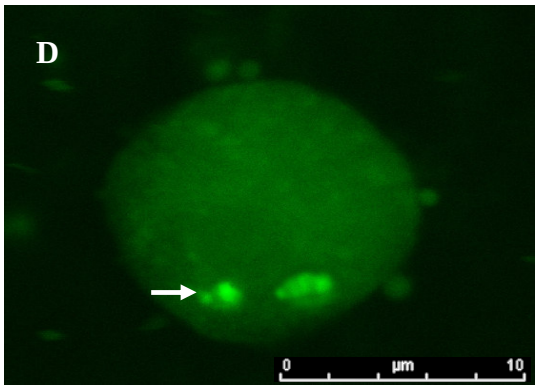
Experimental groups	Log RQ
Control	0
Diabetic	-6.5 ± 0.77 [a]
D + I	-3.4 ± 0.57 [b]
D + C	-2.4 ± 0.21 [b]
D + V	-3.0 ± 0.58 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consist of 6-8 rats. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic group, . D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats

Figure-158
Acetylcholinesterase expression in the pancreas of control and experimental rats



→ **Acetylcholine esterase**



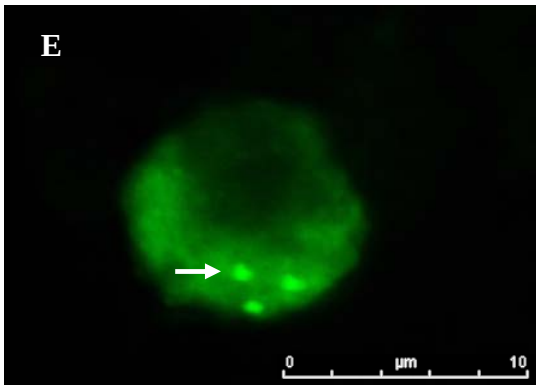


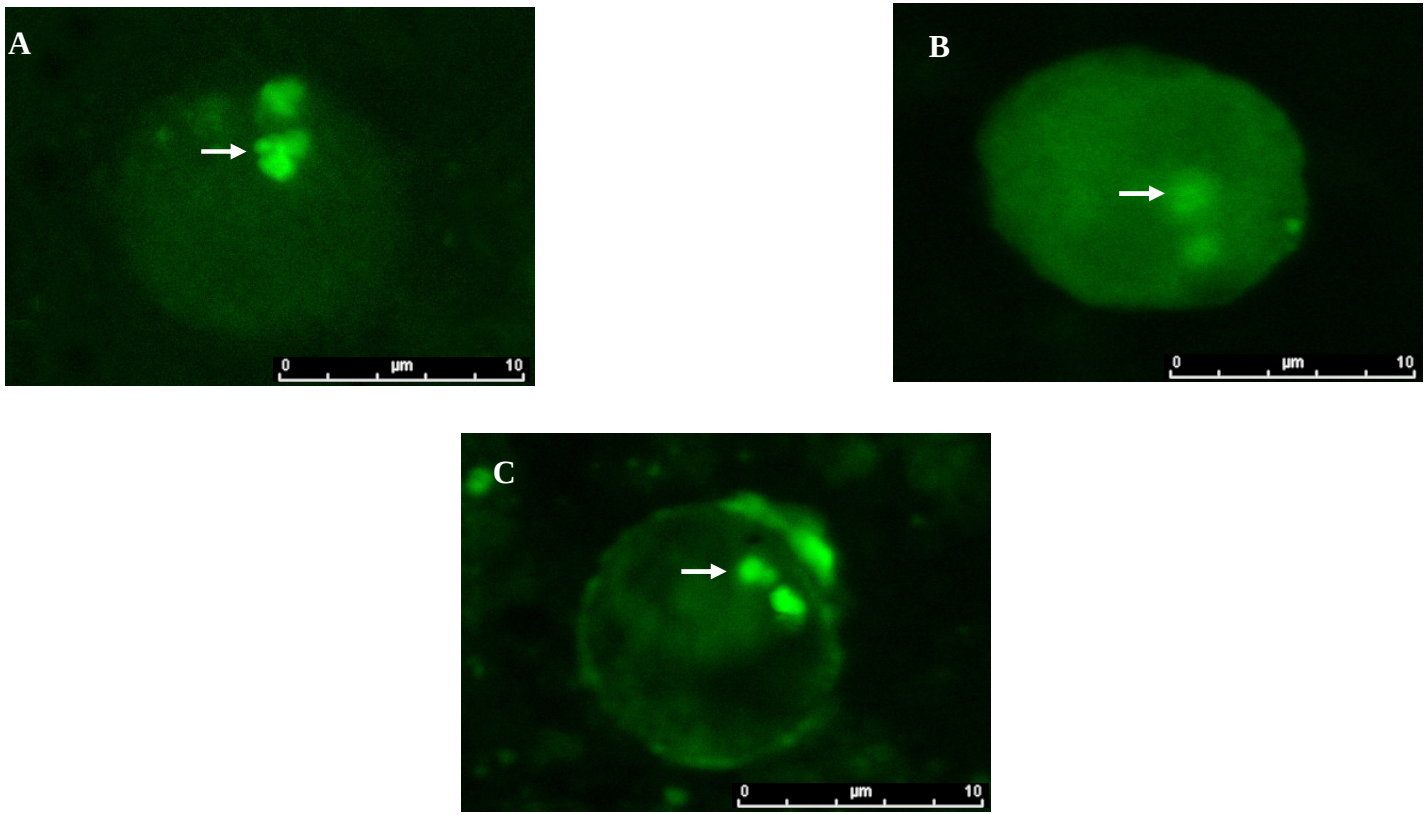
Table-158

Acetylcholine esterase expression in the pancreas of control and experimental rats

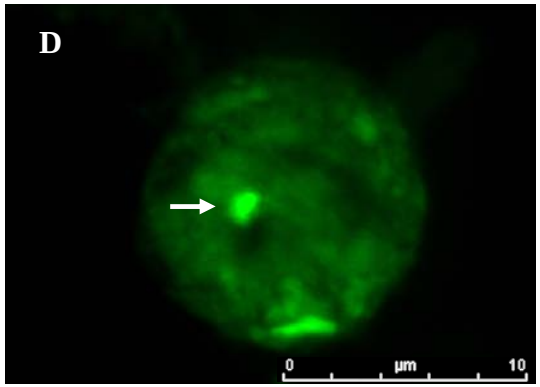
Condition	Mean pixel value
Control	79 ± 2.4
Diabetic	20 ± 3.0 [a]
D + I	60 ± 6.8 [b]
D + C	62 ± 4.2 [b]
D + V	65 ± 5.0 [b]

Confocal image of Acetylcholine esterase expression in the pancreas of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent acetylcholine esterase specific primary antibody and FITC as secondary antibody. (⟶) in white shows acetylcholine esterase. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 10 µm.

Figure-159
Muscarinic M1 receptor expression in the pancreas of control and experimental rats



→ **Muscarinic M1 receptor**



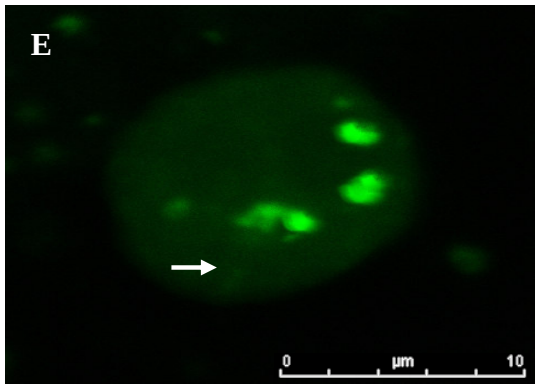


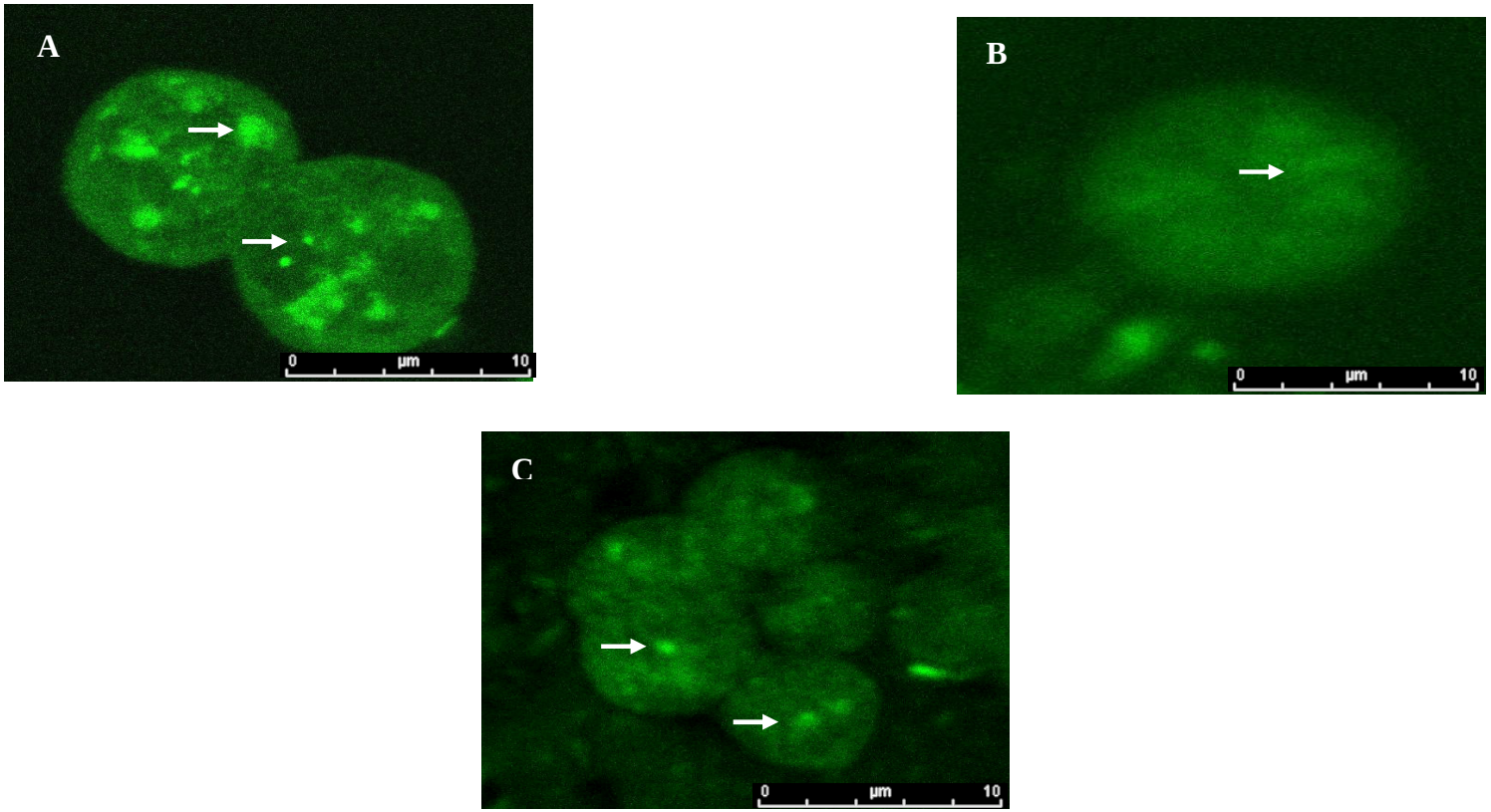
Table-159

Muscarinic M1 receptor expression in the pancreas of control and experimental rats

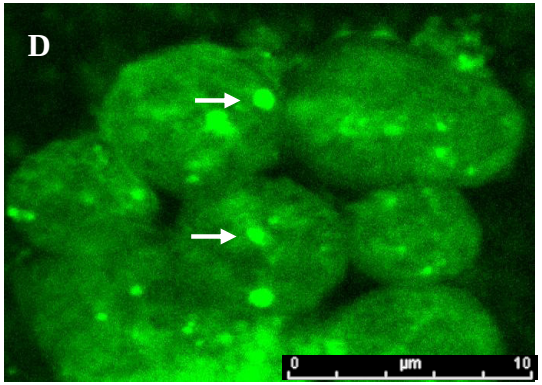
Condition	Mean pixel value
Control	78 ± 5.0
Diabetic	46 ± 4.3 [a]
D + I	72 ± 6.2 [b]
D + C	80 ± 6.4 [b]
D + V	75 ± 2.0 [b]

Confocal image of muscarinic M1 receptors in the cerebral cortex of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M1 receptors. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 10 µm.

Figure- 160
Muscarinic M3 receptor expression in the pancreas of control and experimental rats



→ **Muscarinic M3 receptor**



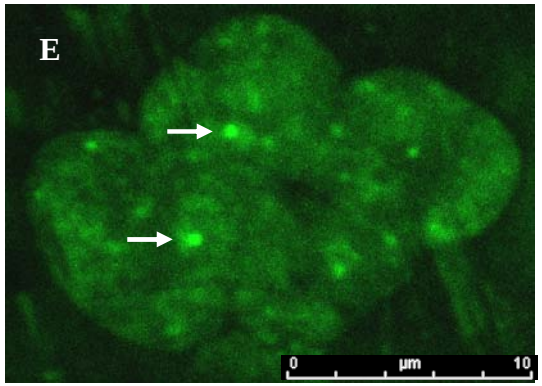


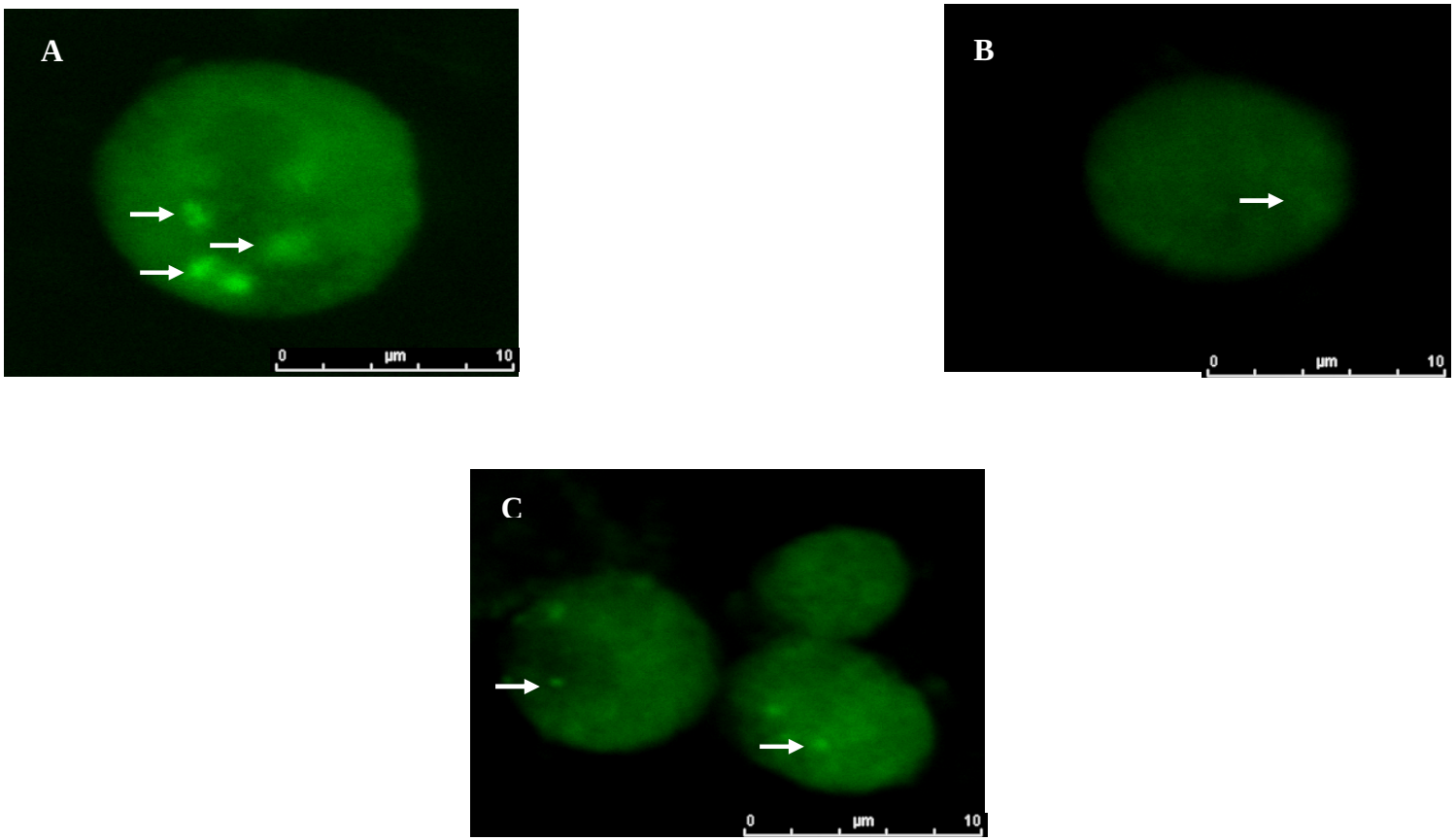
Table-160

Muscarinic M3 receptor expression in the pancreas of control and experimental rats

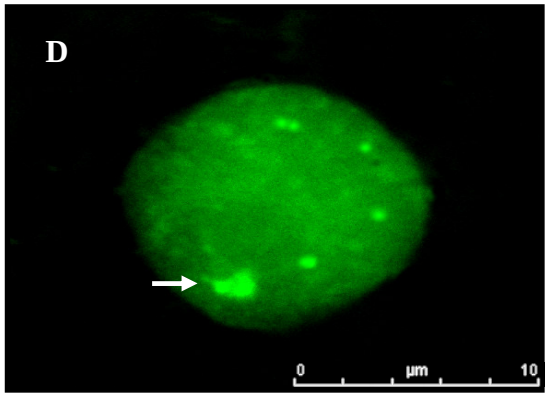
Condition	Mean pixel value
Control	88 ± 4.7
Diabetic	22 ± 1.9 [a]
D + I	67 ± 2.5 [b]
D + C	89 ± 3.4 [b]
D + V	70 ± 3.6 [b]

Confocal image of muscarinic M3 receptors in the pancreas of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (⟶) in white shows muscarinic M3 receptors. Scale bar = 50 µm. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 10 µm.

Figure-161
Vesicular acetylcholine transporter Expression in the pancreas of control and experimental Rats



→ Vesicular acetylcholine transporter



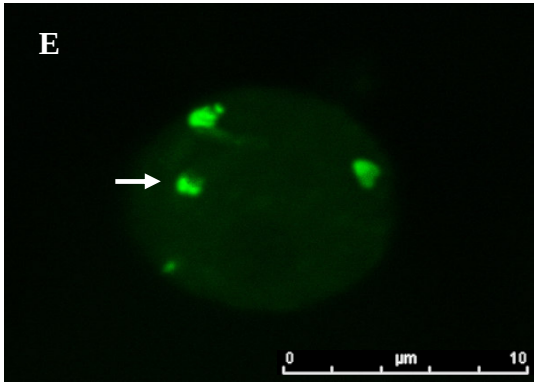


Table-161

Vesicular acetylcholine transporter expression in the pancreas of control and experimental rats

Condition	Mean pixel value
Control	66 ± 5.8
Diabetic	20 ± 3.5 [a]
D + I	55 ± 4.1 [b]
D + C	72 ± 5.2 [b]
D + V	55 ± 2.6 [b]

Confocal image of vesicular acetylcholine transporter in the pancreas of control (A), Diabetic (B), D + I (C), D + C (D) and D + V (E) rats using immunofluorescent vesicular acetylcholine transporter specific primary antibody and FITC as secondary antibody. (⟶) in white shows vesicular acetylcholine transporter. [a] $P<0.001$ when compared to control, [b] $P<0.001$ when compared to diabetic. D + I- Insulin treated diabetic rats, D + C- Curcumin treated diabetic rats and D + V- Vitamin D_3 treated diabetic rats. Scale bar = 10 µm.