Discussion

BLOOD GLUCOSE & BODY WEIGHT

Increased blood glucose and decreased body weight during diabetes is similar with previous reports as a result of the marked destruction of insulin secreting pancreatic islet β-cells by streptozotocin (Junod et al., 1969). 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. Our results showed that administration of Aegle marmelose and Costus pictus leaf extracts to STZ diabetic rats normalizes blood glucose levels. The glucose lowering activity of Aegle marmelose leaf extract confirmed the previous reports (Ponnachan et al., 1993).

Glucose tolerance test was carried out to find out the effective dose (the quantity of leaf extract that can bring down the glucose level in the blood to the control level of Costus pictus leaf extract. Costus pictus leaf extract was administered to diabetic rats at a dose of 250mg/Kg body weight and showed a significant glucose lowering activity. From this data it is clear that Costus pictus leaf extract has anti hyperglycemic activity. As far as the molecular action as well as pharmacological activities of Costus pictus leaf extract is concerned, no previous reports have been demonstrated. This is the first scientific study to demonstrate the anti hyperglycemic activity of Costus pictus leaf extract. The results suggest that the mode of action of the plant extract is probably mediated by an enhanced secretion of insulin and enhanced tissue glucose utilization. The decreased body weight in the diabetic rats is due to excessive breakdown of tissue proteins. Treatment of diabetic rats with insulin,
*Aegle marmelose* and *Costus pictus* leaf extracts improved body weight significantly which indicate prevention of muscle tissue damage due to hyperglycemic condition.

**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 *Aegle marmelose* and *Costus pictus* leaf extracts treated diabetic rats attribute to the stimulation of the surviving beta cells by the extracts, which in turn exerts an antihyperglycemic action. Reports are available to show that antidiabetic plants are known to increase circulating insulin levels (Lamela *et al.*, 1985). Thus, it can be suggested that the *Aegle marmelose* and *Costus pictus* leaf extracts induce the release of insulin thereby potentiating its effect. A possible mechanism of action is that the extracts stimulate the residual pancreatic β-cell function or produced the antihyperglycemia through an extra-pancreatic mechanism, probably increasing peripheral utilization of glucose. This data confirmed the antihyperglycemic activity of *Aegle marmelose* and *Costus pictus* leaf extracts.

**CENTRAL ACETYLCHOLINE ESTERASE ACTIVITY**

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 (Goodman & Soliman, 1991; Ellman *et al.*, 1961)). It is well recognized that diabetes mellitus results in
altered membrane functions in several tissues (Alberti et al., 1982; Osterby., 1988 and Striker et al., 1993). Membrane alterations have been recognized as the underlying primary biochemical defect (Alberti et al., 1982). It has been well established that there is a marked change in the acetylcholine esterase 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. Thus central cholinergic activity will be implicated in the insulin secretion.

Central cholinergic activity was studied in experimental rats after using AChE as marker. Our results showed an increase in $V_{max}$ and decrease in $K_m$ in cerebral cortex and hypothalamus of diabetic rats when compared to control group. In brainstem there was an increase in $V_{max}$ of diabetic group without a change in $K_m$ when compared to control rats. This study support the reported delayed nerve transmission and impaired brain functions (Bartus et al., 1982; Davis et al., 1983; Clements, 1979; Carrington et al., 1991). In corpus striatum of diabetic rats there was decrease in activity of enzyme when compared to control group. Decreased $V_{max}$ without alteration in $K_m$ for erythrocyte AChE from diabetic patients has been reported (Suhail & Rizvi, 1989). The decrease was observed to have a negative correlation with the blood glucose level.

The activation of central cholinergic system by administration of cholinergic agonist into the third cerebral ventricle reported to produce hyperglycemia in rats (Iguchi et al., 1985). 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 (Iguchi et al., 1986). In insulin treated, Aegle marmelose and Costus pictus leaf extracts treated diabetic rats
AChE activity was reversed back to near control value. Our results showed that diabetic state clearly influenced the kinetic properties of AChE enzyme and the reversal of AChE activity to near control value found in the insulin, *Aegle marmelose* and *Costus pictus* leaf extracts treated diabetic rats brain regions is a compensatory mechanism to maintain the normoglycemic level.

**CENTRAL MUSCARINIC RECEPTOR ALTERATIONS**

Over the past decade, the role of muscarinic receptors in health was given much scientific study. The potential therapeutic value of various cholinergic agonists and antagonists have 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 M1 muscarinic 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 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). Cholinergic agonist carbachol normalized glucose-stimulated insulin secretion and glucose tolerance in mice subjected to a high-fat diet. Carbachol also potentiated glucose-stimulated insulin secretion from isolated islets with higher efficiency in high fat-fed mice (Ahren et al., 1999).

Binding studies using [³H]QNB and muscarinic general antagonist atropine revealed that total muscarinic receptors are decreased in the cerebral cortex during diabetic condition. In insulin, Aegle marmelose and Costus pictus leaf extracts treated diabetic rats, binding parameters were reversed to near control values. In these groups, the animals maintained the near control glucose and circulating insulin levels.

Central cholinergic neurons participate in the complex neural events responsible for the hyperglycemic response to neurocytogluccopenia 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., 1999). The downregulation 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 [³H]QNB. Muscarinic M1 receptors were decreased in diabetic rats, with a decrease in Kd indicating an increase in the affinity of receptors during diabetic state. In insulin, Aegle marmelose, Costus pictus leaf extracts treated diabetic rats binding parameters are reversed to near control values.
Muscarinic receptors are reported to be involved in the release of NE in the central nervous system (Appasundaram et al., 1998). In the PC cell lines addition of cholinergic stimulation results in the release of NE and muscarinic M1 receptors are involved in the NE release. 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. Real Time-PCR analysis also revealed a down regulation of the muscarinic M1 receptor mRNA level during diabetic condition. This is in concordant with our receptor binding studies.

Corpus striatum

Densities of M1 receptor subtype were highest in the corpus striatum (Oki et al., 2005). The corpus striatum is the largest component of the basal ganglia. Corpus striatum regulates endocrine functions indirectly through the secretion of other hormones like thyroxin. Binding studies using \(^{3}H\)QNB revealed that total muscarinic receptors decreased in corpus striatum during diabetic condition. In insulin treated and *Aegle marmelos* and *Costus pictus* leaf extracts treated diabetic rats binding parameters were reversed to near control values. Muscarinic M1 receptor changes were studied in experimental rats using subtype specific antagonist pirenzepine. Muscarinic M1 receptors are increased during diabetic state. In insulin, *Aegle marmelos* and *Costus pictus* leaf extracts treated diabetic rats binding parameters are reversed back to near control values. RT-PCR analysis also revealed an up regulation of the muscarinic M1 receptor mRNA level during diabetic condition. This is in concordant with our receptor binding studies.

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Hypothalamus

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). 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 (PVN) serves as the major neuroendocrine and autonomic output centre. In the PVN information from all over the brain is integrated and there are several other hypothalamic nuclei that also feed their information into this nucleus. Microinjection of HgCl₂ and neostigmine into the third ventricle under anesthesia caused marked hyperglycemia. In medulloadrenalectomized and atropine-coadministered rats, no marked hyperglycemia was induced by HgCl₂ or neostigmine. These results show that the muscarinic cholinergic system participates in the HgCl₂-induced central hyperglycemic effect through the function of the adrenal medulla (Takahashi et al., 1994).

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 were involved in increasing the plasma glucose and epinephrine levels (Honmura et al., 1992). The muscarinic antagonist atropine suppressed the hyperglycemia induced by hippocampus administration of neostigmine in a dose-dependent manner, suggesting the involvement of muscarinic receptors of the VMH in the glucoregulation (Iguchi et al., 1991).

General muscarinic antagonist, [\textsuperscript{3}H]QNB binding showed that total muscarinic receptors are increased in the hypothalamus during diabetes with a significant decrease in the $K_d$ when compared to control group. 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). In insulin treated, \textit{Aegle marmelose} and \textit{Costus pictus} leaf extracts treated diabetic rats, $B_{\text{max}}$ reversed to near control values. Receptor binding studies using muscarinic M1 subtype specific antagonist pirenzepine showed that M1 receptors decreased during diabetes with an increase in affinity of the receptors when compared to control group. In insulin treated, \textit{Aegle marmelose} and \textit{Costus pictus} leaf extracts treated diabetic rats, binding parameters were reversed back to near control values. The increased activity of the total muscarinic receptors and down regulation of Muscarinic M1 receptors could help the maintenance of normoglycemia. RT-PCR studies showed that the receptor mRNA decreased during diabetic condition. Previous studies demonstrated that the distribution of mRNA of muscarinic receptor generally parallels with the distribution of their protein.
Brain stem

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 brain stem are found to be increased during diabetic condition. Muscarinic M1 receptor changes were studied in experimental rats using subtype specific antagonist pirenzepine. Muscarinic M1 receptors are decreased during diabetic state. In insulin treated, Aegle marmelose and Costus pictus leaf extracts 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). The insulin was reported to be mitogenic and stimulated pancreatic β-cell proliferation in vitro. RT-PCR analysis also revealed a down regulation of the muscarinic M1 receptor mRNA level during diabetic condition. This is in concordant with our receptor binding studies.

MUSCARINIC M1 RECEPTORS ALTERATIONS IN THE PancreAS

Expression of muscarinic receptors in rat islets, RINm5F cells, and INS-1 cells was established by reverse transcriptase-polymerase chain reaction (RT-PCR) 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).

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. Several different neurotransmitters are stored within the terminals of these nerves, both acetylcholine and noradrenalin, 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. It stimulates \( \text{Ca}^{2+} \) influx through the voltage dependent L-type \( \text{Ca}^{2+} \) channel that is primarily activated by glucose. Studies showed that M1 and M3 are the major muscarinic receptors present in the pancreas (Lismaa et al., 2000). During diabetic condition M1 receptors are decreased. The muscarinic M1 receptors are decreased in number during diabetes with an increase in affinity.

Muscarinic M1 receptor changes were studied in experimental rats using subtype specific antagonist pirenzepine. Muscarinic M1 receptors are decreased in diabetic rats while \( K_d \) was increased when compared to control group. In insulin treated, *Aegle marmelose* and *Costus pictus* leaf extracts treated diabetic rats, binding parameters were reversed back to near control values.

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 also prevented by pretreatment with the M1 antagonist,
pirenzepine, or the M1 + 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 M1 receptors are involved in the glucose induced insulin secretion. In insulin treated, Aegle marmelose and Costus pictus leaf extracts treated diabetic rats Muscarinic M1 receptor status reversed to near control level. It helps to increase the insulin secretion from remaining β-cells to maintain the normal glucose level. RT-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.

STIMULATION OF INSULIN SYNTHESIS AND SECRETION FROM PANCREATIC β-CELL IN VITRO BY Aegle marmelose & Costus pictus LEAF EXTRACTS

To understand the mechanisms by which Aegle marmelose and Costus pictus ameliorates hyperglycemia, in vitro insulin secretion study using rat primary islet culture was carried out. Signal-transduction in the pancreatic β-cell and thereby the insulin secretory process is regulated by a sophisticated interplay between glucose and a plethora of additional factors including other nutrients, neurotransmitters, islet generated factors and systemic growth factors. The coupling of glucose metabolism to electrical activity remains central in all models of β-cell stimulus-secretion coupling. The resting membrane potential of the β-cell is set by the ATP-sensitive potassium
(KATP) channel (Ashcroft & Rorsman, 1990). Incubation of the pancreatic β-cells with stimulatory glucose concentrations leads to the activation of a cascade of reactions, which ends in the exocytosis of stored insulin. This complex of processes starts with the uptake of glucose by the β-cell high-\(K_m\)/low affinity glucose transporter GLUT2 and proceeds with the conversion of glucose into glucose-6-phosphate by the β-cell isoform of glucokinase (Randel, 1993; Matschinsky, 1996). Metabolism of glucose in glycolysis and the Krebs cycle results in the generation of ATP. Elevation in the ATP/ADP ratio leads to closure of the KATP, which in turn results in depolarization of the plasma membrane. The subsequent opening of voltage-gated L-type \(\text{Ca}^{2+}\) channels leads to an increase in the cytoplasmic free \(\text{Ca}^{2+}\) concentration, \([\text{Ca}^{2+}]_c\), which promotes insulin secretion (Berggren & Larsson, 1994).

Isolated pancreatic islets were incubated for one hour with five different concentrations (0.25, 0.5, 1, 2 & 5 mg/ml) of \textit{Aegle marmelose} and \textit{Costus pictus} leaf extracts separately in the presence of 4mM and 20mM glucose concentration, which would represent normal and diabetic conditions respectively. In one hour pancreatic islet cell culture, \textit{Aegle marmelose} and \textit{Costus pictus} leaf extracts enhanced glucose stimulated insulin secretion significantly at both the concentrations (4mM and 20mM) of glucose when compared to control.

Twenty four hours islet cell culture was done to study the long-term effect of \textit{Aegle marmelose} on insulin synthesis and release from the isolated islets. Long-term insulin secretion studies showed that all the concentrations of \textit{Aegle marmelose} except in higher concentration (5mg/ml) enhanced glucose stimulated insulin secretion significantly at both the concentrations (4mM and 20mM) of glucose when compared to control. In 24 hour culture \textit{Costus pictus} leaf extract enhanced glucose stimulated insulin secretion significantly at both the concentrations (4mM and 20mM) of glucose when compared to control.

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These experiments revealed that aqueous extract of *Aegle marmelose* and *Costus pictus* enhanced insulin secretion. The enhancement of insulin secretion by the *Aegle marmelose* and *Costus pictus* extracts correlates with the blood glucose and circulating insulin level data and can be attributed to the stimulation of the surviving beta cells by the extracts, which in turn exerts an antihyperglycemic action. Similar reports have been observed from some previous studies (Gray & Flatt, 1997; 1999).

**MUSCARINIC STIMULATION OF INSULIN SYNTHESIS AND SECRETION FROM PANCREATIC β-CELL IN VITRO**

Activation of the parasympathetic branch of the autonomic nervous system has long been known to increase insulin secretion and peripheral glucose uptake (Porte & Woods, 1990). Cholinergic stimulation of pancreatic β-cells increases insulin secretion. This effect is mediated by muscarinic receptors.

Isolated pancreatic islets were incubated for one hour with four different concentrations (0.5, 1, 2 & 5 mg/ml) of *Aegle marmelose* leaf extract separately in the presence 10⁻⁷M carbachol and four different concentrations (0.5, 1, 2 & 5 mg/ml) of *Costus pictus* leaf extract separately in the presence 10⁻⁷M carbachol in 4mM and 20mM glucose concentration. Carbachol at low concentration (10⁻⁸M) stimulated insulin secretion. In one hour pancreatic islet cell culture all the four concentrations (0.5, 1, 2 & 5 mg/ml) of *Aegle marmelose* and *Costus pictus* leaf extracts in the presence of 10⁻⁷M carbachol enhanced glucose induced insulin secretion when compared to 10⁻⁷M carbachol alone.

Twenty four hours islet cell culture was done to study the long-term effect of cholinergic agonist carbachol and *Aegle marmelose*, *Costus pictus* leaf extracts on insulin synthesis and release from the isolated islets. The presence of insulin
synthesis/secretion stimulators in the 24 hours islet cell cultures showed that they capacitate the ability of the viable cells to synthesise and secrete the insulin. Cholinergic agonist showed stimulatory effect in the long-term studies also (Renuka et al., 2006). Twenty four hours islet cell culture also showed similar results as in one hour in vitro culture.

Acetylcholine stimulation-insulin secretion coupling is mediated by complex mechanisms of signal transduction and several factors are involved. 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). IP$_3$ exerts its action through receptors that are ligand-activated, Ca$^{2+}$ selective channels. IP$_3$ receptors have been localized to the endoplasmic reticulum, nucleus and insulin granules (Yoo et al., 1990).

PKC plays an important role in mediating insulin secretion in response to cholinergic stimulation (Persaud et al., 1989; Wollheim & Regazzi, 1990). PKC also mediates densensitisation in many cell types. Activation of PKC by carbamylcholine leads to densensitisation and TPA (phorbol 12-myristate 13-acetate) treatment inactivates PKC leading to the inhibition of the densitisation process in islets (Verspohl & Wienecke, 1998). It is also reported that the desensitisation of PLC – coupled muscarinic receptors is mediated by PKC (Haga et al., 1990). The inhibition of insulin secretion by the addition of high concentration of carbamylcholine is the result of the receptor desensitisation by PKC.

In the present in vitro study we observed an increase in insulin release when islets were incubated with various concentrations of the Aegle marmelose and Costus
pietus leaf extracts and the combinations of these plants extracts with cholinergic agonist carbachol. This finding agrees with in vivo results thus strengthening the evidence that the extract acts as a stimulator of insulin secretion. Similarly insulin secretoagogue effect has been reported in plants such as Agaricus campestris (Gray and Flatt, 1998); Viscum album (Gray and Flatt, 1999) and Urtica dioica (Farzami et al., 2003) in isolated islets. Our study confirmed that the regulatory activity of these plant extracts on insulin secretion is through Muscarinic receptors.

GLUCOSE UPTAKE STUDY

The plasma glucose level is tightly controlled throughout life in the normal individual. The stability of the plasma glucose level is a reflection of the balance between the rates of whole body glucose production and glucose utilisation. Each of these processes is tightly regulated by the levels of hormones and substrates in blood (Alan, 1999). The liver plays a major role in insulin-regulated glucose homoeostasis through the balance between glucose utilization and glucose production, both processes being tightly coordinated (Nevado et al., 2006; Carmen et al., 2005). The glucose dependence of liver glucose uptake is influenced by the route of glucose delivery and the prevailing insulin levels (Chen et al., 2004).

Fourteen days after the STZ injection, liver and cerebral cortex slices of control, diabetic, insulin treated, Aegle marmelose and Costus pictus treated diabetic rats were incubated with 14C glucose for 30 minutes and one hour. In the liver and cerebral cortex of diabetic rats there was a significant reduction in the glucose uptake activity when compared to control group. Treatment with insulin, Aegle marmelose and Costus pictus extracts enhanced glucose uptake in the liver and cerebral cortex.
One of the underlying mechanisms of glucose lowering activity is suggested to be due to stimulation of peripheral glucose utilization. Since STZ induced diabetes was accompanied by insulin resistance, *Aegle marmelose* and *Costus pictus* leaf extracts treatment can act by improving sensitivity. In diabetes, the decrease in body weight is associated with decreased rate of glucose utilization and impaired carbohydrate metabolism. *Aegle marmelose* and *Costus pictus* leaf extracts treatment seems to have regulated these disturbances at the cellular level.

The incorporation of $^{14}$C - glucose was found to be significantly decreased in the liver slices of diabetic rats. The present result of *in vitro* $^{14}$C - glucose uptake in diabetic rat liver are in accordance with the reports that glucose utilisation is inhibited in diabetic conditions due to lack of insulin that in turn decreases transport of glucose across the cell wall of hepatocytes (Chaikoff, 1951; Hemendex & Sols, 1963). *Aegle marmelose* and *Costus pictus* leaf extracts treatment of diabetic rats significantly enhanced the *in vitro* $^{14}$C - glucose uptake in liver slices which could possibly be due to the regulation caused by these plant extracts at the level of glucose transport system. The incorporation of $^{14}$C - glucose was found to be significantly decreased in the cerebral cortex slices of diabetic rats. Tuonq *et al.*, (1984) reported that slices from rat cerebral cortex incubated in the presence of 2-deoxy[$^{3}$H]glucose accumulate the sugar mainly in the form of its phosphorylated derivative.

**ELECTROPHYSIOLOGICAL CHANGES DURING DIABETES**

Neuroelectrophysiological recordings represent a non-invasive and reproducible method of detecting central and peripheral nervous system alterations in diabetes mellitus (Morano *et al.*, 1996). Neurophysiological alterations have been described in animal models of diabetes, in particular in rats. Deficits in both motor
and sensory nerve conduction velocity (MNCV and SNCV, respectively) can be detected within weeks after the onset of diabetes and increase up to 2–3 months after diabetes onset, remaining relatively stable thereafter (Moore et al., 1980; Cameron et al., 1986; Brismar et al., 1987; Kappelle et al., 1993). Studies of MNCV and SNCV in diabetic rats have made important contributions to the elucidation of the pathogenesis of the effects of diabetes on the PNS, as well as in the development of putative pharmacotherapy.

The control, diabetic, insulin treated, Aegle marmelose and Costus pictus treated diabetic rats underwent EEG analysis. The diabetic rats showed a change in the EEG pattern compared to the control rats. Treatment with insulin, Aegle marmelose and Costus pictus brought the wave patterns to the near control levels.

Diabetes mellitus is associated with chronic complications such as nephropathy, angiopathy, retinopathy and peripheral neuropathy. In diabetic patients, hyperglycemia may precipitate seizures, and in experimental diabetes, indications for an increased neuronal excitability have been found (Anderson et al., 2006). These changes in the diabetic cause the altered wave patterns. In our study it was found that the neurological disturbances can be reduced by the administration of Aegle marmelose and Costus pictus leaf extracts.

Thus our results suggest that the acetylcholine acting through muscarinic and specifically muscarinic M1 receptor subtype of receptors regulate the glucose homeostasis. Aegle marmelose and Costus pictus leaf extracts have a potential role in the insulin synthesis and secretion from the pancreatic β-cell, mediating its function through muscarinic receptors of acetylcholine. This has immense clinical significance in the management of diabetes.