5.1. Phytochemistry

Spectral analysis of the compound from *Tinospora cordifolia* in this study revealed the compound to be a polysaccharide, which was eluted from active fraction 3 of methanol extract. The second compound was identified as catechin, which is a flavanoid isolated from active fraction 4 of methanol extract of *Cassia fistula*.

Plants have an almost limitless ability to synthesize aromatic substances (Geissman, 1973). Most are secondary metabolites, of which atleast 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). These substances serve as plant defense mechanisms and a few give plants their colour. There is less than 1% of some 250,000 higher plants have been screened in-depth for their phytochemistry or pharmacology. The ethnomedical approach to plant drug discovery is practical, cost-effective and logical.

5.2. Hypoglycemic effect of *Tinospora cordifolia* and *Cassia fistula*

Diabetes mellitus is the most severe metabolic pandemic of the 21st century, affecting essential biochemical activities in almost every cell in the body and increasing the risk of atherosclerosis, myocardial infarction, neuropathy, nephropathy, etc. These complications have long been assumed to be related to chronically elevated glucose level
in blood.Conventionally, insulin-dependent diabetes mellitus is treated with exogenous insulin (Felig et al., 1995) and non insulin-dependent diabetes mellitus with synthetic oral hypoglycemic agents like sulphonylureas and biguanides (Rosak, 2002). However, the hormone fails as a curative agent for complications of diabetes (Mukherjee et al., 1966). Though insulin therapy is used for the management of diabetes mellitus but there are several drawbacks like insulin resistance (Peidrola et al., 2001), anorexia nervosa, brain atrophy and fatty liver (Yarura-Tobias et al., 2001) along with requirement for refrigeration of the drug and skilled technician as well as of its cost, which are not affordable in poor economic community. Synthetic oral drugs produce adverse health effects (Raheja, 1977). Chronic treatment with sulfonylureas and biguanides are also associated with side effects (Rang et al., 1991). Thus, managing diabetes without any side effects is still a challenge (Radermecker and Scheen, 2007). Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation. Different medicinal systems are using the active plant constituents, which discovered as natural hypoglycemic medicine, came from the virtue of traditional knowledge. Herbal drugs are considered free from side effects than synthetic one. They are less toxic, relatively cheap and popular (Momin, 1987: Valiathan, 1998).

*Tinospora cordifolia* and *Cassia fistula* crude extracts and compounds isolated from the effective hexane extract tested in the
present study brought about significant hypoglycemic activity in STZ-induced hyperglycemic rats. These extracts and compounds improved the biochemical parameters assessed and the expression of Glut-4 mRNA and protein.

5.2.1. Restoration of plasma Glucose

There is a wealth of experimental data demonstrating that either streptozotocin or alloxan is used to induce experimental diabetes (Leatherdale et al., 1981; Lamela et al., 1986; Day et al., 1990; Karunanayake et al., 1990; Hameda et al., 2006; Aslan et al., 2010). But streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Papaccio et al., 2000) and induces severe and irreversible, hyperglycemia (Mitra et al., 1996; Robert et al., 2001; Kanter et al., 2003; Akbarzadeh et al., 2007). The number of functionally intact β-cells in the islet organ is of decisive importance for the development, course and outcome of diabetes mellitus. The renewal of β-cells in diabetes has been studied in several animal models. The total β-cell mass reflects the balance between the renewal and loss of these cells (Nagappa et al., 2003; Jetton et al., 2005; Peshavaria et al., 2006).

Significant increase in the blood glucose level after a single (60 mg/kg b.wt) intraperitoneal injection of STZ in the present study shows that experimental diabetes is induced. Daily administration of Tinospora cordifolia and Cassia fistula crude extracts for 90 days
significantly reduces the blood glucose level to near normal in STZ-induced diabetic rats. This emphasizes the hypoglycemic effect of the plant extracts. The decrease in blood glucose was considerably greater in the methanol crude extract treated diabetic rats than in hexane, ethyl acetate, and aqueous extract treated rats. It is worth to recall the report of Noor et al., (2008), who postulated two possible mechanisms for antidiabetic activity of Aloe vera in streptozotocin-induced diabetic rats. First, A. vera may exert its effect by preventing the death of β-cells and/or second, it may permit recovery of partially destroyed β-cells. Burcelain et al., (1995) reported that the hypoglycemic action of the extract of herbal plants in diabetic rats may be possible through the insulin mimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles.

The present study showed that polysaccharide from Tinospora cordifolia and catechin from Cassia fistula, isolated from methanol extracts significantly reduced the plasma glucose level. This reduction may be achieved by either insulin release from pancreatic β-cells, inhibited glucose absorption in gut, stimulated glycogenesis in liver, increased glucose utilization by the body or insulin like activity of the compounds.

In concordance with our results, oral administration of aqueous extract of leaves of G. sylvestre normalized blood sugar levels of
diabetic animals through β–cell regeneration (Shanmugasundaram et al., 1981, 1983, 1988, 1990 a & b). Kamble et al., (1998) demonstrated that the Coccinia grandis extract mimics insulin like activity and improved the functional status of enzymes in glycolytic pathway and lypolytic pathway. Rajsekaran et al., (2004) reported a prominent reduction in the fasting blood glucose along with improved plasma insulin level of diabetic rats by the administration of ethanolic extract (300mg/kg b.wt) of Aloe vera. Aqueous leaf-extract of Annona squamosa Linn significantly reduced the levels of blood glucose and increased the activity of plasma insulin and antioxidant enzymes (Kaleem et al., 2006). The methanolic extracts of Sinularia firma and Sinularia erecta were found to be effective in lowering blood glucose level at the dose of 250mg/kg body weight (Tamrakar et al., 2008). These studies supported our results that plant extract treatment would decrease the plasma glucose level through insulin secretion by the regenerated β-cells of the pancreas and performing insulin mimetic activity.

5.2.2. Changes in body weight, food and water intake levels

According to Furuse et al. (1993), the body weight is reduced in the diabetic state whereas the food intake is increased and this recovers during the exposure of hypoglycemic treatment. Due to insulin deficiency, protein content was decreased in muscular tissue by proteolysis (Vats et al., 2004). In the present study, a reduced level of body weight and elevated level of food and water intake were
observed in STZ-induced diabetic rats. Oral administration of methanolic extracts of *Tinospora cordifolia* and *Cassia fistula* crude extracts for 90 days restored these levels significantly (p<0.001) towards the normal control than other three extracts treated rats at the same level of doses. Similar kind of restoration was observed in polysaccharide and catechin treated animals also. This effect may be due to increased secretion of insulin from the regenerated β-cells of the pancreas and insulin mimetic activity of the compounds; this anabolic effect might also have resulted in increased synthesis of proteins.

### 5.2.3. Oral Glucose Tolerance Test (OGTT)

In the present study, the oral glucose tolerance pattern of the compounds treated (for 60 days) STZ-induced diabetic animals was observed to be better. The oral glucose tolerance test of STZ-induced diabetic rats showed a significant rise in the basal plasma glucose concentration. After 180 minutes of oral glucose load, the glucose level did not reach the basal level as seen in control animals suggesting the impaired glucose tolerance which appears to be the result of insulin deficiency–mediated impairment of glucose oxidation. The oral glucose tolerance test of the polysaccharide and catechin treated animals was similar to that of the normal rats in terms of plasma glucose value under basal condition and 180 minutes after oral glucose load suggesting the importance of the compounds in glucose homeostasis. This justified the efficacy of the compounds to control elevated blood
sugar levels. This effect would have been brought about by stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilization of glucose (Whitton and Hems, 1975). The results of the present study are similar to the one in which Eriobotrya japonica seeds effectively improve glucose tolerance in the KK-Ay mice (Tanaka et al., 2008). Similarly the glucose tolerance report of Jie Yang et al., (2009) states that, treatment with Potentilla discoulour extract in alloxan-induced diabetic mice could significantly increase the rate of glucose disposal.

5.2.4. Hemoglobin (Hb) and glycosylated hemoglobin (HbA1c)

Glycosylated hemoglobin is considered to be a good measure to indicate the average blood glucose concentration over the preceding weeks while a single glucose determination gives a value which is true only at the time the blood sample is drawn (Goldstein et al., 1982; Karunanayake et al., 1990; Murray et al., 2000; Chen et al., 2001). Glycosylated hemoglobin has been found to increase in patients with diabetes mellitus (Koenig et al., 1976; Baskaran et al., 1990) and the magnitude of this increase is directly proportional to the fasting blood glucose level (Jackson et al., 1979; Al–Yassin and Ibrahim, 1981).

Increased non-enzymatic and auto-oxidative glycosylation is one of the possible mechanisms linking hyperglycemia and the vascular complications of diabetes. Measuring the glycosylated hemoglobin will reflect the blood glucose equilibrium 6-8 weeks prior to sampling (Kennedy et al., 1981; Kameswara Rao et al., 2003). Glycosylated
hemoglobin is formed progressively and irreversibly over a period of time and is stable till the life of the RBC and is unaffected by diet, insulin or exercise on the day of testing. Therefore, glycosylated hemoglobin can be used as an excellent marker of overall glycemic control. Since it is formed slowly and does not dissociate easily, it reflects the real blood glucose level over a period of time (Bunn et al., 1976, 1978, Bunn, 1981; Guoyan, 1992).

In our study, the diabetic rats had a decrease in the hemoglobin content with a proportionate increase in the glycosylated hemoglobin content. The increased glycosylated hemoglobin in the diabetic control rats indicates that erythrocytes are more prone to oxidative stress in diabetes. These alterations were recouped back near to normal in methanolic extract of *Tinospora cordifolia* and *Cassia fistula* treated rats. Treatment of crude extracts for 90 days resulted in improvement of overall blood glucose which in turn would reduce the glycosylation of hemoglobin. Therefore, at the end of the treatment the level of hemoglobin is improved and the glycosylated hemoglobin level is decreased. Restoration of hemoglobin and glycosylated hemoglobin contents was observed in polysaccharide and catechin treated rats also. Similar results were noted by Chandramohan et al., (2008), when the diabetic rats treated with 3-hydroxymethyl xylitol brought back Hb and HbA1c values to near normal levels. Administration of bark extracts of *Heliceres isora* (100, 200mg/kg) to diabetic rats decreased
the level of glycosylated haemoglobin and increased the level of hemoglobin (Kumar and Murugesan, 2008).

5.2.5. Liver and skeletal muscle glycogen

Liver plays an important role in buffering the postprandial hyperglycemia and is involved in the synthesis of glycogen. Diabetes mellitus is known to impair the normal capacity of liver to synthesize glycogen (Osborn et al., 1953; Spiro et al., 1958; Steiner and King, 1964; Hornbrook, 1970; Migliorini, 1971; Anderson, 1974; Whitton and Hems, 1975; Ponnachan et al., 1993; Bhavapiya et al., 2001; Chakrabarti et al., 2003; Mutalik et al., 2005; Ramesh and Pugalendi, 2006). Diabetic rats treated with Tinospora cordifolia and Cassia fistula crude extracts for 90 days and their compounds for 60 days increased the skeletal muscle and liver glycogen content. This restoration of the depleted glycogen in the liver and muscles might possibly be due to stimulation of insulin release from β–cells (Lolitkar and Rao, 1966; Grover et al., 2000; Chakrabarti et al., 2003). This increased glycogen content may be due to insulin mimetic activity of the extracts and compounds. In accordance with the present study, other investigators also reported similar findings (Sharma et al., 2003; Babu and Prince 2004; Mutalik et al., 2005; Stanley et al., 2006; Daisy et al., 2007; Chandramohan et al., 2008).

5.2.6. Plasma insulin and C-peptide

The pancreatic Islets of Langerhans are the sites of production of insulin. Most of the central areas of the Islets are composed of the
insulin-producing β-cells. These account for about 80% of the Islets. Insulin is initially synthesized as larger "prepro-insulin" and "pro-insulin". Pro-insulin is cleaved to give insulin, comprised of 2 polypeptide chains, A (21 amino acids) and B (30 amino acids), and C-peptide or "connecting peptide", a single chain of 31 amino acids. C-peptide serves as a linker between the B and A chains of insulin, facilitating appropriate folding and formation of the insulin disulphide bridges (Steiner and Oyer, 1967; Steiner and Dodson 1998; Steiner et al., 2001). It is of interest because C-peptide is released simultaneously with the hormone. C-peptide makes insulin appear more rapidly in the circulation and enhance its stimulatory effect on glucose utilization (Shafqat et al., 2006). Both insulin and C-peptide follow two-compartment kinetics, although single-compartment descriptions have been frequently used in the literature (especially for insulin) (Caumo et al., 2007). It has repeatedly been stated that positive effects of C-peptide cannot be detected in healthy humans or animals.

The pancreatic β-cell possesses the ability to respond to minor increase in the plasma glucose levels, thereby keeping the blood glucose level within a very narrow range. Progressive destruction of pancreatic β-cell leading to decreased insulin production and subsequent hyperglycemia is observed in all forms of diabetes mellitus. Similar observation could be seen in the streptozotocin-induced animals of the present study, which is also due to the
progressive destruction of pancreatic β-cell. In accordance with these observations other studies also reported that the serum insulin level is dropped down in streptozotocin-induced diabetic rats (Benwahhoud et al., 2001; Abdel-zaher et al., 2005). Since β-cell releases equimolar amounts of insulin and C-peptide, it is clearly observed that the C-peptide level also dropped down proportionally. In this respect, the reports of Johansson et al., (1996, 2000) would be of particular interest who demonstrated a reduction in the plasma C-peptide level in type-1 diabetic patients.

The C-peptide content was recovered to near normal level in the Tinospora cordifolia methanol extract and polysaccharide treated animals when compared to other extracts. But no significant recovery of insulin proportional to C-peptide was observed in the same group of animals. This disproportionate level of insulin and C-peptide even after their equimolar release from the β-cell may be due to degradation of released insulin at the level of liver.

One can follow insulin secretion by measuring the level of C-peptide which has a half-life of about 30 minutes. C-peptide is serving as a surrogate marker for insulin release and has no biological activity of its own. In this regard, it is very much clear that an equimolar amount of insulin and C-peptide must have been released after the treatment with Tinospora cordifolia methanol extract and polysaccharide. This could be possible by the regenerated β-cells in the pancreas. In agreement with this study, other researchers have
also reported that plant extracts stimulate insulin secretion from regenerated β-cells and enhance peripheral utilization of glucose (Karunanayake et al., 1984; Cakici et al., 1994; Kameswara Rao et al., 2003; Abdel-zaher et al., 2005; Sridhar et al., 2005; Dimo et al., 2007; Franch, 2007; Subash Babu et al., 2007; Chandramohan et al., 2008; Kumar and Murugesan., 2008; Gireesh et al., 2009).

In case of Cassia fistula extracts and catechin treated animals no marked recovery of insulin and C-peptide could be seen. This observation shows the inefficacy of the plant extract and compound to regenerate β-cells of the pancreas to release insulin and C-peptide. Muruganandan et al., (2005) suggested that the effectiveness of drugs to treat diabetic condition might rely on the actions other than pancreatic β-cells insulin release, when a high dose level of streptozotocin (60mg/kg) is used to induce diabetes which can effectively destroy the β-cells. In this regard, the report of Gray and Flatt, (1999) would be of particular interest, which reveals that plant and herbal compounds possess properties that mimic insulin action, preferably by interacting with the insulin receptor and enhanced glucose uptake by cells.

From this observation, it is clear that polysaccharide and catechin would serve as better oral drug for type-1 diabetes than insulin injection, though the mode of action differs.
5.2.7. Kidney function test

Renal injury in diabetes mellitus is an important clinical as well as biological problem. Approximately 30% of patients with type-1 or insulin-dependent diabetes mellitus (Andersen et al., 1985) and 5 to 10% of patients with type-2 or non-insulin-dependent diabetes mellitus (Fabre et al., 1982) will develop chronic renal insufficiency requiring treatment in an end-stage renal disease program. The clinical manifestation of diabetic nephropathy is the development of microalbuminuria. Gomes et al., 1997 observed that untreated diabetic animals developed albuminuria, which may be due to leakage of albumin by damaged glomerular membrane. The metabolic renal alterations in experimental diabetes, leading to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis have already been reported (Pathak and Dhawan, 1998; Bhavapriya et al., 2001).

Streptozotocin diabetes-induced renal damages can be ameliorated by plant extracts and their active principles and herbal formulas (Kim et al., 2008; Lee and Ku. 2008; Liu et al., 2008; Yang et al., 2008; Liu et al., 2009). Rao and Nammi, (2006) described the improvement of renal functions by the administration of chloroform extract of Terminalia chebula for 8 weeks in STZ-diabetic rats.

Urea is the major nitrogen containing metabolic product of protein metabolism. As protein catabolism is a major metabolic disturbance in diabetes mellitus, the blood urea levels increased
significantly in STZ–induced diabetic rats. Treatment–related increase in blood urea concentrations are variables used not only to indicate impairment in kidney function, but also clinical chemistry end points to detect treatment-related toxic effects of compounds on the kidney in rats (Travlos et al., 1996; Braunlich et al., 1997; Hwang et al., 1997; Bwititi et al., 2000; Bhavapriya et al., 2001; Nagappa et al., 2003).

Creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of glomerular filtration rate (Burtis and Ashwood, 1996). Creatinine, a marker of renal function (Travlos et al., 1996; Braunlich et al., 1997; Hwang et al., 1997; Bwititi et al., 2000; Bhavapriya et al., 2001; Toora and Rajgopal, 2002; Nagappa et al., 2003) is significantly increased in the diabetic control animals (Ponnachan et al., 1993; Katoh et al., 2000; Grover et al., 2003).

Significant reduction in the protein and increase in the urea, uric acid and creatinine levels in the present study emphasizes that STZ-induced experimental diabetes can lead to renal damage. Restoration of protein and diminution of urea, uric acid and creatinine levels in the Tinospora cordifolia and Cassia fistula crude extracts and their compounds treated rats suggests that the extracts and compounds recovered the kidney function. In this regard, it is wise to recall the report of El-Demerdash et al. (2005), who showed that administration of onion and garlic extracts to alloxan–diabetic rats not only decreased urea and creatinine levels, but also restored them to
normal values when administered for 4 weeks. Bhavapriya et al., (2001) also suggested that Aavirai kudineer (a herbal formulation) treatment could restore the protein content in alloxan diabetic rats.

5.2.8. Lipid parameters

Cholesterol is a vital substance that the body uses to produce such things as digestion-aiding material, hormones, and cell membranes. It is both produced by the body and absorbed from some of the foods. Cholesterol and triglycerides are transported in the blood by combinations of lipids and proteins called lipoproteins. HDLs, the so-called “good” or “healthy” cholesterol, are lipoproteins made mostly of protein and little cholesterol. HDLs can help to clear cholesterol deposits in blood vessels left by another blood component called low-density lipoproteins, or LDLs. The level of serum lipids is usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart disease (Davidson, 1981; Shanmugasundaram et al., 1990b; Jaiprakash et al., 1993; Al-Shamaony et al., 1994; Dwivedi and Aggarwal, 1994; Kim et al., 2006; Rajasekaran et al., 2006).

According to Ravi et al., (2005) abnormalities in lipid profile are one of the most common complications in diabetes mellitus found in 40% of diabetic cases. Diabetes causes an increase in the cholesterol, triglycerides, LDL and VLDL (Soltani et al., 2007). The abnormal high concentration of serum lipids in the diabetic subjects is due mainly to the increase in the mobilization of free fatty acids from the peripheral
fat depots, since insulin inhibits the hormone-sensitive lipase. Insulin deficiency or insulin resistance may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This results in increased production of cholesterol-rich LDL particle (Balasee et al., 1972; Taskimen, 1987; Murali et al., 2002). With longer insulin deficiency, the liver converts free fatty acids into ketone bodies (Basso and Havel, 1970; Bainton et al., 1992) and reduces the lipoprotein lipase activity resulting in impaired clearance of VLDL and chylomicrons from blood (Bagdade et al., 1968; Nikkila et al., 1977; Taskimen, 1987; Chakrabarti et al., 2003). VLDL, which is a major carrier of plasma triglycerides in blood, becomes rich in cholesterol and acts as a carrier of cholesterol (Mizuguchi, 1968; Miller, 1980; Shanmugasundaram et al., 1983).

Insulin inhibits lipolysis with an increase in the uptake of fatty acids into adipose tissue and triglyceride synthesis. Shirwaikar et al., (2004) suggested that, the concentration of serum free fatty acids is elevated in insulin-deficient diabetes, as a result of free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification–triglyceride lipolysis cycle is displaced in favor of lipolysis. HDL is an antiatherogenic lipoprotein. It transports cholesterol from peripheral tissues to the liver and thereby acts as a
protective factor against coronary heart disease. The level of HDL-cholesterol, which increased, might be due to the increase in the activity of lecithin cholesterol acyl transferase (LCAT), which may contribute to the regulation of blood lipids (Patil et al., 2004).

Lowering of serum lipid concentration through reduced dietary intake or drug therapy seems to be associated with a decrease in the risk of vascular diseases (Rhoads et al., 1976; La Rosa et al., 1990; Shanmugasundaram et al., 1990b; Huttunen et al., 1991; Guan and Zhao, 1995; Sheela et al., 1995; Ahmed and Sharma, 1997; Chen et al., 2001). Significant lowering of total cholesterol and rise in HDL-cholesterol is a desirable biochemical state for prevention of atherosclerosis and ischaemic conditions (Miller, 1980; Reaven, 1988; Schwenke and Carew, 1989; Luc and Fruchart, 1991; Mitra et al., 1995; Sachdewa and Khemani, 2003). Several studies show that an increase in HDL-cholesterol is associated with a decrease in coronary risk and most of the drugs that decrease total cholesterol also decrease LDL-cholesterol (Wilson, 1990; Carlsen et al., 1996; Andallu and Radhika, 2000; Kameswara Rao et al., 2003; Nagappa et al., 2003).

In the present study, streptozotocin- induced diabetic rats had an elevation in the serum lipids. Oral administration of Tinospora cordifolia and Cassia fistula crude extracts and their compounds and insulin significantly (p<0.05) decreased the serum cholesterol, triglycerides, LDL and VLDL and increased the HDL-cholesterol. In
accordance with the present study, lipid lowering effects of other plant extracts in STZ-induced diabetic rats have also been demonstrated (Mathur et al., 1996; Fontbonne et al., 1989; Pushparaj et al., 2000 & 2006; Hannan et al., 2003; Kameswara Rao et al., 2003; Nagappa et al., 2003; Sachdewa and Khemani, 2003; Al-Amin et al., 2006; El-Hillay et al., 2006; Park et al., 2006; Dimo et al., 2007; Bhatia et al., 2008; Sangameswaran and Jayakar, 2008; Aguilar-Santamaria et al., 2009; Gupta et al., 2009)

This hypolipidemic effect may be due to an increase insulin secretion that ultimately led to a decrease in the synthesis of cholesterol and fatty acids. The mechanism of action of plant extracts and its compounds appears to be through an increase in insulin level, which increased the activity of lipoprotein lipase and decreased fatty acid synthesis.

5.2.9. Serum amino-transferase levels

According to Ohaeri, (2001) STZ-induced experimental diabetes would cause liver necrosis. In the present study, elevation in the AST, ALT, ALP and ACP levels indicates the presence of diabetes-induced liver damage in diabetic rats. This elevation may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993), which indicates the hepatotoxic effect of STZ.

Streptozotocin treatment has a significant role in the alteration of liver function since the activity of AST and ALT were significantly
higher than the normal values. Serum concentration of AST is in proportion to the amount of cellular leakage or damage. It is released into serum in larger quantities when the tissues are damaged. Administration of *Tinospora cordifolia* and *Cassia fistula* crude extracts improved the liver function by decreasing the serum ALT, AST, ALP and ACP levels in both normal as well as diabetic rats. In accordance with these findings Chandramohan *et al.*, (2008) reported that administration of 3-hydroxymethyl xylitol lowered the serum AST, ALT, ALP activities in diabetic rats. Treatment of the diabetic rats with the polysaccharide and catechin caused reduction in the activity of these enzymes in plasma compared to the diabetic group and this result is in accordance with the report of Eidia *et al.* (2006) who showed an increase in the activity of AST, ALT and ALP by the administration of *Allium sativum* in normal and streptozotocin-induced diabetic rats. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew *et al.*, 1987; Maiti *et al.*, 2005).

### 5.2.10. Enzymes of Carbohydrate metabolism in serum and tissues (liver and kidney)

Liver functions as a “glucostat” and plays a vital role in the maintenance of blood glucose level and is the candidate organ involved in glucose homeostasis. It is the site for glycolysis, a process where glucose is degraded and gluconeogenesis, where glucose is
synthesized from lactate, amino acids and glycerol. Bhavapriya and Govindasamy, (2000) reported that these are the two important complementary events that balance the glucose load in our body. Glucokinase is the prime enzyme catalysing glucose phosphorylation. The first step in glycolysis (Vestergoard, 1999) is severely impaired during diabetes (Sheela and Augusti, 1992; Sato et al., 1998; Prince and Menon, 2000; Bhavapriya et al., 2001). Impairment of glucokinase activity leads to the impaired oxidation of glucose via glycolysis leading to its accumulation resulting in hyperglycemia.

Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase and phosphofructokinase. Glucokinase catalyses the conversion of glucose to glucose 6-phosphate and plays a central role in the maintenance of glucose homeostasis. In the liver, this above enzyme is an important regulator of glucose storage and disposal (O'Doherty et al., 1999). It is the most sensitive indicator of the glycolytic pathway in the diabetic state (Steiner and King, 1964; Shanmugasundaram et al., 1983; Prince et al., 1997; Vats et al., 2003).

In the present study, decreased activity of glucokinase was observed in STZ-induced diabetic rats. The enzymatic activity was increased with Tinospora cordifolia and Cassia fistula crude extracts and their compounds polysaccharide and catechin treatment. Similar observations were also recorded by other investigators
(Shanmugasundaram et al., 1981, 1983; Prince and Menon, 2000; Singh et al., 2001). These observations imply that entry of glucose into the cells is facilitated by the plant extracts and compound treatment, which in turn would stimulate the activity of this enzyme.

Glucose-6-phosphate is a pivotal point in the synthesis of glucose and glycogen and in glycolysis and pentose phosphate pathway. The status of glucose-6-phosphate denotes the direction in which mobilization proceeds and also indicates the metabolic status of glucose. Fructose-1, 6-bisphosphatase and glucose-6-phosphatase are important regulatory enzymes in gluconeogenesis. In diabetic animals the levels of these enzymes were observed to increase (Arathi and Sachdanandam, 2003). The increased activities of glucose 6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney of the streptozotocin-induced diabetic rats may be due to insulin insufficiency. Studies suggest that the activity of gluconeogenic enzyme glucose-6-phosphatase is enhanced during diabetes (Sheela and Augusti, 1992; Prince et al., 1997, Prince and Menon, 2000; Vijayvargia et al., 2000). Fructose-1, 6-bisphosphatase (FBPase) is a target for the development of novel diabetes therapeutics focused on lowering hepatic glucose production.

In the present study, a marked increase in the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in serum and tissues was observed in STZ-induced diabetic rats. On the other hand, oral administration of Tinospora cordifolia and Cassia fistula
crude extracts for 90 days and their compounds polysaccharide and catechin for 60 days dropped down the activity of glucose-6-phosphatase and fructose-1, 6-bisphosphatase indicating that gluconeogenesis is inhibited in extract-treated diabetic rats. The efficacy of plant extracts and compounds in altering the hepatic key enzymes of carbohydrate metabolism has been illustrated (Shanmugasundaram et al., 1983; Reddy et al., 1995; Latha and Pari, 2003; Pari and Latha, 2005; Kumar et al., 2006; Leelavinothan Pari and Narayanasamy Rajarajeswari., 2009). The inhibition of glucose-6-phosphatase activity after administration of the extracts suggests that glucose-6-phosphate is not utilized for the synthesis of glucose in the gluconeogenic pathway, but may be used as a substrate for glycogenesis or in the HMP pathway (Reddy et al., 1995).

Similar effect observed in the insulin treated rats indicates that the plant extracts and compounds have the potency to stimulate regeneration of β-cells and thereby insulin secretion or insulin mimetic activity.

5.2.11. Glycogen synthase and glycogen phosphorylase levels

Leloir et al. (1959) suggested that glycogen synthetase (EC 2.4.1.11) is the key enzyme involved in the synthesis of glycogen from glucose in skeletal muscle. According to Gutman, (1986) glycogen synthase activity is subject to multiple regulatory factors. However, covalent phosphorylation and the allosteric effector G-6-P are the major regulatory contributors for glycogen synthase (Roach,
Breakdown of glycogen is limited by the activity of glycogen phosphorylase. The enzyme exists in two forms, designated as phosphorylase-a and phosphorylase-b, both of which can stimulate glycogenolysis. In resting muscle under aerobic conditions, phosphorylase-a is more active than b and thus the form mainly responsible for glycogenolysis under most circumstances (Morgan and Parmeggiani, 1964).

Reduction in glycogen synthase and a concomitant elevation in the glycogen phosphorylase activities during diabetic condition have been reported (Chang, 1972; Prasannan, 1973; Stearns and Camillo, 1977; Narendhirakannan et al., 2006). Our observations in the present study also go along with the above reports. Studies suggest that this fall in the enzyme level should be due to the low insulin in the diabetic state, which would result in the inactivation of glycogen synthetase system (Villar-Palasi and Larner, 1961; Bishop, 1970; Tan and Nuttall, 1976; Witters and Auruch, 1978; Golden et al., 1979; Hauguel and Cedard, 1979; Naik et al., 1991; Perfumi et al., 1991). Lack of insulin may lead to glycogenolysis which results in the reduced liver glycogen content in diabetic rats.

Previous studies have demonstrated the efficacy of plant extracts on the recovery of glycogen metabolizing enzymes (Stanley et al., 2006; Anand et al., 2008). Similar effects were obtained in the present study after the oral administration of polysaccharide and catechin for 60 days and in insulin treated rats. A significant increase
in hepatic glycogen levels in STZ-diabetic rats after the treatment may possibly be due to the reactivation of glycogen synthase system as a result of increased insulin secretion from the regenerated β-cells or insulin mimetic activity.

5.2.12. Erythrocyte and tissue ATPase levels

Diabetes has a marked effect on the metabolism of a variety of tissues and since Na\(^+\), K\(^+\)-ATPase is critical for the membrane potential and many transports, a change in its activity in diabetes would have profound consequence in these tissues. Streptozotocin- and alloxan-treated or genetically susceptible diabetic rodents are animal models used to assess metabolic and physiological changes induced by insulin-dependent diabetes. Among the diabetes-induced metabolic changes, disturbance of Na\(^+\), K\(^+\)-ATPase activity has been widely reported (Sima and Sugimoto, 1999).

Several alterations in erythrocytes have been reported in type-1 and type-2 diabetes mellitus (Watala, 1993). A relationship was found between blood glucose levels and erythrocyte membrane ATPases activity (Adamson et al., 1986). An altered activity of erythrocyte membrane Ca\(^{2+}\)-ATPase is seen in diabetic conditions (Gronda et al., 1986; Gonzalez Flecha et al., 1990). It has been reported that glycosylation of erythrocyte membrane proteins significantly inhibits Ca-ATPase activity (Davis et al., 1985). The decreased Ca\(^{2+}\)-ATPase activity in diabetes may also be due to increased glycosylation of erythrocyte membrane protein. Kempaiah and Srinivasan (2006) have
reported increased oxidative stress in high-fat fed rats which contributed to decrease in Ca$^{2+}$ and Mg$^{2+}$-ATPase activity. The inhibition of these transport systems in the cell may result in a sustained increase in cytosolic Ca$^{2+}$ concentrations producing over-stimulation of cellular processes leading ultimately to cell death (Ver et al., 1999).

In the present study, loss of enzyme activity, found as a consequence of STZ-induced diabetes, may be linked to lack of insulin, which was shown to be a potential stimulator of membrane-bound enzymes Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ ATPases. C-peptide was considered as a biologically inert molecule. But according to Claire et al. (2008), C-peptide can bind to a cell surface receptor which initiates multiple cellular effects including the stimulation of Na$^+$ K$^+$-ATPase. It is an ubiquitous membrane-associated protein complex that uses energy from the hydrolysis of ATP to drive the counter-transport of sodium and potassium across the plasma membrane.

Dufayet et al. (1998) found that the activity of erythrocyte Na$^+$,K$^+$-ATPase was consistently lower in patients with type-1 diabetes and complete C-peptide deficiency than healthy controls and subsequently, shown that infusion of C-peptide into patients with type-1 diabetes resulted in an increase in plasma cGMP and erythrocyte membrane Na$^+$, K$^+$-ATPase activity. In the present study, the marked increase in Na$^+$, K$^+$-ATPase activity with a corresponding
increase in the activities of total ATPase, Ca$^{2+}$ and Mg$^{2+}$ ATPases in polysaccharide treated animals reveals that there should be an involvement of C-peptide. This confirms the regeneration of β-cells from the islets of pancreas and also validates the β-cell regenerative efficacy of polysaccharide.

But a similar effect for all the membrane bound enzymes was found in the catechin treated animals also, which indicates the insulin mimetic effect of catechin.

5.2.13. Plasma and tissue glycoproteins levels

Glycoproteins are carbohydrate-linked protein macromolecules found in the cell surface, which form the principal component of animal cells. Hexose, hexosamine and sialic acid are the basic components of the glycoproteins. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to the cell surface, the secretion and absorption of macromolecules (Mittal et al., 1996). Impaired metabolism of glycoproteins plays a major role in the pathogenesis of diabetes mellitus (Knecht et al., 1990). Several investigations have suggested that elevated levels of glycoproteins in plasma, liver and kidney tissues in the diabetic condition could be a consequence of impaired carbohydrate metabolism. The increase in plasma glycoprotein components has been associated with the severity and duration of diabetes. The raised levels of glycoproteins in diabetics may also be a predictor of angiopathic complications (Konukoglu et al., 1999) and
insulin deficiency and high levels of plasma glucose in the diabetic condition may result in an increased synthesis of glycoproteins (Youngren et al., 1996; Patti et al., 1999).

Many traditionally important medicinal plants have been tested for their efficacy against impaired glycol protein levels in diabetes (Ramkumar et al., 2007; Kumar and Murugesan, 2008). In this study, increased levels of hexose, hexosamine, fucose and sialic acid were observed in the plasma and tissues of streptozotocin-induced diabetic rats. Treatment with compounds such as polysaccharide and catechin results in decreased level of glycoproteins which proves the protective role of these compounds which may be due to their influence on regeneration of β-cells and insulin secretion or insulin mimetic effect.


Studies suggest that normal serum insulin is essential to maintain glucose homeostasis by enhancing glycolysis and glycogenesis in skeletal muscle (Ritcher et al., 1984: Mandarino et al., 1987) with concomitant decrease in the glycogenolysis in the liver and skeletal muscles (Shimazu, 1987). Insulin regulates Glut-4 gene expression (Jones and Dohm, 1997). A family of glucose transporters (Glut) mediate glucose transport across cell membrane and Glut-4 is predominant in skeletal muscle (Fukumoto et al., 1989; James et al., 1989; Pessin and Bell, 1992). It has been established that (Cushman and Wardzala, 1980; Susuki and Kono, 1980) the regulation of Glut-4 by insulin involves rapid translocation of the
transporter from the intracellular vesicles to the plasma membrane, although changes in the transporter’s intrinsic activity may also occur (Kahn et al., 1988; 1989). In addition to the regulation of Glut-4 by insulin, expression of the Glut-4 gene appears to be hormonally and metabolically regulated.

Previous reports suggest that insulin dependent (including STZ–induced) diabetes, which could alter the plasma insulin level and its counter regulatory hormones, drastically lower Glut-4 mRNA and protein levels (Berger et al., 1989; Garvey et al., 1989; Sivitz et al., 1989; Charron and Kahn, 1990). Glut-4 gene expression is down regulated in STZ-induced diabetes, a state of insulin deficiency, suggesting that insulin act as a positive regulator of gene expression (Charron et al., 1999). A decrease in the Glut-4 mRNA and protein expression was observed in STZ-induced diabetic animals (Berger et al., 1989; Garvey et al., 1989; Ramlal et al., 1989; Kahn et al., 1991) which accounts for the impaired glucose disposal. These reports support the present study, which demonstrated a decrease in Glut-4 mRNA and protein expression in the skeletal muscle of STZ-induced diabetic rats.

Gastrocnemius and the triceps are the muscle types with high glucose utilization capacity. The gastrocnemius muscle is anaerobic and glycolytic type (Holloszy and Coyle, 1984; Thayer et al., 1993) and triceps is oxidative and glycolytic (Ariano et al., 1973). Glucose transport into the skeletal muscle is the first rate-limiting step for
glucose utilization under physiological condition (Watson et al., 2004). Glut-4 exists exclusively in insulin-sensitive tissues mainly skeletal muscles and adipose tissues and is thus the major transporter protein responsible for insulin-mediated whole-body glucose uptake (Shepherd and Kahn. 1999). In general, the rate of synthesis of protein (mRNA translation) is directly related to the amount of encoding mRNA and the efficacy of translation of that specific mRNA (Granner and Scott, 2005).

It has been shown that the marked reduction in Glut-4 mRNA and protein expression in the skeletal muscle of diabetic rats could be restored back with insulin treatment (Kahn et al., 1991; Schalin-Jantti et al., 1994; Seung-Soon et al., 2006). Similar restoration was observed in the insulin treated animals of the present study. This confirms that insulin promotes the expression of Glut-4 mRNA and protein.

Glut-4 mRNA and protein expression were significantly increased in diabetic animals treated with polysaccharide for 60 days. At the same time, an elevation in the circulatory C-peptide and an equivalent insulin secretion was also observed indicating, the insulin secretory effect of the polysaccharide. In view of this, it can be concluded that the polysaccharide-induced high levels of insulin could have contributed for the restoration of Glut-4 mRNA and protein expression in the skeletal muscles. In this regard, it is worth to recall
the earlier reports which demonstrated the restoration of Glut-4 mRNA and protein expression on STZ-induced diabetic rats treated with plant extracts and the active principle from the extracts (Baque et al., 1998; Min-Lu et al., 2000; Maleppillil Vavachan Vijayakumar et al., 2005; Siddiqui et al., 2006). However, the circulating C-peptide and insulin levels were not restored back to normal in catechin treated rats. But here also an equal increase in the Glut-4 mRNA and protein expression could be observed as that of the polysaccharide treated rats suggesting that catechin might have mimicked the insulin activity in the restoration of Glut-4 mRNA and protein expression. Aqueous extracts of Sambucus nigra and Langerstroemia speciosa have been demonstrated to have an insulin like effect on glucose uptake facilitated by Glut-4 translocation in-vitro (Gray et al., 2000; Liu et al., 2001).

5.2.15. Glucose Oxidation

Glucose oxidation is an important process which provides energy to the cells to perform various functions. The rate of glucose oxidation in a cell depends on the rate of entry of glucose into the cell. Insulin stimulated glucose transport is achieved by translocation of the major insulin responsive glucose transporter Glut-4 from the intracellular vesicle storage site to plasma membrane (Munoz et al., 1995; Ploug et al., 1998). Defect in glucose transport can account for a reduced glucose disposal in diabetes (Zierath and Kawano, 2003).
In the present study, glucose oxidation is increased in the skeletal muscle of polysaccharide and catechin treated diabetic rats. The transport of glucose from the extracellular fluid into the cell is effected by a family of proteins known as glucose transporters (Joost et al., 2002). Therefore, in the present study polysaccharide and catechin-induced elevation in the skeletal muscle glucose oxidation appears to be the result of increased expression of Glut-4. Liu et al. (2006) also recorded a similar increase in glucose oxidation by isoferulic acid in STZ-induced diabetic rats.

**5.3. HISTOLOGICAL CHANGES IN THE ISLETS OF LANGERHANS**

**5.3.1 Light microscopical studies**

The use of light microscopy to study the normal and experimentally or pathologically altered pancreatic islets of Langerhans from the morphometric properties provides data pertaining to the number, size and distribution of the cell types (Remacle et al., 1977; Saito et al., 1978a, b, 1979; Sato and Herman, 1981; Ahlawat and Sahi, 1985; Ferri et al., 1987; Ashizawa, 1997). The islets of human beings and animals exposed to toxic chemicals introduced into the environment are known to undergo destruction particularly in respect to their β–cells. Similarly, under experimental conditions too, β–cell cytotoxicity has been reported. Loss of islet mass is associated with experimental diabetes brought about by chemicals. β–cells underwent conspicuous regression after treatment with STZ (Bora et al., 1989; Das et al., 1996; Szkudelski, 2001). As compared
to a homogeneously normal configuration in non-diabetic rats, the islet tissues of diabetic animals depict profound distortion in its structural organization. STZ-diabetes results in degenerative and lytic changes in the islets of Langerhans of the pancreas. The islet is considerably reduced and shrunken, there is destruction of some β-cells with central hyalinization, a few cells show pyknotic nuclei and the number of cells is lower (Chatterjee et al., 1980; Bora et al., 1985, 1989; Shanmugasundaram et al., 1990a; Kavalali et al., 2003). Histopathological examination of pancreas in streptozotocin-induced diabetic rat treated with D-400 (a herbomineral formulation) revealed that the treatment restored the activity of the islets of Langerhans (Mitra et al., 1995, 1996).

In the present study, oral intubation of Tinospora cordifolia crude extracts to STZ-induced diabetic rats brought about an improvement in the histoarchitecture of the islets. Histopathological abnormalities in the islets of Langerhans of STZ-induced diabetic rats were reversed to normal condition i.e. increased the cell mass of the islets by the administration of Tinospora cordifolia methanol extracts better than the other crude extracts.

Histopathological abnormalities in the islets of Langerhans were not reversed to normal condition in Cassia fistula extracts treated STZ-induced diabetic rats suggesting its ineffectiveness to regenerate β-cells.
5.3.2. Ultrastructural studies

Electron microscopic studies have played a key role in the evolution of our understanding of the biology of pancreatic islets. In most tissue systems, basic cellular composition has been defined by the light microscopic studies of the past century. However, only with the use of electron microscope the variety of cell types comprising the pancreatic islets has been appreciated (Munger et al., 1965; Like and Orci, 1972; Slavin et al., 1977; Sato and Herman, 1981; Polak and Bloom, 1992; Delfino et al., 1993; Bertelli et al., 1994; Mythili et al., 2003). The total volume of the endocrine part of the mammalian pancreas is only a small percentage of the whole gland and consists of different types of parenchymal cells dispersed in small clusters throughout the pancreas. The endocrine pancreas is represented by the islets of Langerhans (Langerhans, 1869), small clusters of endocrine cells (Larsson et al., 1976; Jorns et al., 1988) and by single endocrine cells scattered throughout the exocrine tissue (Aponte et al., 1985; Falkmer, 1985; Bendayan, 1987; Gepts and Veld, 1988; Johnston et al., 1988; Samols, 1991; Oertell et al., 1992; Park and Bendayan, 1992; Fawcett, 1994). The islets of Langerhans have been studied in detail. Usually, the pancreatic islets consist of all endocrine cell types of the pancreas, but it is not rare to find some islets composed of only one or two cell types. The ratio between the different cell types can vary in the islets according to the pancreatic lobe. Appropriate fixation and staining techniques reveal the presence of several cell types. The two most common are the larger, flame
shaped β-cells, which constitute about 20%, and the smaller β-cells which constitute about 75% of the islet cells. The β-cells are sometimes absent in the smaller islets and, when present tend to be located peripherally (Like, 1967; Larson et al., 1976; Pelletier and Leclerc, 1977; Baetens et al., 1979; Jorns et al., 1988).

The β-cells were originally characterized as having a uniform population of extremely electron-opaque secretion granules (Like, 1967; Jorns et al., 1988; Bertelli et al., 1994). The β-cells are roughly same size, the only distinguishing feature being the nature of the core. In all primates studied, core of the granules of the β-cells has two characteristic components, an extremely electron-opaque central spherical mass located asymmetrically with respect to the limiting membrane and a granular material of moderate electron opacity filling the compartment between the electron-opaque component and the limiting membrane. Thus, the granule of primate β-cells resembles an eccentrically shaped bull’s eye. The other mammals have β-cells granules characterized by a clearly demarcated spherical, electron-opaque core and an electron-lucent space separating the core from the limiting membrane. The structure of β-cells granules appears to be a relatively consistent characteristic among all mammals. Clusters of granular endoplasmic reticulum are commonly observed in the β-cells. The cytoplasm of β-cells contains a well-developed Golgi complex, a moderate amount of rough endoplasmic reticulum and free ribosomes. A few small filamentous mitochondria are present in the
cytoplasmic matrix. The nucleus of β-cells tends to be deeply indented or lobular (Lacy, 1972; Like and Orci, 1972; Unger, 1976; Kodama, 1983; Jorns et al., 1988; Yamamoto and Kataoka, 1988; Bertelli et al., 1994).

The β-cells are the easiest cells to identify in electron micrographs in that they usually have very distinctive cytological characteristics (Lacy, 1962). The β-cells of most of the animals are characterized by the presence of an electron-opaque para-crystalline granule core. This electron-opaque, somewhat angular, mass is separated from a granular limiting membrane by an electron-lucent space. These characteristic secretion granules are usually massed towards the secretory pole. The cytoplasm of β-cells between the numerous secretion granules contains the organelles including the Golgi apparatus, rough and smooth endoplasmic reticulum, mitochondria, microtubules and cytoplasmic microfilaments (Greider et al., 1969; Orci, 1974, 1985; Orci et al., 1973a, b, 1975; Aponte et al., 1985; Bendayan, 1987; Pipeleers, 1987, 1992; Sasaki et al., 1991; Delfino et al., 1993; Pai et al., 1993).

The pancreatic islets receive their blood supply through a complex system of fenestrated capillaries. These fenestrates are probably induced by the presence of endocrine cells, since capillaries between an islet and an exocrine acinus contain approximately five times as many fenestrae on the endocrine side when compared with
the exocrine side (Henderson and Moss, 1985; Hart and Pino, 1986; Lukinus et al., 1995; Samols et al., 1998).

Insulin-dependent diabetes mellitus (IDDM) is a disease caused by progressive destruction of the insulin secreting β-cells. Despite meticulous insulin therapy, the appearance of micro- and macro-angiopathy complications after 15 to 20 years of the disease is difficult to prevent in some patients. Presently, the only option to achieve permanent normoglycemia in diabetic patients is renewal of the β-cells (Robertson, 1992, 1993).

The ultrastructure of pancreatic islets in Vinca rosea flower and leaf–treated diabetic rats showed considerable improvement in β-cells activity. This is probably due to regeneration and rejuvenation of β-cells leading to increased insulin production and secretion (Ghosh and Suryawanshi, 2001). As mentioned earlier, in the present study also, there was a complete loss of β-cell secretion granules in STZ-treated rats. Oral administration of the Tinospora cordifolia crude extracts brought about an increase in insulin granules in STZ–induced diabetic rats. The all extracts of Tinospora cordifolia were found to improve the β-cell activity in a dose-dependent manner but methanol extract of Tinospora cordifolia is the most promising one. This confirms that the methanol extract of Tinospora cordifolia possesses regenerative efficacy.
But the oral administration of *Cassia fistula* crude extracts did not produce the secretary granules. This implies the inefficacy of *Cassia fistula* crude extracts to regenerate the β-cells.

### 5.4. *In-silico* Analysis

Computer-Aided Drug Design (CADD) allows us to simulate screening before performing experimental assays, which helps researchers prioritize lead candidates before synthesis. According to Brooijmans *et al.*, 2003 *in-silico* molecular docking is one of the most powerful techniques to discover novel ligands for receptors of known structure and thus play a key role in structure-based drug design. Molecular Docking continues to hold great promise in the field of Computer-based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. As a result, novel ligands for receptors of known structure are designed and their interaction energies are calculated using the scoring functions (Irwin *et al.*, 2002). There is a wealth of reports signifying the successful application of CADD in developing specific drugs in different therapeutic areas. In this regard, the finding of Singh *et al.* (2003) would be particular interest, which showed the role of HTS-466284/LY-364947, a 27nM inhibitor against type-1 TGF-receptor kinase using virtual screening by Biogen IDEC. Sawyer *et al.* (2003) worked on the same enzyme in cell based high-throughput screening. According to Becker *et al.* (2006), totally 31 compounds were successfully developed, of which a novel, potent,
and selective anti-anxiety, anti-depression 5-HT\textsubscript{1A} agonist, is at the clinical trial stage after lead optimization.

In the present study, the ligand orientations obtained from the docking was likely to represent more valid and reasonable binding modes of the receptors and was validated with the docking parameters of Discovery Studio. The docked results further more explain that ligands were fit with a specific optimal orientation exactly to the active site cavities of the receptors. Yu et al. (2007) suggested that, docking of 21 receptors spanning diverse protein families would give consistently accurate results with all ligands, if they docked within a 2Å\textsuperscript{RMSD}. The low RMS deviation (RMSD) between the docked receptor and crystal ligand in the present docking study indicate that there is a good alignment of the experimental and calculated positions. So it is clearer that the present docking results are accurate.

The steric and H-bonding intermolecular function called the PLP scores plays an important role in validating the docking results. In this regard, it is wise to remember the report of Gehlhaar et al. (1999), who imply that higher PLP scores indicate stronger receptor-ligand binding. In our present study, polysaccharide and catechin produced a higher PLP scores with both the receptors when compared to TZD. Libdock score also plays a vital role in validating the docking results. Generally, summing pairwise interaction terms and the overall inter atomic pairs of the receptor-ligand complex which includes sum of five
interaction terms namely Lipophilic interactions, Polar attractive interactions, Polar repulsive interactions, Solvation of the protein and ligand, an entropy term for the ligand (Jain, 1996). PMF developed based on statistical analysis of the 3D structures of protein-ligand complexes will give the Libdock score. A high Libdock score indicates a stronger receptor-ligand binding affinity (Muegge, 2006; Muegge et al., 1999). A high Lobdock score obtained in the present docking study confirms that there is a stronger receptor-ligand binding affinity between polysaccharide and catechin with the two receptors.

A total of 12 contacts and 3 hydrogen bonds at ARG 288, GLU 343 and GLU 295 are formed when TZD is docked with the Peroxisome proliferator-activated receptor gamma. The TZDs interact with insulin receptor forming 5 hydrogen bonds at THR 1154, ARG 1155, ARG 1155, ARG 1155 and GLY 1152 along with a total of 24 contacts showing that TZD form a good complex at the ligand binding domain of insulin receptor and PPAR.

The design of dual agonist is of major importance. Polysaccharide formed 4 hydrogen bonds with Insulin receptor at ASP 1150, THR 1154, GLY 1152 and HIS 1130 with a total of 6 contacts. At the same time it interacted with Peroxisome proliferator-activated receptor gamma with a total contact of 7 of which 6 are hydrogen bonds (ARG 288, GLU 291, GLU 291, GLU 291, GLU 291 and ARG 280).
Catechin interacted with LYS 1030, LYS 1030 and GLU 1047 of Insulin receptor and at LEU 228, SER 342 and LEU 340 of Peroxisome proliferator-activated receptor gamma. These interactions of polysaccharide and catechin with the proteins reveal that they can also form a good complex at the ligand domain of the proteins. This proves that polysaccharide and catechin can be a better agonist for the two receptors chosen for the study.

As the in-vivo (animal studies) results coincide with the validated in-silico data, it is clear that polysaccharide and catechin can serve as an anti-diabetic agent and can be used for further drug discovery approaches. It is therefore essential to perform docking experiments, which can help in validating a target and add support to the in-vivo studies.

5. 5. Mode of Action of the Chosen Plants and their Compounds

_Tinospora cordifolia_ and _Cassia fistula_ extracts and their compounds possess similar effect in lowering plasma glucose level and re-establishing all the altered metabolic impairments of STZ-induced diabetes. But data of the present investigation reveal that their mechanism of glucose lowering effect differs.

In the present study, almost all β-cells were degranulated, degenerated or necrosed in the STZ-treated rats leading to a decrease in insulin secretion and increase in blood glucose concentration. Oral administration of _Tinospora cordifolia stem_ extracts for 90 days and
polysaccharide for 60 days, the pure compound isolated from the methanol extract of *Tinospora cordifolia* caused a significant elevation in the plasma C-Peptide level with a proportionate increase in insulin level. The electron micrographs of the pancreatic sections reveal restored β-cells in the plant extracts treated islets suggesting regeneration of the insulin producing β-cells. From these results of the present study, it is evident that the treatment of plant extracts for 90 days had caused regeneration of β-cells, and thereby had stimulated insulin release. This suggests that the mode of action of *Tinospora cordifolia* and polysaccharide is neither insulin-like nor similar to oral hypoglycemic agents but it is only by regeneration of β-cells to bring down the plasma glucose level.

Plasma insulin and C-peptide levels were not restored back by treatment with *Cassia fistula* stem bark extracts and catechin, pure compound isolated from the methanol extract. β-cell granules could not be seen in the electron micrographs of the pancreatic sections in the plant extracts treated islets suggesting lack of regeneration of the insulin producing β-cells. This indicates that the mode of action of *Cassia fistula* and catechin is neither regenerative nor similar to oral hypoglycemic agents but it is only by insulin mimetic action to bring down the plasma glucose level.