DISCUSSION

5.1. Phytochemistry

The compound isolated from the active fraction 3 of the petroleum ether extract of *Elephantopus scaber* (leaves) of the present study was subjected to spectral analysis and it revealed that the compound is to be lupeol.

Plants produce a wide range of secondary metabolites. These plant specific compounds represent very important quality traits of plants. They are involved in defense against pests and diseases and responsible for color, taste, flavor and health effects of our food. Many secondary metabolites are economically important fine chemicals such as drugs, flavors, fragrances, dyes, antioxidants and insecticides.

5.2. *Elephantopus scaber* (leaves) as hypoglycemic plant

Diabetes is a condition primarily defined by the level of hyperglycemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life. These complications have long been assumed to be related to chronically elevated glucose level in blood. Near-normalization of blood glucose concentrations in patients with insulin-dependent diabetes mellitus can be achieved
safely by intensive insulin therapy (Fanelli et al., 1993). However, the hormone fails as a curative agent for complications of diabetes (Mukherjee and Mukherjee, 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 hypoglycemic agents like sulphonylureas and biguanides are used to treat non insulin-dependent diabetes mellitus. But they cause adverse health effects (Raheja, 1977). Chronic treatment with sulfonylureas and biguanides are also associated with side effects (Rang et al., 1991). Therefore, finding other anti-diabetes agents, especially those made from natural sources is an important area of research.

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Herbal drugs are considered less toxic, relatively cheap and popular (Momin, 1987: Valiathan, 1998) than synthetic one. As per traditional claims, E. scaber is used as a medicinal plant to treat various diseases and clinical conditions. Crude (leaves) extracts and lupeol isolated from the effective petroleum ether (leaves) extract tested in the present study brought about significant hypoglycemic activity in STZ-induced hyperglycemic rats. These
extracts and compound improved the biochemical parameters assessed and the expression of Glut-4 mRNA and protein.

5.2.1. Experimental diabetes

Generally, experimental diabetes is induced either by streptozotocin or by alloxan (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. Papaccio et al., 2000 stated that interferes with cellular metabolic oxidative mechanisms. It 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. Significant increase in the blood glucose level after a single (60 mg/kg b.wt) intra-peritoneal injection of STZ in the present study shows that experimental diabetes is induced.

5.2.2. Plasma Glucose reduction

Significant reduction in the blood glucose level to near normal in STZ-induced diabetic rats could be observed after the daily administration of Elephantopus scaber (leaves) crude extracts for 90 days. This put emphasis on the hypoglycemic effect of the plant extracts. The decrease in blood glucose was considerably greater in the petroleum ether (leaves) crude extract treated diabetic rats than in
hexane, ethyl acetate, and aqueous extract treated rats. Burcelain et al., (1995) reported that the hypoglycemic action of the plant (leaves) extract 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 reduction of blood glucose in the present study may be achieved by either insulin release from pancreatic $\beta$-cells, inhibited glucose absorption in gut, stimulated glycogenesis in liver, increased glucose utilization by the body or insulin like activity of the compounds.

A number of investigations go in accordance with our results. Oral administration of aqueous extract of leaves of *G. sylvestre* was found to normalize blood sugar levels of diabetic animals through $\beta$–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.

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

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. As Vats et al., (2004) stated, this reduction in body weight may be due to insulin deficiency, which leads to proteolysis in muscular tissue. This condition was significantly reversed towards the normal after the oral administration of Elaphantopus scaber (leaves) crude extracts for 90 days. Similar kind of restoration was observed after lupeol treatment also. In this regard, it is wise to consider the statement of Furuse et al. (1993), which states that, the body weight is reduced in the diabetic state whereas the food intake is increased and this recovers during the exposure of hypoglycemic treatment. 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.

5.2.4. Oral Glucose Tolerance Test (OGTT)

In the present study, the oral glucose tolerance pattern of STZ-induced diabetic rats showed a significant rise in the basal
plasma glucose concentration after the glucose load. 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. But the oral glucose tolerance pattern of the lupeol 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 lupeol in glucose homeostasis. It shows that the oral glucose tolerance pattern of the lupeol treated (for 60 days) STZ-induced diabetic animals to be better. This justified the efficacy of lupeol to control elevated blood sugar levels. The results of the present study are similar to the studies of Tanaka et al., (2008) who established the improved glucose tolerance in KK-Ay mice after the treatment with Eriobotrya japonica seeds and Jie Yang et al., (2009) who reported the glucose tolerance pattern of alloxan-induced mice after the treatment Potentilla discolor extract.

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

In diabetic condition glycosylated hemoglobin has been found to increase (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). 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. 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).

Determination of 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). 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). Therefore, glycosylated hemoglobin can be used as an excellent marker of overall glycemic control.

In the present study, a decrease in the hemoglobin content with a proportionate increase in the glycosylated hemoglobin content could be observed. 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 Elephantopus scaber (leaves) extract 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. Best result could be observed in petroleum ether extract treatment. Therefore, at the end of the treatment the level of hemoglobin is
improved and the glycosylated hemoglobin level is decreased. Lupeol treatment also produced the same trend. This result goes in accordance with the result of Kumar and Murugesan, (2008) who established a decreased the level of glyosylated haemoglobin and increased the level of hemoglobin after oral administration of bark extracts of *Helicteres isora* (100, 200mg/kg) to diabetic rats. 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.

### 5.2.6. Liver and skeletal muscle glycogen

One of an important role of liver is the buffering of the postprandial hyperglycemia leading to the synthesis of glycogen. In general, diabetes is associated with hyperglycemia and is known to impair the normal functions of liver including capacity 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; Bhavapiiya *et al.*, 2001; Chakrabarti *et al.*, 2003; Mutalik *et al.*, 2005; Ramesh and Pugalendi, 2006). In the present study, oral administration of *Elephantopus scaber* (leaves) crude extracts for 90 days and lupeol for 60 days increased the skeletal muscle and liver glycogen content in STZ-induced diabetic rats. 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). 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).

5.2.7. 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 STZ-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 *Elephantopus scaber* petroleum ether extract and lupeol 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 *Elephantopus scaber* (leaves) petroleum ether extract and lupeol. This could be possible by the regenerated β-cells in the pancreas. In concurrence 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; Sindurani and Rajmohan, 2000; 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). From this observation, it is clear that lupeol would serve as better oral drug for type-1 diabetes than insulin injection.

**5.2.8. Kidney function tests**

One of the common clinical as well as biological problems in diabetes mellitus is renal injury. 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 micro-albuminuria. 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).

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).

According to Burtis and Ashwood, (1996) creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of glomerular filtration rate. 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).

Streptozotocin diabetes-induced renal damages could 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.

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 *Elephantopus scaber* (leaves) crude extracts and lupeol 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. Bhavapiya et al., (2001) also suggested that Aavirai kudineer (a herbal formulation) treatment could restore the protein content in alloxan diabetic rats.

5.2.9. Lipid parameters

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 (cholesterol, triglycerides, LDL and VLDL) 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; Ravi et al., 2005; Kim et al., 2006; Rajasekaran et al., 2006; 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).

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).

In the present study, streptozotocin- induced diabetic rats had an elevation in the serum lipids. Oral administration of Elephantopus scaber (leaves) crude extracts and lupeol significantly 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; Sachdeva 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, Akanksha et al., 2010)
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 lupeol appears to be through an increase in insulin level, which increased the activity of lipoprotein lipase and decreased fatty acid synthesis.

5.2.10. Serum amino-transferase levels

Ohaeri, (2001) reported that 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 Elephantopus scaber (leaves) 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 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.
In addition, treatment of the diabetic rats with lupeol 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 Chandramohan et al., (2008) who reported that administration of 3-hydroxymethyl xylitol lowered the serum AST, ALT, ALP activities in 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).

5.2.11. 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 (Vestergaard, 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.

In the present study, decreased activity of glucokinase was observed in STZ-induced diabetic rats. The enzymatic activity was increased with *Elephantopus scaber* (leaves) crude extracts and lupeol 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 (leaves) extracts and lupeol 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. Arathi and Sachdanandam, (2003) reported an increase in the levels of these enzymes in diabetic animals. 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 *Elephantopus scaber* (leaves) crude extracts for 90 days and lupeol 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. Many reports have established the efficacy of various plant extracts and compounds in altering the hepatic key enzymes of carbohydrate metabolism (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). Reddy et al., (1995) suggested that 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.
Similar effect observed in the insulin treated rats in the present study, indicates that the plant extracts and lupeol have the potency to stimulate regeneration of β-cells and thereby insulin secretion.

5.2.12. Glycogen synthase and glycogen phosphorylase levels

According to Leloir et al. (1959) glycogen synthetase is the key enzyme involved in the synthesis of glycogen from glucose in skeletal muscle. Gutman, (1986) suggested that glycogen synthase activity is subject to multiple regulatory factors. 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 lupeol 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-cell.

5.2.13. Erythrocyte and tissue ATPase levels

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 because diabetes has a marked effect on the metabolism of a variety of 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. These results go in accordance with the report of Ohinishi et al., (1982). 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, Ca²⁺ and Mg²⁺ ATPases in lupeol 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 lupeol.

5.2.14. Plasma and tissue glycoproteins levels

Mittal et al., (1996) suggested that glycoproteins 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 hexose, hexosamine and sialic acid are the basic components of the glycoproteins. They are carbohydrate-linked protein macromolecules found in the cell surface, which form the principal component of animal cells. Knecht et al., (1990) stated that impairment in the metabolism of glycoproteins plays a major role in the pathogenesis of diabetes mellitus. 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 the present study, increased levels of hexose, hexosamine, fucose and sialic acid were observed in the plasma and tissues of STZ-induced diabetic rats. Treatment with lupeol 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.

5.2.15. Expression of glut-4 mRNA and protein.

Insulin is the most important peptide hormone in the human body. It not only regulates the carbohydrate metabolism, but also stimulates lipogenesis, diminishes lipolysis, and increases amino acid transport into the cells. 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; SchalinJantti et al., 1994; SeungSoon 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.

Oral administration of lupeol for 60 days in STZ-induced diabetic rats could cause a significant raise in the GLUT-4 mRNA and protein expression. 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 lupeol. In view of this, it can be concluded that the lupeol-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).

5.2.16. 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 lupeol treated diabetic rats. The transport of glucose from the extracellular fluid into the cell is affected by a family of proteins known as glucose transporters (Joost et al., 2002). Therefore, in the present study lupeol-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 *Elephantopus scaber* (leaves) 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 *Elephantopus scaber* (leaves) petroleum ether extract better than the other crude extracts.

### 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 (Larson *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,
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; Orci et al., 1973a, b, 1975; Aponte et al.,
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 Elephantopus scaber (leaves) crude extracts brought about an increase in insulin granules
in STZ–induced diabetic rats. The all extracts of *Elephantopus scaber* (leaves) were found to improve the β-cell activity in a dose-dependent manner but petroleum ether extract of *Elephantopus scaber* (leaves) is the most promising one. This confirms that the petroleum ether extract of *Elephantopus scaber* (leaves) possesses regenerative efficacy.

5.4. In-silico Analysis

Improving the oral bioavailability of a compound already optimized for potency is the most time consuming, labor intensive and variable part of the preclinical drug discovery process (Lipinski, 1999). These observations led to the creation of the ‘Rule of 5’. Lipinski compared drug-like compounds with compounds not presumed to be drug-like and determined that compounds with excessive log P, molecular weight (MW), and H-bond donors or acceptors were more likely to have solubility and or permeability problems that would lead to poor oral bioavailability (Lipinski, 1997). Austel, (1989), demonstrated that membrane permeability can be predicted for some compounds with reasonable accuracy based solely on physicochemical parameters. In general dissolution is determined by the highly interdependent influences of aqueous solubility, and lipophilicity (octanol/water log P or log D7.4). Furthermore, log P is a crucial factor governing passive membrane partitioning, influencing permeability opposite to its effect on solubility (i.e. increasing log P enhances permeability while reducing solubility). In connection to
these contexts, in the present study, lupeol satisfied Lipinski’s rule of five.

Lipinski defined oral drug-likeness as ‘compounds that have sufficiently acceptable ADME properties and toxicity properties to survive through the completion of phase I clinical trials’ (Lipinski, 2000). Hence ADMET related discovery screening can be implemented ever earlier into the discovery process.

Avdeef, (1998), proposed that solubility plays an essential role in drug disposition, since the maximum rate of passive drug transport across a biological membrane, is the main pathway for drug absorption. Poor solubility has been identified as the cause of numerous drug development failures. The aqueous solubility level was calculated using Accord for Excel. Chen and Merz, (2003), described that solubility level ‘3’ denotes ‘good solubility’ whereas level ‘4’ indicates ‘optimal solubility’ and level ‘5’ represents ‘very soluble’ nature of compound. It is wise to connect here that, lupeol is good soluble in nature since its solubility was calculated as 3.

Li, (2001), stated that for a chemical to enter the systemic circulation after oral administration, it needs to survive intact through the low pH environment of the stomach, and then absorbed in the duodenum and the small intestines. Drug absorption is greatly influenced by physiological factors such as transit time, fluid contents and membrane properties. All these factors impact the ability of the compound to dissociate, to dissolve and to associate with the lipid
bilayer of the intestinal epithelium. In Accord for Excel, the human intestinal absorption level (HIA) '0' indicates good absorption (Egan and Lauri, 2002). In connection to this concept, lupeol found to have good absorption.

Li, (2002), proposed that drug toxicity is a major problem in drug development. A large number of drugs, in spite of extensively preclinical animal safety studies and clinical human trials, have been found to cause severe human toxicity, leading to market withdrawal or severe use limitations. Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another noninfectious agent. According to Weinshilboum, (2003), the liver, located between the absorptive surface of the gastrointestinal tract and drug targets throughout the body, is central to the metabolism of virtually every foreign substance. Most drugs are lipophilic, enabling them to cross the membranes of intestinal cells. Drugs are rendered more hydrophilic by biochemical processes in the hepatocyte, yielding water-soluble products that are excreted in urine or bile.

Hepatotoxicity may be predictable or unpredictable (Kaplowitz, 2004). Zimmerman, (1978), suggested that predictable reactions typically are dose-related and occur in most persons who are exposed shortly after some threshold for toxicity is reached. Unpredictable hepatotoxic reactions occur without warning, are unrelated to dose, and have variable latency periods, ranging from a few days to 12
months. The clinical presentations of hepatotoxicity that are most readily distinguished are acute hepatocellular injury and cholestatic liver disease.

Cells within the liver may be the target of drug injury or serve as modulators of an incipient reaction. For example, Kupffer’s cells activate cytokines that may amplify injury, and fat-storage cells (stellate cells) or macrophages may augment injury, produce fibrosis, or form granulomas (Jonsson et al., 2000). Chemotherapeutic agents can injure sinusoidal endothelial cells, a process that can lead to veno-occlusive disease (DeLevee et al., 2002).

Dixon, (1999), stated that hepatotoxicity model predicts potential organ toxicity for a wide range of structurally diverse compounds using Accord for Excel Thus lupeol has satisfied both the Lipinski’s rule of five and ADMET, achieving the status of ‘oral drug-likeness’.

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 2A°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, lupeol 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 et al., 1999; Muegge, 2006). A high Lobdock score obtained in the present docking study confirms that there is a stronger receptor-ligand binding affinity of lupeol with the receptor.

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. The design of dual agonist is of major importance. Lupeol formed 2 hydrogen bonds with Insulin receptor at ALA 1028, and GLU 1077 with a total of 16 contacts.

The reference drug thiazolidinedione, chosen for the present study has 117.12 dalton molecular weight, 1 hydrogen bond donors, 5 hydrogen bond acceptors, A log P value is 7.24. It has log P value greater than 5, indicating lower solubility but due to its lower molecular weight, it has medium permeability. Thazolidinedione, has HIA level as ‘0’ representing very good absorption and it has hepatotoxicity value as ‘1’ indicating ‘toxic effect’. It has energy value of about 83.855 for drug-receptor complex which denotes higher energy value and the libdock score is 125.036.

Thiazolidinedione is widely used in the treatment of type-2 diabetes mellitus. However, it should be noted that thiazolidinedione violates some of the molecular properties. Nonetheless, phytocompound lupeol satisfy both Lipinski’s rule of five and ADMET properties. It also has produced good results for molecular interaction with the receptor 1IRK in docking models. It is wise to recall here that insulin receptor mediated transactivation of IRS, frequently accounts for the activation of PKC and Akt cascade through PI3 Kinase. This world in-turn translocate the GLUT to the membrane and transporting glucose in and out of the cells thus leading to a
reduction in the blood glucose level. Hence diabetic condition could be managed. In connection to this context, lupeol has molecular interaction with receptor insulin receptor, indicating it has ability to activate insulin receptor, thus it may result in GLUT-4 translocation, modulation of several metabolic pathways and leading to the management of diabetes.

As the *in-vivo* (animal studies) results coincide with the validated *in-silico* data, it is clear that lupeol 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. Lupeol loaded Chitosan-alginate (CS-ALG) nanoparticles**

Nanotechnology has opened up new vistas in the field of drug delivery with potential clinical applications (Kayser *et al.*, 2005). The development of alginate based drug delivery systems makes use of the ability of this polymer to undergo gelation in presence of divalent cations. However, a critical adjustment in the relative concentrations of alginate and the cation results in a pregel state, *i.e.*, alginate nanoparticles that can be harvested by high-speed centrifugation. This principle was initially exemplified taking doxorubicin as a model drug (Rajaonarivony *et al.*, 1993) and the same technique was employed for encapsulating lupeol in the present study.
The “incorporation method” for drug-loading purposes was followed. Encapsulation of the model drug lupeol into the hydrophilic CS-ALG nanoparticulate system can be better understood from similar research done earlier (Li et al 2008a). Incorporation of an ethanol solution of lupeol into calcium chloride aqueous solution resulted in nanocrystals of lupeol. As described in the Methods section, before the pre-gelation step free lupeol was separated from lupeol incorporated calcium chloride solution by centrifugation at lower speed (3000 rpm) so that only undissolved lupeol in the aqueous phase precipitated. The light yellow coloration of the supernatant shows the presence of lupeol as nanocrystals. Pre-gel of calcium alginate as discrete NPs along with lupeol nanocrystals were embedded when cross-linked with the polycationic polymer CS, and a nanodispersion of lupeol-loaded ALG-CS-NPs was obtained. Thus, water miscibility of ethanol and lupeol behavior in aqueous phase are the two important factors causing successful loading of hydrophobic lupeol to hydrophilic CS-ALG NPs.

The efficiency of drug encapsulation/loading depends on the type of drug and the resultant drug-polymer interactions. Hence, the drug encapsulation efficiency ranged from 80%-90% for lupeol. The results were better than that previously obtained for alginate microspheres (30%-70%) (Qurrat-ulAin et al., 2003; Pandey and Khuller, 2004). This is because nanoparticles possess a higher surface area to volume ratio as compared to microspheres. The nanoparticles
mediated drug delivery has been found to greatly enhance the therapeutic index of a drug. A drug when administered inside the body along with a high molecular weight polymer performs better in terms of its increased bioavailability with reduced side effects (Verma et al., 2005).

The drug mashed with the polymer would be available for interaction with other blood factors and it is released slowly in a controlled fashion as the polymer swells or degrades in aqueous media. The gelation nanoparticles were applied as drug delivery vehicles for steady and controlled release of lupeol in aqueous media. The entrapment efficacy of the CS-ALG nanoparticle was found to be 50% and the drug release was approximately 30%. In the present study, the drug lupeol was entrapped in biodegradable CS-ALG nanoparticle of size 600nm and its release was studied over 72h in PBS 7.4 and the drug release was found to be higher in body temperature than room temperature. This results go in accordance with the report of Gadspy, (2002), who studied the in-vitro release of drug from a mixed micelles in PBS 7.4 at different temperatures. The difference in release from dialysis bag for both free and lupeol loaded CS-ALG nanoparticle is evidently attributed to the prolonged release function of the nanoparticle (Li et al., 2008b). This implies that lupeol loaded in CS-ALG can be released slowly and kept at a constant concentration for long period both in-vitro and in-vivo, which is very important for the clinical application.
Drug loading capacity measurement revealed a high loading of lupeol. According to a previous research, the strong hydrophobicity of polymers enables a large quantity of a drug to be trapped in the polymeric nanoparticles, which strengthens the stability of the drug delivery system (Senthilkumar et al., 2008a). In-vitro release study further demonstrated the strong affinity between lupeol and CS-ALG. In addition, one thing needs to be clarified that the release of free lupeol in-vitro is actually the diffusion rate of free lupeol through the dialysis bag and lupeol loaded CS-ALG nanoparticle release in-vitro is the sum of lupeol release from nanoparticles and diffusion rate of lupeol through the dialysis bag. As the in-vitro release data showed, lupeol was released in a sustained manner from the core-shell structure of polymeric nanoparticles. Thus, the slow dissociation of lupeol from the polymeric micelles to the targeted tissues also may provide a more sustained release of the drug.

The sustained release of formulated lupeol would be less likely to cause systemic toxicity than the continuous systemic administration of conventionally formulated lupeol during similar periods of time. It has been demonstrated in other studies that the in-vivo toxicity in the various drug delivery systems was found to be significantly lower than that of a conventional formulation of paclitaxel (Zhang et al., 1997; Li et al., 1999).
5. 6. Mode of Action of the Chosen Plant and lupeol isolated from petroleum ether extract

*Elephantopus scaber* (leaves) extracts and lupeol are found to possess plasma glucose level lowering effect and re-establishing all the altered metabolic impairments of STZ-induced diabetes.

In the present study, intra-peritoneal injection of STZ caused degranulation and degeneration of almost all β-cells, leading to a decrease in insulin secretion and thereby increases in blood glucose concentration. Oral administration of *Elephantopus scaber* (leaves) extracts for 90 days and lupeol, the pure compound isolated from the petroleum ether extract of *Elephantopus scaber* (leaves), for 60 days, 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. In particular petroleum ether extract caused higher level β-cell regeneration and further insulin release. This suggests that the mode of action of *Elephantopus scaber* (leaves) and lupeol the pure compound isolated from the petroleum ether extract of *Elephantopus scaber* (leaves), 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.