Discussion
The currently available drug regimens for management of diabetes mellitus have certain drawbacks and, therefore, there is a need to find safer and more effective anti-diabetic drugs (UGDP, 1974; Knatterud et al., 1978; Grover et al., 2000). Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, neuropathy, nephropathy, etc. These complications have long been assumed to be related to chronically elevated glucose level in blood. Methanolic and aqueous extracts of the plants tested in the present study brought about significant hypoglycemic activity in alloxan-induced hyperglycemic rats. These extracts improved the biochemical parameters assessed and also brought about regeneration of β-cells of pancreas.

5.1 Biochemical Parameters

5.1.1 Blood Glucose

In most of the experimental studies hyperglycemia was induced by streptozotocin or alloxan (Leatherdale et al., 1981; Lamela et al., 1986; Day et al., 1990; Karunanayake et al., 1990). It is well known that alloxan selectively destroys insulin-secreting β-cells in the islets of Langerhans and the effects are irreversible (Fischer, 1985; Cakici et al., 1994). Alloxan causes a massive reduction in insulin release by the destruction of β-cells of the islets of Langerhans and thereby induces hyperglycemia (Dunn et al., 1943; Goldner and Gomori, 1943; Lazarow, 1954; Lazarus et al., 1958; Butt, 1962; Marquis et al., 1977, Chakravarthy et al., 1982; Owerbach et al., 1982; Halliwell and Gutteridge, 1985; Joseph, 1985; Taha, 1991; Al-Shamaony et al., 1994; Taha and Raza, 1996; Jafri et al., 2000; Prince and Menon, 2000; Sabu et al., 2002; Siyem et al., 2002; Beppu et al., 2003). 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). The hypoglycemic effect of aloe and its bitter principle in alloxan-induced diabetic mice may be mediated through enhanced synthesis and/or release of insulin from the β-cells of Langerhans (Ghannam et al., 1986; Ajabnoor, 1990; Bolkent et al., 2004). The lowering of blood sugar level in streptozotocin-induced hyperglycemic animals through the administration of Cassia alata leaf extract may be due to a stimulating effect on insulin release from regenerated β-cells of the pancreas or increased cellularity of the islet tissues and regeneration of the granules in the β-cells (Palanichamy et al., 1988).

Flavonoid fraction of Pterocarpus marsupium has been shown to cause pancreatic β-cell regranulation and may explain the antidiabetic activity of the plant (Chakravarthy et al., 1980, 1981, 1982; Sheehan et al., 1983). Epicatechin, a pure flavonoid isolated from the ethanolic extract of P. marsupium bark has also been shown to possess significant antidiabetic effect in alloxan-diabetic rats supporting the possibility of β-cell regeneration (Chakravarthy et al., 1982; Hii and Howell, 1984; Ahmad et al., 1991; Rizvi et al., 1995; Kameswara Rao et al., 2001a). Leaf and flower extracts of Vinca rosea were shown to cause regeneration of β-cells in alloxan-induced diabetic rats (Ghosh and Suryawanshi, 2001) and similar effects in streptozotocin treated diabetic animals were induced by pancreas tonic (Rao et al., 1998),
ephedrine (Xiu et al., 2001) and Gymnema sylvestre leaf extracts (Shanmugasundaram et al., 1990a). 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, 1990a). EMC-D (plaque purified from mouse heart passaged M variant) virus–induced diabetes in mice provides a new model to study the process of regeneration in pancreatic β–cells and reversal of diabetes. Such a spontaneous regeneration has been reported in streptozotocin–induced diabetes in mice, which provides another model to investigate the mechanisms and promoting factors of such restorative processes (Bhonde, 1996). Methanolic and aqueous extracts of Terminalia catappa exhibited significant anti-hyperglycemic activity in alloxan-induced hyperglycemic rats (Nagappa et al., 2003). The damage of pancreas in alloxan–treated diabetic control rats and regeneration brought about by the extracts of Terminalia catappa fruit may be due to β–carotene, which was reported to be a constituent of T. catappa fruit (Duke, 1992). Beneficial role of β–carotene in reducing diabetic complications like glycosylation in diabetic rats has been reported (Aruna et al., 1999; Olmedilla, 1999; Ladeji et al., 2003; Nagappa et al., 2003).

Aqueous extract of kernels of Eugenia jambolana exerts a dual effect, namely a combination of mechanism of action of sulfonylureas and biguanides in alloxan-induced diabetic rats (Grover et al., 2000, 2002a). Extract of kernels of E. jambolana (200 mg/kg), administered for 50 days in streptozotocin–induced diabetic mice, reduced the plasma glucose level and tail flick latency but gastric emptying rate was increased (Grover et al., 2001,
Oral administration of 2.5 and 5 g/kg body weight of the aqueous extract of the seed for 6 weeks resulted in a reduction in blood glucose in alloxan-induced diabetic rats. The possible mechanism by which the seed extract brought about the hypoglycemic action may be by potentiation of the insulin effect by increasing either the pancreatic secretion of insulin from β-cells of islets of Langerhans or its release from the bound form (Prince et al., 1998). Feeding of diets containing neutral detergent fibre and water soluble gummy fibre isolated from *E. jambolana* seeds to alloxan-induced diabetic rats for 21 days indicated that the water soluble gummy fibre exhibited pronounced hypoglycemic effect, whereas neutral detergent fibre was ineffective (Phyllis, 1994; Pandey and Khan, 2002). Ethanolic extract of the seeds of *E. jambolana* was investigated in alloxan-induced diabetic rabbits. The mechanism of action appeared to be both pancreatic (Bansal et al., 1981) and extra-pancreatic since reduced insulin degradation was noticed after the administration of the extract of the seeds because of insulinase inhibition (Achrekar et al., 1991). This shows that *E. jambolana* is effective in decreasing blood glucose not only in NIDDM but also in IDDM (Grover et al., 2000, 2002a; Sharma et al., 2003).

Daily administration of methanolic extract of *Phyllanthus emblica* (Sabu and Kuttan, 2002) and aqueous extracts of *P. acidus* and *P. emblica* (Daisy et al., 2004a, b) brought about a reduction in blood glucose level. In the present investigation daily administration of aqueous and methanolic extracts of *Elephantopus scaber* (root/leaf), *Eugenia jambolana* (seed/bark), *Clitoria ternatea* (leaf/flower) and *Phyllanthus* fruits (*emblica/acidus*) resulted in a decrease in blood glucose level in alloxan-induced diabetic rats. The
decrease in blood glucose was considerably greater in the aqueous extract-treated diabetic rats than in methanolic extract-treated ones.

5.1.2 Glycosylated Hemoglobin

Glycosylated hemoglobin is produced through glycosylation of hemoglobin. Increased non-enzymatic and autooxidative glycosylation is one of the possible mechanisms linking hyperglycemia and the vascular complications of diabetes. Measurement of glycosylated hemoglobin reflects the blood glucose equilibrium 6-8 weeks prior to sampling (Kennedy et al., 1981; Kameswara Rao et al., 2003). 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 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 (Bunn et al., 1976, 1978; Bunn, 1981; Guoyan, 1992).

In this study, the diabetic rats had higher levels of glycosylated hemoglobin in comparison to normal rats. This indicates the poor glycemic control (Al-Shamaony et al., 1994; Mitra et al., 1995; Trejo-Gonzalez et al., 1996; Prince et al., 1998; Prince and Menon, 2000; Vijayvargia et al., 2000; Andallu and Varadacharyulu, 2002; Pari and Latha, 2002). The increased
glycosylated hemoglobin in the diabetic control rats indicates that erythrocytes are more prone to oxidative stress in diabetes. The abnormal hemoglobin is associated with a reduction in red cell life span (Allen, 1964). 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).

Momordica cymbalaria (Kameswara Rao et al., 2003), Artemisia herba alba (Al-Shamaony et al., 1994), Enicostemma littorale Blume (Vijayvargia et al., 2000), D-400, a herbomineral formulation (Mitra et al., 1995), fenugreek seeds (Sharma et al., 1996a) and mulberry leaves (Andallu and Vardacharyulu, 2002) reduced the glycosylated hemoglobin level in diabetic rats. A significant reduction in glycosylated hemoglobin was noted with GS₄ (water soluble extract from Gymnema sylvestre leaf) supplementation in diabetic patients (Shanmugasundaram et al., 1990b). Ethanolic extract of seeds of Eugenia jambolana exhibited significant reduction in glycosylated hemoglobin in alloxan-induced diabetic rabbits (Sharma et al., 2003). In accordance with these reports, the diabetic rats in the present study had shown higher levels of glycosylated hemoglobin than in untreated rats, indicating the poor glycemic control. The significant decrease of glycosylated hemoglobin in alloxan–induced diabetic rats due to ESR, ESL, EJS, EJB, CTL, CTF, PEF and PAF therapy indicates that the overall blood glucose control is improved which must be due to improvement in insulin secretion.
5.1.3 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; Bhavapriya et al., 2001; Chakrabarti et al., 2003). Insulin plays a crucial role in lowering blood glucose level by enhancing glycogenesis in liver and muscles. Assessment of glycogen serves as a marker for studying insulinomimetic activity. Glycogen content of skeletal muscles and liver markedly decreases in diabetes (Prasannan and Subramanyam, 1965; Losert et al., 1966; William and Goldberg, 1967; Murphy and Anderson, 1974; Whitton and Hems, 1975; Khandelwal et al., 1977; Welihinda and Karunanayake, 1986; Hikino et al., 1989; Ferrannini et al., 1990; Grover et al., 2000; Annapurna et al., 2001; Huang et al., 2000; Vats et al., 2003). Decrease in glycogen synthetase during diabetic condition has been reported (Chang, 1972; Prasannan, 1973; Stearns and Camillo, 1977). The decrease observed in the present study is probably due to the lack of insulin in the diabetic state, which results 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).

Muscle glycogen content was low in diabetic control animals. It increased several fold in diabetic animals treated with Catharanthus roseus (Singh et al., 2001), Aegle marmelos leaf powder (Ponnachan et al., 1993),
*Trigonella foenum graecum* (Vats *et al.*, 2003), D-400, a herbomineral formulation (Mitra *et al.*, 1996), *Momordica charantia* (Kedar and Chakrabarti, 1982) and *Momordica cymbalaria* (Kameswara Rao *et al.*, 2003). Treatment of alloxan induced diabetic animals with *Gymnema sylvestre* leaves resulted in an increase in glycogen content in liver and skeletal muscle. This may be due to an increase in glucose uptake by the tissues following *G. sylvestre* administration, increased glycogen synthesis or a combination of both, probably mediated through the action of insulin (Shanmugasundaram *et al.*, 1983). Treatment with ethanolic extract of seeds of *Eugenia jambolana* prevented the depletion in liver and skeletal muscle glycogen content in alloxan-induced diabetic rabbits (Sharma *et al.*, 2003).

Similar to the above findings, an increase in skeletal muscle and liver glycogen content was found in ESR, ESL, EJB, CTL, CTF, PEF and PAF-treated diabetic rats. This prevention of glycogen depletion 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).

### 5.1.4 Serum Insulin

The serum insulin level decreases in diabetic animals (Benwahhoud *et al.*, 2001). The serum insulin level decreased in diabetic animals, whereas diabetic rats treated with ethanolic extract of *Hibiscus rosa-sinensis* flower had an increase in their serum insulin level (Sachdewa and Khemani, 2003). *Urtica dioica* leaf extract treatment brought about a marked increase in serum insulin in streptozotocin-induced diabetic rats (Farzami *et al.*, 2003). The hypoglycemic activity of the leaves of *Globularia alypum* was reported to be
due to an elevation of plasma insulin level (Skim et al., 1999a, b; Jouad et al., 2002). Administration of aqueous extract of *Enicostemma littorale* increased the serum insulin level in alloxan–induced diabetic rats suggesting its possible action by increasing insulin release as well as activating insulin gene transcription (Efrat et al., 1991; Maroo et al., 2002). It was found that administration of yacon tea (*Smallanthus sonchifolius* leaves) increased the circulating plasma insulin levels (Aybar et al., 2001). This increase may be a consequence of the stimulation of insulin synthesis and secretion, and/or inhibition of insulin degradation, since many compounds present in plants have been demonstrated to produce these effects (Venkateswaran and Pari, 2002, 2003). For instance, benzoic acid-related molecules inhibit insulinase and enhance insulin effects (Marles and Famsworth, 1995; Peungvicha et al., 1998; Aybar et al., 2001).

Serum insulin level was increased in *Gymnema sylvestre* administered diabetic rabbits suggesting the insulinotropic activity of *G. sylvestre* leaf extract (Shanmugasundaram et al., 1981, 1983). The aqueous extract of *Momordica charantia* fruit possesses significant hypoglycemic activity on long-term treatment in diabetic rats. The hypoglycemic activity of *Momordica charantia* is associated with an increase in plasma insulin levels, suggesting the insulinogenic activity of the extract. The increased levels of insulin in extract-treated diabetic rats indicated that *M. charantia* extract stimulates insulin secretion from regenerated β-cells (Karunanayake et al., 1984; Cakici et al., 1994; Kameswara Rao et al., 2003). In the present study also, serum insulin level of diabetic animals treated with the extracts of *Elephantopus scaber* (roof/leaf), *Eugenia jambolana* (seed/bark), *Clitoria ternatea*
(leaf/flower) and *Phyllanthus* fruits (*emblica/acidus*) increased when compared to the diabetic controls.

### 5.1.5 Glucokinase

Diabetes mellitus is characterized by partial or total deficiency of insulin, resulting in derangement of carbohydrate metabolism and a decrease in the activities of glucokinase and hexokinase, thereby depleting liver and muscle glycogen (Murphy and Anderson, 1974; Chang et al., 1977; Storey and Bailey, 1978; Hikino *et al.*, 1989; Grover *et al.*, 2000; Singh *et al.*, 2001). Glucokinase, an insulin-dependent enzyme, 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). The activity of this enzyme is decreased during diabetes, leading to reduced conversion of glucose as glucose-6-phosphate (Sheela and Augusti, 1992; Prince and Menon, 2000; Bhavapriya *et al.*, 2001).

The activity of glucokinase is increased on treatment with *Catharanthus roseus* extracts *in vitro* (Singh *et al.*, 2001). Administration of *Tinospora cordifolia* root extract (Prince and Menon, 2000) and leaves of *Gymnema sylvestre* (Shanmugasundaram *et al.*, 1981, 1983) to diabetic animals increased the activity of glucokinase in liver. The increased activity of glucokinase can cause an increase in glycolysis and, thereby, utilization of glucose for energy production. The extract-induced decrease in the concentration of blood glucose in alloxan-treated rats may be the result of increased glycolysis (increased glucokinase activity) (Prince and Menon, 2000). In the present study, the increased activity of glucokinase in
Elephantopus scaber (root/leaf), Eugenia jambolana (seed/bark), Clitoria ternatea (leaf/flower) and Phyllanthus fruits (emblica/acidus)–treated diabetic rats implies that entry of glucose into the cells is facilitated by the plant extracts, which in turn would stimulate the activity of this enzyme. This glucose influx could be due to the insulin releasing effect of the plant extracts through β-cell regeneration.

5.1.6 Glucose-6-Phosphatase

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). 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. Aavirai Kudineer treatment suppressed the hepatic gluconeogenesis by reducing the activity of glucose-6-phosphatase (Bhavapriya et al., 2001). The enzyme activity was brought down to almost normal in moderately diabetic rats and decreased to a significant extent in the case of severe diabetic rats on treatment with the aqueous extract of Enicostemma littorale Blume (Vijayvargia et al., 2000). Administration of Cassia auriculata (Pari and Latha, 2002), Hibiscus rosa-sinensis flower extract (Sachdewa and Khemani, 2003) and Gymnema sylvestre leaf extract (Shanmugasundaram et al., 1983) decreased the activity of glucose-6-phosphatase in the liver of diabetic rats.
The activity of glucose-6-phosphatase decreased in diabetic mice after the administration of sepia shell extract (Reddy et al., 1995). During the extract-induced hypoglycemia, the blood glucose was reduced and there was an enormous increase in liver glycogen content. This may be due to mobilization of blood glucose towards liver glycogen reserve. The decrease in the activity of glucose-6-phosphatase indicates that the extract decreased the endogenous production of glucose from the intermediate metabolites viz., glucose-6-phosphate and pyruvate (Shanmugasundaram et al., 1983). The inhibition of glucose-6-phosphatase activity after administration of the extract 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). In the present investigation also it was found that the activity of glucose-6-phosphatase increased in the liver of diabetic rats. On the other hand, administration of aqueous and methanolic extracts of *Elephantopus scaber* (root/leaf), *Eugenia jambolana* (seed/bark), *Clitoria ternatea* (leaf/flower) and *Phyllanthus* fruits (*emblica/acidus*) decreased the activity of glucose-6-phosphatase indicating that gluconeogenesis is inhibited in extract-treated diabetic rats.

### 5.1.7 Serum Cholesterol

Insulin deficiency or insulin resistance is associated with hypercholesterolemia and hypertriglyceridemia (Tchobroutsky, 1978; Rodrigues et al., 1986; Durrington, 1993; Sharma et al., 1996b; Kameswara Rao et al., 2003; Sachdewa and Khemani, 2003). 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.,
High levels of total cholesterol and, more importantly LDL-cholesterol, in blood are major coronary risk factors (Fredrickson et al., 1967; Mizuguchi, 1968; Kannel et al., 1971; Levy et al., 1972; Epstein, 1992; Schwartz et al., 1993; National Cholesterol Education Program Expert Panel, 1994; Vaidya, 1994; Bhavapriya et al., 2001; Hannan et al., 2003).

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 particles (Balasee et al., 1972; Taskimen, 1987; Murali et al., 2002). On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may, therefore, be regarded as a consequence of the stimulatory action of lipolytic hormones in the fat depots (Goodman and Gilman, 1985; Goodman-Gilman et al., 1990; Al-Shamaony et al., 1994).

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). Administration of soluble dietary fibre fraction of *Trigonella foenum graecum* to diabetic rats lowered total cholesterol (Hannan et al., 2003). Aqueous extract of *Suaeda fruticosa* brought about a significant reduction in plasma cholesterol in diabetic rats (Benwahhoud et al., 2001). Root of *Withania somnifera*, when administered to diabetic patients, brought about a significant decrease in serum cholesterol (Andallu and Radhika, 2000). Tincture of panchpama, a polyherbal formulation, brought about a decrease in serum cholesterol level in alloxan-induced diabetic rats (Annapurna et al., 2001). The observed hypolipidemic effect may be due to decreased synthesis of cholesterol and fatty acid (Bopanna et al., 1997). Earlier studies have reported a similar lipid lowering activity of some medicinal plants like *Terminalia arjuna* tree bark (Ram et al., 1997) and *Caesalpinia bonducella* seed (Sharma et al., 1997). Ethanolic extract of seeds of *Eugenia jambolana* brought about hypocholesterolemic effect in alloxan-induced diabetic rabbits (Shamria et al., 2003). The hypocholesterolemic effect of *Phyllanthus emblica* fruit juice has been shown in a number of studies (Tariq et al., 1977; Thakur and Mandal, 1984; Bhandari, 1989; Mathur et al., 1996).

In the present study also, alloxan-induced diabetic rats had an increase in serum total cholesterol. Administration of aqueous and methanolic extracts of *Elephantopus scaber* (root/leaf), *Eugenia jambolana* (seed/bark), *Clitoria ternatea* (leaf/flower) and *Phyllanthus* fruits (emblica/acidus) brought about a significant reduction in serum cholesterol in diabetic rats. The aqueous extracts of these plants were more effective in decreasing the serum
cholesterol than the methanolic extracts. This hypolipidemic effect may be due to an increase in insulin secretion that ultimately led to a decrease in the synthesis of cholesterol and fatty acid.

5.1.8 Serum Triglyceride

Coronary artery disease, as a result of premature atherosclerosis, is a major cause of death both in type-1 and type-2 diabetes (American Diabetes Association, 1989; Fontbonne et al., 1989; Carlsen et al., 1996; Reed et al., 1999; Pushparaj et al., 2000; Chakrabarti et al., 2002). Although the exact cause of premature atherosclerosis in diabetes is not well understood, several independent risk factors such as hypertriglyceridemia and hypertension may contribute to coronary artery disease (Gjesdal et al., 1976; Reinila, 1981; Ferrannini et al., 1987; Zavaroni et al., 1987; Bainton et al., 1992; Schwartz et al., 1993; National Cholesterol Education Program Expert Panel, 1994; Reed et al., 1999). The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Shanmugasundaram et al., 1990b; Jaiprakash et al., 1993; Khan et al., 1995; Mitra et al., 1995; Sharma et al., 1996b; Andallu and Radhika, 2000; Chen et al., 2001; Pepato et al., 2002; Kameswara Rao et al., 2003). Hypertriglyceridemia is also associated with metabolic consequences of hypoinsulinemia, insulin resistance and glucose intolerance (Zavaroni et al., 1989; Ginsberg, 1994; Burcelin et al., 1995; Jouad et al., 2003; Sachdeva and Khemani, 2003). The development of hypertriglyceridemia in uncontrolled diabetes is a consequence of a number of metabolic abnormalities that occur sequentially. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased production of VLDL-triglyceride in the liver (Balasse et
al., 1972; Murali et al., 2002; Kameswara Rao et al., 2003). With longer insulin deficiency, the liver converts free fatty acids into ketone bodies (Basso and Havel, 1970; Bainton et al., 1992). At the same time, lipoprotein lipase activity decreases 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).

Repeated administration of the leaf extract of *Hibiscus rosa-sinensis* decreased serum triglyceride in diabetic rats (Sachdewa and Khemani, 2003). Ethanolic extract of *Helicteres isora* caused a significant reduction in serum triglyceride in diabetic mice (Chakrabarti et al., 2002). Repeated administration of *Cinnamomum tamala* leaf extract prevented the elevation of both total cholesterol and triglyceride in diabetic rats (Sharma, 1996b). The hypolipidemic effect may be due to decreased fatty acid synthesis (Bopanna et al., 1997; Chakrabarti et al., 2003). The strong hypolipidemic effect brought about by the extract is in accordance with the earlier findings (Ahmed and Sharma, 1997; Sheela et al., 1995). *Averrhoa bilimbi* leaf extract reduced the triglycerides by decreasing the serum non-esterified fatty acids (NEFA) in diabetic rats (Pushparaj et al., 2000). Administration of ethanolic extract of seeds of *Eugenia jambolana* brought about hypotriglyceridemic effect in alloxan-induced diabetic rabbits (Sharma et al., 2003). Administration of *Phyllanthus emblica* fresh juice reduced serum triglycerides in cholesterol-fed rabbits (Thakur and Mandal, 1984; Bhandari, 1989; Mathur et al., 1996).
In the present study, a significant increase in serum triglyceride was observed in alloxan-induced diabetic rats. Aqueous and methanolic extracts of *Elephantopus scaber* (root/leaf), *Eugenia jambolana* (seed/bark), *Clitoria ternatea* (leaf/flower) and *Phyllanthus* fruits (*emblica/acidus*) brought about a significant reduction in serum triglyceride level of alloxan-induced diabetic rats. The aqueous extracts brought about more decrease in serum triglycerides than methanolic extract. The mechanism of action of the plant extracts appears to be through an increase in insulin level, which increased the activity of lipoprotein lipase and decreased fatty acid synthesis.

### 5.1.9 HDL-cholesterol

Significant lowering of total cholesterol and rise in HDL-cholesterol is a very 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; Sachdeva 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 alloxan-induced diabetic rats, the rise in blood glucose is accompanied by an increase in serum cholesterol and triglyceride and decrease in HDL-cholesterol. Treatment with aqueous extract of *Momordica cymbalaria* decreased serum cholesterol and triglycerides and increased HDL-cholesterol level (Kameswara Rao *et al.*, 2003). Methanolic and
aqueous extracts of *Terminalia catappa* fruit increased the serum HDL-cholesterol in alloxan-induced diabetic rats (Nagappa *et al.*, 2003). Soluble dietary fibre fraction of *Trigonella foenum graecum*, while lowering total cholesterol, increased the HDL-cholesterol significantly (Hannan *et al.*, 2003). Ethanolic extract of *Hibiscus rosa-sinensis* flower significantly lowered total cholesterol and increased HDL-cholesterol in diabetic rats (Sachdewa and Khemani, 2003). Leaf extract of *Averrhoa bilimbi* increased HDL-cholesterol in diabetic rats and, thus, it has the potential to prevent the onset of atherosclerosis and coronary heart disease which are the secondary diabetic complications of severe diabetes mellitus (Fontbonne *et al.*, 1989; Pushparaj *et al.*, 2000). When atherogenic diet was given to rabbits along with *Phyllanthus emblica* fruit juice, HDL-cholesterol was higher than in rabbits given the atherogenic diet alone (Mathur *et al.*, 1996).

Ethanolic extract of seed of *Eugenia jambolana* exhibited hypocholesterolemic and hypotriglyceridemic effects while increasing the HDL–cholesterol (Sharma *et al.*, 2003). In the present study, HDL–cholesterol was decreased in diabetic rats. However, administration of the plant extracts brought about a significant increase in HDL–cholesterol. Aqueous and methanolic extracts of *Elephantopus scaber* (root/leaf) brought about a greater increase in HDL–cholesterol level in alloxan–induced diabetic rats than by *Eugenia jambolana* (seed/bark), *Clitoria ternatea* (leaf/flower) and *Phyllanthus* fruits (*emblica/acidus*).
5.1.10 Total Protein

Renal disease is one of the most common and severe complications of diabetes (Siperstein et al., 1973; West, 1981; Serrano et al., 1983; Shanmugasundaram et al., 1990a; Das et al., 1996; Kakkar et al., 1997; Sassy-Prigent et al., 2000). Distinct metabolic renal alterations are demonstrable in experimental diabetes, leading to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis (Pathak and Dhawan, 1998; Bhavapriya et al., 2001). Insulin is a physiological factor, which plays an important role in the maintenance of protein balance, since it not only stimulates the uptake of amino acids and protein synthesis, but also inhibits protein degradation. Neuropathy of peripheral neurons, increased proteolysis and decreased protein synthesis, vascular dysfunction and decreased carbohydrate metabolism are some of the typical features of induced experimental diabetes (Jasra and Talesara, 1986). There is a decline in total protein content of blood in alloxan-induced diabetic rat. The protein content increased in Aavirai Kudineer-treated diabetic rats (Bhavapriya et al., 2001).

The total protein was restored to the normal range in Vinca rosea flower- and leaf-treated diabetic rats (Ghosh and Suryawanshi, 2001). Alloxan-induced diabetic rats had a decrease in serum protein content. Methanolic and aqueous extracts of Elephantopus scaber (root/leaf), Eugenia jambolana (seed/bark), Clitoria ternatea (leaf/flower) and Phyllanthus fruits (emblica/acidus) brought about a significant increase in serum protein content of alloxan–induced diabetic rats.
5.1.11 Serum Urea

The blood urea levels increased significantly in alloxan–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). Indeed, a relationship between treatment–related alterations in urea concentration and histopathology of the kidney has been reported in rats (Ponnachan et al., 1993; Travlos et al., 1996; Bolkent et al., 2004). *Enicostemma littorale* prevented kidney dysfunction in diabetic rats by bringing down the serum urea concentration (Murali et al., 2002). *Aavirai Kudineer* treatment brought down urea levels in alloxan–induced diabetic rats, indicating its positive effect on renal function (Bhavapriya et al., 2001). A decrease in blood urea with the onset of *Gymnema sylvestre* leaf therapy was recorded. It suggests that the onset of secondary complications, microangiopathy or diabetic kidney disease, may be delayed by herbal therapy (Shanmugasundaram et al., 1990b). Methanolic and aqueous extracts of *Terminalia catappa* fruits produced significant decrease in serum urea in alloxan–induced diabetic rats (Nagappa et al., 2003). In *Aegle marmelose* leaf extract–treated diabetic rats, the blood urea was brought back to the control level (Ponnachan et al., 1993). In the present study also there was an increase in serum urea in alloxan-induced diabetic rats. The extract administration brought about a significant decrease in serum urea. Aqueous and methanolic extracts of *Elephantopus scaber* (root/leaf), *Eugenia*
jambolana (seed/bark), Clitoria ternatea (leaf/flower) and Phyllanthus fruits (emblica/acidus) brought about a decrease in serum urea concentration in alloxan–induced diabetic rats. Increase in serum urea concentration, which is considered as a marker of kidney dysfunction, has been rectified by administration of these extracts in alloxan–induced diabetic rats.

5.1.12 Serum Creatinine

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; Grover et al., 2003). An increase in creatinine level was shown in diabetic rodents (Katoh et al., 2000; Grover et al., 2003). In the early phase of diabetic nephropathy, there is hyperfiltration and an increase in creatinine clearance resulting in no change in creatinine levels but in the later stages the creatinine level starts increasing (Grover et al., 2003). In diabetes, there is a relationship between glucose homeostasis and renal damage (Rasch, 1979 a, b; Bolkent et al., 2004). This is very important because many reports have suggested that therapeutic intervention can delay the development of end-stage renal disease (Mathiesen et al., 1994). Diabetic rats indicated a significant increase in serum creatinine levels, but treatment with Enicostemma littorale significantly decreased the creatinine level (Murali et al., 2002). Seeds of Brassica juncea also brought about a significant decrease in serum creatinine level (Grover et al., 2003). Aavirai Kudineer treatment appreciably normalized creatinine levels in diabetic rats, revealing its positive effect on renal function (Bhavapriya et al., 2001). Methanolic and aqueous extracts of Terminalia
catappa fruits brought about a marked decrease in serum creatinine level of alloxan-induced diabetic rats (Nagappa et al., 2003).

Serum creatinine was higher in diabetic rats than in non-diabetic controls, and kernels of Eugenia jambolana brought about a decrease in serum creatinine level in diabetic rats (Grover et al., 2001). In the present study, an increase in serum creatinine level was observed in alloxan–induced diabetic rats. The aqueous and methanolic extracts of Elephantopus scaber (root/leaf), Eugenia jambolana (seed/bark), Clitoria ternatea (leaf/flower) and Phyllanthus fruits (emblica/acidus) brought about a decrease in the serum creatinine level of diabetic rats. Since creatinine measurement is an important tool to check kidney function, the present investigation reveals that the administration of plant extracts improves the kidney function by bringing down the serum creatinine level in alloxan-induced diabetic rats.

5.1.13 Biochemical Parameters: A Sum-Up

The data presented provide evidence for a decrease in blood glucose levels in alloxan-induced diabetic rat treated with the aqueous/methanolic extracts. The extracts were not only effective in bringing about a decrease in blood glucose but also caused a decrease in glycosylated hemoglobin. The observed decrease in glycosylated hemoglobin is due to decrease in blood glucose. Insulin-dependent tissues, like skeletal muscle and liver, showed an increase in glycogen after the extract treatment. This indicates that the peripheral free glucose is stored in the skeletal muscle and liver in the form of glycogen by increasing glycogenesis and this is due to an increase in insulin level. Treatment with plant extracts increased the activity of glucokinase in
the liver, indicating an overall increase in glucose influx. Thus, the extracts seem to have an overall effect in increasing glucose utilization. Inhibition of glucose-6-phosphatase activity after the administration of the plant extracts suggests that glucose-6-phosphate is not the substrate for the synthesis of glucose in gluconeogenic pathway, but may be used as a substrate for glycogenesis or in the HMP pathway.

The plant extracts brought about hypocholesterolemic and hypotriglyceridemic effects while at the same time increasing HDL-cholesterol. The underlying mechanism of this pharmacological effect appears to depend upon insulin secretion. Treatment-related increases in serum urea and creatinine concentrations are variables used not only to indicate impairment of kidney function, but also clinical chemistry end points to detect treatment-related toxic effects of compounds on the kidney in rat. The plant extracts chosen for the present investigation do not induce any toxic effect in the kidney as shown by a decrease in serum urea and creatinine levels. This alteration in carbohydrate, lipid and protein metabolism in alloxan-induced diabetic rats after the administration of the extracts of all the five plants (aqueous/methanolic) is due to an increased insulin secretion suggesting possible regeneration or repair of the islets of Langerhans. The extracts assume significance because of their ability to increase the insulin level in diabetic rats by enhancing insulin secretion from pancreatic β-islets and also by accelerating glucose uptake and peripheral utilization of glucose.

The results of the biochemical analyses indicate that all the five plants are capable of bringing about improvement of the glycemia. Between the
aqueous and methanolic extracts, the former is more potent than the latter. Hence, only the aqueous extract–treated rats were considered for histological, and electrophoretic studies.

5.2 Histological Changes in the Islets of Langerhans

5.2.1 Light Microscopy

The use of light microscopy to study the morphometry of normal and experimentally or pathologically altered pancreatic islets of Langerhans provides data pertaining to the number, size and distribution of the cell types (Remacle et al., 1977; Saito et al., 1978a, b; Saito et al., 1979; Sato and Herman, 1981; Ahlawat and Sahi, 1985; Ferri et al., 1987; Ashizawa, 1997).

The islets of man 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–cells 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 streptozotocin (Bora et al., 1989; Das et al., 1996; Szkudelski, 2001). As compared to a homogenously normal configuration in non-diabetic rats, the islet tissues of diabetic animals depict profound distortion in its structural organization. Streptozotocin 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).

Oral intubation of lectin isolated from the seeds of *Urtica pilulifera* prevented the cellular damage of the islets. The proposed mechanism of action on hypoglycemic action, is to increase either the pancreatic secretion of insulin from β-cells of islets or release of insulin from the bound form (Kavalali et al., 2003). A decrease in blood sugar level was observed in streptozotocin-treated animals when treated with *Cassia alata* leaf extract. The lowering of blood sugar level was suggested as due to a stimulating effect on insulin release from regenerated β-cells of the pancreas or increased cellularity of the islet tissues and regeneration of the granules in the β-cells (Palanichamy et al., 1988).

Sections of pancreatic islets of *Gymnema sylvestre* leaf extract-treated diabetic rats showed an increase in β-cell numbers. Therefore, the extract assumes significance because of its ability to regenerate the damaged endocrine tissue partially (Shanmugasundaram et al., 1990a, b). Photomicrographic data showed regeneration of beta cells in the pancreas of *Terminalia catappa* fruit extract-treated diabetic rats. This effect may be due to β-carotene, which is a constituent of *T. catappa* fruit (Duke, 1992; Nagappa et al., 2003). Histopathological abnormalities in the islet of Langerhans of alloxan–induced diabetic rabbits were reversed to almost normalcy on administration of *Eugenia jambolana* seed extract (Sharma et al., 2003).
In the present study, oral intubation of *Elephantopus scaber* (root/leaf), *Eugenia jambolana* (seed/bark), *Clitoria ternatea* (leaf/flower) and *Phyllanthus* fruits (*emblica/acidus*) to alloxan-induced diabetic rats brought about an improvement in the histoarchitecture of the islets in the pancreas of alloxan-induced diabetic rats.

### 5.2.2 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 α-cells, which constitute about 20% and the smaller β-cells, which constitute about 75% of the islet cells. The α-cells are sometimes absent in the smaller islets and, when present tend to be located peripherally (Like, 1967; Larsson et al., 1976; Pelletier and Leclerc, 1977; Baetens et al., 1979; Jorns et al., 1988).

The α-cells, as contrasted to the β-cells, were originally characterized as having a uniform population of extremely electron-opaque secretion granules (Like, 1967; Jorns et al., 1988; Bertelli, 1994). The α- and β-cell secretion granules are roughly the same size, the only distinguishing feature being the nature of the core. In all primates studied, core of the granules of the α-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 α-cells resembles an eccentrically shaped bull's eye. The other mammals have α-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 α-cell granules appears to be a relatively consistent characteristic among all mammals. The cytoplasmic organelles present in the α-cells are similar to those of the β-cells with minor exceptions. Clusters of granular
endoplasmic reticulum are commonly observed in the α-cells. The cytoplasm of the α-cell 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 the α-cell 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, 1957, 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 an agranular 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; Delfino et al., 1993; Pai et al., 1993; Sasaki et al., 1991).

A third cell type encountered less frequently (about 5 %) contains small granules. These cells are designated δ-cells. The δ-cells have not been extensively studied in many species but it has been described in some to contain numerous membrane-bound granules of moderately low density
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; Samols et al., 1988; Lukinus et al., 1995).

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).

After administration of alloxan and incubation of pancreas in vitro, the β-cell nuclei show initial chromatin clumping followed by pyknosis and karyolysis. There is gradual shrinkage of the nucleus and progressive aggregation and concentration of interchromatinic material, while the nucleoli are unaltered. The ultrastructure of alloxan-diabetic pancreas shows considerable reduction in β-cell granules and granular endoplasmic reticulum (Wellmann, 1967; Rerup, 1970; Lazarus and Shapiro, 1973; Ghosh and Suryawanshi, 2001).
The ultrastructure of pancreatic islets in *Vinca rosea* flower- and leaf-treated diabetic rats showed considerable improvement in β-cell activity. The number of insulin granules was high in the β-cells. 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 alloxan-treated rats. Oral administration of the aqueous extracts of *Elephantopus scaber* (root/leaf), *Eugenia jambolana* (seed/bark), *Clitoria ternatea* (leaf/flower) and *Phyllanthus* fruits (*emblica/acidus*) brought about an increase in insulin granules in alloxan-induced diabetic rats. The aqueous extracts of *Elephantopus scaber* (root/leaf) were more effective in improving the β-cell activity.

### 5.2.3 Immunocytochemical Studies

Cytochemistry is the visualization of chemical compounds in tissue sections by staining. This predominantly qualitative discipline has gradually developed into a quite sophisticated science employing a variety of micro-assays for the analysis of isolated tissue elements and tissue sections (Lacy, 1962; Gepts and Gregoire, 1971; Rhoten and Hall, 1982; Stemberger, 1986). Cytochemistry, in this modern sense, is indispensable in the exploration of complex tissues composed of a variety of cells (Gossner, 1963; Sasaki *et al.*, 1991). The endocrine pancreas is such a tissue, and it is the contribution of cytochemists that has enabled us to understand its specific functions (Grodsky and Forshan, 1966; Park and Bendayan, 1992). Application of reliable and specific immunocytochemical techniques has allowed clear
identification of the major islet cell types; insulin containing β-cells, glucagon containing α-cells and somatostatin secreting δ-cells (El-Naggar et al., 1993 Elayat et al., 1995; Higdon et al., 2001; Rebecca et al., 2004; Koyuturk et al., 2005).

The immunocytochemical findings show that there is a species-specific arrangement of cell types in rat islets (El-Naggar et al., 1993; Rebecca et al., 2004). The difference in the distribution may reflect a functional significance and may be related to the embryonic development of the pancreas (Wolfe-Coote and Du Toit, 1987; Rebecca et al., 2004). In rat pancreas, the endocrine islets of different sizes are present, each containing from several to several thousands of cells. In the round or elongated compact islets, the insulin-positive cells (β-cells) represent 60-80% of the endocrine cell population and are mainly disposed in the more central area of the islets (Brelje et al., 1989; Joms, 1994). In large islets, cells lying in the most central area exhibit weaker insulin immunostaining than cells lying more at the periphery (Ogneva and Nikolov, 1994; Wieczorek et al., 1998).

Immunocytochemical staining of the islets of control rats for alloxan diabetes revealed that the islets are well granulated, and the insulin positive β-cells form the majority of the islet cells and are located at the center (Wieczorek et al., 1998; Rebecca et al., 2004). Islets from the diabetic animals showed lack of insulin response, with degenerated β-cells (Sasaki et al., 1991; Higdon et al., 2001). In the present investigation also, islets from untreated rat showed well granulated β-cells with a majority of the β-cells being insulin-positive. Diabetic rat did not show any insulin-positive cells in
the islets. Oral intubation of the aqueous extracts of *Elephantopus scaber* (root/leaf), *Eugenia jambolana* (seed/bark), *Clitoria ternatea* (leaf/flower) and *Phyllanthus* fruits (*emblica/acidus*) to alloxan-induced diabetic rats showed several insulin positive β-cells in the islet of Langerhans with extensive granulation. The findings indicate that the increase in beta cell population is due to repopulation of secretion granules in beta cells, and would account for the increase in the serum insulin level. The extracts of the five plants assume significance because of their capacity to regenerate at least partially the damaged endocrine tissue, such that the insulin content/β-cell number increased by the extract treatment.

5.3 The Probable Mechanism of Action of the Plant extracts

The plant extracts may contain some principles, which act to regenerate the damaged beta cells. It was shown that polyphenolic part of BYC (Bai-Yu-Cha tea) is the latter’s anti-diabetic principle (Zhu *et al.*, 1990). The water-soluble polysaccharide fraction of the green tea is responsible for its antidiabetic effect (Shimizu *et al.*, 1988; Isigaki *et al.*, 1991). β-carotene is the active hypoglycemic principle in *Terminalia catappa* fruit (Duke, 1992; Nagappa *et al.*, 2003). The hypoglycemic principle present in *Pterocarpus marsupium* is (-) epicatechin, which brings about β-cell regeneration (Chakravarthy *et al.*, 1980, 1981, 1982). Many plant polysaccharides have been reported to exhibit hypoglycemic effects (Marles and Farnsworth, 1995; Alarcon-Aguilra *et al.*, 2000). The active principle responsible for the hypoglycemic activity in *Urtica pilulifera* is lectin (Kavalali *et al.*, 2003).
Flavonoids are frequently found in hypoglycemic plants (Harborne \textit{et al.}, 1974; Oliver-Bever, 1980; Alcaraz and Ferrandiz, 1987; Lamba \textit{et al.}, 2000; Andrade-Cetto and Wiedenfeld, 2001; Anjaneyulu and Chopra, 2004). The extract of \textit{Hibiscus rosa-sinensis} flower contains flavonoids and glycoside type of active principle. Such principles have already been reported to exhibit hypoglycemic activity in diabetic animals (Ivorra \textit{et al.}, 1989; Geetha \textit{et al.}, 1994; Cherian and Augusti, 1993, 1995; Seetharam \textit{et al.}, 2002; Sachdewa and Khemani, 2003; Anjaneyulu and Chopra, 2004). Preliminary phytochemical analysis of the aqueous extract of \textit{Rubus fructicosus} revealed the presence of essential oils, flavonoids and tannins as the major constituents. These natural compounds could act separately or synergistically to cause hypoglycemic effect (Marles and Farnsworth, 1995; Jouad \textit{et al.}, 2002). Preliminary phytochemical analysis of the aqueous extract of \textit{Suaeda fruticosa} revealed the presence of flavonoids, the active principles responsible for the hypoglycemic activity (Benwahhoud \textit{et al.}, 2001). The active principles in the aqueous extract of \textit{Spergularia purpurea} are flavonoids, saponins and tannins. Flavonoids are considered as the active principles in many medicinal plants (Wollenweber, 1988) and natural products with positive effect for human health (Jouad \textit{et al.}, 2001). On the other hand, oral administration of saponins from some medicinal plants significantly reduced the triglycerides and cholesterol levels in rat. The use of diet with high saponin content was suggested to reduce heart diseases (Cheeke, 1971; Oakenfull, 1981; Hostettman and Marston, 1995; Jouad \textit{et al.}, 2003).

Phytochemical screening of aqueous extract of \textit{Abutilon indicum} leaf extract revealed the presence of saponins, flavonoids and glycosides. Since
flavonoids are known to regenerate the damaged pancreatic β-cells and
glycosides stimulate the secretion of insulin in β-cells of the pancreas, the
presence of both these chemicals in the aqueous extracts may be responsible
for the hypoglycemic activity (Chakravarthy et al., 1980; Cherian and Augusti,
1993; Seetharam et al., 2002).

Anthocyanosides, a plant flavonoid are believed to act by improving the
vascularisation of the pancreas. Vascular troubles develop gradually during
the disease (diabetic angiopathy) entailing thickening of the basal membrane
of the walls of the small blood vessels, and increase of their permeability
causing disturbance of metabolic exchanges. Thus the improvement of
diabetes itself obtained with some plants rich in anthocyanosides could
possibly be due to recovery of the vascularisation of the pancreas. Other
plant flavonoids, which also appear to act on the capillaries could have a
similar action (Pourrat, 1977; Pourrat et al., 1978; Oliver-Bever, 1980).

Preliminary phytochemical investigations of Elephantopus scaber
revealed the presence of sesquiterpene lactones, alkaloids, flavonoids and
triterpenes (Hayashi et al., 1987; Geetha et al., 2003). Eugenia jambolana
seeds contain flavonoids, glycoside, alkaloids, and tannins, whereas, the bark
contains tannins, resin and proteins (Chopra et al., 1956; Oliver-Bever, 1980;
Rastogi and Mehrotra, 1995; Prince et al., 2003). On preliminary
phytochemical screening Clitoria ternatea leaves showed positive results for
glycosides, flavonoids and terpenoids. On the other hand, flowers showed
positive test for anthocyanins, glycoside, robinin, flavonoids and glucoside
investigation of *Phyllanthus emblica* fruit revealed the presence of tannins, crude, cellulose, cytokines, alkaloids and flavonoids (Gulati *et al.*, 1995; Bhattacharya *et al.*, 1999; Khandelwal *et al.*, 2002; Ram *et al.*, 2002), whereas *Phyllanthus acidus* fruits contain terpenoids, tannins and flavonoids (Rizk, 1987; Unander *et al.*, 1990).

From the preliminary phytochemical investigation, it is evident that the five plants chosen for the study contain flavonoids, glycosides and several other active components. Flavonoids are known to regenerate the damaged pancreatic β-cells in alloxan-diabetic animals and glycosides stimulate the secretion of insulin in β-cells of pancreas. Such groups have already been reported to exhibit hypoglycemic activity, in diabetic animal model. In addition to flavonoids, glycosides the plants also contain several other active principles, each with a single or a diverse range of biological activities, which could have acted synergistically with the flavonoids and glycosides to bring about the hypoglycemic effect.

The five plants chosen in the present study produced hypoglycemic activity. The aqueous extract of the plants are more effective than the methanolic extract in bringing about the hypoglycemic effect in alloxan-diabetic rats. This may be due to the fact that the active principles responsible for hypoglycemic activity probably polar in nature, being more soluble in water than in methanol. Among the five plants, the aqueous extract of *Elephantopus scaber* (root/leaf) is the most effective in bringing about the hypoglycemic effect. Between them, ESR treatment is more effective than ESL. *Eugenia jambolana* extract also brought about
hypoglycemic effect in diabetic rats. Its hypoglycemic activity is, however, greater than that of *Clitoria ternatea* extract and lesser than that of *Elephantopus scaber* extracts. EJS extracts produce a greater hypoglycemic activity than EJB extracts. CTL-treatment produces a greater hypoglycemic activity than CTF treatment. *Phyllanthus* fruits (*emblica/acidus*) also possess hypoglycemic activity. PEF treatment is superior to PAF in bringing about hypoglycemic effect. The biochemical mechanism of action of these plants appears to be through regeneration of the damaged β-cells and, thereby, increase the insulin level of the serum. The metabolic alterations found in the diabetic rats due to insulin deficiency were rectified. The increase in insulin secretion, decrease in blood glucose and glycosylated hemoglobin, replenishment of glycogen in liver and skeletal muscles, increase in the activity of glucokinase, decrease in the activity of glucose-6-phosphatase, decrease in total blood cholesterol and triglycerides, increase in HDL-cholesterol and decrease in urea and creatinine were observed. The plant extracts did not cause any harmful effect on liver and kidney as evident from the biochemical studies. The acinar portion of the pancreas was also not affected, as evident from the histological studies. The active principles in the plants flavonoids, glycosides and others through their action appear to regenerate the damaged endocrine tissue and thereby stimulate the secretion of insulin in β-cells as revealed by insulin assay, light microscopy, transmission microscopy and immunocytochemical staining.