Chapter-II

REVIEW OF LITERATURE
Diabetes mellitus, a metabolic disorder, is characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins with an increased risk of vascular disease (Pickup and Williams, 2003). The minimum defining characteristic feature to identify diabetes mellitus is chronic and substantiated elevation of circulating glucose concentration (Keen and NgTang, 1982; Ziv et al., 1999). It is a complex heterogeneous assemblage of disorders having the common feature of appearance of glucose in the urine. The characteristic symptoms that are recognized clinically include polydipsia, polyuria, pruritus, weight loss, or one or more of the many complications associated with or attributable to the disease. Diabetes mellitus may present as a relatively sudden, potentially lethal metabolic catastrophe or it can be associated with few symptoms or signs and may escape detection for many years. These extremes of clinical manifestations constitute the basis for subdividing diabetes mellitus into the insulin dependent (IDDM) and the non-insulin dependent (NIDDM) types (Norris, 1985).

The major problems associated with diabetes are retinopathy, nephropathy, the cardiovascular system with accelerated arteriosclerosis and neuropathy (Lacy and Kissane, 1977). The pathogenic mechanisms of diabetes mellitus responsible for the diminished available insulin are multiple, but they
usually are related either to destruction of islets secondary to severe pancreatic or to selective degeneration of islet cells (Thomson. 1989).

Diabetes mellitus has been studied extensively in different experimental and domestic animals. Diabetes mellitus was reported in rabbits (Roth and Conaway, 1982). dogs (Meier. 1960) and several species of rodents like guinea pigs (Lang et al., 1976). Chinese hamster (Meier and Yerganian, 1959; Like et al., 1974), rats (Schmidt-Nielson et al., 1963) and mice (Boucher and Notkins, 1973).

In case of dogs, diabetes mellitus is a common endocrine disorder with a reported incidence of 1 in 200 (Meier. 1960). Most cases of spontaneous diabetes occur in mature dogs and in females approximately twice as often as in males (Wilkinson. 1960; Kaneko. 1980). It is characterized by disturbances of carbohydrate, lipid and protein metabolism and glucose intolerance (Milne. 1987). There is a relative or absolute deficiency of insulin resulting in hyperglycemia (Nelson, 1985). According to Nelson (1985) the four classic symptoms of a dog affected by diabetes mellitus are polyuria, polydipsia, polyphagia and weight loss.

Sandhu et al. (2000) have reported that alloxan-induced diabetes mellitus in dogs is characterized by vomiting, polydipsia, polyuria, inappetence, dehydration, hypothermia, dullness, depression, hindleg weakness and recumbency followed by death. Development of diabetes mellitus in young dogs may be associated with idiopathic atrophy of the pancreas, acute pancreatitis with necrosis and haemorrhage, or aplasia of pancreatic islets. The overall size of the pancreas with idiopathic atrophy is reduced to a third of normal or less, and there is evidence of both endocrine and exocrine deficiency. Aplasia of pancreatic islet has been a cause of diabetes mellitus in dogs from two to three months of age. The islets are absent, but the pancreatic acini and ducts are
present and functional (Thomson, 1989). Histopathological examination of alloxan-induced diabetic dogs has revealed vacuolation, necrosis of pancreatic cells along with hyalinization and degeneration, exfoliation of tubular epithelium, coagulative necrosis in the kidneys and degenerative changes in the brain, liver, heart and intestines (Sandhu et al., 2000).

Cats with diabetes mellitus usually have specific degenerative lesions localized selectively in the islets of Langerhans, whereas the remainder of the pancreas appears to be normal. The selective deposition of amyloid in islets, with degenerative changes in α and β cells, is the most common pancreatic lesion in cats with diabetes. However, scattered amyloid deposits in the pancreatic islet occur in many cats without development of overt diabetes mellitus (Johnson et al., 1970). A common islet lesion in cats with diabetes mellitus is hydropic (vacuolar) degeneration of β and α cells. Vacuolar degeneration with glycogen accumulation in cats appears to develop in β cells as a response to long term over stimulation (exhaustion) because of insulin resistance (Thomson, 1989).

The diabetes mellitus in rabbits is essentially similar to the insulin independent diabetes mellitus in humans (age dependent diabetes) (Roth and Conaway, 1982). Experimental studies of diabetes mellitus in rabbits have shown an increase in biochemical parameters such as increased blood urea and serum creatinine vis-à-vis a decrease in β cell number (Dubey et al., 1994; Rastogi et al., 1998). The histopathological examination of pancreas shows, differently to other species, a hypergranulation of the β cells. Due to this, a malfunction of the insulin secretion is assumed the reason of this disease (Conaway et al., 1981). The naturally occurring diabetes mellitus in rabbits seems to be caused by a genetic predisposition but also other still unknown environmental influences might be the reason for the occurrence of the disease.
(Conaway et al., 1980; 1981). The disturbances of the insulin secretion (Roth et al., 1980) are assumed to be due to hypergranulation of the β cells, which result in decrease of serum insulin levels. The diabetic symptoms of rabbits as reported by Roth et al. (1982) include polyphagia, polydipsia, polyuria, glycosuria and the development of cataracts.

In case of rats, several studies regarding diabetes mellitus have been made to understand the diabetic complications such as cataract (Rawal et al., 1986), peripheral motor neuropathy (Narama and Kino, 1989) and granular lesions (Tago et al., 1991). Aged male rats of WBN/Kob strain with spontaneous diabetes frequently suffer long term hyperglycemia and glycosuria, and it has been suggested that they are useful animal models for type II–non-insulin dependent diabetes mellitus (Nakama et al., 1987; Mori et al., 1988).

**Diabetogenic Activity of Alloxan**

Since the discovery of its diabetogenic property, it has been extensively used for induction of diabetes mellitus in experimental animals through different routes (Rerup, 1970; Copenhaver et al., 1975; Duckworth et al., 1979; Chakravarthy et al., 1980; Rawal et al., 1986; Agarwal et al., 1987; Dubey et al., 1994; Rastogi et al., 1998) causing hyperglycemia. Alloxan exerts its diabetogenic action when it is administered parenterally, intravenously, intraperitoneally or subcutaneously. The dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human islets are considerably more resistant to alloxan than those of the rat and mouse (Eizirik et al., 1994). The most frequently used intravenous dose of this drug in rats is 65mg/kg b.w. (Gruppuso et al., 1990; Boylan et al., 1992). When alloxan is given intraperitoneally or subcutaneously its effective dose must be 2-3 times higher. Fasted animals are more susceptible to alloxan (Szkudelski et al., 1998), whereas increased blood glucose provides partial protection.
(Bansal et al., 1980; Szkudelski et al., 1998). The action of alloxan in the pancreas is preceded by its uptake by the B cells (Boquist et al., 1983). Rapid uptake by insulin-secreting cells has been proposed to be one of the important features determining alloxan diabetogenicity. Another aspect is the formation of reactive oxygen species (Heikkila et al., 1976). The formation of reactive oxygen species is preceded by reduction of alloxan. In beta cells of the pancreas its reduction occurs in the presence of different reducing agents. Since, alloxan exhibits a high affinity to the SH-containing cellular compounds, reducing glutathione (GSH), cysteine and protein-bound sulphhydryl groups including SH-containing enzymes are very susceptible to its action (Lenzen and Munday, 1991). Alloxan is vulnerable to glucokinase, which is one of the SH-containing compound essential for proper glucose-induced insulin secretion (Lenzen et al., 1987).

Once alloxan is reduced in the body it forms dialuric acid which is then re-oxidized back to alloxan establishing a redox cycle for the generation of superoxide radical (Munday, 1988). The dialuric acid formed undergoes auto-oxidation to yield detectable amounts of hydrogen peroxide (H₂O₂), superoxide anion and hydroxyl free radicals (Fischer, 1985). Superoxide radicals are able to liberate ferric ions from ferritin and reduce them to ferrous ion. Fe³⁺ can also be reduced by alloxan radicals (Sakurai and Ogiso, 1995).

One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in B cells exposed to alloxan (Sakurai and Ogiso, 1995). DNA damage stimulates poly ADP-ribosylation, a process participating in DNA repair. Some inhibitors of poly ADP-ribosylation can partially restrict alloxan toxicity. This effect is, however, suggested to be due to their ability to scavenge free radicals rather than to a restriction of poly ADP-ribosylation initiated by alloxan (Sandler & Swenne, 1983). Further, like beta cells, all cells take up glucose for metabolism but also have special
monitoring glucose transporters. A specific surface glucose transporter (GLUT2) has been characterized on the surface of beta cells which is exploited by alloxan and streptozotocin (Wang and Gleichmann, 1998).

Another diabetogenic action of alloxan includes disturbances in intracellular calcium homoeostasis elevating cytosolic free $\text{Ca}^{2+}$ concentration in pancreatic B cells (Kim et al., 1994). This effect arises due to alloxan-induced calcium influx from extracellular fluid, exaggerated calcium mobilization from intracellular stores and its limited elimination from the cytoplasm. The calcium influx may result from the ability of alloxan to depolarize pancreatic B cells (Dean and Mathews, 1972). Depolarization of the cell membrane opens voltage-dependent calcium channels and enhances calcium entry into cells. The effect of alloxan on intracellular calcium concentration seems to be mediated, at least partially, by hydrogen peroxide which itself exerts a similar effect on calcium concentration in B cells (Park et al., 1995).

**Diabetogenic Activity of Streptozotocin**

The biological action of streptozotocin corresponds closely to alloxan being both labile and hydrophilic. Streptozotocin has thus been used to induce diabetes mellitus in experimental animals (Zysset et al., 1987; Jamshid et al., 1988; Wang et al., 1994; Mulder et al., 1997; Hardikar et al., 1999; Shenoy et al., 2002). The action of streptozotocin in B cells is accompanied by characteristic alterations in blood insulin and thereby glucose concentrations. Two hours after injection, the hyperglycemia is observed with a concomitant drop in blood insulin. About six hours later, hypoglycemia occurs with high levels of blood insulin. Finally, hyperglycemia develops and blood insulin levels decrease (West et al., 1996). These changes in blood glucose and insulin concentrations reflect abnormalities in B cell function. Streptozotocin impairs glucose oxidation (Bedoya et al., 1996) and decreases insulin biosynthesis and
secretion (Bolaffi et al., 1987; Nukatsuka et al., 1990). It was reported that streptozotocin at first abolishes the B cell response to glucose. Temporary return of responsiveness then appears which is followed by its permanent loss and cells are damaged (West et al., 1996).

Intracellular action of streptozotocin results in changes of DNA in pancreatic B cells comprising its fragmentation (Morgan et al., 1994). During the decomposition of streptozotocin, highly reactive carbonium ions are formed, which causes alkylation of DNA bases (Doux et al., 1986). Streptozotocin may also damage the B cell membrane and break the DNA strand which leads to the activation of poly (ADP-ribose) synthetase. NAD depletion and further reduction of the ATP content (Heller et al., 1994) and ultimately to cell death (Okamato, 1981; Portha et al., 1989). The concept of unfavourable consequences of augmented poly ADP-ribosylation as a result of streptozotocin action was confirmed by experiments revealing that the inhibition of this process prevents the toxicity of this diabetogenic agent. It was found that 3-aminobenzamide, a strong inhibitor of poly (ADP-ribose) synthetase, protected against the action of streptozotocin in rats, even when this substance was administered 45-60 min after streptozotocin (Masiello et al., 1990). Another inhibitor of poly (ADP-ribose) synthetase is nicotinamide which is also scavenging oxygen free radicals, exerted best protection when it was administered shortly after streptozotocin (Masiello et al., 1990).

Being nitric oxide (NO) donor, streptozotocin has been found to bring about the destruction of pancreatic islet cells and contributing DNA damage (Morgan et al., 1994). The participation of NO in the cytotoxic effect of streptozotocin was confirmed experimentally (Kroncke et al., 1995). Pancreatic B cells exposed to streptozotocin manifested changes characteristic for NO action, i.e., increased activity of guanyl cyclase and enhanced formation of cGMP (Turk et al., 1993). Streptozotocin is, however, not a spontaneous nitric
oxide donor but this molecule is liberated when streptozotocin is metabolized inside cells (Kroncke et al., 1995). On the other hand NO scavengers lowered NO concentration in pancreatic islet cells by inhibitions of the inducible form of nitric oxide synthase and partially counteracted DNA cleavage induced by streptozotocin (Kroncke et al., 1995).

Beta cells of islets of Langerhan's posses high activity of an enzyme called xanthine oxidase. Augmented ATP dephosphorylation caused by streptozotocin increases the supply of substrate for xanthine oxidase and enhances the production of uric acid which is the final product of ATP degradation (Nukatsuka et al., 1990a). Xanthine oxidase in turn catalyses reaction in which the superoxide anion is formed (Nukatsuka et al., 1988). As a result of superoxide anion generation hydrogen peroxide and hydroxyl radicals are formed (Nukatsuka et al., 1990a).

Calcium, which may also induce necrosis, does not seem to play a significant role in the necrosis evoked by streptozotocin since calcium channel antagonists do not protect B cells against streptozotocin, as they do in the case of alloxan (Katsumata et al., 1992).

**Pathology/Complications of Diabetes Mellitus**

The consequences of hyperglycemia are almost in every tissue and organ of the body which undergoes biochemical, functional and structural alterations that occur in pancreas (Gepts. 1965; Kloppel et al., 1985; Haward and Van, 1986), kidneys (Mauer et al., 1979; O'Donnel, 1986), liver (Zysset & Tlach, 1987), bladder (Lincoln et al., 1984; Kolta et al., 1985; Longhurst and Belis, 1986; Uvelius, 1986), fat cell (Chiappe De Cingolani, 1986), cardiovascular system (Vadlamudi et al., 1982; Latifpour and McNeill, 1984; Macleod and McNeill, 1984; McNeil, 1985), eyes (Kinoshita et al., 1965; Rawal and Gandhi, 1986), reproductive system (Balasubramanian et al., 1991) and nerves (Buck et
accounting for the major complications in diabetes. Possibly these complications are related to the severity of hyperglycemia since control of blood glucose from clinical point of view is associated with minimizing the development of complications (Mohan, 2000). Extensive studies with respect to biochemical alterations in respect of sialic acid, acetylcholinesterase, surface glycoproteins and key glycolytic enzymes of diabetic red cell membrane (IDDM) have been made (Suhail and Rizvi, 1989; Suhail et al., 1992).

Islet Damage

A number of pathological changes have been demonstrated in the islets in association with diabetes. Many of the structural islet lesions reflect important pathologic events in the pancreas (Opie, 1901). Distinct differences exist in the pathologic changes observed in the pancreas of individuals with classic juvenile- and maturity-onset diabetes. In recent-onset insulin-dependent diabetes mellitus, no significant reduction of the pancreatic size is found (Maclean and Ogilivie, 1959; Gepts, 1965) whereas in long standing insulin-dependent diabetes mellitus there is often, but not always, a significant reduction of the pancreatic weight (Maclean et al., 1959) accompanied by interstitial fibrosis of the exocrine tissue (Doniach and Morgan, 1973; Rahier et al., 1983). In type 1 diabetes mellitus there is often a qualitative reduction in the number of islet per area (Gepts, 1965; Doniach and Morgan, 1973) but in quantitative studies there is considerable overlap with the normal pancreas (Junker et al., 1977). In long-standing insulin dependent diabetes mellitus the islets are small (Rahier et al., 1983) and there is a major reduction of the islet volume (Maclean and Ogilivie, 1959; Gepts, 1965), which depends largely on the almost complete loss of islet β cells (Gepts, 1965). In spite of the pronounced β cell loss, a few β cells are commonly found in many cases of insulin dependent diabetes mellitus of long duration. In contrast to IDDM, the
pancreas in non-insulin dependent diabetes mellitus (NIDDM) is usually of normal size (Westermark et al., 1978; Rahier et al., 1983) but with a tendency to fatty infiltration (Westermark et al., 1978) most probably due to the obesity present in many of these patients. Arteriosclerotic changes are the rule and diffuse or focal fibrosis is common. A pronounced β cell loss as seen in IDDM does not occur in NIDDM. The β cells in NIDDM are rich in granules and not degranulated as in IDDM. There are contradictory results concerning the α cell mass in NIDDM, with both reduced (Saito et al., 1979; Kloppel et al., 1985) and increased mass reported (Rahier et al., 1983). In a majority of individuals, many of the islets show pathologic alterations, particularly amyloid deposits. The β cell volume in obese NIDDM patients has been reported twice as that of non-obese diabetic individuals (Kloppel et al., 1985).

Glycogen is deposited in β cells of the islets when there is persistent hyperglycemia for long period of time. Previously this lesion was called hydropic degeneration of β cells since the cells appeared greatly vacuolated and it was assumed that the vacuoles contained water. The use of special stains demonstrated that the vacuoles contained glycogen. Glycogenesis of β cells occurs in diabetes in man as well as in experimental animals with diabetes (Toreson, 1951). The deposition of glycogen is attributable to a change in the intracellular metabolism of glucose that shifts the metabolism to the deposition of glycogen (Lacy and Kissane, 1977).

Electron microscopic studies have revealed a second pathologic change in β cells of dogs with experimental diabetes induced by the administration of growth hormone and glucose. This lesion is called "ballooning degeneration" since multiple vacuoles are present in the cytoplasm, the vacuoles do not contain glycogen, and the cells appeared to be undergoing degeneration. This
degenerative change may represent the initial stages in the destruction of β cells during prolonged hyperglycemia (Lacy and Kissane, 1977).

**Diabetic Nephropathy**

Diabetic nephropathy is the most important cause of death in type I diabetic patients, of whom 30-40% eventually develop end-stage renal failure (Giorgino et al., 2004). Studies have shown that good metabolic control is beneficial in slowing the progression of nephropathy in diabetes, and if the duration of diabetes is prolonged before reinstitution of normoglycemia, nephropathy is not easily reversed (Floretto et al., 1998; Renu et al., 2004). In type II diabetic patients, nephropathy is also one of the major complications leading to death (Dubey et al., 1994; Mohan, 2000). The development of diabetic nephropathy is characterized by a progressive increase in albuminuria and a late decline in glomerular filtration rate, leading eventually to end-stage renal failure (Salah et al., 2004). This severity of renal disease in diabetic patients correlates with the levels of blood urea and serum creatinine (Dubey et al., 1994). Diabetic nephropathy accounts for considerable morbidity and mortality even in patients with well controlled blood sugar values (Grenfel, 1991). In diabetic nephropathy different types of lesions have been described namely glomerular lesions, renal vascular lesions, principally arteriosclerosis, pyelonephritis including narcotizing papillitis (Kumar et al., 2001) and tubular lesions or Armanni-Ebstein lesions (Mohan, 2000; Kumar et al., 2001). The most important glomerular lesions are capillary basement membrane thickening, diffuse glomerulosclerosis and nodular glomerulosclerosis.

The most common features of vascular lesions in diabetic patients are renal glomerular degeneration. Previous studies on the long-term effects of diabetes in experimental animals showed that the effects were not only on the peripheral capillaries but they also induced the glomerular nephropathy along
with tubular and interstitial abnormalities (Rasch, 1979; Hirose et al., 1982). Glomerular basement membrane thickening, an indicator of diabetic microangiopathy may be seen in patients with a two year history of diabetes, whereas it increases by 30% after 5 years of diabetes (Qsterby and Gunderson, 1989). The short-term effects of diabetes have been suggested to be the increased number of glomerular mesangial cells and interstitial alterations (Seyer-Hansen et al., 1980) other than the increased basement membrane thickness, which are the indicators of long term effects of diabetes (Qsterby and Gundersen, 1979). In the very early phase of human and experimental diabetes, renal and glomerular growth is a consistent observation along with increased glomerular filtration rate (Hosteller et al., 1981; Seyer-Hansen, 1983), and this early diabetic hypertrophy-hyperfunction syndrome may contribute to the later development of overt diabetic kidney disease (Brenner et al., 1981; Mogenson and Christensen, 1984). Glomerular filtration rate (GFR) is found to be elevated on average by 20 to 40% above that of age matched normal subject to both in adults and children with IDDM (Mogenson, 1971) which is a clinically silent phase of variable duration after diagnoses of diabetes. Approximately 25% of patients with IDDM have a GFR exceeding the upper limit of the normal range. Renal plasma flow (RPF) has been reported to be elevated, normal or reduced in IDDM (Mogenson, 1971; Ditzel and Junker, 1972) although more recent work shows an elevation of RPF ranging between 9 and 14% (Christiansen, 1984). The increased GFR and RPF are accompanied by an increase in kidney size of approximately 20%, and a good correlation between GFR and kidney volume has been described in patients with IDDM (Mogensen and Anderson, 1973; Christiansen et al., 1981). Approximately 40% of patients with IDDM have kidneys that are larger than normal. A large kidney is a prerequisite for the occurrence of the GFR above the upper limit of the normal range, but normal GFRs can be found in patients with large kidneys (Wiseman and Viberti, 1983).
Histologically, vacuolations and abundance of mucopolysaccharides have been reported in kidneys of streptozotocin-induced diabetic rats (Tedong et al., 2006). In alloxan-induced diabetic dogs, the kidney sections has been reported to show exfoliation of epithelial lining and degeneration of glomerular and tubular epithelium (Bansal et al., 1994). Bulut and his associates (2001) have reported that glomerular capillaries entirely fill the renal corpuscle along with mesangial cell proliferation and hypertrophy in alloxan-induced diabetic rabbits. In diabetic dogs, degeneration of glomeruli and tubular epithelium along with the presence of hyaline casts, mildly sclerotic glomerulus and coagulative necrosis of tubular epithelium has been reported (Sandhu et al., 2000). Mir and Baqui (2005) have reported structural alterations in kidney sections of experimentally induced diabetic rabbits.

**Bladder Dysfunction**

Besides the above complications associating with diabetes mellitus, bladder dysfunction is also a common complication of diabetes mellitus. Ellenberg and Weber (1967) reported that there is an 83% incidence of neurogenic bladder dysfunction in diabetics who have signs of peripheral neuropathy. Histochemical and functional studies have shown that there is a diabetes-induced alteration in the cholinergic innervation and/or change in the response of bladder of smooth muscle to cholinergic agonists in both humans (Buck et al., 1976) and experimental animals (Kolta et al., 1985; Longhurst et al., 1986; Jamshid et al., 1988).

**Liver Damage**

Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homoeostasis and is severely affected during diabetes (Seifter and England, 1982). Liver participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and
triglycerides. During diabetes a profound alteration in the concentration and composition of lipid occurs (Sochor et al., 1985). Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (Baquer, 1998). Structural alterations in the diabetic liver of experimental animals showed dilatation of sinusoids, disruption, degeneration, congestion and necrosis of hepatic architecture has been reported (Sandhu et al., 2000). Bansal and his associates (1994) have reported fatty changes in liver of diabetic dogs. Further, a reduction in volume of hepatocytes, their nuclei and sinusoids in rat liver by streptozotocin injection have been reported (Noorafshan et al., 2005). A reduction in the mean volume and weight of the liver by 15% and 12% respectively has been reported.

Lesions of the liver are characterized by hyperplasia of the ductules, together with dilatation with granular, brownish material. Hepatomegaly, due to either fatty metamorphosis or cirrhoses often occurs in diabetic dogs (Thomson, 1989). The enlarged liver is friable and soft. Palpation can lead to its disruption, rupture and intra-abdominal haemorrhage. The accumulation of fat in the liver is the result of increased fat mobilization in diabetic patients. In addition, parenchymal cells in the liver are injured when ketonemia is present, resulting in decreased utilization of fats (Thomson, 1989).

**Diabetic Retinopathy**

Diabetic retinopathy is present in virtually all patients with IDDM with nephropathy (Parving et al., 1988). Visual impairment, sometimes even total blindness, is one of the more feared consequences of long standing diabetes (Kumar et al., 2001). The ocular involvement may take the form of retinopathy, cataract formation (Rawal et al., 1984), or glaucoma. (Mohan 2000. Kumar et al., 2001). The development of lesions (Boyd, 1970) caused due to changing patterns of blood flow through the retina (Rawal et al., 1986) result in
ischaemia, microaneurysm in the retinal capillaries, new formation of the retinal capillaries, new formation of the capillaries within of the retina, subsequent haemorrhage into the vitreous, and formation of granulation tissue (Lacy and Kissane, 1977). The development of these lesions requires many years with a varying degree of severity in individual patients and long periods of remissions with no further impairment of vision.

Alloxan induced cataractous lenses have shown an increase in glutathione reductase (GSH-R) activity and fall in glutathione (GSH) content in blood, aquous humor and lens indicating that alloxan interferes with intracellular oxidation reduction process in the lens and thus lead to cataract formation (Rawal and Gandhi, 1984). Further, in alloxan- induced cataractous lenses, an increase in sodium, calcium, water content and a fall in potassium content and total proteins have been reported (Rawal and Gandhi, 1986). Gabbay (1973) has reported that cataract formation in the diabetic patient is related to the unique sorbitol pathway by which glucose is metabolized in the lens.

Cardiovascular Damage

The prevalence and severity of atherosclerosis is higher among diabetic patients, particularly coronary artery disease (CAD) that is a major contributor to mortality and morbidity among type II diabetic subjects (Pyorala et al., 1987). Diabetic subjects have been shown to have a higher risk for CAD compared to the non-diabetic population (Keen et al., 1999). Further, independent of the cardiovascular risk factors seen among non-diabetic subjects, diabetes specific factors also contribute to the increased CAD risk and also to vulnerability of plaque ruptures (Nesto and Rutter, 2002). The pathophysiological process of atherosclerosis in diabetic subjects is accelerated by several factors such as hyperglycemia, insulin resistance, abnormal lipid profile, oxidative modification of lipoproteins, increased blood pressure, altered rate fibrinolysis (Arvind et al., 2002).
Diabetic Foot

The greatest fears of the diabetic patient are loss of eyesight and amputation. In the United States, 50,000 major non-traumatic amputations (above-knee and below-knee) are performed each year, 30,000 of them involving patients with diabetes (Kahn et al., 1994). Diabetes mellitus accelerates the development of arteriosclerosis with a resultant earlier onset of coronary arteriosclerosis and atherosclerosis in general (Lacy and Kissane, 1977). The arteriosclerotic process also involves the vessels to the lower extremity with resultant production of gangrene of the toes and feet. The precipitating causes of gangrene of the lower extremities are usually mechanical, thermal or chemical trauma resulting in ulceration, infection and subsequent gangrene. Tropic disturbances, such as ulceration of the fingers or toes and neuropathic arthropathy may develop as complication of diabetic peripheral neuropathy and susceptibility to infections all tend to promote gangrene of the extremities in diabetics (Anderson, 1985).

Diabetic neuropathy

A great number of anatomical, functional and biochemical alterations have been described in the nervous system of diabetic animals (Tomlinson et al., 1992; Ozturk et al., 1996). This variety of alterations, generally called diabetic neuropathy, affects the brain, spinal cord and peripheral nerves (Gallego et al., 2003). They were reported as degenerative changes in the autonomic nervous system of diabetic rats, with widespread degeneration of ganglionic tissue, reduction of axonal calibre and demyelinization (Tomlinson and Yusof, 1983; Schmidt and Pulard, 1986; Kniel et al., 1986). In the central nervous system, diabetes reduces brain weight and neocortical volume, which is associated with a reduction of the number of cortical neurons (Jokobsen et al., 1987). These central and peripheral changes indicate decreased neuronal activity.
Biochemical changes in diabetic neuropathy are more widespread than anatomical changes. The plasma and tissue catecholamine levels are increased, decreased or unchanged based on the selection of tissue, severity or duration of diabetes (Fushimi et al., 1984; Bitar et al., 1986; Hilsted, 1995). Gallego et al. (2003) have reported that diabetes alters the catecholaminergic system in a very specific manner. The dopamine content is reduced only in the dopaminergic nigrostriatal system. Norepinephrine is altered, increased or decreased in the sympathetic nervous system, but not in the central nervous system, and epinephrine is only altered in the adrenal gland and serum.

Drug Treatment of Diabetes Mellitus

Insulin was first isolated by Banting and Best from dog pancreas in 1921 (Banting and Best, 1922), and the first injection of insulin was given to a patient with diabetes at the Toronto General Hospital on January 12, 1922 (Best, 1956). Insulin promotes the storage of fat as well as glucose within specialized target cells and influences cell growth and tissues (Katzung, 1989). Transient and partial recovery of β cell function occurs in many IDDM patients during the first few months of conventional insulin treatment, and is manifested by increased circulating C-peptide concentrations, symptomatic remission and a decline in insulin requirement (Wallensteen et al., 1988). Type II diabetes mellitus is a progressive disorder, and maintenance of near-normal glycemic control has been demonstrated to reduce the risk of its associated long-term vascular complications, and this treatment goal can be achieved in most patient with use of single oral agents, combination of oral agents, or insulin (Buse, 2000). Oral hypoglycemic agents include the sulphonylureas, the biguanide metformin, the α-glucosidase inhibitor acarbose and in some cases anti-obesity drugs such as D-fenfluramine are useful (Pickup and Williams, 1997). In NIDDM, drugs correct the underlying metabolic disorders, namely, insulin resistance and inadequate insulin secretion, and they should be used to modify
the patients lifestyle, particularly restriction of fat and total energy intake and increased physical exercise (Pickup and Williams, 1997). The UK Prospective Diabetes Study (UKPDS) in 1998 showed that, compared with conventional diet and life style modification, more intensive therapy with metformin, sulphonyluria, or insulin was associated with a 0.9% absolute reduction in HbA1c value over 10 years, a 25% reduction in microvascular endpoints, and a 16% reduction in microvascular disease.

Sulphonylurea Treatment

Sulphonylurea has represented the backbone of NIDDM therapy for more than 30 years, yet there is still much controversy about its mode of action and specifically whether they lower blood glucose through extra-pancreatic mechanisms other than stimulation of insulin secretion (Groop, 1992). In vitro studies using the perfused rat pancreas and in-vivo studies using the hyperglycemic clamp have demonstrated that sulphonylurea stimulate insulin secretion in a biphasic fashion (Grodsky et al., 1977; Groop et al., 1987). The insulinotrophic effect of sulphonylurea is augmented by glucose, and they apparently increase β cell sensitivity to glucose and non-glucose stimuli (Pfeifer et al., 1980). Glipizide, glyburide and glibenclamide are potent second generation sulphonylurea drugs that improve glucose tolerance by augmenting insulin secretion and enhancing insulin action (Loubatieres, 1957; Pfeifer et al., 1980; Groop et al., 1985). Glyburide appears to exert greater effect to lower the fasting plasma glucose concentration, whereas glipizide has a greater effect on meal-stimulated insulin secretion (Groop et al., 1985). In addition, glibenclamide seems to suppress hepatic glucose production more effectively than glipizide when examined at identical plasma concentrations (Groop et al., 1987), whereas glipizide results in greater postprandial glucose excursions than glibenclamide (Sonkson et al., 1981; Groop et al., 1985).
Hypoglycemia is the most common and severe side effect of sulphonylurea and in elderly subjects it can lead to permanent neurological damage and death (Ferner and Neil, 1988; Jenning et al., 1989). Glibenclamide has been reported to possess hypoglycemic activity (Mishra et al., 1982). Most cases of severe and fatal hypoglycemia have been reported with the long acting sulphonylureas, chlorpropamide and glibenclamide (Berger, 1985; Femer and Neil, 1988; Jenning et al., 1989). About 20% of patients treated with sulphonylureas in the UK reported at least one episode of symptomatic hypoglycemia during the previous six months (Jenning et al., 1989).

Biguanide Treatment

Biguanides have been reported to improve the sensitivity for insulin without stimulating its production (Bailey and Turner, 1996). Synthetic biguanide (Metformin and Phenformin) were introduced in the late 1950s for the treatment of NIDDM (Pickup and Williams, 1997). Phenformin was withdrawn in most countries because of its association with lactic acidosis (Katzung, 1989; Pickup and Williams, 1997), but is only given to type II diabetic who is allergic to sulphonylurea and insulin and who fails diet therapy (Katzung, 1989). Metformin has remained in use throughout Europe and in many other countries and was recently approved by the Food and Drug Administration (FDA) for introduction in the USA (Pickup and Williams, 1997). Metformin is the only biguanide registered, since phenformin causes the serious adverse effect of lactic acidosis (Misbin, 1977; Misbin et al., 1998) and it has been demonstrated that treatment with metformin in obese patients with type II diabetes is accompanied by a significant decrease in glycosylated haemoglobin. A number of studies have confirmed that metformin is as effective as sulphonylureas in reducing fasting plasma glucose concentrations (Clarke and Duncan, 1968; Herman, 1979). Dunn and Peters (1995) have studied that the increase in body weight did not take place during metformin
therapy as is normally observed when glycemic control is improved. This makes metformin pre-eminently suitable as primary drug for obese patients with type II diabetes with insufficient glycemic control in spite of diet (Stades et al., 2000). Various studies have reported a 20 to 25% increase in peripheral glucose uptake during treatment with metformin (Widen et al., 1994), mostly due to an increase in non-oxidative glucose metabolism. DeFronzo et al. (1991) have found that peripheral glucose uptake increased only under hyperglycemia conditions. Bailey and Turner (1996) and Pickup and Williams (1997) have reported that hypoglycemia during metformin monotherapy is rare and has been therefore, considered as an antihyperglycemic rather than a hypoglycemic agent. Buse (2000) has reported that metformin restores glycemic control in highly insulin resistant obese patients without increasing peripheral insulin levels, largely by suppressing hepatic glucose output. One recent study (Aviles-Santa et al., 1999) showed a marked additive impact of metformin with insulin therapy after 6 months of treatment among type II diabetes patients with poor glycemic control. In this trial, treatment with insulin plus metformin resulted in a lower 

\[ \text{HbA1c} \]

level than insulin plus placebo (6.7% Vs 7.7%) at a lower dose of insulin (96 U/day Vs 129 U/day) (Aviles-Santa et al., 1999).

**Treatment of Drug-induced Diabetes Mellitus**

In experimental diabetes, the use of herbal medicine is widespread. More than 400 traditional plant treatments for diabetes mellitus have been recorded, but only small members of these have received scientific and medical evaluation to assess their efficiency (Bailey and Day 1989; Satyavati et al., 1987). There are various medicinal plants in the world, which are the potential sources of the drugs and most of the herbs are reported to possess some degree of antidiabetic activity (Marles and Farnsworth, 1996).

**Gymnema:** *Gymnema sylvestre* has a long history of use in India for controlling diabetes and is commonly named as gurmar meaning "sugar-destroying"
because of the plants antisacharogenic property (Suppresses the taste of sugar). Chewing the leaves actually deadens the sense of sweat tastes and also the bitterness of bitter substances (Nadkarni, 1976). In diabetic rabbit model, administration of G. sylvestre was shown not only bringing about blood glucose homoeostasis, but also increasing the activities of enzymes involved in glucose utilization (Shanmugasundaram, et al., 1983). Additionally, the investigators reported that glycogen depletion in the liver and lipid accumulation in the diabetic animals was reversed as a result of Gymnema sylvestre therapy.

**Garlic and Onions:** Garlic and onions contain sulphur compounds, which are believed to be responsible for many of the plants reported health benefits, including antidiabetic properties. S-allyl cysteine sulfoxide (SACS), a compound present in garlic, was reported to decrease fasting blood glucose and lower serum cholesterol levels in diabetic rats in a manner similar to the effects of glibenclamide and insulin (Sheela and Augusti, 1992). Jelodar et al. (2005) have reported that garlic was able to reduce blood sugar level in alloxan-induced diabetic rats.

**Fenugreek:** Fenugreek seeds (*Trigonella foenum graccum*) have been demonstrated to posses hypoglycemic properties in both animal and human studies, thus, lending support to its traditional use (Ribes, 1986; Sharma, 1986). Research further suggests that fenugreek has a lowering effect on plasma cholesterol and triglyceride levels (Bordia et al., 1997).

**Bitter Gourd:** Bitter gourd (*Momordica charantia*) also known as balsam pear, is a tropical vegetable widely cultivated in parts of Asia, Africa and South America, which has been extensively used in folk medicine as a remedy for diabetes (Welihinda et al., 1982). The antidiabetic action of the fresh juice or extract of the unripe fruit has been established in both animal and human studies (Karunanayake, et al., 1990; Ali et al., 1993; Welihinda et al., 1986).
**Syzygium malaccense:** The astringent bark of Malay apple i.e. *Syzygium malaccense* an indigenous plant is recommended as a local remedy for a variety of disorders like cough, constipation, headache, diabetes, antibacterial activity, diuretic, abortifacient etc. Further, it has been reported that the extracts of *Syzygium malaccense* with their beneficial effects on blood sugar and hyperlipidemia associated with diabetes could serve as good adjuvant to other oral hypoglycemic agents (Bairy *et al.*, 2005).

**Azadirachta indica (Neem):** In India neem is widely used as a medicinal plant for thousand of years. Extracts of ripe leaves, tender leaves, fruits and flowers of *Azadirachta indica* have been reported to possess antidiabetic activity (Bhattacharji *et al*., 1953). The hypoglycemic effects of neem has been well studied (Murty *et al*., 1978). Recently, it has been reported in diabetic rats that *A. indica* leaves and *Abroma augusta* roots when given together as water extract posses hypoglycemic action and had better effect than given alone (Halim, 2003).

**Cassia auriculata:** *Cassia auriculata* Lin., commonly known as Tanner's senna, is a common highly branched shrub with large bright yellow flowers distributed widely in dry regions of the central provinces and western peninsula of India (Kirtikar and Basu, 1981). The plant as a whole has been used as antidiabetic, antidysentric, antimicrobial and for various skin diseases from ancient times (Chaterjee, 1997). In Ayurveda, its seeds are used to treat various gastrointestinal disorders (Chaterjee, 1997). The flowers of the plant are used as folk remedy for the treatment of diabetes mellitus in Southern parts of India (Nadkarni and Nadkarni, 1982). In streptozotocin-induced diabetic rats its flower extract has been reported to suppress the elevated blood glucose and lipid levels and a dose of 0.45g/kg has been found to be comparable to glibenclamide (Pari and Latha, 2002). Recently the ethanol extract of *Cassia auriculata* flowers has been reported to possess antidiabetic activity which is
attributed to the presence of sterols, triterpenoids, flavonoids and tannins (Hatapakki et al., 2005).

**Aralia cachemirica Decne:** It is known as Khoree in Kashmiri and is found distributed in the temperate himalayas from Kashmir to Sikkim at 2100 to 4000m (Asolkar et al., 1992). The alcoholic extract of *Aralia cachemirica Decne* roots have been reported to show anti-hyperglycemic activity and an enhanced glucose tolerance activity (Bhat et al., 2005).

**Nigella sativa:** *N. sativa* L. is a spice plant containing black seeds which possess more than 30% of a fixed oil and 0.40-0.45% w/w of a volatile oil (Aqel and Shaheen, 1996). The volatile oil has been shown to contain 18.4 – 24% thymoquinone and a total of 46% of many monoterpenes (El-Tahir et al., 1993), which have diuretic and hypotensive activity (Zaoui et al., 2000). The aqueous extract of *N. sativa* L. seeds has been reported to decrease the diabetes-induced disturbances of heart rate and some haematological parameters of alloxan-induced diabetic rabbits (Meral et al., 2004).

**Hedera helix:** *Hedra helix* L. is an evergreen woody climber widely distributed in India, Nepal, China and Pakistan (Stewart, 1972; Bahijri et al. 1984) and is locally claimed to possess hypoglycemic properties. Zafar et al. (2002) have reported that oral administration of ethanolic extract of *Hedera helix* has a beneficial effect on the alloxan-induced diabetic rabbits by lowering the blood glucose level through extra-pancreatic actions rather than by stimulated insulin release.

Rao et al. (1998) have reported that for clinical management of alloxan-induced diabetic dogs, oral antidiabetic drug glibenclamide @ 5mg daily alongwith dietetic adjustment consisting of 40 gm each of Rajmah and Bengal gram Dal are recommended. Shenoy et al. (2002) have reported that treatment with perindopril prevented streptozotocin-induced hyperglycemia and
decreased the elevated blood pressure in both Wistar diabetic and spontaneously hypersensitive diabetic rats. Babu et al. (2002) have reported antihyperglycemic activity of Cassia kleinii leaf (alcohol extract) in both glucose-fed hyperglycemic and alloxan-induced diabetic rats. Kakuda et al. (1996) reported the hypoglycemic effects of Lagerstroemia speciosa known by the Tagalog name of banaba in the Phillipines in a study using hereditary diabetic mice (type II. KK-A^y). Dubey et al. (1994) have reported that D-400, a herbomineral preparation, showed favourable response against alloxan-induced renal damage and hyperglycemia. Schauburger et al. (1977) have reported that pretreatment with n-butanol protects mice from alloxan-induced diabetes by the indirect mechanism of producing hyperglycemia at the time of alloxan administration.

The effective way of clinical management of diabetes mellitus includes exercise and diet control apart from anti-diabetic drugs (Giri et al., 1986). Diet has been recognized as a corner stone in the management of diabetes mellitus (Sharma and Raghuram, 1990). Dietary restrictions are similar for both maturity-onset diabetes (MOD) and juvenile-onset diabetic patients although a reduction in total caloric intake is necessary for overweight MOD patients. Specific diets must be determined individually for every patient. Carbohydrates are still essential to the diabetic diet but need to be in the form of polysaccharides (e.g. starch). Mono- and disaccharides are usually avoided because of rapid uptake and resultant hyperglycemia soon after ingestion. The ingestion of simple sugars is less a problem for the MOD patient as the pancreas can respond with some insulin release (Norris, 1985).