Chapter-V

DISCUSSION
Alloxan-induced diabetes mellitus in rabbits was confirmed by elevated blood sugar (F) level on first week after intraperitoneal administration of alloxan, followed by persistent hyperglycemia during the entire period of the experiment. Keen and NgTang (1982) reported that the minimum defining characteristic feature to identify diabetes mellitus is chronic and substantiated elevation of circulating glucose concentration. Establishment of diabetes mellitus in rabbits in the present study, induced by alloxan administration, might be attributable to specific irreversible toxic effects of alloxan on β cells of pancreas (Dunn et al., 1943; Lokenes, 1948). Fisher and Herman (1982) reported that alloxan is rapidly reduced in the body forming dialuric acid, that undergoes auto-oxidation yielding detectable amounts of hydrogen peroxide, superoxide anion (−O₂⁻) and hydroxyl free radicals (−OH); the latter being produced by metal catalyzed Haber-Weiss reaction. These reduced species of oxygen, particularly the extremely reactive OH radical, are believed to initiate alloxan based attack on β cells. The deleterious effects of alloxan causing hyperglycemia, might be due to rapid inhibition of insulin secretory mechanism (Grodsky et al., 1982). Malaisse (1982) suggested that alloxan and its metabolites have a tendency to concentrate in pancreatic islet tissue relative to some other tissue and that the selective cytotoxicity of alloxan was due to the function of three factors: efficient uptake, oxidant production by redox coupling of the drug with intracellular reductant (ascorbate and thiols) coupled with low levels of glutathione peroxidase in the islets of Langerhan's. In vivo and in
vitro experiments have demonstrated that alloxan elevates cytosolic free Ca^{2+} concentration in pancreatic beta cells (Kim et al., 1994; Park et al., 1995). This effect arises due to alloxan-induced calcium influx from extra cellular fluid, exaggerated calcium mobilization from intracellular stores and its limited elimination from the cytoplasm. The calcium influx might result from the ability of alloxan to depolarize pancreatic beta cells (Dean and Mathews, 1972). Depolarization of the cell membrane opens voltage-dependent calcium channels and enhances calcium entry into cells. A stimulatory effect on mitochondrial Ca^{2+} efflux with simultaneous inhibitory action on Ca^{2+} uptake by mitochondria was also found to be exerted by alloxan (Nelson and Boquist, 1982; Lenzen et al., 1992). The effect of alloxan on intracellular calcium concentration seems to be mediated, at least partially, by H_2O_2 since it exerts a similar effect on calcium concentration in beta cells (Park et al., 1995). The exaggerated concentration of Ca^{2+} contributes to supraphysiological insulin release and together with reactive oxygen species, causes damage of pancreatic beta cells (Kim et al., 1994).

Fisher (1985) reported that a number of agents such as radical scavengers (e.g., Dimethyl urea); chelators of metal catalysts, a variety of relevant enzymes (e.g., Superoxide dimutase). nicotinamide and its analogues given to animals prior to alloxan administration prevented the diabetogenic effect of alloxan. Further, adrenergic agents (e.g., epinephrine. and clonidine) were shown to protect alloxan-induced diabetes (Nakadate et al., 1983). Schauburger and his associates (1977) have observed that methanol. ethanol. n-propanol and n-butanol pretreatment of mice protects the animals from alloxan-induced diabetes. Pretreatment of mice with n-butanol causes hyperglycemia at the time of alloxan administration (Heikkila et al., 1976) and glucose administration is known to protect animals from alloxan-induced β cell necrosis (Bhattacharya, 1953; Rossini et al., 1975).
The four doses of intraperitoneal administration of alloxan given to fasted rabbits caused elevation of blood glucose up to sixth weeks. In the seventh week, the blood glucose was almost constant. The multiple low doses of alloxan given at periodical intervals might be responsible for destruction of beta cells and establishment of diabetes mellitus. Similar methods for establishment of diabetes in fasted rabbits were followed by earlier workers (Rastogi et al., 1998; Baqui et al., 2005). Katsumata et al. (1992) reported that the intraperitoneal dose below 150 mg/kg b.w. is insufficient for inducing diabetes in the rat. Further, fasted animals have been reported to be more susceptible to alloxan (Katsumata et al., 1992; Szkudelski et al., 1998). The multiple low doses of alloxan were given because too high doses of alloxan administration cause loss of animals due to kidney tubular cell necrotic toxicity (Lenzen et al., 1996). The blood sugar level in all the rabbits was on peak in the sixth week, plateaued on the seventh week and later on started to show fluctuations with a decreasing tendency. Alloxan, in low doses, has been reported to produce non-insulin dependent diabetes mellitus (NIDDM) like state which can progress to a gradual recovery or to an insulin dependent diabetes mellitus (IDDM) stage (Cooperstein and Watkins, 1981; Bailey and Flatt, 1991).

The changes in other biochemical parameters viz. blood urea and serum creatinine in alloxanized diabetic rabbits also increased consistently with blood sugar level. Blood urea and serum creatinine levels were on peak up to sixth week in consonance with blood sugar level and later on showed fluctuations. The changes in blood urea and serum creatinine have been reported in rabbits following alloxan administration by other workers (Dubey et al., 1994; Baqui et al., 2005). These biochemical changes, which are indicative of renal damage, might be due to increased renal threshold for hyperglycemia. Deekert and Grenfel (1991) reported that the severity of renal disease positively correlates with the levels of blood urea and serum creatinine. Salah et al. (2004) have
characterized the development of diabetic nephropathy by a progressive increase in albuminuria and a late decline in glomerular filtration rate, leading eventually to end stage renal failure.

The clinical signs of hyperglycemia in rabbits, as observed in the present study were polyuria, polydipsia, general weakness, lethargy and decreased physical activity. These findings are in agreement with earlier observations recorded by other workers in sheep (McCandlers et al., 1984), goats (Prasad et al., 1985), dogs (Nelson et al., 1990; Rao et al., 1998) and rats (Balasubramanian, 1991). The signs of polyuria and polydipsia might have been caused by the excessive fluid intake required to carry the increased glucose levels in the blood and exceeding the renal threshold (Doxy et al., 1985). Similarly, the absorption of water by kidneys is inhibited by the osmotic diuresis, thus, resulting in polyuria. Nelson (1985) had observed the symptoms of a diabetic dog as polyuria, polydipsia, polyphagia and weight loss. Sandhu et al., (2000) characterized alloxan induced diabetes mellitus in dogs by vomiting, polydipsia, polyuria, inappetence, dehydration, hypothermia, dullness, depression, hind leg weakness and recumbency followed by death.

The reduction in body weight, as observed in alloxan induced diabetic rabbits, is in consonance with earlier reports (McCandlers et al., 1984; Prasad et al., 1985; Mir et al., 1995). The decrease in body weight might be due to insulin insufficiency leading to decreased accumulation of body reserve and an increased mobilization of endogenous energy store particularly fat (Edward, 1977). The alterations in body weight of diabetic rabbits were along with the changes of blood sugar, blood urea and serum creatinine throughout the experimental study. The disturbances in carbohydrate, lipid and protein metabolism are characteristic of diabetes mellitus (Milne, 1987).

Histopathological examination that revealed necrosis, degeneration and vacuolation of beta cells of islets of Langerhan's was due to the cytotoxicity of
alloxan. These pathological features of beta cells induced by alloxan have been experimentally observed in animals (Sandhu et al., 2000; Szkudelski, 2001; Mir et al., 2005). The action of alloxan in the pancreas is preceded by its rapid uptake by the beta cells (Boquist et al., 1983). Rapid uptake by insulin-secreting cells has been proposed to be one of the important features determining alloxan diabetogenecity. The other histomorphological changes in five-month-old diabetic rabbits showing chronic pancreatitis, haemorrhage, proliferation of fibroblasts and disorganization of pancreatic acini have been reported earlier (Thomson, 1989). Further, in long standing diabetes mellitus interstitial fibrosis of the exocrine tissue has been reported (Doniach et al., 1973; Rahier et al., 1983 a).

The kidneys, which excrete the waste products of metabolism and regulate the body concentration of water and salt, indicated impaired structural and functional activity in alloxan induced diabetic rabbits. Nephrosis, occlusion of tubules and degenerative changes in kidney observed in the present study are in agreement with other workers (Nakayama et al., 1986; Bansal et al., 1994; Mir and Baqui, 2005; Mir et al., 2005). Thickening of the glomerular basement membrane and capillaries of diabetics have been reported earlier (Heidland et al., 1996; Rabkin et al., 1996) and might contribute to end stage renal damage. In the present study the long-term effects of diabetes on kidneys indicated chronic nephritis, interstitial nephritis and chronic changes in medullary sites. Previous studies on the long-term effects of diabetes in experimental animals show glomerular nephropathy along with tubular and interstitial abnormalities (Rasch, 1979; Hirose et al., 1982). Further, histologic studies on kidneys showed proliferation of polygonal cells and resulted in occluding the lumen at some sites. 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). The structural changes in kidneys could be attributed to altered metabolism in diabetes (Rasch, 1980) and the subsequent effects on the increased renal threshold for hyperglycemia (Mir et al., 2005). Further, 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 reinsertion of normoglycemia, nephropathy is not easily reversed (Floretto et al., 1998; Renu et al., 2004). Contrary to it diabetic nephropathy accounts for considerable morbidity and mortality even in patients with well-controlled blood sugar values (Grenfal, 1991).

In the present study histomorphological changes of liver in alloxan-induced diabetic rabbits showed degenerative changes such as hepatosis, biliary hyperplasia and chronic hepatitis. The pathoanatomical changes in liver of alloxan and streptozotocin induced diabetic animals has been previously reported (Herman et al., 1999; Sandhu et al., 2000). Liver, an insulin dependent tissue, playing a pivotal role in glucose and lipid homoeostasis, is severely affected during diabetes (Seifter and England, 1982). There is a profound alteration in the concentration and composition of lipid (Sochor et al., 1985). Changes in glucose metabolism such as decreased glycolysis, impeded glycogenesis and increased gluconeogenesis in diabetic liver have been reported (Baquer, 1998). Further studies suggest that untreated diabetic liver result in hyperglycemia which in turn is known to activate isoforms of protein kinase C (PKC) in several tissues (Porte and Schwartz, 1996) and in hepatocytes, PKC is an intermediate step in the insulin transduction pathway that activates mitogen activated protein kinase (Adachi et al., 1996).
activated protein kinase leads to decreased apoptosis and hyperplasia and finally results in diabetic hepatomegaly (Herman et al., 1999).

The histopathological changes in heart such as histocyte proliferation, haemorrhage and myocarditis (inflammation of cells) observed in the present study could be attributed to the subsequent effects of hyperglycemia which induces degenerative changes in the tissues along with cardiomyopathy and nephropathy by oxygen free radicals (Oberley, 1988). Mechanisms that contribute to the formation of free radicals in diabetes mellitus include not only increased non-enzymic and auto-oxidative glycosylation, but also metabolic stress resulting from changes in energy metabolism, the levels of inflammatory mediators, and the status of antioxidant defense systems (Griesmacher et al., 1995). The evidence indicates that oxidative stress is increased in diabetes due to over production of reactive oxygen species and decreased efficiency of antioxidant defenses. Oxidative stress as well as non-enzymic glycosylation, is considered as a major factor contributing to the extent of chronic diabetic complications (Yaki, 1984; Griesmacher et al., 1995; Gul et al., 2000). Previous studies in diabetic patients had shown the higher prevalence and severity of atherosclerosis compared to non-diabetic population (Keen et al., 1999) contributing to mortality and morbidity among diabetic subjects (Pyorala et al., 1987). Furthermore, in diabetic subjects hyperglycemia, insulin resistance, abnormal lipid profile, oxidative modification of lipoproteins, increased blood pressure and altered rate fibrinolysis have been found to accelerate pathophysiology (Arvind et al., 2002).

The present experiment demonstrated degenerative changes in neurons and edema in brain sections of alloxan-induced diabetic rabbits. 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). The variety of alterations, called diabetic neuropathy, affects the brain.
spinal cord and peripheral nerves (Gallego et al., 2003). Diabetes aggravates brain damage in experimental and clinical subjects, accelerates maturation of neuronal damage, increases infarct volume and induces post-ischemic seizures (Muranyi et al., 2003). Diabetic neuropathy has been related to excessive generation of sorbitol by aldose reductase due to maintained hyperglycemia, altered metabolism of phosphoinositides and reduced Na/K-ATPase activity (Greene et al., 1987; Tomlinson et al., 1992).

The histomorphological study of lung in the present study demonstrated significant alterations. These included edema, haemorrhage, bronchial hyperplasia and emphysema in the five month old diabetic rabbits. These findings were correlated with the severity of diabetes mellitus. There is the possibility that these morphological alterations observed in the lung after the alloxan treatment might be due to the direct action of alloxan per se, since it has been reported that a large dose of alloxan produced pulmonary oedema (Houssay, 1947; Aufdermaur, 1948) and changes in both the capillary endothelium and the alveolar epithelium of the lungs (Cottrell et al., 1967). Sandhu et al. (2000) observed edema, collapse of alveoli, congestion and haemorrhage in lung sections of alloxan-induced diabetic dogs. The pulmonary emphysema observed in the present study is an additional observation. Therefore, the alteration in the lung of the rabbits treated with multiple doses of alloxan are most likely due to severity of hyperglycemia for a prolonged period. Previous studies have demonstrated that diabetic lungs in rats show depressed glucose oxidation (Morishige et al., 1977) and a reduced rate of glucose incorporation into neutral lipids and phospholipids (Moxley and Longmore, 1975). These observations suggest that the disorder of glucose metabolism in diabetes mellitus may lead to a disturbance of the synthesis of the pulmonary surfactant in the lungs (Sugahara et al., 1981). The present findings in the lungs of diabetic rabbits indicate pulmonary dysfunction in diabetic animals.
The present study further, revealed histomorphological changes in the
gut of diabetic rabbits such as intestinal congestion and gastritis which might be
attributed to the development of both macrovascular and microvascular
complications reported in diabetes (Brownlee, 2001). Hypertrophy and
hyperplasia of intestinal epithelium in diabetic animals are linked to absorptive
abnormalities (Younoszai et al., 1993). Previous studies in alloxan and
streptozotocin induced diabetes in experimental animals have reported
degeneration and necrosis at the tips of intestinal villi (Sandhu et al., 2000) and
greater intestinal weight with higher tissue water content (Schedl and Wilson,
2005).

Alloxan-induced diabetes mellitus serves as pathological model for
detecting various diseases that are associated with diabetes. Experimental
models of diabetes developed from chemical induction with alloxan have been
most widely used (Rerup, 1970). Administration of alloxan to different animals
produces, via necrosis of islets, several features common to those observed in
human diabetes (Lukens, 1948; Gaulton et al., 1985; Quan et al., 2001). In the
present study elevation of glucose in the blood established at the beginning of
experiment and monitored in the course of the experiment was a conclusive
proof of the experimentally produced pathological condition with the help of
alloxan. Similarly, the increase in blood urea and serum creatinine of alloxan
induced diabetic rabbits confirms the subsequent effects of hyperglycemia on
the biochemical alterations of kidneys and indicates that effects could extend to
other organs/tissues of animals. The decrease in body weight observed in the
present experiment by alloxan is indicator of insulin insufficiency leading to
decreased accumulations of fat and increased lipolysis. When comparing the
biochemical parameters for glucose, blood urea and serum creatinine, and body
weight of alloxanized diabetic rabbits with those of the saline-treated normal
rabbits, statistical evaluation of the variations of all the parameters showed that
the changes had been significant both at the beginning and at the end of the experiment. Further, excess of the renal threshold for glucose leading to polyuria and polydipsia and other behaviour changes such as dullness, lethargy etc observed throughout the experimental study, also gives evidence of the diabetes production. Furthermore, the subsequent effects of hyperglycemia due to complete destruction of beta cell of pancreatic islets caused deleterious effects on other tissues of the animals. The pathomorphological features observed in pancreas, kidneys, liver, heart, lungs, brain and gut caused by alloxan were significant in contrast to normal rabbits.

Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Papaccio et al., 2000). Intravenous administration of streptozotocin (65 mg/kg b.w.) in the present study effectively induced diabetes mellitus in rabbits and is in consonance with earlier methods of induction (Kedar and Chakrabarti, 1983; Tawfeeg and Sherif, 2001). The elevation of blood sugar level on day 2nd confirmed the establishment of diabetes mellitus in rabbits which is attributed to its selective cytoxicity on beta cells and subsequently impairs glucose oxidation (Bedoya et al., 1996). Two hours after injection of streptozotocin, the hyperglycemia is observed with a concomitant drop in blood insulin followed by hypoglycemia about six hours due to decrease in blood insulin levels (West et al., 1996). The blood sugar level of the rabbits was on peak on day 2nd after streptozotocin administration followed by changes with a decreasing tendency. The changes in blood glucose and insulin concentrations reflect abnormalities in beta cell function (Bedoya et al., 1996). The fluctuations in the blood sugar might also be attributed to the sensitivity to streptozotocin that varies with species, strain, sex and nutritional state and there are batch differences in activity (Okamato, 1981). When administered intravenously, plasma levels of
Streptozotocin rapidly decrease within 15 minutes and concentrate in the liver and kidneys (Sicor Pharmaceuticals, 2003). Twenty percent of the drug is metabolized and/or excreted by the kidneys (Sicor Pharmaceuticals, 2003). The changes in blood urea and serum creatinine observed in the present study could be attributed to the functional and/or morphological changes in the kidneys (Alderson et al., 2004). Kedar and Chakrabarti (1983) had reported elevated levels of blood sugar to 340 mg percent associated with glycolysis, ureamia, hypercholesterolemia, hypertriglyceridemia and loss of body weight in rabbits by a single intravenous injection of streptozotocin (65 mg/kg). Further, a significant increase of total protein excreted, albuminuria, glycosuria, and urinary urea levels indicated impaired renal function (Alderson et al., 2004).

Streptozotocin effectively induced diabetes in rabbits characterized by polydipsia, polyuria, weight loss, decreased physical activities and hyperglycemia, which is in agreement with earlier findings (Calabresi and Chabner, 1985; Shenoy and Goyal, 2002). In streptozotocin induced diabetes there is excess of fatty acids in the serum, which promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins. The abnormal high concentration of serum lipids in the diabetic subject is due, mainly to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase (Bopanna et al., 1997).

The decrease in cellularity within islets of Langerhan's observed in the present study reflects the cytotoxicity of streptozotocin (Papaccio et al., 2000; Szkudelski, 2001). The reduction in the number of beta cells was also confirmed in rabbits using special stains. Streptozotocin destroys beta cells selectively and a single adequate dose produces lasting hyperglycemia and insulin deficiency (Szaleczky et al., 1999). Previous studies have reported that
streptozotocin enters the beta cells via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD⁺ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of super oxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, beta cells undergo destruction by necrosis (Szkudelski, 2001). Other studies indicated that cytotoxic effects of streptozotocin are dependent upon DNA alkylation by site-specific action with DNA bases (Benneth and Pegg, 1981) and by free-radical generation during streptozotocin metabolism (Bolzan and Bianchi, 2002). In the present study beta cells in some islets were found to be fusiform. The change in the shape of cells can be attributed to the partial damage of streptozotocin due to inadequate dose. Aybar et al., (2001) have reported that use of lower dose of streptozotocin produced an incomplete destruction of pancreatic beta cells even though rats became permanently diabetic.

The histomorphological study of the lungs observed in the present study indicated alterations such as congestion and haemorrhage in alveoli and bronchioles. Lung damage in streptozotocin induced diabetic hamsters has been reported (Popov and Simionescu, 1997). It is postulated that hyperglycemia affects the lungs by damaging capillaries and by the non-enzymatic glycosylation of collagen (Bell et al., 1988). Hyperglycemia appears to cause cellular stress by a number of mechanisms, which could be detrimental to the lung (Brownlee, 2001). Firstly, hyperglycemia increase movement of glucose through polyol pathway and sorbitols are produced which in turn causes
osmotic stress to cells and dihydronicotine amide adenine dinucleotide phosphate (NADPH) is consumed, depleting intracellular glutathione. Secondly, hyperglycemia increases concentrations of advanced glycation end products. These glycosylated proteins are formed by non-enzymatic reactions, and changes in protein structure may alter their cellular functions. Thirdly, glucose activates various isomers of protein kinase C which in turn affects the expression of nitric oxide, endothelin, nuclear factor kappa B and plasminogen activator inhibitor. Finally, hyperglycemia increases the flux of glucose through the hexosamine pathway effecting inflammatory mediators and insulin resistance. The combined effect of the four mechanisms results in overproduction of mitochondrial superoxides, causing cellular stress and damage (Brownlee, 2001).

The morphological study in kidneys of streptozotocin induced diabetic rabbits did not show any significant alteration. It has been reported that streptozotocin does not possess any significant nephrotoxic potential (Floretto et al., 1998). However, the kidney sections showed congestion in the present study, which can be attributed to altered metabolism in diabetes (Rasch, 1980). The changes in the liver in diabetic rabbits induced by streptozotocin have been reported earlier (Mitra et al., 1996). The diabetic liver showed degeneration and congestion. In diabetes, degradation of liver glycogen and gluconeogenesis are increased while glucose utilization is inhibited. Glucose 6-phosphatase increases in the liver, facilitating glucose release into the blood. The opposing enzymes which phosphorylate glucose is hexokinase, which is unaffected by insulin and glucokinase, which decrease in diabetes. As a result, the liver continues to produce glucose even with severe hyperglycemia. Under these circumstances the normal liver would shut off and deposit glycogen (Sheila and James, 1993).
The histopathological changes in the heart of streptozotocin-induced diabetic rabbits showed haemorrhage and cardiomyopathy which could be attributed to the hyperglycemia, which by the formation of oxygen free radicals induces degenerative changes in the tissues along with cardiomyopathy and nephropathy (Oberley, 1988).

In the present study the nervous system of streptozotocin-induced diabetic rabbits showed mild neuronal damage. Diabetes accelerates maturation of neuronal damage, increases infarct volume, and induces postischemic seizures (Muranyi et al., 2003).

Furthermore, histomorphological study of alimentary canal did not show any significant alteration. However, stomach sections showed proliferation of yeasts. Although, the association between diabetes mellitus and increased susceptibility to infection is not supported by strong evidence (Wheat, 1980; Thornton, 1971) but many specific infections are more common in diabetic patients, and some occur almost exclusively in them (Joshi et al., 1999). Further, there is evidence that improving glycemic control in patients improves immune function, which is exemplified by the fact that the efficiency of intracellular billing of microorganisms improves with better glycemic control (Gallacher et al., 1995).

Streptozotocin induced diabetes mellitus in many animal species has been reported to resemble human hyperglycemic nonketotic diabetes mellitus (Weir et al., 1981). This effect has been extensively studied and appears to be mediated through a lowering of beta cell nicotinamide adenine dinucleotide (NAD\(^+\)) and results in histopathological alteration of pancreatic islet beta cells (Karunanyake et al., 1974). The present experiment, thus, confirms that a single intravenous injection of streptozotocin is capable of inducting diabetes mellitus in rabbits leading to biochemical, behavioural and structural alterations.
A significant improvement of biochemical indicators viz. blood sugar, blood urea and serum creatinine along with the amelioration of histomorphological changes in alloxan-induced diabetic rabbits by the oral administration of antidiabetic herbal/allopathic drugs was observed in the present study in comparison to saline treated diabetic rabbits. The normoglycemia in the rabbits observed in the present study by the administration of either *Abroma augusta* or *Syzygium jambolanum* might be due to the increased uptake of glucose peripherally and increased sensitivity of insulin (Habib *et al*., 2005). In a number of studies the antihyperglycemic activities of the *Abroma augusta* and *Syzygium jambolanum* either alone or in combination with other drugs have been reported (Das and Basu, 1970; Mukherjee and Shah, 1977; Halim, 2003; Bairy *et al*., 2005). Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances (Collier *et al*., 1987), some may inhibit insulinas activity (Bhide and Aiman, 1963) and others may increase beta cells in the pancreas by activating regeneration of these cells (Shanmugasundaram *et al*., 1990; Abdel *et al*., 1997). The fiber of plants may also interfere with carbohydrate absorption, thereby affecting blood glucose (Nelson *et al*., 1991). Other studies have reported that administration of herbal products block the absorption of sugar molecules in the intestine and improve the body’s ability to use sugar which would help to reduce blood sugar levels (Meir and Yaniv, 1985).

Previous laboratory studies have shown that Abromine, the active constituent of *Abroma augusta* identified as betaine is responsible for antihyperglycemic activity (Das and Basu, 1970; Mukherjee and Shah, 1977). The leaves of the plant contain octacosanol, terasxerol, β-sitosterol acetate and mixture of long chain fatty diols (Mukherjee and Shah, 1977 and 1978).

An antihyperglycemic effect has been reported in experimental and uncontrolled clinical studies on the seeds (Bansal *et al*., 1981; Nair and
Santhakumari, 1986), fruit (Shrotri et al., 1963; Achrekar et al., 1991) and leaves (Sepaha and Bose, 1956; Soares, 2000) of Syzygium. Its chemical composition consists of tannins, resins (gambol), terpans (α-pigeon, β-pigeon, limenene), acids (gallic, palmitic, stearic, oleic), steroids (phytosterol), saponinicy glycosides (antimelin) and flavanols (Albuquerque, 1989; Correa et al., 1998).

A decline of biochemical indicators such as blood sugar, blood urea and serum creatinine of alloxan-induced diabetic rabbits following glimepiride (Sulphonylurea) treatment observed in the present study is in total agreement with earlier workers (Takada et al., 1996; Krauss et al., 2004). Sulphonylurea bind to specific receptors on beta cells resulting in closure of potassium ATP channels and subsequently open calcium channels leading to an increase in cytoplasmic calcium that stimulates insulin release (Pilipson and Steiner, 1995). Glimepiride (a newer sulphonylurea) appears to have a more rapid onset than previous sulphonylureas (both glyburide and glipizide) and consequently less risk of hypoglycemia (Geisen, 1988). Other studies suggest that glimepiride has a potent extra pancreatic effect on glucose metabolism and may directly stimulate glucose transport activity through phospholipid signaling pathway (Takada et al., 1996).

In the present study the improvement in blood urea and serum creatinine of diabetic rabbits following treatment therapies can be attributed to the recovery of renal function (Tedong et al., 2006), which is explained by the regenerative capability of the renal tubules (Kissane, 1985). 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 re instituted of normoglycemia, nephropathy is not easily reversed (Florretto et al., 1998; Renu et al., 2004). Tedong et al. (2006) have reported that the normoglycemia in diabetic rats with treatment therapies could ameliorate the
glomerular and tubular lesions that characterize diabetic nephropathy and subsequently recover renal morphology and function.

The significant increase in the number of beta cells in the islets of Langerhan's by the application of antidiabetic drugs in comparison to saline treated diabetic rabbits can be attributed to the regenerative effect of plants on pancreatic tissue (Chakravarthy et al., 1980; Shanmugasundaram et al., 1990; Abdel et al., 1997). Increase in pancreatic beta cells mass may result from mitotic proliferation of pre-existing islet cells, or islets may bud off from the ductal system of the pancreas (Slack, 1995), or arise from transformation of the acini into new islets, or may even be derived from the centro-acinar cells (Jindal et al., 1995). There is strong evidence that islet stem cells may exist in the pancreatic duct and that these ductal epithelial cells may be switched into a proliferative/regenerative phase leading to nesideoblastosis (neogenesis of islets) (Hellerstrom, 1984; Bonner-Weir et al., 1993). According to Waguri et al. (1997) the beta cells can regenerate either through differentiation of the precursor cells from the pancreatic duct, or proliferation from existing or surviving mature beta cells. Lipsett and Finegood (2002) reported beta cell neoformation from precursor cells in the pancreatic duct of diabetic animals. Schossler et al., (2004) reported the regeneration of insulin producing cells in the pancreatic duct wall of *Syzgium cumini* treated alloxan-induced diabetic rats. Chakravarthy (1980) reported that *Pterocarpus marsupium* Roxb. acts as hypoglycemic agent by a selective regeneration of beta cells of alloxan damaged pancreas and that its presence can protect the beta cells against the necrotic effect of subsequently administered alloxan. Such evidences corroborate the suggestion that the drugs used in the present study possess the chemical substances that stimulate precursor cell differentiation causing regeneration of beta cells. Rastogi et al. (1988) reported β-cell regeneration with homoeopathic drug *Cephalendra indica* Q in diabetized rats.
The pathological changes of diabetic organs are caused due to the production of oxygen free radicals (Oberley, 1988). Mechanisms that contribute to the formation of free radicals in diabetes mellitus include not only increased non-enzymic and auto-oxidative glycosylation, but also metabolic stress resulting from changes in energy metabolism, the levels of inflammatory mediators, and the status of antioxidant defense systems (Griesmacher et al., 1995). Free radicals meet many of the criteria required for a role in the pathogenesis of diabetic syndrome (Giron et al., 1999). The reversal of oxidative damage shown as a measure of antioxidant enzymes with the antidiabetic compounds indicates that they have possibly antioxidant properties that play a crucial role in the defense against oxygen free radicals (Kaleem et al., 2005). The slight changes observed in the present study in different organs viz. pancreas, kidneys, liver, lungs and heart of group III and Group IV rabbits were significant in comparison to Group II rabbits. The amelioration of histomorphological changes can be attributed to the normoglycemia caused by the chemical substances therapeutic properties that mediate the stimulation of regeneration process and revitalization of remaining beta cells (Diatewa et al., 2004).

Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes (Seifter and England, 1982). Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (Baquer, 1998). The amelioration of histomorphological changes in the diabetic rabbits following treatment therapies as observed in the present study can be attributed to the increase in glycogen level in liver by an increase in glycogenesis and/or a decrease in glycogenolysis (Tedong et al., 2006). Kamalakkanan et al., (2003) reported that in liver the prevention of depletion of glycogen is possibly due to stimulation of insulin release from beta cells that activate the glycogen synthase system. Herbomineral preparations have been
reported to reverse histopathological changes in pancreas and liver partially by scavenging the free radicals and increasing the islet cell super oxide dimutase activity (Mitra et al., 1996).