7. Summary of the Thesis

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The World Health Organization (WHO) estimated 135 million diabetics in 1995 and this number would increase to 300 million by the year 2025. USA and developing countries like china, India have high epidemic rates of obesity and type 2 diabetes. Global figures are predicted to rise from 150 millions cases in 2000 to 221 million in 2010. Person suffering from diabetes have socioeconomic burden of diabetic complications and decrease his life expectancy as well as quality of life. It is advisable to maintain normoglycemia with the help of drug available in the market for the better quality of life and to reduce diabetic complications. These therapies have certain limitation in terms of side effects and higher cost. India is a rich source of herbal medicine and many of them known for its antidiabetic property. One of such plant is *Enicostemma littorale* Blume (EL), known in Indian literature for its antidiabetic potential and is currently being used for the treatment. Previously our lab has shown that EL is having good hypoglycemic, hypolipidemic and antioxidant activity; in diabetic animals as well as NIDDM patients. Insulin resistance and diabetic complications are socioeconomic burden to diabetic patient. Hence presence study was an effort to evaluate efficacy of *Enicostemma littorale* Blume extracts in animal models of insulin resistance and diabetic complications.

We observed that aqueous extract of EL was able to reduce insulin resistance in high fructose fed rats by correcting hypertriglyceredimia, which is causative factor for insulin resistant condition. EL treatment not only prevented insulin resistance but it also helped in ameliorating platelet dysfunction, blood coagulation abnormalities as well as development of androgen dependent hypertension in these rats. It also corrects vascular abnormalities thus, prevents hypertension. We can say that EL is effective drug for the treatment of type 2 diabetes and related complications.

As we know that type 2 and type 1 diabetes leads to progressive loss of B-cell and worsens the diseases condition. B-cells have poor antioxidant defense system as compared to other cells of the body, thus prone to oxidative stress induced damage. Our aim was to evaluate the efficacy of EL extract in prevention of B-cell loss by apoptosis. Oxidative stress is one of the main responsible factors for B-cell apoptosis. We selected H_2O_2-induced apoptosis model for our study. Apoptotic dose of H_2O_2 had been standardized and apoptotic events were confirmed by different apoptosis markers like PS/PI staining, DNA damage and Caspase-3 activation. These islets were having high fluorescence when stained with dye DCF-DA, which indicates oxidative stress in those cells. Activity of antioxidant enzymes were low in H_2O_2 treated cells. Islets were pre-incubated with methanolic extract of EL and then challenged with H_2O_2. These islets were protected against H_2O_2 -induced apoptosis manifested by decreased DNA damage, Caspase-3 activity and by improving antioxidant defense system of cells. Thus indicates protective effect of EL on islets of langerhans.

Further studies were undertaken to evaluate the efficacy of methanolic extract of EL in different disease condition like drug induced nephrotoxicity as well as diabetic nephropathy. Kidney failure or end stage renal disease is a major leading cause for mortality in the population. Each and every person undergoes antibiotic treatment at many stages of his life, and many of them suffer from nephrotoxicity induced by these antibiotics. Oxidative stress is major cause of drug induced nephrotoxicity. EL is known to have good antioxidant activity so we thought to check effect of EL in drug induced nephrotoxicity. Gentamicin-induced nephrotoxicity in rat is well established model for the study of nephrotoxicity as well as being used for the screening of compounds for its prevention. In present study we observed generation of oxidative stress in mitochondrial as well as post-mitochondrial fraction of kidney tissue. Oxidative stress was more in mitochondrial fraction as compared to post-mitochondrial fraction. Simultaneous EL treatment with Gentamicin injections significantly reduces oxidative stress more in mitochondrial fraction as compared to post-

mitochondrila fraction. Our study was further strengthen by Vit C treatment individually to gentamicin injected rats to prove that antioxidant treatment can prevent drug induced nephrotoxicity. Similarly protective role of EL was evaluated in diabetic nephropathic condition in rat. Uninephrotomized rat injected with alloxan (XD) chosen as a model system. These rats develops nephropathic lesions faster than diabetic rats with both the kidney intact. Diabetic nephrectomized rats showed all the signs of kidney dysfuctions like increased serum creatinine and urea levels. They also showed dislipidemia, platelet dysfunction and abnormal blood coagulation. These vascular changes can develop atherosclerosis and thrombotic plague formation. All these metabolic changes suggest development of microvascular complications of diabetes.

Biochemical changes in kidney tissue of these rats showed increased Na-K ATPase activity as well as aldose reductase activity. Elevated Na-K ATPase activity suggests more sodium retention in kidney which might be one of the responsible factor for the development of hypertension along with atherosclerotic and thrombotic conditions. High AR activity indicates more glucose flux in polyol pathway and leads to decreased GSH pool via reduced NADPH content of the cell. Oxidative stress is another hallmark of the disease condition. In present study we observed that amount of oxidative stress generated in mitochondrial as well as post-mitochondrial fractions were equal. Diabetic nephrectomized rats treated with methanolic extract of EL showed improvement in kidney function markers, as well as dislipidemia, platelet function and blood clotting parameters. EL treated animals also showed improvement in kidney Na-K ATPase and AR activities. All these metabolic changes were able to prevent hypertension in these rats. Kidney mitochondrial and post-mitochondrial oxidative changes were improved by EL treatment and prevented damage of kidney cells. Thus, our study explores efficacy of EL treatment in prevention of both drug induced nephrotoxicity and diabetic nephropathic condition.

Neuropathy is another diabetic microvascular complication undertaken for the study. Animals were made diabetic with the help of diabetogenic compound alloxan. Animals with hyperglycemic condition for 45 days developed neuropathic symptoms. These animals showed thermal hypoalgesia and hyperalgesia in formalin induced paw irritation test. Biochemical parameters were evaluated in sciatic nerve. Polyol pathway marker AR activity was high in the sciatic nerve while Na-K ATPase activity was low. Oxidative stress was also high in this nerve. EL treatment reduces the blood glucose level and there by decreases flux of glucose into polyol pathway. EL also increases insulin secreation from remaining islets. EL treatment also reduces oxidative stress in the sciatic nerve by increasing the activity of antioxidant enzymes and protects nerves from damage. EL extract is having AR inhibitory activity also which helps in ameliorating the disease condition. It is reported by others that EL is having anti-nociceptive activity. Thus EL have complete package of insulin screatogouge activity, antioxidant and hypolipidemic required for the better drug candidate for diabetes and diabetic complications.

As EL extract is efficacious in preventing diabetic neuropathic condition, we hypothesized that it should also protect diabetic animals from reproductive dysfunctions as peripheral neuropathy is one of the causative factors for its development. Male as well as female diabetic patients suffer from reproductive dysfunction. In male it causes erectile dysfunction and infertility. Prolong hyperglycemic condition for 45 days leads to atrophy of reproductive organs in alloxan-induced diabetic animals. It causes decreased steroidogenesis marked by decreased 17β-HSD and 3β-HSD activity in testis and leads to decreased serum testosterone levels, which is required for spermatogenesis. AR activity was high in testis, epididymis, seminal vesicles and prostate tissue, while fructose content increases in prostate and seminal vesicles. GSH level were less and lipid peroxidation levels were high in testis and epididymis indicating generation of oxidative stress in these tissues. Prostate and epididymal Vit C content was decreased due to oxidative stress. Vitamin C is also responsible for

steroidogenesis thus decreased vitamin C may lead to low steroidogenesis. Oxidative stress in these two tissue leads to decreased spermatogenesis, sperm viability and sperm maturation in diabetic rats. One group of diabetic animals was treated with methanolic extract of EL for 45 days. These rats showed improved testicular enzyme activities of 17β-HSD and 3β-HSD evident by increased serum testosterone level. Decreased AR activity could be due to inhibitory effect of EL as shown by others and also by decreased glucose flux through this pathway. EL treatment also decreases levels of glycosylated hemoglobin indicating reduced level of AGE formation and its related metabolic derangements. Thus our study on efficacy of EL on male reproductive dysfunction suggest that EL can prevent atrophy of reproductive organs by reducing oxidative stress as well as increasing serum testosterone level required for the growth and maintenance of these organs. Hence, it increases spermatogenesis and prevent sperm function abnormalities.

Above studies indicated protective effect of EL on microvascular complications in diabetic rats. Another study was carried out to evaluate the efficacy of EL on macrovascular complications of diabetes that is cardiomyopathy. Diabetic rats after 45 days of hyperglycemic condition showed the sign of cardiovascular complications like dislipidemia, increased enzymatic serum marker of cardiac function, increased platelet hypersensitivity, blood coagulation abnormalities, depressed cardiac Na-K; Ca-ATPase activity, bradycardia, hypertension and generation of oxidative stress in heart tissue. Diabetic animals treated with methanolic extract of EL correct, all metabolic abnormalities as well as vascular abnormalities. It also improves antioxidant status equally in both mitochondrial and post-mitochondrial compartment of heart tissue. Thus, EL has good efficacy in protecting diabetic rats from development of cardiovascular complications.

Efficacy of EL in different disease condition was comparable to standard drugs glibenclamide and rosiglitazone. This could be because both the standard

drugs are having good antioxidant activity along with hypoglycemic/insulin sensitizing activity.

Conclusively our study indicates that EL is having good hypoglycemia, hypolipidemic, insulin secreatogouge, insulin sensitizing and antioxidant activity. These activities can improve insulin resistance, microvascular and macrovascular complications of diabetes in rat models.