Abstract
Hypertension is reported to be more prevalent among diabetics than non-diabetic subjects and it is reported to aggravate the cardiovascular complications of diabetes. Both hypertension and diabetes mellitus are multifaceted and dynamic expressions of pathological disequilibria, which are closely related and even intermingled by common factors such as obesity, hyperinsulinaemia, micro and macrovascular disease and cardiac risk factors. Many of the antihypertensive drugs used are known to produce adverse metabolic effects. However, angiotensin converting enzyme inhibitors (ACEI) are free of adverse metabolic effects and can correct some of the untoward metabolic actions of diuretics such as hypokalaemia and hypercholesterolaemia. Therefore, ACEI appear to be more effective in preventing long-term complications of hypertension when associated with diabetes mellitus including cardiovascular and renal complications. It has been reported from various laboratories including that from ours that ACEI causes improvement in insulin sensitivity and prevent renal dysfunctions in various experimental models of diabetes mellitus and hypertension. Although ACEI are more effective in controlling or managing diabetic conditions, their use is limited because of disadvantages like unproductive cough and angioedema. These difficulties have been overcome by the introduction of specific angiotensin receptor antagonist. Losartan is the prototype non-peptide AT1 receptor antagonist, potent, orally active and highly selective in nature. By inhibiting the binding of angiotensin II at AT1 receptors, losartan and its active metabolite (E 3174) block the vasoconstrictor and aldosterone secreting effects of angiotensin II regardless of the source of secretion of angiotensin II. Losartan is reported to be more effective than ACEI in lowering blood pressure and does not produce any deleterious effect on insulin sensitivity, glucose metabolism and serum lipids. Reports on the effect of losartan on insulin sensitivity in STZ-induced NIDDM rat model and diabetes induced renal dysfunction in IDDM and NIDDM rat models are either not available or scanty. In the light of this the objective of the investigation was to study the effect of Losartan on insulin sensitivity, glucose
tolerance, lipid levels and renal functions using STZ-induced IDDM and NIDDM models of rat.

Diabetes was induced by a single tail vein injection of Streptozotocin (STZ, 45 mg /kg) in female Wistar rats weighing 200–250 gm. Control group received saline. Animals showing glucosuria (>2%) 48 hours after injection of STZ were considered as rats with insulin dependent diabetes mellitus (IDDM). Type II diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM) was induced by intraperitoneal injection of STZ (70mg/kg) to a group of 5-day-old pups. Another group of pups received only saline. Three months after the injection of STZ, the animals were checked for fasting glucose levels. The rats showing fasting glucose levels >120 mg /dl were considered as NIDDM.

These animals were randomly divided into four sub-groups: - 1) Control; 2) Control treated with losartan 3) Diabetic control and 4) Diabetic treated with losartan. Losartan (2mg/kg) was administered as a single dose daily, p o. for six weeks. All the groups were maintained for six weeks with food and water provided ad libitum throughout the study period. During the study period they were observed for changes in their behavior, water intake, food intake, body weight and mortality recorded at third and sixth week. At the end of six - weeks, blood samples were collected from the retro-orbital plexus, serum was separated and stored at -20°C until analysed for glucose, cholesterol, triglyceride, creatinine, urea and blood urea nitrogen levels using spectrophotometer. Serum insulin was measured by radioimmunoassay method. At the end of 6 weeks we did insulin sensitivity tests like OGTT and KTT. We also carried out measurement of blood pressure and heart rate, estimations of urine electrolyte and nucleic acids in the kidney including histopathological study of kidney.

Injection of STZ (45mg/kg) into the adult rats produced cardinal signs of diabetes mellitus like loss of body weight, polyphagia, polydipsia, polyuria and glucosuria. Treatment of rats with losartan prevented the loss of body weight. However, the increase in food intake and water intake was significantly reduced.
partially by the treatment with losartan. Blood pressure in IDDM rats was found to be higher as compared to control animals. Treatment with losartan significantly prevented STZ–induced increase in blood pressure. Blood pressure of control rats treated with losartan was not significantly different from control. STZ-diabetic rats also showed bradycardia which was significantly prevented by treatment with losartan.

Injection of STZ (45mg/kg) to adult rats produced hyperglycemia and it was associated with hypoinsulinemia as compared to control animals. Treatment with losartan partially but significantly reduced hyperglycemia. However, insulin levels remained low in STZ–rats treated with losartan. Glucose and insulin levels of control rat treated with losartan were comparable to untreated controls. Oral administration of glucose (1.5g / kg) produced a time dependent increase in glucose and insulin levels over the period of two hours. The calculated $AUC_{glucose}$ and $AUC_{insulin}$ were found to be significantly increased and decreased respectively in the STZ-diabetic rats. Treatment of rats with losartan could not produce any change in STZ–induced alterations of $AUC_{glucose}$ and $AUC_{insulin}$. Injection of insulin (0.2 U/100g) produced time dependent decrease in glucose levels over a period of one hour. The calculated $K_{ITT}$ value of IDDM animals was comparable to that of control animals. Treatment with losartan also failed to produce any significant effect on $K_{ITT}$ value of IDDM as well as control and control treated with losartan. These results indicate that losartan may not alter insulin release. The decrease in glucose levels by losartan may be explained on the basis of effects of losartan on insulin sensitivity. IDDM rats however, donot show any altered insulin sensitivity. Results of NIDDM (mentioned below) further indicate that losartan produces an increase in insulin sensitivity in NIDDM models of rat.

Injection of STZ (70 mg/kg) to 5 day old pups developed diabetes (NIDDM) in their later life. Both fasting as well as fed glucose levels were significantly higher in STZ-treated rats as compared to controls. These hyperglycemic (NIDDM) animals also showed hyperinsulinemia. Treatment with losartan significantly decreased both fasting as well as fed glucose levels of NIDDM rats. This was associated with a
decrease in insulin levels, suggesting that losartan may increase the insulin activity in STZ-treated NIDDM rats. Oral administration of glucose (1.5g / kg) produced a time dependent increase in glucose and insulin levels over the period of two hours. The calculated AUC$_{\text{glucose}}$ and AUC$_{\text{insulin}}$ were significantly high in NIDDM control animals as compared to control animals. Treatment with losartan produced significant decrease in both AUC$_{\text{glucose}}$ and AUC$_{\text{insulin}}$ values thereby substantiating the hypothesis that losartan treatment increases insulin sensitivity. Injection of insulin (0.2 U/100g) produced time dependent decrease in glucose levels over a period of one hour. The calculated $K_{\text{ITT}}$ value of NIDDM control rats showed a significant decrease in $K_{\text{ITT}}$ value as compared to control animals thereby suggesting that these animals are insulin resistant. Treatment with losartan significantly increased $K_{\text{ITT}}$ value.

NIDDM control animals showed a significantly retarded gain in body weight. However, there was no change in food intake or water intake in these rats as compared to age matched controls. Treatment with losartan prevented loss of body weight as observed in STZ–treated IDDM rats. NIDDM control rats had high blood pressure as compared to control animals. Treatment with losartan significantly prevented the rise in blood pressure. There was no significant difference between heart rate of NIDDM control as compared to the age matched control rats. Losartan treatment did not produce any effect on heart rate of these animals.

STZ induced IDDM as well as NIDDM rats showed hypercholesterolemia and hypertriglyceridemia as compared to control animals. Treatment with losartan significantly decreased cholesterol as well as triglyceride levels. However, there was no significant difference in cholesterol triglyceride levels of control and control treated with losartan.

There was a significant increase in serum creatinine, serum urea and blood urea nitrogen (BUN) levels in STZ-diabetic rats as compared to controls. Treatment with losartan significantly prevented the rise in serum creatinine, serum urea and BUN levels. This suggests nephroprotective action of losartan in diabetic rats. This
was substantiated by further experiments. Creatinine clearance of IDDM animals was significantly less as compared to control rats. Treatment with losartan significantly improved creatinine clearance in STZ-diabetic rats. STZ-diabetes caused a significant increase in sodium excretion for 6 hours as compared to control animal. Similar change was not seen in potassium excretion. Treatment with losartan produced further increase in sodium in STZ-diabetic rats. STZ also produced a significant hypertrophy of kidney in rats as indicated by kidney weight / body weight ratio and increase in protein content in the kidney. Treatment with losartan prevented STZ-induced hypertrophy. The STZ-induced hypertrophy is also supported by the observation that, there was a significant increase in RNA and decrease in DNA contents of kidney obtained from STZ-diabetic rats. Both these changes were prevented by six-weeks treatment with losartan in STZ-diabetic rats. There was a significant increase in protein / DNA ratio and RNA / DNA ratio of STZ-diabetic animals as compared to control. This was decreased by treatment with losartan.

Increase in serum creatinine and BUN levels were also observed in NIDDM rats. Losartan treatment significantly decreased creatinine as well as BUN levels in these animals.

*It can be concluded from the above mentioned results that losartan produces a number of beneficial effects in diabetic models of rat. It not only prevents STZ-induced hypertension and hyperlipidemia but also nephropathy. Prevention of long term complications of hypertension associated with diabetes mellitus, by losartan appear to be through the improvement in insulin sensitivity.*

**Effect of *Enicostemma littorale* in experimental models of diabetes mellitus**

In our country use of medicinal plants and folk remedies is very common in Ayurvedic and Unani system of medicine. These are used either as a whole extract,
isolated constituents or mixture of various plant extracts (Polyherbal preparations). Different formulations like leaf powder, pastes, decoctions and infusions etc. are being used as antidiabetic therapy. Plants like Azaridicta indica, Eugenia jambolana, Momordica charantia, Swertia chirata, Gymnema sylvestre etc. have shown great potential as antidiabetics and they have been included in various polyherbal preparations. Although number of plants claimed to possess antidiabetic property, systematic study in this regard is either not available or scanty.

*Enicostemma littorale* is a herb reported to possess antidiabetic property. It is a glabrous perennial herb belonging to the family Gentianaceae. This plant has been used as the folk medicine for the treatment of diabetes mellitus in Western and Southern India. *Enicostemma littorale* has been shown to produce antihyperglycemic activity in various hyperglycemic models of rats. Although the crude extract of *Enicostemma littorale* has been used to indicate its antidiabetic property, reports on insulin sensitivity and diabetes induced metabolic alterations are not available. We have also undertaken an investigation to study the effects of chronic treatment with *Enicostemma littorale* on insulin sensitivity and glucose intolerance in STZ-induced IDDM and NIDDM models of rat. Attempts were made to study the effect of *Enicostemma littorale* on lipid profile and kidney functions. The study was then extended to the clinical settings where the effect of *Enicostemma littorale* was studied on hemodynamics, lipid profile and kidney functions in patients with hypertension and diabetes mellitus.

Both IDDM and NIDDM rat models of diabetes were developed using STZ as mentioned earlier. These animals were randomly divided into four sub-groups:- 1) Control; 2) Control treated with *Enicostemma littorale* 3) Diabetic control and 4) Diabetic treated with *Enicostemma littorale*. *Enicostemma littorale* (2g/kg) was administered as a single dose daily, p.o. for six weeks. All the groups were maintained for six weeks with food and water provided *ad libitum* throughout the study period. During the study period they were observed for changes in their behavior, water intake, food intake, body weight and mortality recorded at the interval
of ten days. At the end of six-weeks, blood samples were collected from the retro-orbital plexus, serum was separated and stored at -20°C until analysed for glucose, cholesterol, triglyceride, creatinine, urea and blood urea nitrogen levels using spectrophotometer. Serum insulin was measured by radioimmunoassay method. At the end of 6 weeks we also carried out insulin sensitivity test like OGTT, K\textsubscript{ITT}, measurement of blood pressure, heart rate and histopathological study of kidney.

As mentioned earlier, single tail vein injection of STZ produced the development of diabetes (IDDM) characterized by a significant loss of body weight, polyuria and polyphagia as compared to control rats. Like losartan chronic treatment with \textit{Enicostemma littorale} also prevented STZ–induced loss of body weight, polyuria and polyphagia in these animals. Similarly in case of NIDDM model also there was a significant reduction in weight gain of NIDDM control animals as compared to age matched control animals and treatment with \textit{Enicostemma littorale} caused a significant increase in the weight gain in these animals.

Treatment with \textit{Enicostemma littorale} also significantly prevented hyperglycemia without effecting insulin levels in IDDM rats. There was increase in not only fasting as well as fed glucose levels in NIDDM animals but also there was increase in insulin levels in NIDDM animals as compared to control. Treatment with \textit{Enicostemma littorale} significantly prevented the raise in glucose as well as insulin levels. Results with \textit{Enicostemma littorale} on STZ-induced increase in AUC\textsubscript{glucose} and decrease in AUC\textsubscript{insulin} were similar to that of losartan. Treatment with \textit{Enicostemma littorale} did not alter these values significantly however, like that of losartan treatment with \textit{Enicostemma littorale} significantly decreased both AUC\textsubscript{glucose} and AUC\textsubscript{insulin} values in NIDDM rats. It also prevented decrease in K\textsubscript{ITT} value in NIDDM rats. All these results suggest that \textit{Enicostemma littorale} improves insulin sensitivity in diabetic animals. Treatment with \textit{Enicostemma littorale} also produced a significant decrease in cholesterol and triglyceride levels in both IDDM and NIDDM rat models.
It was further interesting to note that the results of animal studies obtained with *Enicostemma littorale* could be extrapolated to the clinical setting. We conducted the clinical study during which a total 119 cases were registered. Out of 119 patients, included in the study 45% had only diabetes mellitus whereas 27% of patients had diabetes mellitus as well as hypertension. Rest of the subjects (28%) were non-diabetic and non-hypertensives. The age, race, height and weight distributions were similar in all the groups. All patients were within the range of ideal body weight. Body mass index (BMI) was higher only in two groups of diabetic patients than non-diabetics (those receiving sulfonylurea or anti-diabetic along with antihypertensives). However, hip/Waist ratio was found to be higher in most of the diabetic patients as compared to control value.

Initial glucose levels of diabetic patients were significantly high as compared to non-diabetic control. It was interesting to note that, insulin levels were significantly higher in all groups of diabetic patients except those on biguanides or receiving some antihypertensive therapy. One month treatment with *Enicostemma littorale* produced not only significant decrease in glucose but also insulin levels in all groups of diabetic patients.

Hypercholesterolemia was found in all groups of diabetic patients except for those on antihypertensive therapy. After one month treatment of patients *Enicostemma littorale* showed a significant decrease in cholesterol level as compared to the initial value in these patients. Patients receiving either insulin or antihypertensives along with antidiabetics did not show any significant alteration.

Hypercholesterolemia was associated with hypertryglyceridemia in diabetic/hypertensive patients as compared to non-diabetics. One month treatment with *Enicostemma littorale* significantly decreased triglyceride levels as compared to their respective initial value. Both hypertensive and diabetic patients also showed a significantly higher creatinine levels as compared to those of non-diabetic group. After
one month treatment with *Enicostemma littorale*, there was a significant reduction in creatinine levels of these patients.

*It can be concluded that Enicostemma littorale possesses potential antidiabetic activity. In addition to decrease in serum glucose and lipids it can also preserve kidney dysfunction in diabetic patients. Enicostemma littorale may be considered as drug of choice in diabetic patients with compromised kidney function.*