DISCUSSION AND CONCLUSIONS
ANIMAL STUDIES

Intravenous Injection of STZ produces fragmentation of β-cell-DNA of pancreas which stimulate poly (ADP-ribose) and deplete NAD ultimately leading to destruction of β-cells of pancreas and it is evidenced by clinical symptoms of hyperglycaemia and hypoinsulinaemia in Wister rats (Kawashima 1978, Rodrigues et al 1986, Goyal et al 1987). Loss of body weight in diabetic animals may be due to dehydration and catabolism of fats and proteins (Oakley 1968). Further, symptoms of polyphagia, polyuria and polydipsia are observed in the STZ-treated rats. All these findings are consistent with those reported earlier (Hoffeizer 1973, Shah et al, 1995; Sevak et al.1996). *E. littorale* treatment significantly prevented the loss in body weight in diabetic animals. There was normal gain in body weight in nondiabetic animals. Treatment with *E. littorale* significantly prevented polydipsia and polyphagia in STZ-diabetic (IDDM) rats. STZ-diabetic rats had significantly higher serum glucose levels and AUC\textsubscript{glucose}. *E. littorale* treatment significantly reduced both the serum glucose levels and AUC\textsubscript{glucose}. Serum insulin levels of STZ-diabetic rats were significantly low. $K_{ITT}$ of STZ-diabetic rats was found to be almost same as that of Wistar control. Both these (insulin levels and $K_{ITT}$ of STZ-diabetic rats) were found to be unaffected by the treatment with *E. littorale*. Thus, it is speculated that *E. littorale* may be increasing the glucose utilization at peripheral tissues or facilitates for glycogen synthesis and thereby decreases glucose level and insulin might not be involved in producing these decreased glucose levels in IDDM rats.

Administration of STZ in to neonatal rats produces a condition of NIDDM in later life. It was reported that STZ (90 mg/kg Body weight) when given intraperitonially to 2-day old male Sprague-dawley rat pups produced transient hyperglycemia in the neonates, followed by normoglycemia until 5 weeks of age, and hyperglycemia (15–20 mg/dl) emerged, which was maintained through 13 weeks (Weir et al 1981). When STZ at dose of 90 mg /kg body weight was administered to 5-day old pups in our laboratory, a high
mortality (70–90%) was observed. We administered STZ (70 mg/kg body weight) i.p. to 5-day old pups to induce NIDDM as per our previous reports (Gokhale et al. 1998). In neonatal STZ–diabetic model, STZ destroys pancreatic cells, but neonatal cells are able to regenerate. However, this regeneration is incomplete and cell mass remains reduced. Consequently, hyperglycemia gradually develops as rat grows (Weir et al. 1981). Thus, NIDDM rats showed significantly high fasting as well as fed glucose and insulin levels as compared to nondiabetic Wister control rats which were decreased significantly by the treatment with E. littorale. In contrast to IDDM, food and water intakes were found to be same in NIDDM rats and Wistar control rats and further, these values remained unaffected by the E. littorale treatment.

Hyperinsulinemia with low hepatic excretion and hypersecretion of beta cells is reported in mild glucose intolerant obese subjects (Bonora et al. 1983). E. littorale treatment significantly prevented the raise in fasting as well as fed glucose levels and insulin levels in NIDDM animals. These results suggest the improvements of insulin sensitivity by E. littorale. The high insulin concentration found in neonatal STZ-diabetic (NIDDM) rats need not be only of pancreatic origin. It could be due to metabolic alterations at extra pancreatic levels. In these rats, the metabolic clearance rate of insulin might have been altered. Insulin degradation following hormone receptor binding (Gilemann and Sonne 1978) and reduced binding of insulin to its receptor has been reported in mild glucose intolerance (Olefsky 1981). Therefore, hyperinsulinemia in neonatal STZ-diabetic rats could be due to either decreased hepatic excretion of insulin or decreased number of insulin receptors, resulting in decreased insulin binding and lowered insulin degradation. In addition to high insulin levels, the AUC_{glucose} and AUC_{insulin} of NIDDM rats were found to be significantly higher indicative of the insulin resistance in NIDDM rats. Treatment with E. littorale was found to decrease serum insulin, AUC_{glucose} and AUC_{insulin} significantly. This further suggests that E. littorale may not alter the release of insulin, but in conditions like hyperinsulinemia, it improves insulin sensitivity for effective glucose disposal.
Kit value of NIDDM was significantly lower as compared to control rats indicative of insulin resistance. The specific mechanisms responsible for insulin resistance are heterogeneous and may include a receptor defect (decrease in insulin sensitivity) or post receptor defect (decrease in responsiveness to insulin) or combination of both (Kahn 1978, Crettaz and Jeanrenand 1980). *E. littorale* treatment significantly increased Kit value suggestive of improvement in insulin sensitivity. Further, the time required to reach 50% of initial glucose level (t1/2) of NIDDM rats was significantly higher which significantly decreased after treatment with *E. littorale*.

It has been documented that diabetes mellitus is associated with changes in lipid metabolism. STZ-treated rats have increased plasma levels of triglycerides, cholesterol, free fatty acids and phospholipids (Rodrigues et al 1986). In our study, we have observed a significant elevation in cholesterol levels in STZ-diabetic animals. Insulin deficiency or insulin resistance may be responsible for dyslipidemia. Hypoinsulinemic condition may be responsible for elevation of cholesterol levels in diabetic animals because insulin has an inhibitory action on HMG-CoA reductase, an essential enzyme acting as a rate-limiting factor in the metabolism of cholesterol rich LDL particles. Serum cholesterol was found to be increased significantly in the STZ-diabetic rats (IDDM group). *E. littorale* significantly prevented the STZ-induced increase in cholesterol without altering insulin levels or insulin sensitivity in IDDM rats. In NIDDM rats as insulin levels were significantly higher, serum cholesterol levels were found to be almost same as those of the control rats and treatment of *E. littorale* did not produce any marked changes in the serum cholesterol levels of NIDDM rats.

The mechanism responsible for the development of hypertriglyceridemia in uncontrolled diabetes in humans (possibly in insulin deficient STZ-diabetic rats) are due to number of metabolic abnormalities that occur sequentially. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-triglyceride from liver (Balasse et al 1972). With longer insulin
deficiency, liver converts free fatty acids into ketone bodies and VLDL-triglyceride secretion diminishes (Basso and Havel 1970). At the same time, lipoprotein lipase activity falls (Nikkila et al. 1977) resulting in impaired clearance of VLDL and chylomichrones from plasma (Bagdade et al. 1968). Reaven (1988) proposed that insulin resistance in diabetic or nondiabetic subjects led to compensatory hyperinsulinemia, which is associated with, increased LDL-cholesterol and reduced HDL-cholesterol concentrations. In the present study both IDDM and NIDDM rats showed hypertriglyceridaemia and the treatment with *E. littorale* significantly decreased this corroborating the hypothesis of improvement of insulin sensitivity by *E. littorale*.

Kidney dysfunction is indicated by significant elevation in serum creatinine levels in patients with diabetes mellitus (Thomson et al. 1989). Hyperglycemia leads to elevated glucose levels in mesangial cells, which activates protein kinase C (Kriesberg et al. 1994) and increases the synthesis of fibronectin, laminin and type (IV) collagen (Kriesberg et al. 1994). This results in imbalance in matrix proteins leading to development of mesangial hypertrophy and eventually nephropathy. Hyperfiltration and microproteinuria, resulting from glomerular leakage are hallmarks of diabetic nephropathy (Corry and Tuck 1996). Decrease in Glomerular Filtration Rate (GFR) and Renal Plasma Flow (RPF) have been reported in type 1 (IDDM) patients (Mogensen et al. 1979) as well as in variety of experimental models of renal disease in rat including experimentally induced diabetes mellitus (Zatz et al. 1986). We also found significant increase in serum urea and BUN levels in IDDM and NIDDM rats. It has been reported that serum creatinine, urea and BUN levels are significantly increased in patients with diabetes (Mulec 1990).

Renal dysfunction can also be defined in terms of creatinine clearance. It is an accurate and useful measure of the GFR and the excretory capacity of the kidney (Godkar 1994). In the present study, STZ-diabetic rats showed a significant decrease in creatinine clearance as compared to control rats. The decrease in creatinine clearance may be due to hyperglycemia, which causes osmotic diuresis and depletion of extracellular fluid volume (McCance and Widdowson 1939). Treatment with *E. littorale* significantly prevented the raise-
in creatinine, urea and BUN levels and thus preventing the kidney damage due to diabetes in animals.

**CLINICAL STUDIES**

**Baseline Demographics and risk factors**

We conducted an open, multicentric clinical study based on parallel group design. Out of total 203 registered cases, there were 45 nondiabetics (26 males and 19 females) and 158 diabetics (94 males and 64 females). The average age of these patients was 57.2±0.9 years. Data are scarce on the incidence of NIDDM in the population below age 30. In the German Democratic Republic, where diabetes is a registered disease, the incidence rates of NIDDM in ages below 30 have been in the range of 5-30 per 100000 per year (Michaekus et al 1981). In Framingham study, the individuals who fulfilled National Diabetes Data Group (NDDG) criteria for NIDDM or impaired glucose tolerance (IGT) were considered as incident case of diabetes (Wilson et al 1981). The rates increased from 230/1000000 per year at ages (40-49), to 465/1000000/year at ages (50-59) years. In other studies also, it was found that the prevalence of NIDDM increases markedly with age (US Govt. 1967, Melton et al 1983).

Both hypertension and type 2 (non-insulin-dependent) diabetes mellitus are associated with overweight (Fuller 1985, Sims and Berchtold 1985, National diabetes data group 1979). Persons with Body mass index (>23 kg/m²) are considered to be overweight (Singh et al 1998). Montoye et al (1965) gave table for the ideal weight and height for the specific age and sex. Females with BMI (>25 kg/m²) and males with BMI (27 kg/m²) are considered to be equivalent to 120% of desirable body weight (Coopan and Flood 1985). Females having BMI more than 26 kg/m² and males with BMI more than 27 kg/m² are considered as obese (Ferrannini et al 1991, Gupta et al 1994, Burton et al 1985). Out of total 26 nondiabetics males, 10 subjects were
having normal weight with BMI 20.9±0.61 kg/m², 12 nondiabetics were having overweight (BMI 24.8±0.3 kg/m²) while 4 nondiabetics were obese (BMI 32.4±1.3 kg/m²). In case of the diabetic males (n=94), 37 patients were having normal weight (BMI 20.74±0.19 kg/m²), 41 patients were having overweight (BMI 24.9±1.7 kg/m²) and 16 patients were obese (BMI 30.1±0.17 kg/m²). Out of 19 female nondiabetic subjects in the present study, there were 5 normal weighed (20.50±0.59 kg/m²), 8 overweight (BMI 24.7±0.3 kg/m²) and 6 obese (BMI 32.2±0.8 kg/m²). Out of 64 diabetic females, there were 16 diabetic females having normal weight (BMI 20.5±0.59 kg/m²), 26 having overweight (24.4±0.18 kg/m²) and 22 diabetic females were having obese type of the body frame (BMI 30.2±0.8 kg/m²). Obesity and NIDDM are classic states of insulin resistance (DeFronzo and Ferrannini 1982). Thus, based on BMI, it appears that 61% males and 75% of females in our present study are found to be having insulin resistance.

Animal studies have shown that in the development of obesity, fat cells undergo an increase in number as well as in size and lipid content. In obese human also a greater number of fat cells with varying degrees of lipid content were found (Hirsch and Knittle 1970, Hirsch et al 1976). Subjects with childhood obesity tend to have greater degree of fat cell hyperplasia. If obesity occurs during puberty or adolescence or in later life, adipose cell hypertrophy makes a greater contribution to the obesity. This led to hypothesis that once fat cells have increased in number, no change can be effected, other than decrease in size, no matter how much weight is lost by diet or other measures. Knittle et al (1977) noted that, after the age of 2 years, one can detect significant differences in adipose tissue growth and development between obese and non-obese children. Obese children may attain adult levels per fat cell size by 2 years of age and thereafter enlarge the fat stores exclusively by increasing the number of fat cells. In non-obese children, there is no major change in the fat depots until around puberty when both cell size
and number increase. The underlying mechanism for this cell hyperplasia or hypertrophy is not unknown. Diabetes, hypertension, respiratory, gall-bladder diseases are more frequently linked to those who are obese than to those in the non-obese general population (Mann 1974, Bortz 1969).

Ratio of waist-to-hips (WHR) is an indicator of central obesity (fat distribution) (Krotkiewski et al 1983, Shutterly et al 1993). Acromegaly, overweight and obesity (indicative of hyperactivity of pituitary) are well-known predisposing factors in diabetes (Tyler 1960). An obese person can have an apple-shaped fat distribution (also known as central type or android obesity or visceral fat distribution with WHR>0.85) or can have a pear-shaped fat distribution (abdominal fat distribution with WHR<0.7) in the body (Casimirri et al 1989). Persons with the central obesity are having a significantly higher insulin resistance than an equally overweight person who have characteristic pear-shaped obesity with a WHR<0.7 (Lipsky and King 2000, Muller et al 1993, Kissebah et al 1987, Fujimoto et al 1994). Out of 45 nondiabetics, 39 subjects had WHR more than 0.85 (0.96±0.018) and only 6 subjects had WHR less than 0.85 (0.82±0.01). While out of total 158 diabetics, 147 patients had WHR more than 0.85 (0.98±0.01) and 11 patients had WHR less than 0.85 (0.81±0.01). Thus, considering WHR as a parameter, 93% diabetic patients in the present study were found to be having WHR more than 0.85 indicating the presence of central obesity, and thereby indicating the presence of insulin resistance. Further, WHR of diabetic females in our study was found to be significantly higher than nondiabetic females indicating significantly higher central obesity, which makes them more prone to related abnormalities.

It has been reported that a person with lesser physical activity is at a major risk of developing diuresis, which ultimately leads to diabetes mellitus (Shashtri 1981 and 1983). Physical activity can be assessed by determining both occupational (Narsingrao et al 1989) and spare time activities (Paffenberger et al 1993). Household activities for women were considered as occupational in the present study. Accordingly, when a person in his/her
routine life walks less than 14.5 km/week or climbs less than 20 flights of stairs per week or performs no moderately vigorous sports or exercises is considered to have sedentary life style (Narsingrao et al 1989, Paffenberger et al 1993, WHO 1993). In the present demographic data, it was found that, among nondiabetics 56% were having medium type of routine physical work whereas among diabetic patients 57% reported to have lighter type of routine physical work. Only 6% of patients having heavy type of routine physical work were found to have diabetes mellitus. Further, it was observed that more than 60% of both diabetics were having a routine habit of taking sleep during daytime. This clearly indicates the detrimental effects of sedentary lifestyle and beneficial effects of exercise or vigorous physical activities. It has been demonstrated that physical exercise improves insulin sensitivity and lowers plasma insulin levels in nondiabetic and NIDDM subjects (Trovati et al 1984, Devlin and Horton 1985). Increased physical activity leading to decrease in central obesity gives benefit in the hypertension (Singh 1997). Further, it was observed that chronic constipation was more prevalent among diabetics as compared to nondiabetics in the present study. This can be considered as a manifestation of prevalence of lesser physical work among diabetics resulting in decreased gastric motility.

In the present study, habit of smoking was more prevalent among diabetics as compared to nondiabetics. Smoking is a major risk factor for cardiovascular morbidity and mortality (Kannel et al 1987, Welin et al 1993). Smokers exhibit a number of characteristics that are established risk factors for CVD. These include a larger WHR (waist-hip ratio) (Lissner et al 1992, Shimokata et al 1989), elevated fibrinogen (Ernst an Resch 1993), elevated triglycerides and lower HDL (high-density lipoprotein) cholesterol levels (Craig et al 1989, Facchini et al 1992). An acute effect of cigarette smoking is to increase the activity of the sympathetic nervous system and the levels of circulating catecholamines (Cryer et al 1976). Since catecholamines are potent antagonists of insulin actions (Lager et al 1986), smoking may be linked to insulin resistance. Smoking can impair insulin action (Attvall et al 1993). In a transectional study, Facchini et al (1992) reported that chronic smokers exhibit traits of impaired insulin action, including higher triglyceride
and lower HDL-cholesterol levels, than a matched group of nonsmoking individuals. Consequently, cigarette smoking has been recognized as an independent risk factor for non-insulin-dependent diabetes mellitus (NIDDM) in both men and women (Feskens and Kromhout 1989, Rimm et al 1993). In a large group of healthy male smokers, the amount of nicotine smoked per day is related to degree of insulin resistance. Thus, the established effect of smoking on various risk factors for CVD such as elevated triglyceride and lower HDL-cholesterol levels (Craig et al 1989, Facchini et al 1992) and higher PAI-I activity (Ologddon et al 1989) can to a large extent be attributed to a concomitant insulin resistance in smokers (Elliasson et al 1994). The findings that the incidence of NIDDM is also higher in smokers (Feskens and Kromhout 1989, Rimm et al 1993) further substantiate this conclusion. Several prospective studies have shown that the degree of insulin resistance is a major predictor of future risk of NIDDM. Several studies have shown that smokers have higher cholesterol levels (Craig et al 1989). Smoking and sedentary lifestyles are the major risk factors, which predisposes CAD (Singh et al 1997). Stress and anxiety mediated through the hypothalmo-pituitary adrenal axis have been reported to aggravate the diabetic condition (Thomsen 1938, Wallach et al 1957). Diabetic patients under present study are found to be having higher percentage of stress.

Thus, the risk factors (sedentary lifestyle and smoking) for development of diabetes were significantly higher among diabetics as compared to nondiabetics.

**General and biochemical indices of diabetes mellitus**

The frequency of night micturation among diabetics was significantly higher as compared to nondiabetics. The frequency of day micturation was found to be higher among diabetics as compared to nondiabetics but it was not statistically significant. Polyuria is one of the cardinal symptoms of diabetes. The frequency of night micturation was found to be correlated with the disturbed night sleep. The diabetics were found to be having significantly higher percentage of disturbed night sleep as compared to nondiabetics.
Diabetics in the present study had higher feeling of pain in upper and lower arms, prevalence of polyphagia and polydipsia as compared to nondiabetics. Numbness and paresthesias are the most common symptoms of peripheral neuropathy in the diabetic person. These symptoms are believed to be the clinical manifestations of changes in nerve electrophysiology, and to reflect altered morphologic and biochemical properties of the axon and the myelin sheath (Moorhouse 1976). Decreased sensory and motor nerve conduction velocities are found in untreated, newly diagnosed diabetics (Ward et al 1979, Graf et al 1979) and similar abnormalities rapidly develop in animals rendered diabetic (Greene et al 1975). Decreased conduction velocity in myelinated neurons is thought to be caused by diminished internodal resistance, which would reduce current density at the nodes and delay excitation (Eliasson 1969, McDonald 1963). Such alterations in internodal resistance could be produced by changes in myelin composition. Morphologic studies of peripheral nerves from diabetics characteristically show segmental demyelination (Clements 1979). The extent and degree of these pathological findings in rats are directly correlated with the severity and duration of hyperglycemia (Chopra et al 1977, Yagihashi et al 1979). Segmental demyelination either may reflect primary metabolic impairment of Schwann cells or, alternatively, may be secondary to prior axonal dysfunction (Clements 1979, Yagihashi et al 1979). In either case, loss of myelin would contribute to the slowing of nerve conduction velocity. Treatment to correct glucose intolerance improves nerve function in both animals and humans. (Ward et al 1979, Greene et al 1975, Gregersen 1967, Graf et al 1979).

Blood glucose was found to be significantly higher in all the diabetic patients and this increase was found associated with glucosuria. During prolonged fasting state, a decrease in insulin sensitivity may occur to mobilize other energy substrates from peripheral tissues so that metabolic function can be maintained (Baba and Neugebauer 1993). Poor glycemic control is responsible for the initiation of diabetic complications (Reichard and Rosenqvist 1989, Hansen et al 1986). Hyperglycemia adversely influences the release of cytokines because of the changes in the osmotic barrier and...
increased generation of advanced glycosylated end products (Cohen and Ziyadeh 1994). The interaction of glycated albumin with endothelial cells, which have been shown to possess dose responsive, saturate receptors, limits cell replication and triggers maladaptive biosynthetic programs, and it may contribute to degenerative macrovascular disease in diabetes (Cohen et al 1995). It is also likely that glucose intolerance exacerbates the cardiovascular risk factors associated with hypertension in the diabetic patients (MacMohan et al 1990).

Diabetic patients in our clinical study showed the presence of hyperinsulinemia. Studies in UK have shown that Asians have higher IRI (Immuno reactive insulin) responses compared to Europeans matched for obesity and Age (Mohan et al 1986, McKeigue et al 1992). Urban Indians were found to have significantly higher IRI than rural Indians (Snehalatha et al 1994). Insulin resistance is a primary defect of Syndrome- X: the combination of hyperinsulinemia, glucose intolerance, abnormal lipid profile and hypertension (Reaven 1988). Fasting hyperinsulinemia may reflect mainly hepatic insulin resistance, whereas impairment of glucose disposal indicated resistance to metabolic effects of insulin in skeletal muscle (Gokhale et al 1998). Insulin resistance is one of the common characteristics in individuals with NIDDM (DeFronzo et al 1985, Groop et al 1989, Kolterman et al 1981). Type 2 diabetics have considerable preservation of the beta cell mass (Maclean and Ogilvie 1955, Westermark and Wilander 1978, Holman and Turner 1979) and often secrete substantial quantities of insulin into the circulation (Bar et al 1979, Kolterman et al 1981). Studies of islet cell morphology have suggested that beta cell mass of the patient with NIDDM have about 50% in the normal level (Maclean and Ogilvie 1955, Westermark and Wilander 1978). A positive correlation was found between serum insulin and systolic blood pressure ($r=0.21$, $P<0.05$) as well as diastolic blood pressure ($r=0.22$, $P<0.05$) respectively. Welborn et al (1966) documented that hyperinsulinemia is associated with high blood pressure. The raise in blood pressure due to insulin may be due to a direct sodium retaining effect of insulin (DeFronzo and Cooke 1975; Kichner 1988) or due to an increase in sympathetic activity (Rowe et al 1981). Insulin, by acting on insulin like growth
factors, causes an increase in vascular smooth muscle cell growth in vitro (King and Goodman 1985, Banskota and Tabu 1989). It is possible that chronic hyperinsulinemia may cause vascular hypertrophy and this may lead to narrowing of lumen of resistant vessels, consequently raising vascular resistance and blood pressure.

Hypertension is reported to be more prevalent among diabetic than nondiabetic individuals (Fuller 1985, Sims and Berchtold 1985, National diabetes data group 1979). Approximately 80% of diabetic patients are found to be hypertensive (Stamler et al 1993). Among diabetic employees of the Dupont Company, Pell and D’Alonzo (1967) reported a 54% higher prevalence of hypertension compared to age, sex and employment matched nondiabetic controls. Moreover, the prevalence in diabetic women was higher than in men. In the Framingham Study, the blood pressure of diabetics was significantly higher than in matched nondiabetics (Garcia et al 1974). In the present study initial systolic blood pressure was found to be significantly higher among diabetics as compared to nondiabetics while initial diastolic blood pressure as well as pulse rates of diabetics were found to be comparable with that of nondiabetics. The occurrence of hypertension among diabetics in our study was variable. Out of 158 diabetic patients, only 6 patients developed hypertension simultaneously or within first 6 months only. Hypertension is commonly seen in association with NIDDM, occurring at a frequency of 30-58% within the different diabetic population (Diabetes Drafting Group 1985, Turner 1985, Sparfka et al 1986, Klein et al 1985, Vaishnava and Blasin 1969). Satia et al (1997) reported the prevalence of 47.85% of hypertension within age and weight matched diabetic population. We found that about 36% of diabetic patients had hypertension by the time they had diabetes for a period more than 5 years. A significant positive correlation was found between systolic blood pressure of diabetic patients and duration of diabetes (r=0.182, P<0.05) while the increase in diastolic blood pressure was not found to be consistent with the increase in duration of diabetes. The percentile of occurrence of hypertension among diabetes increases with the increase in duration of diabetes. Stamler et al (1993) conducted a survey and found that approximately 80% of diabetic patients are hypertensive and
between 5-25% of hypertensive individuals are diabetic after 12 years of duration.

Hypertension aggravates various complications of diabetes (Rodrigues and McNeill 1986). Furthermore, patients with hypertension tend to have dyslipidemia with higher plasma triglyceride concentrations and low concentrations of HDL-cholesterol (Ferrannini et al 1991). Autopsy studies have repeatedly demonstrated that atherosclerosis, in diabetic individuals, is more extensive and accelerated (LeCompt 1955). Diabetes is associated with hyperlipidaemia (high serum triglycerides or total cholesterol concentrations or both) and ketoacidosis (Rodrigues and McNeill 1986a, Nikkila 1984, Stamler et al 1975). The prevalence of hyperlipidemia is variable, depending on the type and severity of diabetes, glycemic control, nutritional status, age and other factors. Blood lipids have commonly been reported to be increased in ambulant diabetic patients also. The most characteristic lipid abnormality in the diabetic is hypertriglyceridemia; with/without associated increase in plasma cholesterol (Ganda 1980, Brunzell et al 1978, New et al 1963, Simpson et al 1979, Reaven 1988, Nikkila 1974). In comparison with age-matched nondiabetic populations, the dominant abnormality in both IDDM and NIDDM is hypertriglyceridemia (Winocour et al 1989, Winocour and Laker 1990). Type 2 diabetic patients were found to have characteristic dyslipidemia with lower HDL-cholesterol and elevated triglycerides (Reaven 1988) and lower HDL-cholesterol than in the average nondiabetic person (Durrington 1993). Further, diabetes is frequently associated with increased concentrations of LDL-cholesterol and reduced concentrations of free fatty acids and phospholipids (New et al 1963, Rodrigues and McNeill 1986, Sosenko et al 1980). Initial total cholesterol, serum triglycerides and ratio of total cholesterol to serum HDL-cholesterol and ratio of serum LDL-cholesterol to serum HDL-cholesterol of diabetics in our study were found to be significantly higher than the nondiabetics. Further, it was found that initial serum HDL-cholesterol of diabetics was significantly lower than that of the nondiabetics. The disturbed lipid profile of the diabetic patients in the present study was an indicator of presence of insulin resistance among them. In the present study, a significant positive correlation was also observed between systolic blood pressure and serum triglycerides of diabetic patients (r=0.163,
Similarly a significant positive correlation was observed between diastolic blood pressure and serum triglycerides of diabetic patients \( (r=0.11, \ P<0.05) \). Derangement in lipid metabolism may occur concurrently with elevated blood pressure. Diabetes is associated with hyperlipidaemia and ketoacidosis (Rodrigues and McNeill 1986a). Thus, controlling blood pressure in diabetics is positively more beneficial as far as progression of diabetic complications are concerned. Increase in serum insulin was also associated with a significant increase in serum cholesterol \( (r=0.19, \ P<0.05) \), serum triglycerides \( (r=0.32, \ P<0.05) \), and ratio of total cholesterol to serum HDL-cholesterol \( (r=0.18, \ P<0.05) \). A significant negative correlation was observed between serum insulin of diabetic patients and serum HDL-cholesterol \( (r=-0.13, \ P<0.05) \). Insulin resistance worsens the lipogram. Insulin plays a significant role in the production as well as the removal of triglyceride-rich proteins and therefore insulin deficiency and/or insulin resistance can lead to either defective removal and/or overproduction of one or more lipoproteins thereby producing dyslipidemia. There is a strong evidence for defective removal of triglyceride-rich lipoproteins in insulin deficient animals and humans (Sosenko et al 1980). Thus, hyperinsulinaemia may be the common element accounting for the association of obesity, NIDDM and hypertension (Modan et al 1985). Insulin resistance and hyperinsulinemia play major role in the etiology of hypertension especially when associated with obesity (Ferranini et al 1987). Correlation with insulin resistance, hyperinsulinemia and blood pressure has been found in obese and lean hypertensive subjects (Berglund et al 1982).

Serum creatinine levels of diabetic patients were found to be above normal \((>2 \text{ mg/dl})\) in diabetics indicative of the kidney dysfunction due to diabetes. Rash (1979a) reported that the kidney and the glomeruli become enlarged in rats with high plasma glucose concentrations maintained for 6 months. Furthermore, glomerular basement membrane becomes thicker (Rasch 1979b) and the mesangial regions enlarged with increased amounts of basement membrane like material (Rasch 1980). The renal disease associated with chronic diabetes is manifested clinically by continuous
proteinuria (total urinary protein excretion >0.5 g/day or urinary albumin excretion >300mg/day), a significant decrease in glomerular filtration rate (GFR) (Mogensen 1971 and 1976) and increase in the size of the kidney (Mogensen and Andersen 1971). Patients with IDDM and diabetic nephropathy manifested by proteinuria have a 100 fold greater risk of mortality as compared to nondiabetic population (Borch-Johnsen et al 1985, Dorman et al 1984). IDDM patients without proteinuria have only a two-fold increase in their relative mortality (Anderson et al 1983). The death in such patients is likely to occur from macrovascular disease or from renal failure (Dorman et al 1984, Mathieson et al 1989, Messent et al 1992, Horl et al 1989). Patients with heavy proteinuria and marked renal histology changes have the worst prognosis (Watkins et al 1972). There is a correlation between the severity of these clinical features and the degree of glomerular basement membrane (GBM) thickening. The width of GBM in diabetics is normal at the onset of the disease but after several years of sustained metabolic derangement, it becomes increasingly thicker (Osterby 1971 and 1973). This continual thickening over many years causes progressive occlusion of glomerular capillaries, leading to chronic renal failure. Thus, the accumulation of basement membrane material that has altered filtration properties constitutes the ultimate structural and functional abnormality underlying diabetic nephropathy. With the passage of time, the nephrons gradually close down, giving rise to kidney failure. Often the blood pressure may increase. The urinary tract infection aggravates kidney failure. The kidneys mainly excrete the products of protein breakdown like urea and creatinine. Hence, these waste products accumulate in blood when the kidney function deteriorates.

Serum creatinine of diabetics in the present study was found to be positively correlated with serum triglycerides levels (r=0.12, P<0.05), ratio of total cholesterol to serum HDL-cholesterol (r=0.2, P<0.05) and ratio of serum LDL-cholesterol to serum HDL-cholesterol (r=0.15, P<0.05). Further, a significant negative correlation was observed between serum creatinine of diabetic patients and serum HDL-cholesterol (r= -0.25, P<0.05). Hyperlipidemia is an important factor that accelerates the loss of kidney
function in renal disease. Different types of experimental animal models develop accelerated glomerulosclerosis when given a high cholesterol diet (French et al. 1967; Kasiske et al. 1990), while reduction in cholesterol, seems to protect kidney function in a variety of glomerulopathies. Mulec (1990), also found that cholesterol is one of the major risk factors for the deterioration of kidney function in diabetic nephropathy, which is characterized by increased levels of serum creatinine, urea and BUN levels. Lack of good metabolic control of diabetes is an important risk factor for the development of nephropathy and the later deterioration of kidney function. Hypertensive patients also develop nephropathy (Rosenstock and Raskin 1986).

Effect of treatment of *E. littorale* in NIDDM patients

The study of baseline demographic data, analysis of various indices of diabetes mellitus and their interrelation indicate that the patients of diabetes mellitus in India are more prone to develop hypertension. Further, as reported recently by other epidemiological studies, high triglyceride levels, low levels of serum HDL-cholesterol and hyperinsulinemia appear to cluster with risk factors of diabetes mellitus among these patients. It was also observed that traditional antidiabetic therapy (sulphonylurea, biguanides and/or insulin) could not bring adequate control of blood glucose levels. Further, many of these patients showed dyslipidaemia and elevated serum creatinine levels. Treatment, of these diabetic patients, with the ghanvatis of *E. littorale* for a period of nine months showed significant reduction of blood glucose levels. Decrease in blood glucose in the patients started from the first month and then continued onwards until the end of the nine months. Initial value of blood glucose of diabetic patients (204±10 mg/dl) was reduced to 141±6 mg/dl at the end of nine months. *E. littorale* appears to possess a potential antihyperglycemic action. Untreated-normotensive-diabetics (who were not taking any antidiabetic and/or antihypertensive therapies) showed the decrease of blood glucose from the initial value of 251.6±28.8 mg/dl to 144.5±14.8 mg/dl. The reduction of blood glucose among the normotensive-diabetics (taking antidiabetic therapies), when co-administered with *E. l.
littorale, was found to be from an initial value of 208.7±14.7 mg/dl to 149.4±8.4 mg/dl after nine months. Initial blood glucose was 177.7±10.7 mg/dl, among the diabetic-hypertensives (taking both antihypertensives and antidiabetics), and the co-administration of E. littorale among these patients decreased blood glucose to 129±8.3 mg/dl at the end of nine months. Thus, decrease in glucose level by E. littorale was dependent on initial glucose level (before therapy). Greater decrease in blood glucose was found if there was severe hyperglycaemia before starting the therapy with E. littorale.

It was also observed that, the dosage of antidiabetic drugs (which the diabetic patients were taking before starting the treatment with E. littorale) was significantly reduced. Out of 72 patients, there were 48 patients taking sulphonylureas. The initial dose of sulphonylurea used by these patients was 11.5±0.71 mg/day and they had blood glucose 195.1±9.8 mg/dl. After the treatment with E. littorale for nine months the average dose of sulphonylurea was reduced to 6.5±0.72 mg/day and the blood glucose level reduced to 139.5±7.6 mg/dl among these patients. There were 27 patients taking biguanides. A significant decrease in the dose of biguanides (from 887±73.2 mg/day to 497.2±96.3 mg/day) and the blood glucose (from 172.6±11.5 mg/dl to 135.6±6.8 mg/dl) was observed when the concurrent treatment of E. littorale was given along with biguanides in these patients for nine months. Even patients taking insulin injections (n=8) along with other medications had to reduce the dose of insulin injections (from 32.5±4.1 units/day to 24.5±3.5 units/day) and blood glucose levels became 171.5±12 mg/dl from 233.8±38.1 mg/dl after the continued use of E. littorale for 9 months. Thus, E. littorale not only reduced blood glucose levels but also the dose of oral and/or injectable antidiabetics among these patients.

Out of 158 diabetic patients, 22 patients had glucose levels in the range (≥110 mg/dl, 90.9±2.8) but they were taking some antidiabetic drugs. Out of these 22 patients with initial controlled blood glucose, 7 patients continued the treatment of E. littorale until the end of nine months. Out of these 7 patients there were 5 patients taking sulphonylureas along with other
medications. The dose of sulphonylurea before starting the treatment with *E. littorale* was (10.5±2.5 mg/day) which was reduced to 2.5±1.7 mg/day at the end of first month of treatment with *E. littorale*. Further, at the end of 2nd month of treatment with *E. littorale*, all these 5 patients had to stop their sulphonylureas. *E. littorale* thus, substituted the sulphonylureas among such diabetic patients and they had normoglycaemia and normal lipogram. All these data suggest a careful titration of dosage of *E. littorale* alongwith other antidiabetics used by the patients.

Initial serum insulin levels of diabetics were significantly higher than the nondiabetics and the treatment of *E. littorale* consistently and significantly decreased them from first month to ninth month in all diabetics. Initial serum insulin was 104±10 mcU/ml which was reduced to 68±3 mcU/ml at the end of ninth month. This indicates that the circulating endogenous insulin in the body was better utilized when *E. littorale* is administered to the patients. The corresponding decrease in blood glucose alongwith decrease in insulin indicates that *E. littorale* produces improvement in insulin sensitivity.

Improvement in the insulin sensitivity due to *E. littorale*, indicated by decrease in the blood glucose and serum insulin, was associated with beneficial effect *E. littorale* on the other biochemical parameters such as disturbed lipogram and elevated creatinine levels. Diabetes induces insulin resistance among the patients, which in turn leads to hypertension as well as dislipidaemia. When insulin resistance decreases, the elevated blood pressure and the disturbed lipid profile tends to become normal. Treatment with *E. littorale* for nine months produced significant reduction in systolic blood pressure (from 139±3 mmHg to 128±2 mmHg), diastolic blood pressure (from 87±1.4 mmHg to 82±0.9 mmHg), pulse rate (from 85±1.5 beats/min to 77±1.5 beats/min), serum triglycerides (from 336±23 mg/dl to 170±7.3 mg/dl), serum cholesterol (from 239±8 mg/dl to 203±5 mg/dl), serum LDL-cholesterol (from 144±7.6 mg/dl to 128±4 mg/dl), ratio of total cholesterol to serum HDL-cholesterol (from 9.3±0.5 to 5.3±0.2) and ratio of serum LDL-cholesterol to serum HDL-cholesterol (from 5.6±0.4 to 3.5±0.2). Low levels of initial serum
HDL-cholesterol of diabetics were significantly increased (from 28±1 mg/dl to 40±1 mg/dl) with the treatment of *E. littorale* for nine months. These results provide further evidence that *E. littorale* reduces insulin resistance in diabetic patients.

Treatment of diabetic patients with *E. littorale* significantly decreased the elevated levels of serum creatinine and it was found that by the end of first month of administration of *E. littorale*, serum creatinine levels were comparable with those of the nondiabetic subjects and the decreasing trend further continued for the rest of the period upto nine months. Initial serum creatinine levels of diabetics were 2.0±0.1 mg/dl which were reduced to 0.9±0.4 mg/dl at the end of nine months. Hyperglycaemia among diabetics leads to renal dysfunction and correction of blood glucose among the diabetics tends to normalize the renal functions. *E. littorale* also reduced other elevated kidney functions and at the end of nine months of treatment with *E. littorale*, serum urea was 26.4±2.1 mg/dl, urine urea was 4.8±0.5 g/l and urine creatinine was 0.71±0.15 g/l in diabetic patients. Thus, treatment of *E. littorale* may preserve kidney functions in diabetic patients.

The hypoglycemic effect of *E. littorale* was suggested to be due to increased peripheral utilization of glucose (Vyas et al 1979). Vijayvargia et al (2000) have reported that *E. littorale* reduces plasma glucose levels, glycosylated hemoglobin and liver glucose-6-phosphatase in alloxan-diabetic rats. Still further studies are required to find the exact mode of antidiabetic action of *E. littorale*.

In conclusion, our data suggest that insulin resistance is highly prevalent among type 2 diabetic patients in India. All the cardinal symptoms of diabetes are present among the patients. *E. littorale* possesses a potent antidiabetic action. It reduces elevated blood glucose and serum insulin levels of type 2 diabetic patients. Further, it not only reduces the dose of antidiabetic therapy (sulphonylurea, biguanides and insulin injections) taken by the patients but also maintains normoglycemia among diabetic patients. Improvement in
insulin sensitivity among diabetics due to *E. littorale* is correlated with the normalization of hypertension and hyperlipidaemia and elevated creatinine levels. *E. littorale* can be used alone or as a supplementary therapy in type 2 diabetic patients.

**PHYTOCHEMICAL STUDIES**

Aqueous extract of *E. littorale* was extracted using different solvents with increasing polarity viz. petroleum ether, toluene, chloroform, ethylacetate and finally n-butanol. The extractive value of the n-butanol extract was found to be higher (0.14%) as compared to all the other extracts, minimum being that of the petroleum ether extract (0.002%). Preliminary phytochemical analysis of all the extracts was carried out using different reagents. Chloroform extract tested positive with Dragendorff's reagent, which indicated the presence of alkaloids. Retnam and DeBritto (1998) detected alkaloids in the petroleum ether, benzene, chloroform and methanol extract of *E. axillare*. Prasad and Bhusan (1954) separated a crystalline alkaloid (m.p. 79°-80° C) from the alcoholic extract of the plant. Gentianine, a crystalline alkaloid (molecular formula C_{10}H_{9}O_{2}N) with m.p. 82°-83° C was isolated by successive extraction using ether and ethanol (Rai and Thakar 1965). Erythrocentuarin, enicoflavine and gentiocrucine are the monoterpene alkaloids isolated from the plant and their identity were established by chemical transformations and spectral (UV, IR, NMR and Mass spectra) evidence (Ghosal et al 1974).

Terpenoids were found to be present in all the extracts (maximum in chloroform and ethylacetate extracts) except petroleum ether extract. Betulin, a triterpene sapogenin, was isolated from *E. littorale* (Rai and Thakar 1966, Desai et al 1966). Steroids were found to be absent in all the extracts in the present study. Daniel and Sabnis (1978) also reported the absence of steroids in *E. littorale*. Swertiamarin a secoirridoid glycoside was isolated from *E. littorale* and its structure was established (Rai and Thakar 1966, Desai et al 1966). Swertiamarin can be converted to an alkaloid gentianine (Rai and Thakar 1966, Ghosal et al 1974). Glycosides were reported in *E. littorale* (Prasad and Bhusan 1954). None of the extracts in the present study was
found to contain anthraquinone glycosides. In our study, flavonoids were found to be present in chloroform extract. Seven flavonoids (apigenin, genkwanin, isovitexin, swertisin, saponarin, 5-O-glucosylswertisin and 5-O-glucosylisoswertisin) were isolated and their structures were elucidated (Ghosal and Jaiswal 1980). The leaves were found to contain glycoflavone and xanthone, that are subtypes of flavonoids (Daniel and Sabnis 1978). The distribution of some common flavonoids such as acacetin, apigenin, chrysin, kampferol, quercetin, baicalein, isorhamnetin, moratin and isovitexin were also reported in this plant (Retnam and Debrito 1998). Phenols and tannins were found to be present in abundance in ethylacetate extract in our study. Various phenolic acids (Vanillic, Syringic, P-hydroxybenzoic, protocatechuic, p-coumaric and ferulic) were reported to be present in the leaves of this plant (Daniel and Sabnis 1978).

It is interesting to note that although the extractive values of both the chloroform and ethylacetate extracts are low, they contained more number of different chemical groups. However, n-butanol extract that showed mainly triterpenoids is found to have the highest extractive value.

Much work has been performed on the identification and isolation of different chemical constituents from *E. littorale* but the HPTLC fingerprint profile has not been worked out till date and we have tried to provide it in the present study. HPTLC is fast emerging as one of the major tools by which the quality control of herbs added in the formulations can be maintained. Trivedi et al (1997) have quantitatively estimated epicatechin from *Acacia catechu*, andrographolide from *Andrographis paniculata* and phyllanthin from *Phyllanthus niruri* using this technique. Lee et al (1981) have reported a procedure for the two-dimensional TLC separation of ginsenosides from the roots of *Panax trifoliius* L. For TLC evaluation, different solvent systems were experimented with, to find suitable solvent system/s for the different extracts, to obtain good resolution of the components. The plates were scanned in UV 254 and 366 nm. It was found that petroleum ether, toluene, chloroform, ethylacetate, n-butanol and the remaining water extract contained 3, 7, 7, 7, 5 and 3 components respectively in UV 254. Petroleum ether, toluene,
chloroform, ethylacetate, n-butanol and the remaining water extract contained 1, 6, 5, 4, 5 and 3 fluorescent components respectively in UV 366. The number of bands in a chromatogram observed under UV light was generally found more than the number of bands of the same plate observed after the derivatization with anisaldehyde-sulphuric acid reagent.

Depending on the resolution, as observed in UV light at 254 nm, one most suitable solvent system was identified for each extract. The reproducible chromatogram for each extract in the particular most suitable solvent system forms a marker for further studies on *E. littorale*, since the original aqueous extract was used for preparing ghanvatis which showed good activity in animal and clinical studies for antidiabetic activity. Solvent system containing n-Hexane:ethylacetate (80:20) was found more suitable for petroleum ether extract indicating presence of 3 different chromophores. The solvent system containing toluene:chloroform:methanol (45:50:5) was found more suitable for toluene and chloroform extracts with maximum components (7) being resolved in toluene and chloroform extracts. Resolution of chloroform extract in the solvent system composed of chloroform:acetone:formic acid (75:16.5:8.5) is also equally better and the extract got resolved into seven bands, all of which were having spectra with different $\lambda_{\text{max}}$. Solvent system containing acetone:chloroform:water (70:30:2) was found suitable for ethylacetate extract showing presence of eight components. For n-butanol extract and water extract (left after extraction with all five organic solvents) good resolution was obtained in the solvent system containing ethylacetate:methanol:water (77:15:8) indicating the presence of 5 and 3 different components in n-butanol extract and water extract respectively. Thus, based on the resolution obtained in UV 366, UV 254 and derivatization with anisaldehyde-sulphuric acid reagent, the solvent system composed of ethylacetate:methanol:water (77:15:8) should be considered the best solvent system for *E. littorale*.

The absorption spectra of all the bands were compared. Total six sets of spectra were found to be overlapping with one another, indicating the
presence of six same or closely related components present in the total six extracts. Toluene and chloroform extracts appear to be containing six and seven components respectively. Out of them four components appear to be same or closely related in both the extracts (based on the overlapping pattern of the spectra and λ_max of the bands of both the extracts). Virtually all these six components, though present in different extracts and separated by different solvent systems, the pattern of their absorption spectra are almost same, and their λ_max was found same or nearly same. The TLC profile, the resolution of the components of different extracts in different solvent systems, their Rf values, the spectral details, the λ_max, when observed in UV 254 and 366 nm and in the plates derivatized by anisaldehyde-sulphuric acid reagent, represent, in toto, the fingerprint profile of the plant. These details attain importance in view of the fact that the aqueous extract of *E. littorale*, from which different extracts were prepared, showed significant antidiabetic activity in both animal models and clinical studies. Pharmacological evaluation of these different fractions and a further activity guided fractionation of these extracts is expected to lead to the isolation of active principles responsible for the antidiabetic activity of the plant.

CONCLUSIONS

The results of animal and clinical studies suggest that *E. littorale* is a potential herbal antidiabetic medication having remarkable insulin sensitizing action. It provides nephroprotection and normalizes the disturbed lipid profile.

HPTLC fingerprint profile was established for different fractions of the aqueous extract of *E. littorale*. The solvent system composed of ethylacetate:methanol:water (77:15:8) was found to be the best solvent system for *E. littorale*.