INTRODUCTION
2. INTRODUCTION

Diabetes mellitus is a group of diseases characterized by increase in fasting and post-prandial blood glucose resulting from defects in insulin production, insulin action, or both. Diabetes mellitus is one of the most common chronic endocrine disorders affecting millions of people worldwide. Diabetes is now recognized as serious global health problem (WHO 1985, King et al., 1993). Only in U.S.A it is estimated that 17 million people- 6.2% of the population have diabetes, out of which 11.1 millions are diagnosed and 5.9 millions are undiagnosed. In India it is estimated that 33 million people are diabetic. Westernized culture and change in life style have resulted in a sharp rise in the cases of non-insulin dependent diabetes mellitus (Bennet and Knowler 1980; Zimmet 1982). It is estimated that more than 300 million people in world will have diabetes by the year 2025 (Harris and Zinman 2000). It is projected that by year 2025 there will be 60-80 million people suffering from diabetes in India (Shah 2000). In diabetes there occurs derangement in carbohydrate, lipid and protein metabolism and the development of long term complications such as microangiopathy, macroangiopathy, atherosclerosis, retinopathy, neuropathy, cardiomyopathy and autonomic neuropathy etc (Hamby 1970; Lancaster 1980; Knowler et al., 1980; Marble et al, 1985; Nathan 1983; Rahman et al., 2000). Diabetes is also associated with increased incidence of morbidity and mortality due to cardiac and renal complications.

Although insulin appears to be prime factor responsible for diabetes, 90 % of diabetics seem to suffer from non-insulin dependent diabetes mellitus (NIDDM) (Kannel 1985). Patients with NIDDM may have few or none of the classic symptoms of diabetes when first diagnosed. They are not dependent on exogenous insulin for survival and are not prone to the development of ketoacidosis. In general clinical practice patients, with NIDDM are treated with sulfonylureas like glibencamide, glipizide, glyclazide etc. however an anti-diabetic agent that can maintain normoglycemia for longer duration (3-5 years) in diabetic patients remains a challenge (The diabetic controlled and complications trial research group, 1995). On long term therapy with sulfonylurea, a NIDDM patient may require injection of insulin for adequate control of glucose level. Further, in spite of anti-diabetic therapy patients may still suffer from dyslipidaemia with increase in circulating triglycerides, very low-density lipoprotein (VLDL) and hence an increased
Introduction

morbidity and mortality due to diabetes induced cardiovascular complications (Winocour et al., 1989). The prevalence of hypercholesterolemia (> 6.5 mmol / lit) is considerably higher in both IDDM (27 %) and in NIDDM (50 %). Levels of HDL-cholesterol tend to be lower in diabetics that matched non-diabetic subjects (Durrington 1993).

Since insulin resistance is the pathogenic feature of both type I and type II diabetes and an underlying cause of the accompanying cardiovascular risk profile, newer anti-diabetics should be such that they combat insulin resistance. Previously, biguanides were the only class of drugs that could improve the insulin sensitivity. However, recently a newer class, thiazolidinediones also known as glitazones has been shown to enhance the sensitivity of muscle and adipose tissue to the action of insulin (Bailey 2000). They are found to enhance glucose uptake in muscle and adipose tissue, reduce gluconeogenesis and glycogenolysis in the liver. These agents improve sensitivity to insulin by binding to nuclear peroxisome proliferator activated receptors-γ (PPAR γ) which acts in conjunction with retinoid X receptor by de-repression to increase transcription of certain insulin sensitive genes (Spiegelman 1998). Therapeutic efficacy of PPAR γ agonist has been found to be promising clinically but the first one troglitazone was withdrawn from the market as result of idiosyncratic hepatotoxicity (Saleh et al., 1999). Although, this has not been observed with rosiglitazone or pioglitazone. Long term benefits versus safety are yet to be observed.

The efficacy of currently used antidiabetic drugs is compromised in several ways. Individual oral agents act only on a part of the pathogenic process of diabetes mellitus and hence they may not produce any cure and may not prevent all the complications of diabetes mellitus (Bailey and Turner 1996; Lebovitz 1998; Day 1999; DeFronzo 1999; Howlett and Bailey 1999; Lebovitz 1999). They do not reinstate normal insulin sensitivity or normal β-cell function. In addition, these agents do not prevent gradual β-cell loss and hence their usefulness depends upon a critical mass of functional β-cells remaining. Thus, although existing classes of anti-diabetic agents offer a variety of actions that can be combined in a complementary and additive manner, few patients maintain the recommended targets for the good glycemic control, and a normal physiological pattern of glucose homeostasis is rarely reinstated. This emphasizes the urgent need for newer and better therapeutic approaches. The search for improved anti-
diabetic drug therapies must take account of the multiplicity of endocrine and metabolic disturbances and attendant risks and complications of diabetic state. In many countries it is traditional to use herbal medicine to control diabetes.

WHO has approved the use of traditional medicines as a part of health programme. To pursue research in these systems of medicine, several USA agencies and institutions such as FDA and National Institute of Health have setup separate wings. According to the WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for the primary health care needs. In almost all the traditional medicine, the medicinal plants play a major role and constitute the backbone of the traditional medicine.

The potential of plant as a source for new drugs is yet to be unexplored systematically. Among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemically (Verpoorte et al., 1998; Cragg et al., 1997; Balandrin et al., 1985). A popular herbal shop in East Los Angeles, herbs of Mexico, which serves a large Mexican American population markets a capsule that contains 18 plant products used in Mexico as hypoglycemic. Practitioners of traditional Chinese medicine, believing that individual herbs work in concert with others herbs for a given benefit prepare remedies using mixtures of various plant products (Bensky and Gamble 1986). In India, herbal agents in current use for diabetes were indicated for this purpose in Ayurvedic medicinal texts within some 2,500 years ago (Ajgaonkar 1979).

India has an ancient heritage of traditional medicine. Materia medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is based on various system including Ayurveda, Siddha and Unani. Also China and UK have got their own traditional system of medicine. These traditional systems have their uniqueness no doubt but there is a common thread running through these systems in their fundamental principles and practices. With the emerging interest in the world to adopt and study the traditional system and to exploit their potentials based on different healthcare systems, the evaluation of the rich heritage of the traditional medicine is essential.
India has about 45,000 plant species: several medicinal properties have been assigned to several thousand. Plants are source of extremely wide range of chemical components. At least hundred chemical substance of known structure are extracted from plants that are being used as drugs throughout the world. In India, a sound knowledge of use of plants for medicinal purpose has come from Ayurveda, Siddha and Unani Systems of Medicine and these systems are still being practiced in all parts of the country. In many countries it is traditional to use medicinal plants, either a single herb or a polyherbal formulations, to control diabetes. The antihyperglycaemic effect of several plants extracts or herbal formulation that are used as a antidiabetic remedies has been confirmed (Sharma et al., 1992). A database of natural hypoglycemics collected by researchers in Mexico lists almost 800 plants (Lozoya 1994). Researchers in India have documented the use of 150 plants in families with reported hypoglycemic activity (Handa and Chawla 1989). A recent cross-cultural compendium cites 1,200 medicinal plants used for diabetes (Marles and Farnsworth 1995). Synthetic hypoglycemic agents can produce serious side effects including hematological effects, coma and disturbances of the liver and kidney. In addition, they are not suitable for use during pregnancy (Lamner 1985). Compared to synthetic drugs, herbal preparations are frequently considered to be less toxic with fewer side effects (Momin 1987). Therefore the search for more effective and safer antihyperglycemic agents has become an area of current research.

Hundreds of products are marketed in India as “natural” agents for lowering blood sugar and decreasing long term complications. These include antibetic, Alphabetic, Diabets, DB-7, Diabetica, Diabetiks, Dia-Comp, DiaVite, GlucoCare, Glucotize, GlycoNase, SugarMax, and Sugar Loss. These formulations are typically the combination products containing the individual components presented here along with others. Various cultures have remedies unique to their own population, such as neem (Azadirachta indica) in India (Chattopadhyay 1996), Pedra hume caa (Myrica unijlora) in brazil (Russo et al., 1990), tronadora (Tecoma stans) in Mexico (Lozoyz-Meckes 1985).

Enicostemma littorale Blume (Gentianaceae) is a glabrous perennial herb belonging to the family Gentianaceae (Kirtikar and Basu 1935). It grows throughout India up to 1.5 feet in height and more frequently near the sea. It is called Chota-kirayata or Chota chirayata in Hindi, Mamejavo in Gujarati, Nagajivha in Bengal and Vellarugu or Vallari in Tamil. E. littorale has been used as a folk medicine for the treatment of
diabetes mellitus in Western and Southern India (Gupta et al., 1962). *E. littorale* contains catechins, sterols, saponins, steroids, triterpenoids, alkaloids and volatile oil (Natarajan and Prasad 1972; Retnam and DeBritto 1988). Some important chemical constituents include betulin, a triterpene sapogenin, a secoiridoid glycoside swertiamarin (Rai and Thakar 1966; Desai et al., 1996), monoterpene alkaloids like enicoflavine and gentiocruicine (Ghosal et al., 1974; Chaudhuri et al., 1975).

Various Ayurvedic formulations containing *E. littorale* as one of the ingredients have been shown to produce antihyperglycemic activity in various hyperglycemic rat models (Gupta et al., 1962). It has been reported that “Phaki” (mixture of twelve indigenous plants of which *E. littorale* is a constituent) at a dose of 50 to 200 mg/kg on i.v. administration in dogs produced a dose dependent % reduction in blood sugar between 39 to 55 % (Ainapure et al., 1984). Ethnomedical studies of North Gujarat (India) reveal the use of hot aqueous extract of *E. littorale* by the tribal inhabitants for the treatment of diabetes, fever, stomach ache, dyspepsia and malaria in interior part of Gujarat. Earlier we have reported antidiabetic activity of crude extract of *E. littorale* in type II diabetic rats (Murali et al., 2002). Antidiabetic activity has also been reported on alloxan-induced diabetes (Maroo et al., 2002). Data from our laboratory have shown that aqueous extract produces antidiabetic effect at dose of 2 g/kg p.o. in NIDDM rats (Murali et al., 2002). Although the crude extract of *E. littorale* has been shown to possess antidiabetic activity on several occasions a detailed investigation of the effect of this plant on the metabolic alterations in diabetes had not been attempted. Thus, the first objective of the present investigation was to perform activity guided phytopharmaceutical analysis of *E. littorale* with special reference to diabetes mellitus using STZ-induced type I and type II rats. Diabetes is a group of diseases characterized by high levels of blood glucose resulting from defects in insulin production, insulin action, or both. Diabetes is associated with serious complications like cardiomyopathy, nephropathy, retinopathy and various other macrovascular and microvascular complications. Hence, while studying anti-diabetic activity of aqueous extract of *E. littorale* and its different fractions we also investigated their effects on lipid profile, kidney function and liver function in STZ-induced type I and type II rats.

Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound
analysis using modern analytical techniques like HPTLC and HPLC. Although several formulations available for diabetes, very limited attempts have been made to evaluate their pharmacological aspects and verify therapeutic efficacy. Moreover, because of resurgence of interest on herbal drugs, it is also important to ensure that only quality products enter the market. Efforts are being made by various government agencies and research laboratories to maintain the quality of herbal drugs by proper identification and detailed pharmacognostic, phytochemical investigations and standardization. However, in spite of the continuing efforts, there are no standard methods available for quality control of herbal drugs, which is the main hurdle for India to enter into the multi-million dollar international market. Further, the composition of plant material can vary and it is known to be influenced by the place of origin, soil, climate, season, time of collection, post harvesting conditions, temperature changes, moisture which affect tremendously the quality and therapeutic efficacy of the drug. Therefore, the quality and efficacy of the herbal drugs need to be established through systematic pharmacognostic, phytochemical and pharmacological evaluation and standardization of the drug. In herbal research, it is also essential to authenticate the plant and to establish phytochemical standardization with help of reliable instruments like HPTLC. Before undertaking pharmacological work, we also carried out pharmacognostic and phytochemical standardization of *E. littorale* crude extract and its various fractions using HPTLC fingerprinting.

Iridoids, a widely distributed class of natural product have shown encouraging biological activities including hepatoprotective activity. Swertiamarin a secoiridoid glycoside is one of the major compounds reported in *E. littorale*. There are no pharmacological data available to substantiate the therapeutic value of *E. littorale*. We made a successful attempt to isolate (7.7 %) and characterize the compound swertiamarin from *E. littorale*. We then studied the effect of swertiamarin in STZ-induced type I diabetic rats. While studying the anti-diabetic activity of various fractions and the compound isolated from *E. littorale* (Swertiamarin) it was found that *E. littorale* also possess impressive antihyperlipidaemic and hepatoprotective activity. Thus, we extended our study to investigate the effect of aqueous extract of *E. littorale* on cholesterol fed hyperlipidemia in rats and CCL₄ induced liver injury. In the end we also made an attempt to find out the mechanism of action of various activities investigated.