INTRODUCTION AND OBJECTIVE
Diabetes mellitus is one of the most common chronic endocrine disorders affecting millions of people worldwide (Geirch 1989, WHO 1994). Diabetes is characterized by an increase in fasting and post-prandial blood glucose levels, insulin deficiency and/or decreased insulin actions. Because of the sustained high glucose levels, glycosylated hemoglobin increases in diabetics (Bunn et al 1976 and 1978) which in turn causes thickening of the capillary basement membrane throughout the body leading to microangiopathy, macroangiopathy, nephropathy and retinopathy. (Marble et al 1985, Ronald 1994). Thus, in diabetes mellitus there occurs derangement in carbohydrate, lipid and protein metabolism (Kar et al 1999) and the development of long-term complications (Lancaster 1980) such as microangiopathy, macroangiopathy, atherosclerosis, retinopathy (Knowler et al 1980), nephropathy (Rosenstock and Raskin 1986, Deekert and Grenfel 1991, Nathan 1993), cardiomyopathy (Hamby 1970), autonomic neuropathy (Rahman et al 2000) etc. Diabetes mellitus is also associated with increased incidence of morbidity and mortality due to cardiac and renal complications.

Diabetes mellitus is now recognized as serious global health problem (WHO 1985, King et al 1993). Westernized cultures (Harris 1982) and populations experiencing rapid acculturation are showing a sharp rise in non-insulin-dependent diabetes mellitus (Bennett and Knowler 1980, Zimmet 1982). The prevalence of NIDDM is increasing exponentially (King et al 1998). It is estimated that more than 300 million people in the world will have diabetes by the year 2025 (Harris and Zinman 2000). Only in U.S.A. there are 8 million diagnosed diabetic patients, another 8 to 12 million undiagnosed diabetic individuals and still an additional 23 million Americans with pre-diabetes or impaired glucose tolerance (IGT) exists (Stuart 1999). Diabetes is one of the few leading causes of death in not only developed countries but also in many developing and under developing nations (Zimmet 1992). Various epidemiological studies in India have shown that the prevalence and manifestations of diabetes is very high (Verma et al 1986, Ramchandran et al 1988 and 1992, Ramaiya et al 1990). At present approximately 18-20 million people are diabetic in India and it is projected that by 2025 there will be 60-80 million diabetics in India and it will be the second country to have the largest
Prevalence of diabetes appears to be low in rural India (<3% in >20 year old) while it is high (>10%) in urban India (Yajnik 1997).

Although insulin appears to be the prime factor responsible for diabetes mellitus, 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 mellitus 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, sulfonylureas like glibenclamide, glipizide, gliburide, glyclazide etc.) are prescribed to such patients. However, an anti-diabetic agent that can maintain normoglycaemia for a longer duration (>3-5 years) in a diabetic patient remains a challenge (The diabetes controlled and complications trial research group 1995). On long term therapy with sulfonylurea, an NIDDM patient requires insulin injections for adequate control of glucose levels. Further, inspite of anti-diabetic therapy patient may suffer from dyslipidaemia with an increase in circulating triglycerides, very low density lipoproteins (VLDL) (Winocour et al 1989, Winocour and Laker 1990). The prevalence of hypercholesterolaemia (>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 than matched nondiabetic subjects (Durrington 1993).

Himsworth (1949) put forward the notion that the insulin insensitivity and not the insulin deficiency is more common in diabetes. This concept received increasing support and insulin resistance is now recognised as one of the characteristic features of NIDDM patients (Reaven et al 1976). Insulin resistance is defined as an impairment in biological response to exogenous or endogenous insulin on glucose homeostasis. It occurs due to decrease in sensitivity to insulin characterized by decrease in the maximum response to insulin or a combination of decreased sensitivity and decrease responsiveness (Yalow et al 1960, Butterfield and Whichelow 1965, Martin et al 1968, Alfrod et al 1971a, Jackson et al 1973, Kahn 1978 and 1998, Reaven 1980 and 1983, DeFronzo et al 1983). Although insulin resistance exerts
numerous effects, (regulates fat and protein metabolism, iron transport, cell
growth and cell differentiation), insulin sensitivity has been considered mainly
in the context of glucose metabolism (Ferrannini et al 1992). NIDDM patients
may be hyperinsulinaemic and develop insulin resistance (Bonora et al 1998,
Mehta et al 1999). Insulin resistance appears to be promoted by an abdominal
obesity (Pollare et al 1990), lipoprotein lipase deficiency,
hypertriglyceridaemia, reduced HDL-cholesterol, increased re-absorption of
sodium, blood volume expansion (Dechatel et al 1977, DeFronzo et al 1981b),
hypertension and diabetes mellitus (Ferrannini et al 1987). Epidemiological
studies in native Indians and migrant Indians have shown high susceptibility of
Indians for insulin resistance and increasing trends in prevalence of NIDDM.
Thus the correction of insulin resistance has great therapeutic potential for the
treatment of NIDDM and so recently efforts are now being made to develop
drugs that can improve insulin sensitivity.

There is a tremendous interest in the application of alternative system
of medicine, in the world over, as part of its available therapeutic
armamentarium. WHO has already approved the use of traditional medicines
as part of health programme. Herbs have been an integral part of natural
medicine prevalent all over the world. A herbal medicine is defined as a
finished, labelled medicinal product that contains active ingredients as aerial
or underground parts of plants or other plant materials or combinations
thereof (Thatte 1998). Approximately 3300 million people in the under
developed countries use medicinal plants on a regular basis while there has
been a great fascination for the herbal medicines and dietary food
supplements in the developed countries (Dobriyal and Narayan 1998). India
has a rich heritage of usage of medicinal plants in the Ayurvedic and Unani
systems with a mention of about 45,000 plants (Hakim et al 1995, Deshmukh
1998). Inter-regional workshop organised by WHO on “The selection and the
use of “Traditional Remedies in Primary Health care” unanimously accepted
the need of development of herbal formulation for diabetes mellitus
(Chowdhary 1992). Diabetes mellitus has recently been identified by Indian
council of medical research (ICMR) as one of the refractory diseases for
which satisfactory treatment is not available in modern allopathic system of
medicine and a suitable herbal preparation is required to be investigated (Palimbo 1976). Although a large number of plant preparations have been reported to possess antidiabetic activity over last several decades (Chaudhary and Vohra 1970, Bever and Zahand 1979, Oliver 1980, Satyavati 1984, Patnaik and Dhawan 1986, Nagarajan et al 1986, Ivorra et al 1989, Atta-ur-Rahman and Zaman 1989, Handa et al 1989, Chatterjee 1996) there has been a question on the reproducibility of results probably because many of the herbal formulations have been studied on IDDM model. Further in-depth studies are required to establish their mode of action and therapeutic efficacy to make best use of available plant resources for the treatment of diabetes mellitus.

According to Ayurvedic texts, a combination of substances is used to get the enhanced desired action and eliminate unwanted side effects (Nadkarni 1982, Satyavati et al 1987). An exhaustive study of 40 polyherbal antidiabetic formulations sold in the Indian market has been carried out and the ingredients present in them were compared. It was found that few plants are repeatedly used among most of the formulations. *Enicostemma littorale* is one such plant found in most of the formulations. The percentage of *E. littorale* present in various formulations is listed in Table 1. *E. littorale* is one of the plants that have been mentioned in various ancient Indian literatures to possess antidiabetic activity (Sharma 1991). *E. littorale* is one of the plant that has been mentioned in various ancient Indian literature to possess antidiabetic activity (Shaligram 1904, Vaidhya 1965, Shodhal 1972, Sharma 1991). It is a glabrous perennial herb belonging to the family Gentianaceae (The Wealth of India 1952, Mayuranathan 1994, Trease 1996).

Plate 1, 1a and 1b are the photographs of the of *E. littorale*. *E. littorale* grows upto 10-50 cm in height and branching from the base. It is a procumbent rainy season herb. It is commonly found in wet places, throughout the greater part of India; more frequently near the sea as well as on hilly slopes at 1500 feet altitude. It is known as chota-chirayata in Hindi (because it is used as the substitute of chirayata), Mamejavo in Gujarati,
## Table 1

**Polyherbal Antidiabetic Formulations containing* Enicostemma littorale* as one of the ingredient**

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of the Product</th>
<th>Name of the Manufacturer</th>
<th>% of <em>E. littorale</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dia Care Herbal Powder</td>
<td>Admark Herbals Ltd. Dhoraji, Gujarat</td>
<td>22.2</td>
</tr>
<tr>
<td>2</td>
<td>Saptirangyadi Vati</td>
<td>Govt. of Gujarat Supply, Ahmedabad and Gujarat Ayurved Vikas Mandal, Junagadh, Gujarat</td>
<td>21.7</td>
</tr>
<tr>
<td>3</td>
<td>Gluconil–R capsule</td>
<td>Hindustan Medi System, Ahmedabad</td>
<td>17.7</td>
</tr>
<tr>
<td>4</td>
<td>Tribhang Shila tablet</td>
<td>Zandu Pharmaceutical Works Ltd. Bombay</td>
<td>12.9</td>
</tr>
<tr>
<td>5</td>
<td>Madhuripu churna</td>
<td>Lallubhai Vrajlal Gandhi, Ahmedabad</td>
<td>10.0</td>
</tr>
<tr>
<td>6</td>
<td>Madhurin powder</td>
<td>Dhanvantry Pharmacy, Jamnagar, Gujarat</td>
<td>10.0</td>
</tr>
<tr>
<td>7</td>
<td>Dicon capsule</td>
<td>Solite, Ahmedabad</td>
<td>10.0</td>
</tr>
<tr>
<td>8</td>
<td>Madhu Sanjivani Churna</td>
<td>Navjivan Herbal Remedies, Kheda, Gujarat</td>
<td>09.3</td>
</tr>
<tr>
<td>9</td>
<td>Sugoleless capsule</td>
<td>Ayucar Pharmaceuticals Pvt. Ltd. Ahmedabad</td>
<td>08.2</td>
</tr>
<tr>
<td>10</td>
<td>Diatic powder</td>
<td>Ethichem Laboratories, Ahmedabad</td>
<td>08.1</td>
</tr>
<tr>
<td>11</td>
<td>Amrut Jivan Garanule</td>
<td>Zeal Pharmaceuticals, Ahmedabad</td>
<td>07.5</td>
</tr>
<tr>
<td>12</td>
<td>Hyponid tablet</td>
<td>Charak Pharmaceuticals (India) Ltd. Samlkha, Haryana</td>
<td>07.4</td>
</tr>
<tr>
<td>13</td>
<td>Madhuri tablet</td>
<td>Raka Laboratories, Ahmedabad</td>
<td>07.0</td>
</tr>
<tr>
<td>14</td>
<td>Glucomap tablet</td>
<td>Mahirishi Ayurveda Corporation Ltd. New Delhi</td>
<td>06.7</td>
</tr>
<tr>
<td>15</td>
<td>Madhumehari churna</td>
<td>Govt. of Gujarat Supply, Ahmedabad and Shree Mahanarayan Ayurvedic Pharmacy, Ahmedabad</td>
<td>05.7</td>
</tr>
<tr>
<td>16</td>
<td>Madhunashinyadi Churna</td>
<td>Zeal Pharmaceuticals, Ahmedabad</td>
<td>05.0</td>
</tr>
<tr>
<td>17</td>
<td>Panvelley Forte pills</td>
<td>Panvelley Herbal Products, Rajkot, Gujarat</td>
<td>05.0</td>
</tr>
<tr>
<td>18</td>
<td>Shugrol tablet</td>
<td>Yogi Pharmacy, Haridwar, Uttar Pradesh</td>
<td>05.0</td>
</tr>
<tr>
<td>19</td>
<td>Limit capsule</td>
<td>Ayucare, Rajkot, Gujarat</td>
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</tr>
<tr>
<td>20</td>
<td>Madhuna powder</td>
<td>Jan Kalyan Aushadhi Nirmanshala, Bombay</td>
<td>03.0</td>
</tr>
<tr>
<td>21</td>
<td>Madhurin pills</td>
<td>Dhanvantry Pharmacy, Jamnagar, Gujarat</td>
<td>02.5</td>
</tr>
</tbody>
</table>
Enicostemma littorale
Family- Gentianaceae
Enicostemma littorale
Family - Gentianaceae
Nagajivha in Bengali (may be because the appearance of its leaf is like snake's tongue), and Vellaragu in South India.

The stem is procumbent, yellowish green in colour and sub-quadrangular or subterete, possessing four bulges as wings at the four corners near the bases, however, the bulges become quite indistinct. Nodes are prominent and covered by annular membranous ring joining lamina of leaves on either side. The internodes are short. The stem bears numerous leaves and small flowers all around. The leaves are simple, sessile, opposite, decussate and variable, 3.2-6.3 cm long and 3-16 mm broad. They are elliptic, lanceolate, obtuse, entire, glabrous, 3- nerved, mid nerve strong and the marginal nerves often obscure. The upper surface is glaucous. Flowering season is July to September. The flowers are small, white and sessile, in axillary whorled clusters, all along the stem. The clayx is 3 mm long, lobes as long as the tube (1.5mm) obtuse, ovate-oblong, with narrow membranous margin. The corolla is white in colour, 6-8 mm long, tubular, lobes 2-5 mm long, lanceolate and acute. The stigma is large, two lobed. Stamens are five, attached to the corolla tube, epipetalous. Fruiting season is July to September. The fruits are capsule. They are 4 mm long, ellipsoid, slightly narrowed at the base, rounded at the apex, apiculate with the remains of the style and contain numerous seeds. The seeds are small. The root is creeping, filliform and white in colour. All parts of the plant are bitter in taste. (Cooke 1904, Kirtikar and Basu 1935, Uniyal et al 1975). More details of the plant have been mentioned in the review of literature.

Blood Sugar level in anterior pituitary extract induced hyperglycemic rats was found to be decreased when treated with Tribhang Shila, an Ayurvedic formulation, (Zandu Pharmaceuticals Ltd., Bombay), containing *E. hyssopifolium* as one of the ingredients, (Gupta et al 1962). The antihyperglycaemic activity of Tribhang Shila was found comparable to that of tolbutamide (Gupta and Seth 1962). *E. littorale* is claimed to be antidiabetic when used along with Shilajeet (Trivedi 1969). Aqueous extract of *E. littorale* (500 mg/kg) caused insignificant reduction of normal blood glucose level but in alloxan-induced diabetic rabbits it was lowered significantly (P<0.02). Thus,
E. iittorale did not seem to produce hypoglycemic effect in normal rabbits but it produced significant reduction in blood sugar in diabetic rabbits. The hypoglycemic effect of E. iittorale was suggested to be due to increased peripheral utilization of glucose (Vyas et al 1979). It has been reported that a 'Phaki' (mixture of twelve indigenous plants, of which E. iittorale is one of the constituents) showed hypoglycemic activity in diabetic rats. It did not show any hypoglycemic activity in normal rats. Aqueous extract of the 'Phaki' in doses ranging from 50 to 200 mg/kg on I.V. administration in dogs produced a dose dependent reduction in blood sugar (Ainapure et al 1984). Tribal inhabitants of North Gujarat are found to be routinely using hot aqueous extract of E. iittorale for the treatment of diabetes (Shah and Gopal 1985). Barot et al (1975) reported E. iittorale to be effective in lowering blood sugar in diabetics (in 57% of patients) producing clinical improvement by reducing the feeling of thirst and hunger.

In the light of the facts mentioned above the objective of the present investigation was to screen and evaluate E. iittorale as an antidiabetic herbal preparation. The results of animal studies were extended into clinical situation. We have undertaken an open, multicentric clinical study based on parallel group design, for the use of an aqueous extract of E. iittorale administered to the patients in the form of Ghanvati, an ayurvedic form of pills, prepared as per Ayurvedic Pharmacopoeia (1966).

Dymock et al (1893) reported that aerial part of the plant gave 34% of dry alcoholic extract and 15.7% of ash while the subterranean part gave 15.5% of dry alcoholic extract and 10.4% of ash. The presence of a bitter glycoside and ophelic acid in E. litorale was reported by Wehmer (1935). One alkaloid (m.p. 79°-80° C) and one phytosterol were isolated from the petroleum ether extract while qualitative estimation of benzene and alcoholic extracts revealed the presence of glycosides, tannins and reducing sugars (Prasad and Bhusan 1954). A crystalline alkaloid (C_{10}H_{18}O_{2}N, m.p. 82°-83° C) was isolated from E. litorale and was found to be identical with the alkaloid Gentianine, first isolated from Gentiana kirilowi (Iyer et al 1956, Govindachari
Water-soluble ash of the whole plant was found 2.08% while acid insoluble ash content of the whole plant was found 15.7%. Qualitative analysis of the ash revealed the presence of iron, potassium, sodium, calcium, magnesium, silica, phosphate, chloride, sulphate and carbonate. The presence of n-hexacosanal, heptacosane and nonacosane in the alcohol-insoluble portion of unsaponifiable matter and myristic, stearic and oleic acids in the saponifiable matter of the petroleum ether extract was recorded (Mehta and Devani 1959). With the help of partition chromatography on buffered filter paper and buffered cellulose columns two alkaloids were isolated from *E. littorale* by partition chromatography (Sharma and Jain 1960). A non-bitter white crystalline compound (m.p. 224°–226° C) was obtained from the ethylacetate insoluble fraction after crystallisation from ethanol. Betulin, a triterpene sapogenin (m.p. 252°–254° C) was isolated from the unsaponifiable portion of ether extract after saponifying it with 5% sodium hydroxide solution and chromatographing over alumina.

Swertianmarin, an extremely bitter pale yellow amorphous compound (m.p. 108°–111° C) was isolated from the green viscous mass obtained from an alcoholic extract of the drug treated with ether followed by ethylacetate (Rai and Thakar 1966, Desai et al 1966). Five alkaloids (one water-soluble and four chloroform-soluble), two sterols and volatile oil were detected in *E. littorale* (Natranjan and Prasad 1972). Swertianmarin can be converted into the alkaloid Gentianine by treatment with ammonia. Phylogenetic significance of the co-existence of erythrocentaurin and a number of structurally related monoterpene alkaloids in *E. hyssopifolium* have been established. Petroleum ether extract of *E. hyssopifolium* was extracted with citric acid and again that citric acid extract was further extracted with ether, which yielded brown crystals of erythrocentaurin. Two monoterpene alkaloids (enicoflavin and gentiocrucine) were isolated from *E. hyssopifolium* (Ghosal et al 1974, Chaudhuri et al 1975). Saponins and tannins were found to be absent in *E. littorale* while flavonoids (glycoflavone i.e. C-glycosides of flavones) and xanthones were found to be present. Six phenolic acids (vanillic acid, syringic acid, p-hydroxy benzoic acid, protocatechuic acid, p-coumaric acid and ferulic
acid) were found present in *E. hyssopifolium*. (Daniel and Sabnis 1978). Seven flavonoids were isolated from alcoholic extract and their structures were identified as apigenin, genkwanin, isovitexin, swertisin, saponarin, 5-O-glucosylswertisin and 5-O-glucosylisowsertisin (Ghosal and Jaiswal 1980). Methanol extract of *E. littorale* was found to be containing alkaloids, phenols, flavones, catechins, saponins, anthraquinones, steroids, triterpens, sugars and different aminoacids. Among amino acids L-glutamic acid, tryptophane, alanine, serine, aspartic acid, L-proline, L-tyrosine, threonine, phenyl alanine, L-histidine monohydrochloride, methionine, iso-leucine, L-arginine monohydrochloride, DOPA, L-Glycine, 2-amino butyric acid and valine were found to be present in *E. axillare* (Retnam and De Britto 1998).

Thus, there is a confusion on the phytochemical analysis of *E. littorale*. Since, our pharmacological results indicated potential of this plant as antidiabetic; we have also undertaken detailed phytochemical studies of this plant including HPTLC fingerprinting.