

Synopsis of the thesis on

**Combined effects of selected medicinal plants  
along with *Enicostemma littorale* Blume on  
diabetes mellitus – A systematic study.**



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## **Introduction**

Diabetes mellitus describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Prevalence of diabetes mellitus in adults worldwide was estimated to be 4 % in 1995 and to rise to 5.4 % by the year 2025. According to a recent report published by WHO, the countries like India, China and US has the largest number of people with diabetes (King et.al., 1998).

Both types of diabetes (Type 1 & Type 2) are characterized by hyperglycemia, the major hall mark of the disease. Insulin is the primary hormone responsible for signaling the storage and utilization of glucose and other nutrients and inhibition of glucose production. Hence, any defect in insulin action in diabetes has potential long term complications that can effect eyes (retinopathy), heart and blood vessels (angiopathy), kidney (nephropathy) and nerves (neuropathy).

Diabetes mellitus is associated with an increased risk of cardiovascular disease. Several studies have demonstrated that increases in chronic blood glucose concentration can contribute to macrovascular injury and atherosclerotic changes (Kawamori, 1998) in Type 1 (insulin-dependent/IDDM) & Type 2 (non-insulin dependent/NIDDM) diabetes mellitus patients.

The preponderance of evidence indicates that hyperglycemia increases oxidative stress, defined as the production of reactive oxygen species (free radicals; ROS) beyond the protective capability of the antioxidant defenses. Enhanced ROS concentrations can cause general damage to proteins through cross-linking, fragmentation, lipid oxidation and also mediate some of the changes associated with the development of atherosclerosis, such as, activation of coagulation, vasoconstriction, increased expression of adhesion molecules, and oxidative modification of low-density lipoprotein (Watts & Playford, 1998). Hyperglycemia activates several major, well characterized biochemical pathways such as advanced glycation end products (AGEs), receptors for AGE (RAGE) (Brownlee, 1995), protein kinase C (PKC) (Koya & King, 1998), and the polyol pathway (Stevens et.al., 2000) playing significant role in diabetic complications. Late complications of diabetes mellitus are also coupled with impaired activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Godin et.al., 1988).

Available oral anti-diabetic agents for the treatment of diabetes mellitus include the sulfonylureas, metformin, and the alpha-glucosidase inhibitors. Insulin has traditionally been used in various forms with an aim to mimic physiological insulin secretion patterns. Combinations of any of these treatment classes have also been utilized for their additive effects. All of these options have specific advantages and disadvantages, making them ideal for certain patients and less ideal for others (Hsia & Davidson, 2002). Moreover they are mainly targeted to counter hyperglycemia and efforts are going on in designing therapy leading to amelioration of – oxidative stress and dyslipidemia.

In recent times, focus on plant research has increased all over the world. More than 100 medicinal plants are mentioned in the Indian system of medicines including folk medicines for the management of diabetes. As an alternative approach, medicinal herbs with antihyperglycemic activities are increasingly sought by diabetic patients and health care professionals (Dey et.al., 2002). Adverse effects are indeed a cause of concern (Gupta & Raina, 1998), however, available evidence suggests that herbal medicines are relatively safe (Bailey & Day, 1989). The basis of diabetes treatment is management of diabetic complications by different approaches with the aim of providing a healthy life to diabetics. Many natural products have been used in traditional medicine and combination therapy appears more useful for the treatment of diabetes rather than the use of a single compound (Suji & Sivakami, 2003) to tackle the devastating manifestations of the disease. **Though there are a lot of medicinal plants which show hypoglycemic, antioxidant and hypolipidaemic properties, the potency of these can be increased when given in combination (Suji & Sivakami, 2003) but keeping in check on its toxic effects, if any.**

Present study is mainly targeted to study antidiabetic potential of herbal combination of medicinal plants selected – *Curcuma longa*, *Embllica officinalis*, *Trigonella foenum-graecum* and *Enicostemma littorale*.

***Curcuma longa* Linn.** – Belongs to the family Zingiberaceae and is a perennial herb widely cultivated mainly in India and China. In India, popularly known as “Haldi”. It contains a variety of sesquiterpenes. Curcumin is the yellow pigment and its derivatives extracted from the rhizome is a potent anti-inflammatory product (Mukophadhyay et.al., 1982), has good antioxidant and inhibits lipid peroxidation and maintains the activities of antioxidant enzymes such as – superoxide dismutase,

catalase, glutathione peroxidase (Pulla Reddy & Lokesh, 1992) and is also capable of scavenging oxygen free radicals (Pulla Reddy & Lokesh, 1992). The effect of turmeric and its active principle curcumin was found to significantly decrease reduced blood sugar, Hb and glycosylated hemoglobin levels along with oxidative stress encountered by alloxan-induced diabetic rats (Arun & Nalini, 2002) and a distinct tendency to counter change elevated levels of lipid fractions in streptozotocin-induced diabetic rats (Babu & Srinivasan, 1997).

***Emblica officinalis* Gaertn.** – The widely used part is its fruit, commonly known as Indian Gooseberry or “Amla” in Hindi, comes under family Euphorbiaceae with the ingredient 'phyllembin'. The hypoglycemic and hypolipidaemic properties of its flavonoids was reported (Anila & Vijayalakshmi, 2000). A clinical study supplemented with *Emblica officinalis* in diet of hypercholesterolaemic men aged 35-55 years showed a significant decrease in serum cholesterol levels (Jacob et.al., 1988). The investigation on the antioxidant activity of tannoid principles of *Emblica* consisting of emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%) showed significant modulation of antioxidant enzymes and decreased lipid peroxidation levels in rat brain (Bhattacharya et.al., 1999).

***Trigonella foenum-graecum* Linn.** – An erect 2 to 3 foot tall annual herb with light green leaves and small white flowers and commonly known as fenugreek or “methi” in Hindi. The plant of family Fabaceae, its brownish-yellow seeds has a bitter taste mainly due to furostanol glycosides. Blood glucose was reduced by an aqueous extract of *Trigonella* leaf in rats whereas ethanolic extract was only active by ip administration (Abdel-Barry et.al., 1997). The seeds have 4-hydroxyisoleucine, which increases glucose-induced insulin release, at 100-1000 microM and is ineffective at low (3 mmol/l) or basal (5 mmol/l) glucose concentrations (Sauvaire et.al., 1998). An increase in food intake, plasma insulin and a decrease in total cholesterol and VLDL levels was also observed in rats fed with fenugreek seeds (Petit, 1993). The hypolipidaemic activity is mainly attributed to the saponins present which inhibit taurocholate and deoxycholate absorption (Stark & Madar, 1993). Fenugreek seeds has also showed normalization of disrupted free radical metabolism in diabetic animals its supplementation in the diet by decreasing lipid peroxidation and increasing antioxidant status (Ravikumar & Anuradha, 1999).

***Enicostemma littorale* Blume** – It is a small perennial herb of family Gentianaceae, the whole plant is used for medicinal purposes. It is commonly known

as “chota chirayita” in Hindi or “mamejua” in Gujarati. It is very bitter and pungent mainly due to the presence of the alkaloid gentianine and the glycoside swertiamarin (Govindachari, 1966; Natarajan et.al., 1972; Ghosal & Jaiswal, 1980). Our lab had reported its glucose lowering effect (Vijayvargia et.al, 2000; Maroo et.al., 2003a) and antioxidant effect (Maroo et.al., 2003b) in alloxan-induced diabetic rats. We had also reported the possible mechanism of glucose lowering effect of aqueous extract of *E. littorale* to be associated with potentiation of glucose-induced insulin release through K(+)-ATP channel dependent pathway but did not require Ca(2+) influx (Maroo et.al, 2002). Its effect on increasing insulin sensitivity, normalizing dyslipidaemia and providing nephroprotection in streptozotocin-induced (NIDDM) diabetic rats was also reported (Murali et.al., 2002).

Oral antidiabetic monotherapies directly address only one defect as their primary mechanism of action, and do not control blood glucose sufficiently well to meet current glycaemic targets. An insulin sensitiser and an insulin secretagogue represent a rational oral antidiabetic combination, as they address the dual endocrine defects of insulin resistance and impaired beta-cell function in type 2 diabetes (Howlett et.al., 2003) along with antioxidative and hypolipidaemic properties. The present study was an attempt to further investigate the antidiabetic effects of *Enicostemma littorale* and increase the antidiabetic potency by combining the commonly known antidiabetic herbs (*Curcuma longa*, *Emblica officinalis*, *Trigonella foenum-graecum*) along with *E. littorale* and thus to design a potent antidiabetic therapy combating hyperglycemia, dyslipidemia and oxidative stress simultaneously.

#### **Objectives of the study –**

- Clinical evaluation of antidiabetic efficacy of *E. littorale* aqueous extract in insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) patients.
- To study the hypoglycemic effect of aqueous extract of herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in alloxan-induced diabetic rats.
- To study the antioxidant and hypolipidaemic effect of aqueous extract of herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in alloxan-induced diabetic rats.

- To study the comparative antioxidant and hypolipidaemic effect of aqueous extracts of *E. littorale* and herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in cholesterol fed rats.

#### **Summary of the work done:**

#### **❖ Clinical evaluation of antidiabetic efficacy of *E. littorale* aqueous extract in insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) patients:**

The *E. littorale* Blume dry plant material was procured from the local market, identified at Botany department (M.S. University of Baroda), cut into small pieces, crushed, soaked and then boiled twice for 20 minutes. The filtrates were combined and concentrated to get the concentration as 1g dry plant equivalent/ml.

Diabetic volunteers with fasting blood glucose levels between 150 & 250 mg/dl and postprandial blood glucose levels between 200 & 300 mg/dl were selected after taking informed written consent for the treatment with aqueous extract. Study protocol was approved by the ethical committee of Baroda Medical College, Vadodara. Presence of any systemic diseases and body mass index (BMI) less than 19 were considered as exclusion criteria. **In the first phase**, a total of 60 diabetes mellitus patients (IDDM : 12; NIDDM : 48) were randomized. IDDM patients were asked to take the aqueous extract of *E. littorale* orally (two divided doses half an hour before meal i.e. 5 g aqueous extract per single dose for a period of 5 months), along with insulin (EL + I). NIDDM patients taking the aqueous extract were further classified into two – those taking other oral hypoglycemic drugs (OHDs) like sulphonylureas (EL + OHDs) and those who were controlling the disease by diet control, exercise and other conventional therapies but not on any OHDs (EL). Out of these, 37 successfully completed the trial (EL + I : 8; EL + OHDs : 14; EL : 15). Healthy controls (n=10) including the author himself, participated in the study. Blood glucose levels were estimated every month and glycosylated haemoglobin levels every two months. Serum lipid profile and antioxidant parameters were estimated at the beginning and end of the study.

At the end of the clinical trial, though there was a decrease in fasting (14%) and postprandial (13%) blood glucose and glycosylated Hb levels (13%) in EL + Insulin group, it was not significant statistically. EL + OHDs and EL group showed

significant decrease in fasting ( $P < 0.01$ ), postprandial ( $P < 0.01$ ) blood glucose and glycosylated Hb levels ( $P < 0.05$ ) at the end of the study.

**In the second phase**, a total of 20 newly diagnosed NIDDM patients were randomized, out of which 11 successfully completed the trial and were taking only the plant extract and no other drugs. This study was conducted for a period of 2 months. There was a significant decrease in fasting, postprandial and glycosylated Hb levels and an increase in serum insulin levels at the end of the trial.

Comparing both the phases, it was seen that the extract was more effective in controlling hyperglycemia in newly diagnosed NIDDM patients but the extract showed potent antioxidant and hypolipidaemic effect in all the groups studied in both the phases, with no significant change in toxicity parameters.

❖ **Hypoglycemic effect of aqueous extract of herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in alloxan-induced diabetic rats.**

*C. longa* dried rhizome, *E. officinalis* dried fruit, *T. foenum-graecum* dried seeds and *E. littorale* whole dry plant was taken in equal proportions, crushed, soaked and boiled twice for 20 minutes. The filtrates were combined and concentrated to get the concentration as 1g dry plant equivalent/ml. This was used as aqueous extract in the study and the dose given was 1.5g dry plant equivalent aqueous extract/100g body weight.

***In vivo* Experiments**

a) Short term experiments – In short term experiments, plasma glucose and serum insulin levels were monitored for 8 hrs after administering the dose of 1.5g dry plant equivalent aqueous extract/100g body weight in normoglycaemic and alloxan-induced diabetic rats. Extract of herbal combination showed decrease in plasma glucose levels along with an increase in serum insulin levels within 2 hrs of administration, as against EL extract alone which showed a delayed plasma glucose lowering and a delayed increase in serum insulin levels (after 4 hrs of administration) in alloxan-induced diabetic rats and thus showing an increased potency of the combination.

b) Long term experiments – In long term experiments, aqueous extract of herbal combination was administered for a period of 20 days (1.5g dry plant equivalent aqueous extract/100g body weight) in normoglycaemic and alloxan-induced diabetic rats. Plasma glucose levels were estimated on 0, 10<sup>th</sup> and 20<sup>th</sup> day, glycosylated Hb and serum insulin levels on 0<sup>th</sup> and 20<sup>th</sup> day. Combined extract showed 59 % decrease

in plasma glucose levels and 87 % increase in serum insulin levels on 20<sup>th</sup> day. Thus herbal combination extract showed increased potency when compared to EL extract alone. Toxicity studies (liver toxicity – SGPT & ALP, Kidney toxicity – Serum creatinine) did not show any toxic effect at this particular dose.

❖ **Antioxidant and hypolipidaemic effect of aqueous extract of herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in alloxan-induced diabetic rats.**

#### ***In vivo Experiments***

The aqueous extract of herbal combination was administered to normoglycaemic and alloxan-induced diabetic rats for a period of 20 days (1.5g dry plant equivalent aqueous extract/100g body weight). Antioxidant parameters such as erythrocyte CAT, SOD and GPx activity, LPO and blood GSH levels were estimated on 0<sup>th</sup> and 20<sup>th</sup> day. Tissue (liver and kidney) CAT, SOD and GPx activity and LPO and GSH levels were estimated on 20<sup>th</sup> day after sacrificing the animal. Increased oxidative stress is commonly seen in diabetic mellitus. An increased CAT, SOD and GPx activity along with LPO levels and decreased GSH levels was seen in untreated diabetic rats. The extract treated groups showed improvement in the antioxidant parameters at the end of study by decreasing the antioxidant enzymes activity, LPO levels and by increasing GSH levels as compared to diabetic controls.

A single report on the hypolipidaemic effect of EL on streptozotocin-induced diabetic rats was published earlier (Murali et.al., 2002). Hypolipidaemic effect of EL extract was seen for the first time in diabetic patients (as shown in the first chapter of this synopsis). Based on these reports, hypolipidaemic effect was evaluated in aqueous extract of EL alone along with aqueous extract of herbal combination in alloxan-induced diabetic rats. Lipid profile like total cholesterol, triglycerides and HDL cholesterol were estimated in serum on 0<sup>th</sup> and 20<sup>th</sup> day. Herbal combination was again more effective than EL alone in controlling the dyslipidemia in treated diabetic rats.

#### ***In vitro antioxidant Experiments***

To understand the mechanism, comparative DPPH (2,2-diphenylpicrylhydrazine) free radical scavenging activity was tested *invitro* in both EL and aqueous extract of herbal combination. EL and herbal combination demonstrated 50% inhibition at 4 mg and 2 mg dry plant equivalent weight

respectively. The EL alone and herbal combination showed invitro nitric oxide and hydroxyl radical scavenging activity but superoxide radical scavenging was demonstrated only by herbal combination.

#### ***Exvivo antioxidant Experiments***

Exvivo experiments with EL and aqueous extract of herbal combination in liver homogenate after LPO induction showed 50 % inhibition at 150 µg and 100 µg dry plant equivalent weight respectively. Similarly, both the extracts also showed significant decrease in nitric oxide release, LPO levels and increased GSH levels in alloxan-treated isolated pancreatic islets.

#### **❖ Comparative antioxidant and hypolipidaemic effect of aqueous extracts of *E. littorale* alone and herbal combination (*C. longa*, *E. officinalis*, *T. foenum-graecum*, *E. littorale*) in cholesterol fed rats.**

*E. littorale* aqueous extract and herbal extract at a dose of 1.5g dry plant equivalent aqueous extract/100g body weight was administered to male charles foster rats who were simultaneously given an atherogenic diet. The study was conducted for a period of 6 weeks. Blood was collected on 0<sup>th</sup>, 3<sup>rd</sup> and 6<sup>th</sup> week and serum lipid profile and antioxidant parameters like – erythrocyte CAT, SOD and GPx activity, LPO levels and blood GSH levels were estimated. On 6<sup>th</sup> week, animals were sacrificed, liver and kidney excised off and antioxidant parameters, liver HMG Co A reductase activity were estimated. Cholesterol and triglycerides content in liver and kidney were measured. Rats fed on atherogenic diet showed increased levels of serum cholesterol, triglycerides and increased oxidative stress, which was significantly controlled in both EL and herbal combination treated groups. Moreover, both the extracts were able to amicably check the increase in body weight as compared to untreated cholesterol fed rats.

In case of tissue (liver & kidney), untreated cholesterol fed rat's showed increased cholesterol and triglyceride content along with increased LPO levels and decreased GSH levels; with no significant change in CAT, SOD and GPx activity as compared to untreated non-atherogenic rats. Group of rats which received EL and herbal combination could ameliorate the increase in tissue cholesterol and triglyceride levels and brought the antioxidant parameters to nearly normal. The decrease in cholesterol levels can be partially explained by the extract's property of inhibiting

HMG CoA reductase enzyme – the rate limiting enzyme in cholesterol synthesis. Comparing, EL alone extract and herbal combination extract, the latter was found to be more potent with respect to hypolipidaemic and antioxidant property in cholesterol fed rats.

**The above results shows that combination therapy would be most helpful and useful for therapeutic purposes against diabetes mellitus. It can increase the potency and therapeutic value by combating hyperglycemia, oxidative stress and dyslipidemia simultaneously and significantly. Drugs can be designed after systematic study so as to tackle diabetes by a single therapy, which can increase both patient compliance and efficacy. But care must also be taken to check about any possible herb-herb interactions. The present study was an effort to evaluate the increased antidiabetic potency by combining some selected known common antidiabetic medicinal plants.**

#### **Research papers published / accepted:**

**Title** – “Antidiabetic effect of *Enicostemma littorale* Blume aqueous extract in newly diagnosed non-insulin-dependent diabetes mellitus patients (NIDDM): A preliminary investigation”

**Authors** – Vihās T Vasu, C Ashwinikumar, Jyoti Maroo, Sharad Gupta, Sarita Gupta.

**Journal** – *Oriental Pharmacy and Experimental Medicine* 3: 84 – 89; 2003.

**Title** – “Antidiabetic efficacy of *Enicostemma littorale* methanol extract in alloxan induced diabetic rats.”

**Authors** – Jyoti Maroo, Arna Ghosh, Rajni Mathur, Vihās T. Vasu, Sarita Gupta.

**Journal** – *Pharmaceutical Biology* 5: 388 – 391; 2003.

**Title** – “Dose dependent hypoglycemic effect of aqueous extract of *Enicostemma littorale* Blume in alloxan induced diabetic rats.”

**Authors** – Jyoti Maroo, Vihās T. Vasu, Sarita Gupta.

**Journal** – *Phytomedicine* 10: 196 – 199; 2003.



**Title** - "Glucose lowering effect of aqueous extract of *Enicostemma littorale* Blume in diabetes : A possible mechanism of action."

**Authors** – Jyoti Maroo, Vihās T. Vasu, Ravi Aalinkeel and Sarita Gupta.

**Journal** – *Journal of Ethnopharmacology* 81: 317 – 320; 2002.

**Title** – "Antioxidative and antihyperglycemic action of *Enicostemma littorale* Blume in diabetes mellitus"

**Authors** – Vihās T Vasu, Jyoti Maroo, C Ashwinikumar, Sharad Gupta, Kantaben H. Patel, Sarita Gupta.

**Journal** – *In Proceedings of the 2<sup>nd</sup> international symposium on molecular medicine held on 20-23<sup>rd</sup> January 2002 by M. S. Univ. of Baroda, Vadodara, Gujarat (India), State University of NewYork at Buffalo, NY, USA and Sun Pharma Advanced Research Centre (SPARC), Vadodara, Gujarat (India) at M. S. Univ. of Baroda, Vadodara, Gujarat (India) 390002. (In Press)*

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