

Chapter VII

Summary

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In traditional practice medicinal plants are being widely used in many countries for the treatment of various diseases. There is a wide spread belief that natural plant products are healthier than synthetic medicines (Gesler, 1992) and chances of experiencing an adverse event are less as compared to allopathic drugs. Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential (Alarcon-Aguilara et.al., 1998). A wide array of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of diabetes (Bailey and Day, 1989). Plants are a potential source of antidiabetic drugs but this fact has not gained enough momentum in the scientific community due to lack of belief among the practitioners of conventional medicine over alternative medicine, possibility of quacks practicing such medicine, their ill defined content, quality and safety (Grover et.al., 2002). Although, oral hypoglycemic agents/insulin are the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of diabetic complications (Rang and Dale, 1991). Moreover, diabetes mellitus is a syndrome characterized by hyperglycemia leading to oxidative stress and dyslipidemia. As the knowledge of heterogeneity of this disorder increases, there is need to look for more efficacious agents with lesser side effects and no modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones had shown to modify the course of diabetic complications. Hence, therapies against

diabetes mellitus are to be targeted for amelioration of hyperglycemia, hyperlipidaemia and oxidative stress.

Oral antidiabetic monotherapies directly address only one defect as their primary mechanism of action, and hence therapy must be designed so as to control various complications with a combinatorial drug. Combination therapy improves therapeutic effect by skillful symptom improvement and enduring function of decreasing blood sugar and thus can enhance therapeutic efficacy and shorten therapeutic course. It was also discovered that the medicinal properties of many herbs required certain other herbs to be present to act as a catalyst. This in turn, increases patient compliance and efficacy when compared to single therapies for various manifestations. Animal studies (Nishizawa et.al., 1995; Suzuki et.al., 1998) and clinical studies (Angelova, 1984; Mozersky, 1999) are also being carried out with combination of herbs for diabetes mellitus. Majority of the herbal products available in the market for the treatment of diabetes are a combination of medicinal plants and multiple constituent nature of botanical products has made standardization a challenging task. Future research may need to more precisely define targeted diabetic populations with regard to disease classification, severity, optimal adjunctive interventions, and perhaps nutrient deficiencies. It will also be important to further elucidate mechanisms of action so that applicability to type 1 or type 2 diabetes can be clarified. The present study was an attempt to further investigate the antidiabetic effects of *Enicostemma littorale* Blume and increase the antidiabetic potency by combining the commonly known antidiabetic herbs – *Curcuma longa* Linn., *Emblica officinalis* Gaertn., *Trigonella foenum-graecum* Linn. – along with *Enicostemma littorale* Blume.

Earlier our lab had reported the hypoglycemic action of aqueous extract of *E. littorale* with no effect on normoglycemic rats (Vijayvargia et.al., 2000; Maroo et.al, 2003b) and antioxidant effect of the methanolic extract of *E. littorale* in alloxan-induced diabetic rats (Maroo et.al., 2003a). Other workers had reported the hypolipidaemic effect of the herb in streptozotocin-induced NIDDM rats (Murali et.al, 2002). In the present study, efficacy of *E. littorale* aqueous extract was investigated in both insulin-dependent (IDDM) and non-insulin dependent (NIDDM) diabetic patients. With a dose of 5 g aqueous extract per single dose, twice a day half an hour before meal (self administered), healthy volunteers did not show any effect in the glycemic parameters, thus supporting the fact that the hypoglycemic effect of the aqueous extract of *E. littorale* is dependent on blood glucose concentration (Maroo et.al., 2003b) with no effect on toxicity parameters studied. The aqueous extract treatment in IDDM patients, who were taking insulin and other OHDs therapy did not show any significant change in the glycemic parameters, though a decrease was seen. But, NIDDM patients who were taking OHDs when treated with the aqueous extract of *E. littorale* showed a significant decrease in fasting and postprandial blood glucose and a decrease of glycosylated Hb levels and NIDDM patients who were not on OHDs but other conventional therapies also showed significant changes. This effect may be attributed to increased insulin release from pancreatic islets as shown by Maroo et al., 2002 and/or due to it's effect on increasing insulin sensitivity as shown in streptozotocin-induced NIDDM rats (Murali et.al., 2002). The lesser effect observed in IDDM patients could be due to the fact that, they were suffering from diabetes since long duration and the extract was not able to induce more insulin release from the already exhausted pancreatic islets. This view can be further strengthened because newly

diagnosed NIDDM patients treated with *E. littorale* extract for 2 months, demonstrated significant decrease in glycaemic parameters,

Apart from the hypoglycaemic property, a significant decrease in serum cholesterol and triglycerides levels were also seen with an increase in HDL cholesterol levels and thus the extract was able to control the hyperlipidaemic state of the NIDDM patients. This could be due to direct effect of some of the chemical constituents present in *E. littorale* on lipid metabolism. Free radicals and peroxides are clearly involved in the pathogenesis of diabetes mellitus (Wolff, 1993). In the present study, significant decrease in erythrocyte CAT activity and LPO levels and an increase in GSH levels were also observed after the administration of extract in the diabetic patients, which could be attributed to the effect of the aqueous extract of the herb as a potent free radical scavenger. Since hypolipidaemic and antioxidant property has been observed in control as well as in IDDM patients, these effects are not secondary but primary. Thus, the present study demonstrated that even when administered alone the extract was very effective in newly diagnosed NIDDM patients and was able to decrease the hyperglycaemic and hyperlipidaemic condition significantly and also improved the antioxidant parameters without any toxic effect at this particular dose.

The unidirectional therapeutic approach in the management of diabetes does not appear to be the way to address this problem. Hence, aqueous extract of herbal combination (*Curcuma longa.*, *Emblica officinalis.*, *Trigonella foenum-graecum* and *Enicostemma littorale*) was administered to alloxan-induced diabetic rats and compared with *E. littorale* aqueous extract treated rats. A single dose of 1.5 g dry plant equivalent extract/100g body weight was used for the study, as this was found to be the effective

dose of *E. littorale* as reported earlier (Maroo et.al., 2003b). In the short term experiments, normoglycaemic rats after 8 hrs did not show any hypoglycemic effect as reported earlier (Vijayvargia et.al., 2000) and in alloxan-induced diabetic rats, herbal combination showed a significant decrease in blood glucose levels from 2nd hr of administration as against *E. littorale*, which showed a delayed response from 4th hr. The above results shows that by combining the selected medicinal plants, the delayed response which was shown by *E. littorale* alone, was decreased and thus the potency of the hypoglycemic effect increased. Even in long term experiments, there was no significant change in glycaemic parameters both the extract treated normoglycaemic rats, where as in diabetic rats, both the extract treatment for 20 days demonstrated significant decrease in blood glucose and glycosylated haemoglobin levels and a significant increase in serum insulin levels and comparatively the effect was seen more in the herbal combination. Cogent db, a polyherbal drug, where fenugreek and turmeric are part of the components had showed significant reduction in blood glucose, glycosylated haemoglobin and increased plasma insulin in alloxan-induced diabetic rats (Pari and Saravanan, 2002). There was also no significant change in the toxic parameters studied and hence the extracts were not toxic at the particular dose used. To understand the possible mechanism of hypoglycemic action, *ex-vivo* experiments with isolated rat pancreatic islets incubated with *E. littorale* and herbal combination with 11.1 mM glucose were carried out. Results showed significant increase in insulin release in both treated groups, at 10 and 60 minutes, which was more in 20 µg herbal combination. 4-hydroxy isoleucine isolated from fenugreek seeds had demonstrated enhanced glucose induced insulin release from isolated pancreatic islets at 11.1 mM glucose (Sauvaire et al,

1998). The glucose lowering effect of aqueous extract of *E. littorale* must be associated with potentiation of glucose-induced insulin release through K⁺-ATP channel dependent pathway (Maroo et.al., 2002). Other mechanisms could be by modulation of carbohydrate metabolizing enzymes as demonstrated by fenugreek (Vats et.al., 2003) and by inhibiting sorbitol dehydrogenase as shown by *C. longa* (Arun and Nalini et.al., 2002).

Abnormalities in lipoproteins are very common in both individuals with non-insulin-dependent diabetes (NIDDM) and insulin-dependent diabetes (IDDM). Hypercholesterolemia is considered a major risk factor in the progression of coronary atherosclerosis and is associated with an increase in the incidence of myocardial ischemia and cardiac event. Experiments were carried out in alloxan-induced diabetic rats and cholesterol fed rats and administration of aqueous extracts of *E. littorale* and herbal combination for 20 days and 6 weeks respectively demonstrated significant decrease in serum cholesterol levels, serum triglycerides, LDL cholesterol, VLDL cholesterol and increased levels of HDL cholesterol. Lipid profile and body weight of hypercholesterolemic rats at 6th week were almost equal to the normocholesterolemic (NC) rats who were on standard diet devoid of atherogenic diet. The effect was more seen in the herbal combination probably because fenugreek, emblica and turmeric, the components of the extract are known potent hypolipidaemic agents. *E. littorale* was reported to show hypolipidaemic effect in streptozotocin-induced NIDDM rats (Murali et.al., 2002). The present study is a first case report of hypolipidaemic effect of *E. littorale* in cholesterol fed rats. Administration of curcumin, from *Curcuma longa* to streptozotocin-induced diabetic rats had decreased serum cholesterol, triglycerides and phospholipids which were elevated in untreated diabetic rats (Babu ans Srinivasan, 1997)

and in rats fed high fat diet (Asai and Miyazawa, 2001). Animal studies have also demonstrated the hypoglycemic and hypolipidemic effects of fenugreek in diabetic (Petit et.al., 1995) and hypercholesterolemic rats (Stark and Madar, 1993). Similarly, serum and tissue lipid levels were significantly decreased by administration of *E. officinalis* in cholesterol fed rats (Mathur et.al., 1996). There are also reports of various polyherbal formulation on cholesterol fed rats, such as Caps HT2 (Mary et.al., 2003), Liposem (Mary et.al., 2002) and Fibernat (Venkatesan et.al., 2003) where administration caused reduction in cholesterol content, clearance of circulating atherogenic LDL, VLDL and significant increase in HDL cholesterol.

The mechanism of lipid lowering could be by inhibiting HMG CoA reductase activity, or by increasing elimination of cholesterol. In fact, *C. longa* and fenugreek had been shown to increase cholesterol elimination by interacting with cholesterol to bile salt conversion (Srinivasan and Sambaiah, 1991; Stark and Madar, 1993). *E. officinalis* treated hypercholesterolemic rabbits excreted more cholesterol and phospholipids, suggesting that the mode of absorption was affected (Mathur et.al., 1996) and its flavonoids significantly inhibited hepatic HMG CoA reductase activity in hyperlipidaemic induced rats (Anila and Vijayalakshmi, 2002). In the present study, treated groups showed further decrease in HMG CoA reductase activity and the effect was seen more in herbal combination (HC + ALL) treated hypercholesterolemic rats and were comparable to Lovastatin (HC + L) treated rats, thus suggesting a possible interaction with the enzyme and thus effects lipid lowering effect.

Excess oxidative stress has captured considerable attention as a potential mechanism for the increased vascular disease in diabetics. The fact that LDL is extremely

susceptible to oxidative damage is known (Steinberg, 1997). Also, oxidation of LDL plays a significant role in atherogenesis. Hence, amelioration of oxidative stress is equally important as controlling hyperglycemia and dyslipidemia. When aqueous extracts of *E. littorale* and herbal combination was administered to alloxan-induced diabetic rats and hypercholesterolemic rats, significant amelioration in erythrocyte CAT, SOD, GPx activities and increased blood GSH levels along with decrease in erythrocyte LPO levels has been observed. The effects were same in the case of tissue antioxidants as well. It was observed that the herbal combination was more potent as an antioxidant than *E. littorale* alone. *C. longa* contains diarylheptanoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin which are good free radical scavengers (Song e.al., 2001) and curcumin had been reported to inhibit lipid peroxidation and maintained the activities of antioxidant enzymes such as – superoxide dismutase, catalase, glutathione peroxidase (Pulla Reddy & Lokesh, 1992). *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). Fenugreek seeds has also showed normalization of disrupted free radical metabolism in diabetic animals by decreasing lipid peroxidation and increasing antioxidant status (Ravikumar & Anuradha, 1999). The antioxidant effect of a methanolic extract of *E. littorale* was reported earlier by our lab (Maroo et.al., 2003b). Also polyherbal combinations like Caps HT2 and Liposem not only demonstrated hypolipidaemic effect as mentioned above, but also antioxidant activity by scavenging hydroxyl and superoxide radicals (Mary et.al., 2003; Mary et.al., 2002).

To understand the antioxidant mechanism of action, *invitro* studies were carried out and it was seen that *E. littorale* showed DPPH free radical, hydroxyl radical and nitric oxide radical scavenging property, whereas herbal combination also showed DPPH free radical, hydroxyl radical, superoxide radical and hydroxyl radical scavenging properties but the ED₅₀ was much decreased than the former, again projecting the increased efficacy of the herbal combination. The free radical scavenging effect of extracts seems to be one of the reason, that there is a decreased activity of antioxidant enzymes in treated diabetics as compared to untreated diabetic rats. *Ex-vivo* experiments using liver homogenates were carried out by inducing LPO, it was seen that both *E. littorale* and herbal combination extracts exhibited protection against induced LPO and were able to protect auto-oxidation of GSH. Reports have shown that the antioxidant status of the islets is weak (Tiedge et.al., 1997). The antioxidant effect of aqueous extracts of *E. littorale* and herbal combination evaluated in the isolated rat pancreatic islets incubated with alloxan showed a decreased LPO levels and NO production, and protected against GSH oxidation, mainly in those groups where the extract were pre-incubated. *E. littorale* showed good efficacy in 20 µg, where as the herbal combination was effective in both 5 and 20 µg concentrations. Herbal combination showed more efficacy due to various properties of herbs as mentioned earlier.

Thus, *E. littorale* was reported to show antioxidant and hypolipidaemic effect in cholesterol fed rats for the first time. Also, hypoglycemic, antioxidant and hypolipidaemic efficacy have been evaluated for the first time in NIDDM and IDDM patients. Combining the selected medicinal plants was able to potentially increase the hypoglycemic, antioxidant and hypolipidaemic efficacy and thus paving way for a

combinatorial therapy in controlling dyslipidemia and diabetes mellitus, a complicated syndrome. Combination therapy might be an effective means of answering some of its serious outcomes and thus a potential candidate for therapeutic purposes.