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

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SUMMARY

The antidiabetic efficacy of *Enicostemma littorale* Blume (chhota chirayata) aqueous extract was examined in newly diagnosed non-insulin-dependent diabetes mellitus (NIDDM) patients taking only the extract and was administered as two divided doses, half an hour before meal as 5g of aqueous extract per single dose. Out of the 20 patients volunteered, 11 successfully completed the 2 month trial and a significant decrease in fasting & postprandial blood glucose and glycosylated haemoglobin levels were observed along with a significant improvement in the antioxidant parameters of the patients. There was also a significant increase in serum insulin levels in 7 patients after extract treatment as compared to levels before treatment. Serum total cholesterol and serum triglyceride levels were decreased significantly with a significant increase in serum HDLCholesterol levels. Other vital parameters remained stable and no side effects were observed. This is the first report showing the hypoglycemic, antioxidant and hypolipidemic properties of the aqueous extract of *E. littorale* Blume in non-insulin dependent diabetes mellitus patients.

Key words: *Enicostemma littorale* Blume; Hypoglycemic effect; Antioxidant effect; Hypolipidemic effect; Non-insulin dependent diabetes mellitus

INTRODUCTION

Diabetes mellitus is a heterogeneous group of disorder, characterized by an increased plasma glucose levels (Lebovitz, 1999). The syndrome is mainly classified into insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) (Kahn and Gordon, 1998). The role of hyperglycemia in causing oxidative stress and diabetic complications had been proved (Ihara, 1999).

Although there are a large number of hypoglycemic drugs available in the market, more and more people are approaching for an alternative treatment for diabetes mellitus in the form of herbal medicine (Bruce and David, 1999). To date, over 400 traditional plant treatments for diabetes have been reported (Bailey and Day, 1989), out of which only a small number have received scientific and medical attention to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes (Dey *et al.*, 2002). There are reports of using herbal extracts for the treatment of diabetes mellitus in humans (ICMR, 1998). Adverse effects are indeed a cause of concern (Gupta and Raina, 1998), however, available evidence suggests that herbal medicines are relatively safe (Bailey and Day, 1989).

Enicostemma littorale Blume, commonly known as chhota chirayata in Hindi or mamejua in Gujarati, is a perennial herb of Gentianaceae family with sessile lanceolate leaves. The presence of alkaloid gentianine and the bitter glycoside swertiamarin had been identified (Govindachari *et al.*, 1966). The effect of *E. littorale* on Daltons ascitic lymphoma

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(Kavimani and Manisenthlkumar, 2000) and its anti-inflammatory activity (Sadique *et al.*, 1987) had also been reported. We had reported the hypoglycemic effect of the aqueous and methanol extract of *E. littorale* in the alloxan induced diabetic rats (Vijayvargia *et al.*, 2000; Maroo *et al.*, 2003a; Maroo *et al.*, 2003b). Based on this, the present study was undertaken.

This study is a preliminary investigation of the hypoglycemic, antioxidant and hypolipidemic properties of the aqueous extract of *E. littorale* Blume in newly diagnosed NIDDM patients.

MATERIALS AND METHODS

Patients

A total of 20 newly diagnosed non-insulin-dependent diabetes mellitus patients (NIDDM) were randomized, out of which 3 dropped out of the trial, 2 were excluded because of uncontrolled blood glucose levels (FBS>230 mg/dl, PP2BS>250 mg/dl) and 4 were not eligible due to non-compliance in taking the extract. The number of patients who successfully completed the trial was 11 who were taking only the extract. Healthy controls (n=10) also participated in the study.

Plant material

The *E. littorale* Blume dry plant material was procured from the local market and identified at the Botany department, M.S. University of Baroda, Gujarat, India. Voucher specimen [Oza 51, 51(a)]. The whole plant aqueous extract was prepared as reported earlier (Vijayvargia *et al.*, 2000). In brief, fine powder of the plant was made in the electric grinder. The powder was soaked in thrice the amount of water for 2 hr and then boiled for 20 min. Then it was passed through a fine cotton cloth to get the filtrate. The filtrate was concentrated to 1 g/ml by boiling. The yield of the extract was found to be 26%. This was used as aqueous extract in the study. The qualitative chemical analysis of the aqueous extract was done. Efforts are going on in the lab to identify and characterize the active component(s) present in the extract.

Experimental procedure

Information on general clinical history and diet of the patients volunteered were obtained. Diabetics with fasting blood glucose levels between 140 & 230 mg/dl and post prandial blood glucose levels between 230 & 270 mg/dl were selected for treatment with the aqueous extract. Presence of any systemic diseases and body mass index (BMI) less than 19 were considered as exclusion criteria. Subjects randomized to the treatment groups were to be orally self administered aqueous extract of *E. littorale* in two divided doses, half an hour before meal as 5g of aqueous extract per single dose. The period of study was for 2 months and monthly assessments were scheduled. The study protocol was approved by the ethics committee of Baroda Medical College, Vadodara, Gujarat, India. All subjects participating in the study gave their written consent.

Monthly assessments were done for plasma glucose levels (GOD-POD Kit method) (Trinder, 1969). Glycosylated haemoglobin (HbA_1c) levels (Parker *et al.*, 1981), serum insulin levels (RIA Kit, BRIT, Mumbai), lipid profile parameters serum total cholesterol (Zlatikis *et al.*, 1953), serum triglycerides (Gottfried and Rosenburg, 1973), HDL-Cholesterol (Burnstein *et al.*, 1970) and antioxidant parameters such as erythrocyte lipid peroxidation (LPO) levels (Beuge and Aust, 1978), catalase (CAT) activity (Aebi, 1984) and reduced glutathione (GSH) levels (Beutler *et al.*, 1985) were evaluated before and after the two months of extract treatment. Serum glutamate pyruvate transaminase (SGPT) (Reitman and Frankel, 1957) & serum alkaline phosphatase (ALP) (Bowers and McComb, 1975) and serum creatinine (Henry *et al.*, 1974) were also evaluated as indicator of liver and kidney function tests respectively. Blood pressure, pulse rate and body weight were monitored at every visit.

Statistical analyses

Statistical evaluation of analytical data was done by Student's t test using the statistical software-GraphPad Prism 3.0. In all comparisons two-tailed $P \leq 0.05$ was considered significant. Results are expressed as mean ± SE.

RESULTS

The qualitative analysis of the chemical extract showed the presence of various compounds as

Table 1. Qualitative analysis of the aqueous extract of *Enicostemma littorale* Blume

Class of compounds	*Enicostemma littorale* Blume aqueous extract
Phenolic	+ +
Tannins	+ + +
Anthroquinone	+ +
Flavonoids	+ +
Glycosides	+ + +

++ - present in low to moderate levels, +++ - present in high levels

presented in Table 1.

There was no significant change in the blood glucose and glycosylated haemoglobin levels of healthy controls. Though a decrease in serum lipid profile and a slight improvement in the antioxidant parameters were observed, it was statistically not significant (data not shown).

Glycemic parameters in NIDDM patients

Newly diagnosed NIDDM patients taking only the aqueous extract of EL showed significant reduction in all the glycemic parameters after treatment. Observed percent reductions at the end of the study were 31.12% ($P<0.001$) for fasting blood glucose (FBS), 36.34% ($P<0.001$) for postprandial blood glucose (PP_2BS) levels (Fig. 1) and 25.73% ($P<0.05$) for HbA_1c levels (Table 2). Out of the 11 patients studied, 7 patients showed a 48% ($P<0.05$) increase in serum insulin levels as compared to baseline values (Table 2), whereas the increase was not significant in the remaining 4 patients.

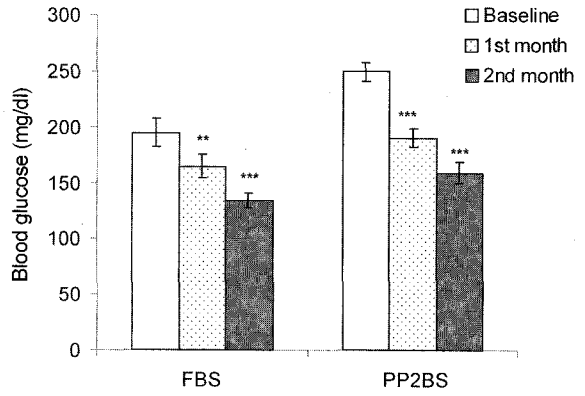

Fig. 1. Hypoglycemic effect of *E. littorale* Blume aqueous extract in NIDDM patients. Values presented as Mean±SE (n = 11).
**$P<0.01$, ***$P<0.001$ as compared to value before treatment.

Table 2. Hypoglycemic effect of *E. littorale* Blume aqueous extract in NIDDM patients

	Before treatment	After treatment
Glycosylated Haemoglobin (%) (n = 11)	10.06 ± 0.57	8.28 ± 0.53*
Serum Insulin levels (mU/ml) (n = 7)	27.86 ± 3.07	41.29 ± 3.48*

Values presented as Mean±SE. *$P<0.05$ as compared to values before treatment.

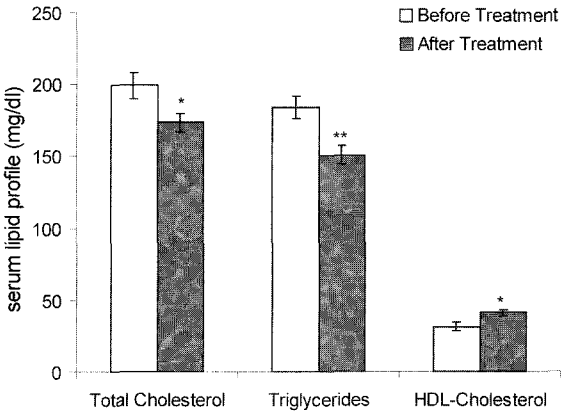

Fig. 2. Hypolipidemic effect of *E. littorale* Blume aqueous extract in NIDDM patients. Values presented as Mean±SE (n=11).
*$P<0.05$, **$P<0.01$ as compared to value before treatment.

Lipid parameters in NIDDM patients

There was significant decrease in serum total cholesterol and serum triglycerides levels and a significant increase in serum HDLCholesterol levels at the end of the study (Fig. 2).

Antioxidant parameters in NIDDM patients

There were also significant changes in the antioxidant parameters of the patients. At the end of study, as compared to initial levels, there was a decrease of 42.98% ($P<0.001$) in erythrocyte CAT activity; a decrease of 14.79% ($P<0.05$) in erythrocyte LPO levels and an increase of 29.61% ($P<0.01$) in blood GSH levels (Table 3).

Toxicity parameters in NIDDM patients

There was no significant change in the other parameters such as serum GPT, ALP and serum

Table 3. Antioxidant effect of *E. littorale* Blume aqueous extract in NIDDM patients

	Before treatment	After treatment
Erythrocyte CAT activity (k/g Hb)	290.4 ± 10.78	165.6 ± 7.30***
Erythrocyte LPO levels (nmoles of MDA formed/g Hb)	210.9 ± 6.02	179.7 ± 11.31*
Blood GSH levels (mg/dl)	21.75 ± 1.10	28.19 ± 1.76**

Values presented as Mean±SE (n = 11). *P<0.05, **P<0.01, ***P<0.001 as compared to value before treatment.

Table 4. Effect of *E. littorale* Blume aqueous extract on serum GPT, ALP and creatinine levels in NIDDM patients

	Before treatment	After treatment
SGPT (IU/L)	39.4 ± 8.43	38.62 ± 7.44 ^{ns}
ALP (IU/L)	84.6 ± 4.90	90.70 ± 8.03 ^{ns}
Creatinine (mg/dl)	0.74 ± 0.13	0.88 ± 0.29 ^{ns}

(IU=μM product formed/min). Values presented as Mean±SE (n=11). Ns=non-significant as compared to values before treatment

creatinine (Table 4) levels. Also, there was no appreciable change in the body weight of patients and none of the patients reported any side effects ascribable to *E. littorale*.

It was interesting to note that 72% (n=8) of the volunteers undergoing extract treatment, experienced a sense of well being and 36% (n=4) reported a decrease in fatigued condition.

DISCUSSION

There was a significant decrease in all the glycemic parameters studied in NIDDM patients after aqueous extract (*E. littorale*) treatment suggesting its usefulness in controlling the hyperglycemic state. The decrease in plasma glucose levels in NIDDM patients was observed in both fasting and postprandial levels, with no significant change in blood glucose levels in healthy controls, thus suggesting the fact that the hypoglycemic effect of the aqueous extract of *E. littorale* is dependent on blood glucose concentration. This is supported by our earlier studies in normoglycemic healthy rats, which did not show glucose lowering effect after *E. littorale* treatment (Vijayvargia *et al.*, 2000). Further, when long term effect of the extract was evaluated, it was demonstrated that glycosylated haemoglobin levels were significantly decreased in treated diabetics suggesting a better long term control by the extract. Decrease in glycemic parameters may be attributed to increased glucose uptake and metabolism within cells and by potentiating insulin release from pancreatic beta cells, which is shown in the present results, where there is an increase in serum insulin levels in extract treated diabetic patients. This effect is further supported by our earlier *invitro* studies on isolated rat pancreatic islet cells which were incubated with 11.1 mM glucose along with aqueous extract (20 μg dry plant equivalent) and showed an enhanced glucose induced insulin release (Maroo *et al.*, 2002). Since, the aqueous extract has more than one active constituents, it is difficult to say which particular component is responsible for the respective property. However, there is a large amount of glycosides present in *E. littorale* aqueous extract (Table 1) and the hypoglycemic effect could be attributed to these class of compounds as reported by other workers (Wang and Ng, 1999).

It is known that diabetes is often associated with hyperlipidaemia. A significant decrease in serum cholesterol and triglycerides levels were 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 by affecting absorption, synthesis or utilization of cholesterol. Further studies are required to understand exact mechanism.

Free radicals and peroxides are clearly involved in the pathogenesis of diabetes mellitus (Wolff, 1993). Catalase (CAT) activity, lipid peroxidation (LPO) and reduced glutathione (GSH) levels in red cells give the measure of the extent of free radical damage inflicted (Pippenger *et al.*, 1998). 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. Preliminary *invitro* studies in our lab with the aqueous extract of *E. littorale* had shown DPPH (2,2-diphenyl picrylhydrazyl) free radical scavenging activity (Vasu *et al.*, 2002), which could be attributed to the effect of the aqueous extract of the herb as a potent free radical scavenger.

The present study shows that even when administered alone the extract was very effective in newly diagnosed NIDDM patients and was able to decrease the hyperglycemic and hyperlipidaemic condition significantly and also improved the antioxidant parameters without any toxic effect at this particular dose. These properties may be attributed due to the presence of various components present in the extract as shown in Table 1. Hence, it is a potential candidate for the development of a novel therapeutic agent for NIDDM patients.

The above preliminary investigation reports the hypoglycemic, hypolipidemic and antioxidant nature of aqueous extract of *E. littorale* for the first time in NIDDM patients.

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