

Evaluation of effect of aqueous extract of *Enicostemma littorale* Blume in streptozotocin-induced type 1 diabetic rats

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The present investigation was undertaken to standardize and study the dose-dependent effect of three weeks treatment with hot and cold aqueous extract of *E. littorale* (0.5, 1 and 2 g/kg, po) on streptozotocin (STZ) induced type I diabetic (confirmed by histopathology) rats (45 mg/kg, iv single dose). Treatment of rats with STZ produced cardinal signs of diabetes-mellitus like a significant loss of body weight, polyuria and polydipsia. There was also a significant increase in fasting blood glucose levels and AUC_{glucose} associated with decrease in insulin levels and AUC_{insulin} in STZ-diabetic rats. Treatment with *E. littorale* hot extract (1 and 2 g/kg) significantly reduced the elevated food intake and water intake, glucose and AUC_{glucose} levels of diabetic rats. There was also a significant increase in serum cholesterol, serum triglyceride in the STZ diabetic rats. Treatment with *E. littorale* hot extract (1 and 2 g/kg) significantly decreased all these elevated levels in diabetic rats. Hot aqueous extract of *E. littorale* at 0.5 g/kg produced a significant decrease in serum glucose and triglycerides. At this doses serum cholesterol and AUC_{glucose} were not found to be altered significantly. TLC finger-print profiles were established for the aqueous extract using HPTLC. Swertiamarin, which was used as a chemical marker, was found to be one of the major components in the hot extract while it was absent in cold extract. The results suggest that *E. littorale* possesses potential antidiabetic activity and improves lipid profile at a small dose of 0.5 g/kg.

Keywords : Diabetes, *Enicostemma littorale*, IDDM, STZ, Swertiamarin.

In diabetes mellitus antihyperglycaemic effect of several plants, extracts and herbal formulations that are being used as antidiabetic remedies has been confirmed¹. Compared to synthetic drugs, herbal preparations are frequently considered to be less toxic with fewer side-effects². Therefore the search for effective and safer antihyperglycemic agents has become an area of current research all over the world.

Enicostemma littorale Blume (Gentianaceae), commonly known as Chota-kirayata or Chota chirayata (Hindi) and Mamejavo (Gujarati), is being used as a folk medicine for the treatment of diabetes mellitus in Western and Southern India and more so the tribal inhabitants of North Gujarat use its hot water extract for the treatment of diabetes, fever, stomach-ache, dyspepsia and malaria. Various Ayurvedic formulations containing *E. littorale* as one of the ingredients have been shown to produce

antihyperglycemic activity in various hyperglycemic rat models³. The plant contains catechins, sterols, saponins, steroids, triterpenoids, alkaloids and volatile oil^{4,5}. Some important chemical constituents of the plant include betulin, a triterpene sapogenin, a secoiridoid glycoside swertiamarin^{6,7}, monoterpene alkaloids like enicoflavine and gentiocrucine^{8,9}. Antidiabetic activity of crude extract of *E. littorale* in type II diabetic rats has been reported¹⁰. Maroo *et al* reported antidiabetic activity on alloxan-induced diabetes¹¹. However, it was a single dose study with a very high dose of 15 g dry plant extract which works out to be about 5.4 g extract/kg body weight. Data from an earlier study showed that aqueous extract produces antidiabetic effect at a dose of 2 g/kg po in NIDDM rats¹⁰. In the present study the dose dependent effect of aqueous extract of *E. littorale* have been investigated in IDDM rats and showed that aqueous extract of *E. littorale* at a lower doses 0.5 g/kg and 1 g/kg is useful. TLC finger-print profiles for the extract were also established using swertiamarin as a chemical marker.

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Materials and Methods

Plant material—Whole plant material of *E. littorale* was collected from Gujarat during August–September at the end of flowering season. The plant was identified and authenticated by Prof. O. P. Saxena, Head, Botany Department, Gujarat University, Ahmedabad, and a voucher specimen was deposited. The plant material was cleaned and dried in shade and stored at 25°C. Standard swertiamarin was a gift sample from Prof. Fumihiko Yoshizaki, Tohoku Pharmaceutical University, Sendai Japan.

Preparation of aqueous extract of *E. Littorale*—Shade-dried herb 1 kg containing all vegetative and reproductive parts of *E. littorale* was powdered in an electric grinder and for preparation of hot extract powder treated with 4 L water for 8 h at 55°–60°C and filtered while cold extract prepared at room temperature. The filtrate was then concentrated to dryness under reduced pressure (yield 85.23 g).

Preliminary phytochemical screening, TLC fingerprinting—The hot aqueous extract of *E. littorale* was subjected to various preliminary phytochemical tests for detecting different classes of compounds. Standard solution of swertiamarin was prepared by dissolving 8 mg swertiamarin in 10 ml methanol. An accurately weighed 0.1 g of powder of the whole herb of *E. littorale* was extracted with (100 ml) water under reflux for 8 hr at 55°–60°C, the extract was filtered and water was removed under reduced pressure. The residue was dissolved in 10 ml of methanol. 30 µl of this sample extract and 10 µl of standard swertiamarin solution were applied on precoated silica gel 60 F254 HPTLC plate (E. Merck) using CAMAG Linomat IV Automatic Sample Spotter. The plate was developed in a solvent system of ethyl acetate:methanol:water (7.7:2.0:0.5). The plate was dried at room temperature and scanned using CAMAG TLC scanner 3 at UV 254 and 366 nm and R_f values and absorption spectra of the resolved bands were recorded. Further, the plates were derivatised by spraying anisaldehyde sulphuric acid reagent followed by heating at 110°C for 5 min, and the R_f and colours of the bands resolved were recorded.

Pharmacological evaluation for antidiabetic activity

Animals—Male Sprague Dawley rats (weight 200–250 g) were used for the study. They were maintained under standard environmental conditions

and were fed a standard pellet diet purchased from Pranav Agro. Ind. Ltd. with water *ad libitum*.

Induction of diabetes—Diabetes was induced in the rats by injection of STZ (45 mg/kg) into the tail vein. Animals showing glucosuria more than 2% (Diastix, Bayer Diagnostics, India) or blood glucose level more than 140 mg/dl 48 h after STZ injection were selected for the experiment. Animals were divided into following 4 groups: normal control, control treated, diabetic control and diabetic treated (n=6-7). Treatment groups received hot extract of *E. littorale* at the dose of 0.5, 1 and 2 g/kg, po daily and cold extract of *E. littorale* at the dose of 1 g/kg po daily for three weeks. Control group received the vehicle.

Blood sampling and biochemical analysis—At the end of three weeks treatment, blood samples were collected from the tail vein into centrifuge tubes and allowed to clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at -20°C until analysis was done. Serum samples were analyzed spectrophotometrically for glucose, cholesterol, triglyceride (Bayer Diagnostics Kits, India). Serum insulin levels were estimated by radioimmunoassay method using the kit from Bhabha Atomic Research Center, Mumbai.

Oral glucose tolerance test (OGTT)—Rats were fasted for 12 hours and glucose administered at the dose of 1.5 g/kg, po. Blood samples were collected at 0, 30, 60 and 120 min. Serum was separated immediately and analyzed for glucose and insulin. The results of OGTT are expressed as integrated areas under the curves (AUCs) for glucose and insulin over a period of 0–120 min. and calculated by trapezoid rule [$AUC = C_1 + C_2/2 \times (t_2 - t_1)$]. Changes in glucose and insulin concentrations during OGTT were expressed as AUC_{glucose} (mg/dl/min) and AUC_{insulin} (µU/ml/min) respectively.

Statistical analysis—Results were expressed as mean ± SE. Significance was evaluated using analysis of variance (ANOVA) followed by Tukey's test in all the experiments ($P < 0.05$ was considered significant).

Results

Preliminary phytochemical screening—Preliminary phytochemical screening of aqueous extract showed the presence of triterpenoids, flavonoids, alkaloids and coumarins.

TLC fingerprint profile—TLC of hot aqueous extract showed 4 quenched bands in UV 254 nm at

R_f 0.19, 0.36, 0.57, 0.77 (Fig. 1) and 7 bands in UV 366 nm at R_f 0.11 (green fluorescent), 0.19 (light yellow), 0.27 (light green fluorescent), 0.35 (light blue), 0.80 (light blue), 0.83 (light blue), and at 0.89 (light blue) while 7 bands after derivatisation at R_f 0.11 (orange red), 0.22 (light green), 0.36 (pink), 0.47 (light orange), 0.79 (light yellow), 0.82 (light pink), 0.88 (purple). Comparison of absorption spectrum of the band in the sample track with that of standard swertiamarin (R_f 0.57) by overlapping confirmed the presence of swertiamarin in the hot extract sample which is absent in cold extract and it was found to be one of the major components of hot extract.

Effect on general parameters—Diabetes (IDDM) produced a significant loss of body weight as compared to control rats. Treatment with hot aqueous extract of *E. littorale* at dose of 0.5 g/kg failed to produce significant change in body weight, water intake and urine output of STZ-induced diabetic rats. STZ-induced significant polyphagia and polydipsia in diabetic control animals. These were significantly

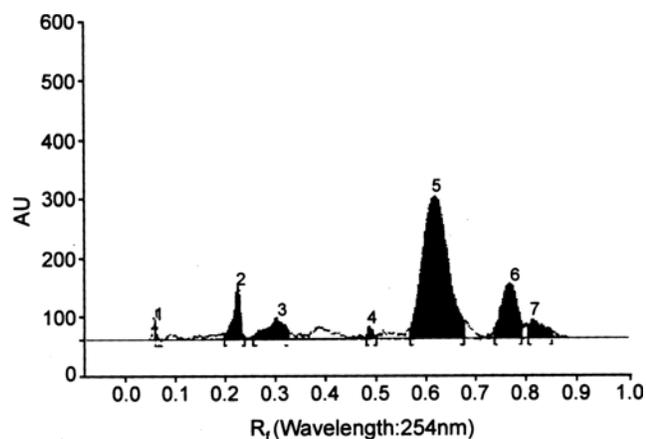


Fig. 1—HPTLC chromatograms of hot aqueous extract of *E. littorale* (R1) scanned at 254 nm.

reduced by *E. littorale* hot aqueous extract treatment at 2 g/kg and 1 g/kg po (Table 1), while cold extract of *E. littorale* failed to show any activity.

Biochemical parameters—STZ-rats exhibited a significant hyperglycemia and hypoinsulinemia as compared to control animals. Treatment with hot water extract of *E. littorale* at all doses significantly prevented STZ-induced hyperglycemia without affecting STZ-induced change in insulin levels (Fig. 2). Glucose and insulin levels of control animals treated with *E. littorale* at 2 g/kg hot water extract were comparable with those of the control. The decrease in glucose levels by *E. littorale* in diabetic animals was not dose dependent and in the doses tried highest fall was seen with 0.5 g/kg. (Table 1).

STZ-injection caused a significant increase in AUC_{glucose} associated with a significant decrease in AUC_{insulin} values in diabetic control animals as compared to control. Treatment with hot aqueous extract of *E. littorale* at all doses significantly prevented STZ induced increase in AUC_{glucose} but did not show any effect on AUC_{insulin} . The decrease in AUC_{glucose} at 500 mg/kg was not statistically significant (Fig. 2a).

STZ diabetic rats produced significant hypercholesteremia and hypertriglyceridemia as compared to control animals. Treatment with hot extract *E. littorale* showed dose dependent decrease in both cholesterol and triglyceride levels. The decrease in serum triglycerides as well as serum cholesterol was maximum at 1 g/kg, and there was no significant change in cholesterol at 500 mg/kg (Table 1). Cold extract had not shown any significant activity on biochemical parameters (results not shown).

Discussion

In the present investigation we found the presence of triterpenoids, alkaloids, flavonoids and coumarins

Table 1— Effect of *E. littorale* hot aqueous extract treatment on general and biochemical parameters in STZ induced diabetic rats [Values are mean \pm SE from 6-7 animals in each group]

Parameter	Control	Control treated (2g/kg)	Diabetic control	Diabetic treated (0.5 g/kg)	Diabetic treated (1 g/kg)	Diabetic treated (2 g/kg)
Body weight (g)	284 \pm 12	271 \pm 8	180 \pm 9*	177 \pm 6	183 \pm 11	191 \pm 8
Food intake (g/day/rat)	21 \pm 3	24 \pm 2	58 \pm 3*	52 \pm 4	43 \pm 2.5**	45 \pm 2**
Water intake (ml/day/rat)	55 \pm 4	51 \pm 3	108 \pm 6*	88 \pm 4	76 \pm 2**	68 \pm 7**
Serum glucose (mg/dl)	70 \pm 2	76 \pm 3	373 \pm 15*	285 \pm 9.3**	269 \pm 8.2**	259 \pm 8.5**
Serum insulin (IU/ml)	45 \pm 3.2	46.8 \pm 3.1	31.9 \pm 2.78*	30 \pm 2.46	32.5 \pm 2.9	32.4 \pm 2.8
Serum cholesterol (mg/dl)	64 \pm 4.3	55 \pm 3.8	191 \pm 4.8*	178 \pm 3	138 \pm 4.4**	153 \pm 5.3**
Serum triglyceride (mg/dl)	72 \pm 5.8	59 \pm 6	264 \pm 14*	123 \pm 7.9**	100 \pm 4.9**	160 \pm 3.8**

*significantly different compared with SD control.

**significantly different compared with diabetic control $P < 0.05$ (One-way ANOVA followed by Tukey test).

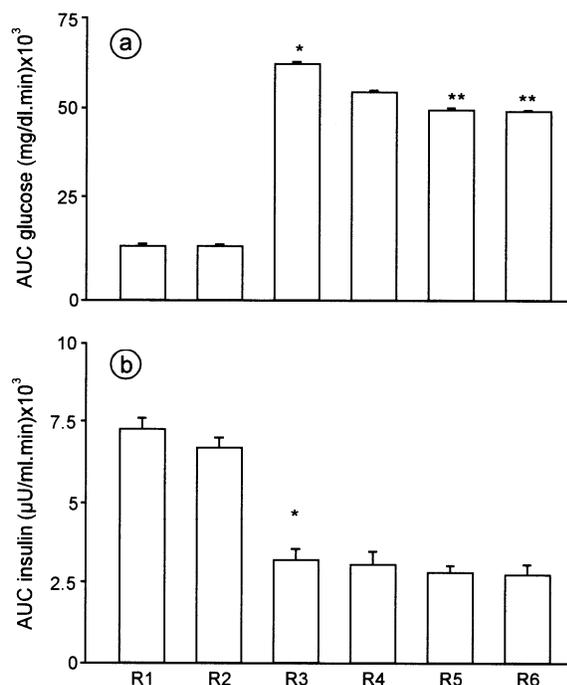


Fig. 2—Effect of *E. littorale* hot aqueous extract treatment on (a) AUC_{glucose} and (b) AUC_{insulin} in STZ induced diabetic rats. Each bar represents mean \pm SE. from 6-7 animals in each group. R1=control, R2=control treated with *E. littorale* (2 g/kg), R3=diabetic control, R4=diabetic treated with *E. littorale* (0.5 g/kg), R5=diabetic treated with *E. littorale* (1 g/kg), R6=diabetic treated with *E. littorale* (2 g/kg). Significantly different from *non-diabetic control, **diabetic control, $P < 0.05$.

in the aqueous extract of *E. littorale*. Rai and Thakar⁶ also reported the presence of triterpenoids and glycosides in *E. littorale*. The presence of alkaloids and flavonoids has also been shown by various workers^{4,5}. Swertiamarin was isolated and characterised by Vishwakarma *et al*¹³. In the present studies we found swertiamarin to be one of the major compounds in the hot water extract of *E. littorale* and hence can be considered as the marker compound for standardization. Along with TLC fingerprint profiles the presence of swertiamarin in the sample hot water extract was confirmed in co-chromatography by R_f 0.57, λ_{max} 240 nm and overlay of absorption spectra.

Hot water extract of *E. littorale* treatment significantly increased body weight in diabetic animals at dose of 2 g/kg. There was practically no change (slight decrease) in body weight of non-diabetic animals. Treatment with hot water extract caused slight decrease in food consumption and water intake. STZ diabetes also produced a significant increase in glucose levels associated with decrease in insulin levels.

It was observed that hot extract did not alter insulin levels in control or diabetic rats. The extract produced a significant decrease in glucose levels and had no effect on insulin in diabetic rats as compared to STZ-diabetic controls, suggesting that the decrease in glucose levels may not be due to increase in insulin release. There was no dose dependent effect on serum glucose levels with the extract. The present results do not support the findings of Maroo *et al*¹¹, who reported an increase in insulin release by *E. littorale* by using 5 g/kg dose of the cold extract; glucose and insulin levels remained unchanged in diabetic rats.

Earlier studies have shown that in STZ-diabetic rats, insulin deficiency is associated with hypercholesterolemia, hypertriglyceridemia and release of free fatty acids from adipose tissue¹⁵. Ketone body formation¹⁶ due to the action of insulin on HMG-Co A reductase and lipase¹⁷. Insulin action on lipoprotein lipase results in impaired clearance of VLDL and chylomicrons from plasma¹⁸. In the present study *E. littorale* significantly decreased both cholesterol and triglyceride level. Decrease in triglyceride was found to be greater at 1 g/kg of *E. littorale*. This may be advantage because triglycerides are considered to be more responsible than cholesterol for heart diseases. Triglycerides levels were brought to near normal levels by the treatment with *E. littorale*. However, decrease in cholesterol was relatively less with treatment as compared to decrease in serum triglycerides. Biochemical parameters of animals treated with cold water extract of *E. littorale* which do not contain swertiamarin at dose 1 g/kg were not statistically significant from control diabetic animals. This suggests that swertiamarin is responsible for change in lipid profile and anti-hyperglycemic activity of *E. Littorale* hot water extract.

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