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Comparative antidiabetic activity of different fractions of *Enicostemma littorale* Blume in streptozotocin induced NIDDM rats

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SUMMARY

Aqueous extract of *Enicostemma littorale* is reported to have antidiabetic activty. In the present investigation, we studied the effect of aqueous extract of *E. littorale* and its different fractions *i.e.*, toluene, chloroform, ethyl acetate, *n*-butanol fractions and remaining residual fraction in streptozotocin (STZ)-induced neonatal type 2 diabetic rats. Fasting glucose and insulin levels in NIDDM were significantly ($P<0.05$) higher than control rats and they were significantly decreased by treatment with aqueous extract of *E. littorale* and its *n*-butanol and ethyl acetate fractions. Results of oral glucose tolerance test (OGTT) showed that aqueous extract and its *n*-butanol and ethyl acetate fractions significantly ($P<0.05$) decrease both $AUC_{glucose}$ and $AUC_{insulin}$ values in NIDDM treated groups. Insulin sensitivity (K_{ITT}) index of NIDDM control was significantly lower as compared to normal control and this was significantly ($P<0.05$) increased after treatment with aqueous extract, its *n*-butanol and ethyl acetate fractions. Treatment with aqueous extract of *E. littorale* and its *n*-butanol and ethyl acetate fractions lowered the elevated cholesterol and triglyceride levels observed in NIDDM rats. Treatment with aqueous extract of *E. littorale* and its *n*-butanol fraction showed significant decrease in creatinine, urea, SGPT and SGOT levels as compared to NIDDM control rats. However ethyl acetate fraction showed significant changes only in creatinine and SGOT levels, and not in the levels of urea, and SGPT as compared to NIDDM control rats. Treatment with toluene, chloroform and residual fractions of *E. littorale* did not produce any effect on glucose, insulin, triglyceride, cholesterol, creatinine, urea, SGPT or SGOT levels as compared to NIDDM control rats. Our data suggest that *n*-butanol and ethyl acetate fractions contain the active compounds which may be responsible for the above activity and associated complications in NIDDM diabetes mellitus.

Key words: *Enicostemma littorale;* Streptozotocin; NIDDM

INTRODUCTION

Indigenous herbs, used as remedies against diabetes in the traditional Indian Systems of Medicine or in ethnomedicinal practices, have not produced any good marketable anti-diabetic medicines. This failure may be attributed to the incorrect pharmacognosy of the medicinal plants, followed by incomplete extraction procedures, or use of insensitive and / or inadequate animal models (Chandrasekhar *et al.*, 1988).

Enicostemma littorale Blume (Gentianaceae) known as Nagajihva or Mamejava in Ayurveda, is a glabarous perennial herb (Kirtikar and Basu, 1935). The plant contains catechins, sterols, saponins, steroids, triterpenoids, alkaloids and volatile oil (Natarajan and Prasad, 1972; Retnam and DeBritto, 1988). Some important chemical constituents include betulin, a triterpene sapogenin, swertiamarin (Rai and Thakar, 1966; Desai *et al.*, 1966), monoterpene alkaloids like enicoflavine and gentiocrucine (Ghosal *et al.*, 1974; Chaudhuri *et al.*, 1975) six

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phenolic acids *viz.*, vanillic acid, syringic acid, *p*-hydroxy benzoic acid, protocatechuic acid, *p*-coumaric acid and ferulic acid (Daniel and Sabnis, 1978), seven flavonoids *viz.*, apigenin, genkwanin, isovitexin, swertisin, saponarin, 5-*O*-glucosylswerisin and 5-*O*-glucosylisoswertisin (Ghosal and Jaiswal, 1980). Methanol extract of *E. littorale* was found to contain different amino acids like L-glutamic acid, tryptophane, alanine, serine, aspartic acid, L-proline, L-tyrosine, theronine, phenyl alanine, L-histidine monohydrocholoride, methionine, isoleucine, L-arginine momohydrocholoride, DOPA, L-glycine, 2-amino butyric acid and valine (Retnam and DeBritto, 1988).

E. littorale is being used as a folk medicine for the treatment of diabetes mellitus in Western and Southern India (Gupta *et al.*, 1962). Various Ayurvedic antidiabetic formulations contain *E. littorale* as one of the herbal ingredients and has been reported to produce antihyperglycemic activity in various hyperglycemic rat models (Gupta *et al.*, 1962). Ethnomedical studies of North Gujarat (India) reveal the use of hot aqueous extract of *E. littorale* by the tribal inhabitants for the treatment of diabetes, fever, stomach ache, dyspepsia and malaria in interior parts of Gujarat.

Aqueous extract of *E. littorale* has recently been reported from our labotary to possesses glucose lowering and insulin sensitising activity (Murali *et al.*, 2002). The present investigation was undertaken to study the effect of various fractions of aqueous extract of *E. littorale* in STZ-induced NIDDM rats.

MATERIALS AND METHODS

Plant material

Whole plant material of *E. littorale* was collected from Gujarat (India) in August September (2000) at the end of flowering season. The plant was identified and authenticated by Prof. O. P. Saxena, Head, Botany Department, Gujarat University, Ahmedabad, India and a voucher specimen was deposited. The plant material was cleaned and dried in shade and stored at 25°C.

Extraction and fractionation

Six kilogram of shade-dried herb of *E. littorale* was powderd and boiled with 24 lit water for 8 h. The aqueous extract was concentrated under reduced pressure (yield=635.2 g). Aqueous extract (545.0 g) was further fractionated using solvents of varying polarity *viz.*, petroleum ether [60-80°C] (0.90 g), toluene (16.2 g), chloroform (20.2 g), ethyl acetate (29.2 g) and *n*-butanol (128.2 g), the extract remained after the fractanation with *n*-butanol was residual extract (350.3 g). The extracts were concentrated under reduced pressure and air dried to remove the solvent completely.

NIDDM rat model and treatment protocol

Healthy albino rats of Sprague Dawley strain were kept for breeding. To induce NIDDM a single dose of injection of STZ (90 mg/kg: *i.p.*) [Sigma Chemical Co., St. Luis, MO, USA] was given to 2 day old pups. Another group of pups received only saline. The animals were weaned at 30 days and after a period of 3 months, checked for fasting glucose levels to confirm the status of NIDDM. The animals showing fasting glucose levels of >140 mg/dl were considered as diabetic. The pups that received saline were considered as control animals. The experimental animals were divided into three groups, six animals in each group (1) control, (2) NIDDM control, and (3) NIDDM treated with *E. littorale* extract. Treatment was given daily for three weeks. The control group received an equal volume of the vehicle. The third group was subdivided into eight groups as follows :

1. NIDDM treated with aqueous extract of *E. littorale* (0.5 g/kg)
2. NIDDM treated with aqueous extract (1 g/kg)
3. NIDDM treated with aqueous extract (2 g/kg)
4. NIDDM treated with toluene fraction (0.5 g/kg)
5. NIDDM treated with chloroform fraction (0.5 g/kg)
6. NIDDM treated with ethyl acetate fraction (0.5 g/kg)
7. NIDDM treated with *n*-butanol fraction (0.5 g/kg)
8. NIDDM treated with residual fraction (0.5 g/kg)

During the study standard food and water were provided *ad libitum*. Changes in body weight, food intake and water intake were recorded.

Oral glucose tolerance test (OGTT)

At the end of three weeks study rats were subjected to an oral glucose tolerance test (OGTT). Glucose (1.5 g/kg) was administered to 12 h fasted rats and the

blood samples were collected from tail vein at 0, 30, 60, 120 min. Serum was analyzed for glucose by GOD-POD method by using diagnostic reagent kits (Bayer Vadodara, India. Ltd) and insulin by RIA kits (Bhabha Atomic Research Center, Bombay, India). The results were expressed as integrated area under the curve for glucose ($AUC_{glucose}$) and insulin ($AUC_{insulin}$), that was calculated by trapezoid rule $AUC=(C_1+C_2)/2\times(t_2-t_1)$.

Insulin tolerance test (K_{ITT})

Insulin tolerance test (Alford, 1971) is used to assess peripheral insulin resistance. This test measures insulin sensitivity using K_{ITT} as an index of insulin mediated glucose metabolism. Rats were fasted for 6 h before giving insulin challenge. Neutral insulin injection (Actrapid Novo, Ahmedabad, India) was diluted with 0.9% saline to a final concentration of 0.2 U/ml, and then administered in the dose of 0.2 U/100 g body weight by slow *i.v.* injection through tail vein. Blood samples were collected at 0 min, and then at 10, 20 and 30 min after administration of insulin injection. Serum was separated and subjected to glucose estimation. K_{ITT} was determined from the slope of a linear portion of the regression line of natural logarithm of glucose *vs* time (Alford, 1971) and K_{ITT} was calculated by following formula (Lundbaeck, 1962).

$$K_{ITT} = \frac{0.693}{t_{1/2}} \times 100$$

where $t_{1/2}$ represents the half-life of plasma glucose decay. The half-life of plasma glucose was obtained by plotting plasma glucose concentration *vs* time on semilogarithmic graph paper.

Effect on other biochemical parameters

At the end of three week treatment blood samples were collected from tail vein. Serum was separated and analyzed spectrophotometrically for cholesterol, triglyceride, creatinine, urea, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) using diagnostic reagent kits (Bayer, India. Ltd).

Preliminary phytochemical screening

The aqueous extract of *E. littorale* and its different fractions were subjected to various preliminary phytochemical tests (Ravishankara *et al.*, 2002) for the presence or absence of various classes of compounds.

HPTLC finger printing

TLC fingerprint profiles of aqueous extract and its *n*-butanol and ethyl acetate fractions were established using HPTLC. Suitably diluted stock solution of aqueous extract, *n*-butanol and ethyl acetate extract were spotted on pre-coated silica gel 60 F_{254} TLC plates (E. Merck) using CAMAG Linomat IV Automatic Sample Spotter and the plate were developed in the following solvent systems:

1. For aqueous extract : Ethyl acetate : methanol : water (7:7: 2.0: 0.5)
2. For *n*-butanol fraction : Ethyl acetate : methanol : water (7:7: 1.5: 0.5)
3. For ethyl acetate fraction : Choloroform : methanol (9.5 : 0.5) respectively

The plates were dried at room temperature and scanned using CAMAG TLC scanner 3 at UV 254 and 366 nm and R_F values, absorption spectra of the resolved bands were recorded. Further, the plates were derivatised by spraying with 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.

Statistical analysis

All the experimental values were expressed as mean±SEM. Results were analyzed statistically using analysis of variance (ANOVA) followed by Tukey's test ($p<0.05$).

RESULTS

Effect on general parameters

STZ treated rats had a significantly reduced weight gain, increase in water intake and food intake as compared with the control animals. Treatment with aqueous extract or its various fractions failed to produce significant change in the body weight, food intake or water intake of these animals (Table 1).

Effect on glucose and insulin levels

Fasting glucose levels were significantly higher in

Table 1. Effect of three week treatment with aqueous extract of *E. littorale* on general and biochemical parameters in STZ diabetic rats

Parameters	Control	NIDDM Control	NIDDM 500mg/kg	NIDDM 1g/kg	NIDDM 2g/kg
Body weight (g)	248.2 ± 6.4	187.6 ± 9.5*	192.3 ± 12.0	208 ± 11.6	205.2 ± 8.1
Water intake (ml/day/rat)	13.8 ±2.4	35 ± 2.4*	25.5 ± 3.0	24.0 ± 3.2	23.6 ± 4.2
Food intake (g/day/ rat)	20.2 ± 4.3	39.3 ± 2.4*	36.5 ± 5.2	31.6 ± 7.2	33.4 ± 4.5
K_{ITT} (per min)	8.6 ± 0.1	4.2 ± 0.4*	6.0 ± 0.1**	7.2 ±0.2**	7.0 ± 0.2**
Serum Creatinine (mg/dl)	1.4 ± 0.1	3.6 ± 0.1*	2.6 ± 0.1**	2.60 ± 0.2**	2.56 ± 0.2**
Serum Urea (mg/dl)	43.4 ± 2.2	58.4 ± 3.2*	46.2 ± 2.9	40.2 ± 2.2**	41.3 ± 2.9**
Serum SGPT (mg/dl)	17.3 ± 2.3	41.5 ± 3.8*	30.9 ± 4.71	26.8 ± 2.4	24.6 ± 1.91**
Serum SGOT (mg/dl)	40.2 ± 2.9	62.4 ± 2.3*	54.3 ± 6.4	46.2 ± 3.8**	42.4 ± 2.5**

Each value is mean ± SEM. (n = 6)
* Significantly different from control groups (P< 0.05).
* * Significantly different from NIDDM control groups (P< 0.05).

NIDDM rats as compared with those in control animals which was associated with hyperinsulinemia in NIDDM control animals (Fig. 1B). Treatment with aqueous extract produced a dose dependent decrease in glucose levels. Aqueous extract produced significant decrease in glucose levels which was little greater at 1 g/kg (Fig. 1A). Treatment with *n*-butanol and ethyl acetate fractions also significantly lowered both glucose and insulin levels (Fig. 1A & 1B). However toluene, chloroform and residual fractions failed to produce any change in glucose or insulin levels.

Both $AUC_{glucose}$ and $AUC_{insulin}$ were significantly higher and K_{ITT} was lower in NIDDM control as compared with control animals. Treatment with aqueous extract and its *n*-butanol and ethyl acetate fractions significantly lowered both $AUC_{glucose}$ and $AUC_{insulin}$ values (Fig. 2A and 2B) and increased

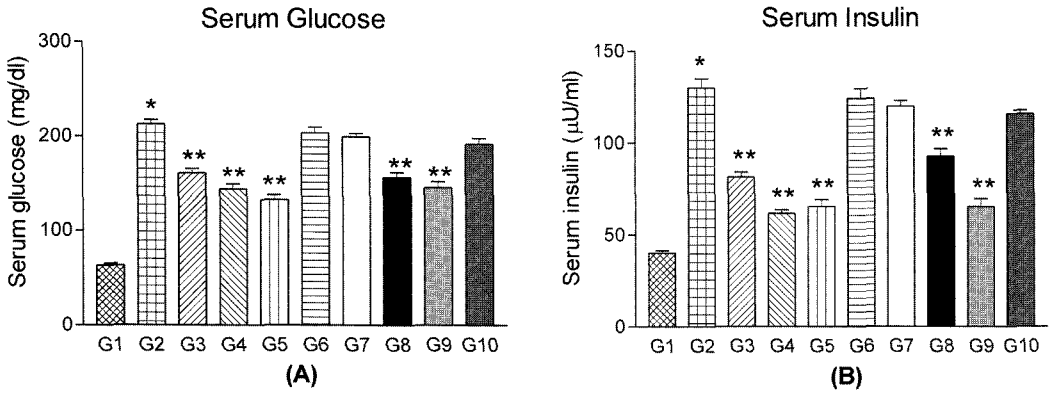

Fig. 1. (**A**) Effect of three week treatment with aqueous extract of *E. littorale* and its various fractions on fasting glucose level in NIDDM rats. *Significantly different from non diabetic control ($P<0.05$). **Significantly different from diabetic control ($P<0.05$). Each bar represents mean±SEM. Number of animals in each group=6. (G1) Control, (G2) Diabetic Control, (G3) Diabetic treated with aqueous extract (0.5 g/kg), (G4) Diabetic treated with aqueous extract (1 g/kg), (G5) Diabetic treated with aqueous extract (2 g/kg), (G6) Diabetic treated with toluene fraction (0.5 g/kg), (G7) Diabetic treated with chloroform fraction (0.5 g/kg), (G8) Diabetic treated with ethyl acetate fraction (0.5 g/kg), (G9) Diabetic treated with *n*-butanol fraction (0.5 g/kg), (G10) Diabetic treated with residual fraction (0.5 g/kg), **(B)** Effect of three week treatment with aqueous extract of *E. littorale* and its various fractions on fasting insulin level in NIDDM rats. *Significantly different from non diabetic control ($P<0.05$). **Significantly different from diabetic control ($P<0.05$). Each bar represents mean±SEM. Number of animals in each group=6. (G1) Control, (G2) Diabetic Control, (G3) Diabetic treated with aqueous extract (0.5 g/kg), (G4) Diabetic treated with aqueous extract (1 g/kg), (G5) Diabetic treated with aqueous extract (2 g/kg), (G6) Diabetic treated with toluene fraction (0.5 g/kg), (G7) Diabetic treated with chloroform fraction (0.5 g/kg), (G8) Diabetic treated with ethyl acetate fraction (0.5 g/kg), (G9) Diabetic treated with *n*-butanol fraction (0.5 g/kg), (G10) Diabetic treated with residual fraction (0.5 g/kg).

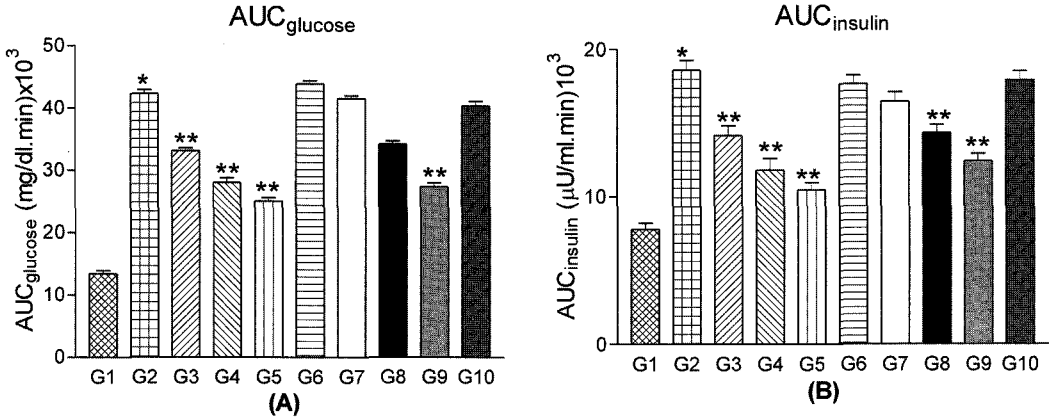

Fig. 2. (**A**) Effect of three week treatment with aqueous extract of *E. littorale* and its various fractions on $AUC_{glucose}$ level in NIDDM rats. *Significantly different from non diabetic control ($P<0.05$). **Significantly different from diabetic control ($P<0.05$). Each bar represents mean±SEM. Number of animals in each group=6. (G1) Control, (G2) Diabetic Control, (G3) Diabetic treated with aqueous extract (0.5 g/kg), (G4) Diabetic treated with aqueous extract (1 g/kg), (G5) Diabetic treated with aqueous extract (2 g/kg), (G6) Diabetic treated with toluene fraction (0.5 g/kg), (G7) Diabetic treated with chloroform fraction (0.5 g/kg), (G8) Diabetic treated with ethyl acetate fraction (0.5 g/kg), (G9) Diabetic treated with *n*-butanol fraction (0.5 g/kg), (G10) Diabetic treated with residual fraction (0.5 g/kg). (**B**) Effect of three week treatment with aqueous extract of *E. littorale* and its various fractions on $AUC_{insulin}$ level in NIDDM rats. *Significantly different from non diabetic control ($P<0.05$). **Significantly different from diabetic control ($P<0.05$). Each bar represents mean±SEM. Number of animals in each group=6. (G1) Control, (G2) Diabetic Control, (G3) Diabetic treated with aqueous extract (0.5 g/kg), (G4) Diabetic treated with aqueous extract (1 g/kg), (G5) Diabetic treated with aqueous extract (2 g/kg), (G6) Diabetic treated with toluene fraction (0.5 g/kg), (G7) Diabetic treated with chloroform fraction (0.5 g/kg), (G8) Diabetic treated with ethyl acetate fraction (0.5 g/kg), (G9) Diabetic treated with *n*-butanol fraction (0.5 g/kg), (G10) Diabetic treated with residual fraction (0.5 g/kg).

K_{ITT} values in NIDDM rats (Table 2). All these changes were significantly greater with 1 g/kg and 2 g/kg aqueous extract, *n*-butanol and ethyl acetate fractions also produced decrease in $AUC_{glucose}$ and $AUC_{insulin}$ along with increase in K_{ITT}. However toulene, chloroform and residual fractions failed to produce any change in $AUC_{glucose}$ and $AUC_{insulin}$ or K_{ITT} values.

Effect on other biochemical parameters

STZ-induced NIDDM rats showed significant hypercholesteremia and hypertriglycemia as compared with control animals (Fig. 3A and 3B). Treatment with aqueous extract and its *n*-butanol and ethyl acetate fractions significantly lowered cholesterol and triglyceride levels as compared to NIDDM

Table 2. Effect of three week treatment with different fractions of aqueous extract of *E. littorale* on general and biochemical parameters in STZ diabetic rats

Parameters	NIDDM Toluene fraction 500 mg/kg	NIDDM Chloroform fraction 500 mg/kg	NIDDM Ethyl acetate fraction 500 mg/kg	NIDDM *n*-Butanol fraction 500 mg/kg	NIDDM Residual fraction 500 mg/kg
Body weight (g)	185.1 ± 11.3	193.6 ± 9.6	201.3 ± 11.0	210 ± 7.8	198.3 ± 6.4
Water intake (ml/day/rat)	33 ± 4.3	26.5 ±3.3	27.5 ± 2.0	27 ± 2.8	32.5 ± 3.6
Food intake (g/day/rat)	37.8 ± 6.3	36.1 ± 3.4	31.6 ± 3.1	36.8 ± 4.8	42.2 ± 5.5
K_{ITT} (per min)	5.1 ± 0.8	5.3 ± 0.7	6.2 ± 0.2**	6.94 ± 0.1**	5.1± 0.8
Serum Creatinine (mg/dl)	3.2 ± 0.1	3.2 ± 0.17	2.8 ± 0.15**	2.8 ± 0.1**	2.9 ± 0.2
Serum Urea (mg/dl)	56.0 ± 2.3	56.2 ± 2.9	50.1 ± 1.3	43.6 ± 2.2**	49.3 ± 3.3
Serum SGPT (mg/dl)	40.3± 1.9	42.8 ± 7.6	26.1 ± 2.6	22.4± 4.3 **	38.6 ± 4.8
Serum SGOT (mg/dl)	58.3 ± 4.8	57.3 ± 2.1	45.3 ± 4.6**	41.2 ± 1.9**	57.6 ± 5.4

Each value is mean±SEM. (n=6)
*Significantly different from control groups ($P<0.05$).
**Significantly different from NIDDM control groups ($P<0.05$).

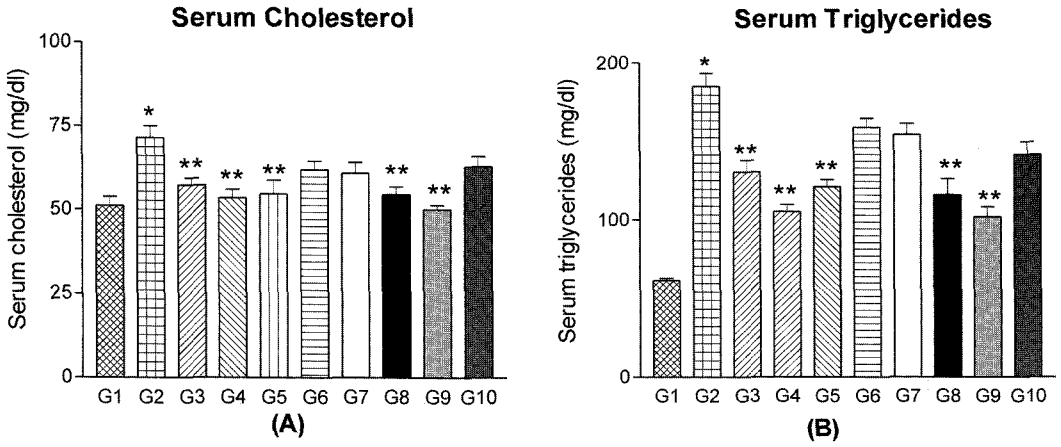

Fig. 3. (**A**) Effect of three week treatment with aqueous extract of *E. littorale* and its various fractions on serum cholesterol level in NIDDM rats. *Significantly different from non diabetic control ($P<0.05$). **Significantly different from diabetic control ($P<0.05$). Each bar represents mean±SEM. Number of animals in each group=6. (G1) Control, (G2) Diabetic Control, (G3) Diabetic treated with aqueous extract (0.5 g/kg), (G4) Diabetic treated with aqueous extract (1 g/kg), (G5) Diabetic treated with aqueous extract (2 g/kg), (G6) Diabetic treated with toluene fraction (0.5 g/kg), (G7) Diabetic treated with chloroform fraction (0.5 g/kg), (G8) Diabetic treated with ethyl acetate fraction (0.5 g/kg), (G9) Diabetic treated with *n*-butanol fraction (0.5 g/kg), (G10) Diabetic treated with residual fraction (0.5 g/kg). (**B**) Effect of three week treatment with aqueous extract of *E. littorale* and its various fractions on serum triglyceride level in NIDDM rats. *Significantly different from non diabetic control ($P<0.05$). **Significantly different from diabetic control ($P<0.05$). Each bar represents mean±SEM. Number of animals in each group=6. (G1) Control, (G2) Diabetic Control, (G3) Diabetic treated with aqueous extract (0.5 g/kg), (G4) Diabetic treated with aqueous extract (1 g/kg), (G5) Diabetic treated with aqueous extract (2 g/kg), (G6) Diabetic treated with toluene fraction (0.5 g/kg), (G7) Diabetic treated with chloroform fraction (0.5 g/kg), (G8) Diabetic treated with ethyl acetate fraction (0.5 g/kg), (G9) Diabetic treated with *n*-butanol fraction (0.5 g/kg), (G10) Diabetic treated with residual fraction (0.5 g/kg).

control (Fig. 3A and 3B). Other fractions of the aqueous extract did not produce any change in cholesterol or triglyceride levels.

NIDDM control rat showed a significant increase in serum creatinine and urea, SGPT and SGOT levels as compared with control animals (Table 2). Treatment with aqueous extract and its *n*-butanol fraction significantly prevented STZ-induced increase in these levels (Table 2). These levels in animals treated with different doses of aqueous extract were not significantly different from each other. Treatment with ethyl acetate fraction showed significant decrease in creatinine and SGOT levels but no significant difference was found in urea and SGPT levels (Table 2). Other fractions of the extract did not produce any change in creatinine, urea, SGPT and SGOT levels.

Preliminary phytochemical screening

Preliminary phytochemical screening of aqueous extract and its *n*-butanol and ethyl acetate fractions showed the presence of triterpeniods, flavonoids, alkaloids and coumarins while saponins, anthraquinone, tannins and phenols were absent.

HPTLC finger print profile

TLC of aqueous extract showed 4 quenched bands in UV 254 nm at R_F 0.19, 0.36, 0.63, 0.77; *n*-butanol fraction showed 8 quenched bands at R_F 0.11, 0.28, 0.49, 0.55, 0.65, 0.78, 0.86, 0.95 and ethyl aceate fraction showed 5 quenched bands at R_F 0.13, 0.20, 0.26, 0.34, and at 0.41.

TLC of aqueous extract showed 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). *n*-butanol fraction showed 7 bands in UV 366 nm at R_F 0.11 (green fluorescent), 0.62 (light green), 0.68 (light blue), 0.75 (blue fluorescent), 0.79 (blue fluorescent), 0.88 (blue fluorescent), and at 0.95 (blue fluorescent). Ethyl acetate fraction showed two bands under UV 366 nm at R_F 0.26 (light blue), and at 0.25 (light blue).

TLC of aqueous extract showed 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). *n*-butanol fraction showed 8 bands after dervatisation at R_F 0.18 (light green), 0.27 (purple), 0.37 (violet), 0.49 (purple), 0.66 (light yellow), 0.75 (light yellow), 0.88 (pink), and at 0.97 (brown). Ethyl acetate showed 8 bands after derivatisation at R_F 0.24 (light green), 0.27 (pink), 0.29 (violet), 0.35 (blue), 0.37 (purple), 0.50 (blue), 0.51 (blue), and at 0.57 (light pink).

DISCUSSION

In the present study, NIDDM control rats showed significantly higher levels of fasting glucose levels as compared with non-diabetic control rats. This is consistent with earlier reports (Weir *et al.*, 1981; Gokhale *et al.*, 1998). Hyperinsulinemia with low hepatic excretion and hypersecretion of beta cells are also reported in mild glucose intolerant obese subjects (Bonora *et al.*, 1983). We also found increase in insulin levels and $AUC_{insulin}$ after glucose load in neonatal STZ-diabetic rats. The high insulin concentration found in neonatal STZ-diabetic rats need not be of pancreatic origin. It could be due to metabolic alterations at extra panceratic levels. In these rats, the metabolic clearence rate of insulin might have been altered. Insulin degradation following hormone receptor binding (Gliemann and Sonne, 1978) and reduced binding of insulin to its receptor have been reported in mild glucose intolerance (Olefsky, 1981). Therefore, the hyperinsulinemia in neonatal STZ-diabetic rats could be due to either decreased hepatic clearance of insulin or decreased number of insulin receptors, resulting in decreased insulin binding and lowered insulin degradation. When animals were subjected to OGTT, the $AUC_{insulin}$ of NIDDM control rats was significantly greater as compared with the non-diabetic rats. However, $AUC_{insulin}$ of NIDDM rats treated with aqueous extract and its *n*-butanol and ethyl acetate fractions was found to be lower as compared with NIDDM control rats, while toluene, choloform and residual fractions were ineffective in lowering $AUC_{glucose}$ and $AUC_{insulin}$ at the dose used. This suggests that in normal animals *E. littorale* does not alter the release of insulin, but in conditions like hyperinsulinemia, it increases the insulin sensitivity for effective glucose disposal. The K_{ITT} was found to be significantly lower in neonatal STZ-diabetic rats as compared with controls. This indicates that NIDDM rats are insulin resistant. The specific mechanism underlying the insulin resistant states are hetrogeneous and may include a receptor defect (decrease in insulin sensitivity) or post receptor defect (decrease in responsiveness to insulin) or combination of both (Khan, 1978; Crettaz and Jeanrenand, 1980). Treatment with aqueous extract and its *n*-butanol and ethyl acetate fractions significantly increased K_{ITT} values, while toluene, cholroform and residual fractions showed no significant change in K_{ITT} values.

ITT, which represents the response to exogenously administered insulin on blood glucose, has been used to estimate insulin sensitivity (Alford *et al.*, 1971). ITT is a simple, reasonably accurate and rapid method for screening insulin resistance (Grulet *et al.*, 1993). ITT indicates net result of resistance to insulin action at a target level including receptor and post receptor defect. In the present investigation the rate of glucose disposale was signifcantly decreased in NIDDM control rats as compared with control rats.

As reported earlier (Durrington, 1993) IR or insulin deficiency is associated with hypercholesteremia and hypertriglyceridemia. STZ-diabetes showed increased plasma levels of cholesterol, triglyceride, free fatty acid and phospholipid (Rodrigues *et al.*, 1986). Insulin deficiency or IR may be reponsible for dyslipidemia because insulin has an inhibitory action on HMG-COA reductase, a key rate-limiting enzyme reponsible for the metabolism of cholesterol rich LDL particles. The mechanisms reponsible for the development of hypertriglyceridemia in uncontrolled diabetes in humans (possibly in insulin deficient STZ-diabetic rats) are due to a number of metabolic abnormalities that occur sequentially. Acute insulin deficiency initially causes an increased free fatty acid mobilization from adipose tissue, resulting in increase in secretion of VLDL-triglycerides from liver (Balasse *et al.*, 1972). With longer insulin deficiency liver converts free fattyacids into ketone bodies and VLDL-triglycerides secretion diminishes (Basso and Havel, 1970). At the same time, lipoprotein lipase activity falls

(Nikkila *et al.*, 1977) resulting in impaired clearance of VLDL and chylomicrones from plasma (Bagdade *et al.*, 1968). Reaven (1988) proposed that IR in diabetic (or non-diabetic) subjects leads to compansatory hyperinsulinamia, which is associated with increased LDL and reduced HDL concentrations. In our study also NIDDM rats showed hypercholesterolemia and hypertriglyceridemia and treatment with aqueous extract and its *n*-butanol and ethyl acetate fractions significantly decreased both cholesterol and triglyceride levels. These findings also support the hypothesis that *E. littorale* causes improvement in insulin sensitivity. Toluene, chloroform and residual fractions showed no significant change in insulin sensitivity at the doses tested.

In NIDDM rats increase in serum creatinine and serum urea levels are observed (Murali *et al.*, 2002). In our study also NIDDM rats showed increase in creatinine and urea levels and treatment with aqueous extract and its *n*-butanol fraction significantly decreased both creatinine and urea levels. Toluene, choloform and residual fractions showed no significant change in creatinine and urea levels at the doses tested.

Our studies show in general that *E. littorale* lowers serum glucose, insulin, lipids, creatinine and urea levels in diabetic rats. Preliminary phytochemical tests showed that the aqueous extract, *n*-butanol and ethylacteate fractions contain triterpeniods, alkaloids, flavonoids and coumarins. It is reported that triterpeniods possess lipid lowering activity (Khanna *et al.*, 1969; Nityanand and Kappor 1973; Arora *et al.*, 1973; Tiwari *et al.*, 1990; Pathak *et al.*, 1990; Shaila *et al.*, 1997) and flavonoids have antioxidant activity (Middleton *et al.*, 2000), which may be responsible for the observed beneficial effects of *E. littorale*.

In conclusion, our data suggests that aqueous extract of *E. littorale* and its *n*-butanol fraction and ethyl acetate fraction have potential anti-diabetic activity. In addition to decreasing the serum glucose and lipids, it can also prevent kidney dysfunction and liver dysfunction in STZ-diabetic rats. Our data also suggests that the active compounds responsible for the above activity might be present in *n*-butanol and ethyl acetate fractions which can be isolated by further fractionation.

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