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A STUDY OF ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITY OF *ENICOSTEMMA LITTORALE*, (BLUME) IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

The concerned study reveals the experimental investigation of the biological activity of *Enicostemma littorale,*(Blume) (Family: Gentianaceae) used as a traditional anti diabetic and hypolipidemic agent in traditional medicine. To study the effect of *Enicostemma littorale* in both normal and *Streptozotocin* induced diabetic rat, the ethyl acetate active fraction (EAAF) of *E.littorale* leaf extract at the dose of 200 mg kg body weight was administered orally once a day to the groups for 30 days. The fasting blood glucose, cholesterol, HDL-cholesterol and serum triglyceride content were estimated in both normal and *Streptozotocin* induced diabetic rats. The fasting blood glucose, cholesterol and serum triglyceride content were found to be significantly reduced ($p<0.05$) in treated rats whereas the extract also showed the potent elevation in the level of serum HDL-cholesterol. The study reveals that *Enicostemma littorale* has significant anti diabetic activity and a hypolipidemic activity in *Streptozotocin* induced and normal fasting rats.

Keywords: Anti diabetic, Diabetes mellitus, *Enicostemma littorale*, Hypolipidemic activity.

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Introduction

Diabetes mellitus is a group of metabolic alterations characterized by hyperglycemia resulting from defects in insulin secretion, action or both[1]. It has already been established that chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels[2]. A multitude of herbs spices and other plant materials have been described for the treatment of diabetes throughout the world[3]. The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as a dietary supplement to existing therapies[4]. Drug in insulin secretion, insulin action both resulting impaired interaction occurs from the additive effect when used metabolism of glucose and other energy-yielding fuels concomitantly with hypolipidemic agent. Some of the plants which are being used for the treatment of diabetes have received scientific or medicinal scrutiny and even the WHO expert committee on diabetes recommends that this area warrant further attention[5]. Diabetes federation estimated that the

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prevalence of diabetes increased from 100 to 135 million affected adults worldwide in 1994 – 1995 to approximately 336 million in 2011, and is expected to raise 439 million by 2030. Thus making it the country with the highest number of diabetic people in the world[6]. There are medicinal plants which are scientifically proved to process anti-diabetic activities.

Enicostemma littorale Blume, Gentiaceae is found in Talakona forest, Tirupathi. The leaves are used as a traditional folk medicine to treat the Diabetes mellitus, rheumatism, abdominal ulcers, anti bacterial, anti fungal anti inflammatory etc. The present study is aimed to investigate the antibiotic effects of ethyl acetate active fraction (EAAF) of whole plant of *E.littorale* on antidiabetic and hypolipidemic activity of *Enicostemma littorale, (*Blume) in streptozotocin induced diabetic Rats.

Materials and methods

Plant Material

E.littorale Blume is a small herb, belonging to family Gentianceae, collected from Talakona forest, Tirupathi. The leaves are washed with water; shade dried, coarsely powdered and kept in air tight.

Preparation of Plant extract for phytochemical screening and anti diabetic studies

Ethyl acetate active fraction was prepared by cold maceration; extract was dried in vacuum desiccators to obtain constant weight. The concentrated extract was subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures[7].

Experimental animals

Adult male rats of Wistar strain weighing approximately 110- 180 g were obtained Mahaveera Enterprises, a licensed breeding centre at Hyderabad, India. All the animals were kept and maintained under laboratory conditions of temperature (22^0C±2), humidity (45±5%), and 12 hour day: 12 hour night cycle; and fed with commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. All the animal experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines and carried out in a licensed research centre with the following registered number (1221/a/08/CPCSEA).

Experimental induction of diabetes

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared Streptozotocin (STZ) (30 mg kg^{-1} b.w.) in 0.1 M citrate buffer (pH – 4.5) in a volume of 1 ml /kg b.w. Rats were supplied with 5% glucose solution for 48 h after STZ injection in order to prevent hypoglycemia. The control animals were treated with citrate buffer (pH – 4.5). Diabetes was developed and stabilized in these STZ treated rats over a period of 7 days. The control animals were treated with citrate buffer (pH-4.5). After 7 days of STZ administration, plasma glucose levels of each rat were determined. Rats with fasting plasma glucose (FPG) range of 280–350 mg/dl were considered diabetic and included in the study. Blood was collected by sinocular puncture.

Fasting Plasma Glucose and plasma insulin levels

Effect of *E. littorale* ethyl acetate active fractions on Fasting Plasma Glucose and plasma insulin levels in STZ-induced diabetic rats was studied for a period of 15 days**.** Rats were divided in to 5 groups of 6 rats each.

Experimental design

Group 1-Normal rats treated with vehicle alone (Dimethylsulfoxide [DMSO] 0.5%; 1ml/kg b.w)
Group 2- Normal rats + ethyl acetate active fraction (200 mg/kg b.w)
Group 3- STZ induced diabetic rats treated with vehicle alone
Group 4- STZ induced diabetic rats + *Ethyl* active fraction (200mg/kg b.w)
Group 5- STZ induced diabetic rats + Glibenclamide (600 µg/kg b.w)

Results

Fasting Glucose Test

From the data recorded in Table 1, it is understood that on Day one, the animals belonging to Group IV (200 mg/ kg. b. w) expressed high levels of fasting glucose (442.5). For the same group of animals the glucose level on the 45th day was very much reduced (128.6). In comparison with the commercial drug, Glibenclamide (600µg/kg b.w) given to the Group V (121.5), the values of the Group IV were very close, suggesting that the plant drug was highly effective in reducing the fasting glucose.

The cholesterol in the serum of untreated diabetic rats in Group III was elevated (91.35) in comparison with the control group of animals (78.49). Similar trend was noticed for the levels of free fatty acid too. On the contrary Triglycerides came down drastically in Group III. But in the treated group of animals in Group IV and Group V the lipid profile data came closer to that of the control group of animals in Group I.

Assessment of Serum HDL, LDL and VLDL levels in control and STZ- diabetic rats (Table 3): In this experiment the effect of *Enicostemma littorale ethyl acetate fractionI* (200mg/kg b.w) *on* Serum HDL, LDL and VLDL levels in control and STZ-diabetic rats was studied. In comparison with the value as shown in Table:3, the untreated diabetic rats in Group III showed lower levels of Serum HDL and higher levels of LDL and VLDL. In Group V, it was observed that the animals treated with Glibenclamide, the commercial drug showed similar values as that of the control group of animals belonging to Group I. From the results obtained for Group IV, it is evident that the effect of plant drug normalized the levels of Serum HDL, LDL and VLDL.

Discussion

Phytochemical screening of EAAF of *E.littorale* leaves revealed the presence of phenolics, flavanoids, tannina, terpenoids and saponins. *E.littorale* leaves is having much importance in diabetic control and hypolipidemic activity, since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, phenols, saponins, tannins and anthocyanins. Several authors reported that flavonoids, steroids, terpenoids and phenolic acids are known to be bioactive anti diabetic principle[8]. Diabetes mellitus is a group of metabolic diseases characterized by hypoglycemia resulting from defects in insulin secretion, insulin action or both in conventional therapy type 1 Diabetes is treated with exogenous insulin and Type II with oral hypoglycemic agent[9]. The single dosed study of EAAF of *E.littorale* doses at 200 mg/kg shows no significant hypoglycemic effect in normal rats but *E.littorale* extract 200 mg/kg significantly decreased blood in STZ induced diabetic rats. In glucose tolerance test the ethyl acetate active fraction of *E. littorale* 200/kg decreased blood glucose level in normal and STZ induced diabetic rats. Hence the extract showed glucose utilization and improves tolerance in glucose loaded rats. The experiment showed that glucose tolerance test (GTT) measures the body ability to use glucose, the body's main source of energy[10] this test can be used to diagnose pre diabetic and diabetes. Glucose lowering effects were found after oral administration of EAAF of *E. littorale* in rats. This may be due to the presence of hypoglycemic flavonoids saponins. The anti hyperglycemic effect of *E. litttorale* may result from potentiation of insulin from existing β-cells of the islets of langerhans. The blood glucose lowering effect was compared with Glibenclamide have been used for many years to treat diabetes and stimulates insulin secretion from pancreatic β-cells[11].

Total cholesterol in the plasma was estimated by the enzymic method described by[12]. Triacylglycerol in the plasma was estimated using the diagnostic kit based on the enzymic method described by Mc Gowan *et al.* (1983)[13]. Free fatty acids in the plasma was estimated by the method of Falholt *et al.*(1973)[14]. Free fatty acids were extracted with chloroform-heptane-methanol mixture to eliminate interference from phospholipids and the extract was shaken with a high density copper reagent at pH 8.1. The copper soaps remained in the upper organic layer from which an aliquot was removed and copper content determined colourimetrically by treating with diphenyl carbazide.

HDL-cholesterol was estimated using the diagnostic kit based on the enzymic method described by[15]. The VLDL and LDL fractions of plasma samples were precipitated using phosphotungstic acid and then HDL in the supernatant was separated by centrifugation and measured for its cholesterol content. Estimation of VLDL- and LDL-cholesterol these were calculated using the formula[16] VLDL cholesterol = TG/5; LDL cholesterol = Total cholesterol – (HDL cholesterol + VLDL cholesterol); The values were expressed as mg/dL of plasma.

Conclusion

It is concluded that the administration of EAAF of *E.littorale* leaves promotes glucose tolerance. The ethyl acetate fraction of *E.littorale* at the dosage of 200mg/kg b. w. exhibited significant hypoglycemic activity and the same dosage of the extract also

showed considerable amount of hypolipidmic activity. Characterization of the isolated active principle must be taken up for future research.

Table No. 01: Effect of *E.littorale* ethyl acetate active fraction on fasting blood glucose in normal and STZ-diabetic rats.

	Group I	Group II	Group III	Group IV	Group V
Day 1	80±6.05	83.6±3.90[a]	403.5±5.79	442.5±6.99[b]	438.5±4.71[b]
Day 15	81±6.13	82.16±2.91[a]	400.3±7.3	322.5±6.9[b]	268.16±7.42:
Day 30	81.5±5.79	80.5±6.8[a]	379.8±5.39	147.3±7.11[a]	131.5±7.95[a]
Day 45	81.83±8.27	82.6±4.81[a]	356±7.23	128.6±4.71[a]	121.5±6.02[a]

Each value is mean ± SD for 6 rats in each group .a: $p<0.05$ by comparison with normal rats.
b: $p<0.05$ by comparison with Streptozotocin diabetic rats.- : No significance

Table No. 02: Effect of of *E.littorale* ethyl acetate active fraction on serum HDL, LDL and VLDL (mg/dl) levels in control and STZ-diabetic rats.

	Group I	Group II	Group III	Group IV	Group V
Total cholesterol	78.49±6.83[b]	74.75±7.51b	91.35±7.74	62±6.88 [b]	67.4±8.39 [b]
Triglycerides	112±7.23	110.66±7.63b	45±5.01	110.83±6.64b	112± 7.23b
Free fatty acids	70.3±4.22	75.83±7.40 b	123.6±7.04	92.5±5.64 b	81.6±7.43 b

Each value is mean ± SD for 6 rats in each group .a: $p<0.05$ by comparison with normal rats.
b: $p<0.05$ by comparison with streptozotocin diabetic rats.- : No significance

Table No. 03: Effect of of *E.littorale* ethyl acetate active fraction on serum HDL, LDL and VLDL ((mg/dl) levels in control and STZ-diabetic rats.

	Group I	Group II	Group III	Group IV	Group V
HDL	56.16±2.99	43±3.88 [a]	30.5±1.87	44.6±4 [a]	49.3±3.82 [a]
LDL-Cholesterol	26.16±4.25	24.5±3.94 [a]	74.3±4.67	32.5±6.6 [:]	28.6±6.18 [a]
VLDL-Cholesterol	20.6±3.85	19.16±2.26 [a]	42.8±6.22	23.83±5.78 [a]	22.6±4.53 [a]

Each value is mean ± SD for 6 rats in each group .a: $p<0.05$ by comparison with normal rats.
b: $p<0.05$ by comparison with streptozotocin diabetic rats.- : No significance

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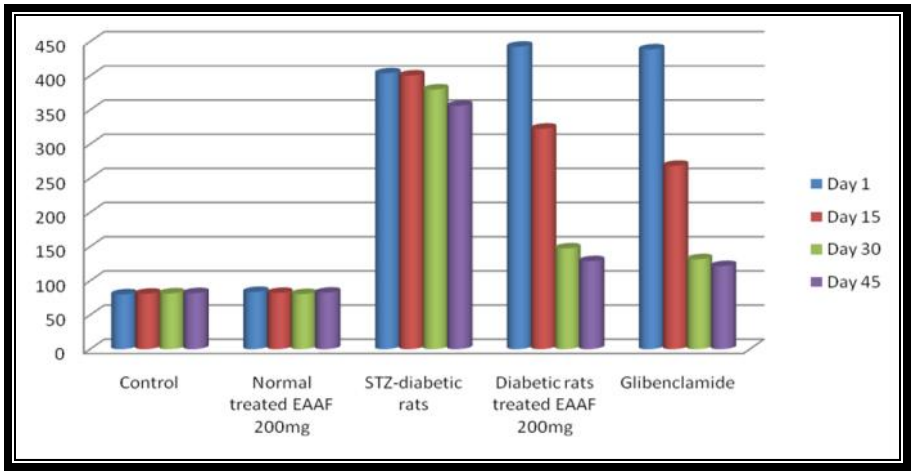

Fig. No. 01: Effect of *E.littorale* ethyl acetate active fraction on fasting blood glucose in normal and STZ-diabetic rats.

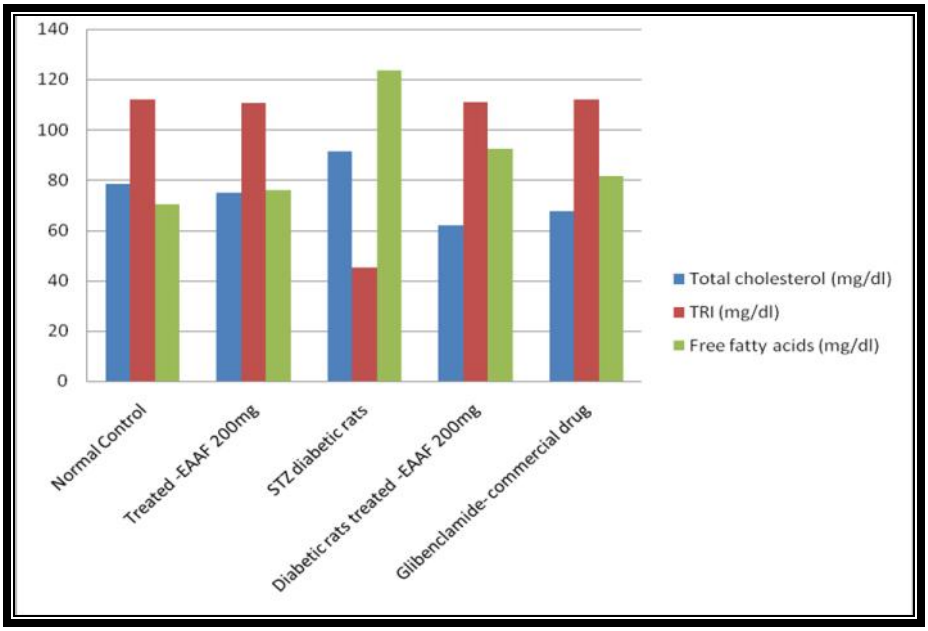

Fig. No. 02: Effect of *E.littorale* ethyl acetate active fraction on Lipid profile in serum of normal and STZ-diabetic rats.