



Antidiabetic Activity Of *Enicostemma Littorale* (Mamajjaka) Leaf Extracts In Streptozotocin-Induced Diabetic Rats

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The antidiabetic activity of *Enicostemma littorale* (Mamajjaka) leaf extracts was investigated in Streptozotocin-induced diabetic albino rats. A comparison was made between the action of different extracts of *Enicostemma littorale* (Mamajjaka) and known antidiabetic drug glibenclamide (600 µg/kg b. wt.). An oral glucose tolerance test (OGTT) was also performed in experimental diabetic rats. The petroleum ether, chloroform, alcohol and aqueous extract of *E. littorale* were obtained by simple maceration method and were subjected to standardization using pharmacognostical and phytochemical screening methods. Dose selection was made on the basis of acute oral toxicity study (50-5000 mg/kg b.wt.) as per OECD guidelines. *E. littorale* aqueous extract (ELAE) and ethonolic extract (ELEE) showed significant antidiabetic activity. In Streptozotocin -induced model, blood glucose levels of these extracts on 7th day of the study were 132.00±4.955 mg/dl (ELAE) and 163.3±28.69 mg/dl (ELEE) in comparison of chloroform extract (210.8±14.91 mg/dl). In glucose loaded rats, ELAE exhibited glucose level of 176±3.724 mg/dl after 30 min. and 110.33±6.687 mg/dl after 90 min, whereas the levels in ELEE treated animals were 166.66±3.403 mg/dl after 30 min. and 148.83±4.615 mg/dl after 90 min. These extract also prevented body weight loss in diabetic rats. The drug has the potential to act as an antidiabetic drug.

Key words: Antidiabetic activity, acute toxicity, Inj. Streptozotocin, *Enicostemma littorale*, Mamajjaka.

Introduction:

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances in carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin. Besides hyperglycemia, several other factors including dyslipidemia or hyperlipidemia are involved in the development of micro and macrovascular complications of diabetes, which are the major causes of morbidity and death.^[1] It is still in top ten list of diseases causing death. Now India is having the largest number of diabetics in the world and gets the name 'Diabetic capital'.^[2]

According to WHO (World Health Organization) projections, the prevalence of diabetes is likely to increase 35% by 2020. Currently, there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetics in the world.^[3] Reasons for this include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span, etc.

Evaluation of plant product products to treat diabetes mellitus is of growing interest as they contain many bioactive substances with therapeutic potential. In recent years, several authors have reported the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals.^[4-9]

Although a large number of medicinal plants have been already tested for their antidiabetic effect, several other Indian medicinal plants remain to be investigated.^[10] *Enicostemma littorale* (Mamajjaka) [Family: Gentianacea] is found throughout India up to height of 1500 feet. This is a perennial herb attaining height of 5-20 inches. It produces yellow or white coloured flowers, which are arranged in clusters.^[11]

This herb is popularly known as 'Mamajjaka' in Sanskrit, 'Nahi' in Hindi and 'Kadu nai' in Marathi. The leaves of *E. littorale* have been use in Ayurvedic medicine as anthelmintic, antipyretic and antidiabetic.^[12]

E. littorale is rich in bitter principle (swertimarine), two alkaloids (one gentianine and other's name not confirmed), ophelic acid and tannins.^[11]

The rationale behind using Mamajjaka (*E. littorale*) in prameha(diabetes) is due to its tikta rasa, katu vipaka, ushna and deepana and pachana properties.^[12] To the best of our knowledge, some scientific data regarding the antidiabetic effect of *E. littorale* are available except in treatise of Ayurvedic medicine. Thus the present study was undertaken to evaluate the antidiabetic effect of *E. littorale* leaves in Streptozotocin - induced diabetic rats.

Material And Methods:

Animals:-

Wistar stain albino rats of either sex weighing between 100-150 gm obtained from animal suppliers were used in the study. They were housed in the animal house of N.I.A., Jaipur. The control and experimental animals were fed with specific rat feed and tap water was given. Also they were maintained under suitable climate conditions such as light and temperature. All the animal experiments were conducted according to the ethical norms approved by CPCSEA (committee for the purpose of control and supervision on experiments on animals).

Chemicals:

Following is the list chemical used.

A] **Inj. Streptozotocin** (Sigma Life Science, United States.).

An injection of Streptozotocin is more uniformly effective and widely used method for induction of Diabetes in animals. Streptozotocin (STZ; N-nitro derivative of glucosamine) which is also called as Izostazin or Zanosar (STZ) is a synthetic anti-neoplastic agent that is classifiably an anti-tumour antibiotic and chemically is related to other nitrosureas used in chemotherapy. It is particularly toxic to the pancreatic and insulin producing beta cell in mammals.^[13,14]

Induction of experimental diabetes in the rat using Streptozotocin is very convenient and simple to use.

Streptozotocin injection leads to the degeneration of the Langerhans islets beta cells.

Clinically, symptoms of diabetes are clearly seen in rats within 2-4 days following single intravenous (IV) or intraperitoneal (IP) injection of 45mg/kg STZ.

B] **Glibenclamida** (Aventis Pharma Ltd., verna, Goa),

C] Sodium citrate buffer solution (Jaipur Labs, India).

Accu-chek® Active Glucometer, Roche Diagnostic Corporation, Germany. Blood gluco-strips (Roche Diagnostic Pvt. Ltd., Mumbai). All other chemicals and reagents used were of analytical grade.

Plant Material:

Leaves of *E. littorale* were collected in and around the local forest area of Nanded district, Maharashtra, and authenticated by the chemist-cum-pharmacist Prof. U.K. Halade, Head, Drug testing laboratory, Govt. Ayurvedic & Unani Pharmacy, Govt. Ayurved college, Nanded.

The collected leaves were dried under shade at room temperature (25⁰C) for 7 days and powdered to a coarse consistency in a grinder mill. The powder was passed through 40# mesh particle size and stored in an airtight container at room temperature.

Preparation of plant extract:

2.5 kg of the fresh, air-dried, powder crude drug of *E. littorale* was extracted with petroleum ether (60-80⁰C), chloroform, 95% ethanol and chloroform water (i.p.) by adopting simple maceration procedure at room temperature for 7 days in a conical flask with occasional shaking and stirring. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites.^[15]

The yield of the extracts was 1.79%, 4.63%, 20.86% and 18.54% w/w for petroleum ether, chloroform, ethanol and water, respectively. All the extracts were preserved in refrigerator till further use. Preliminary phytochemical analysis was carried out in all four extract by different methods of phytochemical analysis.^[16]

A known volume of extract was suspended in distilled water and was orally administered to the animals by gastric intubation using a gavage during the experimental period.

Acute oral toxicity studies:

The acute oral toxicity studies^[17] of extracts were carried out as per the OECD guidelines.

Administration of the stepwise doses of all four extract of *E. littorale* from 50 mg/kg b.wt. up to a dose of 5000 mg/kg b.wt. caused no considerable signs of toxicity in the tested animals. One-tenth of the upper limit dose was selected as the level for examination of antidiabetic activity.

Experimental models:

The preparation of an experimental model consists of two vital steps:

Oral glucose tolerance test^[18]

Fasted rats were divided into six groups of six rats in each. Group-I served as normal control and received distilled water with Tween 80. Group-II received standard drug Glibenclamide as an aqueous suspension at a dose of 600 µg/kg b.wt. Group-III to VI received the different extract at a dose of 500 mg/kg b.wt. as a fine Tween 80 suspension. After 30 min. of extract administration, the rats of all groups were orally treated with 2 g/kg of glucose. Blood samples were collected from the rat tail vein just prior to glucose administration and at 30, 60 and 90 min after glucose loading. Blood glucose levels were measured immediately by using Glucometer.

Streptozotocin-induced diabetic model^[19,20]

Solution of Inj. streptozotocine was prepared with buffer solution as per the routine procedure. The dose of Inj. Streptozotocin according to literature regarding animal experiments is 45 mg/kg. The solution was prepared by addition of 5 ml of buffer solution in 45 mg of Inj. Streptozotocin. So, 0.5 ml of solution will be having 4.5 mg of Inj. Streptozotocin. Dose was calculated according to the weight of each rat. Insulin syringe was used for an induction of Inj. Streptozotocin. An injection was given intraperitoneally (IP).

Blood sugar estimation was done with the help of Glucometer after 24 and 48 hrs of induction. Animals which developed glucose level more than 250 mg/dl were selected for curative study, the part of an experimental trial. The feed of the rats was noted at weekly interval along with their weights. The diabetic rats were separated and divided into six different groups for experimental study, with each group containing six animals. Group-I were left untreated and *severed* as controls which receive vehical alone. Group-II received Glibenclamide 600 µg/kg, Group-III rats were treated with aqueous extract of *E. littorale* (ELAE) at dose of 500 mg/kg b.wt. Group-IV received ethanolic extract (ELEE) of *E. littorale* at a dose of 500 mg/kg b.wt., Group-V rats were treated with chloroform extract of *E. littorale* (ELCE) 500 mg/kg b. wt. and Group-VI diabetic rats were treated with petroleum ether extract of *E. littorale* (ELPEE) 500 mg/kg b. wt. for 7 days.

Other parameters:

Body weight, urine sugar and lipid profile^[21] of diabetic rats were measured during the course of study period [i.e., before inj. Streptozotocin induction (initial value), on the 1st and 7th days of the treatment period]. After the 7th day of treatment blood was collected retro-orbitally using capillary tubes in fresh vials containing sodium fluoride and sodium oxalate as anticoagulant agents. The serum was separated by using centrifuge at 2000 rpm for 2 min. Total cholesterol (TC), Triglyceride (TG), and high density lipoprotein (HDL) were analyzed using diagnostic kit (Span diagnostic Ltd., Surat, Gujarat, India) using colorimeter. Low density lipoprotein (LDL) and very low density lipoprotein(VLDL) levels were calculated using formula of Friedewald *et al.*^[22] Urine sugar was detected with uristix.

Statistical analysis:

The results of the study were subjected to one-way analysis of variance (ANOVA) followed by *Dunnett's t-test* for multiple comparisons. Value with $P < 0.05$ were considered significant.

Results:

Standardization and phytochemical screening:

Standardization parameters for *E. littorale* leaves were determined and all the parameters were found to be within pharmacopoeial standards limit. Crude powder taken for extraction was of green colour with very bitter taste. Losses on drying, total ash, acid insoluble ash and water soluble ash were 7.74%, 6.18%, 2.34% and 1.07% w/w respectively. Thin layer chromatography of *E. littorale* leaves revealed yellow/ orange spot/ florescence with R_f value 0.54, 0.74, 0.77 and 0.82. Phytochemical screening of all the extract of *E. littorale* showed the presence of various phytochemical constituents like swertimarine, gentianine, ophelic acid and tannins and traces of alkaloids.

Toxicity study:

In acute toxicity study, none of the studied extracts of *E. littorale* leaves showed any significant toxicity sign when observed for the parameters during the first 4hrs and followed by daily observations for 14 day and mortality was also not observed; the drug was found to be safe at the tested dose level of 5000 mg/kg b. wt. One-tenth of this dose level was taken as effective dose. All the extracts were experimented at the same dose of 500 mg/kg b. wt. Since the yield

wass less with petroleum ether and chloroform extract and possibility of active compounds was also lesser in such yield. Reason for this may be simple maceration procedure used for extraction in which the solvent does not penetrate in to the plant cells too well and adequate amount of active compound does not come out in extract; if yield is too less, selecting lesser doses may be ineffective and higher doses may be toxic and noncompliant.

In order to ascertain a scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate experimental design of antidiabetic activity by following glucose tolerance test and Streptozotocin-induced model.

Streptozotocin-induced diabetic model:

As expected in the diabetic control, there was severe hyperglycemia as compared to the normal animals. Compared to the diabetic control, all the four extracts (ELAE, ELEE, ELCE, and ELPEE) lowered the elevated blood glucose level only in sub acute treatment [Table 1]. It was observed that the standard drug Glibenclamide lowered the blood glucose level significantly, bringing it nearly back to normal, whereas ELAE and ELEE significantly ($P<0.01$) decreased fasting blood serum glucose in the diabetic rats on 3rd, 5th and 7th days as compared to initial(0 hr.) blood serum glucose levels. When ELAE and ELEE extract of *E. littorale* were compared for their antidiabetic activity in comparison to active control, particularly Glibenclamide, the result showed that their potential was lesser but significant (** $P<0.01$) than the standard drug at sub acute level.

Oral glucose tolerance test model :

The effect of different extracts on glucose tolerance test in normal rats is shown in Table 2. At 30 min. after glucose administration, the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. The aqueous (ELAE) and ethanolic (ELEE) extracts of the leaves of *E. littorale* exhibited remarkable blood lowering effect at 90 min.

Body weight

In the present study, diabetic rats had lower body weights and high blood glucose level as compared to normal rats. In spite of increased food consumption, loss of body weight due to defect in glucose metabolism and excessive breakdown of tissue protein is a characteristic condition in diabetics. As shown in Table 3, treatment with ELAE and ELEE improved the average body weights of rats, which indicates that control over polyphagia and muscle wasting resulted due to hyperglycemic condition.

Urine sugar and lipid profile:

It was intended to assess the effect of long-term treatment on blood glucose level, urine sugar and associated abnormal lipid profile in Streptozotocin-induced severely diabetic rats. The various parameter of blood lipid profile of severely diabetic rats were estimated before and after 7 days treatment [Table4]. The enhanced levels of TC, LDL cholesterol and TG were brought down significantly ($P<0.001$) after 14 days of treatment. A fall of 70% urine sugar was observed after 14 days of treatment [Table 5]. This may be due to improvement in the glycemetic control mechanisms and insulin secretion from remnant pancreatic β -cell in diabetic rats.

Table 1: Effect of *E.littorale* extracts on blood glucose level of Streptozotocin -induced diabetic rats after subacute treatment.

Group	Treatment ^a	Blood glucose (mg/dl)						
		Basal value (0 hr)	1hr	3hr	5hr	3 rd day	5 th day	7 th day
I	Normal control (vehicle only)	80.00±1.693	80.83±1.721	80.83±1.424	79.83±1.376	81.33±0.988	79.83±0.833	81.16±0.792
II	Glibenclamide(600 µg/kg)	277.3±7.923	206.6±6.280**	174±7.095**	154.8±5.043**	125.3±6.960**	114.3±5.251**	105.6±5.097**
III	Aqueous extract (500 mg ELAE)	266±7.967	253.6±5.383 ^{ns}	239.8±6.030 ^{ns}	223.8±9.181*	206.2±9.860**	132.0±4.955**	132.0±4.955**
IV	Ethanolic extract (500 mg ELEE)	329.5±15.46	290±17.48 ^{ns}	268.1±16.45 ^{ns}	256.5±18.03 ^{ns}	220±23.34**	155.8±11.21**	163.3±28.69**
V	Chloroform extract (500 mg ELCE)	312.8±13.12	281.8±10.54 ^{ns}	272.1±9.548 ^{ns}	260.1±11.46*	237±12.77**	225.5±12.92**	210.8±14.91**
VI	Pet. Ether extract (500 mg ELPEE)	327.8±8.448	299±9.063 ^{ns}	291±9.630 ^{ns}	287.8±9.119*	241.5±12.71**	220±12.33**	155.8±11.21**

^amg/kg/day for 7 days. Values are mean ± SEM; n=6. Values are statistically significant at * $P<0.05$ and more significant at ** $P<0.01$, ns=not significant, ** $P<0.01$ (ANOVA).

Table 2: Effect of *E.littorale* extract on blood glucose level in oral glucose tolerance test in normal rats.

Sample	Blood glucose (mg/dl)		
	0 min (mg/dl)	30 min (mg/dl)	90 min (mg/dl)
Normal control[5% Tween 80+glucose(2g/kg)]	78.83±0.703	167.83±2.301	146.83±2.960

Glibenclamide (600µg/kg)+glucose(2g/kg)	76.50±1.522	171.83±4.214	106.16±4.316
ELAE(500mg/kg)+glucose(2g/kg)	77.50±1.648	176±3.724	110.33±6.687
ELEE(500mg/kg)+glucose(2g/kg)	77.33±2.603	166.66±3.403	148.83±4.615
ELCE(500mg/kg)+glucose(2g/kg)	80.16±2.242	175.16±3.728	160.66±3.393
ELPEE(500mg/kg)+glucose(2g/kg)	77.83±3.978	164.50±6.350	156.50±4.089

One-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM. **P < 0.01 as compared to normal control group

Table 3: Effect of various extract of <i>E.littorale</i> on body weight after treatment in diabetic rats.			
Groups	Treatment(n=6)	Average body weight(g)±SEM	
		Initial value	Day 7
I	Normal control (NC)[Vehical only]	166.33±2.974	182.3±2.525
II	Glibenclamide (600µg/kg) [GLB]	183.5±2.078	205.33±1.65
III	Aqueous extract 500 mg [ELAE]	143.5±1.36	167.8±1.42
IV	Ethanolic extract 500 mg[ELEE]	137.8±3.85	163.3±3.48
V	Chloroform extract 500 mg[ELCE]	147.3±2.616	131.2±3.45
VI	Pet. ether extract 500 mg[ELPEE]	152.3±2.390	170.7±2.376

One-way ANOVA followed by Dunnett's test value. Values are expressed as mean±SEM. **P<0.01 as compared to normal control group; n=no. of animals.

Table 4: Effect of oral administration of the various extracts of <i>E.littorale</i> on serum lipid profile in severe diabetic rats.(mean ± SD)						
Test	Normal	Glibenclamide(600µg/kg)	ELAE	ELEE	ELCE	ELPEE
TC (mg %)	139.25±1.74	144.75±3.09	134.41±6.41	140.50±2.14	142.31±2.24	140.25±5.74
TG (mg %)	86.14±1.84	92.24±2.10	88.24±2.01	94.75±1.48	84.12±3.54	83.52±3.78
HDL (mg %)	43.85±1.86	45.65±2.78	44.98±4.12	41.23±3.78	34.75±1.86	35.31±2.95
LDL (mg %)	72.01±1.54	70.31±2.98	67.32±7.32	83.56±5.64	86.32±2.96	84.14±2.52
VLDL (mg %)	17.32±1.69	16.72±1.95	17.45±6.48	18.13±3.12	20.24±3.10	21.12±3.64

Value are mean ±SEM, ELAE-*E. littorale* aqueous extract, ELEE- *E. littorale* ethanolic extract, ELCE- *E. littorale* chloroform extract, ELPEE- *E. littorale* Pet. Ether extract.

Table 5: Effect of various extracts of <i>E. littorale</i> on urine sugar before and after treatment in diabetic rats			
Group	Treatment (n=6)	Average body weight (g) ±SEM	
		Initial value	Day 7
I	Normal control (NC)[Vehical only]	0	0
II	Glibenclamide (600µg/kg) [GLB]	+4	0
III	Aqueous extract 500 mg [ELAE]	+4	+1
IV	Ethanolic extract 500 mg[ELEE]	+4	+1
V	Chloroform extract 500 mg[ELCE]	+4	+3
VI	Pet. ether extract 500 mg[ELPEE]	+4	+2

DISCUSSION:

Diabetes mellitus, a common heterogeneous metabolic syndrome, is prevalent throughout the world and has been projected to become one of the world's main disablers and killer within the next 25 years. Blood glucose level, urine sugar and body weight have been commonly measured to monitor the glycemetic control mechanism.^[23] In the present study, diabetic rats had lower body weight, high blood and urine sugar levels as compared to normal rats. However, orally administered ELAE and ELEE significantly increased the body weight and decreased the blood glucose level. This could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin. The significant and consistent antidiabetic effect of ELAE and ELEE in Streptozotocin-induced diabetic rats may be due to enhanced glucose utilization by peripheral tissues.

Several authors reported swertiamarine, gentianine alkaloids and Ophelic acid as a bioactive antidiabetic principles. The phytochemical screening of *E. littorale* revealed the presence of various alkaloids, tannins, swertiamarine and

other compounds. Hence the antidiabetic activity of the above mentioned ELAE and ELEE is probably due to the presence of several bioactive antidiabetic principles and their synergistic properties.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart diseases. The marked hyperlipidemia that characterizes the diabetic states may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. Lowering of serum lipid concentration through dietary or drug therapy seems to be associated with a decrease in the risk of vascular diseases. The result of this study reveals that the dose of 500 mg/kg not only lowered TC, TG and LDL, but also enhanced the cardioprotective lipid HDL. The fall of 50% and 75% urine sugar of severely diabetic group after 7 days of treatment with the most effective dose further confirms our finding.

CONCLUSION:

Streptozotocin makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes mellitus in the 2-4 days. The function of the insulin system is suppressed, which leads to high level of hyperglycemia and eventually to death, but ELAE and ELEE showed potent antidiabetic effect in Streptozotocin -induced diabetic rats and reduced the mortality rate significantly. The present investigation has also opened avenues for further research, especially with reference to the different dose studies and development of potent formulation for diabetes mellitus from *E. littorale* leaves. Activity guided fractionation, formulation and its evaluation is in progress and will be available in a short period of time.

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