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RESEARCH ARTICLE

Anti-Diabetic Activity of Ethanolic Extract of *Lactuca Laevigata* (Bl.) Dc. Leaves

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ABSTRACT

Based on the ethnobotanical use, the present study has been carried out to evaluate the anti-diabetic activity of ethanolic extract of *Lactuca laevigata* Blume DC. leaves (EELL) in alloxan and streptozotocin induced diabetic rats. A dose of 2000 mg/kg of EELL was found to be nontoxic in acute toxicity studies. The 100, 200 and 400 mg/kg, p.o. doses of the extract were subjected to evaluate for the anti-diabetic activity against alloxan (100 mg/kg, i.p) and streptozotocin (50 mg/kg, i.p) induced diabetic rats. EELL treated diabetic rats showed significant ($p<0.01$) reduction in blood glucose level and increased biochemical parameters such as cholesterol, triglyceride and liver glycogen level, except for HDL and LDL when compared with diabetic control animals. Histopathological studies of pancreas showed the regeneration of β-cells in extract treated diabetic rats, which support the antidiabetic potentials of the extract by preserving the pancreatic islet cells. The present study revealed that the ethanolic extract of *Lactuca laevigata* Blume leaves was found to be effective against alloxan and streptozotocin induced diabetes and therefore supported the ethnobotanical and traditional belief on antidiabetic effect of the plant *Lactuca laevigata*.

KEYWORDS

Alloxan, Anti-diabetic activity, *Lactuca laevigata*, Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) consists of a group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, and proteins; resulting from defects in insulin secretion, its action, or both. It is a chronic metabolic disorder which affects a significant population worldwide. It is a major cause of morbidity and mortality. Present drugs which are used for the treatment of this disease are mainly insulin, sulphonylureas and biguanides. These drugs are associated with adverse effect and not able to control metabolism adequately. Management of diabetes with agents devoid of any side effects is still a challenge to the medical system.

There is growing interest in herbal remedies due to these reasons.[1] In recent years many hypoglycemic agents have been introduced, still diabetes and its related complications continue to be a major problem not only in the developed countries but also in developing countries. Many Indian medicinal plants are reported to be useful in diabetes.[2,3] Due to the side effects associated with the use of insulin and oral hypoglycemic agents, there is an increased demand to use natural product with anti-diabetic activity.[4,5]

Lactuca laevigata (Bl.) DC belonging to the family Asteraceae is a small herb growing up to 10-90 cm tall, a plant endemic to the tropical

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regions of India. It is commonly known as Khmut Sim and Jhur Kthang in Khasi.

Lactuca laevigata has been used for the treatment of diabetes, high blood pressure and skin infection of the face by the tribal healers of Meghalaya.[6] However, no scientific data are available regarding its usefulness as anti-diabetic agent; hence to ascertain the claim, the present communication deals with the evaluation of anti-diabetic potentials of ethanolic extract of leaves of *Lactuca laevigata* (EELL) in alloxan and streptozotocin induced diabetic rats.

MATERIALS AND METHOD

Collection of Plant Material

Fresh leaves of *Lactuca laevigata* were collected from Khasi and Jaintia Hills Area of Meghalaya. After authentication by botanist, voucher specimens are were deposited in the Pharmacognosy department, Srinivas College of Pharmacy, Valachil, India for future reference. The leaves were shade dried and pulverized separately into coarse powder by a mechanical grinder.

Preparation of Extract

The powdered drug was subjected to extraction by hot percolation method (Soxhlet apparatus) using 95% ethanol as solvent. The extract was evaporated to syrupy consistency and then concentrated using a rotary evaporator at low temperature. The extract was preserved in airtight container and kept at 4-5°C until further use.

Preliminary Phytochemical Screening

Freshly prepared ethanolic extract of leaves of *Lactuca laevigata* was subjected to phytochemical tests for the detection of various constituents.[7]

Experimental Animals

Healthy Wistar albino rats (150–200 g) of either sex were used for the experiment. They were maintained under standard conditions (temperature 27 ± 2ºC, relative humidity 60 ± 5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing paddy husk as bedding. They had free access to standard chow and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol prior to initiation of experiments.

Acute Toxicity Study

Acute toxicity study of ethanolic extract of leaves of *Lactuca laevigata* was determined in Wistar albino rats according to OECD guidelines No. 425.[8] The animals were fasted overnight and the ethanolic extract 2000 mg/kg was administered orally. Animals were observed continuously for first 3 h and monitored for 14 days for mortality and general behavior of animals, signs of discomfort and nervous manifestations.

Anti-Diabetic Activity

Oral Glucose Tolerance Test

The oral glucose tolerance test was performed in overnight fasted normal rats. They were divided into five groups containing six animals each. Group I was given 2% Tween-80 orally and served as normal control. Group II were treated with glibenclamide (10 mg/kg) and group III, IV and V were received *L. laevigata* leaves extract at a dose of 100, 200 and 400 mg/kg respectively. After 30 min, glucose at a dose of 2 g/kg was fed to all groups. Blood was withdrawn from the retro- orbital sinus just prior to the glucose administration and at 30, 60, 120, 180 and 240 min after glucose loading and glucose levels was measured.[9]

Alloxan Induced Diabetes Mellitus

The animals were divided into six groups containing six animals each. Hyperglycemia was induced by single i.p injection of 100 mg/kg of alloxan monohydrate. Fasting blood glucose was determined after depriving food for 16 h with free access to drinking water. Development of hyperglycemia in rats was confirmed by fasting blood glucose estimation 48 h post injection. The rats with fasting blood glucose level above 200 mg/dl were considered diabetic and included in the study.[9,10]

Streptozotocin Induced Diabetes Mellitus

The animals were divided into six groups containing six animals each. Hyperglycemia was induced by single i.p injection of 50 mg/kg of STZ in normal saline. STZ solution was freshly prepared and injected within 5 minute of preparation to prevent degradation. Development of hyperglycemia in rats was confirmed by fasting blood glucose estimation 48 h post injection. The rats with fasting blood glucose level above 200 mg/dl were considered diabetic and included in the study.[11]

Experimental Design for Anti-Diabetic Study

Group I: Normal control (2% Tween 80)

Group II: Diabetic + 2% Tween 80 (diabetic control)

Group III: Diabetic + EELL (100 mg/kg/day p.o)

Group IV: Diabetic + EELL (200 mg/kg/day p.o)

Group V: Diabetic + EELL (400 mg/kg/day p.o)

Group VI: Diabetic + Glibenclamide (10 mg/kg/day p.o) and served as standard.

The treatment (p.o) was started from the same day except diabetic control groups and continued for 28 days. Fasting blood glucose was measured using glucostix with glucometer (Johnson & Johnson). Blood samples were withdrawn under mild anaesthesia from tail tip of the overnight fasted animals on 1st, 7th, 14th, 21st and 28th day.

On 35th day the blood was collected from animals by retro-orbital puncture for biochemical estimations such as LDL, HDL, cholesterol and triglycerides by using a corresponding kit from Agappe Diagnostics Pvt. Ltd. The animals were sacrificed, liver and pancreas was removed for glycogen estimation and histopathological studies respectively.

Histopathological Studies

The animals were sacrificed and the pancreas were removed, washed with ice cold saline and preserved in 10% formalin. Block of tissues were routinely processed and embedded in paraffin. Thin sections were cut using rotary microtome and stained with Haematoxylin and eosin for histopathological studies.

Statistical Analysis

Results of biochemical estimation were reported as mean ± S.E.M. The total variation present in a data was analyzed by one way analysis of variance (ANOVA). $P<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of carbohydrates, glycosides, saponins, tannins and proteins.

There was no mortality and any signs of discomfort amongst the dosed groups of animals suggest that the EELL is relatively safe in or non-toxic to rats and hence 100, 200 and 400 mg/kg, p.o. doses were chosen for the study.

The different doses of EELL significantly ($p<0.01$) suppressed the rise in FBG level after glucose load (2 g/kg) in oral glucose tolerance test groups of animals, at first half an hour and up to 2 h time period as compared with the normal control (Table No.1). It is probably due to its antihyperglycemic effect by retarding the carbohydrate absorption from intestine through the inhibition in α- glucosidase activity.

Ethanolic extract showed significant anti-diabetic activity ($p<0.01$) in both the experimentally induced diabetic models when compared to diabetic control on 7th, 14th, 21st and 28th day. The percentage decrease in the blood glucose was dose dependent (Table No.2 and 3).

The significant antidiabetic effect of extract of the leaves of *L. laeviagata* might be due to the potentiation of serum insulin effect by increasing either the pancreatic secretion of insulin from the existing β-cells or its release from the bound form. The significant increase in the liver glycogen concentration might suggest the increased conversion of glucose into glycogen.[12]

Table 1: Effect of EELL on blood glucose level in OGTT induced normal rats

Groups	Blood glucose level (mg/dl)				
	0 min	30 min	60 min	120 min	240 min
Normal control	71.83±1.74	132.31±3.01	124.17±2.66	117.55±1.17	81.56±2.27
Glibenclamide (10 mg/kg)	72.50±1.47	84.30±1.04**	72.93±1.98**	65.59±1.46***	59.10±1.70***
EELL (100 mg/kg)	70.67±1.68	93.43±1.62*	89.56±1.84**	81.66±1.80**	71.65±5.66**
EELL (200 mg/kg)	76.00±2.20	89.12±2.52 **	82.57±2.94**	77.52±1.07**	69.58±2.94**
EELL (400 mg/kg)	71.67±2.30	87.74±4.70 **	79.45±2.06**	73.67±1.78**	64.35±1.60**

Values are expressed as Mean± S.E.M (n=6). One way ANOVA followed by Dunette's 't' test.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared with normal control group.

Table 2: Effect of EELL on blood glucose level in alloxan induced diabetic rats

Groups	Blood glucose level (mg/dl)				
	Initial	Day 7	Day 14	Day 21	Day 28
Normal control	77.50±1.78	76.17±0.79	79.0±1.065	80.17±2.212	82.33±1.687
Diabetic control	335.2±4.86	360.02±5.33	338.0±5.323	305.3±6.53	256.5±1.500
Glibenclamide (10 mg/kg)	340.2±2.33	178.7±6.20**	139.0±2.89**	131.0±3.48**	95.67±1.520***
EELL (100 mg/kg)	336.5±8.86	252.7±6.24*	200.3±16.29*	152.0±6.65**	130.3±2.591**
EELL (200 mg/kg)	349.8±3.97	208.3±4.59**	169.7±4.02**	144.2±0.73**	113.9±0.881***
EELL (400 mg/kg)	344.7±9.92	183.7±6.55**	153.7±1.52**	132.5±2.25**	106.5±1.501***

Values are expressed as Mean± S.E.M (n=6). One way ANOVA followed by Dunette's 't' test.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to diabetic control.

Table 3: Effect of EELL on blood glucose level in STZ induced diabetic rats

Groups	Blood glucose level (mg/dl)				
	Initial	Day 7	Day 14	Day 21	Day 28
Normal control	82.17±0.945	79.67±1.145	85.50±1.478	88.67±1.926	83.83±0.654
Diabetic control	344.7±14.08	345.0±14.43	377.7±17.87	368.2±6.53	295.2±1.22
Glibenclamide (10 mg/kg)	349.7±6.433	233.7±15.76**	183.3±12.33**	138.2±5.44***	107.7±8.05***
EELL (100 mg/kg)	350.5±6.147	300.0±5.266*	219.3±2.43**	164.3±2.69**	135.7±4.95**
EELL (200 mg/kg)	348.0±11.72	290.3±8.488**	226.0±9.38**	165.0±3.27**	128.5±4.39**
EELL (400 mg/kg)	353.7±7.027	278.0±6.491**	187.0±3.28**	137.3±3.50**	116.5±2.11***

Values are expressed as Mean± S.E.M (n=6). One way ANOVA followed by Dunette's 't' test.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to diabetic control.

Insulin deficiency leads to various metabolic aberrations in the rats; the rise in blood glucose level is accompanied by increase cholesterol, triglyceride, ALP, transaminases and urea level. In alloxan-induced diabetic rats, the rise in blood glucose level is accompanied by an increase in the serum cholesterol and triglyceride level. The level of glycemic control is the major determinant of serum triglyceride level. Near normalization of blood glucose results in reduction of serum triglyceride level. In the present study, EELL treated groups of animals showed significant ($p<0.01$) reduction in cholesterol level whereas there was increase in triglyceride level. HDL and LDL levels were decreased compared to that of diabetic control group (Table No. 4 and 5).

Histopathological studies of pancreas also supported our findings. Photomicrographs showed normal acini, and normal cellular population in the islet of langerhams in pancreas of normal control rats. The islets were extensively damaged in the diabetic control group. Glibenclamide treated group restored the normal cellular size of islets. EELL treated group showed possible restoration of the cells islet of langerhams to normal texture and cells were partly preserved. The presence of glycosides, saponins, tannins and other polyphenolic compounds in EELL may attribute the antidiabetic potential of the extract. However, saponins, tannins and other polyphenolic compounds are known to possess antidiabetic activity in animals.[13]

Table 4: Effect of EELL on liver glycogen, serum cholesterol, triglycerides, HDL, LDL and body weight in alloxan induced diabetic rats

Groups	Liver glycogen (mg/gm) wet tissue	Blood				Body weight (g)	
		Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL mg/dl)	LDL (mg/dl)	Day 0	Day 28
Normal control	45.40± 1.208	49.33± 0.843	63.15± 1.876	14.17± 1.302	17.0± 0.577	180.5± 3.215	184.4± 4.055
Diabetic control	12.19± 2.075	67.50± 2.102	57.83± 0.477	16.67± 0.843	35.24± 0.321	183.3± 6.146	143.3± 5.578
Glibenclamide (10 mg/kg)	42.67± 0.653**	47.83± 0.945**	45.33± 1.626**	15.33± 1.406^{ns}	23.88± 1.107**	181.7± 4.773	196.7± 8.433**
EELL (100 mg/kg)	29.36± 0.567**	58.00± 0.816**	87.83± 0.403**	12.17± 0.872*	20.98± 0.919**	193.3± 5.578	175.0± 4.282*
EELL (200 mg/kg)	32.34± 0.824**	54.00± 0.856**	102.7± 0.501**	16.33± 0.614^{ns}	21.28± 0.846**	186.7± 5.578	163.3± 7.601^{ns}
EELL (400 mg/kg)	38.48± 0.379**	51.17± 0.477**	71.04± 0.307**	15.17± 1.014^{ns}	23.21± 1.652**	185.0± 5.627	140.0± 3.651^{ns}

Values are expressed as Mean± S.E.M (n=6). One way ANOVA followed by Dunette's test.
* $p<0.05$, ** $p<0.01$, ns $p>0.05$ when compared with diabetic control group

Table 5: Effect of LLE on liver glycogen, serum cholesterol, triglycerides, HDL, LDL and body weight in STZ induced diabetic rats

Groups	Liver glycogen (mg/gm) wet tissue	Blood				Body weight (g)	
		Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Day 0	Day 28
Normal control	48.20± 0.374	47.17± 0.477	67.57± 0.551	14.50± 0.428	18.52± 0.295	175.5±3 .402	181.85± 4.315
Diabetic control	4.85± 0.737	69.90± 1.122	43.29± 0.705	18.05± 0.365	38.40± 0.428	185.0±4 .482	136.7± 3.333
Glibenclamide (10 mg/kg)	43.0± 1.487**	40.20± 0.969**	47.72± 0.556^{ns}	10.67± 0.667**	25.06± 0.770**	186.7±6 .146	170.0± 2.236**
EELL (100 mg/kg)	62.50± 2.406**	52.50± 2.723**	63.05± 0.616**	13.0± 0.365**	28.67± 1.667**	191.7±6 .009	163.3± 2.278**
EELL (200 mg/kg)	66.33± 4.318**	54.67± 1.430**	80.47± 2.033**	17.17± 0.477^{ns}	22.43± 1.445**	190.0±5 .774	156.7± 2.472**
EELL (400 mg/kg)	78.00± .214**	49.83± 2.868**	101.1± 2.325**	16.67± 0.653^{ns}	21.04± 0.566**	186.7±7 .601	158.3± 2.108**

Values are expressed as Mean± S.E.M (n=6). One way ANOVA followed by Dunette's test.
* p<0.05, ** p<0.01, ns p>0.05 when compared with diabetic control group.

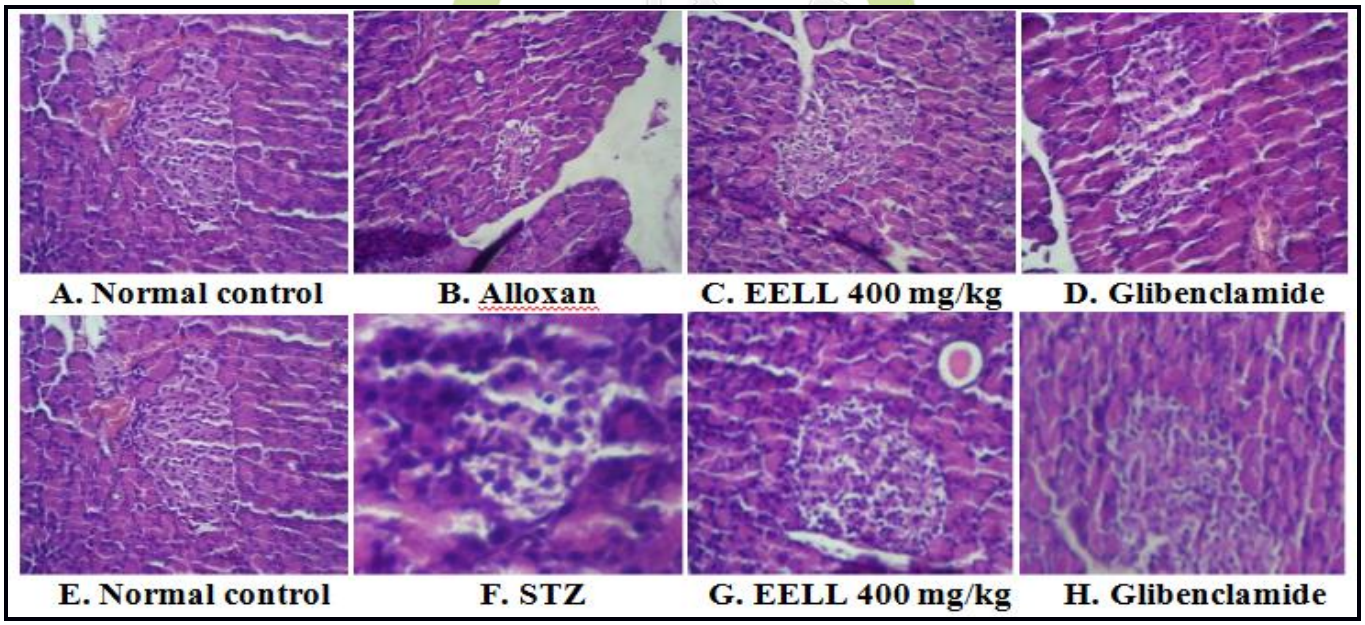

Figure 1: Histopathology studies of pancreas in both alloxan and STZ induced diabetic rats

Normal control (A & E): It showed normal acini and normal cellular population in the islets of langerhans of pancreas. **Diabetic control (B & F):** It suggests extensive damage to the islets of langerhans and reduced dimensions of islets in both alloxan and STZ model respectively. **EELL 400 mg/kg (C & G):** It suggests a possible restoration of repairs of the cells of islet of langerhans, cells are partly preserved in both alloxan and STZ model respectively. **Glibenclamide (D & H):** It suggests restoration of normal cellular size of islets in both alloxan and STZ model respectively.

CONCLUSION

The above experimental data suggest that the ethanolic extract of *Lactuca laevigata* leaves possessed a significant anti-diabetic property as it significantly reduced the fasting blood glucose level in alloxan and STZ induced diabetic rats as compared to diabetic control group. The antidiabetic activity might be probably due to the presence of polyphenolic phytoconstituents present in the extract. Further studies are required to determine the exact mechanism of action and to isolate and characterize the bioactive principles responsible for the claimed activity.

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REFERENCES

1. Pradeepa, R., & Mohan, V. (2002). The changing scenario of the diabetes epidemic: implication for India. *Indian Journal of Medical Research*, 116, 121-132.
2. Kirtikar, K. R., & Basu, B. D. (1995). *Indian Medicinal Plants*, Vol. 1, Dehradun: International Book Distributors, 371-372.
3. Nadkarni, K. M., & Nadkarni, A. K. (1996). *Indian Materia Medica*, Vol. 1, Bombay: Popular Prakashan, 615-616.
4. Holman, R. R., Turner, R. C., Blackwell, Williams, G., & Pickup, S. Oxford. (1997). *Oral agents and insulin in the treatment of NIDDM*. Textbook of Diabetes, 467-469.
5. Rao, K. B., Geri, R., Kesavalu, M. M., & Rao, C. A. (1997). Herbal medicine in the management of diabetes mellitus. *Manphar Vaidhya Patrika*, 1, 33-35.
6. Hynniewta, S. R., & Yogendra, K. (2008). Herbal remedies among the Khasi traditional healers and village folks in Meghalaya. *Indian Journal of Traditional Knowledge*, 7(4), 581-586.
7. Harbone, J. B. (1984). *Phytochemical methods. A Guide to Modern Techniques of Plant Analysis*. 2nd Ed, London: Chapman and Hall, 84-274.
8. OECD. (2001). *Guidelines for testing of chemicals, Acute oral toxicity, Environmental Health and Safety Monograph Series on Testing and Adjustment No*. 425, 1.
9. Ragavan, B., & Krishnakumari, S. (2006). Antidiabetic effect of *Terminalia arjuna* bark extract in alloxan induced diabetic rats. *Indian Journal of Clinical Biochemistry*, 21(2), 123-128.
10. Somani, R. S., & Singhai, A. K. (2008). Hypoglycaemic and anti-diabetic activity of seeds of *Myristica fragrans* in normoglycaemic and alloxan- induced diabetic rats. *Asian Journal of Experimental Science*, 22(1), 95-102.
11. Gangadevi, T., Babu, V., & Subramoniam, A. (2003). Antidiabetic activity of ethanol extract of *Cassia kleinii* leaf in streptozotocin-induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. *Indian Journal of Pharmacology*, 35, 290-296.
12. Gandy, S. E., Buse, M. G., & Crouch, R. K. (1982). Protective role of superoxidase dismutase against diabetogenic drugs. *The Journal of Clinical Investigation*, 70, 650-658.
13. Sabu, M. S., Smitha, K., & Ramadasan, K. (2002). Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *Journal of Ethnopharmacology*, 83, 109-116.

