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ANTIDIABETIC EFFECT OF METHANOLIC EXTRACT OF WHOLE PLANT OF *LINDERNIA CILIATA* (COLSM.) PENNELL. ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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Anti-diabetic
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ABSTRACT: During the past few years many phytochemicals responsible for anti-diabetic effects have been isolated from the plants. Several phyto-constituents such as alkaloids, glycosides, flavonoids, saponins, dietary fibers, polysaccharides, glycolipids, peptidoglycans, amino acids and others obtained from various plant sources that have been reported as potent hypoglycemic agents. In the present study, the effect of methanolic extract of whole plant of *Lindernia ciliata* (LCME) (100, 200 and 400 mg/kg) on glucose levels in streptozotocin (STZ) (45 mg/kg) induced diabetic rats was investigated using glibenclamide 10 mg/kg as the positive control. The effect of the extract on body weight, insulin, total cholesterol, triglycerides, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total protein, serum urea, creatinine and hematological indices in normal, treated and untreated diabetic rats was investigated. Induction of diabetes in male Wistar rats (200-250 g) resulted in increased levels of serum glucose, total cholesterol, triglycerides, SGOT, SGPT, ALP, urea and creatinine and decreased bodyweight, serum insulin, total protein, RBC, WBC and hemoglobin levels. Administration of the extract at three test doses resulted in significant reduction in the levels of serum glucose, total cholesterol, triglycerides, SGOT, SGPT, ALP, urea and creatinine and significant increase in the body weight, insulin, total protein, RBC, WBC and haemoglobin levels which were observed in STZ-induced diabetic rats after 21 days of the treatment with the extract. At 200 mg/kg, LCME showed significant ($p < 0.01$) reduction in plasma glucose levels compared to glibenclamide (10 mg/kg). The results indicate that methanolic extract of whole plant of *Lindernia ciliata* possesses significant antidiabetic activity.

INTRODUCTION: Diabetes is a metabolic disorder characterized by chronic hyperglycemia and alterations in carbohydrate, protein and lipid metabolism with absolute or relative deficiencies in insulin secretion and/or insulin production ¹.

According to the current status of diabetes, the number of diabetic patients is projected to increase from 422 million in 2014 to 642 million by 2040.

Among the people with diabetes, about 15% have type 1 (known as insulin-dependent diabetes); while about 85% have type 2 diabetes (known as non-insulin dependent diabetes) ². The increasing prevalence and diabetes (known as non-insulin diabetes) ². The increasing prevalence and associated complications threaten to reverse economic gains in developing countries.

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Currently available pharmacotherapies for the treatment of diabetes mellitus include oral hypoglycemic agents and insulin. However these current drugs do not restore normal glucose homeostasis and they are not free from side effects³. In view of the adverse effects associated with synthetic drugs and as plants are safer, cheaper and much effective, conventional antidiabetic plants can be explored⁴. Over 400 traditional plants have been reported for the treatment of diabetes⁵. Furthermore, after World Health Organization recommended, investigation of hypoglycemic agents from medicinal plants has become more important⁶. Also, diabetes has been treated orally with several medicinal plants or their extracts based on folklore medicine since ancient times.

The plant *Lindernia ciliata* (Colsm.) Pennell. belongs to the family Scrophulariaceae is a low growing, stoloniferous, mat-forming, annual, herb from 0.13 - 0.20 m high. In India it was found as an insignificant weed, mainly in rice fields. Traditionally it is used as a bitter drug and remedy for gonorrhoea, jaundice, urinary disturbances, bronchitis, headache, liver complaints, spleen diseases, constipation, fever, loss of appetite, asthma, cough, skin diseases⁷.

Preliminary phytochemical screening of methanolic extract of *Lindernia ciliata* revealed the presence of saponins, steroidal/triterpenoids and flavonoids compounds and their glycosides and phenolic compounds⁸. Petroleum ether extract of whole plant of *Lindernia ciliata* contains beta-sitosterol, stigmasterol and lup-20(29)-en-3-beta-ol⁹. There are no scientific reports are available on antidiabetic activity of *Lindernia ciliata*. Phytoconstituents such as alkaloids, glycosides, flavonoids, saponins, terpenoids dietary fibers, polysaccharides, glycolipids, peptidoglycans, amino acids and others obtained from various plant sources that have been reported as potent hypoglycemic agents¹⁰.

Based on the presence of phytoconstituents and traditional use as a bitter drug, the present study was undertaken to investigate the antidiabetic effect of methanolic extract of the whole plant of *Lindernia ciliata* and its effects on some biochemical and hematological parameters in streptozotocin induced diabetic rats.

MATERIALS AND METHODS:

Chemicals: Streptozotocin (STZ) was purchased from Sisco Research Laboratories Pvt. Ltd., Hyderabad, Telangana, India. Glibenclamide was purchased from Sigma-Aldrich Company, Germany. Biochemical analytical kits were purchased from Merk Specialties Pvt. Ltd., Mumbai, India. All other chemicals and solvents used were of analytical grade.

Plant Material and Extract: The plant *Lindernia ciliata* was collected in the month of August 2012, from rice fields of Bhayyaram, Telangana state, India, after the authentication of the plant by Prof. V. S. Raju, Department of Botany, Kakatiya University, Warangal. A voucher specimen of the plant (KU/UCPSC/50) is being maintained in the herbarium of the Department of Pharmacognosy and Phytochemistry, University College of Pharmaceutical Sciences, Kakatiya University, Warangal. The whole plant was air-dried, coarsely powdered and macerated with methanol in a round bottom flask for 7 days with intermittent stirring and filtered after seven days and concentrated under reduced pressure to yield a green semisolid mass. It was given as a code LCME.

Animals: Wistar male albino rats weighing 200-250 g were purchased from Mahaveer agencies, Gatkesar, Hyderabad, India. The animals were housed in the propylene cages and maintained at 27 ± 2 °C with an alternatively 12 h light and 12 dark cycles. Animals were provided with a standard rat pellet diet. They had free access to food and water *ad libitum*. All the experiments on animals were conducted after obtaining permission from Animal Ethical Committee of the institute. (1820/GO/Re/S/15/CPCSEA Date: 01-09-2015).

Acute toxicity study was carried out for the methanolic extract of *Lindernia ciliata* according to the Organization for Economic Co-operation and Development (OECD) 420 guidelines (OECD, 2001). All animals were observed for toxic symptoms and mortality for 72 h.

Effect of LCME on Hypoglycemic Activity in Euglycemic Rats: A total of 30 normal rats fasted for 18 h and were divided into '5' equal groups [I-V] (n=6). Group I served as 5% gum acacia (control group), group II received glibenclamide

10mg/kg b.w (standard group) group III, IV and V received LCME orally at the dose of 100, 200 and 400 mg/kg. Blood glucose levels were determined '0' (Initial fasting blood sample) and 2, 4, 6, 8, 12 and 24 h after the treatment. The samples were analyzed on an auto-analyzer for blood glucose content using glucose oxidase peroxidase method¹¹.

Oral Glucose Tolerance Test (OGTT): Glucose tolerance test is used to determine the rate at which glucose is cleared from the blood after the administration of a massive dose of glucose. Overnight fasted rats were divided into five groups (I-V) of each consisting of six rats and their fasting blood glucose levels were recorded. Group I served as control, received vehicle (5% gum acacia). Group II received a reference drug, glibenclamide (10 mg/kg b.w). Whereas other Groups III, IV and V received a plant extract LCME (100, 200 and 400 mg/kg b.w.) respectively. Then, 30 min after the administration of test samples, the rats of all the groups were loaded with glucose 2 g/kg b.w. and the glucose levels were determined at 30, 60, 90 and 120 min after glucose load. Blood was collected from the tip of the tail vein and fasting blood glucose level was measured using one-touch select simple glucometer (Johnson & Johnson Pvt. Ltd, Mumbai).

Induction of Diabetes: The STZ was dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5) immediately before use and was administered by intraperitoneal route at the dose of 45 mg/kg body weight for each rat and their blood glucose levels were checked after 72 h. The rats whose blood glucose levels were more than 250 mg/dl were considered diabetic.

Sub Acute Treatment: The diabetic rats (glucose >180 mg/dl) were divided into 6 groups of six animals each. Group I served as control received 5% gum acacia, group II served as diabetic control received 5% gum acacia, group III, IV and V received LCME at a dose of 100, 200 and 400 mg/kg and group VI served as standard glibenclamide received at a dose of 10mg/kg. The freshly prepared solutions were administered daily using oral gavage for 21 days. During the study period, the bodyweight of the animals and blood glucose levels were recorded after 7, 14 and 21

days of the treatment. Biochemical parameters such as total cholesterol, triglycerides, SGOT, SGPT, ALP, insulin, total protein, urea and creatinine in serum and hematological parameters such as RBC, WBC, and Hb estimated after 21 days of the treatment.

Histopathological Study: Histological studies were conducted on the pancreas of the rats. The pancreas were excised after sacrificing the animals, weighed and fixed in 10% formalin, dehydrated in a graded series of ethanol and embedded in paraffin wax before sectioning. Thin sections were cut with a rotary microtome stained with hematoxylin and eosin. The sections were examined microscopically for histopathological changes.

Statistical Analysis: All the values were expressed as mean \pm SD. The data were statistically evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's t-multiple comparison test using Graph pad prism 6 computer software, P-value of 0.05 or less was considered to be significant.

RESULTS:

Acute Toxicity Study: The methanolic extract of *Lindernia ciliata* (LCME) did not cause any adverse effects and mortality up to a dose level of 2000 mg/kg b.w.p.o. Hence three doses, 100, 200, 400 mg/kg b.w. of methanolic extract were selected for conducting hypoglycaemic, OGTT and subacute studies.

Effect of LCME on Hypoglycemic Activity in Euglycemic Rats: The results of the study were depicted in **Table 1** and **Fig. 1**. The effect of different doses of LCME on fasting blood sugar levels in normal rats was assessed at different time intervals. LCME produced a significant ($p < 0.05$) hypoglycemic effect after 2 h of the treatment at all the three test doses (100, 200, 400 mg/kg b.w.) and this effect was observed up to 24 h. The three test doses showed maximum hypoglycemic effect ($p < 0.01$) after 6 h of the treatment with the percentage reduction in blood glucose level. However, out of the three test doses LCME at 200 mg/kg b.w. exhibited maximum hypoglycemic effect in blood glucose reduction and it was well comparable with the standard drug, glibenclamide at 10 mg/kg b.w.

Effect of LCME on OGTT: The results of the study are presented in **Table 2** and **Fig. 2**. It is very clear from **Table 2** that 30 min after glucose administration, the blood sugar level of the animals from all the groups was significantly increased and thereafter, the blood glucose level gradually decreased in all groups of the rats treated with LCME and standard drug. LCME at three test doses (100, 200, 400 mg/kg) produced significant ($p<0.01$) clearance of glucose from the blood after 60min of glucose load and improved glucose tolerance up to 120 min of the study. In both the test and standard groups, the blood glucose level of the animals reached to less than normal values after 120 min of the investigation. Out of the three test doses LCME 200 mg/kg showed maximum percentage reduction in blood glucose level, and it was comparable to that of reference drug, glibenclamide (10 mg/kg).

Effect of LCME on Different Biochemical and Haematological Parameters in Sub Acute Study (21 days):

Body Weight: It was observed that there is a gradual diminution in the bodyweight of animals in diabetic control group. The animals treated with LCME and the reference drug, glibenclamide showed a gradual increase in their body weight after 7 days of treatment. The significant ($p<0.01$) effect of the three test doses (100, 200, 400 mg/kg) of LCME on body weight of the animals was observed only after 21 days of the treatment.

Among three test doses, LCME at 200 mg/kg showed significant recovery on body weight of the animals, and it was well comparable to that of reference drug, glibenclamide (10 mg/kg). The results are shown in **Table 3**.

TABLE 1: EFFECT OF METHANOLIC EXTRACT OF *LINDERNIA CILIATA* ON FASTING BLOOD GLUCOSE LEVEL IN NORMAL RATS

Group no.	Treatment	Dose (mg/kg)	Blood glucose level (mg/dl) at different hours						
			0 h	2 h	4 h	6 h	8 h	12 h	24 h
I	Control (5% gum acacia)		87.3±4.1	85.8±4.2	84±4	83.5±2.8	86.6±3.01	84.6±3.6	84.9±4.2
II	Glibenclamide	10	75.8±3.7	48.8±4.9*	40.5±2.8**	38.2±3.3**	39.1±4.5**	46±2.6*	53.6±4.4
III	LCME	100	74.1±3.4	61.3±2.3*	53±3.6**	51.6±2.19**	51.9±3.8**	53.6±3.7*	63.9±2.3
IV	LCME	200	72.3±3.3	51.5±3.01*	43.3±2.3**	41.1±3.4**	41.6±2.6**	49.1±3.1*	54.6±3.9
V	LCME	400	74±3.6	54.3±4.03*	49.3±4.8**	50±1.8**	50.5±4.3**	51.6±3.2*	56.5±2.5

All values are expressed as mean ± SD, n=6; statistically significant * $p<0.05$; ** $p<0.01$ compared with the control group

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF *LINDERNIA CILIATA* ON ORAL GLUCOSE TOLERANCE IN NORMAL RATS

Group no.	Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)				
			0min	30min	60min	90min	120min
I	Control (5% gum acacia)		82.3±4.8	115.8±5.8	106.6±3.9	104.8±4.4	102.8±3.8**
II	Glibenclamide	10	87±3.9	109.3±6.0*	95.8±4.7**	74.5±5.6**	62.6±4.2**
III	LCME	100	80.8±4.7	98.1±6.7*	94.9±3.6**	87.9±5.8**	75.3±5.4**
IV	LCME	200	81.8±5.8	101.6±4.3*	93.5±3.7**	78.5±3.3**	65.1±4.7**
V	LCME	400	80.2±4.0	96.5±3.3*	90.8±2.2**	82.6±2.6**	72.1±3.8**

All values are expressed as mean ± SD, n=6; statistically significant * $p<0.05$; ** $p<0.01$ compared to control at the respective time point

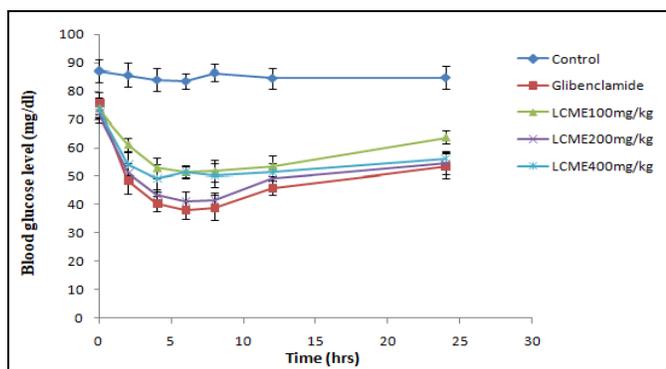


FIG. 1: EFFECT OF METHANOLIC EXTRACT OF *LINDERNIA CILIATA* ON FASTING BLOOD GLUCOSE LEVEL IN NORMAL RATS

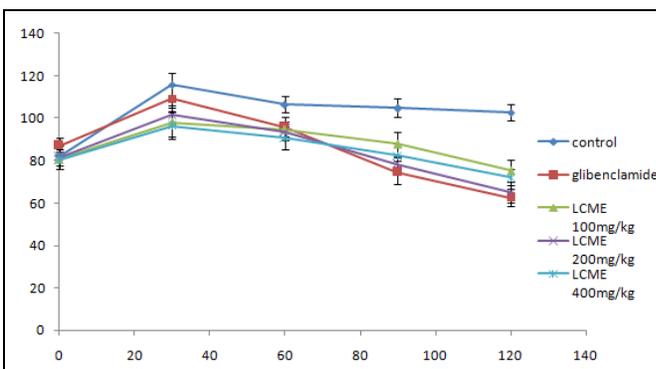


FIG. 2: EFFECT OF METHANOLIC EXTRACT OF *LINDERNIA CILIATA* ON ORAL GLUCOSE TOLERANCE IN NORMAL RATS

Biochemical Changes: LCME and reference drug, lowering the blood glucose level ($p < 0.01$) gradually after 7 days of the treatment and continued the effect up to the end of the study ($p < 0.01$). The maximum effect was observed after 21 days of the treatment. LCME at 200 mg/kg showed significant reduction in blood glucose, and it was well comparable to that of the reference drug, glibenclamide 10 mg/kg at each time interval

of the study. The results are shown in **Table 3**. LCME at 200 mg/kg showed a significant ($p < 0.01$) effect on total cholesterol, triglycerides, SGOT, SGPT, ALP, urea and creatinine level in serum by reducing their elevated level while increasing the diminished serum insulin and total protein levels in STZ induced diabetic rats, and it was comparable to that of the reference drug glibenclamide (10 mg/kg). The results are shown in **Table 4**.

TABLE 3: EFFECT OF LCME ON BODY WEIGHT AND BLOOD GLUCOSE LEVEL IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Group	Bodyweight (g)				Blood glucose (mg/dl)			
	Days of treatment				Days of treatment			
	1	7	14	21	1	7	14	21
Control	270±3.4	271.7±2.9	274.1±4.1	273.4± 3.9	85.2± 5.6	92.4±6.8	87.6±7.2	97.4±6.4
Diabetic control	215.4±5.4	216.1±5.1	218.4±4.9	217.1± 5.2	310.2±9.5	280.4±10.8	290.8±11.4	267.5±12.9
LCME 100 mg/kg	214.9±4.5	218.7±4.9	223.1±4.2*	225.5±5.2**	284.6±8.9	265.1±8.4*	182.2±7.8**	135.5±8.6**
LCME 200 mg/kg	216.5±4.8	222.5±4.6	230.8±4.2*	235.4±3.6**	297.5±8.4	230.4±8.2*	146.1±7.2**	111.7±9.1**
LCME 400 mg/kg	218.1±3.9	220.7±4.1	225.9±2.9*	228.5±3.5**	302.6±6.5	250.2±5.8*	172.1±4.8**	122.7±6.9**
glibenclamide	216.2±4.4	224.4±5.2	235.1±4.9*	240.2±5.5**	295.5±6.4	225.3±4.2*	140.8±7.2**	106.2±5.8**

Statistically significant * $p < 0.05$, ** $p < 0.01$, compared to diabetic control at the respective time point; Data represented as mean \pm SD.

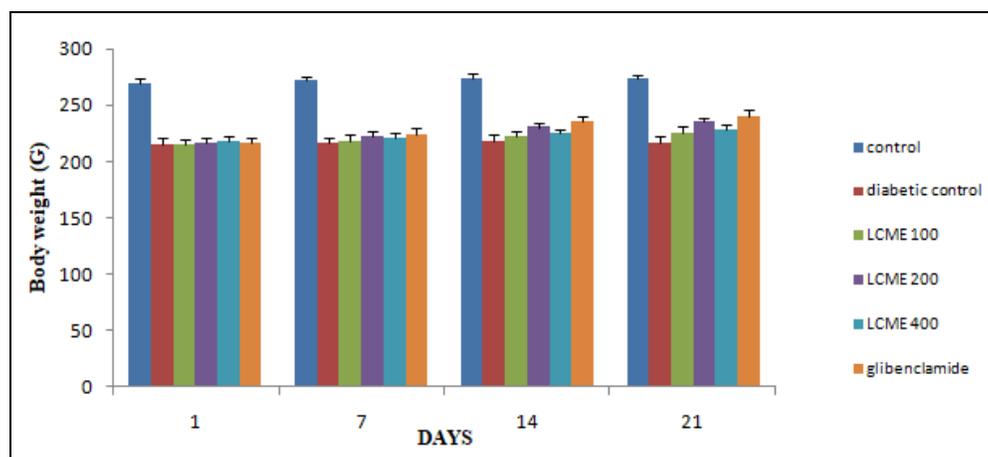


FIG. 3: EFFECT OF LCME ON BODY WEIGHT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

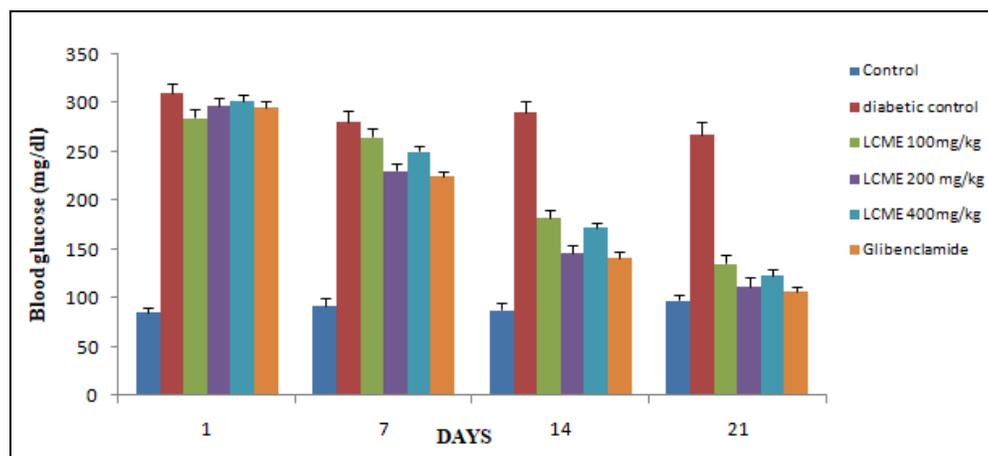


FIG. 4: EFFECT OF LCME ON BLOOD GLUCOSE LEVEL IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Hematological Changes: The daily treatment of streptozotocin induced diabetes in rats with the three test doses (100, 200, 400 mg/kg) and the reference drug for 21 days has shown a significant ($p < 0.01$) improvement in the reduced RBC, WBC, and hemoglobin. Among the three test doses, 200 mg/kg showed a maximum percentage increase in RBC, WBC, and hemoglobin and was comparable to that of reference drug glibenclamide (10 mg/kg). The results are shown in **Table 5**.

TABLE 4: EFFECT OF LCME ON DIFFERENT BIOCHEMICAL PARAMETERS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Groups	Insulin ($\mu\text{IU/ml}$)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total protein (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Control	8.62 ± 0.2	80.2 ± 7.4	87.7 ± 9.1	118.6 ± 3.9	81.2 ± 3.5	159.0 ± 7.5	8.5 ± 0.42	58.9 ± 3.6	35.5 ± 1.5
Diabetic control	3.52 ± 0.42	132.2 ± 6.7	162.3 ± 12.5	220.8 ± 4.01	119.0 ± 5.2	335.5 ± 8.9	4.24 ± 0.53	98.05 ± 7.7	62.25 ± 0.9
LCME	4.81 $\pm 0.1^*$	124.5 $\pm 5.5^*$	140.2 $\pm 7.5^*$	160.0 $\pm 9.8^*$	102.5 $\pm 8.2^*$	230.0 $\pm 7.2^*$	5.12 $\pm 0.42^*$	80.6 $\pm 5.3^*$	55.5 ± 0.95
100 mg/kg LCME	6.42 $\pm 0.50^{**}$	98.9 $\pm 8.8^{**}$	112.9 $\pm 6.4^{**}$	139.8 $\pm 4.2^{**}$	87.1 $\pm 4.7^{**}$	200.5 $\pm 5.2^{**}$	6.92 $\pm 0.25^{**}$	69.1 $\pm 2.0^{**}$	43.1 $\pm 1.0^{**}$
200mg/kg LCME	5.79 $\pm 0.3^{**}$	109.2 $\pm 9.5^{**}$	128.4 $\pm 12.1^{**}$	152.5 $\pm 5.7^{**}$	96.9 $\pm 6.3^{**}$	195.2 $\pm 5.4^{**}$	5.89 $\pm 0.37^{**}$	78.7 $\pm 4.5^{**}$	47.5 $\pm 1.5^{**}$
400 mg/kg LCME	7.02 $\pm 0.47^{**}$	91.2 $\pm 4.2^{**}$	107.2 $\pm 6.4^{**}$	135.5 $\pm 3.6^{**}$	85.8 $\pm 6.5^{**}$	195.2 $\pm 5.4^{**}$	7.54 $\pm 0.18^{**}$	65.1 $\pm 5.1^{**}$	39.2 $\pm 1.9^{**}$
Glibenclamide									

Statistically significant * $p < 0.05$, ** $p < 0.01$, compared to control vs. other groups at the respective time point; Data represented as mean \pm SD.

TABLE 5: EFFECT OF LCME ON DIFFERENT HAEMATOLOGICAL PARAMETERS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Groups	RBC ($\times 10^6/\mu\text{L}$)	WBC ($\times 10^3/\mu\text{L}$)	Hb (g/DL)
Control	7.2 \pm 0.4	12.4 \pm 0.85	15.2 \pm 0.29
Diabetic control	3.8 \pm 0.5	6.72 \pm 0.86	9.02 \pm 0.42
LCME 100mg/kg	4.3 \pm 0.18*	8.2 \pm 0.92*	10.3 \pm 0.32*
LCME 200mg/kg	5.2 \pm 0.4**	8.6 \pm 1.1**	11.2 \pm 0.35**
LCME 400mg/kg	4.8 \pm 0.12**	8.4 \pm 0.42**	10.8 \pm 0.36**
Glibenclamide	5.9 \pm 0.2**	9.2 \pm 0.52**	11.8 \pm 0.24 **

Statistically significant * $p < 0.05$, ** $p < 0.01$, compared to control vs. other groups at the respective time point; Data represented as mean \pm SD

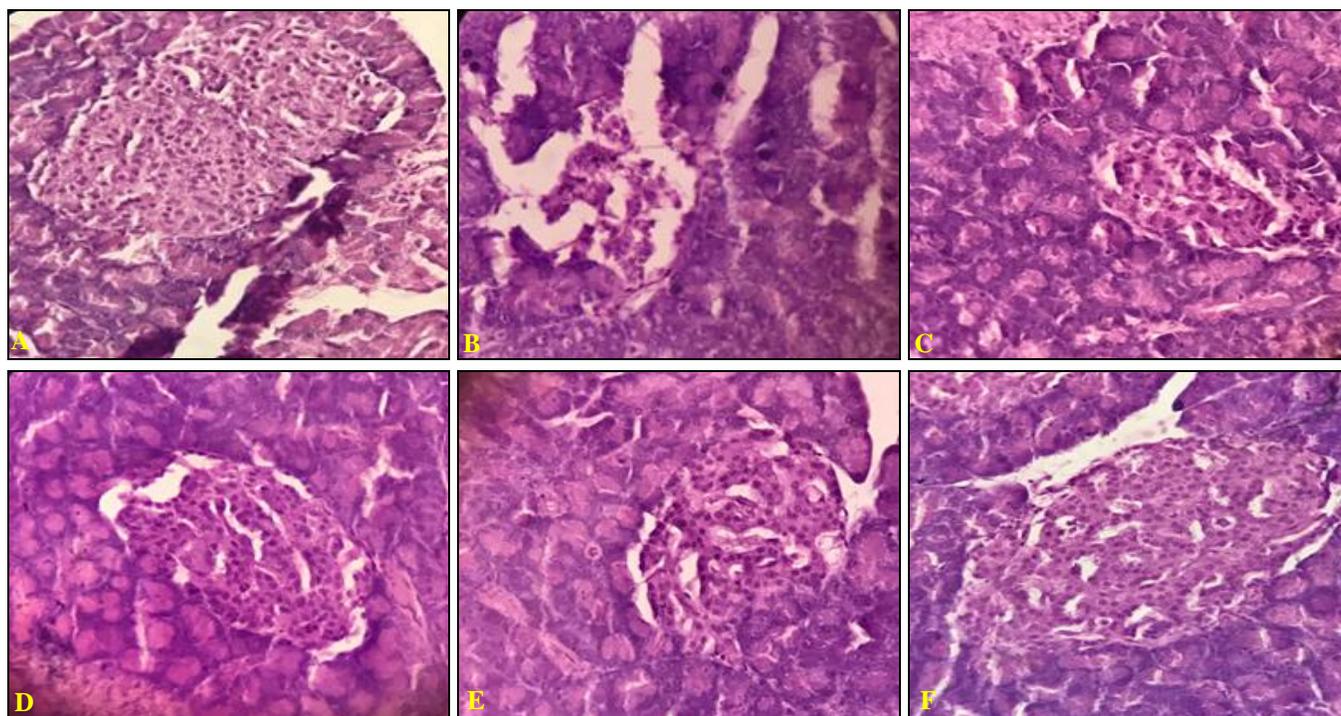


FIG. 5: HISTOLOGY OF RATS (A) NORMAL CONTROL RATS; (B) DIABETIC RATS; (C) DIABETIC RATS TREATED WITH LCME 100 mg/kg; (D) DIABETIC RATS TREATED WITH LCME 200 mg/kg; (E) DIABETIC RATS TREATED WITH LCME 400 mg/kg; (F) DIABETIC RATS TREATED WITH GLIBENCLAMIDE

Histopathological Observations: Fig. 5a-f represents the histopathological changes of the pancreas of the animals of the different groups of the study. The photomicrograph of vehicle-treated normal rats showed normal cellular population in the pancreatic islets of Langerhans, Fig. 5a. The islets of Langerhans in streptozotocin induced diabetic rats showed extensive damage to the pancreas *i.e.* decrease in the number of β -cells and the size of the islets of Langerhans, and the islets appeared as shrunken Fig. 5b. The rats treated with glibenclamide showed a marked increase in size of the islets and intensity of the β -cells Fig. 5c. Administration of three test doses (100, 200, 400 mg/kg) of LCME produced a moderate expansion of islets with an increase in the β -cells intensity. The expansion of islets and intensity of the β -cells were greater in 200 mg/kg Fig. 5d as compared to other groups.

DISCUSSION: The study was undertaken to evaluate the hypoglycemic activity of LCME in normal, glucose loaded hyperglycaemic and streptozotocin-induced diabetic rats. In acute toxicity study, no mortality occurred within 72 h up to a dose of 2000 mg/kg b.w.p.o. with methanolic extract of the whole plant of *Lindernia ciliata*. Hence, antidiabetic studies of this extract were carried out with three graded doses *i.e.* 100, 200, 400 mg/kg b.w.p.o. Hyperglycemia, the primary clinical manifestation of diabetes, is the most responsible factor for the development of various chronic diabetic complications and free radical production¹². Hence, the preliminary investigations were carried out on the methanolic extract of *Lindernia ciliata* to investigate whether these extracts could decrease efficiently the raised blood glucose level or normal blood glucose level in experimental animals in different models *viz.*, hypoglycemic activity in normal rats and glucose loaded hyperglycemic rats.

The results of the hypoglycemic activity of LCME in normal rats revealed that it has significant hypoglycemic activity. The significant ($p < 0.01$) decrease in the blood glucose level was observed after 2 h of administration of three test doses. Among these three test doses (100, 200, 400 mg/kg) LCME at 200 mg/kg showed a maximum percentage reduction in blood glucose level (43.1%) after 6 h administration and it was

comparable to that of reference drug glibenclamide (10 mg/kg). The hypoglycemic effect of LCME observed in the present investigation might be due to changes in the insulin secretion and/or its action or interference in the absorption of carbohydrates in small intestine, leading to the suppression of blood glucose level.

Oral glucose tolerance test is used to determine the altered carbohydrate metabolism during post glucose administration¹³. The three test doses (100, 200, 400 mg/kg) of LCME showed improvement in oral glucose tolerance. Among the three test doses of LCME 200 mg/kg produced a maximum improvement in oral glucose tolerance after 60 min onwards. The blood glucose level reached nearly to normal or less than normal in both the extracts treated and glibenclamide (10 mg/kg b.w) treated groups after 120 min in a similar manner. Increased glucose tolerance in LCME treated rats was due to insulin secretion from β -cells and increased glucose utilization by the tissues.

In view of the promising results obtained in hypoglycemic and OGTT, LCME was evaluated for antidiabetic activity in STZ- induced diabetic rats by subacute study (21 days).

The intraperitoneal administration of STZ damages partially the insulin-secreting β -cells of the pancreas by breaking DNA strands leading to decreased endogenous insulin release which ultimately results in diabetes mellitus.

i) The LCME treated group exhibited a significant reduction in serum glucose levels as compared to the diabetic control group. Administration of LCME to diabetic rats showed a significant reduction in the levels of blood glucose and an increase in the levels of serum insulin. The possible mechanism by which LCME brought about its hypoglycemic action might be by increasing insulin secretion from regenerated β -cells of pancreas. It was further supported by histopathological observations which clearly revealed the presence of shrinkage, necrosis and damaged β -cell population in the endocrine region of pancreas in STZ-induced diabetic rats. The extract (LCME) treated animals showed increase in the number of islets, lesser degree of shrinkage and restoration of necrosis of β -cells of pancreas.

ii) STZ-induced diabetes is known to cause weight loss in animals as a result of proteinuria and insulin deficiency. Insulin deficiency triggers the liver to breakdown protein into amino acids leading to muscle wasting and excessive weight loss¹³ which was observed only after 21 days of the treatment. Among three test doses (100, 200, 400mg/kg) LCME at 200 mg/kg was found to be more effective in maintaining the body weight compared to diabetic control rats and reference drug glibenclamide treated rats, which may be due to their protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and improvement in insulin secretion.

iii) The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia¹⁴. In insulin-deficient subjects, cholesterol biosynthesis increased by increasing the 3- hydroxy- 3- methyl- glutaryl coenzyme A reductase (HMG COA reductase), a key enzyme of cholesterol biosynthesis. In insulin-deficient subjects, it fails to activate the enzyme lipoprotein lipase, (which hydrolyzes triglycerides) and causes hypertriglyceridemia¹⁵. In this study, treatment of diabetic rats with LCME improved the lipid profile by decreasing the serum levels of TC and TG after 21 days of the treatment. Three test doses (100, 200, 400 mg/kg) showed significant reduction in TC and TG. Among the three test doses LCME at 200 mg/kg showed more antihypertriglyceridemic and antihypercholesterolemic activity and it was comparable to that of the reference drug, glibenclamide. Hence, it may be stated that the possible mechanism of reduction of serum lipids levels with LCME may be through insulin release or by enhancing insulin sensitivity in the tissues.

iv) The significant ($p < 0.01$) reduction in SGOT, SGPT, and ALP in LCME treated rats after 21 days of the study and it was well comparable to that of the reference drug, glibenclamide. Increased gluconeogenesis and ketogenesis occur in diabetes, which may be due to high level in the activities of these hepatospecific enzymes. LCME at 200 mg/kg shown more reduction than the other test doses.

v) In both the extract and the reference drug, glibenclamide treated groups; there was a significant ($p < 0.01$) increase in serum total proteins after 21 days of the treatment. In diabetes, changes

in protein metabolism take place, leading to a reduction in serum total proteins which is due to deficiency of insulin. Insulin stimulates uptake of amino acid into muscle and increases protein synthesis¹⁶. Therefore, the increased serum protein levels by the extracts explain their antidiabetic effect.

vi) An increase in serum urea and creatinine level is the most sensitive indicator of kidney injury. Hyperglycemia in diabetes induces elevation of serum urea and creatinine levels and is considered as significant markers of renal dysfunction¹⁷. After the administration of plant extract at three test doses and reference drug for 21 days, elevated urea and creatinine levels in diabetic rats was declined significantly ($p < 0.01$), which indicates in the improvement of functional status of the kidney.

vii) The occurrence of anemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins. Oxidation of these proteins and hyperglycemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to hemolysis of RBC. The levels of RBC and Hb in the diabetic animals were drastically reduced which may be attributed to the infections on the normal body systems¹⁸. In both the extracts and the reference drug, glibenclamide treated groups, there was significant ($p < 0.01$) increase in RBC and Hb after 21 days of the treatment.

(viii) Streptozotocin is a well-known chemical that suppresses the immune system by damaging WBC and certain organs in the body. The intraperitoneal injection of streptozotocin into rats significantly reduced the WBC count. The reduction of these parameters could be correlated to suppression of leukocytosis from the bone marrow which may account for poor defensive mechanisms against infection¹⁹.

Diabetic rats treated with plant extract (LCME) and reference drug, glibenclamide showed improvement in WBC count, which could probably due to the fact that the plant extract contains some phytochemicals that stimulate and/or promote the production of WBCs. LCME at 200 mg/kg showed maximum activity compared to other test doses 100 and 400 mg/kg.

CONCLUSION: The present study concludes that LCME showed potent hypoglycaemic activity in diabetic rats compared to normal rats with significant improvement in body weight, levels of serum insulin and other biochemical parameters. The observed antihyperglycaemic activity of whole plant of *Lindernia ciliata* might be related to the presence of saponins, steroidal/triterpenoids and flavonoids compounds and their glycosides, phenolic compounds and sterols as active constituents. As this is the first report on antidiabetic activity of whole plant of *Lindernia ciliata*. Thorough, phytochemical analysis needs to be executed in order to isolate and characterize the biologically active principles with their mechanism of action for developing *Lindernia ciliata* to be an effective and safe antidiabetic drug.

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