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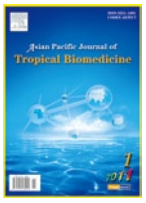

Document heading

Antidiabetic and Hypolipidemic effect of methanol extract of *Lippia nodiflora* L. in streptozotocin induced diabetic rats

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ABSTRACT

Objective: To assess the antidiabetic and hypolipidemic properties of *Lippia nodiflora* (*L. nodiflora*). **Methods:** Acute toxicity test was done to check the toxicity of *L. nodiflora* methanol extract and oral glucose tolerance test was performed in normal rats. *L. nodiflora* methanol extract at three dose levels was administerd orally to streptozotocin (STZ) (40mg/kg bw) induced diabetic rats for 15 days. The various parameters were studied including body weight, fasting blood glucose levels, plasma insulin, lipid profile, glycogen content, glycoslylated hemoglobin (HbA1c) and serum marker enzyme levels in normal, treated and diabetic rats. Histochemical analysis of pancreas was also carried out in normal, treated and diabetic rats. **Results:** The treatment group with the extract at three dose levels showed a significant increase in the liver, muscle glycogen and serum insulin level and a significant decrease in fasting blood glucose, glycosylated hemoglobin levels and serum marker enzyme levels. The total cholesterol and serum triglycerides levels were also significantly reduced and the high density lipoprotein level was significantly increased upon treatment with the *L. nodiflora* methanol extract. Histochemical study of pancreas also confirmed the biochemical findings. Acute toxicity studies revealed the non-toxic nature of the *L. nodiflora* methanol extract. **Conclusions:** The results of the experiments presented here suggest that methanol extract of *L. nodiflora* exerts significant antidiabetic and hypolipidaemic effect in STZ-induced diabetic rats.

1. Introduction

Diabetes mellitus (DM) has been defined as a chronic disease with persistently elevated blood glucose concentration, leading to acute or long term complications[1]. Globally, DM presents enormous and increasingly important public health issues. The prevalence of DM in all age groups was estimated to be 2.8% (170 million) in 2000 and the rate is expected to rise to 4.4% (366 million) in 2030[2]. The pharmacological agents currently used for treatment of type 2 diabetes include sulfonylureas, biguanide, thiazolidinedione and α-glycosidase inhibitors. These agents, however, have restricted usage due to several undesirable side effects and fail to significantly alter the course of diabetic complications[3]. Renewed attention to alternative medicines and natural therapies has stimulated new waves of research interest in traditional practices, and there is a need to look for more efficacious agents with lesser side effects. Presently, there is a growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents for the treatment of diabetes mellitus[4].

Lippia nodiflora (*L. nodiflora*) L. (Verbenaceae) is a creeping perennial herb with small white flowers. It is found in wet grounds and grassy slopes. The plant is distributed throughout India, Srilanka, Baluchistan, and Africa. The whole plant is used traditionally by the local and tribal people of south India for the treatment of bronchitis, hypertension and fever. The herb possesses cooling and diuretic properties and stops knee joint pain. The plant made into a poultice is used as maturant for boils. Antimalarial activity was reported from the herb. Leaves of the plant were reported to possess anti-inflammatory, analgesic antipyretic and antioxidant activities[5]. Previous phytochemical investigation on this plant have resulted in the isolation of several flavones glycosides, including lippiflorin A & B, nodiflorin A & B, alkaloids, essential oil,

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resin, stigmasterol, β-sitosterol, sugars, mono and diflavone sulphates of neptin, jaceosidin, hispidulin and 6-hyfroxy-luteolin[6]. However, there are no reports on the antidiabetic activity of the plant. Hence this study was under taken to evaluate the anti- diabetic activity of methanol extract of *L. nodiflora* (LN) in streptozotocin (STZ) induced diabetic rats.

2. Materials and methods

2.1. Plant material

Whole plant of *L. nodiflora* was collected from Padappai, Kanchipuram district, Tamilnadu, during the month of January, 2007. The plant was identified by Dr. S Somusundaram, National Institute of Siddha, Chennai. The voucher specimen was deposited in the herbarium at Entomology Research Institute, Loyola College, Chennai (ERIP-4).

2.2. Preparation of extracts

The whole plant of *L. nodiflora* was washed thoroughly with water to remove the soil particles, and then was air-dried and powdered. One kilogram of plant material was extracted with methanol solvent (2.5 L for each time). The filtrate was concentrated under reduced pressure at 40 ℃ and the extract was stored in a refrigerator at 4 ℃ for use in subsequent experiments.

2.3. Chemicals

Streptozotocin was obtained from Sigma Chemicals, Bangalore, India. Kits to estimate total cholesterol, triglycerides and HDL- cholesterol kit was purchased from Merck, Mumbai, India. All other chemicals were of analytical grade.

2.4. Preliminary phytochemical screening of the extract

The preliminary phytochemical analysis was carried out for the *L. nodiflora* methanol extract (LN methanol extract) using standard phytochemical methods[7].

2.5. Animals

Healthy adult Wister male albino rats with body weight around (170±5) g at 60-70 days from birth and raised in the animal house at Entomology Research Institute, Loyola College were used for the study. Housed individually in poly propylene cages, maintained under standard conditions in 12 h light and 12 h dark cycle at (25±3) ℃, the rats were fed with standard rat pellet diet (Pranav Agro Industry Ltd., Maharastra) and water *ad libitum*. The study was approved by the Animal Ethical Committee of the Institute (IAEC-ERI-LC-10).

2.6. Acute toxicity study

Healthy adult Wister albino rats of either sex, starved overnight were divided into five groups (n=6) and were orally fed with the LN methanol extract in increasing dose levels of 100 mg/kg bw, 500 mg/kg bw, 1 g/kg bw, 3 g/kg bw and 5 g/kg bw[8]. The animals were observed continuously for 2 h under the following profiles[9] (a) Behavioral profile: Alertness, restlessness, irritability, and fearfulness; (b) Neurological profile: spontaneous activity, reactivity, touch response, pain response and gait; (c) Autonomic profile: defecation and urination.

After a period of 24 and 72 h they were observed for any lethality or death.

2.7. Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight (18 h) fasted normal rats[10]. Rats divided into three groups (n=6) were administered drinking water, LN methanol extract at 50, 100 and 200 mg/kg, respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation at 30, 60 and 120 min of glucose administration and glucose levels were estimated using a glucose oxidase- peroxidase method[11].

2.8. Induction of diabetes mellitus

Diabetes mellitus was induced in overnight fasted adult Wister strain albino rats weighing (170 ± 5) g by single intraperitoneal injection of freshly prepared STZ (sigma - Aldrich, Bangalore) at dose of 40 mg/kg bw in 0.1 M citrate buffer (pH = 4.5). After seven days of STZ administration, blood glucose level was determined. Rats with blood glucose level above 200 mg/dL were considered diabetic and included in the study.

2.9. Experimental design

In the experiment a total of 36 rats (6 normal; 30 STZ diabetic surviving rats) were used. The rats were divided into six groups of six rats each.

Group I: Normal control rats; Group II: Diabetic control rats; Group III: Diabetic rats treated with LN methanol extract at dose of 50 mg/kg bw; Group IV: Diabetic rats treated with LN methanol extract at dose of 100 mg/kg bw; Group V: Diabetic rats treated with LN methanol extract at dose of 200 mg/kg bw; Group VI: Diabetic rats treated with Glibenclamide at dose of 600 μg/kg bw. The extract was dissolved in 2% tween 80 solutions and administered orally in Group III, Group IV and Group V for two weeks .

At the end of the study, the animals were euthanized between 0900 - 1100 h to minimize diurnal variation. Fasting blood glucose level was estimated by glucose oxidase - peroxidase method[11]. The assay of insulin in the plasma of normal and diabetic rats was performed by enzyme-linked immunosorbent assay (ELISA) method. The glycogen level of liver and skeletal muscles was measured by anthrone

method[12]. Glycosylated hemoglobin was estimated by the method of Sudhakar and Pattabiraman, 1981[13]. Lipid profile [total cholesterol, high density lipoprotein (HDL), cholesterol and triglyceride] levels in serum were determined according to the instructions of the manufacturer (Merck, Mumbai, India). Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) was determined by th method of Reitman ande Frankel, 1957[14].

2.10. Histological studies

After blood sampling for the biochemical analysis, the animals were sacrificed, quickly dissected. Small slices of pancreases were taken and fixed in 10% formalin. The specimen were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 6 μm in thickness were prepared and stained with haematoxylin and eosin and subjected to microscopical examination[15].

2.11. Statistical analysis

One- way ANOVA and Student's *t*-test (SPSS program; version 11.5) were carried out to compare the data with the level of significance set at $P \leqslant 0.05$.

3. Results

Phytochemical analysis of LN methanol extract revealed the presence of sterols, saponins, coumarins, quinones, tannins, flavanoids and reducing sugars. The simple quantitative analysis of the extract was based on the intensity of colour change.

Acute toxicity studies showed the non-toxic nature of the LN methanol extract. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period. In OGTT, LN methanol extract, from 30 min onwards showed significant reduction in plasma glucose levels (Table 1).

Table 2 showed the changes in body weight of experimental rats at zero and final day of treatment. Significant decrease in body weight of diabetic control rats were observed when compared with normal control rats. However, significant increase in body weight was observed in LN methanol extract treated groups in dose dependent manner when compared with diabetic control rats.

Inductions of diabetes in the experimental rats were confirmed by the presence of a high fasting blood glucose level. The effect of the LN methanol extract on the fasting blood glucose level of normal and STZ induced diabetic animals were presented in Table 3. At the end of 15th day study LN methanol extract at 200 mg/kg bw treated group reduced the fasting blood glucose level significantly (46.32%) when compared with diabetic control.

Tables 4 and 5 showed the effect of LN methanol extract on serum insulin, glycosylated hemoglobin and liver and muscle glycogen content. Administration of LN methanol extract to diabetic rats for 15 days significantly increased the levels of serum insulin, liver glycogen content, muscle glycogen and significant decrease was observed in glycosylated hemoglobin when compared with diabetic control.

Table 6 showed the increased level of total cholesterol, triglycerides and decreased level of HDL- cholesterol in diabetic rats compared to normal control. Administration of LN methanol extract for 15 days significantly reduced the total cholesterol, triglycerides levels and significantly increased the HDL- cholesterol level when compared with

Table 1
Effect of the LN methanol extract on OGTT in normal rats (Mean ± SEM).

Groups (*n*=6)	Treatment	Blood glucose (mg/dL)			
		0 min	30 min	60 min	120 min
I	Control + 2g/kg bw glucose	68.65 ± 2.41	147.04 ± 1.42	133.59 ± 1.29	123.68 ± 1.88
II	LN 50mg/kgbw + 2g/kg bw glucose	64.97 ± 1.28	134.79 ± 0.84*	116.54 ± 1.09**	103.16 ± 1.88**
III	LN 100mg/kgbw + 2g/kg bw glucose	65.79 ± 1.27	128.01 ± 1.17**	110.56 ± 1.09**	96.34 ± 0.86**
IV	LN 200mg/kgbw + 2g/kg bw glucose	66.14 ± 1.18	123.63 ± 1.56**	100.66 ± 0.90**	74.83 ± 1.76**
V	glibenclamide 600 μg/kg bw + 2g/kg bw	65.82 ± 1.27	123.46 ± 1.05**	113.68 ± 1.22**	78.15 ± 1.12**

* Values deviate significantly from diabetic control; ** Values deviate very significantly ($P \leqslant 0.05$) when compared with diabetic control values.

Table 2
Effect of oral administration of LN methanol extract on body weight in STZ induced diabetic rats after 15 days (Mean ± SEM).

Group (*n*=6)	Treatment	Body weight (g)	
		0 day (g)	15th day (g)
I	Normal control	160.93 ± 4.49	168.18 ± 0.84
II	Diabetic control	168.04 ± 6.03	139.61 ± 1.47
III	Diabetic + LN (50 mg/kg bw)	165.37 ± 2.06	170.52 ± 2.02**
IV	Diabetic +LN (100 mg/kg bw)	149.71 ± 2.21	163.97 ± 3.54*
V	Diabetic + LN (200 mg/kg bw)	148.52 ± .810	167.64 ± 1.38**
VI	Diabetic + glibenclamide (600 μg/kg bw)	159.79 ± 1.72	175.01 ± 2.05**

* Values deviate significantly from diabetic control; ** Values deviate very significantly from diabetic control group ($P \leqslant 0.05$).

Table 3
Effect of oral administration of LN methanol extract on plasma glucose levels in STZ induced diabetic rats (Mean ± SEM).

Groups (n=6)	Treatment	Plasma glucose levels (mg/dL)		
		0 day	7th day	15th day
I	Normal control	75.02 ± 1.23	74.92 ± 1.64	76.29 ± 1.16
II	Diabetic control	212.93 ± 3.26	235.07 ± 2.35	247.36 ± 2.89
III	Diabetic + LN (50 mg/kg bw)	206.99 ± 1.47	185.35 ± 2.25**	167.51 ± 2.20**
IV	Diabetic + LN (100 mg/kg bw)	208.79 ± 1.09	167.05 ± 1.44**	144.31 ± 2.22**
V	Diabetic + LN (200 mg/kg bw)	211.50 ± 1.44	158.33 ± 0.62**	113.52 ± 1.96**
VI	Diabetic + glibenclamide (600 μg/kg bw)	205.68 ± 1.89	156.50 ± 0.78**	101.26 ± 1.70**

** Values deviate very significantly ($P \leqslant 0.05$) when compared with diabetic control values.

Table 4
Effect of oral administration of LN methanol extract on plasma insulin levels in STZ induced diabetic rats (Mean ± SEM).

Groups (n=6)	Treatment	Plasma Insulin (μU/mL)	
		Zero day	Final day
I	Normal control	129.11 ± 0.87	130.09 ± 1.88
II	Diabetic control	49.49 ± 0.81	54.66 ± 1.89
III	Diabetic + LN (50 mg/kg bw)	48.95 ± 1.61	70.30 ± 2.13*
IV	Diabetic + LN (100 mg/kg bw)	51.19 ± 1.96	85.85 ± 1.17**
V	Diabetic + LN (200 mg/kg bw)	50.65 ± 1.73	114.29 ± 1.28**
VI	Diabetic + glibenclamide (600 μg/kg bw)	49.49 ± 1.38	116.70 ± 0.98**

* Values deviate significantly from diabetic control; ** Values deviate very significantly ($P \leqslant 0.05$) when compared with diabetic control values.

Table 5
Effect of oral administration of LN methanol extract on glycosylated hemoglobin, liver glycogen and muscle glycogen in STZ induced diabetic rats after 15 days (Mean ± SEM).

Groups (n=6)	Treatment	Glycosylated hemoglobin (%)	Liver glycogen (mg/g wet tissue)	Muscle glycogen (mg/g wet tissue)
I	Normal control	3.00 ± 0.14	53.02 ± 0.92	8.51 ± 0.15
II	Diabetic control	9.27 ± 0.55	16.94 ± 0.36	2.56 ± 0.17
III	Diabetic + LN (50 mg/kg bw)	6.85 ± 0.12*	22.29 ± 1.24	3.37 ± 0.14
IV	Diabetic + LN (100 mg/kg bw	5.21 ± 0.32*	28.50 ± 0.49**	4.75 ± 0.07**
V	Diabetic + LN (200 mg/kg bw)	3.75 ± 0.25**	39.82 ± 0.67**	6.43 ± 0.18**
VI	Diabetic + glibenclamide (600 μg/kg bw)	3.34 ± 0.22**	46.15 ± 0.61**	7.46 ± 0.17**

* Values deviate significantly from diabetic control; ** Values deviate very significantly ($P \leqslant 0.05$) when compared with diabetic control values.

Table 6
Effect of oral administration of LN methanol extract on lipid profile in STZ induced diabetic rats after 15 days (Mean ± SEM).

Group (n=6)	Treatment	Lipid profile (mg/dL)		
		Total cholesterol	Triglycerides	HDL
I	Normal control	55.10 ± 2.34	44.16 ± 2.58	73.65 ± 2.79
II	Diabetic control	115.82 ± 3.14	133.75 ± 6.52	27.79 ± 1.94
III	Diabetic+ LN (50 mg/kg bw)	106.15 ± 2.65*	95.96 ± 3.82**	45.24 ± 3.08**
IV	Diabetic+ LN (100 mg/kg bw)	99.82 ± 1.16**	90.37 ± 1.12**	70.10 ± 2.88**
V	Diabetic+ LN (200 mg/kg bw)	95.77 ± 1.67**	78.16 ± 5.16**	73.61 ± 1.02**
VI	Diabetic+ glibenclamide (600 μg/kg bw)	94.89 ± 2.21**	110.63 ± 1.48**	74.44 ± 1.01**

* Values deviate significantly from diabetic control; ** values deviate very significantly ($P \leqslant 0.05$) when compared with diabetic control values.

Table 7
Effect of oral administration of LN methanol extract on serum marker enzymes in STZ induced diabetic rats after 15 days (Mean ± SEM).

Group (n=6)	Treatment	Serum marker enzymes (μ/L)		
		SGOT	SGPT	ALP
I	Normal control	61.92 ± 0.96	68.65 ± 1.38	121.34 ± 1.06
II	Diabetic control	152.85 ± 2.05	155.14 ± 1.99	242.09 ± 1.41
III	Diabetic + LN (50 mg/kg bw)	122.69 ± 1.50**	119.99 ± 1.90**	170.42 ± 1.24**
IV	Diabetic + LN (100 mg/kg bw)	94.54 ± 0.72**	92.49 ± 1.27**	139.84 ± 0.77**
V	Diabetic + LN (200 mg/kg bw)	65.09 ± 1.45**	74.77 ± 2.35**	124.92 ± 1.54**
VI	Diabetic + glibenclamide (600 μg/kg bw)	63.11 ± 1.33**	65.80 ± 1.70**	124.11 ± 2.26**

** values deviate very significantly ($P \leqslant 0.05$) when compared with diabetic control values.

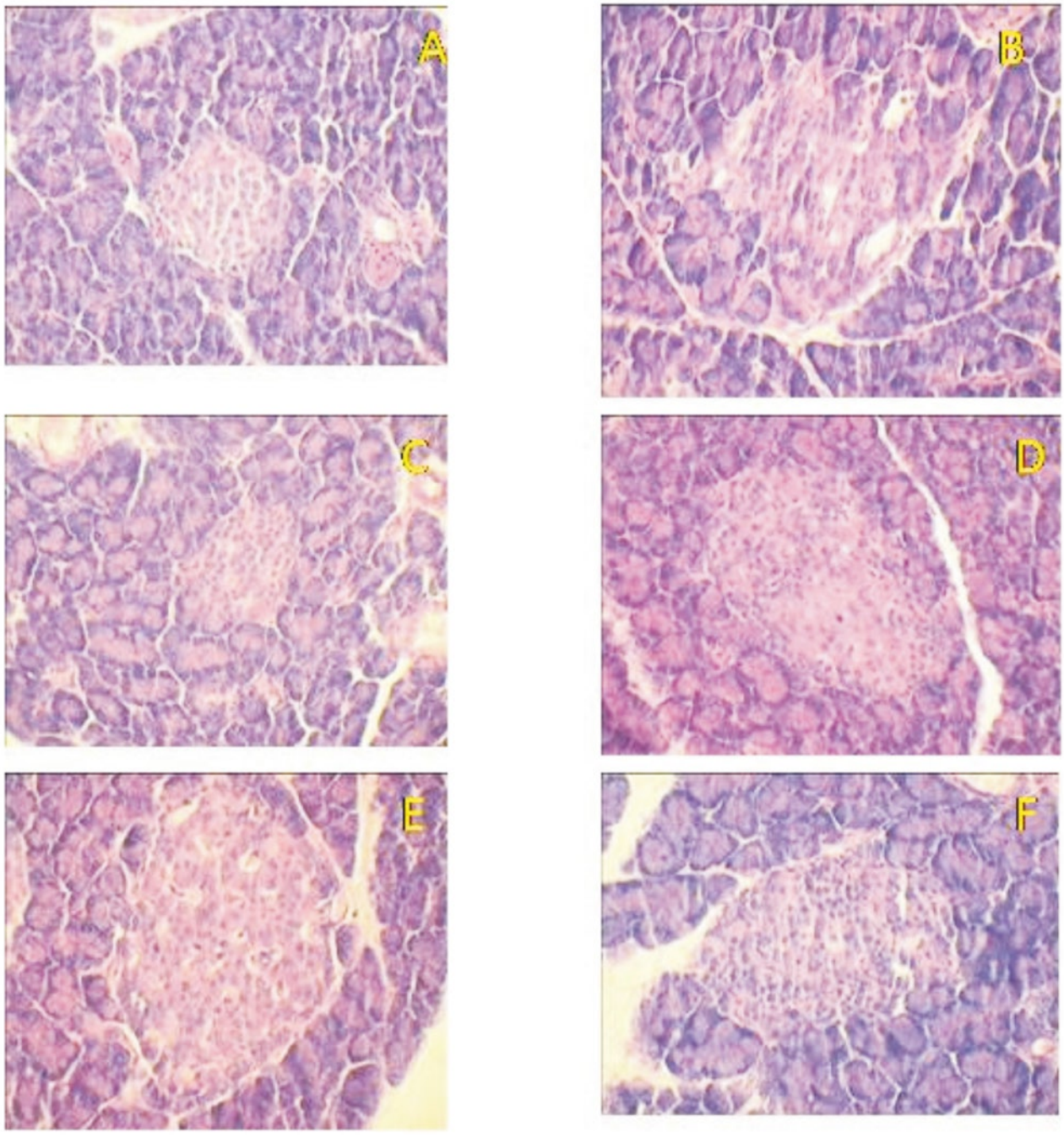

Figure 1. A) normal control; B) diabetic control; C) Diabetic + LN methanol extract (50 mg/kg bw); D) Diabetic + LN methanol extract (100mg/kg bw); E) Diabetic + LN methanol extract (200 mg/kg bw); F) Diabetic + Glibenclamide (600 μg/kg bw).

diabetic rats.

Table 7 showed the effect of administration methanol extract of LN on serum markers enzymes. The concentration of SGOT, SGPT and ALP was increased in diabetes condition when compared with normal control. Administration of LN methanol extract and glibenclamide was found to keep the levels near to normal values.

Histological examination of pancreas showed the normal histology in normal rat (Figure 1A). Hematoxylin-eosin section (400×) showed pancreatic acini, small atrophic islets cells in diabetic control(Figure 1B). Diabetic rats treated with LN methanol extract (50 mg/kgbw) showed expanded and dilated islet cells (Figure 1C). Diabetic rats treated with 100 mg/kg bw LN methanol extract showed mild expansion and absence of dilation (Figure 1D). Diabetic rats treated with LN methanol extract at dose of 200 mg/kg bw showed moderate expansion pancreatic islets showed prominent hyperplastic islets (Figure 1E). Glibenclamide treated rats pancreas (600 μg/kg bw) showed absence of dilation and prominent hyperplastic of islets (Figure 1F).

4. Discussion

The present study for the first time reports the antidiabetic and hypolipidemic effect of methanol extract of LN whole plant in STZ induced diabetic rats. STZ is one of the most commonly used substances to induce diabetic in rats. This

toxin causes the death of pancreatic β－cell by alkylation of DNA resulting in reduced synthesis and release of insulin. Furthermore, it has been shown to be involved in the fragmentation of DNA by means of production of reactive oxygen species[16,17].

Induction of diabetes by STZ leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins[18]. Administration of LN methanol extract for 15 days significantly increased the body weight when compared with diabetic control in dose dependent manner. When LN methanol extract was administered to glucose loaded normal rats fasted for 18 h, hypoglycemia was observed after 30 min. The decline in blood sugar level reached its maximum at 2 h. The observed significant increase in the level of blood glucose and significant decrease in the level of plasma insulin in diabetic rats could be due to the destruction of pancreatic β－cells by STZ[19]. Oral capacity of LN methanol extract to decrease the elevated blood sugar to normal glycemic level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. The possible mechanism by which LN methanol extract bring about its hypoglycaemic action in diabetic rats may be improving glycemic control mechanism and insulin secretion from remnant pancreatic beta cells in diabetic rats[20], as it is evidenced by the significant increase in the level of insulin in treated rats by LN methanol extract (200 mg/kg bw).

Phytochemical investigation of LN methanol extract reveals the presences of sterols, saponins, coumarins, quinones, tannins, flavanoids. These principles are known to be bioactive for the management of diabetes. It is well known that certain flavonoids exhibit hypoglycemic activity and pancreas beta cell regeneration ability. Sterols have also shown to decrease blood sugar in experimental animal models[21]. Thus, the significant antidiabetic effect of aqueous extract of LN may be due to the presence of more than one antihyperglycemic principle and their synergistic properties.

Insulin is the main regulator of glycogenesis in muscle and liver. The decrease of liver glycogen level observed in this study may be due to lack of insulin in diabetic condition or oxidative stress which may inactivate the glycogen synthetase[22]. The marked reduction in liver and muscle glycogen level is observed (15 days) in streptozotocin induced diabetic animals. Treatment with LN methanol extract remarkably increased the glycogen level in liver and muscle. In the view of glycogen level, there may be three possible ways of antidiabetic action; one possible way may be increased insulin level by preventing the inactivation of the glycogen synthetase and by synthesize the glycogen synthatase[23]. The typical characteristics of diabetes is the increase of serum glycated protein such as glycated hemoglobin (HbA1C), which is a parameter for glycemic control where glucose or other reducing sugars react with the amino residues of proteins to form Amadori products such as glycated hemoglobin[24]. Animals treated with methanol extract of LN significantly decreased the glycosylated hemoglobin level which could be due to an improvement in insulin secretion from the remnant pancreatic beta cells in diabetic rats[25].

Since lipid abnormalities accompanying with premature atherosclerosis is the major cause of cardiovascular diseases in diabetic patients, therefore ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. Cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes[26-31]. From this point of view, it is encouraging that the 15 day treatment of methanol extract of LN brought down the elevated level of lipid profile such as total cholesterol, triglycerides to near normal level. There was increase in HDL-cholesterol also, which was desirable feature.

Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. SGOT, SGPT and ALP are reliable markers of liver function. The liver was necrotized in STZ-induced diabetic rats. Therefore an increase in the activities of SGOT, SGPT and ALP in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream which gives an indication of the hepatotoxic effect of STZ[32]. Treatment of the diabetic rats with the LN methanol extract caused reduction in the activity of these enzymes in plasma compared to the diabetic control group and consequently alleviated liver damage caused by STZ-induced diabetes. These results are in agreement with those obtained by Eliza *et al*[33].

Histopathology studies also supported our findings. STZ was suspected to destroy pancreas partially. Diabetic rats showed reduced islet cells, which were restored to near normal upon treatment with the LN methanol extract. No such changes were found in the normal rats.

The findings of this study indicate that consumption of LN methanol extract exerts significant antidiabetic and hypolipidemic effect in STZ-induced diabetic rats. Histopathological studies of the pancreas of LN methanol extract treated diabetic rats show evidence of signs of regeneration of β－cells. Longer duration studies of *L. nodiflora* and its isolated compounds on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent antidiabetic drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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