ORIGINAL ARTICLE

Evaluation of the hypoglycemic activity of aqueous and alcoholic extracts leaves of *Leucas indica* var. nagalapuramiana in normoglycemic and hyperglycemic rats

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Abstract

Background: Plants and their bioactive elements are used to treat diabetes mellitus, particularly in regions where provision to traditional allopathy is limited. There are several reports on the antidiabetic activity of *Leucas* spp. **Objective:** The present work was aimed for investigation of antidiabetic effect of aqueous and alcoholic extracts of *Leucas indica* var. nagalapuramiana leaves on normoglycemic and streptozotocin (STZ)-induced hyperglycemic rats, and their effects were compared with standard oral hypoglycemic drug, tolbutamide (40 mg/kg). **Methods:** Animals weighing 180–200 g were selected for the study and were performed at three dose levels 100, 200, and 400 mg/kg of aqueous and ethanolic extracts in normoglycemic and STZ-induced hyperglycemic rats. Blood glucose levels (BGLs) were monitored at 1, 2, 4, 8, 16, and 24 h post-administration using glucometer. The experimental results were analyzed using ANNOVA followed by Turkey's *post hoc* test to determine the statistical significance among treatment groups. **Results:** The results showed that at all time periods, no statistically significant difference in BGLs was seen when groups treated with varying doses of the extracts were compared to each other and to the standard treatment group. However, a significantly ($P < 0.05$) high percentage glucose reduction was observed with 400 mg/kg of aqueous and alcoholic extracts in normoglycemic rats and 400 mg/kg of alcoholic extracts in hyperglycemic rats, and is comparable to standard, tolbutamide (40 mg/kg).

Key words: Antidiabetic, hyperglycemic, *Leucas indica* var. nagalapuramiana, normoglycemic

INTRODUCTION

Diabetes mellitus (DM) is a common chronic disease characterized by a rise in blood glucose levels (BGLs) that are abnormal. It also causes changes in protein and lipid metabolism as a result of the pancreas' failure to produce enough insulin and/or the body's inability to adequately utilize the insulin it produces.[1,2] It is a disease that's only like a tip of the iceberg and is of two types, Type-I and Type-II. Type-II is most common type of diabetes and accounts for more than 90% of the world cases. Diabetes affects 180 million people globally, according to the World Health Organization, and will reach 300 million by 2025. In adult urban Indians, prevalence rates range from 10% to 18%, however, the prevalence is also increasing in rural areas due to changed lifestyle. It was reported that every 5th individual in the world is diabetic and, in India, every 10th person in metropolitan cities like Mumbai is diabetic.[3] The rate of increase in DM prevalence is concerning, and it is estimated that around 650 million people will be diabetic by year 2040.[4-7] The complications (retinopathy, cardiovascular disease, nephropathy, neuropathy, and other complications) associated with diabetes further make the life miserable for diabetic population. DM can be managed by proper control in diet, regular exercise, healthy lifestyle, and medications,

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namely, insulin therapy and oral hypoglycemic agents.[8] The current treatment for DM focuses on lowering BGLs with oral hypoglycemic medications or insulin injections. However, these medicines have several adverse effects. This insists for the search of alternative therapies that are easily available and have little or no side effects in the treatment and management of diabetes[9] of which herbal therapy makes first choice.

Herbal therapy, where plants and their bioactive elements are used to treat DM, is an age-old practice round the world, particularly in regions where availability of conventional allopathic treatment is limited.[10,11]

The plant *Leucas indica* var. nagalapuramiana belonging to the family, Labiatae, and is abundantly accessible across the Seshachalam hills, Chittoor district, on roadsides, waste fields, riverbanks, and on rocky slopes throughout India. The leaves of this plant have traditionally been used as a vermifuge, stomachic, sedative, and in the treatment of jaundice, inflammation, asthma, dyspepsia, fever, colds, snakebites, and ulcers.[12-14] In the present study, based on literature survey and traditional use, the present study was designed to evaluate the hypoglycemic activity of aqueous and alcoholic extracts leaves of *L. indica* var. nagalapuramiana in normoglycemic and streptozotocin (STZ)-induced hyperglycemic rats.

MATERIALS AND METHODS

Animals

Male albino rats weighing 180–200 g and of 3–4 months of age were selected for used for the study. Animals were placed as groups of six, were housed in a standard polypropylene cage maintained under standard laboratory conditions (22 ± 2°C, light:dark cycle of 12:12 h), and were provided with normal pellet diet and water (4). The experiment was conducted with prior approval of the Institutional Animal Ethical Committee (IAEC Approval No.516/PO/c/01/IAEC/06).

Drugs, Chemicals, and Instruments

STZ, citric acid monohydrate, and trisodium citrate were produced from Sigma-Aldrich, Mumbai, i-QARE DS-W® blood glucose meter and strips (Alliance International, Taiwan). All chemicals used were of analytical grade and were obtained from SD Fine Chemicals, Mumbai.

Plant Collection and Processing

L. indica var. nagalapuramiana plants were collected from forest area of Tirupati, Chittoor district, Andhra Pradesh, India, in the month of June–July. The identity of the plant material was verified by Dr. K Madhava Chetty, Asst. Prof., Dept. of Botany, Sri Venkateshwara University, Tirupati, with voucher specimen number 1748 was deposited at the institute level. Leaves of the collected plants were washed thoroughly with tap water to remove mud, surface dust, and other unwanted materials. The leaves are then allowed to dry for 20–30 days under shade. The dried leaves are then finely powdered. The powdered material was sieved and was used for subsequent extraction process.

Extraction Procedure

In a round bottom flask, 100 g powdered leaf material was combined with 250 mL distilled water and heated under reflux for 3 h to get the aqueous extract. It was then allowed to soak in water for 3 days for complete extraction.[15] Ethanolic extracts were prepared by extracting 100 g of powdered material with 250 mL of ethanol under soxhlation.[16] Extracts were then concentrated under reduced pressure at 45°C in a rotary flash evaporator and processed for lyophilization to obtain crude powder (dry).[17]

Acute Oral Toxicity Studies of the Prepared Extracts

The acute oral toxicity test of the aqueous and alcoholic extracts was done based on the limit and main test recommendations of OECD No. 423 Guidelines. Rats were fasted overnight and were administered with the plant extracts as per the procedure and observed for mortality for 24 h.[18,19]

Preparation of Doses

The aqueous and alcoholic extract suspensions were prepared immediately before administration on respective treatment days. Suspensions of the extracts were prepared at concentrations of 200 mg/ml using carboxymethyl cellulose (CMC) (0.1%) as suspending agent and a homogeneous suspension.

Measurement of BGL

BGLs were measured using DS-W® blood glucose meter. Blood was collected by tail vein puncture and the drops were collected over glucose strips. The measurement was made in triplicate and results were expressed as Mean ± SEM.

Induction of Experimental Diabetes

Animals were fasted overnight, and a single intraperitoneal (i.p.) dose of a freshly prepared solution of STZ at a dose of 50 mg/kg b.w. in 0.1 M cold citrate buffer (pH 4.5) was administered to induce diabetes in rats. Thirty minutes post-injection animals were allowed to have free access to water and food. Further, animals were allowed to drink 5% glucose solution overnight to prevent hypoglycemic shock. Seventy-two hours post-STZ

injection, blood was withdrawn from tail vein of the animals, to measure fasting BGLs. The rats with a BGL >250 mg/dl were selected for the experiment as diabetic rats.

Hypoglycemic Activity of the Extracts in Normoglycemic Rats

Normoglycemic rats were divided into eight groups of six animals each. Baseline BGLs (0 h) were measured in overnight fasted rats, just before administration of extracts. Group 1 and Group 2 act as normal control and standard control and receive 0.1% sodium CMC and naïve tolbutamide (40 mg/kg, p.o.), respectively. Groups 3, 4, 5 and Groups 6, 7, 8 receive 100, 200, and 400 mg/kg, p.o. of *L. indica* var. nagalapuramiana leaves aqueous and ethanolic extracts, respectively. Blood samples were collected from the tail vein under ether anesthesia just before and at intervals of 0, 1, 2, 4, 6, 8, 16, and 24 h, post-dosing, BGLs were measured using a glucose oxidase-peroxidase reactive strips and a glucometer.

Hypoglycemic Activity of the Extracts in STZ-induced Diabetic Rats

STZ-induced diabetic rats were divided into 8 groups of six animals each. Baseline BGLs were measured in overnight fasted rats, before administration of extracts. Groups 1 and Group 2 act as normal control and standard control and receive 0.1% sodium CMC and naïve tolbutamide (40 mg/kg), p.o., respectively. Groups 3, 4, 5 and Groups 6, 7, 8 receive 100, 200, and 400 mg/kg, p.o. of aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves, respectively. Blood samples were collected from the tail vein under ether anesthesia just before and at intervals of 0, 1, 2, 4, 6, 8, 16, and 24 h, post-dosing, BGLs were estimated using glucose oxidase-peroxidase reactive strips and a glucometer.

Statistical Analysis

Data were expressed as mean ± standard error of the mean. Means of all parameters among groups and within a group were compared using one-way ANOVA followed by Tukey's *post hoc* multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Percentage Yield of Aqueous and Ethanolic Extracts of *L. indica* var. Nagalapuramiana Leaves

A total of 100 g of dried leaves powder were processed separately for aqueous and ethanolic extraction process, and at the end of the extraction process, it was found that the percentage yield was 18.54% and 32.43% w/w, respectively, for aqueous and extraction process.

Acute Oral Toxicity Study

The acute oral toxicity study revealed that oral dose of maximum 2000 mg/kg of both the aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves showed no treatment-related signs of toxicity or mortality.

Hypoglycemic Activity of the Extracts in Normoglycemic Rats

The effect of aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves is shown in Table 1. According to our findings, there is no significant change in BGL across the therapy groups. At all time periods, no statistically significant difference in BGL was seen when groups treated with varying doses of the extracts were compared to each other and to the positive control.

Further, within the groups, the results showed that the aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves showed a dose-dependent decrease in BGLs, and with 400 mg/kg dose of aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves, where a percentage glucose reduction of 30.13 and 30.84% reduction in BGL was observed at end of at 6th h, in comparison with baseline BGL. These results are comparable with that of standard treatment (tolbutamide 40 mg/kg) which showed a decrease of 35.12**%** of BGL. The results also showed that there is no significant difference ($P < 0.05$), Figure 1, in mean BGL in standard treatment (tolbutamide 40 mg/kg) and 400 mg/kg of aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves, indicating similar effects and normalization of BGL.

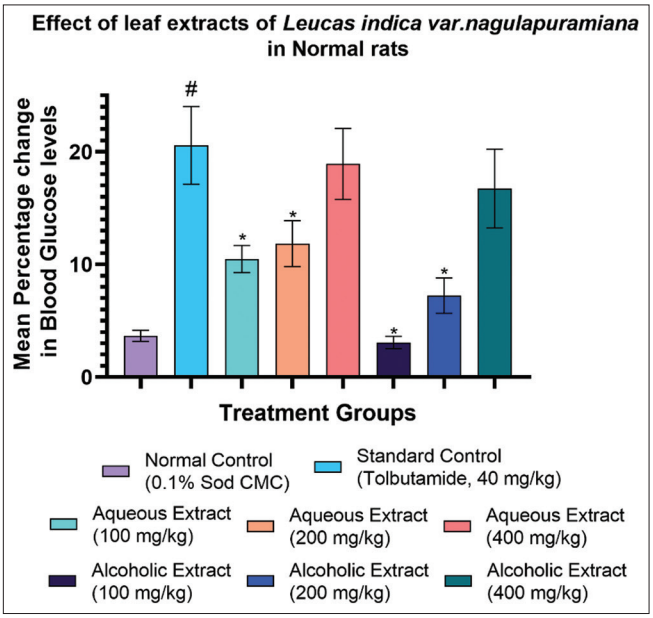

Figure 1: Effect of aqueous and ethanolic extracts of *Leucas indica* var. nagalapuramiana leaves on normoglycemic rats. #P <0.05 vs. Normal control, *P < 0.05 vs. Standard control

Table 1: Hypoglycemic activity of aqueous and ethanolic extracts of *Leucas indica* var. nagalapuramiana leaves in normoglycemic rats

Treatment groups	BGLs (mg/dl)							
	0	1	2	4	6	8	16	24
Normal control (0.1% Sod CMC)	93.37±0.41	91.40±0.53	91.37±0.45	90.61±0.40	89.53±0.34	89.53±0.53	88.65±0.45	88.50±0.47
Standard control (tolbutamide 40 mg/kg)	92.79±0.28	84.69±1.76	79.12±1.63	70.02±1.61[a]	60.20±0.41[a]	66.89±1.27[a]	75.40±0.80[a]	79.54±0.94[a]
Aqueous extract (100 mg/kg)	95.59±0.56	90.40±0.24[b]	85.31±1.71[a]	82.79±1.59[a,b]	81.68±1.64[a,b]	84.02±1.61[a,b]	86.49±1.58[b]	88.07±1.74[b]
Aqueous extract (200 mg/kg)	92.79±1.31	88.81±1.49	82.48±1.17[a]	78.77±1.00[a,b]	74.27±0.95[a,b]	78.86±0.92[a,b]	82.13±0.78[a,b]	87.01±1.82[b]
Aqueous extract (400 mg/kg)	94.54±1.03	87.82±0.9	77.73±1.22[a]	70.95±0.85[a]	66.03±0.61[a,b]	71.05±0.57[a]	77.69±0.84[a]	84.87±0.89
Alcoholic extract (100 mg/kg)	93.43±0.98	92.34±0.93	91.07±0.78[b]	89.53±0.81[b]	88.51±0.93[b]	89.76±0.86[b]	90.78±0.86[b]	91.89±0.82[b]
Alcoholic extract (200 mg/kg)	92.07±0.86	89.34±0.45[b]	85.94±0.92[b]	81.71±1.43[a]	79.49±1.21[a,b]	84.05±1.40[a, b]	87.35±1.04[b]	89.80±1.08[b]
Alcoholic extract (400 mg/kg)	95.84±0.85	88.90±0.70[b]	81.39±1.37[a]	73.90±0.99[a]	66.23±1.14[a,b]	73.79±1.48[a,b]	83.20±2.07[a,b]	90.75±1.31[b]

[a]Compared to the normal control, [b]compared to the baseline BGL. BGL: Blood glucose level, CMC: Carboxymethyl cellulose

Hypoglycemic Activity of the Extracts in STZ-induced Diabetic Rats

The effect of aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves is shown in Table 2. According to our findings, there is no significant change in BGL across the therapy groups. At all time periods, no statistically significant difference in BGL was seen when groups treated with varying doses of the extracts were compared to each other and to the positive control.

Further, within the groups, the results showed that the aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves showed a dose-dependent decrease in BGLs, and with 400 mg/kg dose of ethanolic extracts of *L. indica* var. nagalapuramiana leaves, a percentage reduction of 36.33 and 32.36% in BGL was observed at end of the 6th and 8th h, respectively, in comparison with baseline BGL. These results are comparable with that of standard treatment (tolbutamide 40 mg/kg) which showed a decrease of 39.93 and 36.77% of BGL at end of the 6th and 8th h, respectively. The results also showed that there is no significant difference ($P < 0.05$), Figure 2, in mean BGL in standard treatment (tolbutamide 40 mg/kg) and 400 mg/kg of ethanolic extracts of *L. indica* var. nagalapuramiana leaves, indicating similar effects and normalization of BGL.

DISCUSSION

DM is one of the most serious worldwide health issues of the 21st century. Because present DM drugs have distinct limits, there is a need for alternative treatment strategy

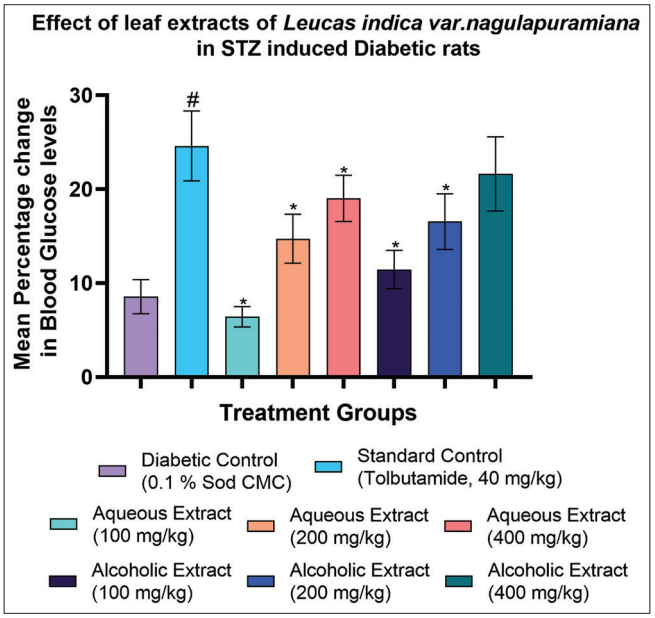

Figure 2: Effect of aqueous and ethanolic extracts of *Leucas indica* var. nagalapuramiana leaves on streptozotocin-induced hyperglycemic rats. #P <0.05 vs. Normal control, *P < 0.05 vs. Standard control

Table 2: Hypoglycemic activity of aqueous and ethanolic extracts of *Leucas indica var.* nagalapuramiana in STZ-induced hyperglycemic rats

Treatment groups	BGLs (mg/dl)							
	0	1	2	4	6	8	16	24
Diabetic control (0.1% Sod CMC)	320.47±4.98	326.34±4.45	333.04±4.67	339.70±4.25	347.23±4.66	354.19±4.69	360.76±4.73	366.67±5.17
Standard control (tolbutamide 40 mg/kg)	319.57±5.15	283.74±7.66[a]	265.88±8.61[a]	239.46±5.32[a]	213.28±6.23[a]	235.41±4.31[a]	263.96±5.78[a]	287.63±6.25[a]
Aqueous extract (100 mg/kg)	319.36±6.24	312.17±5.53	305.36±5.49[b]	295.62±5.57[a,b]	286.78±4.92[a,b]	293.88±5.10[a,b]	300.20±5.47[a,b]	305.82±6.52[a]
Aqueous extract (200 mg/kg)	320.40±6.24	305.43±6.32	293.18±6.79[a]	276.17±7.07[a,b]	251.43±4.35[a,b]	257.70±4.24[a]	262.83±5.05[a]	269.15±4.84[a]
Aqueous extract (400 mg/kg)	319.31±5.15	297.88±6.13	276.45±3.29[a]	260.16±5.32[a]	231.52±3.04[a]	249.28±6.08[a]	254.86±6.27[a]	259.08±6.53[a]
Alcoholic extract (100 mg/kg)	322.24±9.17	304.87±8.13	293.68±9.06[a]	284.00±7.97[a,b]	274.95±6.93[a,b]	282.96±7.70[a,b]	290.90±8.63[a]	299.90±8.67[a]
Alcoholic extract (200 mg/kg)	317.46±10.84	296.56±8.47	278.03±9.56[a]	259.84±7.06[a]	241.30±10.87[a]	260.84±8.91[a]	273.29±10.53[a]	281.02±11.46[a]
Alcoholic extract (400 mg/kg)	321.24±6.54	299.51±4.80	284.04±5.63[a]	265.06±6.31[a]	211.80±7.28[a]	223.78±6.50[a]	245.01±5.10[a]	264.61±7.11[a]

[a]Compared to the diabetic control, [b]compared to the baseline blood glucose level. STZ: Streptozotocin. BGL: Blood glucose level, CMC: Carboxymethyl cellulose

which is safe and pharmacologically effective. Plant-based products, which are freely available and do not require extensive pharmaceutical processing, are being explored for the management of DM.

In the present study, we have evaluated the antidiabetic potential of the aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves, in normoglycemic and STZ-induced hyperglycemic rats. Acute oral toxicity study on rats showed no treatment-related signs of toxicity or mortality for the extracts at oral dose of maximum 2000 mg/kg. STZ-induced diabetes is a well-established model for induction of diabetic in rats. STZ when injected intraperitoneally will induced DM, by damaging beta-cell of pancreatic islets cause hyperglycemia and hypoinsulinemia. This can be confirmed from the results of normal control (0.1% Sod CMC) [Table 2] in STZ-induced hyperglycemic rats where a persistent increase in BGLs was observed during the study period.

In normoglycemic animals, there is no significant change in the baseline BGLs across the groups, however, we have observed reduction in BGL after administration of aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves and standard drug, this indicates that changes induced on BGL were due to treatments received. Both the extracts showed a dose-dependent increase in hypoglycemic activity with best action at 400 mg/kg dose. In normal animals, a percentage glucose reduction was observed with 400 mg/kg dose of aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves, where a 30.13 and 30.84% reduction in BGL was observed at end of the 6th h, in comparison with baseline BGL. These results are comparable with that of standard treatment (tolbutamide 40 mg/kg) which showed a decrease of 35.12% of BGL. Whereas, in STZ-induced diabetic animals, a percentage glucose reduction of 36.33 and 32.36% in BGL was observed at end of the 6th and 8th h, respectively, which was observed with 400 mg/kg dose of ethanolic extracts of *L. indica* var. nagalapuramiana leaves, in comparison with baseline BGL. These results are comparable with that of standard treatment (tolbutamide 40 mg/kg) which showed a decrease of 39.9% of BGL.

Phytochemicals such as alkaloids, phenolic compounds, flavonoids, and terpenoids are responsible for medicinal plants' antidiabetic properties.[14] Flavonoids are known to have insulinogenic and pancreatic beta-cell regenerating activities.[20] Thus, the blood glucose lowering effect of the aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves may be due to the presence of these different secondary metabolites known to have antidiabetic activity with possible additive or synergistic effects. However, detailed pharmacological and biochemical studies are required to identify the exact mechanism for the hypoglycemic and antihyperglycemic effects observed in the study.

CONCLUSION

The present study has revealed the antidiabetic potential of the aqueous and ethanolic extracts of *L. indica* var. nagalapuramiana leaves. However, detailed pharmacological and biochemical studies are required to identify the exact mechanism for the hypoglycemic and antihyperglycemic effects observed in the study and identification and isolation of compound responsible for the antidiabetic activity is required.

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