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Evaluation of the Antidiabetic Potential of Leaves of *Leea rubra* Blume in Streptozotocin-Induced Diabetic Swiss Albino Mice

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ABSTRACT

Leea species are used traditionally to treat diabetes, cancer, rheumatism, and arthritis. This study assessed the antidiabetic potential of *Leea rubra* leaves (LRL). LRL was extracted with methanol, and its antidiabetic potential was evaluated through hypoglycemic screening in normoglycemic mice, oral glucose tolerance test (OGTT), and antihyperglycemic test in streptozotocin (STZ)-induced (45 mg/kg, 3 days) diabetic mice, using two doses of the LRL extract (40 and 80 mg/kg) and glibenclamide (5 mg/kg). For the hypoglycemic and OGTT studies, mice received a single oral dose of LRL extract or glibenclamide, with normal control group receiving vehicle only. For the antihyperglycemic studies, diabetic control mice received vehicle, while treatment groups received daily oral doses of LRL extract or glibenclamide for 15 consecutive days. At the end of treatment, blood glucose levels and body weights were measured. Acute toxicity study, conducted according to OECD guideline 425, showed that the extract is safe with ≥2 g/kg. The extract at dose of 80 mg/kg showed significant hypoglycemic activity ($p<0.001$) and oral glucose tolerance ($p<0.0001$) compared to normal control. In diabetic mice, both doses of the extract showed statistically significant ($p<0.0001$) antihyperglycemic activity at the 15th day of treatment in comparison with the diabetic control mice. The extract at 80 mg/kg showed significant ($p<0.05$) improvement in body weight. These findings confirm the antidiabetic potential of LRL, suggesting that it may serve as a promising candidate for developing novel antidiabetic therapies, and further research is needed to elucidate the underlying mechanisms of action.

Keywords: *Leea rubra*, Streptozotocin, Antidiabetic, Hypoglycemic, Antihyperglycemic.

Introduction

Diabetes mellitus (DM), commonly known as diabetes, is a complicated and pervasive abnormal metabolic condition identified by hyperglycemia, a physiologically unusual situation characterized by continued higher plasma sugar levels.[1] Hyperglycemia due to abnormality in either insulin release or insulin function or both and some problems in the metabolism of carbohydrate, fat, and protein appears in chronic and varied manner.[2] Pancreas is a gland that produces insulin, which plays an important role in controlling blood sugar levels.[3] There are different types of diabetes, such as type 1 diabetes (5-10%), type 2 diabetes (90-95%), gestational diabetes and diabetes caused by other reasons (e.g., monogenic diabetes or drugs).[4] Type 1 diabetes develops when the autoimmune destruction occurs in the beta cells of the pancreas; as a result, the body produces an insufficient amount of insulin. In contrast, Type 2 diabetes is defined by a combination of insulin resistance, a condition when the body's cells do not respond properly to insulin, and relative insulin insufficiency.[5]

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Chronic hyperglycemia and its associated carbohydrate, fat, and protein metabolic dysfunctions exert deleterious effects on various organs and systems in the body, contributing to long-term damage and dysfunction. Organs particularly susceptible to the consequences of sustained high blood sugar levels include the eyes, kidneys, nerves, heart, and blood vessels.[6] The complications arising from diabetes span microvascular and macrovascular diseases, encompassing conditions like retinopathy, neuropathy, nephropathy, and cardiovascular diseases.[7] Diabetes has evolved into a multifaceted global epidemic, affecting individuals across diverse demographic and socioeconomic strata.[8] Factors including genetic predisposition, decisions regarding lifestyle, and environmental influences, play important roles in the improvement and progression of the disease.[9] Modernization-related lifestyle changes, including inactive habits, bad diets, and increasing rates of obesity, further amplify the incidence of diabetes.[10] Globally, in 2021 diabetes affected approximately 537 million people aged 20 to 79 years, and the number is expected to increase to 643 million by 2030 and 783 million by 2045. By 2045, according to the International Diabetes Federation (IDF) 1 in every 10 adults, nearly 783 million, will have diabetes, a rise of 46%. More than 90% of humans with diabetes have type 2 diabetes, which is influenced by socio-economic, demographic, environmental, and genetic factors.[11] Bangladesh is one of the 7 countries in the IDF South-East Asia region; diabetes affects 537 million people worldwide and 90 million people in the SEA region; by 2045 this figure will increase to 151.5 million,[12] and considering substantial regional inequalities in Bangladesh, the combined prevalence of DM linked with hypertension ranges from 6% to 19%.[13] Existing antidiabetic drugs are facing difficulty in providing good glycemic control with limiting side effects such as nausea, abdominal discomfort, metallic taste, flatulence, anorexia, gastrointestinal disturbances, thrombocytopenia, leukopenia, anaphylactic reactions, congestive heart failure, edema, weight gain,

bladder cancer, hepatotoxicity, diabetic macular edema, increased ovulation, and acute kidney injury, as well as ensuring accessibility.[14] Nowadays, the treatment of DM by medicinal plants is being promoted due to its lower cost, easy availability, effectiveness, and fewer side effects.[15] The rising global prevalence of diabetes has accelerated the scientific validation of traditional medicinal plants, with systematic studies confirming the potent antihyperglycemic properties of numerous species.[16] The Vitaceae family includes the genus *Leea*, which is found in multiple regions, such as Australia, Africa, New Guinea, Malaysia, Thailand, Bangladesh, China, and India.[17] *Leea (L.)* has about 70 different species. Among these, Bangladesh is home to 7 species, including *L. asiatica*, *L. guineensis*, *L. indica*, *L. macrophylla*, *L. aequata*, *L. rubra* and *L. alata*. Leaves and roots of *Leea* species, including *L. Indica* and *L. asiatica* have shown antidiabetic effect.[18] The effective antihyperglycemic and hypolipidemic effects of *L. Indica*'s ethanolic leaf extract were demonstrated via reduction of blood sugar and lipid levels in Wistar rats.[19] Additionally, STZ-induced albino rats' pancreatic β-cell damage was repaired by the treatment with the ethanolic extract of *L. Macrophylla* leaves.[20] Chemotaxonomic and molecular phylogenetic data stated that if a few species from genera or families shown previously explained the effect against particular disease conditions, the remaining species would have the same specific biological activity.[21] The explanation for this is that plants under the same phylogenetic groups could possess identical genetics, thus possessing identical chemical-producing abilities, which in turn show identical therapeutic benefits.[21, 22] *L. rubra* was chosen based on this concept. The goal of the study was to estimate the antidiabetic activity of methanolic extract of *L. rubra* leaves (LRL) in streptozotocin (STZ)-induced diabetic Swiss albino mice.

Materials and Methods

Drug chemicals and materials

Streptozotocin (Sisco Research Laboratories Pvt, Ltd, Maharashtra, India), citric acid monohydrate, trisodium citrate dihydrate, distilled water, glucose, glibenclamide (Dibenol, SQUARE Pharmaceutical Ltd) and a glucometer (ACCU-CHEK Active, Germany) were used during the study. Additionally, all the chemicals utilized met analytical grade standards.

Plant materials collection

The leaves of the plant were obtained in December 2021 from Bandarban, a district in the Chittagong Division of South-Eastern Bangladesh. A taxonomist from the Bangladesh National Herbarium in Dhaka identified and authenticated the plant specimen, and a voucher specimen was submitted for future reference (voucher number: DACB66292).

Extraction of the plant material

After thoroughly washing the leaves under running water to remove any dirt, they were exposed to intermittent sunlight while being dried in the shade for 3 weeks. They were dried for 24 hours at 35°C in an oven to facilitate processing. Using a grinding machine, the dried leaf materials were reduced to a coarse powder and kept at room temperature (25±2°C) for future use in the Department of Pharmacy at the University of Rajshahi, Bangladesh. 100% methanol (1.5 liters) was used three times for maceration of 650 grams of powdered leaves. Following filtering through cotton and Whatman No. 1 filter sheets and rotary evaporation at 35°C in accordance with Alam's protocol, an 80-gram concentrated crude methanolic extract (CME) was obtained.[23] This optimized extraction method prepares the way for investigating the medicinal properties of methanolic extract of LRL in herbal remedies.

Animals

A total of 35 Swiss Albino female mice, aged 2 months, weighing 20-30 g, were purchased from the Department of Biochemistry, Jahangirnagar University. Prior to the beginning of experiments, all mice were habituated to their new environmental conditions for one week. The mice were housed with adequate ventilation and kept at a temperature of 25℃ with access to standard pellets from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDRB) and fresh water throughout the experiment. All mice were housed in cages and kept in a 12-hour natural light/dark cycle.

Ethical approval

The study protocol was reviewed and approved by the ethical review committee of the Institute of Biological Sciences, Rajshahi University [ref.72(23)/320/IAMEBBC/IBSc].

Acute oral toxicity study

Acute oral toxicity study of LRL extract was carried out following the OECD 425 guidelines with some modifications.[24] Instead of the sequential dosing protocol, a fixed-dose approach was employed to obtain preliminary toxicity data. After a one-week acclimation period, 5 healthy female mice, each weighing between 20-30 g were used. Two mice were given a 4-hour fast before receiving an oral dose of 2000 mg/kg of the LRL methanolic extract using a gavage procedure. The mouse was closely observed for 24 hours at regular intervals to look out for any immediate toxicological symptoms. The remaining three mice were given the same dose orally for once, and their behaviors were monitored in conjunction with their cages to look out for any discernible changes for a period of 14 days. Then, the dose was increased to 4000 mg/kg, and the similar process was performed on another five fresh female mice.

Grouping and dosing of animals

Twenty mice were categorized into four groups, with five mice in each group. Group I (Normal control, NOC), Group II (Standard, GL-5), Group III (Treatment, CME-40), and Group IV (Treatment, CME-80). Each group was evaluated for the hypoglycemic activity and oral glucose tolerance. Glibenclamide (5 mg/kg) was given to Group II, 40 mg/kg of CME of LRL was given to Group III, and 80 mg/kg of CME of LRL extract was given to Group IV. In the STZ-induced diabetic model, mice were placed into 5 groups, each consisting of 5 mice. All the groups receive intraperitoneal injection of STZ (45 mg/kg) except Group I. Group I was a normal control group (NOC), and while Group II was a negative control (NC) group, both received a dose of Distilled water (DW), Group III was a standard group (GL-5) treated with glibenclamide (5 mg/kg), and Group IV (CME-40) and Group V (CME-80) were treatment groups treated with 40 mg/kg and 80 mg/kg of CME of LRL extract, respectively, for 15 consecutive days.[25]

Measurement of blood glucose level

In each animal model, tail vein blood samples were collected by gently trimming the tip of the tail. Fasting blood glucose level (FBGL) was evaluated using a glucometer and glucose standard strips/kits (ACCU-CHEK Active, Germany). For the examination of the repeated dose effect in the STZ model, BGL in mice was regarded as FBGL when they were fasted for 8 hours.[26] Throughout the fasting period, mice were kept in an empty box to avoid feeding on wood dust for the defined fasting period.

Hypoglycemic activity

Mice with normoglycemia were fasted for 6 hours, during which they had access to water, in order to assess the extract's hypoglycemic effects. A controlled approach was ensured by randomly dividing the animals into four groups, as explained in the grouping and dosing section. With this design, the extract's impact on fasting blood sugar levels under normoglycemic conditions could be precisely examined. This highlights the significance of both the length of the fast and random group assignment for precise evaluations of the extract's possible hypoglycemic effects. Blood samples were taken from animal tail veins at 0, 1, 2, 3, and 4 h following treatment to measure the FBGL.[27] The study utilized an auto-analyzer glucometer and active glucose standard strips/kits sourced from Germany to ensure accurate measurement of FBGL.

Oral glucose tolerance test (OGTT)

OGTT was performed according to the procedure explained by Ayele et al.[25] Tests on mice fasted overnight (14-16 h) were performed to determine the extract's antihyperglycemic capability. The mice were then divided into four groups of five at random after the fasting phase.

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One 2.5 g/kg dose of glucose solution was given orally to the animals just before the treatment. Right after the administration of glucose the Group-I, Group-II, Group-III, and Group-IV were treated with DW, glibenclamide (5 mg/kg), and CME at 40 and 80 mg/kg, respectively and the treatment started with Group-I and ended with Group-IV. Blood samples were drawn from the animals' tail veins in order to measure their FBGL right before treatment and measure their postprandial blood glucose level (PBGL) at 30, 60, and 120 minutes after treatment.[28] % of increment in BGL at the 30th min was measured using the following formula.

% of increment in BGL at 30 min = (M_{30} BGL – M_0 BGL) / M_0 BGL × 100

Where;
M_0 stands for blood glucose level at 0 minutes and M_{30} stands for blood glucose level at 30 minutes.

Induction of diabetes
All procedures for inducing diabetes followed the protocols confirmed by the Animal Models of Diabetes Complications Consortium (AMDCC).[29] Therefore, measurements of both BGL and body weight were taken after female mice underwent an overnight fasting period, prior to starting the induction of diabetes, in order to determine the precise dosage at which STZ may introduce DM in this particular test. It was necessary to starve the mice for 6 hours before injecting them with STZ. Experimental mice were given an intraperitoneal injection of 45 mg/kg STZ dissolved in a newly made 0.1M chilled citrate buffer at a pH set to 4.5 in order to develop diabetes. An injection of 45 mg/kg of body weight was given once daily for 3 consecutive days.[30] The mice had unrestricted access to food and water after thirty minutes of injection. Each animal's plasma fasting blood sugar levels were monitored regularly for three days after the STZ injections. After 72 hours, the FBGL were tested. Animals were considered diabetic if their FBGL reached 200 mg/dL.[24]

Antihyperglycemic activity
Anti-hyperglycemic effect of the extract was evaluated in STZ-induced diabetic mice following repeated dosing. 3 days after STZ injection, diabetic mice were administered DW, a standard drug, or extract once daily for 15 days (from day 0 to day 14) depending on their grouping. The NOC group was likewise given DW for the same duration.
To observe the blood sugar lowering effects, the FBGL was evaluated utilizing an auto-analyzer glucometer on days 0, 5, 10, and 15 following fasting for 8 h. The experimental mice's tail vein was used to draw blood, and the results were presented in mg/dL of blood. This experiment was conducted following the methodology explained by Geetha,[31] and Joseph et al.[32]

Body weight determination
Evaluations were conducted to see the effects of the extract or the standard drug on the body weight changes in STZ-induced diabetic mice. With the use of an electronic balance, the mice's body weight was recorded for the treated and control groups both on 1st day of the treatment and at the 5th, 10th, and 15th day of the therapy in order to track changes in body weight across the treatment period.[33]

Statistical analysis
The results were presented as mean ± SEM using Microsoft Excel 2016 computer program and Graphpad prism version 8. Statistical differences between groups were assessed using one-way analysis of variance (ANOVA) followed by Dunnett test. Results were regarded as significant when *p*-values were less than 0.05.

Results and Discussion

The antidiabetic potential of LRL extract was investigated using a well-established mice model of diabetes, using STZ-induced diabetic mice. The use of STZ to induce diabetes in animals is widely accepted due to its ability to closely replicate many characteristics of human DM.[34, 35] STZ-induced diabetes in rodents is typically associated with symptoms such as hyperglycemia, glycosuria, polydipsia, polyphagia, and significant body weight loss, which differ markedly from the normal physiological conditions of healthy rodents.[35] STZ is a preferred agent in diabetes research, primarily because of its prolonged half-life, which results in sustained hyperglycemia over several days. Unlike other diabetic-inducing agents, STZ is related to a decreased chance of ketosis and reduced death rates.[24] Upon administration, pancreatic β-cells uptake STZ through the GLUT 2 transporter, where it fragments DNA and induces death of β-cells. The production of nitric oxide, DNA methylation through the formation of carbonium ions (CH_3^+), and free radical generation as hydrogen peroxide are major pathways linked to cell death, which cause extensive DNA damage in the β-cells of islets of Langerhans, resulting in β-cell destruction and development of type-2 diabetes.[36]

Acute toxicity study
The acute toxicity test result showed that an oral dose of 2 g/kg of crude methanol extract (CME) of LRL was safe. After 24 hours, it was discovered that the animals tolerated the dose, and within 14 days, no mice exhibited any symptoms of toxicity. During the initial 24 hours and for a maximum of 14 days of investigation at 2 g/kg, CME did not result in any changes in normal behaviors in the current investigation. Two of the five mice passed away at the maximum dose of 4 g/kg after the dosage was increased to that level. Consequently, the extract's oral median lethal dosage (LD_{50}) is between 2 to 4 g/kg.

Hypoglycemic activity
In the current study, the administration of LRL extract at both examined doses (40 and 80 mg/kg) elicited hypoglycemic effect in normoglycemic mice, with CME-80 showing significantly higher hypoglycemic activity than CME-40, comparable to GL-5. The results are presented in Table 1. There was no significant difference in the FBGL between groups prior to administering the test samples at 0 hour. GL-5 reduced the BGL significantly at the 1st hour ($p<0.01$), 2nd, 3rd, and 4th hour ($p<0.0001$) in comparison with NOC. According to previous findings glibenclamide produces a hypoglycemic effect through the enhanced release of insulin from pancreatic β-cells.[37]

Table 1: Hypoglycemic effect of *Leea rubra* leaf methanolic extract on fasting blood glucose level

Groups	FBGL (mg/dL)				
	0 hour	**1st hour**	**2nd hour**	**3rd hour**	**4th hour**
NOC	116.40 ± 3.89	110.99 ± 3.15	107.03 ± 3.10	101.26 ± 2.51	95.86 ± 2.51
GL-5	109.91 ± 3.32$^{A\Phi}$	94.78 ± 2.10$^{A\beta}$	81.08 ± 3.82$^{A\delta}$	69.55 ± 2.71$^{A\delta}$	61.62 ± 2.31$^{A\delta}$
CME-40	120.72 ± 1.27$^{A\Phi}$	110.27 ± 1.84$^{A\Phi}$	103.78 ± 1.08$^{A\Phi}$	97.30 ± 0.57$^{A\Phi}$	92.25 ± 0.67$^{A\Phi}$
CME-80	116.40 ± 3.35$^{A\Phi}$	104.87 ± 3.62$^{A\Phi}$	94.06 ± 3.48$^{A\alpha}$	84.33 ± 3.80$^{A\beta}$	79.64 ± 3.48$^{A\gamma}$

Values are mean ± SEM; n = 5; One way ANOVA was used for the analysis; ^{A}compared to normal control; NOC: Normal control (non-diabetic, vehicle only), GL-5: Glibenclamide at 5 mg/kg, CME-40: Crude methanolic extract at 40 mg/kg, CME-80: Crude methanolic extract at 80 mg/kg; $^{\Phi}p>0.05$, $^{\alpha}p<0.05$; $^{\beta}p<0.01$; $^{\gamma}p<0.001$; $^{\delta}p<0.0001$;

In mice under normal fasting conditions, the hypoglycemic effect of CME-80 became evident after 2 hours of treatment. Throughout the treatment period, LRL extracts exhibited a dose-dependent hypoglycemic activity. The reduction in FBGL by CME-40 was not significant throughout the treatment period in comparison with NOC. On the other hand, CME-80 reduced FBGL significantly at the 2nd hour

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($p<0.05$), 3rd hour ($p<0.01$), and 4th hour ($p<0.001$) in comparison with NOC.

Studies have reported that plant extracts containing various secondary metabolites like alkaloids, phenols, flavonoids, saponins, tannins, polysaccharides, coumarins, and terpenes protect pancreatic beta cells, enhance insulin signaling, reduce oxidative stress and inflammation, activate AMPK, and inhibit carbohydrate digestion and absorption.[38] The observed changes in blood glucose levels across different time points suggest a dynamic influence of the extract. Notably, the mean values for CME-40 and CME-80 hinted at potential hypoglycemic effects compared to the NOC and GL-5. This study provides a foundation for exploring the extract's potential in glycemic control, with promising implications for diabetic and pre-diabetic conditions.

Oral glucose tolerance test (OGTT)

OGTT was conducted to assess impaired glucose tolerance (IGT) and/or type 2 DM (T2DM).[39] To evaluate changes in glucose metabolism following glucose administration, we employed the OGTT model.[25] Elevated blood glucose levels trigger insulin secretion, which promotes glucose utilization and typically restores normal levels within two hours.[27]

In this investigation, after 30 min of glucose administration, all groups exhibited hyperglycemia, where the maximum increase was achieved by NOC (134.22%) and the minimum increase was achieved by the GL-5 (55.87%) group. The normalization of postprandial blood glucose levels to baseline took over 2 hours in the non-treatment group (NOC). Conversely, the administration of the CME-80 and GL-5 achieved the restoration of blood glucose levels to baseline within or less than a 2-hour timeframe. The increase in PBGL was significantly ($p<0.0001$) lower than NOC for GL-5, CME-40, and CME-80 at the 30th & 60th min of glucose loading. At the end of the experiment in the 120th min, a significant decline was noted for CME-40 ($p<0.05$) and CME-80 ($p<0.001$). The findings of OGTT are represented in Table 2.

Table 2: The effect of methanolic extract of *Leea rubra* leaf on oral glucose tolerance test.

Groups	FBGL (mg/dL)	PBGL (mg/dL)			% BGL increment at 30th min
	0 min	30th min	60th min	120th min	
NOC	122.16 ± 1.84	286.13 ± 3.80	207.57 ± 3.39	149.91 ± 1.44	134.22
GL-5	115.32 ± 2.96$^{A\Phi}$	179.75 ± 2.81$^{A\delta}$	146.31 ± 2.70$^{A\delta}$	113.88 ± 2.31$^{A\delta}$	55.87
CME-40	117.12 ± 2.48$^{A\Phi}$	226.67 ± 2.38$^{A\delta}$	178.74 ± 2.09$^{A\delta}$	139.46 ± 3.15$^{A\alpha}$	93.54
CME-80	113.15 ± 3.04$^{A\Phi}$	199.28 ± 1.86$^{A\delta}$	153.88 ± 2.18$^{A\delta}$	130.69 ± 2.35$^{A\gamma}$	76.12

Values are mean ± SEM; n = 5; One way ANOVA was used for the analysis; [A]compared to normal control; NOC: Normal control (non-diabetic, vehicle only), GL-5: Glibenclamide at 5 mg/kg, CME-40: Crude methanolic extract at 40 mg/kg, CME-80: Crude methanolic extract at 80 mg/kg; $^{\Phi}p>0.05$, $^{\alpha}p<0.05$; $^{\beta}p<0.01$; $^{\gamma}p<0.001$; $^{\delta}p<0.0001$; Time is noted after glucose loading.

The OGTT revealed intriguing findings regarding the antihyperglycemic potential of *LRL* extract. The baseline blood glucose levels showed a consistent pattern across the groups at time 0, indicating effective randomization and control. At 30 minutes, the extract-treated groups (CME-40 and CME-80) demonstrated significant decrease in PBGL in comparison with the NOC, suggesting an early positive influence. This trend persisted at 60 and 120 minutes, reinforcing the potential sustained antihyperglycemic effects of the extract. The results align with the premise that *Leea rubra* Blume may possess compounds contributing to improved glucose tolerance.

Antihyperglycemic activity

The administration of STZ results in the destruction of the β-cell, causing a temporary increase in BGL. STZ hinders insulin secretion, inducing a state of insulin-dependent diabetes mellitus. These effects stem from its distinctive chemical properties, particularly its potent alkylating activity.[40] The initial effect of STZ administration is a rapid increase in BGL, which typically continues for 5 to 10 days due to the production of ROS and free radicals that exacerbate cellular injury.[41] After this phase of acute hyperglycemia, the condition generally stabilizes, interventions like LRL extract can be assessed for their antidiabetic activity. A noteworthy decrease in FBGL was observed in all treatment groups in comparison to the NC group. Throughout the 15-day treatment period, the antihyperglycemic effect of LRL extract on FBGL in diabetic mice was evaluated once every 5th day. GL-5 and CME-80 showed significant variation ($p<0.0001$) in FBGL at the 5th day of treatment in comparison with NC. CME-40 also showed a significant difference ($p<0.001$) in FBGL at the 5th day of treatment in comparison with NC. All the treatment groups exhibited significant variation in FBGL ($p<0.0001$) at both the 10th and 15th days of treatment in comparison with NC. GL-5 caused the highest decline in FBGL on the 15th day. CME-80 caused a higher reduction in FBGL than CME-40 throughout the treatment periods. The results of the antihyperglycemic activity test are represented in Table 3. This experiment aligns closely with the hypoglycemic activity test and OGTT, demonstrating the antidiabetic effect of LRL extract. As mentioned earlier, various secondary metabolites like flavonoids, saponins, phenolic compounds, etc., found in different plant species have exhibited potent hypoglycemic and antihyperglycemic effects.

Table 3: Antihyperglycemic activity of *Leea rubra* leaf methanol extract on fasting blood glucose level in STZ-induced diabetic mice

Groups	FBGL (mg/dL)			
	0 day	5th day	10th day	15th day
NOC	116.76 ± 2.81	113.88 ± 2.76	117.84 ± 2.46	106.67 ± 2.98
NC	269.55 ± 2.46$^{A\delta}$	324.69 ± 3.80$^{A\delta}$	344.15 ± 3.25$^{A\delta}$	356.14 ± 3.16$^{A\delta}$
GL-5	260.54 ± 3.15$^{B\Phi}$	268.83 ± 2.87$^{B\delta}$	220.18 ± 2.54$^{B\delta}$	195.68 ± 4.35$^{B\delta}$
CME-40	267.39 ± 0.92$^{B\Phi}$	299.46 ± 2.87$^{B\gamma}$	288.25 ± 3.60$^{B\delta}$	264.38 ± 2.47$^{B\delta}$
CME-80	270.63 ± 3.14$^{B\Phi}$	290.45 ± 2.31$^{B\delta}$	264.34 ± 2.74$^{B\delta}$	232.39 ± 4.18$^{B\delta}$

Values are mean ± SEM; n = 5; One way ANOVA was used for the analysis; [A]compared to normal control; [B]compared to negative control; NOC: Normal control (non-diabetic, vehicle only), NC: Negative control (Diabetic, vehicle only), GL-5: Glibenclamide at 5 mg/kg, CME-40: Crude methanolic extract at 40 mg/kg, CME-80: Crude methanolic extract at 80 mg/kg; $^{\Phi}p>0.05$, $^{\alpha}p<0.05$; $^{\beta}p<0.01$; $^{\gamma}p<0.001$; $^{\delta}p<0.0001$.

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Effect on body weight change

The impact of methanolic extract of LRL on changes in body weight was evaluated in mice with STZ-induced diabetes. The comprehensive analysis of body weight dynamics provides valuable information about the physiological effects of STZ induction and the subsequent interventions. The body weight of diabetic and normal mice was not significantly different up to the 5th day of treatment. The NC group, representing the untreated diabetic mice, exhibited a noticeable reduction in body weight at the 10th (p<0.001) and 15th (p<0.0001) days in comparison with NOC. This aligns with the expected weight loss often associated with diabetes. The observed weight reduction in diabetic mice could be attributed to protein wasting due to a lack of carbohydrates available for energy production.[42] At the 15th day, significant weight change was exhibited by GL-5 (p<0.05) and CME-80 (p<0.05) in comparison with NC, displaying a trend toward reducing this weight loss, showcasing a potential protective effect. Figure 1 illustrates the changes in body weight. This result suggests that methanolic extract of LRL has a sustained influence on body weight dynamics. This study lays a foundation for exploring the extract's role in managing hyperglycemia, offering promising avenues for future research in the realm of antidiabetic interventions. However, further statistical analysis is essential to confirm the significance of these observations.

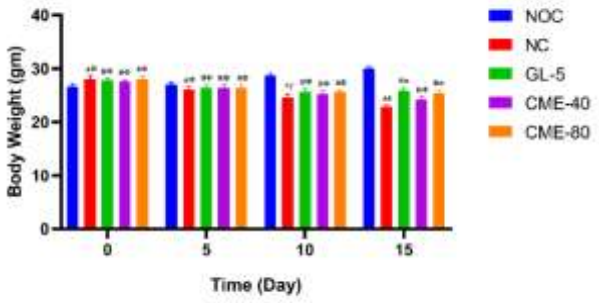

Figure 1: Effect of *Leea rubra* leaf methanol extract on weekly body weight change of diabetic mice after 15 days treatment; Every value implies mean ± SEM; n = 5; Analysis was performed by one way ANOVA; ^{A}compared to normal control; ^{B}compared to negative control; NOC: Normal control (non-diabetic, vehicle only), NC: Negative control (Diabetic, vehicle only), GL-5: Glibenclamide at 5 mg/kg, CME-40: Crude methanolic extract at 40 mg/kg, CME-80: Crude methanolic extract at 80 mg/kg; $^{\Phi}p$>0.05, $^{\alpha}p$<0.05; $^{\beta}p$<0.01; $^{\gamma}p$<0.001; $^{\delta}p$<0.0001.

Conclusion

This investigation highlights the promising antidiabetic effect of *Leea rubra* leaf methanol extract. The hypoglycemic effects observed in mice with normoglycemia and oral glucose loading suggest a potential role in glycemic control. Furthermore, the extract demonstrated notable antihyperglycemic effects in streptozotocin-induced diabetic mice, reflected in significant reductions in fasting blood glucose levels. The observed trends in body weight changes suggest a potential effect against diabetes-induced weight loss. These findings warrant further exploration, including in-depth mechanistic studies and clinical trials, to elucidate the molecular pathways involved and assess the extract's therapeutic viability. *Leea rubra* emerges as a candidate for future investigations in the pursuit of the discovery of novel antidiabetic agents with their mechanism of action.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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