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Exploration of Antidiabetic and Anti-inflammatory Effect of *Eriolaena lushingtonii:* A Phytopharmacological Approach

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Abstract

Background: Research validates *Eriolaena lushingtonii's* pharmacological activities, promoting the investigation of novel plant-derived therapies for chronic diseases like diabetes and inflammatory disorders. **Aim:** To evaluate the antidiabetic and anti-inflammatory activity of *E. lushingtonii's* leaf extract (HEL) in *in vivo* models. **Methods:** The hydroalcoholic extract underwent phytochemical screening. In alloxan-induced diabetic rats, water/feed consumption, body weight, fasting blood glucose, serum lipid profile, kidney function indicators, and glycated haemoglobin were used to assess antidiabetic efficacy. **Results:** Rat models exhibiting paw oedema induced by carrageenan and dextran were used to assess anti-inflammatory effectiveness. HEL was administered at doses of 100 mg/kg and 200 mg/kg, and the results were compared to those of standard drugs such as glibenclamide and diclofenac sodium. The phytochemical analysis revealed the presence of glycosides, terpenoids, tannins, flavonoids, alkaloids, phenolics, and saponins, yielding 15.6% w/w. HEL dramatically decreased ($p < 0.001$) water/feed consumption, fasting blood glucose, Low Density Lipoprotein (LDL), triglycerides, and creatinine, while boosting High Density Lipoprotein (HDL), total protein, and body weight in diabetic rats. The levels of HbA1c were also brought back to normal. In carrageenan and dextran models, HEL200 inhibited oedema dose-dependently at 3 and 6 hours, with 39.22% and 32.89% inhibition, respectively. **Conclusion:** The phytochemical abundance of *E. lushingtonii* may elucidate its potent antidiabetic and anti-inflammatory effects. These findings not only support its historical use but also need further investigation into its prospective therapeutic roles in metabolic and inflammatory disorders.

Major Findings: *Eriolaena lushingtonii* (HEL) leaves Hydroalcoholic extract includes several phytochemicals viz.flavonoids, phenolics and alkaloids. It dramatically decreased fasting blood glucose, HbA1c and water/feed consumption and improved body weight in diabetic rats. HEL improved lipid profile by reducing LDL, triglycerides and raising HDL. It also displayed renoprotective properties by decreasing blood creatinine level and increasing total protein level and dose-dependant anti-inflammatory action with considerable reduction of paw oedema in several mice.

Keywords: Antidiabetic, Anti-inflammatory Activity, Carrageenan, HbA1c, *Eriolaena lushingtonii*, Renal Function

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1. Introduction

Chronic diseases, including diabetes and inflammatory disorders, are major causes of death and sickness across the world. Diabetes mellitus, especially Type 2, is a complicated metabolic illness that causes high blood sugar levels that don't go down because the body can't make enough insulin or the insulin doesn't work[1,2]. The International Diabetes Federation (IDF) predicts that the number of people with diabetes will rise from 537 million in 2021 to 643 million by 2030. The immune system uses inflammation as a main way to fight off things like germs, damaged cells, and irritants[3]. Chronic inflammation is linked to several diseases, including cancer, diabetes, heart problems, and neurological disorders. Chronic low-grade inflammation is strongly

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connected to insulin resistance, which means that inflammation is not only related to diabetes[4]; it is a major cause of the condition. Synthetic drugs like metformin and Nonsteroidal Anti- Inflammatory Drugs (NSAIDs) (like Ibuprofen) are the main ways to treat diabetes and inflammation. These drugs may produce long-term adverse effects, such as discomfort in the stomach, liver damage, and nephrotoxicity[5-8].

Interest in herbal medicine and phytotherapy has increased significantly owing to millennia of traditional knowledge and contemporary scientific confirmation of bioactive plant chemicals[9-14]. Medicinal plants provide an abundant supply of natural compounds such as flavonoids, alkaloids, tannins, glycosides, and terpenoids, many of which have considerable antidiabetic and anti-inflammatory effects[15-20]. Studies indicate that around 80% of the world population depends on traditional plant-based medicines for fundamental healthcare requirements. The Ayurvedic system records the use of many indigenous plants for the treatment of "*Prameha*" (analogous to diabetes) and "*Shotha*" (inflammation), especially in India[21-29].

Eriolaena lushingtonii, a lesser-known but historically important plant, has potential for scientific investigation since it is said to have medicinal characteristics. *Eriolaena lushingtonii* is a tiny tree or shrub that belongs to the *Malvaceae* family[30,31]. It is commonly used in Tribal medicine as it helps cure wounds, kill germs, and stop ulcers from forming. *E. lushingtonii*'s leaves and bark are used in many traditional medicine systems to treat skin ailments, ulcers, digestive issues, and inflammation[32-34]. Local folklore and anecdotal evidence support its use for fever, physical pain, and glycemic management, implying potential anti-inflammatory and antidiabetic effects[35].

Phytochemical examination of *E. lushingtonii* identifies flavonoids, phenolics, terpenoids, saponins, and alkaloids, which are associated with antioxidant, insulin-sensitising, and anti-inflammatory properties in other botanical species[36]. Quercetin and kaempferol, both flavonoids, lower blood sugar by improving insulin action, inhibiting α-glucosidase enzymes, and reducing oxidative stress. Tannins and phenolic acids fight inflammation by stopping pro-inflammatory cytokines like Tumour Necrosis Factor (TNF-α) and Interleukin-6 (IL-6) from working. Terpenoids and saponins effectively modulate immune response and mitigate chronic inflammation[17,37].

Eriolaena lushingtonii may have several pharmacological actions via the modulation of oxidative stress, enhancement of glycaemic control, and attenuation of inflammatory pathways[38]. *E. lushingtonii* is backed by anecdotal and ethnomedicinal evidence, although there is a deficiency of scientific data substantiating its therapeutic efficacy, particularly for metabolic and inflammatory disorders. There has been no substantial study to assess the antidiabetic and anti-inflammatory activities of its leaf extract in standardised experimental models[39,40]. This research aims to integrate traditional knowledge with scientific evidence by examining the antidiabetic and anti-inflammatory properties of *E. lushingtonii* leaf extract via proven *in vivo* methodologies. This research assesses the antidiabetic benefits of *E. lushingtonii* leaf extract in alloxan-induced diabetic mice by analysing fasting blood glucose, HbA1c levels, serum lipid profile, total protein, and renal function parameters. It evaluates the anti-inflammatory effects by carrageenan-induced paw oedema and dextran-induced rat paw oedema. It also validates the pharmacological activities of *E. lushingtonii*, encouraging the investigation of novel plant-derived therapies for chronic ailments. If the findings are good, it would be possible to make standardised herbal remedies for diabetes and inflammation. Help protect and use native medicinal herbs in a responsible way. Encourage more mechanistic investigations and clinical trials to improve translational research. Research into plant-based remedies helps find novel medicines and supports the worldwide push for holistic and integrative treatment.

2. Materials and Methods

2.1 Literature Search

This review reported the previous research on the antibacterial activity of medicinal plant extracts. We followed the Prisma guidelines to perform our literature search[17]. The data were gathered from scientific databases such as Scopus, Web of Science, Google Scholar, and PubMed, focusing on entries published between 2014 and 2024. The keywords explored to get appropriate literature for this review were "*Eriolaena lushingtonii* Dunn" and "Endemic species in India". The literature reported a few

articles, mainly 5 articles and 5 databases, only to give the summary of ethanobotanical information, characteristics of the plant and a few reported pharmacological activities, including antimicrobial, anti-inflammatory, and wound healing activities[13,15].

2.2 Collection and Authentication of Plant Material

Eriolaena lushingtonii Dunn was reported by the International Union for Conservation of Nature (IUCN) as red list of threatened species in the Red Data book found in Andhra Pradesh and Tamil Nadu region[10]. The leaves of *E. lushingtonii* Dunn were collected from Mannanur, Nallamalla forest and Nagarkurnool District of the State of Telangana, in India, during August- September in the year 2023[11]. The collection was done by a taxonomist of the Indian Council of Agricultural Research (ICAR) University under a small expedition for the search of plants, ensured by all permissions and licenses required for collection by Forest officers, Telangana, India, with all permissions and licenses obtained under IUCN policy for collection of threatened species, 1989[12]. The plant was authenticated by Dr Vijaya Bhaskar, Department of Botany, Osmania University (OU), and the specimen was submitted to the Herbarium, Hyderabad, Department of Botany, OU, Hyderabad (Voucher No. OUAS-216). After cleaning the leaves, they were cut into pieces (0.5—1.5×1×0.2 cm3) with the help of scissors and knives. They were left for shade drying on the newspapers for ten days. After that, the leaf pieces were dried in a hot air oven at 40°C for an hour just before starting the extraction process to remove the moisture content.

2.3 Successive Extraction Using Soxhlet Apparatus

Shade-dried leaf powder was obtained through a mechanical grinder and sieved to get the powder of a uniform size. The powder of leaves was extracted successively in the Soxhlet apparatus with a hydroalcoholic solvent system consisting of ethanol and distilled water in a 70:30 (v/v) ratio. This solvent system was selected to facilitate the extraction of both polar and moderately nonpolar phytoconstituents. The Extraction temperatures were adjusted to the boiling points of the solvent to allow a faster rate of cycling of fresh solvent. Six hours of duration were allocated to the solvent for hot continuous and successive extraction. The extract obtained was then concentrated and dried in an oven at 45°C. Then the dried extract was used for the phytochemical screening. The dried extract obtained with solvent was weighed, and the yield was calculated with respect to the air-dried weight of the plant material as in Equation 1. The extract was then subjected to physical evaluation for colour, touch, pH and solubility characteristics.

$$\text{Percentage Yield} = \frac{\text{Weight of the crude(mg/g)}}{\text{Weight of the plant material}} \text{ X } 100 \text{ - (I)}$$

2.4 Screening of Phytochemical

The leaf extracts were subjected to preliminary phytochemical screening for the presence of secondary metabolites. Phytochemical tests were carried out adopting standard procedures. Tests were performed for alkaloids, flavonoids, saponins, steroids and terpenoids, phenolic compounds, tannins, glycosides, coumarins, phytosterols, quinones, anthraquinones and resins.

Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds.

2.5 Experimental Animals

Ten-week-old albino Wistar rats weighing 180 to 250 grams were used in this experiment. The animals were kept in a standard environment at 24±2°C and 30–70% relative humidity. The light and dark cycles lasted 12 hours. The animals were given their usual pellets. Good Laboratory Practice ensured proper care for the animals. The rats acclimated to their new environment for a minimum of seven days before the trials. The Committee for Control and Supervision of Experiments on Animals (CCSEA) granted ethical approval for animal testing. The Institutional Animal Ethics Committee (IAEC) approved the experimental methodology (CCSEA/IAEC/JLS/21/04/24/046).

2.6 Acute Toxicity Studies

The study followed Organisation for Economic Cooperation and Development (OECD) guideline 420 for testing new dosage forms in rats for acute toxicity.

The rats used in the tests weighed between 180 and 250 grams, regardless of their sex. Animals received 2000 mg/kg according to OECD recommendations[27]. For 48 hours, the animals were watched for changes in how they acted, how poisonous they were, and how they moved. According to the results of animal behaviour and toxicology studies, the dose for *E. lushingtonii* has been changed to 100 mg/kg and 200 mg/kg, respectively.

2.7 Antidiabetic Activity

2.7.1 Groups and Experimental Protocols

Ten-week-old albino Wistar male rats, with a body weight ranging from 180 to 250 grams, were utilised. The groups under investigation were Group I, which received normal saline to healthy albino Wistar rats; Group II, which was administered alloxan (120 mg/kg, i.p.), Groups III and IV which were treated with hydroalcoholic extract of *E. lushingtonii* leaves (HEL - 100 and 200 mg/kg, p.o.), and Group V which was given Glibenclamide (10 mg/kg, p.o.). Two doses of HEL were selected based on the literature, and Glibenclamide was utilised as a standard medication.

2.7.2 Water, Feed Weight, and Blood Glucose Monitoring

Throughout the 21-day study period, the water consumption, feed consumption, and body weight gain of all the rats were observed[2,4]. Blood samples were taken every 7 days via retro-orbital plexus puncture, and blood glucose levels were measured using an electronic glucometer (Miles Inc, USA) and glucostix (Bayer Diagnostic India Ltd., Baroda).

2.7.3 Induction of Diabetes and Biochemical Analysis

Alloxan monohydrate (120 mg/kg) was administered intraperitoneally to albino Wistar rats in a single dose to induce diabetics[5]. Alloxan was initially weighed for each animal based on its weight and subsequently solubilised in 0.2ml of saline (154mM NaCl) immediately before injection. Rats exhibiting plasma glucose levels exceeding 200 mg/dl were incorporated into the study two days post-alloxan administration. Treatment with HEL extracts commenced 48 hours post-alloxan injection. Rats exhibiting glucose levels over 200 mg/dl were chosen for a diabetic investigation and categorised into five groups, each comprising six animals. Group I consisted of normal rats, group II consisted of diabetic rats that had not been treated, group III consisted of diabetic rats that were treated with HEL 100 mg/kg, orally, group IV consisted of diabetic rats that were treated with HEL 200 mg/kg, orally, and group V consisted of diabetic rats that were treated with glibenclamide 10 mg/kg, orally. All groups received oral treatment once daily for 21 days. Rats in groups I and II were administered a vehicle. Fasting blood glucose levels were assessed at regular intervals of 1, 7, 14, and 21 days.

Blood samples were collected at weekly intervals until the conclusion of the investigation (i.e., 3 weeks). Fasting blood glucose levels were assessed on days 1, 7, 14, and 21 of the research. On day 21, blood was obtained via heart puncture under moderate ether anaesthesia, and serum was isolated for analysis of total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol. The levels of total protein, creatinine, urea, calcium, and uric acid were assessed.

2.7.4 Examination of Total Protein, Lipid Profile, and Kidney Function Tests

An automated analyser evaluated the levels of triglycerides, HDL cholesterol, LDL cholesterol, and total cholesterol in the blood (Beckman Coulter Inc., Ireland)[9,10]. The total protein in the serum was determined using bovine serum albumin as a reference. The Randox Assay kits were used to find out how much creatinine, urea, calcium, and uric acid were in the blood.

2.7.5 Estimating the HbA1c Level

After being lysed with 5ml of water, the erythrocytes were washed with normal saline and then put in an incubator at 37°C for 15 minutes[14]. After centrifuging the contents and throwing away the supernatant, 0.5 millilitres of saline were added. The mixture was heated to 100 degrees Celsius for four hours after adding 4 millilitres of oxalate hydrochloride solution to 2 millilitres of the aliquot. After that, it was cooled and precipitated with 2 millilitres of 40% Trichloroacetic Acid (TCA). After centrifuging the mixture, 0.5ml of the supernatant was added, along with 0.5ml of 80% phenol and 3ml of concentrated sulfuric acid. The colour that was produced was measured at 480nm after 30 minutes. A one per cent fructose solution was

used to make working standards (10–50µg). After 30 minutes, the working standards were filled again with 0.5ml of 80% phenol and 3ml of concentrated sulphuric acid. The reading was obtained at 480nm. The amount of HbA1c was stated in milligrams per gram of haemoglobin.

2.8 Anti-inflammatory Activity

2.8.1 Groups and Experimental Protocols

A total of 24 Wistar rats of both sexes were segregated randomly into four groups. Every group had access to a total of six animals (three males and three females) housed in separate enclosures. The oral administration procedure was conducted as follows: Individuals in Group 1 received distilled water orally as a control. The second group received Diclofenac sodium (10 mg/kg/bw) (DS10) as a positive control. Participants in Groups III (HEL100) and IV (HEL200) were administered extracts at doses of 100 and 200 mg/kg body weight of *E. lushingtonii,* respectively.

2.8.2 Carrageenan-induced Rat Paw Oedema Method

The acute anti-inflammatory effect was evaluated by carrageenan-induced rat paw oedema according to the method oedema was induced by injection of 1% suspension of carrageenan in 0.9% sterile saline solution into the right plantar region of the rat[15,16]. The plant extract (100 and 200 mg/kg), Diclofenac sodium (10 mg/kg per body weight), or vehicle was administered orally 1 h before injection of carrageenan. Paw volume was measured with a digital vernier calliper 3 and 6 hours after injection. The percentage inhibition was calculated using the formula (2) given below:

$$\text{Percentage inhibition} = \frac{(Ct - Co)\ \text{control} - (Ct - Co)\ \text{treated}}{(Ct - Co)\ \text{control}} \times 100 \text{----} (2)$$

Where Co = Volume of oedema in the control group and Ct = Volume of oedema in the test group

2.8.3 Dextran-induced Rat Paw Oedema Method

The paw oedema was induced in the right hind paw by sub-plantar injection of 0.1mL of freshly prepared 1% dextran solution[19-21]. The plant extract (100 and 200 mg/kg), Diclofenac sodium (10 mg/kg per body weight), or vehicle was administered orally 1 h before injection of dextran. Paw volume was measured with a digital vernier calliper 3 and 6 hours after injection. The percentage inhibition was calculated using the formula (2) given above.

2.9 Statistical Analysis

The raw data collected, analysed and represented as mean ± Standard Error Mean (SEM). The calculations were performed using the statistical program GraphPad Prism (Version 10.2.2). The tests were analysed using Analysis of Variance (ANOVA), followed by both Kruskal-Wallis and Dunnett multiple comparison tests (n=6). ***P < 0.001, **P < 0.01 and *P < 0.05 vs. control. ###P < 0.001, ##P < 0.01 and #P < 0.05 vs. Standard.

3. Results

3.1 Plant Characterisation, Morphology and Botanical Characters

Eriolaena. lushingtonii Dunn in Bull. Misc. Inform. Kew 1915: 88 1915 & in Gamble, Fl. Madras 1: 109.1915; Malick in B.D. Sharma and Sanjappa, Fl. India 3: 417. 1993; Ellis, Fl. Nallamalais 1: 57. 1987.Type: India, Andhra Pradesh, Chelama, A.W. Lushingtons.n. (Holotype: Acc. No. K000380321). Moderate tree; bark greyish; young branches stellately pubescent. Leaves up to 6-9×5-10 cm, orbicular, cordate at base, acute, sometimes younger leaves are slightly lobed towards the apex, irregularly shortly dentate, stellate hairy above, soft tomentose beneath, 7-nerved from the base; petioles 3-5cm long. Flowers ca 2cm long, slender 2-3-flowered racemes, near the ends of the branches, peduncles longer than leaves, pubescent; flowering buds oblong, constricted at the middle; pedicels jointed above the middle; involucral bracts 2-3, 2-4mm long, linear, little distant from the flower, minute, multisect, caduceus as seen in Figure 1. Flowers yellow. Sepals 4 or 5, linear, wider near the base and apex, very acute, 1.5-2 × 0.2-0.3cm long, pubescent within and tomentose without, glandular at the base inside. Petals 4 or 5, obovate, equaling the sepals, white or yellow; glabrous except for a narrowed densely pubescent base, undulate near the apex. Staminal column is 2cm long; anthers 10-25, linear to oblong, basifixed, irregularly arranged near apex; staminodes absent. Ovary pubescent; style as long as staminal column, densely stellate hairy below, sparsely towards apex; stigma 4-7 lobed, sparsely hairy, the lobes revolute. Capsules

woody, ovoid, ca. 4cm long, 4-7 celled, 5-10 valved; valves smooth, more or less pubescent, usually silky villous at the inner angles. Seeds numerous, winged on one side at the top (Figures 2A-D). The plant is an endemic species of Telangana and AP, which lies in a dry deciduous forest and bears flowers and fruits during the months of April and October. The Indian specimens examined widely include: Andhra Pradesh, Prakasham district, Moulatla RF, 13 July 1984, R.K. Mohan 0405 (CAL); Prakasham district, Rollapenta, 16 August 1972, Ellis 42284 (MH); Rollapenta, 12 May 2017, KC Mohan 009413 (BSID); Kurnool dist., Chelama, 1902, Lushington (K000380321) (K: image); Kurnool district, Chelama 04 July 1963, Ellis 16715 (MH); Telangana, Mahabubnagar, Near Mannanur Core zone, 30 June 1988, V.B. Hosagoudar 86610 (BSID). Note: As examined in the Madras herbaria, specimens reported from Karnataka, Tamil Nadu and Kerala states were misapplied.

3.2 Phytochemical Analysis and Percentage Yield of Hydroalcoholic Leaf Extract of *Eriolaena lushingtonii*

The phytochemical screening results show that the hydroalcoholic leaf extract of *E. lushingtonii* has a lot of biologically active compounds in it, such as flavonoids, alkaloids, phenolics, tannins, saponins, terpenoids, and glycosides. These compounds may be responsible for the antidiabetic and antiinflammatory effects seen in other pharmacological studies. It was determined that the hydroalcoholic extract of *E. lushingtonii* leaves had a percentage yield of 15.6% w/w.

3.3 Antidiabetic Activity

3.3.1 Water/ Feed Consumption and Gained Weight

The administration of HEL dramatically decreased ($P < 0.001$) the amount of water and feed intake in diabetic animals, whereas the untreated diabetic rats (Group II) consumed more water. In a similar vein, the untreated diabetic rats displayed polyphagia and ate more feed than the rats in the treatment and control groups. When compared to the control and treatment groups, the weight gain of the untreated diabetic rats was significantly lower ($P < 0.001$). Treatment with 100 and 200 mg/kg body weight of HEL generally compares favourably to those of glibenclamide, a well-known standard medication for diabetes (Figure 1A-C and Table 1).

3.3.2 Fasting Blood Glucose Level

Upon completion of the experiment, it was discovered that the continuous administration of HEL significantly decreased ($p < 0.001$) the fasting blood glucose level in diabetic rats. Further, the impact was more noticeable

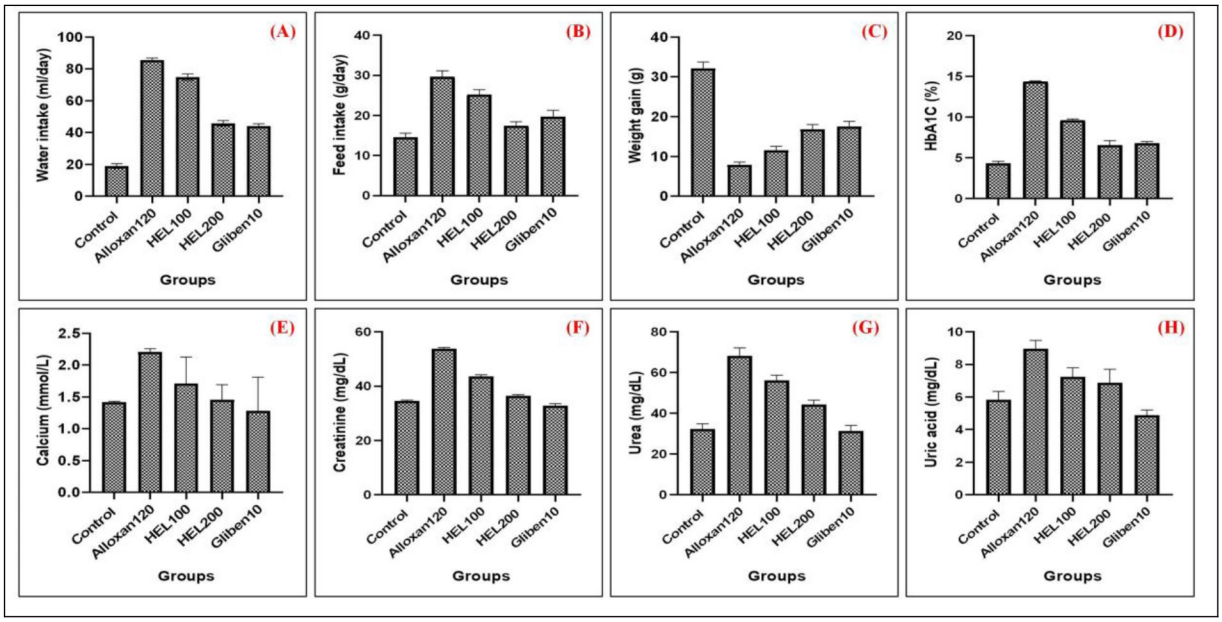


Figure 1. Effect of HEL on water intake, feed intake, weight gain, HbA1C (%), calcium, creatinine, urea, and uric acid in diabetic rats. **(A).** Water intake (ml/day), **(B).** Feed intake (g/day), **(C).** Weight gain (g), **(D).** HbA1C.

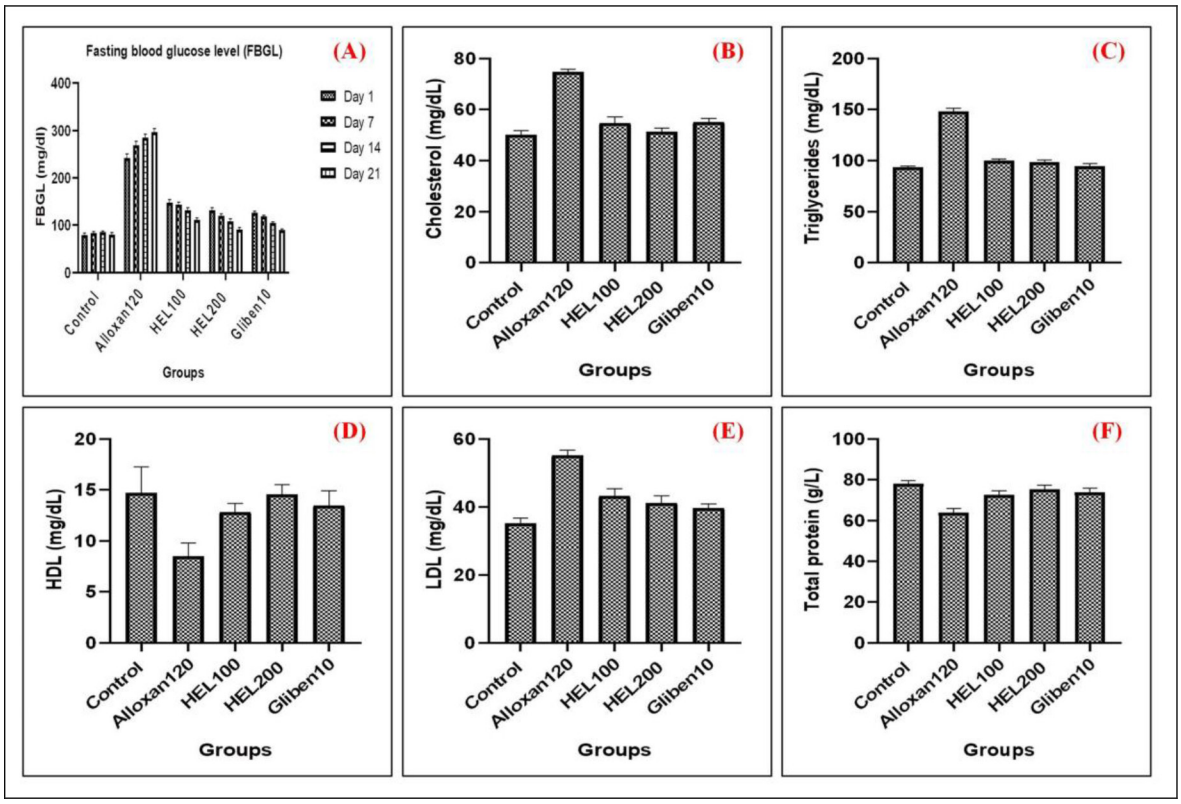


Figure 2. Effect of HEL on fasting blood glucose levels, Cholesterol, Triglycerides, HDL, LDL, and Total protein levels in diabetic rats. **(A).** Fasting blood glucose level (FBG)-mg/dl, **(B).** Cholesterol (mg/dL), **(C).** Triglycerides (mg/dL), **(D).** HDL (mg/dL), **(E).** LDL (mg/dL), **(F).** Total protein(g/L).

Table 1. Effect of oral administration of hydroalcoholic leaf extract of *Eriolaena lushingtonii* on water intake, feed intake, and body weight in diabetic rats

Groups	Water Intake (ml/day)	Feed Intake (g/day)	Weight Gain (g)
Group I - Control (normal saline)	19.12 ± 1.52	14.56 ± 1.02	32.16 ± 1.58
Group II - Alloxan (120 mg/kg, i.p)	85.74 ± 1.23***	29.65 ± 1.47***	7.89 ± 0.72***
Group III - *Eriolaena lushingtonii* (HEL - 100 mg/kg, p.o)	74.89 ± 1.89$^{\#\#\#}$	25.21 ± 1.21$^{\#\#\#}$	11.58 ± 0.98$^{\#\#\#}$
Group IV - *Eriolaena lushingtonii* (HEL - 200 mg/kg, p.o)	45.82 ± 1.78$^{\#\#\#}$	17.41 ± 0.96$^{\#\#\#}$	16.82 ± 1.25$^{\#\#\#}$
Group V - Glibenclamide (10 mg/kg, p.o)	44.23 ± 1.27$^{\#\#\#}$	19.69 ± 1.57$^{\#\#\#}$	17. 53 ± 1.36$^{\#\#\#}$

Data are presented as mean ± SEM (n=6) and analysed using one-way ANOVA followed by Duncan's multiple comparison test. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ compared to control. $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, and $^{\#}P < 0.05$ compared to Alloxan (120 mg/kg, intraperitoneally).

in the rats given 100 and 200 mg/kg bodyweight of the HEL, and it fared better as a standard in the rats which were given glibenclamide (Figure 2A and Table 2).

3.3.3 Serum Lipid Profile and Total Protein

When comparing the diabetic rats to the control group, there was a substantial increase ($p < 0.001$) in the serum concentrations of LDL, triglycerides, and cholesterol, as well as a decrease in HDL and protein. After 21 days of treatment, the levels of blood cholesterol, triglycerides, and LDL were dramatically reduced ($p < 0.001$) by the HEL and glibenclamide, while the concentrations of HDL and protein were elevated to almost normal, as shown in the control group (Figure 2B-F and Table 3).

3.3.4 Kidney Function Parameters

A significant increase ($p < 0.001$) was observed in all the kidney function indices examined in the untreated diabetic rats when compared with the control. The hydroalcoholic extract of *E. lushingtonii* had a positive impact on the

Table 2. Effect of oral administration of hydroalcoholic leaf extract of *Eriolaena lushingtonii* on fasting blood glucose levels in diabetic rats

Groups	Fasting Blood Glucose Level (FBG)			
	Day 1 (mg/dl)	Day 7 (mg/dl)	Day 14 (mg/dl)	Day 21 (mg/dl)
Group I - Control (normal saline)	78.52 ± 4.89	82.47 ± 3.64	85.21 ± 2.58	80.23 ± 3.57
Group II - Alloxan (120 mg/kg, i.p)	241.2 ± 9.65***	268.6 ± 8.57***	284.5 ± 7.58***	295.8 ± 7.96***
Group III - *Eriolaena lushingtonii* (HEL - 100 mg/kg, p.o)	147.5 ± 6.58$^{\#\#\#}$	142.8 ± 5.47$^{\#\#\#}$	131.2 ± 5.89$^{\#\#\#}$	110.7 ± 4.89$^{\#\#\#}$
Group IV - *Eriolaena lushingtonii* (HEL - 200 mg/kg, p.o)	131.4 ± 5.77$^{\#\#\#}$	119.3 ± 5.25$^{\#\#\#}$	108.1 ± 4.82$^{\#\#\#}$	90.58 ± 3.91$^{\#\#\#}$
Group V - Glibenclamide (10 mg/kg, p.o)	125.8 ± 3.54$^{\#\#\#}$	118.7 ± 2.08$^{\#\#\#}$	104.3 ± 2.18$^{\#\#\#}$	89.75 ± 2.03$^{\#\#\#}$

Data are presented as mean ± SEM (n=6) and analysed using one-way ANOVA followed by Duncan's multiple comparison test. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ compared to control. $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, and $^{\#}P < 0.05$ compared to Alloxan (120 mg/kg, intraperitoneally).

Table 3. Effect of oral administration of hydroalcoholic leaf extract of *Eriolaena lushingtonii* on cholesterol, triglycerides, HDL, LDL, and total protein levels in diabetic rats

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Total Protein (g/L)
Group I - Control (normal saline)	50.21 ± 1.57	93.58 ± 1.41	14.72 ± 2.53	35.23 ± 1.51	78.22 ± 1.49
Group II - Alloxan (120 mg/kg, i.p)	74.87 ± 0.98***	148.25 ± 3.08***	8.52 ± 1.28***	55.28 ± 1.43***	63.85 ± 2.06***
Group III - *Eriolaena lushingtonii* (HEL - 100 mg/kg, p.o)	54.73 ± 2.47$^{\#\#\#}$	99.85 ± 1.57$^{\#\#\#}$	12.82 ± 0.86$^{\#\#\#}$	43.21 ± 2.23$^{\#\#\#}$	72.58 ± 2.16$^{\#\#\#}$
Group IV - *Eriolaena lushingtonii* (HEL - 200 mg/kg, p.o)	51.28 ± 1.45$^{\#\#\#}$	98.74 ± 1.91$^{\#\#\#}$	14.58 ± 0.96$^{\#\#\#}$	41.23 ± 2.04$^{\#\#\#}$	75.48 ± 1.84$^{\#\#\#}$
Group V - Glibenclamide (10 mg/kg, p.o)	55.23 ± 1.25$^{\#\#\#}$	94.51 ± 2.59$^{\#\#\#}$	13.48 ± 1.43$^{\#\#\#}$	39.65 ± 1.27$^{\#\#\#}$	73.86 ± 2.08$^{\#\#\#}$

Data are presented as mean ± SEM (n=6) and analysed using one-way ANOVA followed by Duncan's multiple comparison test. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ compared to control. $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, and $^{\#}P < 0.05$ compared to Alloxan (120 mg/kg, intraperitoneally).

Table 4. Effect of oral administration of hydroalcoholic leaf extract of *Eriolaena lushingtonii* on calcium, creatinine, urea, uric acid, and HbA1c in diabetic rats

Groups	Calcium (mmol/L)	Creatinine (mg/dL)	Urea (mg/dL)	Uric Acid (mg/dL)	HbA1C (%)
Group I - Control (normal saline)	1.42 ± 0.01	34.54 ± 0.36	32.33 ± 2.58	5.84 ± 0.51	4.36 ± 0.21
Group II - Alloxan (120 mg/kg, i.p)	2.21 ± 0.05***	53.81 ± 0.47***	68.23 ± 3.84***	8.95 ± 0.52***	14.38 ± 0.04***
Group III - *Eriolaena lushingtonii* (HEL - 100 mg/kg, p.o)	1.71 ± 0.42$^{\#\#\#}$	43.57 ± 0.53$^{\#\#\#}$	56.21 ± 2.58$^{\#\#\#}$	7.24 ± 0.56$^{\#\#\#}$	9.63 ± 0.12$^{\#\#\#}$
Group IV - *Eriolaena lushingtonii* (HEL - 200 mg/kg, p.o)	1.46 ± 0.23$^{\#\#\#}$	36.52 ± 0.31$^{\#\#\#}$	44.27 ± 2.13$^{\#\#\#}$	6.89 ± 0.82$^{\#\#\#}$	6.58 ± 0.54$^{\#\#\#}$
Group V - Glibenclamide (10 mg/kg, p.o)	1.28 ± 0.53$^{\#\#\#}$	32.83 ± 0.74$^{\#\#\#}$	31.45 ± 2.53$^{\#\#\#}$	4.89 ± 0.32$^{\#\#\#}$	6.82 ± 0.18$^{\#\#\#}$

Data are presented as mean ± SEM (n=6) and analysed using one-way ANOVA followed by Duncan's multiple comparison test. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ compared to control. $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, and $^{\#}P < 0.05$ compared to Alloxan (120 mg/kg, intraperitoneally).

kidney function indices of diabetic rats by significantly reducing ($p < 0.001$) the serum concentrations of calcium ion, creatinine, urea, and uric acid as compared to the untreated diabetic rats. The positive impact is, however, more pronounced in the rats treated with 100 and 200 mg/kg body weight extract (Figure 1E-H and Table 4).

3.3.5 Glycated Haemoglobin (HbA1c) Level Assessment

Compared to control animals, HbA1c levels of diabetic animals significantly increased ($p < 0.001$). The HbA1c levels were restored to normal by glibenclamide and HEL (Figure 1D and Table 4).

Table 5. Effect of hydroalcoholic leaf extract of *Eriolaena lushingtonii* on carrageenan-induced rat paw oedema

Parameters		Control (1ml Distilled Water)	DS10	*Eriolaena lushingtonii*	
				HEL100	HEL200
Paw Volume (mm)	3hrs	4.89 ± 0.11	2.83 ± 0.04**	6.07 ± 0.19##	3.99 ± 0.15##
	6hrs	4.42 ± 0.19	2.72 ± 0.13**	3.99 ± 0.05##	3.52 ± 0.32##
% Inhibition	3hrs	1.55 ± 0.25	60.28 ± 5.21**	10.65 ± 2.08##	21.28 ± 2.87##
	6hrs	2.83 ± 0.68	69.54 ± 6.43**	19.67 ± 2.56##	39.22 ± 2.91##

Data were calculated and expressed in mean ± SEM (n = 6), and ANOVA was used to determine the multiple comparisons significance. ***P < 0.001, **P < 0.01 and *P < 0.05 vs. control. ###P < 0.001, ##P < 0.01 and #P < 0.05 vs. Diclofenac sodium (10 mg/kg).

Table 6. Effect of *Eriolaena lushingtonii* extract on dextran-induced rat paw oedema

Parameters		Control (1ml Distilled Water)	DS10	*Eriolaena lushingtonii*	
				HEL100	HEL200
Paw Volume (mm)	3hrs	5.68 ± 0.56	4.88 ± 0.13**	5.09 ± 0.18##	5.22 ± 0.15##
	6hrs	5.23 ± 0.27	4.35 ± 0.08**	4.85 ± 0.24##	4.71 ± 0.18##
% Inhibition	3hrs	1.89 ± 0.54	55.81 ± 4.53**	16.52 ± 1.47##	27.25 ±5.28##
	6hrs	2.55 ± 0.68	59.27 ± 6.21**	19.24 ± 2.28##	32.89 ± 3.32##

Data were calculated and expressed in mean ± SEM (n = 6), and ANOVA was used to determine the multiple comparisons significance. ***P < 0.001, **P < 0.01 and *P < 0.05 vs. control. ###P < 0.001, ##P < 0.01 and #P < 0.05 vs. Diclofenac sodium (10 mg/kg).

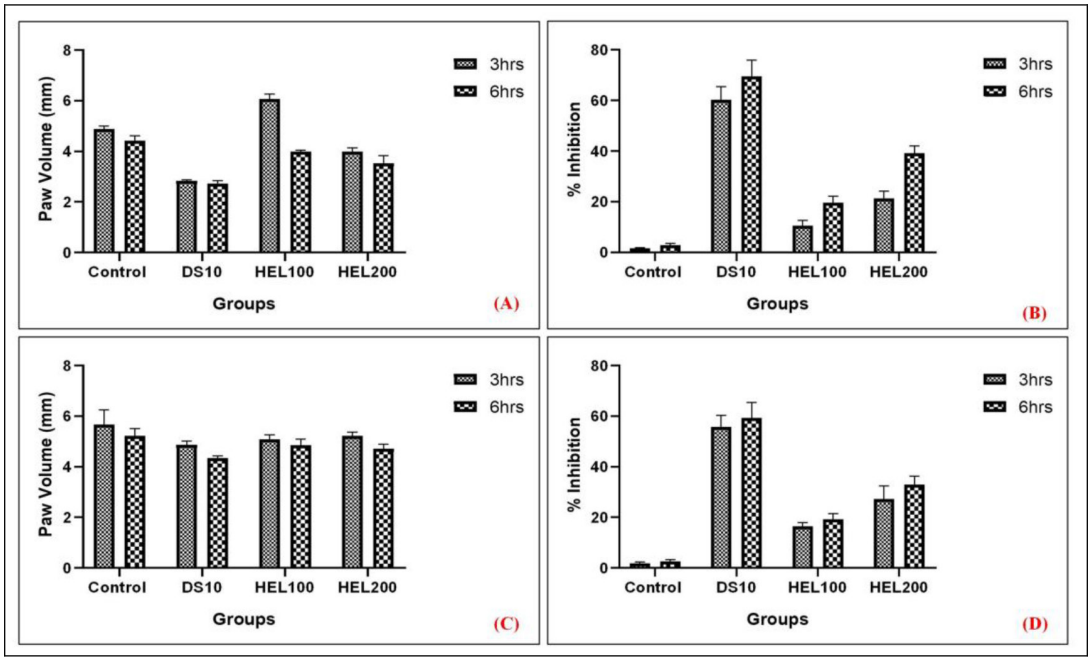


Figure 3. Effect of HEL on carrageenan and dextran-induced rat paw oedema. 1. Carrageenan-induced rat paw oedema: **(A).** Paw Volume (mm) and **(B).** % Inhibition, 2. Dextran-induced rat paw oedema: **(C).** Paw Volume (mm) and **(D).** % Inhibition.

3.4 Anti-inflammatory Activity

3.4.1 Carrageenan-induced Inflammation

In carrageenan-induced animal models, the positive control group (DS10-60.28% (3hrs) and 69.54% (6hrs)) were found to be statistically significant when compared to the control group. The hydroalcoholic extract of *E. lushingtonii* at concentrations 100 and 200 mg/kg inhibited the oedema formation in the third

hour by HEL100-10.65%, and HEL200-21.28%, in a dose-dependent manner, respectively. This effect also extended and significantly increased up to the sixth hour at HEL100-19.67 %, and HEL200-39.22% ($p < 0.001$). All the experimental groups were found to be significant in comparison with the positive control. Results were shown in Table 5 and Figures 3A and 3B.

3.4.2 Dextran-induced Inflammation

In animal models induced by dextran, the positive control group (DS10-55.81% (3hrs) and 59.27% (6hrs)) showed a statistically significant difference compared to the control group. The hydroalcoholic extract of *E. lushingtonii*s at concentrations of 100 and 200 mg/kg showed inhibitory effects on oedema formation in the third hour. The inhibition percentages were as follows: HEL100-16.52%, and HEL200-27.25%. The inhibitory effects were observed in a dose-dependent manner. The effect was observed to persist and intensify up to the sixth hour at the following percentages: HEL100-19.24%, and HEL200-32.89% ($p < 0.001$). All of the experimental groups were shown to be statistically significant when compared to the positive control. The findings were shown in Table 6 and Figures 3C and 3D.

4. Discussion

The purpose of this study was to investigate the potential anti-inflammatory and anti-diabetic effects of the hydroalcoholic leaf extract of *E. lushingtonii* by using experimental models. The findings demonstrated that the extract had powerful pharmacological actions among other plants, lower hyperglycemia and promoted inflammation reduction. It includes bioactive substances, including flavonoids, alkaloids, tannins, phenolics, terpenoids, and saponins. This is probably the reason why it has this effect. *Eriolaena lushingtonii* extract was administered to diabetic rats that had been induced with alloxan. The therapy resulted in a considerable drop in fasting blood glucose levels, an improvement in HbA1c levels, and a partial restoration of body weight in comparison to the diabetic control group. According to traditional pharmacological research, the hypoglycemic effects of a number of medicinal plants have been recorded. These results are consistent with such findings.

The antidiabetic effect may be linked to a variety of mechanisms, one of which is the targeted death of pancreatic beta cells by Alloxan through the use of oxidative stress-induced destruction[7,8]. There is a possibility that the extract, which is abundant in antioxidants, had a role in either the maintenance of beta cells or the promotion of beta-cell neogenesis. Several substances, including flavonoids, have been shown to have an effect on the signalling and release of insulin[9]. Tannins and saponins have the potential to inhibit enzymes such as α-amylase and α-glucosidase, hence delaying the process of glucose absorption. Terpenoids and phenolic compounds might improve the signalling of insulin receptors, which would therefore assist peripheral tissues in absorbing glucose[12]. When compared to a standard anti-diabetic medication like glibenclamide, the extract had glucose-lowering effects that were equivalent to those of the medicine, but with a lesser level of effectiveness. Despite this, the lack of toxicity and the possibility of long-term safety make it more suitable for use as an adjuvant or supportive therapy in the treatment of diabetes[14].

In the carrageenan-induced paw oedema paradigm, which simulates acute inflammation, the *E. lushingtonii* extract dramatically reduced the swelling of the paws at the third and sixth hours. This indicates a reduction in both the early (histamine/serotonin-mediated) and the late (prostaglandin-mediated) inflammatory responses. One of the most significant methods for finding medications that are effective against early-stage inflammation is the dextran-induced paw oedema procedure. The good results imply that *E. lushingtonii* may give advantages in illnesses that are characterised by enhanced mast cell activation, as well as responses that are aided by histamine and serotonin. This is particularly true in cases of allergic reactions, dermatitis, and inflammation that are associated with asthma. The mechanism responsible for the reduction of inflammation is most likely derived from the suppression of pro-inflammatory cytokines, which include TNF-α, IL-1β, and IL-6. These cytokines are often seen at elevated levels in cases of chronic inflammation. By inhibiting the COX pathway[17,19], one may prevent the formation of prostaglandins, which is particularly beneficial in the latter stages of swelling brought on by carrageenan. The process of eliminating Reactive Oxygen Species (ROS) and lowering oxidative

damage, both of which are contributors to the loss of tissue that is brought on by inflammation[19]. Through the modulation of immune cell infiltration and fibroblast proliferation, a decrease in the development of chronic granulomas is achieved. Although the extract demonstrated a statistically significant decrease in both oedema and granuloma weight, it is possible that it might not be as effective as conventional nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac or indomethacin[21]. Not only does the plant extract have the ability to target several pathways, but it also has an enhanced safety profile, which may give significant benefits for the long-term therapy of chronic inflammatory illnesses[22].

The broad phytochemical profile of the extract lends credence to the therapeutic efficiency seen in animals using the extract to treat diabetes and inflammation. Every primary component has a significant influence on the pharmacological effects that are seen[25]. Flavonoids, for instance, possess a multitude of antioxidant properties, an activity that mimics insulin, and anti-inflammatory advantages via reducing the signalling of NF-κB through their actions. The reduction of oxidative stress and the inhibition of enzymes related to inflammation and glucose metabolism are both significant functions that phenolic compounds perform. The way in which the body absorbs glucose and the functioning of the immune system may be influenced by saponins. Tannins and terpenoids have the potential to inhibit the activity of enzymes and alter the function of inflammatory mediators. It is believed that the combined impacts of these compounds are responsible for the extensive spectrum of biological activity that the extract has. Both our results and those of previous research that investigated comparable pharmacological effects in plant species belonging to the family *Malvaceae* are in agreement with one another. For example, *Abutilon indicum* has shown potent anti-inflammatory and anti-diabetic properties, which are believed to be a result of the flavonoid content of the plant. The *Sida cordifolia* plant, which belongs to the family *Malvaceae*, has potent anti-inflammatory and liver-protective characteristics[32]. This lends credence to the hypothesis that *E. lushingtonii* has restorative properties that may be on par with those of other medicinal plants that have been subjected to more research. There is evidence that *E. lushingtonii* has been used in traditional medicine throughout history, which substantiates the effectiveness of this plant. Due to the fact that it grows naturally in a number of regions in India, it is simple to cultivate. In models that are concerned with metabolism and inflammation, it is shown that it effectively functions. When compared to synthetic medications, a natural alternative[34].

The findings are encouraging. Nonetheless, it is important that molecular research was not completely discussed, such as insulin signalling, cytokine regulation, and enzyme inhibition. These may have been investigated further. Before moving further with clinical applications, it is necessary to do research on both the acute toxicity and the long-term safety of the substance. In this work, a crude extract was employed; however, in further research, the focus should be on isolating and characterising specific bioactive components. These conclusions are only based on research conducted on animals and need further verification via clinical studies conducted on humans. Considering the results of this study, it is recommended that future research concentrate on pharmacokinetic and pharmacodynamic studies in order to get a better understanding of absorption, metabolism, and target specificity. The process of ensuring that the extract is consistent and of high quality at all times to facilitate its subsequent formulation into capsules or tablets. The purpose of this *in vitro* study was to shed light on the cellular and molecular pathways. Efficacy and safety in human populations can be validated in further research.

5. Conclusion

The hydroalcoholic leaf extract of *E. lushingtonii* has been shown to have antidiabetic and anti-inflammatory actions, according to the early findings of this study, which are intriguing. In diabetic animal models, the extract was able to considerably lower blood glucose levels, and it was also able to successfully treat both acute and chronic inflammation in traditional in vivo models. Numerous phytochemical components, including flavonoids, tannins, alkaloids, phenolics, and terpenoids, which are recognised for their antioxidant, insulin-sensitising, and anti-inflammatory properties, may be responsible for the pharmacological actions that were discovered. The results not only provide credence to the traditional medicinal

uses that are connected with *E. lushingtonii*, but they also highlight the potential of this plant to serve as a natural, plant-derived alternative or adjunct in the treatment of inflammatory diseases and diabetes. This study establishes a good basis for the creation of innovative herbal products that are produced from *E. lushingtonii*. Although more research is necessary to describe the precise processes, identify active components, and evaluate the long-term safety and efficacy of the product, this research does create a solid foundation. A conclusion may be drawn that *E. lushingtonii* is a potential herb for integrated phytotherapy. Additional research may strengthen its inclusion into evidence-based herbal medicine for the treatment of chronic metabolic and inflammatory disorders.

6. Statement and Declarations

6.1 Ethical Consent

All the permissions for the ethical consent to perform the experiment were approved in the CPCSEA meeting. The protocol was approved, and the experiment was performed within the premises of Gitam School of Pharmacy, Gitam University, Hyderabad, India as per OECD guidelines.

7. Acknowledgment

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Author Contribution

Jyothi Vadthya contributed to the study design, interpretation of results, manuscript revision and Vinyas Mayasa supervised. All authors have read and agreed to the published version of the manuscript.