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Original Article

Antidiabetic potential of leaf extracts of *Ecobolium linneanum* Kurz in streptozotocin-induced diabetic rats

[Potencial antidiabético de extractos de hojas de *Ecobolium linneanum* Kurz en ratas diabéticas inducidas por estreptozotocina]

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Abstract

Context: In a general context, the importance's of several medicinal plants has been explored in treating diabetes mellitus. In this connection, the antidiabetic potentiality needs to be established for one of the medicinally emerging plants *Ecbolium linneanum* Kurz.

Aims: To evaluate the antidiabetic activity of petroleum ether, ethyl acetate, and methanol extracts of *E. linneanum* leaves in streptozotocin-induced diabetic rats.

Methods: Diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/kg BW) in male Wistar rats. Diabetic rats were treated orally with petroleum ether, ethyl acetate and methanol extracts of *E. linneanum* leaves at two doses (100 and 200 mg/kg BW/day) for 14 days. The blood glucose, lipid profile, serum enzyme levels, glycosylated haemoglobin (HbA1c), creatinine and serum urea levels were monitored. Glibenclamide (10 mg/kg BW) was used as a standard hypoglycaemic drug.

Results: The methanolic leaf extract at 200 mg/kg BW showed increasingly substantial ($p<0.001$) antidiabetic activity on day 14 and also significantly reduced ($p<0.001$) SGOT, SGPT, TG, TC, VLDL, LDL, HbA1c and antioxidant enzymes (SOD, CAT and GSH levels) increased significantly ($p<0.001$) compared to diabetic group. Renal parameters also decreased significantly ($p<0.001$) at both doses relative to diabetic control, but petroleum leaf extract and ethyl acetate leaf extract exhibited a less significant effect ($p<0.05$) compared to the methanol extract. All the extracts of leaves showed a protective effect on the oxidative status of the liver at tested concentrations (100 and 200 mg/kg BW).

Conclusions: The present research findings revealed that *E. linneanum* leaf possesses significant anti-hyperglycaemic activity and encourages conventional use in the treatment of diabetes mellitus under the experimental conditions exposed in this study.

Keywords: antidiabetic activity; *Ecbolium linneanum*; oxidative status; phytochemical screening; streptozotocin.

Resumen

Contexto: Se ha explorado la importancia de varias plantas medicinales en el tratamiento de la diabetes mellitus. A este respecto, es necesario establecer la potencialidad antidiabética de una de las plantas medicinales emergentes *Ecbolium linneanum* Kurz.

Objetivos: Evaluar la actividad antidiabética de extractos de éter de petróleo, acetato de etilo y metanol de hojas de *E. linneanum* en ratas diabéticas inducidas por estreptozotocina.

Métodos: Se indujo diabetes mediante inyección intraperitoneal de estreptozotocina (60 mg/kg de peso corporal) en ratas Wistar macho. Ratas diabéticas fueron tratadas por vía oral con extractos de éter de petróleo, acetato de etilo y metanol de hojas de *E. linneanum* en dos dosis (100 y 200 mg/kg PC/día) durante 14 días. Se monitorizaron los niveles de glucosa en sangre, perfil lipídico, enzimas séricas, hemoglobina glicosilada (HbA1c), creatinina y urea sérica. Se utilizó glibenclamida (10 mg/kg de peso corporal) como fármaco hipoglucemiante estándar.

Resultados: El extracto de hoja metanólico a 200 mg/kg de peso corporal mostró una actividad antidiabética cada vez más sustancial ($p<0,001$) en el día 14 y también redujo significativamente ($p<0,001$) SGOT, SGPT, TG, TC, VLDL, LDL, HbA1c y enzimas antioxidantes (niveles de SOD, CAT y GSH) aumentaron significativamente ($p<0,001$) en comparación con el grupo de diabéticos. Los parámetros renales también disminuyeron significativamente ($p<0,001$) en ambas dosis en relación con el control diabético, pero el extracto de hoja de petróleo y el extracto de hoja de acetato de etilo exhibieron un efecto menos significativo ($p<0,05$) en comparación con el extracto metanólico. Todos los extractos de hojas mostraron un efecto protector sobre el estado oxidativo del hígado a las concentraciones probadas (100 y 200 mg/kg de peso corporal).

Conclusiones: Los hallazgos de la presente investigación revelaron que la hoja de *E. linneanum* posee una importante actividad antihiperglucémica y fomenta el uso convencional en el tratamiento de la diabetes mellitus en las condiciones experimentales expuestas en este estudio.

Palabras Clave: actividad antidiabética; *Ecbolium linneanum*; estado oxidativo; estreptozotocina; tamizaje fitoquímico.

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Abbreviations: BW: Body weight; CAT: Chloramphenicol acetyltransferase; CMC: Carboxy methyl cellulose; DM: Diabetes mellitus; GSH: Glutathione; HbA1c: Glycated haemoglobin; LDL: Low density lipoprotein; SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: serum glutamic-pyruvic transaminase; SOD: Superoxide dismutase; STZ: Streptozotocin; TC: Total cholesterol; TG: Triglycerides; VLDL: Very low-density lipoprotein.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease associated with carbohydrate, protein, and fat metabolism owing to extreme or partial insulin release dysfunction both with and without variable levels of insulin resistance (Cressey et al., 2014). It is also linked to impaired glucose metabolism contributing to ketoacidosis and doubles the risk of vascular problems, including cardiovascular disease (Sachan et al., 2009). There might be either genetic or environmental factors responsible for the development of DM (Murea et al., 2012). Insulin therapy is by far the most effective treatment used to counter DM, though several abnormalities, including insulin resistance, fatty liver, brain atrophy, and anorexia nervosa, have been confirmed (Ghosh et al., 2008).

STZ is a glucosamine-nitrosouric molecule derived from *Streptomyces achromogenes* that is therapeutically used as an antineoplastic agent. STZ therapy eventually led to diabetes, impacting pancreatic β-cell degeneration and necrosis (Lenzen, 2008). It is included in the screening of traditional medicines with renowned therapeutic effectiveness in the management of DM (Adeneya and Olagunju, 2009). Natural products are a reservoir of discovering lead molecules and play an imperative role in drug development (Sharifi-Rad et al., 2018). The traditional medicine system has a wide variety of organisms with diverse pharmacological and medicinal properties. Herbs, owing to their potent efficacy, also provide a valuable set of phytochemicals restricting health risks.

Ecbolium linneanum Kurz (*Acanthaceae*) is a well-known medicinal herb, termed 'Blue Fox Tail', and a synonym of *E. ligustrinum* (Vahl) Vollesen (accepted name). *E. linneanum* is highly used for the treatment of jaundice, menorrhea, rheumatism, and anti-inflammatory activity (The Wealth of India, 2006). Anti-helminthic and premenstrual colic effect is found from root juice (Sharma and Sharma, 2010). Traditionally different parts of the plant, like roots, stems, leaves, and whole plant, are used in folklore medicine to treat several ailments, such as cancer, jaundice, and rheumatism. *E. linneanum* is reported to have antimicrobial, cytotoxic, analgesic, antitrypanosomal, anti-inflammatory, anti-plasmodial, antidiarrheal, hepatoprotective, and antioxidant properties (Diyya et al., 2014). Leaves, roots, and flowers contain glycoflavones, orientin, vitexin, isoorientin, and isovitexin, which have been isolated from methanol extract (Ghani, 1998, Diyya and Rao, 2018). Furthermore, a recent study was revealed the antidiabetic and antioxidant properties of flower extracts of *E. ligustrinum* (Rathor et al., 2013). Since the leaves are an abundant source of several phytoconstituents, the existence of traditional claims and findings in literature reports an attempt was made to explore the antidiabetic and antioxidant properties of leaf extracts.

MATERIAL AND METHODS

Chemicals and reagents

Glibenclamide was obtained as a gift sample from Suzikem Drugs Private Limited, Hyderabad, Telangana, India. Streptozotocin was purchased from Sigma-Aldrich, Mumbai, India. Other reagents and chemicals such as total cholesterol, HDL, and triglycerides kit were of analytical grade.

Collection of plant

The leaves of *E. linneanum* were collected from the wastelands of Guntur, Andhra Pradesh, India, (Lat: 16°17'20.87016 N, Lon: 80°22'21.9162 E), as raw material, during the first week of July 2019 and the plant material was authenticated by Dr. K. Madhava Cheety (Rtd.), Plant Taxonomist (IAAT: 3337), Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India.

Preparation of extracts

The collected material was shade dried for a week until leaves became brittle and then were powdered coarsely. About 500 g of the coarsely powdered leaf was taken into a Soxhlet apparatus and successively extracted with petroleum ether (PEEL) (60-80°C) for defatting, ethyl acetate (EAEL), and methanol (MEEL) each for 48 h. The filtrate was concentrated (45°C) via vacuum (rotary evaporator). The obtained concentrated extracts were stored in a refrigerator at 4°C until use.

Preliminary phytochemical screening

The extracts were subjected to qualitative phytochemical tests to confirm the presence of flavonoids, phenols, glycosides, carbohydrates, alkaloids, tannins, sterols, and saponins in the leaf extracts (Trease and Evans, 1989).

Preparation of animals

Healthy adult male Wistar rats weighing about 150-200 g were used for the study. Rats were housed in cages placed in a room with a normal photoperiod (12 h light-darkness cycle), under standard laboratory conditions at 25 ± 2°C and relative humidity of 50 ± 15% (OECD, 2002). During the experimental study, a commercial pellet diet and water were provided *ad libitum*. The study was approved by the Institutional Animal Ethics Committee (IAEC) and the experiments were carried out by following the ethical standards and guidelines of the IAEC. The IAEC reviewed all experimental procedures employed in the study (Regd. No. 1533/PO/a/11/CPCSEA) and were under institutional ethical guidelines.

Acute toxicity

The acute toxicity study was carried out as per OECD guideline No. 420 (OECD, 2002). Wistar rats weighing 150-200 g were used for the study. The animals were randomly divided into one control group and nine treated groups, each containing three animals. They have been retained in animal cubes, supplied with *ad libitum* water, and acclimatized to laboratory conditions for a week before the experiment after fasting overnight. The control group received normal saline and the treated groups received doses of 50, 100, 500, 1000, 2000 mg/kg of PEEL, EAEL and MEEL extracts of *E. linneanum* by intraperitoneal (i.p.) route (OECD, 2002). The animals were monitored continuously for 14 days to observe any behavioural changes, toxicity or death, and other physiological activities.

Oral glucose tolerance test (OGTT)

The OGTT was conducted in healthy adult Wistar rats, which were fasted overnight and randomly assigned into nine groups of six each (Bartoli et al., 2011). Group I was considered to control and received 1% sodium CMC; Group II served as diabetic control; Group III was standard, which was treated with glibenclamide (10 mg/kg BW) by the i.p. route. The test extracts PEEL, EAEL, and MEEL at 100 and 200 mg/kg BW were administered to Groups IV and IX. After 30 mins of drug administration, glucose (3 g/kg BW) solution was administered. Blood samples were drawn at 0 min before glucose load and followed by 30, 60, and 120 min after glucose load by retro-orbital plexus puncture on mild ether anaesthesia (Lopa et al., 2018).

Hypoglycaemic effect

Healthy adult male Wistar rats were fasted over night and randomly divided into nine groups of six in each. Group I received 1% sodium CMC; Group II served as diabetic control; Group III received glibenclamide (10 mg/kg BW) by the i.p. route as a positive control. Test extracts at doses of 100 and 200 mg/kg BW of PEEL, EAEL, and MEEL were administered by i.p. route to Groups IV to IX, respectively. Blood glucose concentrations of animals were assessed after 0, 2, 4, and 6 h of administration of extracts (Ekrem et al., 2005).

Induction of DM by streptozotocin

DM was induced in overnight fasted Wistar rats by intraperitoneal injection of STZ at a 60 mg/kg dose dissolved in citrate buffer (pH 4.5). After 5 h of STZ administration, rats received 2% glucose solution by i.p. route for 24 h to avoid hypoglycaemic mortality. Advancement of diabetes was confirmed after one week of STZ injection by quantifying the blood glucose level in blood samples taken from the retro-orbital plexus puncture in rats. Rats with blood glucose levels above 250 mg/dL were perceived to be diabetic, and those were used in further study. From this point, the experiment was started as it was considered as the 0^{th} day (Mojani et al., 2014).

Treatment protocol

Animals were divided into nine groups; six in each group receiving the treatment scheduled as mentioned below:

- Group I: Control rats receiving vehicle 1% sodium CMC (1.8 mL) orally for 14 days.
- Group II: Rats receiving STZ at a dose of 60 mg/kg BW, i.p.
- Group III: Diabetic induced rats receiving glibenclamide (10 mg/kg BW) for 14 days, i.p.
- Group IV: Diabetic induced rats receiving PEEL 100 mg/kg BW for 14 days, i.p.
- Group V: Diabetic induced rats receiving PEEL 200 mg/kg BW for 14 days, i.p.
- Group VI: Diabetic induced rats receiving EAEL100 mg/kg BW for 14 days, i.p.
- Group VII: Diabetic induced rats receiving EAEL 200 mg/kg BW for 14 days, i.p.
- Group VIII: Diabetic induced rats receiving MEEL 100 mg/kg BW for 14 days, i.p.
- Group IX: Diabetic induced rats receiving MEEL 200 mg/kg BW for 14 days, i.p.

Acute antidiabetic activity

On the 1st day, blood samples were drawn from all groups at 0, 2, 4, 6, and 8 h after the extract was administered. Plasma samples were analysed for glucose levels as indicated above (Viet Hieu et al., 2020).

Sub-acute antidiabetic activity

Blood samples were drawn from the retro-orbital plexus of overnight fasted rats, and basal blood glucose levels were determined using a digital glucometer before and after 1 week of STZ induction (0 days of the experiment). The fasting blood sugar levels of control, standard and treated rats were further tested at 0, 7, and 14 days (Srilakshmi et al., 2019).

Estimation of glycosylated haemoglobin (HbA1c)

HbA1c was analysed from blood samples using the ion exchange resin method, and data was recorded with the help of a semi-automatic analyser (PACE 28, Biomed, Chandigarh, India) (Sherwani et al., 2016). Red blood cells were lysed and passed through a negatively charged resin packed in a column. Positively charged haemoglobin molecules interact with the negatively charged resin, so the negatively charged molecules move at a faster rate. The bound haemoglobins were released by varying solvent conditions injected into the column (e.g., increasing the ion effect of the solvent system by increasing the salt concentration of the solution, increasing the column temperature, changing the pH of the solvent, etc.)

Estimation of lipid profile

Upon completion of the 14th day of treatment with the test extracts, the rats were sacrificed by decapitating them under ether anaesthesia, and blood samples were collected from all group animals, including the control group, which was taken as reference. Serum supernatant was segregated by centrifugation at 2500 rpm for 10 min and used for lipid profile analyses such as total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) measured calorimetrically (Systronics Digital colorimeter) (Neelutpal and Biman, 2017).

Hepatic marker enzymes

Harvested blood was also used to evaluate serum biochemical parameters like SGOT, SGPT (Dumala et al., 2018).

Estimation of liver antioxidant enzymes

Upon sacrificing the rats on the 14th day, the liver was carefully separated from different groups of animals and washed with ice-cold saline. 0.5 g of wet tissue was weighed and homogenized in 0.1 M Tris–HCl buffer, pH 7.4 at 4°C in a semi-auto homogenizer (PACE 28, Biomed, Chandigarh, India) with Teflon pestle rotated at 600 rpm for 30 min. The samples were centrifuged at 2500 rpm by refrigerated centrifuge for 10 min at 4°C. The supernatant was used to test antioxidant enzymes such as reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Czeczot et al., 2006)

Estimation of kidney parameters

Creatinine levels were estimated using the Folin and Wu (1920) process in both serum and urine samples using the creatinine estimation kit as instructed by the manufacturer. Serum urea was measured using the enzymatic UV-kinetic system (Morsy et al., 2010).

Statistical analysis

All the results data obtained were statistically analysed using one-way ANOVA preceded by the Dunnett's test and represented as mean ± standard error of mean (SEM) for six animals from every group. The p-value depicted as $p<0.001$, $p<0.01$, $p<0.05$ was considered statistically significant (Arkkelin et al., 2014).

RESULTS

Preliminary phytochemical screening

In the preliminary phytochemical test, all the solvent extracts of leaf showed the variable distribution of phytoconstituents. Saponins and tannins were commonly present, whereas there was a varying distribution of other phytoconstituents in three solvent extracts. The identified phytoconstituents are listed in Table 1.

Acute toxicity

No behavioural changes or death were observed till the end of the study even at a dose of 2000 mg/kg BW for all rats present in each group.

Oral glucose tolerance test (OGTT)

In normal rats, significant changes in blood glucose levels were observed after glucose loading (3 g/kg BW). The treatment with EAEL and MEEL at 100 and 200 mg/kg BW as well PEEL at 200 mg/kg BW increased the blood glucose levels at 30 min followed by decreased up to 120 min. The EAEL and MEEL extracts at test concentrations showed a significant decrease ($p<0.001$) in blood glucose levels when compared with PEEL at 100 mg/kg BW (Table 2).

Hypoglycaemic effect

All the groups were tested for blood glucose levels at predetermined time intervals (Table 3). PEEL, EAEL, and MEEL at 100 and 200 mg/kg BW were showed a substantial decrease ($p<0.001$) in blood glucose levels after 4 h of treatment, which was equivalent to the impact of glibenclamide.

Acute antidiabetic activity

On single-dose administration of STZ to normal rats, we observed a 3.5 to the 4-fold spike in blood glucose levels and thereby inducing diabetes in rats (Table 4). In STZ-induced diabetic rats which were treated with PEEL, EAEL and MEEL extracts displayed a dose-dependent decline in blood glucose levels after 2 h of treatment, with impact sustaining up to 4 h. Maximal significant ($p<0.001$) reductions of 40.0 and 45.76 % in blood glucose were accomplished by 100 and 200 mg/kg BW of MEEL, respectively after 4 h of treatment. PEEL and EAEL showed similar dose-dependent declines in blood glucose levels when compared among the extracts. EAEL recorded a significant reduction ($p<0.001$) of 34.15 and 40.70% for 100 and 200 mg/kg BW, respectively in blood glucose levels after 4 h. Correspondingly, PEEL developed a significant hypoglycaemic impact ($p<0.001$) with a decline of 28.47 and 35.45%, respectively.

Table 1. Phytochemical screening data of leaf extracts of *E. linneanum.*

Phytochemical	PEEL	EAEL	MEEL
Alkaloids	-	+	+
Carbohydrates	-	-	+
Flavonoids	-	+	+
Glycosides	-	+	+
Phenols	-	+	+
Phytosterols	+	-	-
Saponins	+	+	+
Tannins	+	+	+

PEEL: Petroleum ether extract of *E. linneanum*; EAEL: Ethyl acetate extract of *E. linneanum;* MEEL: Methanol extract of *E. linneanum*; '+' indicates presence; '-' indicates an absence.

Table 2. Effects of *E. linneanum* leaf extracts on oral glucose tolerance test.

Group	Dose (mg/kg)	Blood glucose (mg/dL)			
		0 min	30 min	60 min	120 min
Normal	0.1% Na CMC (1.8 mL)	83.38 ± 1.56	111.85 ± 1.94	93.62 ± 1.64	82.43 ± 1.96
Diabetic control	60 mg/kg STZ	75.36 ± 2.31*	95.73 ± 1.36*	81.52± 1.58*	71.45 ± 1.84*
Glibenclamide	10	77.46 ± 2.31*	98.63 ± 1.36*	85.59 ± 1.58*	73.52 ± 1.84*
PEEL (mg/kg)	100	81.43 ± 1.98**	107.93 ± 2.02**	91.93 ± 1.95**	81.92 ± 2.03**
	200	80.68 ± 1.86**	102.65 ± 2.31**	89.66 ± 1.67*	79.53 ± 2.15*
EAEL (mg/kg)	100	80.93 ± 2.21**	105.56 ± 1.85**	89.52 ± 1.54*	80.18 ± 1.75**
	200	79.13 ± 1.68*	101.72 ± 1.56**	87.12 ± 2.08*	78.78 ± 1.60*
MEEL (mg/kg)	100	79.89 ± 1.85*	103.19 ± 1.48**	88.13 ± 2.16*	78.93 ± 2.05*
	200	78.54 ± 2.34*	99.99 ± 2.11*	86.15 ± 1.78*	77.65 ± 2.17*

Group 1: Normal Control; Group II: Diabetic Control; Group III: Standard; Group IV: PEEL at 100 mg/kg; Group V: PEEL at 200 mg/kg; Group VI: EAEL at 100 mg/kg; Group VII: EAEL at 200 mg/kg; Group VIII: MEEL at 100 mg/kg; Group IX: MEEL at 200 mg/kg. Data are expressed as mean ± SEM (n=6); *p<0.001, **p<0.01 and ***p<0.05 (compared to the control group with the corresponding times).

Table 3. Effects of *E. linneanum* leaf extracts on normal glycaemic animals.

Group	Dose (mg/kg)	Blood glucose (mg/dL)			
		0 h	2 h	4 h	6 h
Normal	0.1 % Na CMC (1.8 mL)	71.39 ± 2.45	76.86 ± 2.98	72.43 ± 2.34	75.98 ± 2.56
Diabetic control	60 mg/kg STZ	75.36 ± 2.31*	95.73 ± 1.36*	81.52± 1.58*	71.45 ± 1.84*
Glibenclamide	10	70.12 ± 2.15*	63.19 ± 1.86*	55.65 ± 2.68*	61.26 ± 2.67*
PEEL	100	71.25 ± 2.18*	69.99 ± 1.95*	65.57 ± 2.10**	69.53 ± 2.48*
	200	70.96 ± 2.34*	67.02 ± 2.07*	62.37 ± 2.84**	64.16 ± 2.38*
EAEL	100	71.02 ± 1.98*	69.28 ± 2.14*	62.66 ± 2.65**	64.53 ± 2.92*
	200	70.91 ± 2.05*	6693 ± 2.64*	59.81 ± 2.87*	62.99 ± 2.47*
MEEL	100	71.08 ± 2.45*	68.52 ± 2.51*	60.12 ± 2.34**	63.64 ± 2.16*
	200	70.43 ± 2.31*	65.62 ± 1.78*	57.53 ± 2.85*	62.12 ± 2.38*

Group 1: Normal Control; Group II: Diabetic Control; Group III: Standard; Group IV: PEEL at 100 mg/kg; Group V: PEEL at 200 mg/kg; Group VI: EAEL at 100 mg/kg; Group VII: EAEL at 200 mg/kg; Group VIII: MEEL at 100 mg/kg; Group IX: MEEL at 200 mg/kg. Data are expressed as mean ± SEM (n=6); *p<0.001, **p<0.01 and ***p<0.05 (compared to the control group with the corresponding hours).

Table 4. Effects of *E. linneanum* on acute antidiabetic activity.

Group	Dose (mg/kg)	Blood glucose (mg/dL)				
		0 h	2 h	4 h	6 h	8 h
Normal	0.1 % Na CMC (1.8 mL)	72.53 ± 1.85	72.98 ± 2.18	73.62 ± 2.54	74.12 ± 2.55	71.92 ± 2.34
Diabetic control	60 mg/kg STZ	268.92 ± 2.58	269.11 ± 2.16	277.52 ± 2.85	288.59 ± 2.45	293.52 ± 2.67
Glibenclamide	10	256.18 ± 2.64* (4.74%)	205.42 ± 2.38* (23.66%)	143.69 ± 298* (48.22%)	190.98 ± 2.69* (33.82%)	228.13 ± 2.51* (22.28%)
PEEL	100	268.43 ± 2.37* (0.20%)	255.04 ± 2.08** (5.23%)	198.52 ± 2.54** (28.47%)	235.16 ± 2.67** (18.51%)	248.53 ± 2.08** (15.33%)
	200	266.16 ± 2.08* (1.03%)	240.19 ± 2.57** (10.75%)	179.14 ± 2.22** (35.45%)	228.52 ± 2.19** (20.81%)	244.62 ± 2.16** (16.66%)
EAEL	100	262.95 ± 2.94* (2.22%)	248.52 ± 2.48** (7.65%)	182.76 ± 2.18** (34.15%)	227.78 ± 2.67** (21.10%)	242.72 ± 2.57** (17.31%)
	200	259.83 ± 2.64* (3.38)	235.62 ± 2.88** (12.44%)	164.57 ± 2.27* (40.70%)	211.61 ± 2.58** (27.70%)	238.56 ± 2.38* (18.60%)
MEEL	100	260.32 ± 2.38* (3.20%)	232.54 ± 2.63** (13.59%)	166.63 ± 2.38* (40.00%)	219.66 ± 2.84** (23.89%)	239.92 ± 2.55** (18.26%)
	200	257.94 ± 2.66* (4.10%)	228.13 ± 2.48** (15.23%)	150.52 ± 2.88* (45.76%)	205.92 ± 2.54* (28.65%)	234.66 ± 2.36* (20.05%)

Group 1: Normal Control; Group II: Diabetic Control; Group III: Standard; Group IV: PEEL at 100 mg/kg; Group V: PEEL at 200 mg/kg; Group VI: EAEL at 100 mg/kg; Group VII: EAEL at 200 mg/kg; Group VIII: MEEL at 100 mg/kg; Group IX: MEEL at 200 mg/kg. Data are expressed as mean ± SEM (n=6); *p<0.001, **p<0.01 and ***p<0.05 (compared to the control group with the corresponding hours).

Sub-acute antidiabetic activity

Throughout the sub-acute diabetic study, the maximum significant decline (p<0.001) of 43.25 and 51.90% in blood glucose was observed for 100 and 200 mg/kg BW of MEEL, respectively, when compared to the standard (66.77%) during the 14-day protocol. EAEL also showed a significant reduction (p<0.001) of 39.25 and 51.16% for 100 and 200 mg/kg BW, respectively for blood glucose levels on the 14th day, and PEEL also produced a significant hypoglycaemic effect (p<0.001) with a decline of 12.94 and 28.36% for both the doses when compared to the standard. On treating the groups with PEEL, EAEL, and MEEL extracts, the data indicates a dose-dependent decline in blood glucose levels on the 7th day but a lesser significant decline in blood glucose levels observed compared to day 14 (Table 5).

Table 5. Effects of *E. linneanum* leaf extracts on sub-acute antidiabetic activity.

Group	Dose (mg/kg)	Blood glucose (mg/dL)		
		1st day	7th day	14th day
Normal	0.1 % Na CMC (1.8 mL)	78.52 ± 2.08	79.86 ± 2.48	77.16 ± 2.66
Diabetic control	60 mg/kg STZ	239.69 ± 2.19	233.42 ± 2.95	228.52 ± 2.17
Glibenclamide	10	230.45 ± 2.33* (3.85%)	155.95 ± 2.31* (33.19%)	102.35 ± 2.74* (55.21%)
PEEL	100	237.53 ± 2.54** (0.90%)	222.69 ± 2.50** (4.60%)	198.96 ± 2.64** (12.94%)
	200	236.96 ± 2.08** (1.14%)	207.57 ± 2.34** (11.07%)	163.72 ± 2.85** (28.36%)
EAEL	100	234.92 ± 2.31* (1.99%)	201.78 ± 2.85** (13.55%)	138.82 ± 2.19** (39.25%)
	200	231.73 ± 2.61* (3.32%)	196.77 ± 2.74** (15.70%)	111.62 ± 2.35* (51.16%)
MEEL	100	232.52 ± 2.55* (2.99%)	198.99 ± 2.15** (14.50%)	129.69 ± 2.09** (43.25%)
	200	230.99 ± 2.46* (3.63%)	181.15 ± 2.33** (22.40%)	109.91 ± 2.53* (51.90%)

Group 1: Normal Control; Group II: Diabetic Control; Group III: Standard; Group IV: PEEL at 100 mg/kg; Group V: PEEL at 200 mg/kg; Group VI: EAEL at 100 mg/kg; Group VII: EAEL at 200 mg/kg; Group VIII: MEEL at 100 mg/kg; Group IX: MEEL at 200 mg/kg. Data are expressed as mean ± SEM (n=6); *p<0.001, **p<0.01 and ***p<0.05 (compared to the control group with the corresponding days).

Table 6. Effects *E. linneanum* leaf extracts on lipid profile.

Group	Dose (mg/kg)	Lipid content (mg/dL)				
		TG	TC	HDL	LDL	VLDL
Normal	0.1 % Na CMC (1.8 mL)	75.42 ± 2.54	80.19 ± 3.08	48.16 ± 2.56	47.39 ± 2.16	16.53 ± 2.38
Diabetic control	60 mg/kg STZ	179.51 ± 2.38	164.59 ± 2.95	20.47± 2.41	115.56 ± 2.34	42.59 ± 2.54
Glibenclamide	10	79.17 ± 2.69*	84.73 ± 2.67*	47.15 ± 2.38*	46.98 ± 2.31*	17.44 ± 2.61*
PEEL	100	111.09 ± 3.04**	114.52 ± 3.12**	29.36 ± 2.16**	72.89 ± 3.05**	27.75 ± 2.08**
	200	97.73 ± 2.68**	98.65 ± 3.06**	38.10 ± 2.84	59.98 ± 2.94**	24.48 ± 2.34**
EAEL	100	101.18 ± 3.15**	102.46 ± 1.99**	35.82 ± 2.91	62.28 ± 2.67**	26.62 ± 2.38**
	200	92.54 ± 3.17**	96.78 ± 3.04**	41.20 ± 2.47*	50.85 ± 2.87*	21.86 ± 2.84*
MEEL	100	96.76 ± 2.67**	94.68 ± 2.94**	38.95 ± 2.65**	56.61 ± 3.07**	24.21 ± 2.92**
	200	82.65 ± 2.58*	88.57 ± 2.48*	43.31 ± 2.38*	42.58 ± 2.68*	19.66 ± 3.08*

Group 1: Normal Control; Group II: Diabetic Control; Group III: Standard; Group IV: PEEL at 100 mg/kg; Group V: PEEL at 200 mg/kg; Group VI: EAEL at 100 mg/kg; Group VII: EAEL at 200 mg/kg; Group VIII: MEEL at 100 mg/kg; Group IX: MEEL at 200 mg/kg. Data are expressed as mean ± SEM (n=6); *p<0.001, **p<0.01 and ***p<0.05 compared to the diabetic group.

Lipid profile

Serum lipid level parameters, such as TG, TC, HDL, LDL, and VLDL, were calculated in all groups and summarized in Table 6. Results showed an increase in TG, TC, VLDL, and LDL values and a decline in HDL values in diabetic-induced rats compared to control group rats. Whereas rats treated with PEEL, EAEL, MEEL, and glibenclamide showed a significant decrease ($p<0.001$) in elevated TG, TC, VLDL, LDL levels and increase in HDL levels when compared with the diabetic group, thereby normalizing the lipid levels.

Hepatic marker enzymes

The levels of SGPT and SGOT in STZ induced diabetic rats were enhanced. High levels of SGPT and SGOT with 200 mg/kg BW EAEL and MEEL were significantly restored ($p<0.001$) and 100 mg/kg BW also significantly restored ($p<0.001$), respectively, but PEEL has a less significant effect ($p<0.01$) relative to the diabetic group (Table 7).

Glycosylated haemoglobin

In diabetic rats, glycosylated haemoglobin level was significantly higher compared to the normal rats. Treatment with 200 mg/kg BW of EAEL and MEEL significantly decreased ($p<0.001$) and 100 mg/kg BW decreased significantly ($p<0.001$), but PEEL showed a much less significant impact ($p<0.01$) compared to the diabetic group and the results are summarized in Table 8.

Liver antioxidant enzymes

The diabetic rats induced by STZ showed a significant decrease in SOD, catalase, and GSH-Px levels compared to control group rats. Treatment with PEEL, EAEL, and MEEL for 14 days resulted in a significant increase in the activity of antioxidant enzymes equivalent to that of diabetic rats. Dose of 200 mg/kg BW of EAEL and MEEL significantly ($p<0.001$) increased, and 100 mg/kg BW also considerably improved ($p<0.001$), but PEEL showed a less significant impact ($p<0.01$) relative to the diabetic group (Table 9).

Kidney parameters

Kidney parameters were assessed in terms of serum urea levels, serum and urinary creatinine levels for normal and diabetic rats treated with PEEL, EAEL, and MEEL leaf extracts. Higher creatinine and urea levels were observed in diabetic rats when compared to normal rats. The creatinine and urea levels significantly ($p<0.001$) decreased in the standard group where glibenclamide was administered and also in PEEL, EAEL, and MEEL at test doses during the 14-day treatment ($p<0.01$) (Table 10).

DISCUSSION

The preliminary phytochemical screening and antidiabetic studies were studied in the current study by considering its traditional claim. Therefore, the study was undertaken to rationalize its reported uses. Initially, the plant material underwent successive Soxhlet extraction with petroleum ether, ethyl acetate, and methanol, resulting in the production of three taken solvent extracts. The extracts were then screened for preliminary phytochemicals using various phytochemical tests and the results suggested that there was a wide equivalent distribution of phytoconstituents three taken solvent extracts. All three extracts confirmed the presence of saponins evenly, whereas other secondary metabolites such as alkaloids, glycosides, flavonoids, sterols, phenols, and tannins were mostly present in EAEL and MEEL, and that might be a factor for the optimum activity of extracts. This is indeed preliminary work, and much more research is required to identify the active ingredients in the extract.

Healthy adult male Wistar rats have been assigned for antidiabetic activity as experimental animals. The extracts were evaluated for antidiabetic activity in STZ-induced rats. Plant extracts demonstrated considerable antidiabetic activity at tested doses, i.e., 100 and 200 mg/kg BW. It was further illustrated by a corresponding decrease in blood glucose levels after the 14th-day concurrent administration of the extract at these concentrations. During this prolonged study, various parameters such as blood glucose, haemoglobin, lipid profile, hepatic marker enzymes, kidney parameters along with enzyme and nonenzymatic activity were also observed.

In the present study, the disproportion between blood glucose levels in the different groups under research revealed a significant improvement in blood glucose levels in the diabetic group when compared to those in the normal group at the end of the 14th day (sub-acute) experimental period. For long-term glycaemic control, haemoglobin is a commonly used as marker. Persistent hyperglycaemia in diabetes is shown to increase the level of HbA1c as a result of haemoglobin glycation. Increased haemoglobin levels are often associated with complications like diabetic retinopathy, nephropathy, and neuropathy. In addition, the relative insufficiency of blood glucose was substantially higher in diabetics than STZ compared to normal insulin, which resulted in reduced protein synthesis in all tissues, which may be due to reduced haemoglobin synthesis in diabetes (Zhong et al., 2019).

Diabetes is connected with proactive alterations in serum lipid, TG, and lipoprotein, which in turn causes an escalating risk of developing cardiovascular disease (Baquer et al., 2011). Elevated levels of serum lipids like cholesterol and TG in diabetic rats could be attributable to the fact that, under normal conditions, insulin stimulates lipoprotein lipase and hydrolyses triglycerides; enhances the absorption of fatty acids into adipose tissue and TG synthesis and prevents lipolysis. If any insulin deficiency is present, lipolysis is not inhibited, leading to hyperlipidaemia.

Table 7. Effects of *E. linneanum* leaf extracts on hepatic marker enzymes.

Group	Dose (mg/kg)	SGPT (IU/L)	SGOT (IU/L)
Normal	0.1 % Na CMC(1.8mL)	44.52 ± 3.54	53.98 ± 3.21
Diabetic control	60 mg/kg STZ	97.63 ± 3.18	105.49 ± 2.98
Glibenclamide	10	45.97 ± 3.67*	55.86 ± 3.15*
PEEL	100	93.33 ± 3.33***	101.77 ± 3.27***
	200	87.51 ± 3.28**	92.65 ± 3.22**
EAEL	100	74.21 ± 3.94**	81.92 ± 3.09**
	200	68.92 ± 3.61*	70.14 ± 2.94*
MEEL	100	63.33 ± 3.36*	73.44 ± 2.87*
	200	54.15 ± 3.42*	67.18 ± 3.08*

Group 1: Normal Control; Group II: Diabetic Control; Group III: Standard; Group IV: PEEL at 100 mg/kg; Group V: PEEL at 200 mg/kg; Group VI: EAEL at 100 mg/kg; Group VII: EAEL at 200 mg/kg; Group VIII: MEEL at 100 mg/kg; Group IX: MEEL at 200 mg/kg. Data are expressed as mean ± SEM (n=6); *p<0.001, **p<0.01 and ***p<0.05 compared to the diabetic group.

Table 8. Effects of *E. linneanum* leaf extracts on glycosylated haemoglobin.

Group	Dose (mg/kg)	HbA1c (%)
Normal	0.1 % Na CMC (1.8 mL)	5.23 ± 1.98
Diabetic control	60 mg/kg STZ	12.71 ± 1.85
Glibenclamide	10	5.14 ± 1.97*
PEEL	100	8.93 ± 1.64**
	200	7.98 ± 1.58**
EAEL	100	7.59 ± 1.76**
	200	6.12 ± 1.47*
MEEL	100	6.92 ± 1.81*
	200	5.85 ± 1.75*

Group 1: Normal Control; Group II: Diabetic Control; Group III: Standard; Group IV: PEEL at 100 mg/kg; Group V: PEEL at 200 mg/kg; Group VI: EAEL at 100 mg/kg; Group VII: EAEL at 200 mg/kg; Group VIII: MEEL at 100 mg/kg; Group IX: MEEL at 200 mg/kg. Data are expressed as mean ± SEM (n=6); *p<0.001, **p<0.01 and ***p<0.05 compared to the diabetic group.

Table 9. Effects of *E. linneanum* leaf extracts on liver antioxidant enzymes.

Group	Dose (mg/kg)	SOD (U/mL)	Catalase (U/mL)	Glutathione peroxidase (U/mL)
Normal	0.1 % Na CMC (1.8 mL)	9.83 ± 2.98	9.32 ± 2.64	5.13 ± 2.68
Diabetic control	60 mg/kg STZ	3.65 ± 2.64	6.72 ± 2.38	3.18 ± 2.54
Glibenclamide	10	9.15 ± 2.88*	8.92 ± 2.51*	5.32 ± 2.19*
PEEL	100	5.46 ± 2.76**	6.12 ± 2.09**	3.76 ± 2.38**
	200	6.98 ± 2.73**	7.57 ± 2.64*	4.16 ± 2.46*
EAEL	100	7.92 ± 2.64*	7.62 ± 2.88*	3.98 ± 2.57**
	200	8.74 ± 2.81*	8.55 ± 2.49*	4.66 ± 2.22*
MEEL	100	8.11 ± 2.55*	8.09 ± 2.67*	4.12 ± 2.94*
	200	8.93 ± 2.61*	8.74 ± 2.84*	4.95 ± 2.87*

Group 1: Normal Control; Group II: Diabetic Control; Group III: Standard; Group IV: PEEL at 100 mg/kg; Group V: PEEL at 200 mg/kg; Group VI: EAEL at 100 mg/kg; Group VII: EAEL at 200 mg/kg; Group VIII: MEEL at 100 mg/kg; Group IX: MEEL at 200 mg/kg. Data are expressed as mean ± SEM (n=6); *p<0.001, **p<0.01 and ***p<0.05 compared to the diabetic group.

Table 10. Effects of *E. linneanum* leaf extracts on kidney parameters.

Group	Dose (mg/kg)	Serum creatinine (mg/dL)	Urinary creatinine (mg/dL)	Serum urea (mg/dL)
Normal	0.1 % Na CMC (1.8 mL)	0.54 ± 1.68	18.92 ± 1.68	24.13 ± 2.08
Diabetic control	60 mg/kg STZ	1.69 ± 1.94	61.92 ± 1.98	65.58 ± 2.15
Glibenclamide	10	0.59 ± 2.08*	20.49 ± 1.77*	26.54 ± 2.11*
PEEL	100	0.85 ± 2.12**	37.45 ± 1.85**	43.42 ± 1.86**
	200	0.73 ± 2.17**	29.18 ± 2.06*	35.16 ± 1.94**
EAEL	100	0.79 ± 1.84*	32.48 ± 2.08**	39.55 ± 1.76**
	200	0.66 ± 1.92*	26.92 ± 2.11*	30.16 ± 1.82*
MEEL	100	0.72 ± 1.86*	27.82 ± 1.97*	34.18 ± 1.84**
	200	0.61 ± 1.79*	23.48 ± 1.88*	28.57 ± 1.68*

Group 1: Normal Control; Group II: Diabetic Control; Group III: Standard; Group IV: PEEL at 100 mg/kg; Group V: PEEL at 200 mg/kg; Group VI: EAEL at 100 mg/kg; Group VII: EAEL at 200 mg/kg; Group VIII: MEEL at 100 mg/kg; Group IX: MEEL at 200 mg/kg. Data are expressed as mean ± SEM (n=6); *p<0.001, **p<0.01 and ***p<0.05 compared to the diabetic group.

The groups administered with plant extracts showed a significant decrease in serum levels of TC, LDL, TG, and VLDL, with a significant increase in HDL levels.

Exceeding high levels of serum biomarker enzymes like SGOT, SGPT noticed in diabetic rats reflect impaired liver function, it might be due to hepatocellular necrosis. While the treatment with extract restored all raised hepatic enzymes to normal levels (Rao et al., 1989). Liver antioxidant enzymes show a decreased level in diabetic rats, which may be due to cellular stress. All the enzymes, SOD, CAT, and GSH levels were restored to normalcy in standard and extract-treated groups, which might be due to the presence of phenolic compounds abundantly in the plant. Renal damage also occurs in diabetes, which was estimated using prognostic markers like serum urea and creatinine (Chen and Fang, 2018). The results in extract-treated groups showed significant restoration compared to the diseased group. Hence, the present study reveals that active control of blood sugar levels could hold back the advancement to diabetic nephropathy.

The report has shown that the extracts of *E. linneanum* could even be incorporated into the resource of herbal formulations that are valuable for the pharmacotherapy of diabetes. The current research also paved the way for further research, in particular concerning the sustainable development of effective formulations for DM from *E. linneanum*.

CONCLUSION

Results displayed that *E. linneanum* extracts possessed antidiabetic activity, particularly methanol extract proving its folklore claim. Additionally, the preliminary phytochemical screening showed the presence of flavonoids, glycosides, phenolic compounds, which might be responsible for the significant activity. Further isolation of active compounds from the extracts and investigations to affirm the activity and clarify the mechanism of action ought to proceed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Srilakshmi N	Narender M	Rajeswari P	Alekhya K
Concepts or ideas	x	x		
Design	x	x		
Definition of intellectual content		x		
Literature search	x			
Experimental studies	x			x
Data acquisition	x		x	
Data analysis	x			x
Statistical analysis			x	x
Manuscript preparation	x			x
Manuscript editing		x	x	
Manuscript review	x	x	x	x

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