ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

Anti-Diabetic Effects of Leucas URTICAEFOLIA -HAM. in STZ-Induced Diabetic Rats

Received: 18 August 2022, **Revised:** 16 September 2022, **Accepted:** 23 October 2022

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Keywords

Leucas Urticaefolia. Pharmacological aspects. anti-diabetic. medicinal plants. STZ-induced diabetic rats

Abstract

Aims/hypothesis The main objective of this study is to explore the anti-diabetic effects of Leucas Urticaefolia (LU) in STZ-induced diabetic rats.

Methods In this research, we executed in vivo tests on a diabetic Wistar rat model. The studies were carried out, and we orally administered a LU extract and a control medication to streptozotocin (STZ)-induced diabetic rats to assess their anti-diabetic properties.

Results We discovered that a LU extract at a therapeutic dose of 400 mg/kg/body weight had a substantial effect on insulin reduction in STZ-induced rats as compared with the control group ($P<0.001$). Our histology results also revealed that the extract significantly improved pancreatic and liver histopathology.

Conclusion/ interpretation In vivo investigations demonstrated that LU has an anti-diabetic effect in STZ-induced diabetic rats.

Abbreviations

LU	Leucas Urticaefolia
OECD	Organization for Economic Cooperation and Development
STZ	Streptozotocin
GHS	Globally Harmonized System
FDA	Food and Drug Administration
IAEC	Institutional Animal Ethical Committee
CPCSEA	

1. Introduction

The Lamiaceae family's genus Leucas is compensated by around 100 species [1]. The genus is represented in the Indo-Pakistani subcontinent by 35 species, the majority of which are shrubs or plants found in hilly or temperate regions [2]. LU is an anthelmintic, haemostatic, stimulant, diuretic and astringent annual plant [3]. Bronchial catarrh, fever, uterine haemorrhages, dysentery, diarrhea, gravel, cystitis, dropsy, skin ailments, calculus, and many forms of mental problems are also treated with the plant [4].

Type 1 diabetes occurs when the pancreas' b-cells failing to create insulin owing to hereditary causes or an autoimmune reaction, whereas type 2 diabetes

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occurs when the pancreas producing insulin but its receptors being unable to function or blocked due to an unhealthy lifestyle and other dynamic variables [5].

The recent study sought to investigate the phytoconstituents of LU as well as its anti-diabetic properties in STZ-induced diabetic rats. We investigated the hypoglycemic/antidiabetic potentials of LU using a hydroalcoholic (70%) crude extract. We discovered that giving this plant extract to rats reduced their blood glucose levels. As a result of our in vitro research, we discovered that this plant has a high antioxidant potential, which might explain its anti-diabetic effects.

2. Methods

Histopathology of Pancreas Control animals' pancreas exhibited islet histopathology and pancreatic parenchymal cell. In diabetic controls, the pancreas revealed considerable hyperplasia and increased voculation, in the pancreatic parenchyma, with fewer islets and modest inflammatory cell infiltration. In diabetic rats treated with plant extracts, pancreas sections revealed moderate congestion of pancreatic parenchyma, islet hyperplasia and an increase in islet number. Glibenclamide enhanced the number of pancreatic islets in diabetic animals.

Histopathology of Liver Control animals' liver histopathology revealed normal hepatocyte morphology. The liver segment under diabetes control displays hepatic congestion at sinusoids and the portal artery, Kuffe cell proliferation, pericentre globular micro-steatosis, mononuclear infiltration and hepatocyte widespread necrosis. The standard control slice demonstrates modest hepatic congestion at the sinusoids and the portal artery, no Kuffe cell growth, pericentre globular micro-steatosis, mononuclear infiltration and slight hepatocyte widespread necrosis. All other low and high dosage of test substance treated groups showed moderate hepatic congestion at portal and sinusoids vessels, decreased Kuffe cell proliferation, pericentre globular micro-steatosis, mononuclear infiltration and minor hepatocyte diffuse necrosis.

Histopathological investigation The pancreas and liver are saline-washed before a little amount of this organ is preserved in 10% formalin. The tissues were subsequently processed using normal histological techniques. Wax bricks were created. Microtome sections then sliced thinly and stained with eosin and haematoxylin.

Acute toxicity class method The acute toxicity study was conducted in line with OECD standard 423. It is a step-by-step process involving three animals of the same sex every phase. Depending on the animal's mortality and morbidity state, some steps may be required to allow judgement on the test material. The strategy is to repair a limited number of animals while creating a suitable database. The approach employs a variety of fixed dosages (2000, 300, 50 and 5 mg/kg bw (body weight)), and the findings permit a chemical to be evaluated and categorized in accordance with the GHS for categorising extract producing toxic effects [6].

The study included three wistar rats were watched hourly for 24 hours after oral injection to assess mortality and identify any variations in behavioural reactions such as respiration, alertness, irritability, aggression, urine, salvation, spontaneous activity, corneal reflex and convulsion, among others. Most crude extracts have an LD50 expected to be high than the fixed weight, this has been utilised as a starting dosage.

For 14 days, the rats were monitored daily to look for fatalities or hazardous indications. Because there was no fatality, the experiment was recurring with the same dose to validate the outcomes. The flow chart of the process used for this method is depicted in Fig. 1.

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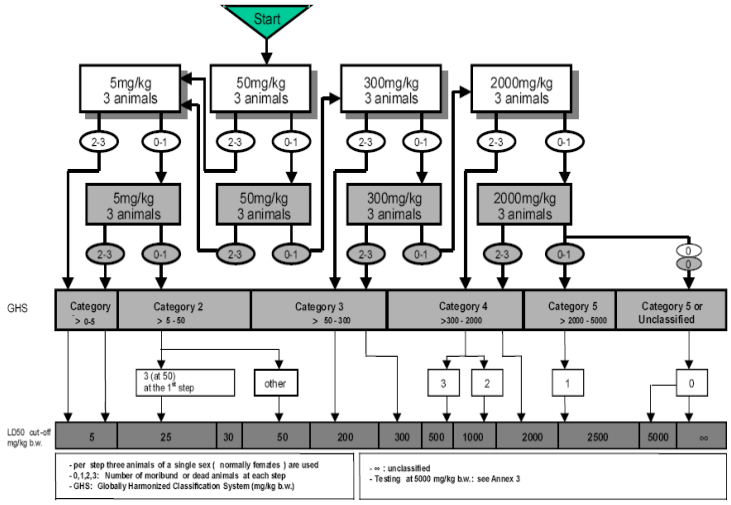

Figure 1 Test procedure with a starting dose of 50mg/kg body weight

Acute toxicity study of EALU and EELU In the acute toxicity trial, EALU and EELU were supplied orally and examination of animals for mortality and central nervous system, and behavioural activities. Even at 2000 mg/kg for the EALU and EELU, no mortality was reported. All of the animals were found to be healthy, and no changes in behavior were seen until the end of the study. In an acute toxicity model research, the EALU and EELU were proven to be very safe up to 2000 mg/kg of body weight, termed as the maximum tolerated dosage (MTD). As a result, 1/5th and 1/10th of MTD were chosen, and efficacious dosages of 200 and 400 mg/kg were determined for additional pharmacological research.

Antidiabetic Study Because of its sensitivity to β-cell toxicity, the STZ-induced diabetic rat other than alloxan [7]. Diabetes might be generated in animals experimented by administering STZ intraperitoneally at a dosage of 60 mg/kg bw. Based on previous research [8], the dosage of STZ used to induce diabetes in the current investigation was selected [9]. Six more rats served as the regular control group.

STZ damages β -cells and induces diabetes in 3 days [10]. The control animals received simply saline injections. The blood glucose concentration was tested 72 hours after STZ injection using a glucometer and a sample of blood from a vein in the tail. Diabetic animals with blood sugar levels of 180 mg/dl [11].

Streptozotocin Induced Acute Toxicity Study and Antidiabetic Study of *Leucas Urticaefolia*

Plant extract used Ethyl acetate extract of *Leucas urticaefolia* (EALU) and Methanol extract of *Leucas urticaefolia* (EELU). Each time the plant extracts were injected, they were suspended in water.

Preparation Glibenclamide is a standard antidiabetic drug used to test the anti-diabetic effects of different hypoglycemic drugs in STZ-induced mild diabetes [12]. It was stored at room temperature.

In distilled water the pills were crushed, suspended and administered at 0.5 mg/kg bw/day for 28 days. According to the FDA, the provided dose was assessed to be comparable to the human therapeutic dose.

Experimental animals Animal home provided healthy Wistar Albino rats weighing 150-200 g of any gender. The IAEC which is approved by the committee for the reason of CPCSEA approved the entire study. The animals were kept in well-ventilated animal homes with a 12-hour light-dark cycle in dry, clean polycarbonate cages. Ad libitum, the animals were fed a regular pellet diet. The animals were fasted overnight but given unlimited access to water throughout the study.

Experimental grouping of animals In the current study, 66 rats (6 normal and 60 diabetes surviving rats) were split into eleven groups of rats to

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test the anti-diabetic of EALU and EELU.

Group I : Rats have unrestricted food and water supplies.

Group II : Diabetic rats have unlimited food and water.

Group III : Diabetic rats were given the usual medication Glibenclamide orally for 28 days at a dose of 0.5mg/kg.

Group IV : EALU 200 mg/kg was given orally to diabetic rats for 28 days.

Group V : EALU 400 mg/kg was given orally to diabetic rats for 28 days.

Group VI : EELU 200 mg/kg was given orally to diabetic rats for 28 days.

Group VII : EELU 400 mg/kg was given orally to diabetic rats for 28 days.

Blood glucose level determination with an electronic glucometer The glucose level was determined using blood drawn from the tail vein. When the bleeding began, the rat was held against the blood glucose test strip, as well as a blood specimen was left to evaporate on the patch, which interacted with the haemoglobin. Gucometer's display reveals the glucose level. Blood sugar levels in control and experimental mice were monitored every 7 days for 28 days. The result was represented in serum mg/dl.

Collection of blood on the morning of the corresponding trial day, at the conclusion of the 4th week of behavior, all rats were slaughtered by cervical dislocation while fasting. Through direct puncture of the heart, Blood was drawn out from the animals. Plasma and serum were centrifuged at 2500 rpm for 10 minutes and kept at –20°C until the biochemical and enzyme assays were done.

Histopathology study Organs from the slaughtered animals, such as the liver and pancreas, were promptly removed and stored in a 10% formaldehyde solution.

Haematological parameters

1. Haemoglobin content (Hb)
2. Glycosylated haemoglobin
3. Glucose

Lipid profile and Serum enzyme

1. Triglycerides (TG)
2. Total cholesterol (TC)
3. Alkaline Phosphatase (ALP)
4. HDL
5. LDL
6. SGOT
7. SGPT

3. Experimental Results

The purpose of this research is to analyse the pharmacological potential of the antidiabetic activity of Methanol and Ethyl acetate exract of Leucas urticaefolia shown in Table 1.

Blood sugar level The level of blood sugar levels in separate groups of animal experiments were monitored at 7-day intervals for a total of 28 days. EELU (200, 400 mg/kg) was shown to be very significant ($p<0.001$) when administered to the experimental groups. The findings are depicted in Table 2.

After treatment, glucose levels in the blood of Streptozotocin diabetic rodents (Group II) were observed to be extremely ($p<0.001$) higher than in normal rats (Group I). Group III through Group VII resulted in a substantial ($p<0.05$; $p<0.01$) decrease in blood sugar level (Group II). Groups V, VI, and VII experienced the greatest drop in blood sugar levels on the 28th day of therapy.

Lipid profile analysis The lipid profiles of many animal experiment groups were examined, and the findings are shown in table 3.

When compared to normal rodents, the quantity of overall cholesterol in the plasma of STZ-induced diabetic rats (Group II) was considerably ($p<0.01$) greater (Group I). When EELU given to the experimental groups (Group VI and Group VII) resulted in extreme substantial ($p<0.001$) decrease (Group II).

EELU administration dosages significantly ($p<0.001$) recovered triglycerides in diabetes caused rats. A high dosage of EELU ($p<0.001$) had a significantly

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significant effect on HDL restoration. In diabetic caused rats, a modest dosage of EELU had a significant ($p<0.01$) impact, whereas a greater dose of EELU had a highly significant ($p<0.001$) effect on LDL.

Biochemical Analysis Table 3 explores the amounts of blood sugar and glycosylated haemoglobin in experimental and normal rats.

Diabetic rodents outperformed normal control rats (Group II) had a substantial rise in blood glucose and glycosylated haemoglobin ($p<0.001$) (Group I). EELU at greater and lower dosages resulted in a highly significant ($p<0.001$) drop in blood glucose and glycosylated haemoglobin levels in diabetic rats.

Liver marker enzymes In (Group II) when compared to normal rodents, the level of liver marker enzymes in blood rose considerably ($p<0.001$) (Group I). On diabetic caused rats, two doses of EALU and EELU recovered the SGOT extremely substantially ($p<0.001$). Two dosages of EELU ($p<0.001$) exhibited a very significant impact in SGPT restoration, but 200 and 400 mg/kg of EALU showed just a modest ($p<0.01$) effect. There was a less significant ($p<0.05$) effect of EALU (200, 400 mg/kg) on ALP reduction, but a greater dose of EELU considerably ($p<0.001$) decreased ALP in diabetes induced rats.

Table 1 Effect of Ethyl acetate, Methanol extracts of Leucas Urticaefolia

Treatment groups	Blood glucose Mg/dl	Triglycerides IU	Total cholesterol IU	LDL IU	HDL IU
Standard control Glibenclamide 0.5 mg/kg	80.35 ± 6.20	2.52 ± 0.17	1.62 ± 0.60	0.81 ± 0.05	1.29 ± 0.02
Normal control	77.55 ± 6.70	2.78 ±0.23	1.80 ± 0.10	0.79 ± 0.10	1.46 ± 0.12
Diabetic control	282.13 ± 2.72	3.20 ± 0.16	3.16 ± 0.05	1.52 ± 0.08	0.70 ± 0.05
Treatment with EALU 200mg/kg	70.71 ± 2.30***	2.13 ± 0.25***	1.86 ± 0.03***	0.83 ± 0.16*	0.93 ± 0.04
Treatment with EALU 400mg/kg	74.08 ± 1.32***	1.56 ± 0.59***	1.46 ± 0.17***	0.78 ± 0.10**	0.87 ± 0.08
Treatment with EELU 200mg/kg	72.18 ± 1.86***	1.81 ± 0.12***	1.41 ± 0.02***	0.79 ± 0.07**	0.80 ± 0.08*
Treatment with EELU 400mg/kg	76.74 ± 3.77***	2.57 ± 0.15***	1.78 ± 0.10***	0.72 ± 0.05*	1.38 ± 0.06***

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Table 2 Determination of blood glucose level

Treatment groups	Blood glucose Mg/dl				
	Treatment days				
	0	7	14	21	28
Standard control Glibenclamide 0.5 mg/kg	208.15 ± 2.53	164.32 ± 2.42	152.45 ± 3.17	136.16 ± 1.50	115.30 ± 2.25
Normal control	81.13 ± 1.56	82.84 ± 1.29	80.78 ± 1.27	81.35 ± 1.68	79.50 ± 1.36
Diabetic control	215.55 ± 4.60	220.33 ± 5.13	2.32.16 ± 3.69	240.75 ± 4.41	231.84 ± 4.60
Treatment with EALU 200mg/kg	265.71 ± 2.42*	225.29 ± 4.17	192.38 ± 2.90	150.12 ± 2.52**	126.22 ± 2.75**
Treatment with EALU 400mg/kg	232.14 ± 2.52*	218.65 ± 2.80	172.08 ± 1.06	136.5 ± 1.94**	119.08 ± 2.62**
Treatment with EELU 200mg/kg	240.70 ± 2.60*	212.52 ± 2.10*	174.38 ± 2.10**	139.15 ± 2.22***	122.65 ± 1.18***
Treatment with EELU 400mg/kg	258.32 ± 2.13**	208.72 ± 1.40*	158.50 ±**	127.52 ± 2.78***	88.50 ± 1.65***

Table 3 Effect of Ethyl acetate, Methanol extracts of Leucas Urticaefolia on haemoglobin and liver enzymes

Treatment groups	Hb	Glycosylated haemoglobin	SGOT	SGPT	ALP
Standard control Glibenclamide 0.5 mg/kg	14.45 ± 0.48	3.28 ± 1.70	26.65 ± 2.40	32.55 ± 5.58	152.08 ± 4.20
Normal control	13.37 ± 0.08	4.72 ± 0.31	26.55 ± 1.77	32.63 ± 1.51	143 ± 2.15
Diabetic control	09.19 ± 0.43	11.08 ± 2.63	58.13 ± 4.28	53.70 ± 2.60	168.78 ± 7.41
Treatment with EALU 200mg/kg	12.30 ± 0.12*	8.10 ± 0.25*	26.42 ± 1.50***	37.26 ± 5.87**	165.22 ± 5.30*
Treatment with EALU 400mg/kg	12.22 ± 2.62*	6.72 ± 1.60**	24.35 ± 1.60***	34.17 ± 0.38**	155.50 ± 4.20*
Treatment with EELU 200mg/kg	13.83 ± 0.50*	5.35 ± 0.30***	28.52 ± 1.49***	35.50 ± 0.60***	148.70 ± 3.60**
Treatment with EELU 400mg/kg	14.75 ± 1.62**	4.40 ± 0.67***	27.33 ± 2.16***	33.33 ± 0.30***	144.32 ± 17.28***

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For each group of six animals, all data are computed as mean ± SEM. ***p<0.001, **p<0.01, *p<0.05 significance between normal and diabetes control groups and medication treated groups.

4. Discussion

Most early medications were based on traditional medical practises, which were then followed by clinical, pharmacological, and chemical research [13]. For thousands of years, plants have been widely recorded for their medical purposes. Over millions of years, they have developed and adapted to endure insects, bacteria, fungus, structurally varied secondary metabolites. They were used as a primary source of pharmaceuticals for preclinical drug development due to their ethnopharmacological properties. The current study aimed to determine the anti-diabetic and acute toxicity potential of LU (70 percent hydroalcoholic) extract in Wistar rats with STZ-induced diabetes.

The extract's nontoxic behaviour was validated by a 2-week acute toxicity study in which no deaths were reported. This analysis confirmed the plant's compatibility and safety for in vivo testing. Thus, the extract's substantial antioxidant capacity and nontoxic nature were adequate reasons to investigate its anti-diabetic potential in a STZ-induced diabetes model.

Diabetes-induced islet cytoplasmic degenerations improved considerably following treatment with LU 400 mg/kg in sections from the diabetes-induced group. The extract can reverse the histopathological damage induced by STZ, as well as restore biochemical and haematological parameters, reduce inflammatory indicators and revitalise endogenous antioxidants. The defensive effects of LU are most likely attributable to its antioxidant capacity and phytochemical profile. The histopathology of Induced model rats revealed indications of acute toxicity, including necrosis, fatty degeneration, fibrosis, vein distortion, cellular hypertrophy, vacuolization, steatosis and haemorrhage. Low and high dosages of LU administration attenuated the harmful effects of STZ and the damage caused by STZ intoxication.

5. Conclusion

Our findings indicate that LU extract has the capacity to alleviate the histopathological lesions caused by STZ, as well as the ability to restore biochemical and haematological parameters, decrease inflammatory indicators and revitalise endogenous antioxidants. All of this supports its preventive and shielding ability to prevent pancreatic damage caused by oxidative stress. The content of polyphenols and/or flavonoids in LU extract was shown to be equivalent to glibenclamide in terms of anti-diabetic activity. We recommend subjecting this plant extract to bioassay-guided isolation in order to identify the active component (s). The extracts might be a promising anti-diabetic ingredient for future formulations.

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