Original Article

Antidiabetic Activity Of Leaf Extract Of Leptadenia Reticulata Against Streptozotocin Induced In Wister Rat

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Abstract

Objective: To examine the effect of a Leptadenia reticulata leaf extracts on glucose concentrations and lipid profiles in rats with and without diabetic. **Material and methods:** Streptozotocin caused diabetes in Wistar rats. Oral administration of Leptadenia reticulata leaf extracts in petroleum ether, ethyl acetate, and ethanol at a rate of 200 mg/kg was performed. Metformin (50 mg/kg), a common diabetes medication, was evaluated for its ability to reduce glucose levels and hypolipidemia. **Results:** According to the findings of the sub-acute toxicity investigation, there was no discernible change in animal behaviour as a result of the lack of toxicity. In diabetic rats, the ethanol extract effectively diminished glucose levels ($P<0.05$), as well as cholesterol and triglyceride levels, and increased HDL levels. **conclusion:** In conclusion, the current study provides scientific support for the traditional usage of Leptadenia reticulata in the treatment of diabetes and cardiovascular disease. Moreover, the ethanol extract of Leptadenia reticulata (ETLR) are found to be more active against hyperglycaemia and hyperlipidaemia.

Introduction

Diabetes mellitus (DM) is a metabolic condition that is mostly brought on by a total lack of or decreased efficacy of insulin and is characterized by hyperglycaemia, glycosuria, and a negative nitrogen balance. It is the most prevalent disease in the world, affecting 25% of the population, and currently affects 150 million people, with an expected increase to 300 million cases by 2025.[1] Type II diabetes, a metabolic disorder characterized by a consistent decline in insulin function, is the most common type of diabetes. Only 20% of the population has type 1 diabetes, an auto - immune disorder that causes the pancreas' insulin-producing beta cells to die, lowering the body's insulin level.[2] Juvenile diabetic, feline diabetes, and gestational diabetes are further types of the disease. In order to maintain glucose homeostasis, the cells often secrete more insulin to overcome insulin resistance. In noninsulin-dependent type 2 diabetes, insulin resistance impairs this cell's function, lowering glucose homeostasis and resulting in impaired plasma glucose (the body's response to high blood sugar, also known as glucose resistance).[3] Diabetes patients who experience hyperglycaemia have altered lipid and glucose metabolism as well

as altered levels of liver enzymes.[4] Atherosclerosis, heart attacks, strokes, and other cardiovascular disorders (CVD) are all known to be significantly increased by diabetes mellitus. CVD is responsible for over 75% of diabetes-related fatalities in men and 57% in women.[5] The current antidiabetic medication is still unable to totally cure diabetes. The only effective treatment for diabetes mellitus is insulin therapy, despite its numerous downsides, including insulin sensitivity, anorexia, neurological dysfunction, and fatty liver when used long-term.[6] There are a number of oral hypoglycaemic medications used therapeutically, however due to some side effects and insufficient efficacy, researchers are looking for more potent medications. Consequently, herbal drugs are being utilised to treat diabetes. The primary advantages of herbal medicine appear to be its low cost, low incidence of major side effects, and purported effectiveness. The treatment of diabetes traditionally uses more than 800 different plants worldwide.[7] Scientific validation of the antidiabetic properties of phytopharmaceuticals and medicinal plants that have fewer negative effects is still absent, nevertheless. The plant species Leptadenia reticulate often known as Jiwanti, is a member of the Asclepiadaceae family. In the sub-Himalayan regions of Punjab, Gujrat, Uttar Pradesh, and the entire Indian peninsula, it grows to 900 metres. Ayurveda claims that Jiwanti is a jeevana tonic that raises bodily energy levels. It is typically advised for people who lack energy or willpower. Jiwanti is described as galactagogues, antimicrobial, stimulant, tonic,restorative, lactogenic and hypotensive in certain sources. [8] Calogenin and tocopherols were produced by hydrolysing a number of pregnane glycosides, including as leptaculatin, deniculatin, and reticulin that were isolated from aerial portions.[9] Leptadenia reticulate leaf extracts were investigated for their possible anti-diabetic effects on streptozotocin (STZ)-induced diabetic Wister rats.

Materials and methods

The medication streptozotocin (STZ) was purchased in Bangalore, India from Sigma Aldrich. Metformin, a popular diabetes medication, was purchased from Sun Pharma in Mumbai, India. Petroleum ether, ethyl acetate, and ethanol are the analytical-grade solvents that were utilised to extract the plant material, and they were bought from Sigma Arch Chemicals Ltd.

Preparation of Extracts of Leptendina reticulata by Hot Continuous Percolation Method

The flask with the selected solvent based on the polarity increase in petroleum ether, ethyl acetate and ethanol respectively. When the vapor from the flask enters the thimble through the syphon tube, it dissolves the substance's active ingredients. Continuous extraction is the name given to this technique. Until all of the soluble components are separated, the process is repeated. At the bottom, the extract was taken and dried at a lower temperature and pressure. Each time, the powdered material was air dried before extraction with various solvents.

200g of dry powder were weighed, properly wrapped in Whatman filter paper, kept in a thimble, and the Soxhlet apparatus was set up. Different solvents with varying degrees of polarity, such as ethyl acetate, ether and petroleum ether were used to extract the powder. The orders of polarities are petroleum ether 0.656, ethyl acetate 4.4, and ethanol 5.2.[10] Here, the extraction solvent's boiling point served as the basis for temperature maintenance. A rotary evaporator was used to extract the solvents under reduced pressure, and they were then kept in a desiccator.[11] The extract had a semisolid consistency. Repeat until extract is obtained at desired consistency (semi solid).

Preparation of Dispersion of Various Extracts of Leptadenia reticulata

200 mg/kg of extract either petroleum ether / ethyl acetate / ethanol was triturated in a glass mortar with 0.5 ml of tween- 80 and add 3 ml of distilled water. The trituration was continued until the dispersion of the extract was achieved. This preparation was used for anti-diabetic activity. The leaves of Leptadenia reticulata extracts are named as PELR for petroleum ether extract, EALR for ethyl acetate extract, ETLR for ethanolic extract.

Experimental Animals

After receiving the necessary consent from the institutional animal ethics committee, the tests were conducted. For a week prior to and throughout the trials, the animals were kept at Central Animal House, Radiant laboratory in Bangalore, India, under standard temperature (272°C), relative humidity (44-56%), and light and dark cycles of 10 and 14 hours, respectively. Animals were given water, adlipidum, and a regular food (Lipton, India). The food

was stopped 18 to 24 hours before to the experiment's start. The ethical standards for the welfare of laboratory animals dictated that all tests be carried out in the morning.[12]

Acute toxicity studies

Petroleum ether, ethyl acetate, and ethanolic extract of LR were tested for acute oral toxicity in accordance with the guidelines of the Organization for Economic Co-operation and Development (OECD - 423).

After being fasted for the previous night and receiving only water, the relevant group of wistar rats were gastric intubated and given the extracts orally at a dose of 5 mg/kg body weight in Tween-80. The animals were kept under observation for 14 days while being fed regular diet. Two or three animals died within 14 days, and the dose is considered hazardous. However, the same dose is given once again for confirmation after one animal dies. The treatment was repeated at greater doses of 50, 300, and 2,000 mg/kg body weight if mortality was not seen, though. For 72 hours, toxic signs such behavioral abnormalities, abnormal movement, convulsions, and even death are detected.[13,14] The acute toxicity evaluation of other extracts used the same methodology.

Induction of Diabetes

In addition to a high-fat diet, STZ at a dose range 50 mg/kg was administrate at intravenously to the fasting rats (HFD). The HFD has been freshly manufactured each day; 15 animals used as controls received standard pellet chow (Lipton, India). For three days, the animals were fed a high-fat diet before even being given a STZ injection to induce diabetes. Prior to use, the STZ were freshly diluted in citrate buffer (0.01 M, pH 4.5). Diabetic rats with blood glucose levels higher than 200 mg/dl were utilized in the experiment one week after receiving STZ.

Experimental protocol

After an overnight fast, six groups of six diabetic and normal rats were separated. Only 1 ml distilled water was given to the rats in group I. Rats in Group II were treated with a high-fat diet and 0.9% saline injections in addition to diabetic animals. Diabetes-related animal fed a high-fat diet and treated with 200 mg/kg of petroleum ether extract in sterile saline was given to group III rats. A diabetic animal administered with 200 mg/kg of ethyl acetate extracts in normal saline was fed a high-fat meal containing group IV rats. Group V rats were given diabetes animals fed a high-fat diet as well as handled with 200 mg/kg of ethanolic extract in sterile saline. Rats in group VI were given 50 mg/kg of metformin.

Assessment of Liver, Kidney and Pancreas Function

After an overnight fast, six groups of six diabetic and normal rats were separated. Only 1 ml distilled water was given to the rats in group I. Rats in Group II received 0.9% saline injections along with diabetic animals and were fed a high-fat diet. Estimations of total cholesterol (CHL), alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and total bilirubin (TBL) were made using an auto analyzer made by Merck (300 TX, Merck- Micro Labs, Mumbai). Before the animals were slaughtered, their pancreas, kidney and liver were removed, weighed, and preserved in 10% formalin for histological studies.

Histopathological Studies

Histopathology Since correct diabetes and other disease diagnosis typically necessitates histological inspection of samples, microscopic analysis of infected tissue is an essential tool in anatomical pathology.[22]

Sliced into 5 mm pieces, the isolated pancreas, kidney and liver were preserved in alkaline formalin solution (10%) for three days before being rinsed under running water for around 12 hours. Dehydration was then followed with alcohol with progressively higher strengths (70, 80, and 90%) for 12 hours each. Absolute alcohol was used for the final dehydration, with 3 changes occurring at 12-minute intervals. Xylin was used for cleaning, and changes were made every 15 to 20 minutes. The parts underwent paraffin infiltration in the automatic tissue processing facility after cleaning. To completely eliminate the formalin, the items were rinsed under running water.

Results and discussion

Acute toxicity studies

In the current study, where ETLR's acute toxicity was investigated using OECD-423 criteria, neither the animals receiving the maximum dose of 2000 mg/kg nor the animals in the control group experienced any fatalities. The sub-acute toxicity study's maximal dose was 200 mg/kg, or 1/10th of 2000 mg/kg.[23] According to the findings of the sub-acute toxicity investigation, there was no discernible change in animal behavior as a result of the lack of toxicity. The rats given ETLR exhibited normal body weights and growth patterns when compared to rats given conventional saline and control treatments.

Antidiabetic activity

As stated previously, the anti-diabetic properties of Leptadenia reticulata leaf extracts were evaluated in high-fat diet (HFD) and streptozotocin (STZ)-induced diabetes models. The fasting levels of blood sugar were measured in Wistar rats 1 week after STZ-induced diabetes. The development of hyperglycemic rats (blood sugar >250 mg/dl) (each group with 6 rats). Three weeks later, the normal control, diabetic control, and treatment groups received daily oral dosages of standard drug hyperglycaemic drug metformin (50 mg/kg), and various leaf extracts of LR (200 mg/kg), respectively. Over the course of 21 days, weight, food, and water intake were recorded daily. The rats were severed from their heads on day 21 after a 16-hour fast, and blood was drawn to assess the levels of LDL-V, TG, FBG, TC, HDL-C, serum creatinine, glycogen, serum urea, ALP, AST and ALT. Separated, weighed, and kept at -70°C were the pancreas, liver, and kidney.

Effect of Leaf Extracts of Leptadenia reticulata on Oral Glucose Tolerance Test (OGTT) in Normal and Diabetic Rats

The effect of different leaf extracts of Leptadenia reticulata on oral glucose tolerance test (OGTT) in normal and diabetic rats presented in Table 1(a) (b) and Figure 1(a)(b) respectively.

Table 1 (a): Effect of Leaf Extracts of Leptadenia reticulata on OGTT in Normal Rats

Treatments	**Minutes before and after administering glucose**					
	-30	**0**	**30**	**90**	**150**	**210**
Normal control	78.4 ± 3.1	78.6 ± 3.4	78.2 ± 3.6	77.4 ± 3.8	78.8 ± 2.8	78.2 ± 3.4
PELR (200mg/kg)	78.7 ± 3.2	75.5 ± 4.9	178.6 ± 2.4	117.6 ± 3.4	109.7 ± 5.9	99.1 ± 1.4
EALR(200mg/kg)	81.4 ± 2.9	79.4 ± 4.7	145.3 ± 1.8	120.4 ± 2.4	105.4 ± 3.4	97.5 ± 4.8
ETLR (200mg/kg)	79.4 ± 2.6	75.4 ± 2.6	133.2 ± 1.9	102.1 ± 2.9	95.6 ± 4.3	81.9 ± 4.6*
Metformin(50mg/kg)	81.9 ± 3.1	77.8 ±2.3	124.1 ± 2.5	96.4 ± 3.2	89.9 ± 3.2	79.8 ± 2.8*

Table 1(b): Effect of Leaf Extracts of Leptadenia reticulata on OGTT in Diabetic Rats

Treatments	**Minutes before and after administering glucose**					
	-30	**0**	**30**	**90**	**150**	**210**

Diabeticcontrol	174.8 ± 5.6	167.8 ± 3.1	234.1 ± 2.4	225.3 ± 1.9	218.3 ± 1.8	214.2 ± 2.4
PELR(200mg/kg)	168.5 ± 3.2	166.7 ± 4.9	247.6 ± 2.4	231.6 ± 3.4	183.7 ± 5.9	178.1 ± 1.4
EALR(200mg/kg)	175.9 ± 2.9	173.1 ± 4.7	239.3 ± 1.8	205.4 ± 2.4	182.4 ± 3.4	174.5 ± 4.8
ETLR(200mg/kg)	180.4 ± 2.6	178.4 ± 2.6	234.2 ± 1.9	190.1 ± 2.9	152.6 ± 2.3**	86.9 ± 3.6**
Metformin (50mg/kg)	171.9 ± 3.1	170.8 ± 2.3	226.1 ± 2.5	143.4 ± 3.2	138.9 ± 3.1**	79.8 ± 2.8**

One-way ANOVA was used for the statistical analysis, which was accompanied by Dunnett's multiple comparison tests (n = 6). When compared to the usual control group, *P<0.05.

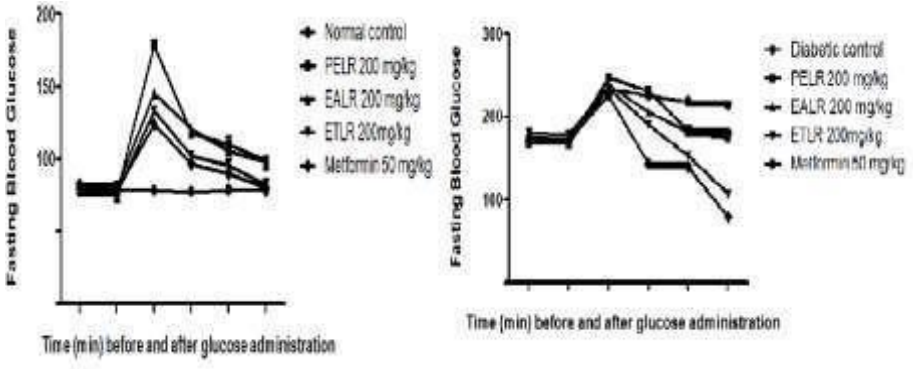

Fig (a) **Fig (b)**

Fig. 1: (a) The impact of Leptadenia reticulata leaf extracts on the OGTT in normal rats (b) Effect of various Leptadenia reticulata leaf extracts on the OGTT in diabetic rats

Compared to the normal control and diabetes control in the OGTT (Table 1), ETLR and metformin exhibited substantial (P<0.05) hypoglycemic action at 210 minutes when compared to diabetic and normal control groups. PELR and EALR did not significantly reduce blood glucose levels compared to diabetic and normal control groups. The mean percentage of glucose levels in the PELR, EALR, ETLR, and metformin-treated groups changed by 28.76%, 25.38%, 17.26%, and 14.60%, respectively, as compared to the normal control group. Blood glucose levels in the PELR, EALR, ETLR, and metformintreated groups have decreased by 64.37%, 63.08%, 55.32% and 51.65%, respectively, as compared to the diabetic control group. PELR is superior to EALR, ETLR, and Metformin in terms of blood sugar decrease percentage.

Effect of Body Weight, Water, and Food Intakes in STZ-Induced Diabetic Rats Prior to and Following Oral Administration with Vehicle, Various Leaf Extracts of Leptadenia reticulata, and Metformin for Three Weeks

The ETLR, metformin-treated group may have had better control of diabetes insipidus than the PELR and EALR treated groups due to the management of blood glucose levels. In STZ-induced diabetic rats, the results demonstrated differences in body weight, water intake, and food intake before and after oral administration of a vehicle, several leaf extracts of LR, and metformin for three weeks. The results are shown in Table 2.

TABLE 2: Effect of Body Weight, Water, and Food Intakes in STZ-Induced Diabetic Rats Prior to and Following Oral Administration with Vehicle, Various Leaf Extracts of Leptadenia reticulata, and Metformin for Three Weeks

Treatment	Body Weight		Water Intake		Food Intake	
			(ml/100g body weight of rat/day)			
	Before	After	Before	After	Before	After
Normal control	183.06 ± 3.18	179.05 ± 2.18	166.08 ± 4.51	170.02 ± 1.43	32.33 ± 1.08	34.46 ± 0.19
Diabetic control	179.18 ± 4.02	108.19 ± 1.09	158.19 ± 1.96	179.12 ± 2.35	33.46 ± 3.19	46.48 ± 2.18
PELR (200 mg/kg)	172.35 ± 1.82	106.24 ± 3.46	157.54 ± 2.76	176.43 ± 6.48	32.46 ± 2.48	44.53 ± 3.6
EALR (200 mg/kg)	176.39 ± 1.74	142.43 ± 2.83	162.47 ± 5.92	174.17 ± 4.36	36.58 ± 3.13	48.35 ± 4.3
ETLR (200 mg/kg)	179.42 ± 3.58	169.64 ± 4.64	151.34 ± 6.23	147.46 ± 2.46	37.13 ± 0.4	39.48 ± 1.5
Metformin (50 mg/kg)	176.35 ± 2.46	189.43 ± 3.86	159.26 ± 3.76	151.02 ± 3.57	35.08 ± 1.48	31.14 ± 2.19

One-way ANOVA was used for the statistical analysis, which was accompanied by Dunnett's multiple comparison tests (n = 6). When compared to the usual control group, *P<0.05.
The body weight, food intake and water intake of the rats did not differ significantly in PELR, EALR, ETLR and metformin treated diabetic groups.

When comparing to the 0-day readings, the mean percentage changes in body weight, food intake, and water intake in the diabetes control groups were 65.74%, 11.73%, and 28.26%, respectively. In contrast, the mean percentage of rat weight, water and food intakes in PELR-treated groups is 62.22 %, 10.79 %, and 27.10 %, while EALRtreated groups are 23.87 %, 5.26 %, and 24.3 %. ETLR treated groups are 5.58 %, 2.72 %, and 5.95 %, while metformin treated groups are 6.87 %, 5.29 %, and 12.65 % when compared to 0-day values. When compared to the same treatment, the metformin-treated group's water intake was much lower. On the other hand, compared to the same treatment, food intake is significantly higher in the ETLR treated groups.

Effect of Various leaf Extracts of Leptadenia reticulata on Blood Glucose Level in STZ Induced Diabetic Rats

The results of the effect of various extracts of Leptadenia reticulata on the blood glucose level in STZ induced diabetic rats are shown in Table 3 and Figure 2.

PELR, EALR, ETLR and metformin treated groups did not showed significant (P< 0.05) decrease in blood glucose level when compared to control group animals. When compared to the normal control group, the mean rate of blood glucose changed from 61.1% in diabetic group to 64.37%, 63.08%, 55.32%, and 51.65% in the PELR, EALR, ETLR, and metformin-treated groups, respectively. In contrast to diabetic control rats, the percentage of all treatment groups was lower. EALR > PELR > ETLR > Metformin is the order of blood glucose % in all treated groups.

TABLE 3: Effect of Various leaf Extracts of Leptadenia reticulata on Blood Glucose Level in STZ Induced Diabetic Rats

Treatment	Fasting Blood Glucose (FBG) (mg/dl)			
	0 day	7day	14 day	21 day
Normal control	75.6 ± 8.6	77.8 ± 1.6	76.4 ± 2.3	78.6 ± 1.4
Diabetic control	68.4 ± 4.1 75.6 ± 6.3	249.7 ± 3.6 263.5 ± 4.8	248.4 ± 4.6 261.8 ± 5.4	237.7 ± 7.8 260.6 ± 3.9
PELR (200 mg/kg) **EALR (200 mg/kg)**	71.8 ± 5.6	258.4 ± 6.7	251.3 ± 4.1	249.1 ± 2.8
	69.9 ± 8.4	271.7 ± 5.2	196.2 ± 3.8	149.6 ± 5.7
ETLR (200 mg/kg) **Metformin (50 mg/kg)**	65.4 ± 2.3	248.7 ± 4.7	184.5 ± 3.9	136.4 ± 2.7

One-way ANOVA was used for the statistical analysis, which was accompanied by Dunnett's multiple comparison tests (n = 6). When compared to the usual control group, *P<0.05.

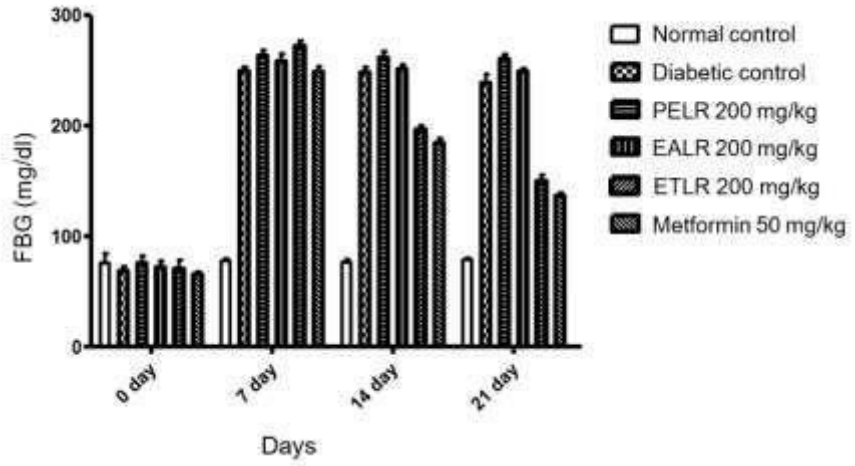

Figure 2: Effect of Various leaf Extracts of Leptadenia reticulata on Blood Glucose Level in STZ Induced Diabetic Rats

Effect of metformin and various Leptadenia reticulata leaf extracts on serum albumin and protein levels

Blood albumin and total protein levels are affected by various Leptadenia reticulata extracts and metformin, as shown in Table 4 and Figure 3.

When compared to the normal control group, the blood albumin and protein levels of the ETLR and metformin-treated groups decreased significantly (P< 0.05). The treated groups for PELR and EALR did not significantly differ from the control group. Compared to the control animal group, the mean percentage of the diabetic control group changed by 11.05%, the PELR group by 10.81%, the EALR group by 9.13%, the ETLR group by 4.51%, and the metformin-treated group by 2.02%.

In all treated groups, the serum albumin and protein percentages are in the following order: PELR > EALR > ETLR > Metformin.

Table 4: Effect of metformin and various Leptadenia reticulata leaf extracts on serum albumin and protein levels

Treatment	Serum albumin (g/dl)	Serum protein (g/dl)
Normal control	3.38 ± 0.17	6.35 ± 0.19
Diabetic control	3.80 ± 0.11	7.19 ± 0.18
PELR (200 mg/kg)	3.79 ± 0.43	7.18 ± 0.14
EALR (200 mg/kg)	3.72 ± 0.15	7.10 ± 0.19
ETLR (200 mg/kg)	3.54 ± 0.18	6.97 ± 0.15
Metformin (50 mg/kg)	3.45 ± 0.12	6.76 ± 0.13

One-way ANOVA was used for the statistical analysis, which was accompanied by Dunnett's multiple comparison tests (n = 6). When compared to the usual control group, *P<0.05.

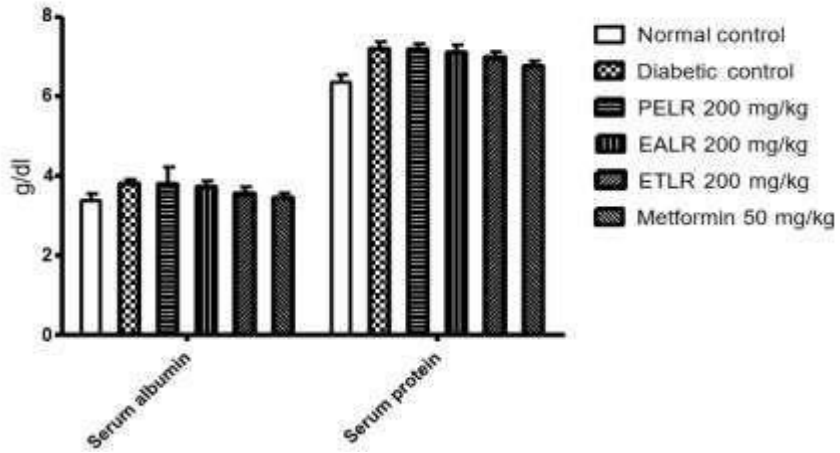

Figure 3: Effect of metformin and various Leptadenia reticulata leaf extracts on serum albumin and protein levels

Effect of Different Leptadenia Reticulata Leaf Extracts and Metformin on Liver Glycogen Level

Information on the effects of metformin and different leaf extracts of Leptadenia reticulata on the amount of glycogen in the liver is shown in Table 5 and Figure 4.

When compared to the normal control, the liver glycogen levels of the ETLR and metformin-treated groups increased significantly (P<0.05), but not those of the PELR and EALR-treated groups. When

compared to the normal control group, the mean percentage of liver glycogen changed from 41.37% in the diabetic control group to 32.25%, 20.58%, 2.12%, and 12.76% in the PELR, EALR, ETLR, and metformin-treated groups, respectively. In all treated groups, the percentage of liver glycogen is distributed as follows: PELR > EALR > ETLR > Metformin.

Table 5: Effect of Various Leaf Extracts of Leptadenia reticulata and Metformin on the Liver Glycogen Level

Treatment	Liver glycogen (mg/100 mg tissue)
Normal control	0.41 ± 0.09
Diabetic control	0.29 ± 0.06
PELR (200 mg/kg)	0.31 ± 0.07
EALR (200 mg/kg)	0.34 ± 0.08
ETLR (200 mg/kg)	0.42 ± 0.01*
Metformin (50 mg/kg)	0.47 ± 0.03*

One-way ANOVA was used for the statistical analysis, which was accompanied by Dunnett's multiple comparison tests (n = 6). When compared to the usual control group, *P<0.05.

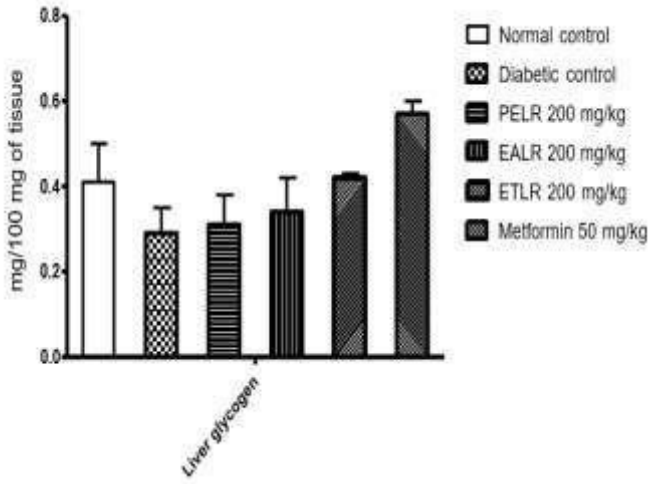

Figure 4: Effect of Various Leaf Extracts of Leptadenia reticulata and Metformin on the Liver Glycogen Level

Effect of Metformin and Different Leaf Extracts of Leptadenia Reticulata on Serum Urea and Creatinine Level

The effects of metformin and various leaf extracts of Leptadenia reticulata on serum urea and serum creatinine levels are shown in Table 6 and Figure 5, respectively.

Serum urea and creatinine levels significantly decreased (P 0.05) in the groups treated with ETLR plus metformin. The mean percentage of serum urea decreased from 21.74% in the diabetic control group to 20.29%, 18.11%, 8.96%, and 1.74% in the PELR, EALR, ETLR, and metformin-treated groups, respectively, as compared to the normal control group. Compared to the normal control group, the mean percentage of creatinine in the diabetic controlled group decreased from 39.59% to 24.68%, 19.04%, 4.04%, and 4.03% in the PELR, EALR, ETLR, and metformin-treated groups, respectively. Serum urea and creatinine percentages are PELR > EALR > ETLR > Metformin across all treated groups.

Table 6: Effect of Metformin and Different Leaf Extracts of Leptadenia Reticulata on Serum Urea and Creatinine Level

Treatment	Urea (mg/dl)	Creatinine (mg/dl)
Normal control group	24.36 ± 4.32	2.38 ± 0.23
Diabetic control group	31.13 ± 6.51	3.94 ± 0.50
PELR (200 mg/kg)	30.69 ± 4.68	3.16 ± 0.57
EALR (200 mg/kg)	29.75 ± 2.79	2.94 ± 0.48
ETLR (200 mg/kg)	26.87 ± 1.38*	2.48 ± 0.19*
Metformin (50 mg/kg)	24.80 ± 6.51*	2.48 ± 0.15*

One-way ANOVA was used for the statistical analysis, which was accompanied by Dunnett's multiple comparison tests (n = 6). When compared to the usual control group, *P<0.05.

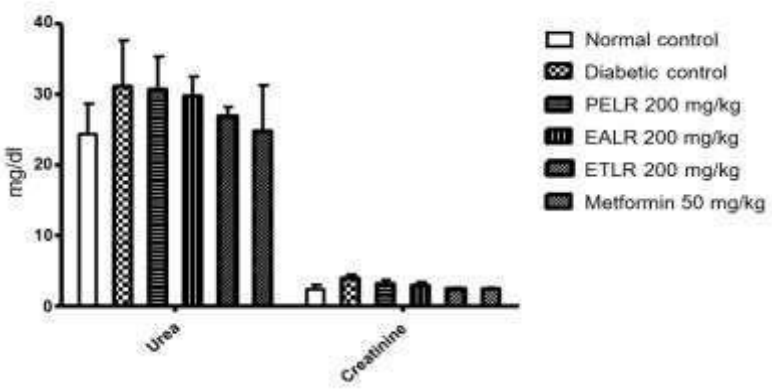

Figure 5: Effect of Metformin and Different Leaf Extracts of Leptadenia Reticulata on Serum Urea and Creatinine Level

Effect of Metformin and Different Leaf Extracts of Leptadenia reticulata on ALP, ALT and AST Level

Table 7 and Figure 6 provide information on how different Leptadenia reticulata extract extracts and metformin affected the levels of AST, ALT, and ALP.

Compared to the normal control, the AST, ALT, and ALP levels reduced significantly (P 0.05) in the ETLR and metformin-treated groups, but not in the PELR and EALR-treated groups. Compared to the normal control group, the mean percentage of AST in the diabetic control group declined by 17.84%, while it reduced by 10.62, 7.51, 1.33, and 0.9% in the PELR, EALR, ETLR, and metformin-treated groups, respectively.

Compared to the normal control group, the mean percentage of ALT in the diabetic control group decreased from 35.48 % to 29.28 %, 25.56 %, 9.17 %, and 6.5% in the PELR, EALR, ETLR, and metformin-treated groups, respectively. The mean percentage of ALP in the diabetic group increased from 28.28% to 61.66%, 45.91%, 12.21%, and 18.01% in the PELR, EALR, ETLR, and metformin-treated groups, respectively, when compared to the normal control group. All treated groups' AST and ALT percentages are in the following order: PELR > EALR > ETLR > Metformin. All treated groups' ALP percentages are distributed as follows: PELR > EALR > Metformin > ETLR.

Table 7: Effect of Various Leaf Extracts of Leptadenia reticulata and Metformin on the AST, ALT and ALP Level

Treatment	ALP (IU/L)	ALT (IU/L)	AST (IU/L)

Normal control	18.59 ± 1.61	99.79 ± 10.81	221.09 ± 12.26
Diabetic control	63.18 ± 2.75	154.67 ± 15.68	249.98 ± 26.42
PELR (200 mg/kg)	48.49 ± 3.84	140.54 ± 11.18	247.38 ± 28.03
EALR (200 mg/kg)	34.37 ± 4.91	133.49 ± 10.14	239.51 ± 21.61
ETLR (200 mg/kg)	20.19 ± 2.57*	109.61±12.6*	224.18 ± 19.08*
Metformin (50 mg/kg)	15.74 ± 2.42*	106.75 ±23.87*	219.11 ± 24.52*

One-way ANOVA was used for the statistical analysis, which was accompanied by Dunnett's multiple comparison tests (n = 6). When compared to the usual control group, *P<0.05.

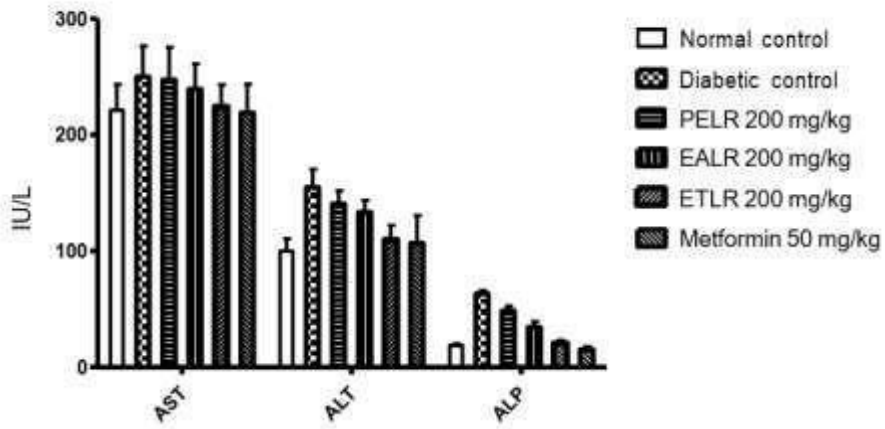

Figure 6: Effect of Various Leaf Extracts of Leptadenia reticulata and Metformin on the AST, ALT and ALP Level

Effect of Various Leaf Extracts of Leptadenia reticulata and Metformin on the Total Cholesterol, Triglyceride, LDL, VLDL and HDL Cholesterol level

Table 8 and Figure 7 provide information on the effects of different leaf extracts of Leptadenia reticulata and metformin on total cholesterol, triglyceride,LDL, VLDL and HDL cholesterol levels.

When compared to the normal control, ETLR-treated groups demonstrated a substantial (P<0.05) reduction in total cholesterol, LDL cholesterol, and VLDL cholesterol, but PELR-treated groups, EALR-treated groups, and metformin-treated groups did not.

When compared to the normal control group, the mean percentage of total cholesterol changed from 31.30% in the diabetic controlled group to 24.12%, 22.76%, 5.59%, and 13.55% in the PELR, EALR, ETLR, and metformintreated groups, respectively. In all treated groups, the proportion of total cholesterol is distributed as follows: PELR > EALR > Metformin > ETLR.

When compared to the untreated control group, triglycerides in the ETLR and metformin-treated animal groups decreased significantly (P<0.05), but not in the PELR and EALR-treated groups. When compared to the normal control group, the mean percentage of triglycerides changed from 21.59% in the diabetic control group to 17.54%, 13.19%, 0.90%, and 1.43% in the PELR, EALR, ETLR, and metformin-treated animal groups, respectively.

The order of triglyceride percentage in all treated groups are PELR > EALR > Metformin > ETLR.

When compared to the normal control group, the mean percentage of LDL-cholesterol decreased in the diabetic control group by 51.34% and in the PELR, EALR, ETLR, and metformin-treated animal groups by 47.13%,

40.57%, 15.17%, and 35.14%, respectively. All treated groups' LDL cholesterol percentages are distributed as follows: PELR > EALR > Metformin > ETLR.

When compared to the normal control group, the mean percentage of VLDL-cholesterol changed from 42.84% in the diabetic controlled group to 37.26%, 29.66%, 2.12%, and 18.57% in the PELR, EALR, ETLR, and metformintreated groups, respectively. In all treated groups, the percentage of VLDL-cholesterol is distributed as follows: PELR > EALR > Metformin > ETLR. When compared to the normal control, the HDL cholesterol in the ETLR treated groups increased significantly ($P<0.05$), while it did not drop significantly ($P> 0.05$) in the PELR, EALR, or metformin treated groups.

When compared to the diabetic controlled group, the mean percentage of HDL-cholesterol changed from 67.77% to 56.03% in the PELR, 28.24% in the EALR, 4.97% in the ETLR, and 28.96% in the metformin-treated groups. All treated groups' HDL-cholesterol percentages are distributed as follows: PELR > EALR > Metformin > ETLR.

Table 8: Effect of Various Leaf Extracts of Leptadenia reticulata and Metformin on the Triglyceride, Total Cholesterol, HDL, LDL and VLDL-Cholesterol level

Treatment	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Normal control	67.72 ± 4.41	128.69 ± 2.86	16.26 ± 3.6	19.68 ± 0.3	27.65 ± 2.54
Diabetic control	98.58 ± 12.58	164.13 ± 23.68	33.42 ± 3.8	34.43 ± 2.8	16.48 ± 1.35
PELR (200 mg/kg)	89.25 ± 5.35	156.08 ± 3.29	30.76 ± 3.7	31.37 ± 2.5	17.72 ± 0.91
EALR (200 mg/kg)	87.68 ± 4.74	148.25 ± 4.47	27.36 ± 5.4	27.98 ± 4.4	21.56 ± 1.74
ETLR (200 mg/kg)	71.73 ± 2.58*	129.87 ± 3.48*	19.17 ± 2.6*	19.27 ± 0.8*	26.34 ± 2.19*
Metformin (50 mg/kg)	78.34 ± 5.42	130.56 ± 5.78*	25.07 ± 1.9	24.17 ± 2.1	21.44 ± 1.26

The results are presented as the mean ±SEM, with six rats per group. One-way ANOVA was used for the statistical analysis, followed by Dunnett's multiple comparison tests. Compared to the normal control group, *$P< 0.05$

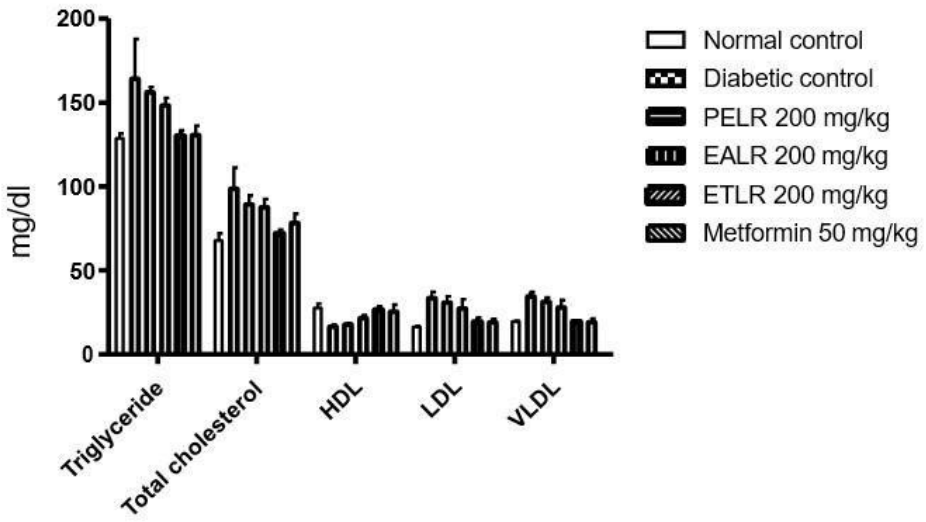

Figure 7: Effect of Various Leaf Extracts of Leptadenia reticulata and Metformin on the Triglyceride, Total Cholesterol, HDL, LDL and VLDL-Cholesterol level

Effect of Various Leaf Extracts of Leptadenia reticulata on Catalase, Reduced GSH (Glutathione) and Lipid peroxidation Levels

Table 9 and Figure 8 provide information on the effects of different leaf extracts of Leptadenia reticulata on catalase, reduced GSH (glutathione), and lipid peroxidation levels.

The catalase levels were significantly higher in the ETLR and metformin-treated animal groups compared to the untreated control animal groups ($P<0.05$). However, the levels of catalase in the mice given PELR and EALR did not significantly change. Reduced GSH levels and an increase in MDA levels were seen in STZ- drug treated animal group's liver. When compared to the control normal group, the ETLR and metformin treated groups displayed a significant improvement ($P< 0.05$) in GSH and MDA levels. GSH and MDA levels did not significantly change in the rats treated with PELR and EALR, respectively.

In the diabetic control animal group, the change in the mean percentage is 53.84%. However, when compare to normal control animal group, the PELR, EALR, ETLR, and metformin treated groups are 34.61%, 24.45%, 0.35%, and 0.71%, respectively.

In the diabetic controlled animal group, there has been a 90.47% shift in the mean percentage of reduced glutathione. However, when compare to the normal controlled animal group, the PELR, EALR, ETLR, and metformin treated groups are 84.62%, 64.38%, 7.14%, and 3.45%, respectively.

In the diabetic controlled animal group, the change in the mean percentage of lipid peroxidation is 33.58%. However, when compare to the normal controlled animal group, the PELR, EALR, ETLR, and metformin treated groups are 39.77%, 19.26%, 2.93%, and 3.52%, respectively.

In all treated groups, the percentages of catalase, reduced glutathione, and lipid peroxidation are in the following order: PELR > EALR > Metformin > ETLR.

Table 9: Effect of Various Leaf Extracts of Leptadenia reticulata on Catalase, Reduced GSH (Glutathione) and Lipid peroxidation Levels

Treatment	Catalase min/mg of protein	Reduced GSH mg/dl	Lipid peroxidation moles/ml
Normal control	280.6 ± 4.60	120.26 ± 2.75	176.25 ± 2.48
Diabetic control	182.45±2.48	63.26 ± 1.24	265.4 ± 5.27

PELR (200 mg/kg)	208.24 ± 3.45	65.56 ± 3.24	246.46 ± 6.43
EALR (200mg/kg)	225.37 ± 2.87	73.14 ± 3.14	218.63 ± 4.67
ETLR (200 mg/kg)	279.4 ± 1.09*	112.87 ± 6.15*	171.74 ± 3.56*
Metformin (50 mg/kg)	282.85 ± 4.30*	116.8 ± 1.96*	170.8 ± 4.19*

One-way ANOVA was used for the statistical analysis, which was accompanied by Dunnett's multiple comparison tests (n = 6). When compared to the usual control group, *P<0.05.

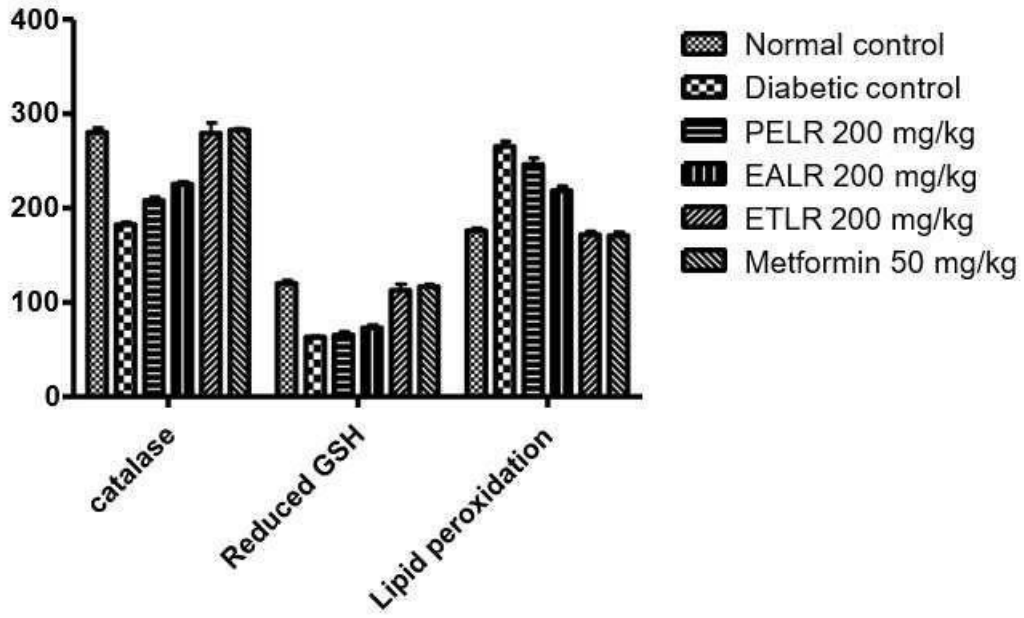

Figure 8: Effect of Various Leaf Extracts of Leptadenia reticulata on Catalase, Reduced GSH (Glutathione) and Lipid peroxidation Levels

Histopathology Examination of Various Leaf Extracts of Leptadenia reticulata Histopathology of Pancreas

According to histopathological studies, the pancreas of healthy control rats contains a sizable islet structure that is encircled by exocrine gland tissue. There are no inflammatory cells present in the islet. The islet form was visible on the pancreas slides from diabetic control rats, and the margins on the left and bottom were lined with exocrine gland tissue. The islet doesn't have any inflammatory cells.

On the lower and left boundaries of the islet structure on pancreatic slides from PELR (200 mg/kg) rat models, exocrine gland tissue was apparent.

Additionally, the islet cells are vacuolated and do not include any inflammatory cells. Exocrine gland tissue could be detected at the upper margin of the tiny islet structure shown on pancreas slides from EALR (200 mg/kg) rats. The islet has a number of inflammatory cells as well as islet cell vacuolation.

Slides of the pancreas from ETLR (200 mg/kg)-treated rats revealed a massive islet formation encircled by exocrine gland tissue. At the islet's margins, there aren't many inflammatory cells to be noticed.

The pancreas of 50 mg/kg Metformin-treated rats demonstrated a large islet development surrounded by exocrine gland tissue. There are no inflammatory cells present in the islet.

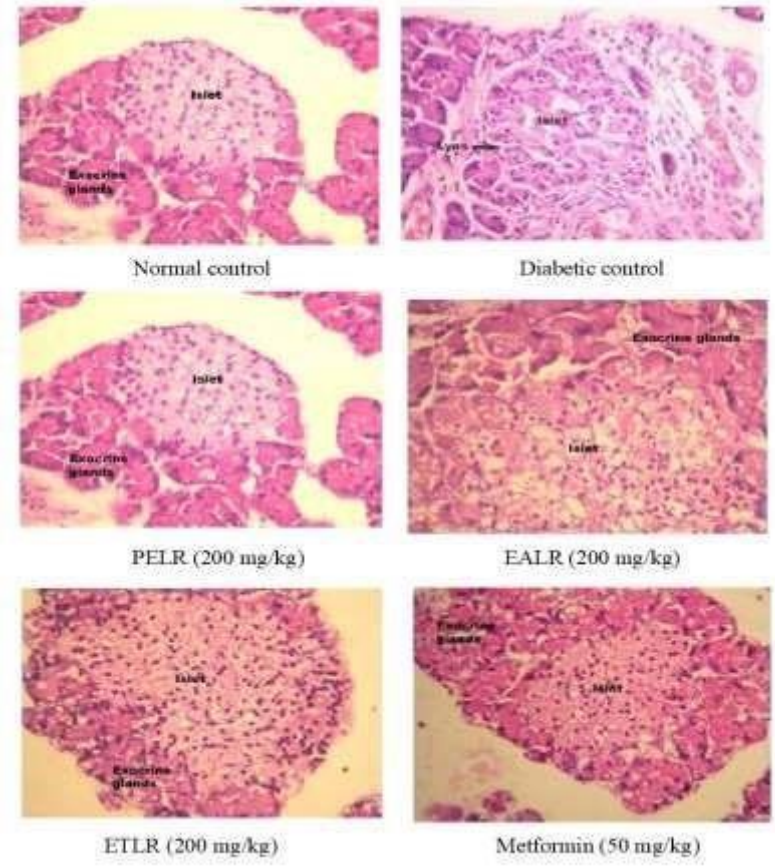

Figure 11: Histopathology of Pancreas

Histopathology of Kidney

Normal glomerular membrane size and cell function were visible on healthy rat kidney slides used during histological analyses. Neither the mesangial matrix nor the glomerular membrane foundation membrane thickened. The tubule count was within the expected range. The mesangial matrix increased, the glomerulus grew larger, and the glomerular membranes basal lamina hardened in diabetic control rat kidney slides. The tubule count was within the expected range.

Rats with PELR (200 mg/kg) and EALR (200 mg/kg)-treated kidney slides demonstrated glomerulus enlargement, extension of the mesangial matrix and thickness of the basement membrane of the glomerulus. The tubules were in the predicted range.

Metformin (50 mg/kg) and ETLR (200 mg/kg)-treated rats' kidney slides demonstrated normal glomerulus size and cellularity, in addition to a little rise in mesangial matrix. Additionally, the basement membranes of the glomeruli are slightly thickened. The range for the tubules was normal.

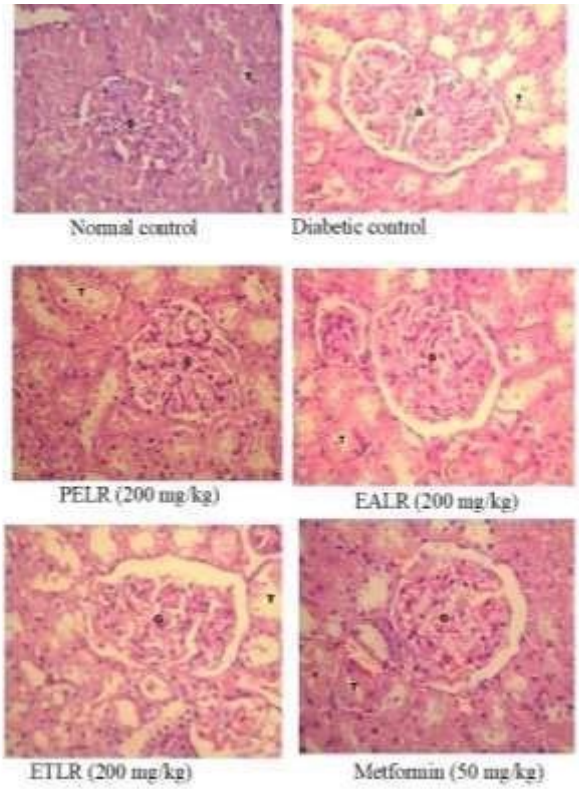

Figure 10: Histopathology of Kidney

Histopathology of Liver

The portal triad area of the liver slides from healthy control rats showed no abnormalities upon histopathological evaluation. The diabetic control rats' livers had hemorrhage, sinusoidal dilatation, necrosis, and inflammatory cell infiltration on liver slides.

Rats receiving (PELR 200 mg/kg, ETLR 200 mg/kg, EALR 200 mg/kg and Metformin 50 mg/kg) revealed no abnormalities in the portal triad area on their liver slides. (Figure 9)

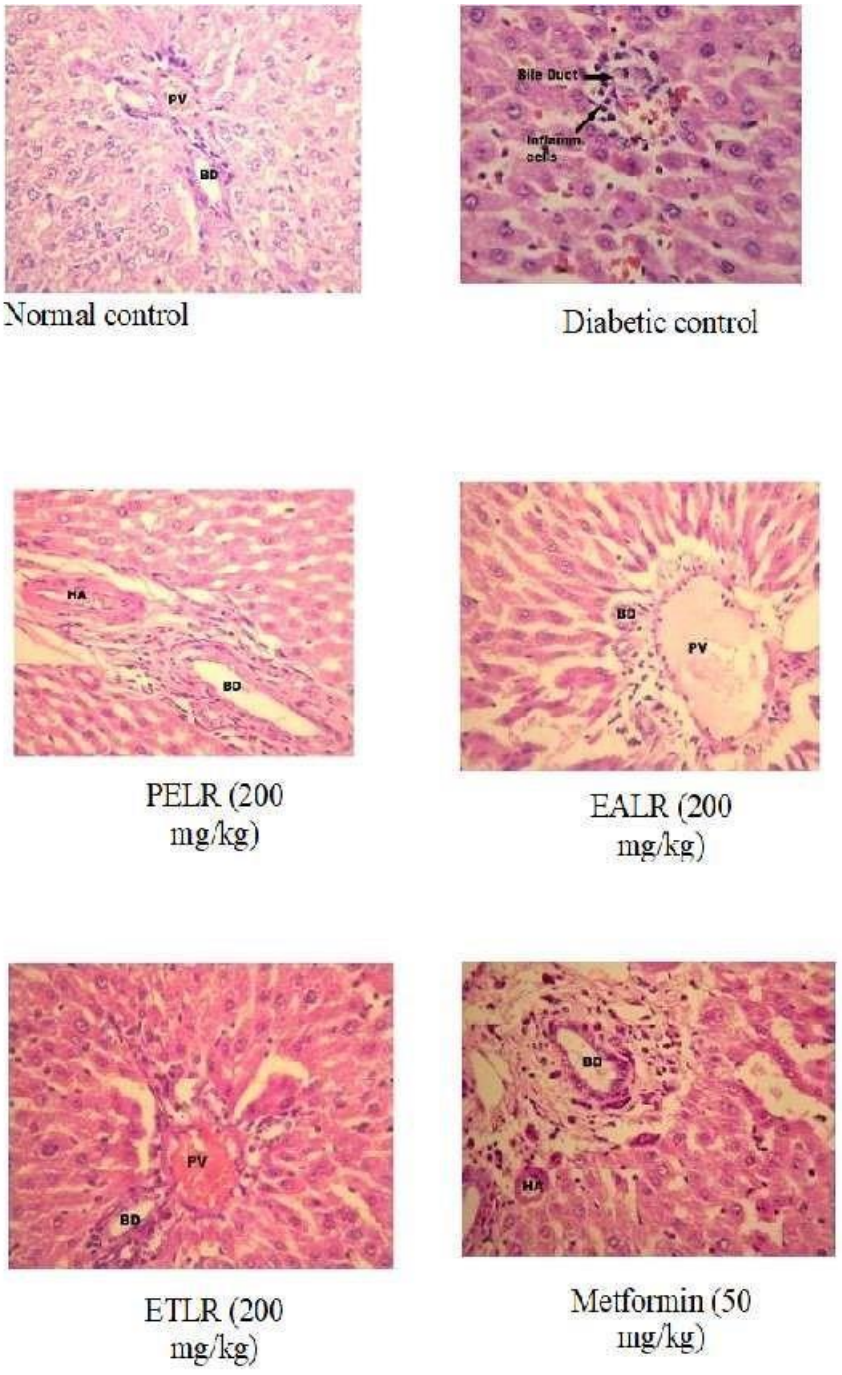

Figure 9: Histopathology of Liver

Discussion

In the current investigation, in which the acute toxicity analysis of ETLR was conducted in line with OECD-423 standards, neither the animals receiving the maximum dose of 2000 mg/kg nor the rats in the normal control experienced any deaths. As a result, 200 mg/kg, or 1/10th of 2000 mg/kg, was chosen as the highest dose for the sub-acute toxicity investigation.[23]

According to the findings of the sub-acute toxicity investigation, there was no discernible change in animal behaviour as a result of the lack of toxicity. Comparing the rats treated with ETLR to control rats and rats treated with regular saline, the ETLR-treated animals had normal growth patterns and body weight. Therefore, variations in body mass can be utilized as a sign of harmful pharmacological and chemical side effects.[24,25] Changes in the

levels of liver-damaging enzymes including ALP, AST, and ALT indicate toxicity. [26] Increases or reductions in serum concentrations of components, which are mostly controlled by synthesis in the liver, indicate liver damage. After administering ETLR for 28 days, the study's findings were evaluated, and it was determined that no concentration of ETLR caused liver damage.

When greater doses of ETLR (500 mg/kg) were given to the treated rats, plasma glucose levels marginally decreased. Analysis of hematological parameters is likely to be used in risk assessment because changes in the hematological system have a better predictive value for human toxicity when information from animal research is extrapolated.[27] After daily administration of ETLR over the period of 28 days of treatment there were no significant variations in the WBC and RBC levels between both the animals in the test group and the animals in the control group. Intriguingly, treatment with ETLR at a higher dose of 500 mg/kg was observed to result in a noticeable increase in hemoglobin levels. One component of ETLR, which may improve iron absorption, is a potential explanation.

The data indicate that ETLR is not harmful to the hematological and leucopoietic systems. Both the hematological and leucopoietic systems are essential indicators of physiologic and pathological status in people and animals, and they are the most susceptible to toxicity.[28] It is safe to presume that the extract is not hemotoxic as a result.

The aforementioned result demonstrates that a dose of 2,000 mg/kg of ETLR is non-toxic. In light of these findings, we will conduct additional research on animals with ETLR.

CONCLUSION

In conclusion, the present study provides scientific support for the traditional usage of Leptadenia reticulata to treat diabetes and cardiovascular disorders. Moreover, the ETLR are found to be more active against hyperglycaemia and hyperlipidaemia. In addition to this study is a platform for our future study to design lead compounds from above mentioned plant.

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