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Antidiabetic, Pancreas Protective and Antioxidant Potential of Ethanol Extract of *Botryocladia leptopoda* (J. agardh) Kylin on Streptozotocin-Induced Diabetic Rats

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ABSTRACT: In this study, ethanol extract of *Botryocladia leptopoda* (J. Agardh) Kylin was subjected to analysis of its antidiabetic activity in STZ provoked diabetes animals. This study was undertaken to investigate the effect of *Botryocladia leptopoda* (Et-Bl) ethanol extract on glucose level, biochemical parameters, and carbohydrate metabolizing enzymes, in vivo antioxidants, and histological study of the pancreas in streptozotocin-induced diabetic rats. Different doses of Et-Bl (100, 200, and 300 mg/kg BW) were administered orally for 60 days. The effects were compared with glibenclamide. Treatment with Et-Bl and glibenclamide for 60 days resulted in a significant alteration in glucose level, biochemical parameters and carbohydrate metabolizing enzymes, in vivo antioxidants, and histological study of the pancreas. The effect at a dose of 300 mg/kg of Et-Bl was more pronounced than that of 100 and 200 mg/kg BW and brought all the parameters to near normal. Thus, the present findings suggest that Et-Bl may be considered an effective therapeutic agent for treating diabetes mellitus.

Keywords: Antioxidants, Antidiabetic, Ethnomedicine, *Botryocladia leptopoda*, Streptozotocin.

INTRODUCTION

Diabetes Mellitus (DM) causes hyperglycemia or reduced insulin secretions Mukhtar *et al*. (2020). Fasting and ketosis cause insulin deficiency. DM is a global pandemic, and the situation in developing countries is deteriorating. DM affects millions globally. DM will affect 300 million in 2025. Since 2000, India's metropolitan areas have improved considerably in Aithal *et al*. (2019). The International Diabetes Federation (IDF) predicts that by 2025, India would have 69.9 million diabetics. The number of diabetic patients is predicted to rise from 171 million in 2000 to 366 million or more by 2030 Abhari *et al.* (2019).

Hyperglycemia causes dysfunctions. These include increased polyol pathway flow, changed cellular redox state, and elevated lipid profile Kang *et al.* (2020). Patients with diabetes mellitus have alterations in metabolic pathways, particularly glucose metabolism Miller *et al. (*2022). Defects in glucose metabolism regulation and physiological efforts to repair the imbalance overwork the endocrine system, deteriorating endocrine control Wishart *et al.* (2019). Persistent endocrine control impairment causes metabolic abnormalities and hyperglycemia Poznyak *et al.* (2020). Gluconeogenesis and glycolysis maintain normoglycemia. This creates a moving therapeutic target that requires different medications to treat the condition.

Increased free radicals and oxidative stress contribute to DM development and late complications. Free radicals are molecules having unpaired electrons. Free radicals can induce cell function interference, oxidative membrane issues, and lipid peroxidation intolerances Ratera *et al.* (2021). Increased lipid peroxidation and reactive oxygen species (ROS) are implicated in the development of several diseases and the toxicity of several substances Vo *et al.* (2020). Antioxidants protect against reactive oxygen damage.

Diabetes mellitus has no cure in current medicine. Insulin therapy is used to manage diabetes, however it can cause insulin allergy, insulin antibodies, lipodystrophy, kidney morphological alterations, and serious vascular consequences Sabbagh *et al*. (2022). Chronic sulfonylurea and biguanide use can cause nausea, vomiting, agranulocytosis, aplastic and haemolyticanemia, dermatological responses, lactic acidosis, and widespread hypersensitivity reaction Recently, hypoglycemic agents based on traditional medicine plants and chemicals.

Conventional diabetes treatment is ineffective and costly. Traditional medicinal plants and their biomolecules have helped manage diabetes for a century. In addition to regular antidiabetic treatment, an antioxidant therapy could improve diabetes. In recent years, the hunt for novel antioxidants in plants and plant-derived products to treat diabetes and related problems has intensified Hano *et al*. (2021). Marine

algae contain flavonoids, terpenoids, carotenoids, fiber, protein, vital fatty acids, vitamins, and minerals. Marine algae are a potential source of bioactive natural compounds. Food, confectionery, textile, pharmaceutical, dairy, and paper use marine algal phytochemicals as gelling, stabilizing, and thickening agents Yesuraj *et al.* (2022).

Marine algal products have antimicrobial, antiviral, antihelminthic, antituberculosis, antimycobacterial, antioxidative, anticoagulant, anti-inflammatory, antipyretic, analgesic, anticancer, insecticidal, antidiabetic, and antiprotozoal bioactivities. Researchers face a great test in identifying new therapeutic medications for life-threatening disorders. Researchers look to natural sources for new chemical discovery Brown N, *et al.*, (2018). Insulin and oral hypoglycemic medicines have undesirable side effects, thus people want natural alternatives. Antidiabetic effect of *Botryocladia leptopoda* in STZ-induced diabetic rats remains untested. The current study assessed carbohydrate metabolizing enzymes, lipid parameters, antioxidants, and pancreatic histopathology in STZ-induced diabetic rats to investigate the antidiabetic effects of *Botryocladia leptopoda* (J. Agardh) Kylin (Family: Rhodymeniaceae) leaves.

EXPERIMENTAL PROCEDURE

Seaweed collection

Botryocladia leptopoda (J.Agardh) Kylin was obtained in September-December 2017 from the rocky intertidal beach of Mandapam, Gulf of Mannar, Tamil Nadu, India. To eliminate the salt, bacteria, and another sediment present in the water, fresh samples were first rinsed with saltwater but then with clean water. After the samples were allowed to dry for two weeks, they were put away at room temperature.

Preparation of the ethanol extracts

Plants were cleaned with fresh water, shade dried at room temperature, and powdered. The powder was soxhlet extracted with ethanol (10 g/400 ml). The extraction was continued until a drop of solvent from the soxhlet siphon tube did not leave any residue when evaporated on a clean glass plate. The solvent was removed from the extract under reduced pressure using a rotary evaporator (VV2000, Heidolph, and Schwabach, Germany). The extract was stored in a -20ºC freezer until use. Ethanol extract of *Botryocladia leptopoda* is represented as Et-*Bl*.

Animals used in research.

Male Wister rats of 170-200 body weight were acquired and maintained at the *In vivo* Biosciences, Bengaluru, India, Fed on a healthy pellet diet (VRK nutrition solution, Maharashtra, India) and water *ad libitum*. The study report was accepted by *In vivo* Bioscience's Institutional Ethical Committee (1165/PO/RcBiBt-S/NRc-L/08/CPCSEA/&09 November 2018/Invivo/118).

Induction of Diabetes Mellitus. DM was experimentally caused by an intraperitoneal administration of Streptozotocin (STZ) (65 mg/kg BW) suspended in 0.1M cold citrate buffer (pH 4.5) in 12-hour fasted rats. After 6h of STZ administration, the rats were treated with a 10% blood sugar solution for the next 24h to avoid hypoglycemia. No mortality or any other adverse reactions have been noted. Rats with modest diabetic issues exhibiting glycosuria and hyperglycemia were chosen for experimental work after having a week of development and aggravation of DM.

Experimental design. Group I: Control rats; Group II: Diabetic induced rats; Group III: Diabetic rats administered orally with Et-*Bl* (100mg/kg bw/rat) for 60 days; Group IV: Diabetic rats administered orally with Et-*Bl* (200mg/kg bw/rat) for 60 days; Group V: Diabetic rats administered orally with Et-*Bl* (300mg/kg bw/rat) for 60 days; Group VI: Diabetic rats administered orally with glibenclamide (600µg / kg bw/rat).

The rats were fasted and sacrificed with cervical decapitation overnight following the last treatment (60 days). The blood was hoarded, and after centrifugation, the plasma was collected. Then, the glucose estimation was done. The pancreas tissues were directly removed from the experimental animals and kept in ice-cold containers. We are then homogenized with an appropriate buffer solution, centrifuged, and stored the supernatant. Biochemical estimates have been made homogeneous on the same day of sacrifice.

Biochemical estimations. Rats of the different groups fasted overnight, and the blood was withdrawn by retro-orbital puncture under light and anesthesia. Blood was withdrawn from the rats on the 0^{th}, 15^{th}, 30^{th}, $45^{th,}$ and 60^{th} day after the induction of diabetes to assess the blood glucose Folin *et al.* (1919). At the termination of treatment, i.e., 60 days, animals were deprived of food overnight. The levels of the following biochemical parameters were estimated according to the respective methods as HbA1C Jeppsson *et al.* (2002), AST, ALT, ALP (King 1965), Protein (Lowry *et al.* 1951), Urea (Natelson *et al.* 1951), Uric Acid(Caraway 1963), Creatinine(Husdan and Rapoport 1968), Glycogen (Carroll *et al.* 1956), Hb (Wintrobe *et al.* 1961), RBC(d'Amour *et al.*, 1948), WBC (Wintrobe *et al.*, 1961), Platelets (Brecher and Cronkite 1950) and Insulin was estimated by using radioimmunoassay kit.

Activities of hepatic hexokinase (Brandstrup and Bruni 1959), glucose-6-phosphatase (Swanson, 1955), glucose – 6- dehydrogenase, fructose-1-6-bisphosphatase (Gancedo and Gancedo 1971) were assayed according to the methods. The levels of lipid peroxidation (LPO) in the tissues were evaluated by Okhawa *et al.* (1979). The superoxide dismutase (SOD) level was assayed by Mishra *et al.* (2013). The level of catalase (CAT) enzyme was evaluated by Chance *et al.* (1952). Glutathione Peroxidase (GSH-Px) was assayed by Rotruck *et al.* (1973). The level of reduced glutathione (GSH) was assessed by the method of Moron *et al.* (1979).

Histological assessment of pancreas by hematoxylin-eosin (H/E) staining. All rats were sacrificed under mild anesthesia after drug treatment. After blood samples were collected, pancreas tissues were fixed in

formalin for 48 hours, dehydrated, and embedded in paraffin. Semi-automated rotator microtome sections were 4 m thick (Suneetha, 1993).

Statistical Analysis. All data are expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) was performed, followed by Tukey's test to compare the differences between treatments. Differences were considered statistically. significant for $p < 0.05$.

RESULTS AND DISCUSSION

Effects of *Botryocladia leptopoda* on blood glucose level in normal & STZ induced diabetic treated rats. Table 1 compares control and experimental animals' blood glucose levels. STZ-induced diabetic rats had higher blood glucose than control rats ($p<0.05$). Et-*Bl* or glibenclamide brought blood glucose near normal. *In vivo* antidiabetic activity of Et-*Bl* was studied. Et-*Bl* extract, an anti-diabetes process, provides the technological basis for this plant's DM efficacy. In STZ-induced diabetic animals, ethanol extract reduced glucose levels Owens III *et al.* (2020). The extract's antidiabetic activity is comparable to glibenclamide's in diabetic rats. The ethanol extract was clearly enriched with energy. The plant normalized animal glucose levels. The drug can boost pancreatic secretion or sugar uptake.

Effects of *Botryocladia leptopoda* on Biochemical Markers. Insulin levels of Group II rats dropped significantly ($p<0.05$) a week after STZ injection (Table 2). Et-*Bl* raised insulin levels in normal rats. Insulin levels of Diabetic rats rose after 60 days of Et-*Bl* and insulin treatment. Group V's insulin level rose 12.030.46 U/ml. STZ-induced diabetic rats had higher HbA1C, AST, ALT, and ALP. All Et-*Bl* treated groups showed a significant decrease in serum AST, ALT, and ALP, suggesting the leaves extract may prevent diabetes-related hepatic injury. Table 2 showed Group I and other experimental groups' proteins, urea, uric acid, and creatinine. Group II had lower glycogen levels than Group V and VI, which received Et-Bl and glibenclamide. Group II rats had different biochemical parameters than Group I rats. Et-Bl and glibenclamide oral administration resulted in levels similar to Group I, with Et-*Bl*'s being more pronounced. Group II rats had lower RBC and Hb levels than Group I rats. Et-*Bl* restored these levels in rats. Both treated and untreated rats had lower WBC counts than normal. Group II had higher platelet counts than normal and drug-treated rats. Platelet count decreases gradually in Groups III, IV, and V (Table 2).

STZ reduces insulin in rats. Insulin levels rose in Et-*Bl*treated STZ-diabetic rats. Et-*Bl* scavenges free radicals, prevents LPO, inhibits STZ-induced oxidative stress, and protects -cells, increasing insulin excretion and reducing blood glucose. Quercetin, the Et-*Bl* aglycone, increased insulin release in STZ-induced diabetic rats. Quercetin reduced oxidative stress and protected pancreatic -cells in STZ-induced diabetic rats. Et-*Bl* stimulated insulin production in diabetic rats' islets of Langerhans, boosting -cells. Quercetin-treated animals had more pancreatic islets.

In diabetes, excess glucose reacts with Hb to form HbA1c. Diabetic rats have lower Hb levels Çelik *et al.* (2022). The rate of glycosylation is proportional to blood sugar at the peak of the glucose tolerance curve, correlates with glycosylation, and improves glycemic control lowers HbA1c. Estimating Hb glycosylation is used in disease management and prognosis. In this study, 100, 200, and 300mg/kg bwrat Et-*Bl* tended to normalize Hb and HbA1c levels.

AST, ALT, and ALP are cytosolic enzymes released into the blood after cell membrane damage that reflect hepatocellular necrosis. AST, ALT, and ALP levels indicate hepatic damage. In the evaluation, free radicals damage the liver, and ALP, ALT, and AST are measured. Membrane damage or necrosis releases the enzymes, so they're measured in serum. High AST levels indicate liver injury, muscle damage, and cardiac infarction. Therefore, ALT is the best biomarker for screening for liver damage. Elevated liver enzymes in serum indicate cellular and membrane damage in the liver Morgan *et al.* (2019). STZ caused hyperglycemia, free radicals, and liver damage in this study. AST, ALT, and ALP levels rose.

STZ-induced diabetic rats have a negative nitrogen balance, developed proteolysis in muscles and tissues, and reduced protein synthesis, which decreases total plasma protein level. As insulin is required for protein synthesis, body protein levels decrease without it Shad *et al.* (2019). In this study, antihyperglycemic property of Et-*Bl*may have increased protein levels in diabetic rats. Catabolism of plasma and liver proteins may increase urea nitrogen production in diabetes. Insulin therapy in diabetes normalizes nitrogen through urea synthesis, according to Othman et al. Reduced nitrogen and protein synthesis increase blood urea Othman MS *et al.*, (2021). Et-*Bl* reduced blood urea in diabetic rats. Diabetic hyperglycemia raises plasma levels of urea, uric acid, and creatinine, kidney dysfunction markers. After Et-*Bl* and glibenclamide treatment, these parameters returned to near normal levels, proving anti-diabetogenic activity of Et-*Bl*. Diabetic rats have high uric acid in this study. This may be due to high xanthine oxidase, LPO, and triglyceride and cholesterol levels in diabetes.

Protein glycation in diabetes may promote muscle wasting and relieve purines. It produces uric acid and xanthine oxidase Polito *et al.* (2021). Et-*Bl* controls purine catabolism by restoring uric acid levels. Creatinine concentration measures kidney impairment. Clinical chemistry aims to find medication-related kidney toxicities in diabetic rats. In this study, creatinine levels in STZ-induced diabetic rats increased significantly. Et-*Bl* treatment prevented creatinine elevation. Et-*Bl* protects the kidneys of STZ-induced diabetic rats, according to this study.

Extracellular glucose concentration and insulin trigger glycogen synthesis in liver cells. Glycogen synthase and glycogen phosphorylase regulate glycogen metabolism in vivo. Reduced glycogen in diabetic rats

decreases glycogen synthase and increases glycogen phosphorylase Gothandam *et al.* (2019). In this study, Et-*Bl*treated diabetic rats restored hepatic glycogen. This may be due to decreased glycogen phosphorylase and increased glycogen synthase. Acute hyperglycemia stress affects hybrid grouper plasma glucose, glycogen content, and glycogen synthase and phosphorylase expression.

Therapeutic substances or plant extracts can alter hematological parameters Enenebeaku *et al.* (2021). Hematological parameters can measure plant extract toxicity and clarify blood-related roles. In this study, diabetic rats had lower RBC and Hb levels than normal rats. Thus, the experimental animals became anemic due to haemoglobin suppression. Et-*Bl* brought these parameters close to normal. The defect may be due to the extract boosting erythropoiesis to increase RBC formation (Van Remoortel *et al.*, 2021) and flavonoids decreasing LPO to inhibit RBC hemolysis. This helps Et-*Bl* control anemia. Both treated and untreated diabetic rats had lower WBC counts than controls. Group II rats had similar platelet levels to Group I. STZ may weaken the immune system, reducing WBCs, causing "leukopenia". Diabetes-induced stress can reduce WBCs, impairing rats' defenses. Diabetic rats treated with Et-*Bl* at 300 mg/kg/rat showed an increase in WBCs, which may be due to the plant's constituents that boost and encourage WBC development Mao C*et al*. (2020), suggesting an immune system guard for rats. Intraperitoneal STZ causes hyperglycemia, reducing platelet count. Long-term platelet deficiency causes internal and external bleeding and death. Et-*Bl* may restore platelet levels in diabetic rats due to phytochemicals that stimulate clotting factor biosynthesis Kumar *et al*. (2021).

Effects of *Botryocladia leptopoda* on Carbohydrate metabolizing enzymes. Fig. 1 compares carbohydrate-metabolizing enzyme levels in control and experimental animals. Hexokinase (Fig. 1a), Glucose-6-Phosphatase (Figure 1b), and Fructose-1-6-Bisphosphatase (Fig. 1d) levels increased ($p<0.05$) in STZ diabetic rats, and Glucose 6 dehydrogenase (Fig. 1c) levels decreased. These changes were normalized by Et-Bl or glibenclamide.

STZ-induced diabetic rats had lower hexokinase levels, which reduced glycolysis and glucose consumption for energyLi *et al.* (2021). Et-Bl maintains enzyme activation, which normalizes diabetes. In this study, diabetic rats had lower GK of Liver than normal rats. Several researchers have shown decreased hepatic GK activity in diabetic animals. The increased range of gluconeogenic enzyme glucose-6-phosphatase in diabetic rats' livers may be due to the enzyme's activation or increased synthesis during diabetes Beidokhti *et al.* (2020).

Et-therapeutic Bl's role may be based on its modulating and regulating the activity of the gluconeogenic enzyme, glucose -6- phosphatase, through 31, 51 - cyclic adenosine monophosphate (cAMP) and any other metabolic activation or inhibition of glycolysis and gluconeogenesis. Glucose-6-phosphate dehydrogenase converts glucose-6-phosphate to 6-phosphogluconate, generating NADPH for bile acid synthesis Schiliro & Firestein (2021). This enzyme controls the pentose phosphate pathway, which supplies NADPH for lipogenesis and ribose for nucleic acid production. Glucose-6-phosphate dehydrogenase, a key glucose-oxidizing enzyme, was altered ($p<0.05$) in group II animals Et-Bl dose-dependently increases glucose-6-phosphate dehydrogenase activity. Standard drugs performed similarly (Group VI).

Fructose 1, 6 bisphosphatase stimulates the rate-limiting step of fructose 1-6-bisphosphate to fructose-6-phosphate. In diabetes, fructose 1, 6-bisphosphatase destruction is reduced, which increases hepatic glucose synthesis. Diabetic rats had impaired carbohydrate metabolic pathways (glycolysis, glycogenolysis, glycogenesis, and gluconeogenesis). Probably due to insulin deficiency Ogilvy-Stuart & Beardsall (2020).

STZ-induced diabetic rats had increased fructose 1,6-bisphosphatase. This can reduce glycolysis and increase gluconeogenesis, increasing blood glucose. Et-*Bl* normalizes diabetes by restoring enzyme activation. In the current study, treated experimental diabetic rats had lower fructose 1, 6 bisphosphate liver activities than Group I rats.

Effects of *Botryocladia leptopodaon* In vivo Antioxidants Activity. Fig. 2 shows TBARS concentrations (Fig. 2a). DM increases tissue TBARS and hydroperoxides compared to normal. Et-Bl reduced diabetic rats' lipid peroxidation. Figures 2b, 2c, and 2d show the functions of SOD, CAT, and GPx in control and experimental rats' pancreas. Diabetes-control rats had lower GPx, CAT, and SOD activity in pancreatic cells than normal rats. Et-Bl increased antioxidant levels in diabetic rats compared to Group I rats. Group II pancreas GSH is reduced (Figure 2e). Groups III and IV diabetic animals had higher antioxidant levels than Group I (Fig. 2e).

Due to STZ's destruction of pancreatic insulin-secreting -tissues, diabetics produce more ROS. Muscle injury and lipid peroxidation improve with higher ROS levels Calkin *et al.* (2022). Islet cellular material is more vulnerable to free-of-charge extreme attacks due to lower antioxidant digestive enzymes like SOD, CAT, and GPx. Infusing STZ damages or impairs most islet tissue. Lack of insulin resistance reimbursement for high blood insulin release raises glucose levels. Diabetes rats have more excellent free radicals, which causes long-term hyperglycemia and damages the antioxidant shield. Our study found an increase in diabetic rats' TBARS tissues. Improved TBARS levels in diabetic rats show peroxidative injuries can cause diabetesSadi G *et al.*, (2018). Et-Bl and glibenclamide reduced TBARS and liver and kidney hydroperoxides in the diabetic control group. Et-effect Bl's promotes antioxidant activity and protects cells from lipid peroxidation.

All types of DM improve free radicals or antioxidant defense alternatives. During DM, enzymatic (SOD, CAT, GPx) and non-enzymatic (ROS) ROS exhaust may be collected (GSH). Diabetic rats' kidneys and

livers have less antioxidant tissue, per a study. Diabetes causes insulin deficiency, which impairs sugar use and increases oxidative stress. STZ-stimulated Group I tissue SOD action decreases. SOD is a protective enzyme that dismutes O2•– to H_2O_2 and molecular oxygen, reducing free radical activity Rowaiye AB *et al.*, (2020). ROS could reduce tissue SOD function, it was suggested Mistry *et al.* (2020). H_2O_2 inactivation or enzyme glycation may reduce SOD activity. Et-Bl increased diabetic rat liver and kidney SOD function, according to this study. Et-Bl can reduce oxygen-free radicals and boost hepatic antioxidizing enzyme activity.

Catalase (CAT) catalyzes hydrogen peroxide and protects tissues from OH radicals Rakesh *et al.* (2021). Decreased CAT may affect enzyme glycation deactivation. CAT reduces the dismutation impulse's H_2O_2 and OH production, protecting peroxisomes from oxidative damage. STZ action on H_2O_2 deposition in rats restored CAT functions, causing harmful effects. Et-Bl boosted diabetic rats in this study. This method uses Et-antioxidant Bl's properties to scavenge free radicals.

GSH, a non-enzymatic antioxidant, prevents free radicals and other ROS directly and indirectly through enzymatic reactions. STZ-stimulated diabetes rats have lower hepatic GSH levels than normal rats. GSH is known for preserving redox homeostasis, squeezing free radicals, and detoxing side effects Kant *et al.* (2022). It's a potent free radical scavenger and glutathione peroxide co-substrate Halim & Halim (2019). The reduction in hepatic GSH was attributed to oxidative stress in all forms of DM. Increased oxidative stress from lipid peroxidation aldehyde products reduces tissue GSH. This review found elevated GSH levels in diabetes-cured rats' liver and kidney. Et-Bl and glibenclamide may improve GSH biosynthesis, reduce oxidative stress, and reduce GSH deterioration, or both.

Glutathione Peroxidase (GPx) is an enzyme that removes peroxide, preventing intracellular hydrogen peroxide buildup Linšak *et al.* (2021). GST acts as a peroxidase, reducing peroxide-caused damage. Reduced GPx activity is due to enzyme inactivation and glycation. Glutathione is a substrate of the GPx enzyme, and increased amounts of glutathione increase GPx's action. As a result, the GPx process scavenges free radicals in diabetic rats. Reduced GPx activity in the liver and kidneys is seen in all forms of DM and can lead to the deposition of toxic chemicals. Diabetes rats' liver and kidney GPx activity is reduced, according to other research. Et-Bl and glibenclamide increased GPx in diabetic rat tissues.

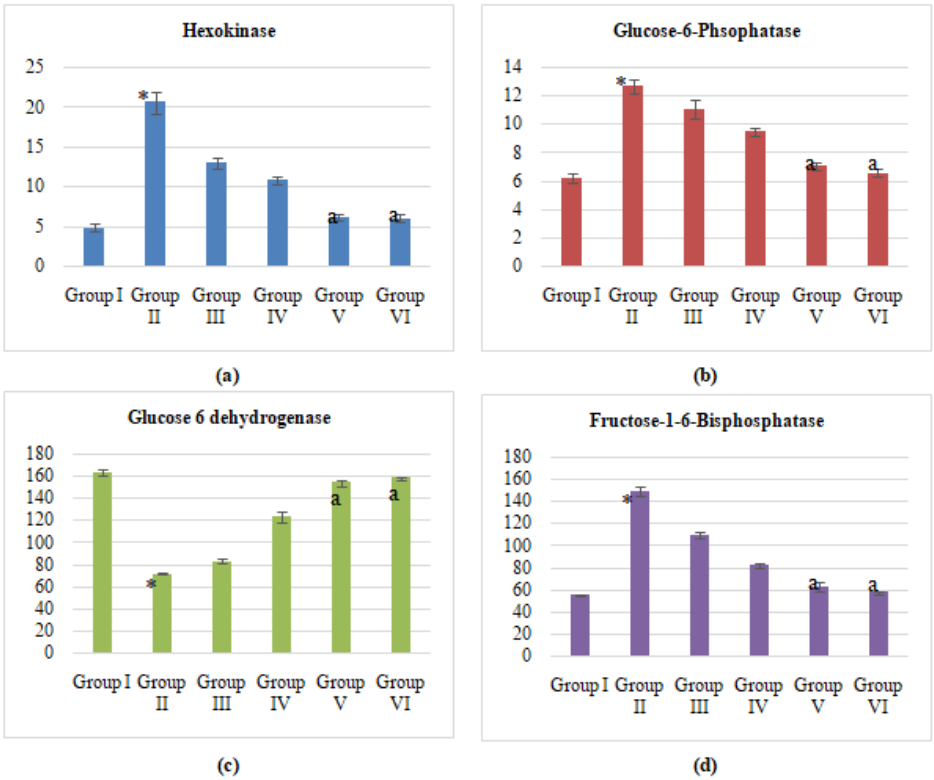
Effect of EFx on histopathology of pancreas. Pancreas Normal control rats exhibited standard histological architecture. Many rounded normal proportions of the islet of Langerhans were found all around the pancreatic acini. Prominent nuclei with well-arranged lobules with surrounding islet cells were found among normal control rats (Fig. 3). Groups that received STZ demonstrated cellular damage to the pancreatic acini and islets, which showed pancreatic β-cell damage and degeneration with asymmetrical vacuoles. Et-Bl treated STZ induced-DM rats showed marked improvement of the cellular injury (Figure 3), as evident from the partial restoration of islet cells, reduced β-cell damage, more symmetrical vacuoles, and an increase in the number of islet cells.

STZ is outstanding for its particular pancreatic islet B-cell cytotoxicity and has been broadly used to initiate type – 1diabetes in an exploratory rodent model Darwish *et al.* (2021). It interferes with the cellular metabolic oxidative mechanism. Escalating signs in both exploratory and clinical examinations suggest that oxidative pressure plays an essential role in advancing and moving the two sorts of DM. Free radicals are shaped excessively in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and oxidative debasement of glycation proteins Zaman *et al.* (2019). Weakened antioxidant defenses usually complement diabetes. Glibenclamide is regularly utilized as a standard antidiabetic medication in STZ actuated diabetes to compare the assortment of hypoglycemic mixes. The present study was conducted to measure the hypoglycemic activity of Et-*Bl* in Group II rats. In diabetic rats of (Group III, IV, V, and VI) treated with Et-*Bl* and Glibenclamide, an increase in the number of Beta-cells in the islets was observed, showing the rejuvenation of beta cells. Also, the intensity of secretary granules in the cells signified that they were stimulated for insulin synthesis.

Table 1: Effect of *Botryocladia leptopoda* on blood glucose level in normal & STZ induced diabetic treated rats.

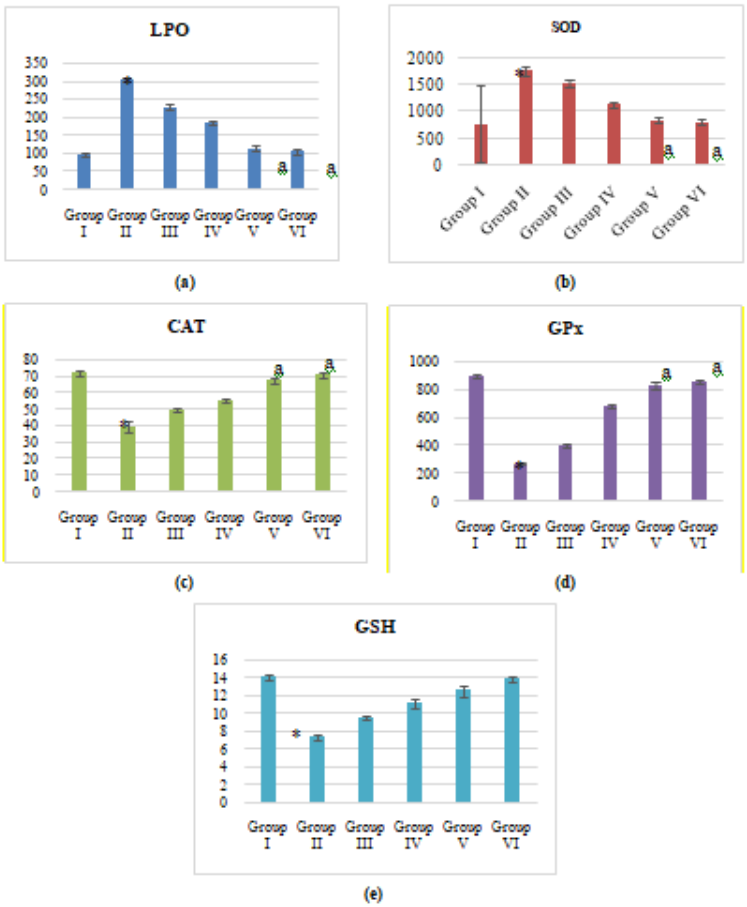
	Group I	Group II	Group III	Group IV	Group V	Group VI
0th day	71.25±2.98	114.64±4.81	113.15±2.06	104.84±2.61	87.16±3.46	77.95±1.06
15th day	77.8±2.44	140.86±10.12*	121.11±2.72	110.97±3.48	89.99±1.8[a]	85±5.76[a]
30th day	76.6±2.37	182.81±1.82*	124.5±3.35	114.16±2.94	93.51±2.82[a]	83.93±3.96[a]
45th day	77.13±1.45	211.5±2.93*	117.57±3.96	106.35±5.15	87.46±1.54[a]	81.95±2.28[a]
60th day	77.83±1.66	252.07±10.4*1	108.92±2.75	96.25±3.88	89.19±3.49[a]	82.8±3.53[a]

Values are Mean±SE, n = 6;* $p<0.05$ statistically significant when compared with Group I; Statistically significant when compared to EAC control group; [a]$p < 0.05$.



Values are Mean±SE, n = 6; * p<0.05 statistically significant when compared with Group I; Statistically significant when compared to EAC control group; [a]p < 0.05.

Fig. 1. Effects of *Botryocladia leptopoda* on Carbohydrate metabolizing enzymes in normal & STZ induced diabetic treated rats.



Values are Mean±SE, n = 6; * p<0.05 statistically significant when compared with Group I; Statistically significant when compared to EAC control group; [a]p < 0.05.

Fig. 2. Effects of *Botryocladia leptopoda* on *In vivo* Antioxidants activity in normal & STZ induced diabetic treated rats.

Table 2: Effect of *Botryocladia leptopodais* on blood glucose level in normal & STZ induced diabetic treated rats.

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Insulin	13.49±0.51	5.09±0.28*	7.96±0.48	9.31±0.49	12.03±0.46[a]	12.11±0.67[a]
HbA1C	5.05±0.72	8.62±0.1*	7.9±0.16	7.45±0.09	6.55±0.35[a]	6.15±0.2[a]
AST	36.24±1.17	86.33±2.08*	59.94±1.56	43.01±0.85	39.3±1.47[a]	38.73±1.66[a]
ALT	48.8±0.62	77.15±0.67*	72.83±0.8	69.97±1.08	51±1.79[a]	50.4±0.69[a]
ALP	112.48±0.47	210.87±0.98*	168.24±3.12	149.48±4.17	122.05±5.02[a]	119.8±3.82[a]
Protein	11.42±0.64	6.7±0.26*	7.77±0.29	8.52±0.37	10.11±0.42[a]	10.86±0.56[a]
Urea	131.48±0.52	335.83±19.63*	259.36±5.77	201±2.23	140.31±5.78[a]	138.65±4.26[a]
Uric Acid	6.95±0.24	16.83±0.44*	13.25±0.36	10.11±0.44	7.73±0.26[a]	7.13±0.12[a]
Creatinine	0.68±0.03	3.63±0.15*	3.14±0.12	2.85±0.15	0.82±0.06[a]	0.71±0.04[a]
Glycogen	61.53±3.34	10.38±0.49*	26.83±0.89	35.14±1.45	53.57±4.18[a]	59.72±1.61[a]
Hb	14.61±0.5	7.58±0.32*	9.04±0.41	11.31±0.37	12.57±0.81[a]	13.05±0.56[a]
RBC	6.73±0.36	3.83±0.32*	4.11±0.17	5.27±0.25	5.87±0.18[a]	6.37±0.18[a]
WBC	4.75±0.4	11.96±0.49*	9.26±0.32	7.29±0.24	5.53±0.21[a]	4.78±0.16[a]
Platelets	173.92±4.44	324.74±9.76*	296.74±13.22	232±4.29	182.07±4.49[a]	180.18±5.05[a]

Values are Mean±SE, n = 6; * p<0.05 statistically significant when compared with Group I; Statistically significant when compared to EAC control group; [a]p < 0.05.

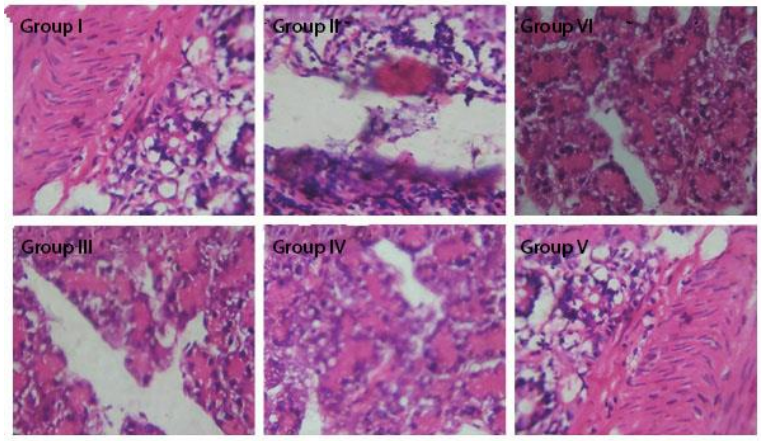


Fig. 3. Effect of *Botryocladia leptopoda* on the histological profile of pancreas in normal, STZ-induced diabetic untreated, and STZ-induced diabetic treated Wistar rats (×100).

CONCLUSIONS

Our study determines effect of Et-*Bl* in diabetic rats. Et-*Bl* reduces hyperglycemia by increasing external glucose consumption and modulating glycolysis and gluconeogenesis. Et-*Bl* was as effective as glibenclamide. Et-*Bl* leaves were taken orally to manage diabetes and prevent complications.

FUTURE SCOPE

In future studies, detailed safety and toxicity evaluations of plant extracts will be conducted. Acute and chronic toxicity studies will be carried out to determine the safety of the extract, including the effect on vital organs, haematological parameters and biochemical markers. Assessments will be made for possible adverse effects or interactions with other medications .

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Conflict of Interest. None.

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