

Antidiabetic activity of extract of *Pterocarpus santalinus* Linn. Seeds on Streptozotocin diabetic rats

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ABSTRACT

The goal of the research purpose was to look into the hypoglycemic and hypolipidemic activity of ethanolic extract of seeds of *pterocarpous santainus* (EESPS), as well as to study its effects on liver and kidney function in diabetic rats induced with streptozotocin (STZ). Thirty male Wistar rats were divided into six groups (n=6) for this experiment. A single dose of STZ at 40mg was used to cause diabetes in four groups. Biochemical markers such as total cholesterol, triglycerides, low and high density lipoprotein cholesterol, and fasting blood sugar (FBS) levels were examined on the final day of treatment. In addition, indicators of liver and kidney function were examined, including Glutamic oxaloacetic transaminase, Glutamicpyruvic transaminase, Alkaline Phosphatase, serum creatinine, and urea levels. After 42 days of treatment, blood sugar levels and triglycerides were found to be significantly lower, indicating that the ethanolic extract at 400 mg/kg body weight (b.w) was influenced significant anti-diabetic efficiency. The HDL levels increased while total cholesterol and LDL levels decreased. Furthermore, the ethanolic extract restored near-normal levels of a major hepatic and renal marker, it indicates that it protects against STZ-induced liver and kidney damage, as well as a rise in body weight. Treatment with EESPS decreased the pancreatic damage caused by STZ and accelerated β -cell regeneration in diabetic rats, according to histopathological study of the pancreas. The current findings point to EESPS's anti-hyperglycemic properties as well as its therapeutic potential. In conclusion Phenols and flavonoid molecules including ferulic acid, umbelliferone, and quercetin may play a key part in the mechanism of action of the EESPS. The extract can manage hyperglycemia and diabetes complications, and it has hypolipidemic activity, according to the findings of this study.

KEYWORDS: Diabetes, Streptozotocin, *Pterocarpussantalinus*, and Metformin .

1. INTRODUCTION

In affluent countries, diabetes mellitus, an endocrine condition, is a major cause of morbidity. Diabetes affects the metabolism of all fuels, including carbohydrates, lipids, and proteins, and individuals with diabetes are more likely to develop lipid problems and develop coronary heart disease, peripheral vascular disease, and cerebrovascular disease [1, 2]. Diabetes is linked to significant changes in plasma lipid and lipoprotein profiles, as well as an increased risk of

atherosclerosis, coronary insufficiency, and myocardial infarction [3]. In diabetes, lipid accumulation is mediated by a protein called lipoprotein lipase. a number of metabolic and regulatory abnormalities, including insulin insufficiency, making the diabetic patient more susceptible to hypercholesterolemia and hypertriglyceridemia [4]. Diabetes mellitus was referred to as "madumeha" by ancient Indian physicians. Many herbal products In ancient literature, various metals and minerals have been documented for the treatment of diabetes mellitus [5]. Ayurveda is a traditional Indian medicine that focuses on plants and plant extracts. The medicinal compounds found in plants are used to treat ailments in this indigenous type of medicine [6]. Plant-based medications are often thought to be less hazardous and have fewer negative effects than manufactured drugs [7]. Many plants have been demonstrated to exhibit hypoglycemic properties in both animals and humans, [8].

The *Pterocarpus santalinus* (Fabaceae) sometimes known as Red Sanders, is an endemic species found only in the southern sections of India's Eastern Ghats, particularly in Andhra Pradesh [9]. The wavy grained heartwood of Red Sanders is highly valued in both the domestic and foreign markets. The red dye derived from the wood is used as a colouring agent for textiles, medicine, and food, in addition to its extensive use in furniture. The wood is used for a variety of problems in traditional and folklore medicine, including diabetes, prickly heat, skin diseases, and a variety of other ailments [10,11].

Despite significant improvements in the treatment of diabetes with oral hypoglycaemic agents, the majority of synthetic medications used in diabetes management are expensive, toxic, and unavailable, particularly to poor and middle income earners. Furthermore, given the high prevalence of diabetes mellitus around the world, there is a need to create indigenous, low-cost botanical sources for diabetes care. This is in order to scientifically validate the plant's traditional use as an anti-diabetic herb; the acute toxicity of *Pterocarpus santalinus* was previously unknown. It has been researched, and the LD50 has been determined to be as high as 2000 mg/kg, making it rather safe. Therefore the purpose of this study is to assess the anti-diabetic impact of *Pterocarpus santalinus* of its solvent fractions in Streptozotocin-induced diabetic rats. [12]

2. METHODS AND MATERIALS:

2.1. Chemicals (AR Grade):

Streptozotocin (STZ) and Metformin were purchased from National scientific, Vijayawada, India. Respectively, Glucose standard strip/kits, and glucometer (Dr. Morepen Gluco One Morepen Laboratories limited Himachal Pradesh). All other chemicals and reagents were purchased from standard commercial suppliers and are of analytical quality.

2.2. Plant material collection and extract processing

P.santalinus seeds were collected in the Vissannapeta area of Andhra Pradesh, India. Dr.K. Madhava Chetty, Department Of Botony, Sri Venkateswara University- Tirupati, taxonomically identified and authenticated them.They were free of virus, and the dried seeds were rinsed in distilled water before being sliced and dried in the lab. The shade-dried sample was ground into a fine powder. This seed powder was kept in an airtight container and utilized in a soxhlet extractor with ethanol for extraction. On the rotary evaporator, the ethanol extract was evaporated to dryness, and the residue was stored in a refrigerator at 2–8 °C for use in later experiments. The sample voucher (SVUH- 0638) Was Deposited In their Herbarium.

2.3. Phytochemical screening:

The EESPS was subjected to grade methods for qualitative testing of the various phyto-constituents.

2.4. Ethical clearance (prior to the start of the study):

The Institutional Animal Ethics Committee (IAEC) accepted the research protocol with the Approval number IAEC/XIII/II/BCOP/2019, and it followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg. no: 1032/PO/Re/S/07/CPCSEA), New Delhi, India.

2.5. Animals in research

Thirty Wistar rats, were obtained from Mahaveera Enterprises in Hyderabad, Telangana, [reg no; 1656POBtS16CPCSEA]. In this investigation, the animals were kept in plastic cages with free access to tap water and a pellet meal, and were kept under conventional conditions (12-hour light/12-hour dark cycles at an ambient temperature of 25 ± 2 °C). Animals were given two weeks to acclimate to laboratory conditions before the start of the trial.

2.6 Investigational Stimulation of Diabetes In Rats

A single intra-peritoneal injection of Streptozotocin (40 mg/kg body weight) diluted in 0.1 M freshly prepared cold citrate buffer pH 4.5 was used to develop diabetes in overnight fasted experimental rats. [13] Blood glucose was tested after 72 hours for the development of diabetes, and rats with fasting blood glucose levels greater than 250 mg/dl were classified as diabetic and employed in this study.

2.7. Design of experiment (DOE)

To assess the Anti-hyperglycemic impact of EESPS in normal and STZ-induced diabetic rats, the animals were Divided into five equal groups (n=6) as follows:

Group 1: Throughout the experiment, normal control rats were provided a baseline diet.

Group 2: STZ-induced diabetic rats were treated with water.

Group 3: Diabetic rats were Treated with Low dose (LD) of EESPS (200 mg/kg). (b.w).

Group 4: Diabetic rats were treated with high dose (HD) of EESPS (400 mg/kg body weight).

Group 5: Diabetic rats were treated the Standard Drug Metformin (Glycomet) (200 mg/kg b.w.) as a control.

Throughout the investigation, body weight and plasma glucose levels were measured on a weekly basis. Glucose levels in the blood were tested using a Dr. Morepen glucometer. To maintain the same dosage throughout the experiment, the extract dosage was adjusted every week to account for changes in body weight. They were administered orally for 42 days using intragastric tube, After 42 days; the rats were fasted overnight and euthanized under anesthesia (Sodium pentobarbitone). The blood was collected with or without anticoagulant sample bottles for plasma and serum respectively.

2.8. Collection of blood and determination of biochemical parameters

Blood was obtained from overnight fasting rats through retro orbital puncture on the 0th (before the start of the experiment), 7th, 14th, 21st, 28th, 35th, and 42nd days. Dr. Morepen's glucometer was used to calculate blood glucose levels [14; 15]. Blood serum was extracted from blood collecting tubes after the complete blood samples were spun at 1,500 rpm for 15 minutes. The serum glucose, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum creatinine (SC), total cholesterol (TC), triglyceride (TG), high-density lipoprotein

(HDL), low-density lipoprotein (LDL), blood urea nitrogen (BUN) level, and albumin levels were all tested using the AUTOLAB-200.

2.9 Histological analysis of Pancreas tissues

The tissue samples from the pancreas were dissected, washed, and fixed in 10% neutral-buffered formalin. After fixing the samples, they were fixed in paraffin and sliced into 5-m sections with microtome. Light microscopy was used to view these sections after staining with haematoxylin and eosin (H&E).

2.10 Statistical analysis

The data was presented as mean \pm SD. Graph pad Instant was used to undertake data analysis using one-way analysis of variance (ANOVA) and Dunnetts multiple comparisons. The statistical significance level was set at $P < 0.05$ to analyze individual differences between the control and treatment groups.

3. RESULTS

3.1. Evaluation of the effect of EESPS on Hyperglycaemia

All of the animal groups were impacted by diabetes, indicating that blood glucose levels had fluctuated slightly. When compared to the other groups, the glucose levels in the negative control (STZ induced) group were considerably higher. At the 21st, 28th, 35th, and 42nd days, blood glucose levels in the Metformin 200 mg/kg treatment group were substantially lower ($P0.05$)*, ($P0.001$) **, and ($P0.0001$) *** when compared to the negative control (STZ) group. When compared to the STZ induced group, the Ethanolic seed extract of EESPS treated groups 200 and 400 mg/kg significantly decreased glucose levels ($P0.001$)** and ($P0.0001$)*** on the final day of the study. EESPS also had more anti diabetic activity starting on the 7th day of the study and the EESPS treated animals 200 and 400 mg/kg significantly decreased glucose levels ($P0.001$)** and ($P0.0001$)***. Table 1 summarizes the outcomes of the experiments.

Table 1 Effect of EESPS on hyperglycemias of diabetic and normal rats the results show in below the table.

Groups	0 th day	7 th day	14 th day	21 st day	28 th day	35 th day	42 nd day
Normal Control	65.17 \pm 1.167***	66.33 \pm 1.498***	66.50 \pm 1.384**	67.00 \pm 1.000***	68.17 \pm 0.7923***	73.83 \pm 0.9458***	82.50 \pm 1.7
Diabetic Control	347.7 \pm 28.91##	329.5 \pm 31.43##	324.5 \pm 30.95##	315.3 \pm 31.16##	310.7 \pm 32.27##	315.0 \pm 31.79##	319.7 \pm 30
Normal(Metformin 200 mg/kg)	436.2 \pm 42.31 ^{ns}	393.0 \pm 42.83 ^{ns}	351.0 \pm 40.49 ^{ns}	277.0 \pm 35.32 ^{ns}	239.7 \pm 28.18 ^{ns}	201.0 \pm 22.94*	162.0 \pm 21
EESPS 200 mg/kg	422.0 \pm 50.02 ^{ns}	382.0 \pm 56.57 ^{ns}	348.5 \pm 59.36 ^{ns}	313.2 \pm 55.19 ^{ns}	296.2 \pm 58.30 ^{ns}	270.3 \pm 52.76 ^{ns}	225.5 \pm 43
EESPS 400 mg/kg	404.2 \pm 67.83 ^{ns}	309.2 \pm 55.81 ^{ns}	328.0 \pm 71.76 ^{ns}	212.2 \pm 29.38 ^{ns}	178.2 \pm 26.66*	144.5 \pm 14.48**	113.0 \pm 16

The results are shown as mean SD, with a P value of 0.05 in an ANOVA with Dunnet's test.

In comparison to untreated normal rats (group 1), there was a significant difference; *. Significant difference as compared to diabetic rats that had not been treated (group 2); #. Significant change from control rats given 200 mg/kg body weight EESPS (group 3);ns. There was a significant difference between diabetic rats treated with 150 mg/kg body weight EESPS (group 4) and diabetic rats treated with 150 mg/kg body weight EESPS (group 4);ns.

3.2. Evaluation of the effect of EESPS on Hyperlipidemia

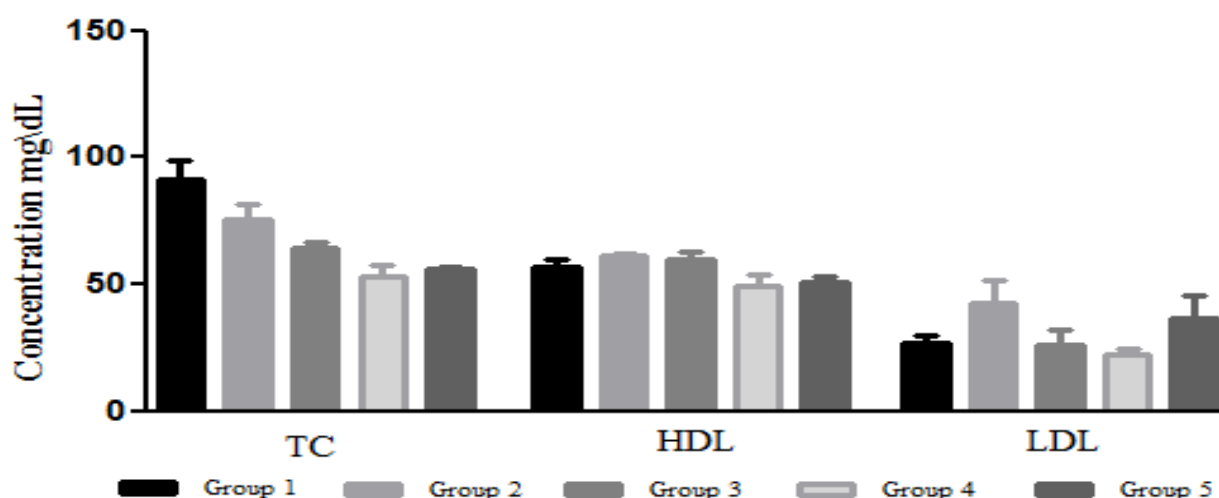
The biochemical data such as total cholesterol (TC), low density lipoprotein (LDL), and high density lipoprotein (HDL) levels were also studied. In the STZ-treated rats had higher TC and LDL levels and lower HDL levels than normal control rats and Metformin-treated rats as observed on the final day. In relation to serum lipid levels. Induction of diabetes for six weeks of therapy with EESPS considerably decreased blood levels of TC and LDL- cholesterol; nevertheless, serum levels of HDL- cholesterol were dramatically elevated when compared to the diabetic control group, according to one-way ANOVA results (Fig. 1).

Table 2: Effect of EESPS on Total Cholesterol, HDL, and LDL Levels in Diabetic Rats Induced by STZ

GROUPS	CHOLESTEROL TOTAL (mg/dL)	CHOLESTEROL HDL (mg/dL)	LDL CHOLESTEROL (mg/dL)
Normal	91.17±7.661 ^{ns}	42.50±2.487 ^{ns}	3.217±0.3563 ^{ns}
Diabetic control	75.67±5.560 ^{##}	45.83±0.8724 ^{##}	5.100±1.102 ^{##}
Standard(Metformin in 200 mg/kg)	64.00±2.708 ^{ns}	45.00±1.949 ^{ns}	3.133±0.7584 ^{ns}
EESPS 200 mg/kg	53.00±4.179 ^{**}	36.83±3.400 [*]	2.700±0.2708 ^{ns}
EESPS 400 mg/kg	55.83±0.9098 [*]	38.17±1.682 ^{ns}	4.350±1.103 ^{ns}

Fig: 1;EESS EFFECT ON TC, HDL, AND LDL LEVELS IN STZ INDUCED DIABETIC RATS

Effect of EESPS on TC, HDL and LDL in Streptozotocin induced diabetes rats



After 42 days of treatment, the effect of EESPS on blood total cholesterol, high-density lipoprotein, and low-density lipoprotein in normal and experimental rats is shown in Fig. 1. In ANOVA using DUNNETS MULTIPLE COMPARISON'S test, the values are reported as mean± SD, P 0.05. Compared to untreated normal rats (group 1), there was a significant difference. #Significant

difference compared to STZ induced rats given with 200 mg/kg body weight of EESPS (group 3);
 *Significant difference compared to untreated diabetic rats (group 2);

3.3. Evaluation of the effect of EESPS on hepatic and renal function markers

The Hepatic (AST, ALT) and renal (serum urea, creatinine, and total protein) function indicators increased in diabetic rats. In diabetes treatment (EESPS 200 mg/kg, 400 mg/kg) groups, the above enzymatic activities were close to normal, and similar results were seen with Metformin. The levels of hepatic and renal function indicators in normal treated rats did not alter significantly. The results of hepatic and renal function markers in all experimental groups are shown in Figs. 2 and Table Fig: 2; Effect of EESS ON ALT and AST level in STZ induced diabetic rats

Effect of EESPS on ALT and AST in streptozotocin induced diabetes rats

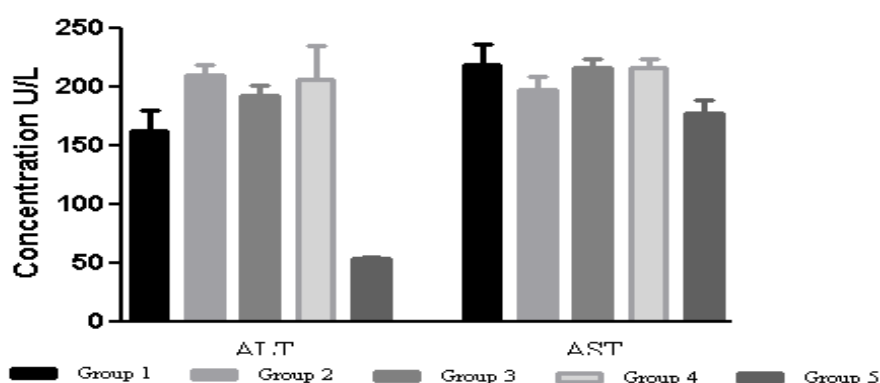
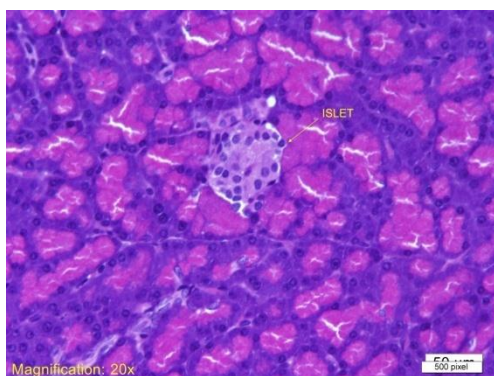


Figure 2 shows the effect of the EESPS on serum glutamic oxaloacetic transaminase and serum glutamicpyruvic transaminase in normal and diabetic rat. The results are presented as mean±SD, with a significance level of $P \leq 0.05$ in an ANOVA with DUNNETS' MULTIPL COMPARISION TEST. ALT: Significant difference from untreated diabetic rats (group 2) *;AST: Significant difference from untreated diabetic rats (group 2)

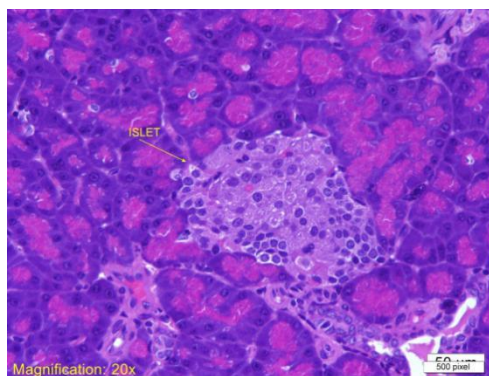
Table 3: Creatinine, serum urea, total protein, and albumin levels in response to EEPSP extract.

GROUPS	CREATININE	UREA	TOTAL PROTEIN	ALBUMIN
Normal	0.6000±0.07746 ^{ns}	54.50±1.317 ^{ns}	7.417±0.2701 ^{ns}	4.183±0.2548 ^{ns}
Diabetic control	0.5167±0.1046 ^{##}	64.88±1.414 ^{##}	7.517±0.1515 ^{##}	3.850±0.1875 ^{##}
Standard(Metformin 200 mg/kg)	0.3333±0.03333 ^{ns}	63.53±3.466 ^{ns}	6.887±0.2535 ^{ns}	3.383±0.1815 ^{ns}
EESPS 200 mg/kg	0.4333±0.07601 ^{ns}	68.62±6.440 ^{ns}	6.967±0.3412 ^{ns}	3.200±0.2380 ^{ns}
EESPS 400 mg/kg	0.3667±0.03333 ^{ns}	53.72±1.341 ^{ns}	7.200±0.1155 ^{ns}	3.533±0.09888 ^{ns}

Fig: 3; Histopathological Study of Pancreas by using EEPSP in STZ Induced Diabetic Rats.



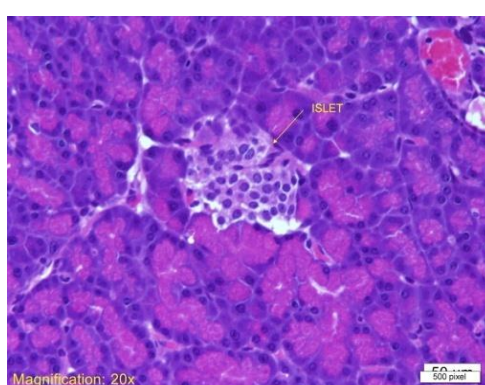
STB -Pancreas, Regressed islet size, regressed BETA cells in islet and irregular islet shape, Grade-severe, 200x



ST1H- Pancreas, progress in islet size and shape as well as β cell population, 200x



ST2T- Pancreas, progress in islet size and shape as well as β cell population, 200x



SST -Pancreas, progress in islet size and shape as well as β cell population, 200x

3.4. DISCUSSION

Diabetic hyperglycemia is the hallmark of the disease. Diabetes and its accompanying secondary consequences such as diabetic nephropathy, neuropathy, retinopathy, cardiovascular disease, and others are caused by persistent hyperglycemia [16]. As a result, in a diabetic patient, maintaining a near-normal glycaemic status is critical. Because blood glucose levels rise quickly following meal digestion and monomer absorption, inhibiting oligo/polysaccharide-hydrolyzing enzymes, such as α -amylase and α -glucosidase, may prolong polysaccharide breakdown. The ethanolic extract of *Pterocarpus santalinus* seeds, Suppressed the activity of hydrolyzing enzymes, implying that it is effective in lowering hyperglycemia [17].

A Low dosage of STZ injection lowers insulin levels quickly while maintaining insulin resistance, resulting in a type-2 diabetic model [18]. The diabetic rat treated with Metformin and the ethanolic extract of *Pterocarpus santalinus* seeds, on the other hand, showed the same results as the control Rats, showing that the diabetic condition was improved or managed. In this study, the body weight of all Type-2 Diabetes rats was increased compared to control Rats. The body weight of diabetic rats reduced after STZ induction compared to the control group. This could be because muscle protein is destroyed in diabetics to give amino acids as gluconeogenesis substrates [19]. Because insulin levels

are low due to STZ-induced diabetes, the cells are unable to transport and use glucose. However, as previously stated, insulin and glucose levels were normalized to the baseline. Because there may be enhanced glucose absorption and hence lower gluconeogenesis in treated diabetic rats, body weight is not reduced in metformin and extract-treated Rats, and thus body weight is not reduced. Type-2 Diabetes Rats plasma lipid profile was altered in this study when compared to Rats from other groups. These findings are consistent with previous research. Plasma TG and TC levels were higher in STZ-induced animals. Diabetes has been linked to an increase in hepatic TG, according to studies. [20]. Increased TG levels cause fat to accumulate in the liver, resulting in non-alcoholic fatty liver disease. [21]. LDL-C and TG levels that are too high have been linked to the development of hypertension and cardiovascular disease. [22,23]. There is a considerable increase in plasma levels of TG and LDL in this study, which could contribute to cardiovascular disease. It's thought to be a sign of dyslipidemia and Associated Diseases. [24]. In this study, diabetic Rats had considerably higher dislipidemia than control, Metformin, and extract-treated Rats, indicating a significant change in diabetic Rats that could contribute to cardiovascular disease. Diabetes is widely known to cause organ malfunction or damage as a secondary consequence. Plasma AST, ALT, and ALP activity were shown to be higher in Type-2 Diabetes Rats than in control Rats in this investigation. T the Liver impairment is indicated by high AST, ALT, and ALP activity. Previous research has shown that in diabetes patients, liver function indicators such as ALT, AST, and ALP elevate, which is consistent with our findings. Diabetic animals given Metformin plus an ethanolic extract of *Pterocarpus santalinus* seeds, Showed The plasma AST, ALT, and ALP activity comparable to control Rats. Similarly, as described in prior studies, renal impairment was exclusively detected in diabetic Rats in our investigation. Finally, diabetic Rats had considerably higher levels of urea, creatinine, total protein, and albumin, indicating kidney injury. Long-term hyperglycemia in diabetics is known to harm the kidneys, resulting in diabetic nephropathy. The plasma concentrations of urea, (blood,urea,nitrogen,)BUN, uric acid, and creatinine in diabetic Rats .

Histopathological study of EEPSP:Streptozotocin (Disease control) treated animals showed regressed islet size, regressed β cell population and irregular shape islet in pancreas. Test drug and marketed drug treated animals (ST1H, ST2H, ASB and SST) were shown progress in islet size and shape as well as β cell population when compare to disease control animals. However, test drug treated animals (ST1H, and ST2H) showed slightly added progress in islet size and shape as well as β cell population when compare to marketed drug treated animals. The test drug showed slightly added efficacy when compare to marketed drug in treating with Streptozotocin induced diabetes.

4. CONCLUSION

The present study indicated that regular administration of EESPS could prevent hyper glycemia and lipidemia, and hepatic and renal diseases. In the flame of these findings consumption of *P. Santalinus* can be useful in the treatment and management of diabetes. Though, the molecular mechanism and the active components such as phenole(s) responsible for this anti-diabetic activity remain to be elucidated, which requires further investigation.

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