Research Article

Blood Glucose Lowering Potency of Hydro-Alcoholic Extract of Roots of *Thespesia populnea* Against Streptozotocin Induced Hyperglycemia in Wistar Rats

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ABSTRACT

Objective: To evaluate the antidiabetic activity of hydroalcoholic extract of roots of *Thespesia populnea* against streptozotocin-induced hyperglycemia in wistar albino rats.

Method: Blood glucose levels of streptozotocin-induced hyperglycemia in wistar albino rats were monitored after the administration of *Thespesia populnea* extract (200 and 400 mg/kg) to diabetic rats for 21 days. Fasting plasma glucose levels, serum lipid profiles, and changes in body weight were evaluated in normal rats while liver glycogen levels and pancreatic TBARS levels were evaluated additionally in diabetic rats.

Results: The diabetic groups treated with the hydroalcoholic root extract were compared with standard metformin. The findings of the study support the antidiabetic claims of *Thespesia populnea*.

Conclusions: The results suggest that the root extract of *Thespesia populnea* possesses antidiabetic activity, which might be a potential source for the isolation of new orally active agents in the treatment of diabetes and its associated complications.

Keywords: *Thespesia populnea*, Hydroalcoholic extract, Metformin, Antidiabetic activity, Streptozotocin.

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INTRODUCTION

One of the most prevalent non-communicable diseases in the world, diabetes mellitus (DM) impairs the quality of life for people of all ages.[1, 2] The illness has developed into a major public health issue that has an impact on a person's socioeconomic standing.[3] It is an old-age, severe heterogeneous disorder characterized by hyperglycemia, altered lipid, carbohydrate, and protein metabolism, and a higher risk of vascular disease consequences.[4] Endocrine control continues to deteriorate, which exacerbates metabolic abnormalities and predominantly causes hyperglycemia.[5]

The secondary pathophysiologic alterations in numerous organ systems brought on by the metabolic dysregulation associated with DM place a heavy strain on both the diabetic and the healthcare system. It often precedes the onset of microvascular and macrovascular illnesses such neuropathy, nephropathy, cardiovascular, and cerebrovascular diseases.[6] Several oral hypoglycemic medications are available to treat diabetes because it is an incurable disease, but they are often ineffective and have a lot of harmful side effects.[7,8] As a result, switching to plant sources is a fresh, optimistic strategy that the World Health Organization's general assembly has long validated.[9] In the case of complex diseases like diabetes and its complications, they may have therapeutic effects. *T. populnea* (Linn.) (Fam. Malvaceae) is a medium-sized, fast-growing, evergreen tree that may reach a height of 10 m. Its yellow, cup-shaped blooms have a maroon center, and it is found in coastal forests across India. It is also commonly grown as a roadside tree. It bears yellow flowers that resemble hibiscus leaves with shiny green colour. Currently, it has become naturalized in tropical regions all over the world. The tree thrives in full sunlight and can withstand dry circumstances. The tree's excellent wind resistance makes it a valuable coastal windbreak. It expands quickly and reproduces easily. The plant profile and its ethnopharmacology will be the main topics of this investigation. *T. populnea* has been discovered to have medicinally beneficial characteristics in a number of different components, including anti-fertility, anti-microbial, anti-inflammatory, antioxidant, purgative, and hepatoprotective activities.[9,10] In order to assess the antidiabetic effect of *Thespesia populnea* hydroalcoholic extract of roots against streptozotocin-induced hyperglycemia in albino wistar rats, the current investigation was conducted.

MATERIALS AND METHODS

1. Collection and Authentication of plant material

Thespesia populnea's fresh roots were procured in Tamil Nadu, India. Dr. S. Mutheeswaran, Scientist, Xavier Research Foundation, Palayamkottai, Tamil Nadu, India,

performed the botanical identification of the plant under voucher number XCH40430. Plant roots were cleaned, shade-dried, cut into small pieces, mechanically ground, sieved through a 40-mesh screen, and then kept in an airtight container for later use.

2. Preparation of Plant Extract

A desiccated extract was created by extracting 100 gms of powdered roots with 70% ethanol in a Soxhlet apparatus, filtering it through muslin fabric, and then drying it out with a rotary evaporator. This desiccated extract was then kept at 4 °C until it was needed. The extract's yield as a percentage was 8.9%. [11]

3. Phytochemical screening

Using established qualitative techniques as outlined by Harborne, the plant material's extracts were examined for the presence of alkaloids, carbohydrates, phenols, gums and mucilage, flavonoids, steroids, proteins, tannins, and saponins. (1973).

4. Animals

Wistar albino rats of either sex, weighing 150–200 grams, were utilized in the investigation. These animals were purchased from the Animal House of the Aditya Bangalore Institute of Pharmacy Education and Research, located in Yelahanka, Bengaluru. The animals were kept in cages at ambient temperature and humidity, with a 12:12 hr dark/light cycle to provide natural illumination. They were provided with a standard food and free water. The Institutional Animal Ethics Committee reviewed the experimental protocol on 4/12/2021, and it gave its approval before the experiment could start (Protocol Proposal No. 70/1611/CPCSEA).

5. Acute toxicity study of the extract

After the animals had fasted for the previous night and only consumed water, a test for acute toxicity of plant extracts was conducted. Each mouse's weight was noted before the extract was given to them. The animals were split into control and treatment groups at random, with six mice in each group. *Thespesia populnea* extracts in 70% ethanol were given orally to each treatment group at doses of 1000, 2000, and 5000 mg/kg while the control group only got the vehicle (1% Tween 80). For the first four hours following the administration of the extract, animals were kept under close observation for any overt toxicities and/or behavioral changes like restlessness, tremor, diarrhea, sluggishness, loss of weight, and paralysis at regular intervals. After that, they were monitored every day for two weeks to look for any changes in general behavior and/or other physical activities. After four hours of administering the extracts, food was accessible. [12]

6. Induction of experimental diabetes

For the investigation, 30 male albino rats weighing between 180 and 200 g were employed. The rats were given a single intraperitoneal injection (i.p.) of 65 mg/kg b.w. of streptozotocin (STZ) diluted in 0.1 mL fresh cold citrate buffer pH 4.5 after being fasted for the previous night. Using a One Touch Glucometer, diabetes was confirmed 72 hours after STZ induction. Rats' tail punctures were used to collect blood samples. After 10 days of STZ induction, animals were deemed diabetic and included in the study as diabetic animals if their fasting blood glucose level was 200 mg/dL or higher.

7. Experimental design

Six rats were divided into five groups at random. As a negative control, group 1 was given normal saline (10 mL/kg), group 2 was given a diabetic control, group 3 was given as the standard, and groups 4 and 5 were given 200 and 400 mg/kg of the root extract, respectively.

8. Determination of blood glucose levels Measurement and weight

On days 0 through 21, blood glucose levels were calculated using a biochemistry analyzer (GOD POD technique). Retro-orbital punctures were used to obtain blood samples from anesthetized (slight ether exposure) animals at the conclusion of the investigation (on 21 days).

9. Hypolipidemic Activity and Biochemical Analysis

Following treatment, blood samples were taken from all rats in all groups at 1, 2, 3, 6, 10, 16, and 24 hours. These samples were then analyzed using a Glucometer to determine the blood glucose content. (Bio Land, Germany). After that, all of the rats were killed, and 1-2 mL of blood was extracted from the heart using disposable syringes. The serum was collected and utilized to calculate the total cholesterol (TC) and serum triglycerides after the blood samples were transferred to centrifuge tubes and centrifuged at 4000 rpm for 10 min. (TG). According to the manufacturer's instructions, serum total cholesterol and triglycerides were calculated at 505 and 546 nm using the CHO/PAP (cholesterol oxidase/ p-amino antipyrine) method and the GPO (glycerol 3-phosphate oxidase) method, respectively.

10. Statistical Analysis

For groups of six animals, all results are shown as means with standard errors of the means (S.E.M), and each set of data was subjected to a one-way analysis of variance (ANOVA) before being compared using the Tukey's Kramer multiple comparison test. $P < 0.05$ was regarded as being significant.

RESULTS

1. Phytochemical screening

Alkaloids, carbohydrates, glycosides, phenols, phytosterols, thiols, gums, mucilage, flavonoids, terpenes, steroids, proteins, tannins, and resins are the principal chemical components of these plants. [13,14]

2. Acute oral toxicity study

According to an acute toxicity investigation, *Thespesia populnea* root extract did not result in any deaths in either dose (2g/kg) over the first 24 hours or for the next 14 days. The experimental rats' physical and behavioral observations also showed no overt toxicity-related symptoms such as lacrimation, lack of appetite, tremors, hair erection, salivation, diarrhea, and convulsion. This indicates that the extract's LD50 is higher than 2 g/kg.

3. Anti-Diabetic Activity

In normal and streptozotocin-induced diabetic rats, the effects of various dosages of *Thespesia populnea* hydroalcoholic extracts on blood glucose (mmol/L), serum total cholesterol, and triglycerides (mmol/L), were examined. As a conventional anti-diabetic medication, metformin HCl (150 mg/mL) was employed.

Effects of hydro-alcoholic extracts of *Thespesia populnea* on Blood Glucose:

A decrease in blood glucose level was observed in animals treated with *Thespesia populnea* at 0, 1, 2, 3, 6, 10, 16, and 24th hr

Following the delivery of glucose, the peak blood glucose level was quickly elevated above the fasting glucose value before decreasing. At 4 hours, both plant extract doses had a discernible reducing effect on blood sugar. In contrast, rats treated with metformin had results that were comparable to those of normal control rats who were given a high dose of the extract (400 mg/kg body weight). *Thespesia populnea* extract's antidiabetogenic efficacy in rat models is demonstrated by the noticeably lower peak blood sugar levels within 4 hours. This result demonstrated a considerable improvement in the glucose tolerance test, which was likely caused by the plant extract's ability to mimic insulin by reversing the delayed insulin response.

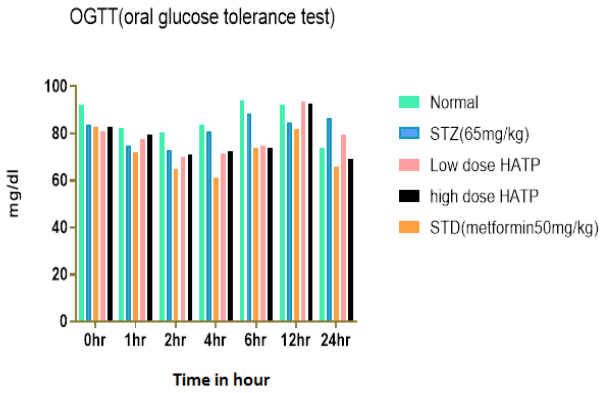

Figure 1: Effect of Hydro-alcoholic extract of *Thespesia populnea* on fasting serum glucose level (OGTT) in STZ induced diabetic rats. (In hours)

All the values are mean ± SEM, n=6, ns= not significant, one-way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test

Table 1: Effect of Hydroalcoholic extract of *Thespesia populnea* (Linn.) on fasting serum glucose level (OGTT) in STZ-induced diabetic rats

Treated group	Fasting serum blood glucose level (OGTT)						
	0hr	1hr	2hr	4hr	6hr	12hr	24hr
Normal	92.00±0.66	82.34±1.06	80.54±1.69	83.84±1.8	93.89±1.4	92.17±1.2	73.8± 1.34
Control STZ (65mg/kg)	83.89±0.52	75.00±1.52	72.83±1.14	81.00±0.32	88.45±0.71	84.85±0.74	86.81±1.25
Standard Metformin (50mg/kg)	82.89±0.53	72.00±1.38	65.00±1.44	61.00±1.02	73.71±1.33	81.8± 1.35	66.12±1.36
HATP (200mg/kg)	81.00±1.73	77.60±1.09	69.92±1.08	71.54±0.84	74.92±1.04	93.70±1.53	79.35± 1.62
HATP (400mg/kg)	83.00±1.74	79.60±1.10	70.92±1.08	72.54±0.85	73.92±1.04	92.70±1.54	69.35± 1.77

All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test, *p< 0.05 and **p<0.01, vs. control group and ^{a}p<0.001, vs normal group. HATP- of Hydroalcoholic extract of *Thespesia populnea* (Linn.)

Table 2: Effect of hydroalcoholic extract of *Thespesia populnea* (on fasting serum glucose level (OGTT) in STZ-induced diabetic rats. (In weeks)

Treated groups	Serum glucose levels at weekly intervals mg/dl				
	0 Day	7th Day	14th Day	21st Day	28th Day
Normal	74.40±0.73	78.17±0.32	77.05±0.65	83.67±0.68	75.73±1.96
Control (STZ 65mg/kg)	214.0±0.27	229.6±0.75	229.0±0.41	249.5±0.75	245.3±1.59
Standard (Metformin)	222.0±0.93	229.3±0.17	192.9±1.66	113.3±0.97	125.3±1.48
HATP (200mg/kg)	219.2±0.12	232.3±1.86	184.1±0.76	167.2±0.22	143.9±1.85
HATP (400mg/kg)	246.0±0.86	234.0±0.42	169.0±0.38	144.8±1.53	107.7±1.367

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *p< 0.05 and **p<0.01, ***p<0.001 vs. control group and ^{a}p<0.001, vs normal group

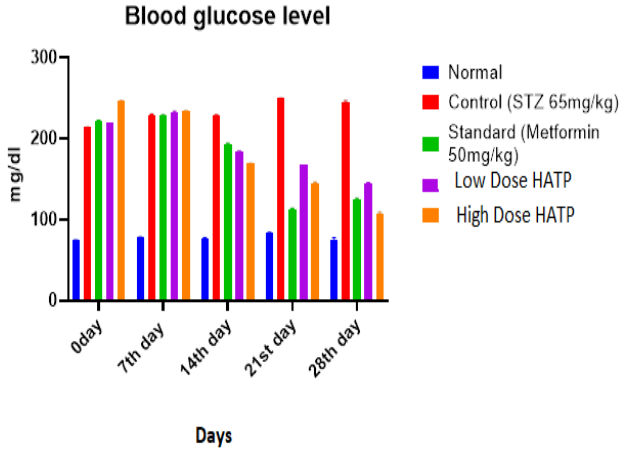

Figure 2: Effect of hydro-alcoholic extract of *Thespesia populnea* on fasting serum glucose level (OGTT) in STZ induced diabetic rats. (In weeks)

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test.

The study's findings made it abundantly evident that when compared to diabetic control mice, the standard and plant extract treated animals showed a considerable decrease in glucose levels. After two weeks of treatment, mice given *thespesia populnea* (400 mg/kg) and metformin (50 mg/kg) returned the hyperglycemia caused by STZ to normal levels.

Effect of hydro-alcoholic extract of *Thespesia populnea* on serum lipid level

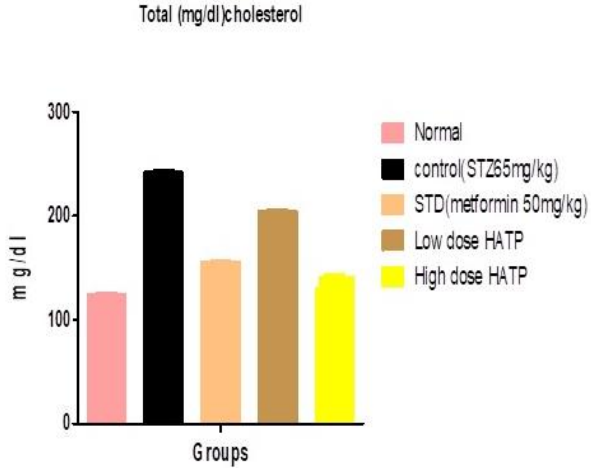

Figure 3: Effect of hydro-alcoholic extract of *Thespesia populnea* on cholesterol in STZ induced diabetic rats

All the values are mean ± SEM, n=6, ns=Not significant, one way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test,

As seen in fig. 3, the diabetic control group's total cholesterol level was higher when compared to that of the healthy controls. When the extract was given to STZ-diabetic rats, the changes in total cholesterol were prevented.

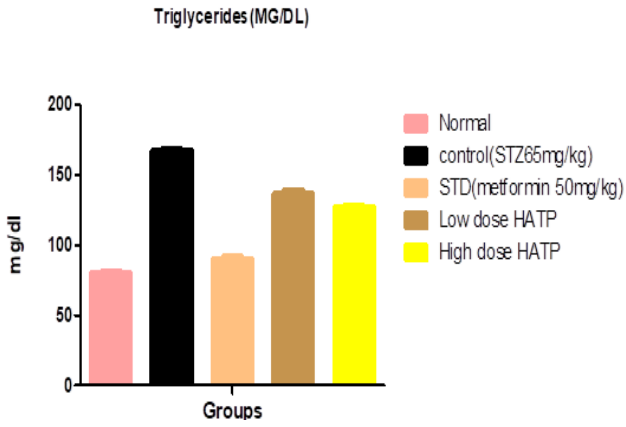

Figure 4: Effect of hydro-alcoholic extract of *Thespesia populnea* on Triglycerides in STZ induced diabetic rats

As shown in fig 4, High doses of *Thespesia populnea* exhibited reduction in TG level were observed in the diabetic control compared with that of normal control.

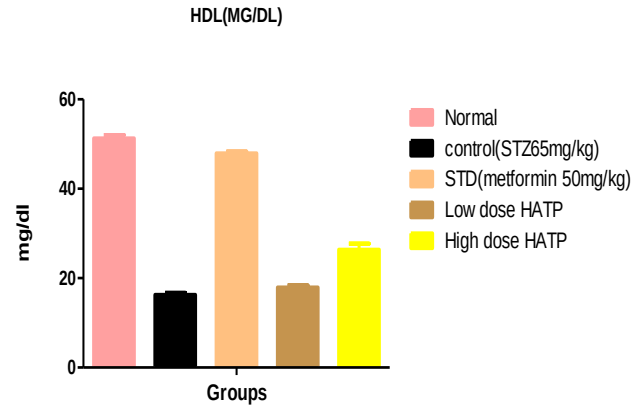

Figure 5: Effect of hydro-alcoholic extract of *Thespesia populnea* on HDL in STZ induced diabetic rats

Treatment of the diabetic rats with hydro-alcoholic extract of *Thespesia populnea* increased HDL level compared to the diabetic group

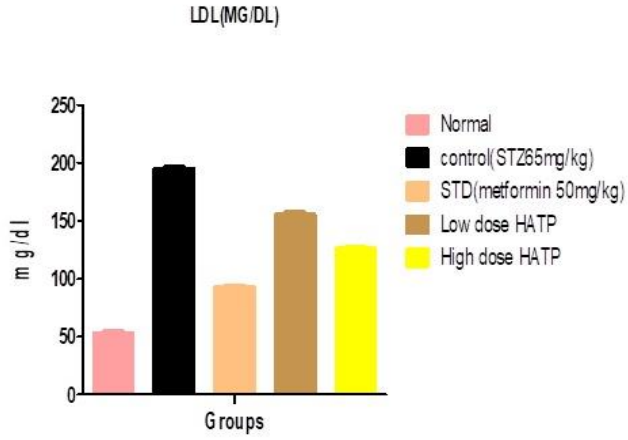

Figure 6: Effect of hydro-alcoholic extract of *Thespesia populnea* on LDL in STZ induced diabetic rats

Treatment of the diabetic rats with hydroalcoholic extract of *Thespesia populnea* reduced LDL level compared to the diabetic group

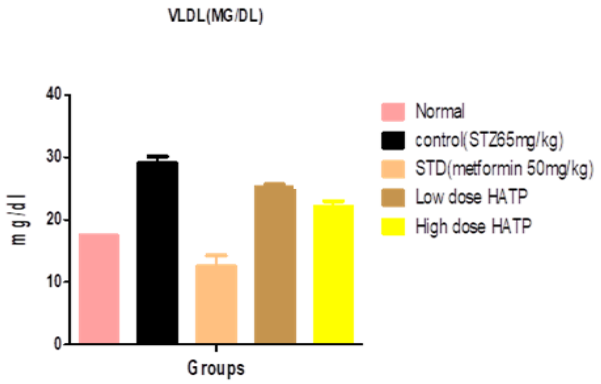

Figure 7: Effect of hydro-alcoholic extract of *Thespesia populnea* VLDL in STZ induced diabetic rats

All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test**.**

When diabetic rats were given a hydro-alcoholic extract of *Thespesia populnea*, their VLDL levels were lower than those of the control group.

Effect of Hydro-alcoholic extract of *Thespesia populnea* on Liver function test

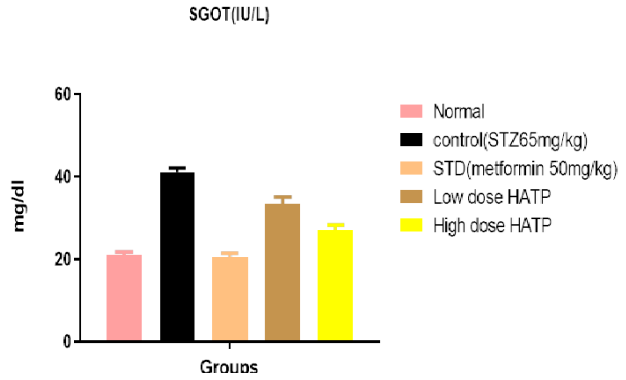

Figure 8: Effect of Hydro-alcoholic extract of *Thespesia populnea* on SGOT in STZ induced diabetic rats

Each value was averaged over its standard error of measurement (SEM), with n=6. Dunett's multiple comparison test was then performed. The hydro-alcoholic extract of *Thespesia populnea* and metformin-treated rats revealed normal liver biomarkers, but the diabetic control group had higher SGOT levels.

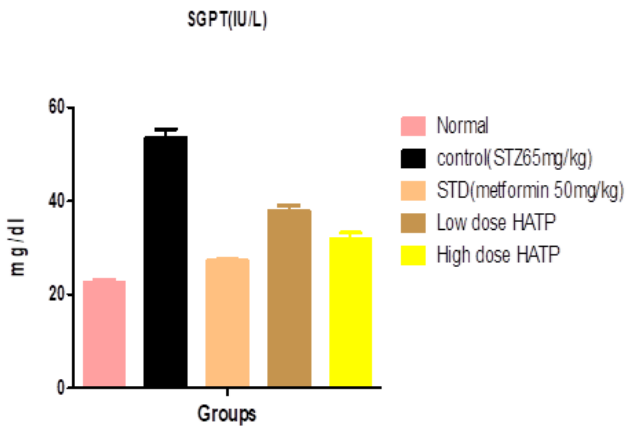

Figure 9: Effect of Hydro-alcoholic extract of *Thespesia populnea* on SGPT in STZ induced diabetic rats

The values were analysed using a one-way analysis of variance (ANOVA) and the Dunett multiple comparison test with n=6, ns=not significant, and mean SEM for all data. The values are all mean SEM, with n=6. The hydro-alcoholic extract of *Thespesia populnea* and metformin-treated rats revealed normal liver biomarkers, but the diabetic control group had higher SGPT levels.

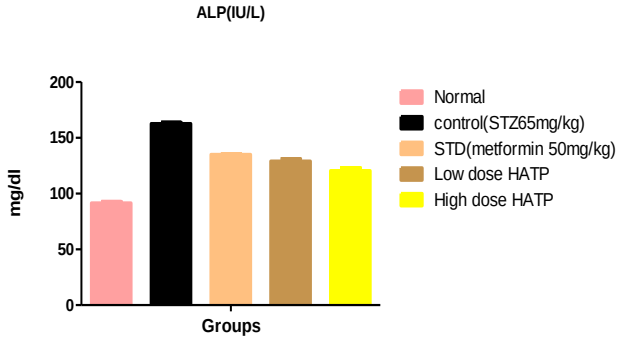

Figure 10: Effect of Hydro-alcoholic extract of *Thespesia populnea* on ALP in STZ induced diabetic rats

Each value was averaged over its standard error of measurement (SEM), with n=6. Dunett's multiple comparison test was then performed. In rats treated with metformin and the hydroalcholic extract of *Thespesia populnea*, liver indicators like ALP were found to be normal, but the diabetic control group had higher ALP levels.

Table 3: Effect of Hydro-alcoholic extract of *Thespesia populnea* on in–vivo Anti-Oxidant Parameter From Liver Homogenate in STZ induced diabetic

Treated Group	SOD (Unit/min/gm)	Catalase (µ m/mg tissue)	LPO (µm of H_2O_2/mg tissue)
Normal	43.87±0.66	72.33±0.81	9.952±0.28
Control (STZ65mg/kg)	25.45±0.83	31.62±0.83	24.38±0.58
Standard (metformin50mg/kg)	40.25±0.34	70.71±0.73	14.64±0.36
HATP (100mg/kg)	36.87±0.70	46.59±0.81	15.83±0.64
HATP (400mg/kg)	34.98±1.66	61.28±1.25	16.61±1.20

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, p<0.05, **p<0.01**, ***p*< 0.001 vs. control group and [a]*p*<0.001, vs Sham-operated normal

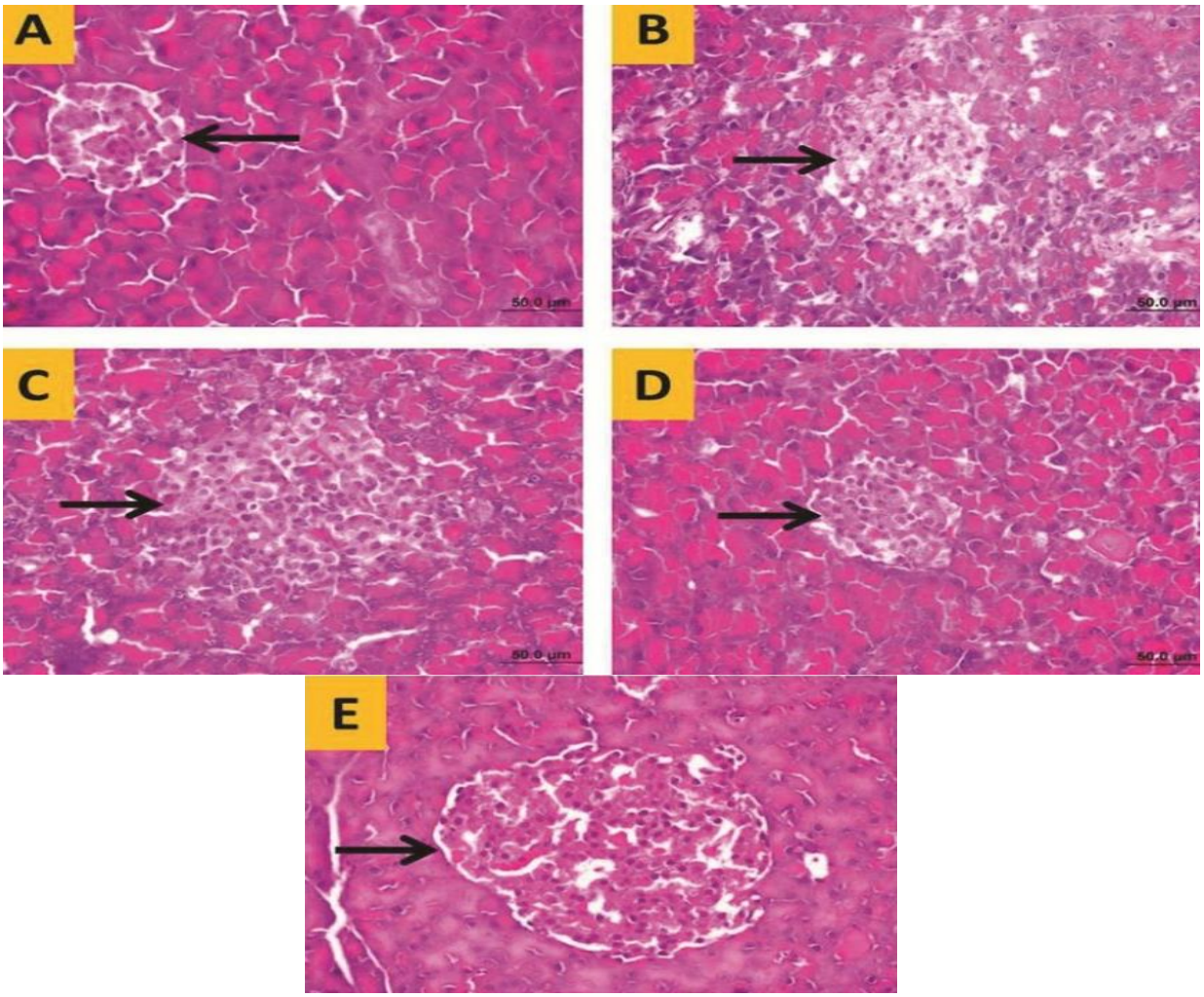

Figure 11: Microphotographs of pancreas tissue examined by routine hematoxylin-eosin of STZ treated animals A: Normal group, B: Control group (STZ 50mg/kg), C: Metformin 10mg/kg, D: HATP 200mg/kg, E : HATP 400 mg/kg

Histopathology of Pancreas

Both the control group and the experimental group of rats displayed the histopathological alterations. The islet cells appeared normally in the pancreas of the control rat. The pancreas damage in the STZ-treated rats was manifested as vacuolization, necrotic alterations, and diminished islet cells. In STZ-treated rats, oral administration of *Thespesia populnea* (Linn.) hydroalcoholic extract at doses of 200 and 400 mg/kg body weight resulted in significantly less necrosis, vacuolization, and decreased islet cells. The pancreas architecture in the reference group, which included STZ and metformin, matched that seen in the control rats. With 400 mg/kg body weight, the greatest therapeutic efficacy against STZ-induced diabetes aberrations was attained.

DISCUSSION

One of the biggest hazards to human health is diabetes mellitus, which is becoming a growing concern for many individuals. The possible hypoglycemic impact of plant aqueous extract has been the subject of numerous investigations, and it has been shown to lower the blood glucose levels of STZ-induced diabetic rats.[13]

Even at a dose of 2000 mg/kg, the mice used in the acute toxicity test for *Thespesia populnea* (Linn.) showed no evidence of toxicity or death, indicating that the extract was well tolerated and the test levels were safe for the animals.[14] *Thespesia populnea* (Linn.) hydroalcoholic extract demonstrated significant hypoglycemic effects ($p<0.01$) against STZ-induced diabetes in rats when administered orally. The extract considerably reduced blood glucose levels.[15]

Thespesia populnea (Linn.) was tested for its impact on fasting blood glucose levels using an autoanalyzer (AccuCheckActive®) glucose kit in STZ-induced diabetic rats to determine its anti-diabetic effectiveness. The fasting blood sugar test monitors blood glucose levels after a fast and is a carbohydrate metabolic test (usually 8–12 h).[16] As a result of the body's stimulation of glucagon secretion during fasting, catabolic processes release glucose into the blood. In order to counterbalance the rise in blood glucose levels, the body normally creates and uses insulin, but in people with diabetes, this mechanism is impaired and blood glucose levels are left high. Both Alloxan and STZ, which are frequently used to induce diabetes mellitus, have a debilitating impact on the beta cells of the pancreas.[17]

Rapid cell death is caused by the cytotoxic action of STZ, which is mediated by reactive oxygen species and massively increased cytosolic calcium concentration.

Because of this, endogenous insulin secretion is reduced, which allows body tissues to use glucose less effectively. and as a result, a rise in blood sugar, a reduction in protein, and an increase in cholesterol and triglycerides.[18]

Thespesia populnea's hydroalcoholic extract is being evaluated in this study for its potential to treat diabetes. (Linn.). In rats with STZ-induced diabetes, the extracts resulted in a dose-dependent decrease in FBG. By damaging pancreatic cells, STZ caused diabetes. This damage was facilitated by the production of cytotoxic oxygen free radicals. These radicals primarily attack the DNA of pancreatic cells, resulting in DNA fragmentation.[19]

After an 18-hour fast, normal rats taking part in the OGTT received a hydroalcoholic extract from *Thespesia populnea* (Linn.) that lowered blood glucose levels after 2 hours. The decline was at its greatest at 24 hours. Based on the variations in the groups' starting and ending fasting blood glucose levels, we found that the diabetic control group saw a considerable increase in blood glucose at the end of the 28-day trial period.[20] After extracts were given to diabetic rats, fasting blood sugar levels significantly dropped and serum insulin levels rose. *Thespesia populnea* (Linn.) hydroalcoholic extract may therefore exert its hypoglycemic effects by potentiating the effects of insulin on plasma, either by increasing the pancreatic secretion of insulin from the body's already-existing beta cells or by causing it to be released from its bound form.[21]

The hydroalcoholic extract of *Thespesia populnea's* high fibre content is one potential mechanism that could explain this. (Linn.) Dietary fibres are advantageous for diabetics, especially type II diabetics, as they play a significant role in decreasing blood glucose levels by slowing the rate of carbohydrate absorption from the intestine.

Triglycerides are hydrolyzed by the enzyme lipoprotein lipase under typical circumstances. Failure to activate this enzyme results in diabetes mellitus, which leads to hypertriglyceridemia. Dietary fibres reduce triglyceride and cholesterol levels. *Thespesia populnea's* high fibre content may therefore be responsible for the considerable control of blood cholesterol levels in the treated groups. (Linn.) When diabetes is induced using STZ, there is a typical decrease of body weight that is brought on by increased muscle wasting. and as a result of tissue protein loss.[22]

The liver is a crucial organ for xenobiotic metabolism, detoxification, storage, and excretion. ALP, SGOT, and SGPT are trustworthy indicators of liver health. The liver of STZ-induced diabetic rats was necrotized. The leaking of these enzymes from the liver cytosol into the blood stream may be the primary cause of a rise in the activity of SGOT, SGPT, and ALP in plasma, which is indicative of the hepatotoxic action of STZ.[23] *Thespesia populnea* (Linn.) hydroalcoholic extract treatment of diabetic rats decreased the activity of these enzymes in plasma compared to the diabetic untreated group, hence reducing liver damage brought on by STZ-induced diabetes. *Thespesia populnea* (Linn.) hydroalcoholic extract treatment of diabetic rats resulted in significant decreases in the activity of several enzymes, indicating the hepatic protective effect in preventing diabetic sequelae.[24]

CONCLUSION

The hydro-alcoholic extract of *Thespesia populnea* may include active ingredient(s) that exert anti-diabetic function, according to the findings of the current study. This explains why this herb has historically been used to treat diabetic mellitus. When compared to the reference standard metformin, plant extract from the plant mentioned in the title exhibits nearly equivalent anti-diabetic action. To isolate, characterize, and biologically assess the active principle(s) of *Thespesia populnea*, further work is still required.

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