Anti-diabetic activity of a novel Phytoformulation of *Tribulus terrestris, Prunus dulcis, Cicer arietinum* and *Azadirachta indica* in streptozotocin-induced diabetic rats

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Abstract

Background and Objective: Selected medicinal plants have been investigated as an alternative source for the treatment of Streptozotocin (Stz) induced diabetes. This aim of current study was to assess the anti-diabetic effects of selected plants formulation, *Tribulus terrestris, Prunus dulcis, Cicer arietinum and Azadirachta indica* in diabetic rats induced by streptozotocin. Plants included in this phytoformulation have shown anti-oxidant, anti-diabetic, and anti-inflammatory properties evident in previous researches.

Material and Methods: Male albino rats were given a single dose of Stz (45 mg/kg) interperitoneally to induce diabetes followed by checking of blood sugar levels, feed and water intake, body weight and various biochemical parameters.

Treatment: Aqueous (4ml/kg) and ethanolic (2ml/kg) extracts of phytoformulation were prepared and for 21 days given orally to the diabetic rat groups.

Results: The treatment showed reduced glucose levels in random and fasting blood glucose tests, increased body weight, considerably decreased levels of ALT, AST, ALP, Hb1AC, serum urea, creatinine, total lipids, cholesterol, triglycerides, LDL and VLDL, whereas significant elevated levels of HDL. Histological analysis of pancreas also indicate that the extract improved the Islets of Langerhans and increased the number of β cells.

Conclusion: The current study has shown that the novel plant composition used has significant anti-diabetic effects and improved various biochemical parameters in rats. It is also suggesting that this plant composition is an easily accessible and cheap alternative medicine for diabetes with least side effects.

Keywords: Diabetes Mellitus, Streptozotocin, Phytoformulation, *Tribulus terrestris, Prunus dulcis, Cicer arietinum and Azadirachta indica*, islets of Langerhans

Introduction

Diabetes mellitus (DM) is an extremely prevalent metabolic condition that influences a large number of people consistently and it is a worldwide medical issue. Diabetes happens due to a reduction in insulin discharge by pancreatic β cells and inadequate insulin receptor response. Severe effects from chronic hyperglycemia include diabetic neuropathy, nephropathy, retinopathy, and cardiovascular disease. Ageing, physical inactivity, obesity, urbanization, a sedentary lifestyle, and poor eating habits all contribute to a rise in diabetes incidence across the world. DM is the fourth biggest cause of mortality, and research suggests it, a pandemic in a number of developing and recently industrialized countries (Sani *et al.*, 2014).

Pakistan, a developing nation, is dealing with a dramatic increase in the prevalence of diabetes (Akhtar *et al.*, 2019). After China and India, Pakistan does have third-highest rate of diabetes globally (WHO, 2021). Diabetes regarded a hazard to public health because the shortage of viable medications for treating the illness, especially in underdeveloped nations, Asia and Africa. Despite the accessibility of different organic and artificial antidiabetic drugs, it is causing serious clinical issue worldwide due to the side effects associated with such synthetic drugs. Biguanides (metformin) may cause stomach problems, anorexia, vomiting, and a B12 deficit if used for an ext ended period of time.

Plant extracts with anti-diabetic action might be utilized as an alternate source for diabetes care due to the drawbacks of conventional medications, specifically in emerging nations, because of their, ease of availability, cheap cost, social acceptance, and lack of harmful consequences. There are 400 or more plants that can treat diabetes (Rashid *et al.*, 2022). Diabetes is a disease that is still not completely curable through currently available anti-diabetic drugs. So medicinal plants are viewed as more effective option for the management of diabetes. Selected medicinal polyherbal formulation studied in current study consists of four plants, *Tribulus terrestris, Prunus dulcis, Cicer arietinum* and *Azadirachta indica*. According to certain reports, each plant possesses unique anti-oxidative and anti-diabetic capabilities.

Tribulus terrestris (TT) plant belongs to the *Zygophyllaceae* family. It is a priceless herb that is widely used as traditional remedies all around the world. Various substances found in TT extract, including alkaloids, flavonoids, oils, saponins, resins, and nitrates, have anti-oxidant, hypolipidemic and anti-hypertensive effects (Hend *et al*., 2015). TT dramatically boosted serum superoxide dismutase activity, whereas blood glucose, triglyceride, and cholesterol levels were considerably decreased (Meena *et al.*, 2019).

Prunus dulcis is a member of the Rosaceae family. The presence of various physiologically active phytochemicals, including tannins, flavonoids, and vitamin E, which contain anti-inflammatory, anti-oxidant, anti-carcinogenic, and anti-diabetic qualities.

Azadirachta indica has gained a lot of interest from the medical world in recent years. It is also known to be useful in treating tumors, syphilis, skin conditions, bronchitis, cough, diabetes, sleepiness, jaundice, and nausea. It is also known to be beneficial in treating obesity. Pharmaceutical firms can use *A. indica* leaf as an adjuvant when producing antidiabetic medications since it has superior hypoglycemic potential (Ezeigwe *et al*., 2020).

Cicer arietinum demonstrated a variety of biological characteristics, including anti-oxidant and anti-diabetic. *Cicer arietinum* (chickpeas) contains bioactive compound including isoflavanoids and diphenolic and phytoestrogens (Soto *et al*., 2021). According to studies, eating chickpeas lowers insulin resistance, which inhibits adipogenesis, raises GLUT-4 levels, and has a favorable impact on adipokines (Kaur and Prasad, 2021).

The goal of the current study was to use *in vivo* testing to determine collective effect these plants, *Prunus dulcis, Tribulus terrestris, Cicer arietinum, and Azadirachta indica*, in streptozotocin-induced diabetic rats.

Material and Methods

Plant material

The four medicinal plants *Tribulus terrestris, Prunus dulcis, Cicer arietinum & Azadirachta indica* which were used to make the polyherbal mixture were bought from a local authentic seller of herbal compound. The dirt was removed and then crushed to fine powder using a mechanical blender.

Ethanolic and Aqueous extracts preparation

Powdered form of herbs was weight with the help of an electronic weighing balance. A 150 gram powdered material was macerated with 99% ethanol (350 mL), 150 grams of powdered material was macerated with distal water (350 mL) and soaked for 14 days. The mixtures were shaken every day. These were almost entirely evaporated after the 14-day and filtered using Whatmann filter paper and dried in a rotating evaporator at 40°C at low heat. The yields were discovered to be 30% ethanolic extract and 30% aqueous extract.

Animal Ethics

The current project involving the use of rats was granted ethical approval by the University of Lahore Ethics Committee (Reference No:12/UoL/2021) and Institute of Molecular Biology and Biotechnology (IMBB) (Lahore, Pakistan) Committee for Ethics in Animal Research involving healthy animal Subjects (Reference No.: 12/21).

The experiments were performed according to the care and use of laboratory animals published by the US National Institute of Health (NIH Publication No. 85 to 23, revised 1985) and established by the Institutional Review Committee of the Institute of Molecular Biology and biotechnology (IMBB), University of Lahore, Pakistan.

Experimental animals

Twenty healthy male albino rats weighing an average of 151g were purchased from the University of Lahore Animal House. Rats were housed in a cage made of stainless-steel wire under normal circumstances. Rats were allowed to acclimatize for one-week under controlled conditions prior to the experiment. They were housed in an ecologically controlled animal house at the University of Lahore and given the same standard food and water while being kept at the right temperature of 25+2°C and with a humidity of 30-70% throughout the 12:12 hours of darkness and light. The rats were fed on the freshly prepared basal diet and tap water for two weeks that met their requirements for growing.

Experimental setup

Before beginning the experiment, experimental rats underwent a week of acclimation. Twenty male albino rats were divided in four groups namely: negative control (NC), positive control (PC), T1 and T2, five rats in each group. Group NC rats were given no treatment and used as negative control group. Group PC rats were given standard dose of stz + normal water and used as a positive group. Group T1 rats were given standard dose of stz & aqueous extract 4ml/kg. Group T2 rats were given standard dose of stz & ethanolic extract 2ml/kg. For 21 days, all of the animals in the treatment groups were given medication via gavage.

Induction of diabetes

Streptozotocin (STZ) was administered intraperitoneally once, at a dose of 45 mg/kg body weight, to overnight-fasted rats to induce diabetes. To avoid early drug-induced hypoglycemia mortality, STZ-injected animals received a 20% Dextrose solution for 24 hours. Standard food and tap water were the only things given to the normal control rats. Diabetes was induced three days later, and glucose strips were used to assess the blood sugar level. Rats with blood sugar levels more than 200 mg/dL were declared diabetic after blood was drawn from the tail vein.

Feed and water intake

To measure the impact of each unique experimental diet, the total feed intake of each rat was computed daily by deducting leftover and collected spilled diet. Water was supplied via drinking bottles, and daily measurements of water intake were made.

Gain in body weight

Throughout the experimental trial period, the growth in body weight for each rat in each group was calculated weekly in order to determine the impact of the extract diet on the weight of the rats using an electronic weighted balance device.

Random and fasting blood glucose level

When the animals were grouped together, fasting blood glucose (FBG) and random blood glucose levels were assessed. Random and fasting blood glucose levels were taken from all the animals in all groups after each seven-day treatment period. A single-touch glucometer was used to assess the blood sugar levels after blood was drawn from the tail vein's tip.

Blood collection

The rats underwent anesthesia after a 12-hour fast and 21 days of treatment. All rats were sacrificed and blood was drawn from cardiac puncture and stored in EDTA & Gel & clot activator tubes in order to test the biochemical parameters.

Histopathology of pancreas

Every animal was slaughtered at the conclusion of the 21st day. For histopathological purposes, the pancreas of each rat was removed using forceps and scissors and kept in 10% formalin. Trimmed longitudinally and processed from pancreatic tissues fixed in formalin. Processes used to prepare the tissue included dehydrating it in progressively stronger alcohol, clarifying it in xylene, and embedding it in paraffin wax. With the Rotary Microtome, 3 mm thick slices of tissue blocks with paraffin wax embedded were created. Hematoxylin & Eosin (H & E) stain was applied to all of the pancreatic slides before they were mounted in DPX and covered with cover slips. To identify histological lesions, the prepared slides were inspected under a microscope.

Glycated hemoglobin HbA1c level

Blood was used to measure glycated hemoglobin (HbA1C). Blood was drawn for this purpose, and the plasma was separated in a bulb containing EDTA. Washed the packed cell pellet six times in regular saline water. Hemolysate was created by adding 1/4-part carbon tetrachloride to the packed cell pellet and centrifuging for 20 minutes at 3000 rpm.

Biochemical parameters determination

A diagnostic kit identified the parameters for the liver, kidney, and lipid profile (DiaSys Diagnostic system) Using a reagent kit, an automated analyzer (Architect c8000 clinical chemistry system) measured the blood levels of albumin, bilirubin, and total protein.

Statistical Analysis

The data were presented as mean ± SE. Applying GraphPad Prism, the data were examined by one-way and two-way Analysis of Variance (ANOVA). The difference was deemed statistically significant at $p<0.05$(Alona and Olha, 2021).

Results

Feed and water intake

In comparison to the negative control group (NC), diabetic control rats significantly increased their consumption of food and water ($p<0.05$). When treatment rats (those on extract administration) were compared to positive control (PC) rats, displayed a considerable reduction in their feed and water intake. Both group T1 and T2 treated with water extract and ethanolic extract, respectively, showed no differences.

Body weight

Effect of polyherbal extract administration on the body weight of rats is depicted in figure 1. Body weight of rats were significantly (*p<0.001*) decreased in STZ- induced diabetic rat's groups with respect to the untreated group (NC). Treatment with the polyherbal treatment enabled the body weight loss return (*p<0.01*) to normal levels. When compared the aqueous extract treated group (T1) and ethanolic extract treated group (T2), there was a minor difference present between them. Rats in the T1 and T2 groups had considerably higher body weights than those in the diabetic group.

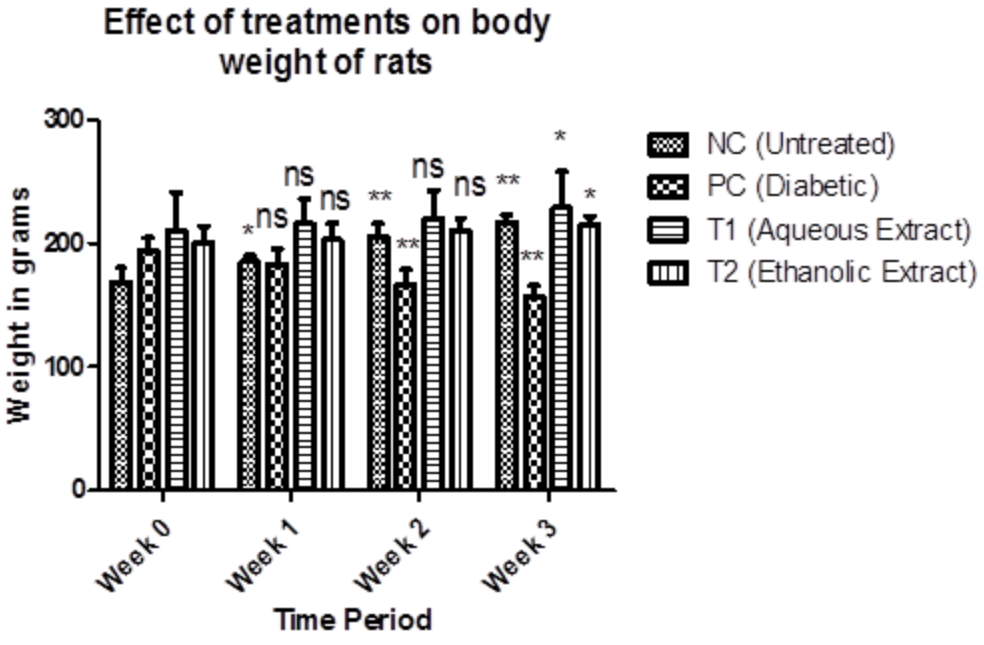


Figure.1 Effects of several treatments on the body weight of experimental rats

Data show standard error (SE) +/- Mean of weight in grams. *$p<0.05$; **$p<0.01$; ***$p<0.001$. ns means not significant. Positive group (PC) compared against negative group (NC) whereas treatment groups (T1 and T2) were compared with the PC to check their antidiabetic effect.

Fasting blood glucose

The use of STZ to successfully induce diabetes resulted in a substantial ($p< 0.001$) rise in the fasting blood glucose levels across the board for all groups, except for the negative control group, which did not receive any STZ. For three weeks, the medication was given. The effects on fasting blood glucose levels were noted at weekly intervals after the various groups of rats received the ethanolic and aqueous extracts (T1 and T2), as shown in figure 2. In comparison to the positive control group (PC) of rats, the treated group of rats given the ethanolic and aqueous extract demonstrated a significant reduction ($p<0.01$) in the FBG levels from week 1 to week 3. The results showed that PC had the highest amount of fasting blood sugar.

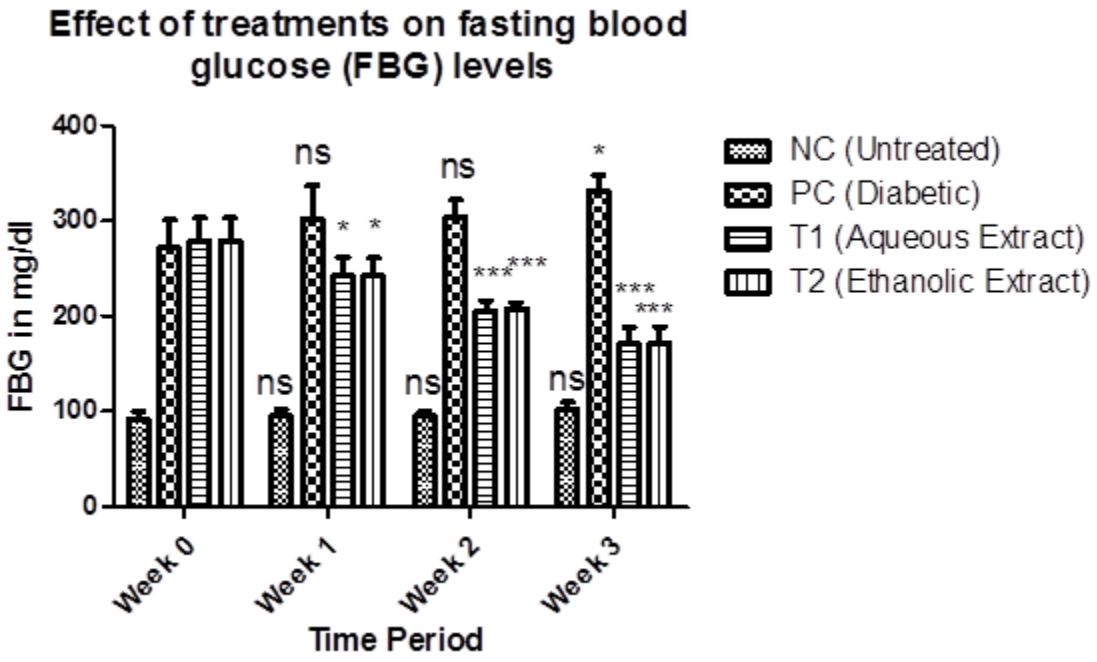


Figure.2 Effects of several treatments on the FBG level in experimental rats

Data show standard error (SE) +/- Mean of glucose level in mg/dl. *$p<0.05$; **$p<0.01$; ***$p<0.001$. ns mean not significant. Positive group (PC) compared against negative group (NC) whereas treatment groups (T1 and T2) were compared with the PC to check their antidiabetic effect.

Random blood glucose

The Random blood glucose (RBG) levels of all group except the NC, which did not receive STZ to cause diabetes, were significantly (*p<0.001*) raised by the induction of diabetes mellitus. Blood sugar levels were significantly lower in the T1 and T2 groups as compared to the PC group (*p<0.01*) as shown in figure 3.

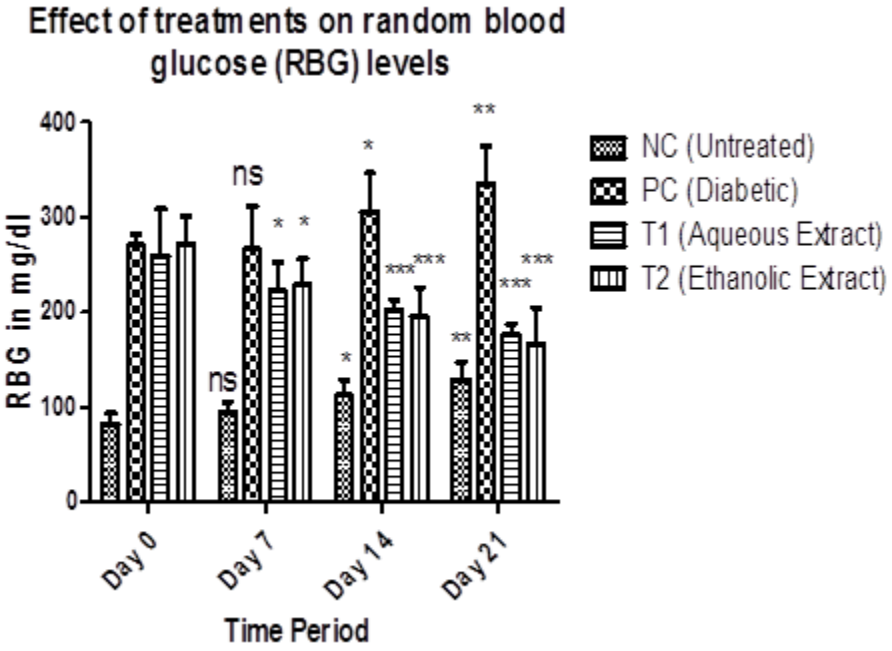


Figure.3 Effects of different treatments on the RBG levels of experimental rats

Data showed standard error (SE) +/- Mean of glucose level in mg/dl. **p<0.05; **p<0.01*; ****p<0.001*. ns mean not significant. Positive group (PC) was compared against negative group (NC) whereas treatment groups (T1 and T2) were compared with the PC to check their antidiabetic effect.

Glycated hemoglobin (HbA1c)

In table 1, the rats' HbAIc values are displayed. Results indicated that the level of HbA1c in the positive control group was substantially greater than that in the negative control group (p< 0.001) But in contrast to diabetic treated group T1 and T2 received extract treatment had significantly(*p<0.01*) recover the HbA1c levels respectively. It was indicated that the extracts decrease the glycated hemoglobin level in T1 and T2 groups.

Kidney parameter

Serum urea and creatinine levels, two kidney parameters, were indicated for each group and displayed in table 1. When compared to the negative group, the blood levels of urea and creatinine

in diabetic rats significantly increased ($p<0.001$). The extract treated groups T1 and T2 group a significantly restore these parameters to near normal levels after the extract is administered.

Table.1 Kidney variables and Hb1AC

Parameters	**Groups**			
	NC	**PC**	**T1**	**T2**
Urea (mg/dl)	23	74	24	29
Creatinine (mg/dl)	0.4	1.5	0.5	0.5
HbA1C %	2.6	6.1	2.7	3.9

Lipid profile

In all groups, lipid parameters including total lipids, cholesterol, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were calculated. Table 2 provides a summary of these results. The findings showed that when compared to the negative group rats, the positive control group cholesterol, triglycerides, VLDL, and LDL levels were much higher while their HDL levels were significantly low. Whereas the extract treated group T1 and T2 showed a significantly decreased ($p<0.05$) in high cholesterol, TG, VLDL and LDL levels and increase in HDL level when compare with diabetic group.

Table.2 Lipid parameters

Groups	**Lipids Parameters**					
	Total Lipid	**Cholesterol**	**TG**	**LDL**	**VLDL**	**HDL**
NC	272	70	55	47	10	38
PC	328	213	138	130	64	10
T1	272	110	87	52	15	44
T2	262	115	74	61	18	48

Liver parameters

When compared to rats under a negative control, serum ALT, ALP, and AST were considerably ($p<0.001$) higher in the STZ-induced diabetic rats. However, there was no change in the bilirubin

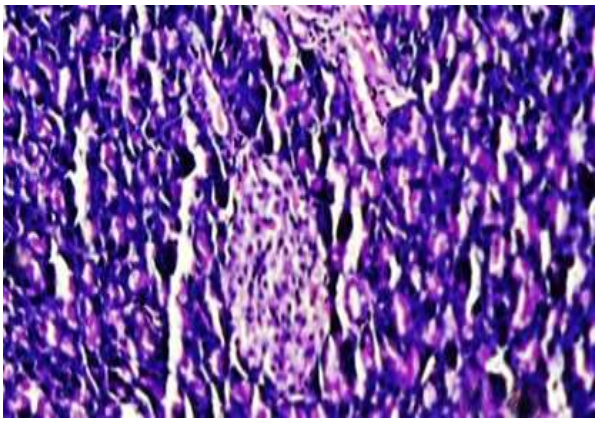
level. Treated T1 and T2 groups showed a significant ($p < 0.05$) decrease and steady improvement toward the normal values. When compared to normal rats, diabetic rats caused by STZ revealed a substantial ($p<0.05$) decrease in protein analyses such as serum total protein, albumin, and globulins. These indicators significantly improved as a result of the administration of plant extracts. Table 3.

Table.3 Liver parameters

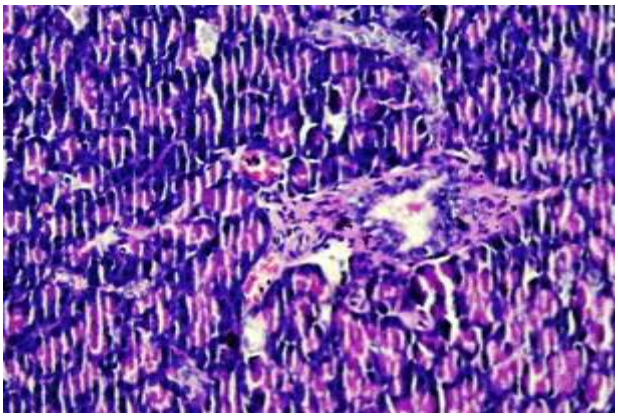
Group	Liver Parameters							
	ALT (U/I)	AST(U/l)	ALP(U/l)	Bilirubin	Albumin	Globulin	Total Protein	A/G %
NC	44	254	171	0.1	3.9	2.3	5.3	1.73
PC	52	320	181	0.1	4.3	2.6	6.5	1.7
T1	45	208	128	0.1	4	2.5	5.2	1.6
T2	46	247	133	0.1	4.1	2.4	5.5	1.5

Histopathology of pancreas

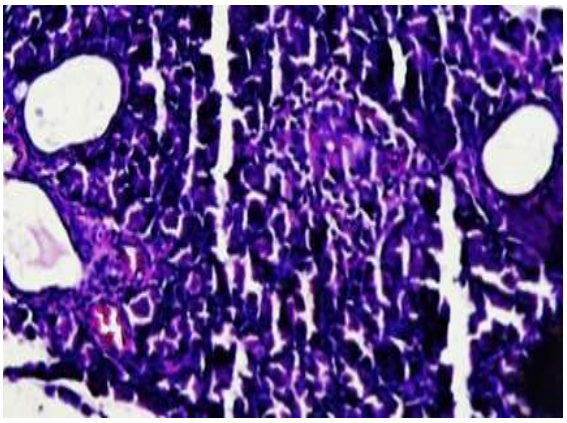
To check out the preventive effects of extract on STZ induced diabetes, the pancreatic islets were examined. Experimental rat groups were compared to check the effect of extracts on rat Pancreas. Figure 4a and 4b showed the islets of Langerhans comparison of negative control with positive control. The pancreatic structure was normal in the negative control group. The pancreatic Islets of positive control group was irregular in shape and relatively small seen in diabetic positive control. Figure 4c and 4d has shown that β cells count increased and pancreatic islet function is improved in extract treated T1 and T2 groups



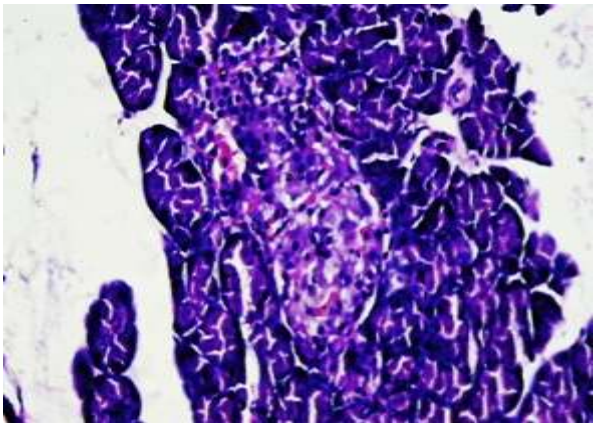
4a) Negative control group



4b) Positive control group



4c) Diabetic and aqueous extract T1



4d) Diabetic and ethanolic extract T2 group

Figure 4 Histopathology of Pancreas in different treatment groups

Discussion

In the current research, the anti-diabetic effects of four distinct plants—Tribulus terrestris, Prunus dulcis, Cicer arietinum, and Azadirachta indica were assessed. Various past researches confirmed that every plant have pharmacological importance and every plant contains antidiabetic properties. Diabetes induced by using STZ injection that degenerate the β cells of pancreas. The primary organ involved in determining the body's energy and nutritional status is the pancreas, which also released insulin in response to elevated blood sugar level (Jayaraju & Ishaq,2021). When compared to allopathic therapy, herbs play a crucial part in the treatment of disorders like diabetes. All across the world, a number of medicinal plants are used to cure diabetes (Elalfy et al., 2019).

The study's chosen plants have a proven record of being used therapeutically as antidiabetic agents. This experiment carried on for twenty- one days on STZ-induced diabetic rat's model. Male albino rats were given an intraperitoneal injection of STZ at a dosage of 45 mg/kg body weight to develop diabetes. One STZ injection can cause diabetes in rodents. Various parameters, including blood sugar, glycated hemoglobin, body weight, liver-related serum biochemical markers, including bilirubin, ALT, AST, and ALP, and kidney-related serum biochemical markers, including urea and creatinine, were also observed throughout this study period. The Pancrease histopathology examination was also a part of this investigation. Both the ethanolic and aqueous extracts of the two polyherbal medicines were administered orally.

The water and feed intake are recorded on daily basis after diabetes induction the water and feed intake were significantly increases in diabetic rats. T1 and T2 are treated with extract showing

significantly decrease water and feed intake. On the first day of the experiment, before and after stz injection, the body weight and blood sugar levels of all the rats were recorded over the research period. All diabetic rats had substantially lower mean body weights ($p< 0.05$) than the rats in the negative control group. Rats given stz have a decrease in body weight due to loss of muscle mass and structural protein damage (Oyedemi *et al*., 2016). Rats who receive the extract exhibit improvement and increase in body weight.

All of the stz diabetic rat groups had significantly ($p<0.05$) higher fasting and random blood sugar (200 mg/dl) than the rats in the negative control group. When Stz is administered, insulin is suppressed, which results in excessively increased blood glucose levels.

After the trial, the extract-treated diabetic rats put on weight. In comparison to the Positive Control group, their body weight increased considerably ($p<0.05$). Similar to this, a drop in the mean fasting and random blood sugar levels of the extract-treated stz diabetic rat groups demonstrates that their blood glucose levels are considerably lower ($p<0.05$) than the untreated positive control group. It was discovered that combined extract therapy was more successful at lowering blood sugar levels in diabetic rats. Plant extracts have a hypoglycemic impact, as evidenced by the improvement in body weight and decrease in blood sugar levels in diabetic rats treated with them. These particular plant extracts may have an insulin-like effect due to the phytochemical components they contain or may stimulate the production of insulin from the pancreatic cells (Noor *et al.,* 2017).

The liver biochemical markers, including ALT, AST, ALP, and bilirubin, were assessed during the liver study after 21 days of treatment. The most used liver function tests for diagnosing liver disease in clinical practice employ these criteria. In contrast to ALT and AST, which are cytosolic enzymes, ALP is a membrane-bound enzyme. The performance of hepatic cells is correlated with bilirubin. The damage to the liver is shown by the greater level of these enzymes (Saleh et al., 2018). All groups of stz-induced diabetic rats used in this investigation had significantly higher blood levels of these liver function markers ($p<0.05$), which is a symptom of liver damage. STZ injection also caused renal injuries like the level of serum urea and creatinine significantly increased. Hepato-renal function may also be affected by STZ injection into experimental animal models (Bouwens and Rooman, 2015). When compared to the diabetic control rat group, the extract significantly reduced the levels of bilirubin, ALT, AST, and ALP in the serum of STZ- induced diabetic rats ($p<0.05$). The plant extract may have reduced blood levels of the above- mentioned enzymes to normal levels due to cell membrane stability and cellular regeneration. (Santillan et al., 2013).

When compared to the positive control group, the serum levels of urea and creatinine were considerably lower ($p<0.05$). During study of lipid profile that includes cholesterol, TG, LDL, HDL and VLDL. All of the Stz diabetic rats had a significant rise in TG, LDL, and VLDL levels as well as a decrease in HDL. By administering the extract, diabetic rats' TG, LDL, and VLDL

cholesterol levels were attenuated. While their HDL levels increased indicates that plant-based formulation had a beneficial effect on the hyperlipidemia. Due to better glycemic control, the extract-treated diabetic rats had HbA1c readings that were close to normal levels. The HbA1c reduction showed the extract's capacity to manage diabetes.

The pancreas of the diabetic rats had necrosis, atrophy, fibrotic alterations, and shrinking of the Islet of Langerhans, according to histopathological results. However, the pancreas of the extract- treated rats had improved necrosis, minor atrophy, fibrotic alterations, and nearly normal Langerhans islets similar to previous research (Jayaraju and Ishaq, 2021).

Conclusion

The findings of this research revealed that, aqueous and ethanolic extract of a particular plant-based formulation may reduce fasting and random blood sugar levels or glycated hemoglobin level, while significantly increasing body weight in T1 and T2 groups relative to the positive control group. The findings of all the parameters were better for the aqueous and ethanolic extract of plants. It is clear from the results that extracts have an anti-diabetic effect in albino rats and might thus be employed as a natural anti-diabetic therapy for the treatment of diabetes and its related problems.

References

Akhtar, M. T., Siddique, A. B., Sultana, N., Irfan, M. I., Qadir, R., Saleem, S., ... & Saadia, M. (2021). Hypoglycemic Potential of Combined Methanolic Extract of Seeds of Nigella sativa (Black Cumin) and Cicer Arietinum (Chickpea). Pakistan Journal of Biochemistry and Biotechnology, 2(2): 261-285.

Asante, D. B., Effah-Yeboah, E., Barnes, P., Abban, H. A., Ameyaw, E. O., Boampong, J. N., ... & Dadzie, J. B. (2016). Antidiabetic effect of young and old ethanolic leaf extracts of Vernonia amygdalina: A comparative study. *Journal of Diabetes Research*, 2016

Elalfy, M. M., Farag, A., Helmy, A. A., Metwaly, Z. E., & Ali, F. R. (2019). Hematological, Biochemical and cytotoxic effect of ethanolic raw Extract of Egyptian Citrullus colocynthis in Sprague Dawley rats. Enz Eng, 8(165): 2.

Ezeigwe, O. C., Okpala, C. O., Okeke, C. S. M., Chiamaka, P., Amuzie, B. A. O., Ikechukwu, C. P. D.,& Nnadi,N. N .Hypoglycemic Property of Different Fractions of Crude Ethanol Extract Azadirachta indica Leaves in Streptozotocin-induced Diabetic Rats.

Gandhi, S., Srinivasan, B. P., & Akarte, A. S. (2013). Potential nephrotoxic effects produced by steroidal saponins from hydro alcoholic extract of Tribulus terrestris in STZ-induced diabetic rats. Toxicology Mechanisms and Methods, 23(7): 548-557.

Hend, M. T., Heba, M. A., Yasmen, S. M., & Nahla, S. E. S. (2015). Efficacy of Tribulus terrestris extract and metformin on fertility indices and oxidative stress of testicular tissue in streptozotocin-induced diabetic male rats. African Journal of Pharmacy and Pharmacology, 9(48): 1088-1098.

Jayaraju, K. J., & Ishaq, B. M. (2021). Evaluation of antidiabetic activity of a novel polyherbal preparation against streptozotocin induced diabetes rat model. pancreas, 1(3).

Jayaraju, K. J., & Ishaq, B. M. (2021). Evaluation of antidiabetic activity of a novel polyherbal preparation against streptozotocin induced diabetes rat model. pancreas, 1(3).

Kaur, R., and Prasad, K. (2021). Nutritional characteristics and value-added products of Chickpea (Cicer arietinum)—A review. Journal of Postharvest Technology, 9(2):1-13.

Meena, P., & Anand, A. (2019). A comprehensive overview of Gokshura (Tribulus terrestris Linn.). Journal of Ayurveda and Integrated Medical Sciences, 4(06): 205-211.

Noor, A., Gunasekaran, S., & Vijay lakshmi, M. A. (2017). Improvement of insulin secretion and pancreatic β-cell function in streptozotocin-induced diabetic rats treated with Aloe vera extract. *Pharmacognosy Research*, 9(Suppl 1): S99.

Quintero-Soto, M. F., Chávez-Ontiveros, J., Garzón-Tiznado, J. A., Salazar-Salas, N. Y., Pineda-Hidalgo, K. V., Delgado-Vargas, F., & López-Valenzuela, J. A. (2021). Characterization of peptides with antioxidant activity and antidiabetic potential obtained from chickpea (Cicer arietinum L.) protein hydrolyzates. Journal of Food Science, 86(7): 2962-2977.

Rashid, M. M., Rahman, M. A., Islam, M. S., Hossen, M. A., Reza, A. A., Ahmed, A. A., ... & Alharbi, H. F. (2022). Incredible affinity of Kattosh with PPAR-γ receptors attenuates STZ-induced pancreas and kidney lesions evidenced in chemicobiological interactions. Journal of Cellular and Molecular Medicine.

Salehi, B., Ata, A., V. Anil Kumar, N., Sharopov, F., Ramirez-Alarcon, K., Ruiz-Ortega, A., ... & Sharifi- Rad, J. (2019). Antidiabetic potential of medicinal plants and their active components. Biomolecules, 9(10): 551.

Sani, I., Oche, O., Chiaka, N. G., & Samuel, N. U. (2014). Antihyperglycemic and antihyperlipidemic effects of aqueous and ethanolic leaf extracts of Vitex doniana in streptozotocin-induced diabetic rats. Research Journal of Medicinal Plant,8(4):178-1.