

IN VIVO EVALUATION OF ANTIDIABETIC HERBAL FORMULATIONS ON PANCREATIC FUNCTION IN ALLOXAN-INDUCED DIABETIC WISTAR ALBINO RATS

¹Bandhana, ²Dr. Abdul Wadood Siddiqui

¹Research Scholar, School of Pharmacy Mangalayatan University, Beswan, Aligarh

²Principal, School of Pharmacy, Mangalayatan University, Beswan, Aligarh

Corresponding author : Bandhana

Received: July 02, 2025

Accepted: July 31, 2025

Published: Aug 06, 2025

Abstract

Aim: The aim of the study is to evaluate the in-vivo activity of herbal formulation on pancreases function in alloxan induced diabetic Wistar albino rats

Methodology: The Polyherbal Formulation was developed by mixing the hydroalcoholic extracts of the stem barks of *Ficus bengalensis*, fruits of *Momordica charantia* and seeds of *Syzygium cumini* & *Trigonella foenum-graecum* in optimized ratio of 1.2:3:5:10 respectively. The acute oral toxicity studies were carried out as per the guidelines of OECD. Albino Wistar male rats is use. Alloxan is widely used as an agent for the development of experimental diabetes to induce selective dysfunction of pancreatic β -cells. Different groups were prepared with normal control, diabetic control group, standard and hydroalcoholic extract of polyherbal formulation. Polyherbal extract (hydroalcoholic) was tested for different parameters like OGTT, Serum Triglycerides, Serum Total Cholesterol, serum High-Density Lipoprotein Cholesterol, HDL assay, ALP, SGOT and SGPT etc.

Result: Result show that polyherbal extract in manifested by decreasing total cholesterol, triglyceride, LDL, VLDL and MDA level. The data propose that the combined extract showed a consistent and ameliorated pancreases function, in the treatment groups. Polyherbal extract (600 mg/kg) have manifested the most effective improvements in diabetic complications. The above results propose that this Polyherbal extract may be a potential source for the development of the new antidiabetic drug.

Conclusion: The proposed activity of the polyherbal extract is supported by various biochemical and histopathologic analyses. By examining its safety and *in vivo* efficacy, the combination of ayurvedic components will give us a novel glimmer of hope for the lifestyle illness.

Keywords: Polyherbal extract, Acute toxicity and Biochemical parameters

1. INTRODUCTION

Diabetes mellitus (DM) is a rising health issue in the world characterized by low levels of insulin production or insulin resistance in a body. This leads to the persistence of high maintenance levels of blood glucose and disorders of nutrient metabolism.^{1,2} Being one of the most complicated public health problems of the 21st century, diabetes, in particular, is a rather difficult issue to tackle in African health systems.³ In Africa, an estimated nearly 19 million people were affected and the continent demonstrated the highest rate of mortality related to diabetes by making 77 percent of deaths related to diabetes. Its high rate can be explained mainly by inaccessibility to healthcare, insulin cost and scarcity, and impact of multisided social, cultural, economic, and ethnic factors.^{4,5}

Diabetes is an increasing disease in the world. The International Diabetes Federation (IDF) reported an estimated number of 463 million adults aged between 20 and 79, having diabetes in 2019.^{6,7} This number is bound to grow tremendously with some 700 million people being likely to fall into this category in 2045. There are two major types of diabetes. In type 1 diabetes, the immune system of the body destroys the β -cells in the pancreas that produce insulin and this causes a old or almost total scarcity of insulin.⁸ Conversely, in Type 2 diabetes the body is resistant to insulin, a hormone that normally assists in glucose uptake in cells.⁹ The burden to prevent or control diabetes is huge in the world, as it takes only eight seconds before a person dies each minute because of diabetes or its complications. Such complications have dire consequences on the performance of different organs and systems in the body.^{10,11} Diabetic cardiomyopathy (DCM) is one of the key consequences and is a chronic state that impacts the heart muscles and causes the dysfunction of the ventricles. The difference between DCM and other types of illness is the fact that DCM does not manifest itself as a symptom of coronary artery illness or hypertension.^{12,13} The other long-term issues that can be very detrimental to a person include diabetic nephropathy (kidney damage), diabetic retinopathy (eye damage), diabetic neuropathy (nerve damage) and diabetic foot ulcers among others. Besides these chronic complications, diabetic mellitus will also be associated with acute and threatening life emergencies that include diabetic ketoacidosis and hyperosmolar hyperglycaemic syndrome.^{14,15}

At present, a number of pharmacological solutions are employed to deal with diabetes. Some of them are insulin treatment and other hypoglycemic drugs. There are some giant drug categories against diabetes: sulfonylureas, biguanide type (such as metformin), thiazolidinediones, insulin sensitizers, and insulin secretagogues.^{16,17} These drugs can help reduce the level of blood sugar and treat the metabolic disorders which are part and parcel of diabetes. Their application, however, is rather restricted because of a set of challenges. Most of these medications are costly and particularly in the low-income setting. Moreover they can have serious side effects like gastrointestinal discomfort, hypoglycemia, weight gain, and sometimes have cardiovascular risks.^{18,19}

It is these shortcomings of the traditional diabetes therapies that have led to efforts of identifying alternative and complementary treatment modalities. Herbal formulations and traditional medicine are some that are undergoing a lot of interest. Several cultures have applied plant-based treatment options to control diabetes and its related conditions over the years.^{20,21} Such herbal medicines are mostly prepared with phytochemicals having certain antioxidant, anti-inflammatory and the insulin mimetic effect, and hence, it is believed that they may aid in controlling the blood sugar level to prevent diseases associated with diabetes. In comparison with the synthetic drugs, herbal medicines are quite more accessible, affordable and tolerative.^{22,23}

Studies on the medicinal plants and polyherbal products have proved thereto encouraging results in the treatment of diabetes. Such remedies are likely to boost the responsiveness of the pancreas, insulin sensitivity as well as uptake in peripheral tissues. Certain plants have been identified to reproduce damaged beta cells or to guard against the effect of oxidative stress. The adverse presence of reactive oxygen species (ROS) can also be mitigated using antioxidant herbs since they are generally hyperactive in diabetic diseases, and they have been identified as causing complications such as neuropathy and nephropathy.^{24,25}

2. MATERIAL AND METHODS

2.1 Collection and Identification of the Plant

The stem bark of *Ficus bengalensis* Linn. was collected from Delhi Road, India, the fruits of *Momordica charantia* Linn., Seeds of *Trigonella foenum graecum* Linn., and seeds of *Syzygium cumini* (L.) Skeels were collected from local market. The plant sample was given for identification in Raw Material Herbarium and laboratory of National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. The plant materials were dried at temperature 40°C. It was pulverized and stored in sealed containers.

2.2 Development of Poly Herbal Formulation:

The Poly Herbal Formulation was developed by mixing the hydroalcoholic extracts of the stem barks of *Ficus bengalensis*, fruits of *Momordica charantia* and seeds of *Syzygium cumini* & *Trigonella foenum-graecum* in optimized ratio of 1.2:3:5:10 respectively. The basis of selection of the individual plant extract was the most effective dose and therapeutic doses were finalized on the basis of Acute Toxicity Studies guidelines.

The Poly Herbal Formulation was prepared in the three different strengths for the purpose of dose optimization and to find out the most effective and safer dose. Formulation (600 mg/kg) were prepared and code names to these formulations were given FMST-1 respectively.

Ingredients	Quantity (mg)
<i>Ficus bengalensis</i> (bark extract)	37.5 mg
<i>Momordica charantia</i> (fruit extract)	93.75 mg
<i>Syzygium cumini</i> (seed extract)	156.25 mg
<i>Trigonella foenum-graecum</i> (seed extract)	312.5 mg

Table 1. Poly Herbal Formulation

2.3 Pharmacological evaluation for anti-diabetic activity

A. Experimental Animals: Albino Wistar male rats of weighing between 150 to 200g. The animals were housed under standard conditions of temperature (25±2°C) and relative humidity (30-70%) with a 12:12 light-dark cycle and acclimatized in the animal house facility. The animals were fed with standard diet and water ad libitum.

B. Induction of Diabetes: The Wistar albino male rats of 150-200 gm were allowed to fast overnight prior to experimentation and rendered diabetic by injecting a single dose of Alloxan 120 mg/kg body weight. It was administered as a 5% w/v solution in distilled water by intra peritoneal route. Since alloxan could evoke fatal hypoglycaemia as a result of massive insulin release, rats received 20% of glucose solution for first 6 hr then simple tap water was given. The rats were then kept for next 24 hr with free access of 5 % glucose solution to prevent hypoglycaemia. After 48 hrs of injection of alloxan, blood glucose level was measured for the evidence of diabetes. The rats which showed blood glucose level more than 200 mg/dl were considered as diabetic and selected. Animals were maintained for 72 hrs in diabetic condition for well establishment of diabetes. Tolbutamide was used as a standard drug. It is an oral hypoglycaemic drug belongs to the category of sulfonylurea. The mechanism of action of tolbutamide is stimulation of β -cells islets tissue to secrete insulin. Tolbutamide lowers blood glucose in Type-2 diabetics and ineffective in patients of Type-1 diabetes. Taking these points into consideration tolbutamide was used as a standard drug for this research work.

C. Experimental Design: Animals were divided into six groups of six each.

Group-I: Normal healthy rats received only the water served as normal control (NC).

Group-II: Alloxan induced diabetic untreated rats served as a diabetic control group (DC) also received water.

Group-III: Standard group (STD) was treated with Tolbutamide 100 mg/kg body weight, per oral.

Group-IV: Diabetic rats treated with hydroalcoholic extract of polyherbal formulation (600mg/kg).

2.4 Oral Glucose Tolerance Test (OGTT)

Insulin and glucose levels was measured on days 1, 8, and 15. In the final week, an oral glucose tolerance test was conducted, involving administration of a solitary oral dosage of glucose solution (2 grams per kilogram of body weight) to assess glucose tolerance. The levels of blood glucose was tested at specific intervals: 0, 30, 60, 90, and 120minutes post-glucose administration.

2.5 Biochemical parameters

2.5.1 Estimation of Serum Triglycerides

Triglyceride detection was achieved using the span diagnostic kit.

2.5.2 Estimation of Serum Total Cholesterol

There are several methods available for measuring serum total cholesterol, including enzymatic methods, colorimetric methods, and direct measurement methods

2.5.3 Estimation of serum High-Density Lipoprotein Cholesterol.:

The span diagnostic kit employed the cholesterol oxidase/peroxidase (CHOD-POD) technique to identify HDL cholesterol.

2.5.4 HDL assay

To determine cholesterol concentration in a serum sample using spectrophotometry.

2.5.5 ALP

Combine 4 ml of buffer substrate with 0.3 ml of serum to initiate the experimental reaction. Give it five minutes at 37°C, followed by an additional 15-minute incubation. Take out the test tube and promptly add 1.8 ml of dilute phenol.

2.5.6 Estimation of SGOT

To estimate SGOT levels, a spectrophotometric method can be employed using a commercially available assay kit.

2.5.7 Estimation of Serum SGPT

To estimate SGPT levels, a spectrophotometric method can be utilized using a commercially available assay kit

2.5.8 LDH

To estimate LDH levels, a spectrophotometric method can be utilized using a commercially available assay kit.

2.5.9 Estimation of Bilirubin

To estimate Bilirubin levels, a spectrophotometric method can be utilized using a commercially available assay kit

2.5.10 GGT

To estimate GGT levels, a spectrophotometric method can be utilized using a commercially available assay kit.

2.6 Serum antioxidant levels

2.6.1 Estimation of MDA

Malondialdehyde (MDA) is a commonly used biomarker for oxidative stress and lipid peroxidation. When MDA reacts with thiobarbituric acid (TBA) in an acidic environment, a complex is formed, which exhibits a characteristic pink colour. This colour change can be quantified spectrophotometrically at a wavelength around 535 nm, providing a measure of MDA concentration in the sample

2.6.2 Estimation of Catalase

Potassium and sodium pH 7.5 phosphate buffer, 50 millilitres: Put 1.1g of Na_3HPO_4 and 0.27g of KH_2PO_4 into 100ml of distilled water to dissolve.

H_2O_2 (20 mm) in a potassium and sodium buffer at 50 mmol/L: Daily dilution and standardization of the solution are carried out at 240 nm using the molar extinction coefficient of 43.6 M. Ammonium molybdate (32.4 mmol/L).

2.7 Histopathology of isolated pancreas and liver: Minute portions of pancreas tissues were accumulated in 10% formalin for proper fixation. Pancreas was fixed in Bouin's fixative (without acetic acid) for histopathological studies. Sections of pancreas (6 microns in thickness) were stained with chromium haematoxylin for differential staining of alpha and beta cells. Photographs depicting the histopathology of liver and pancreas were taken. The photographic figures are given as evidence for the improved architecture. Histopathology of isolated pancreas and liver was carried out.

2.8 Statistical Analysis

Various statistical analyses were performed on the collected quantitative data. To analyse the collected primary data, a variety of statistical instruments and methods were employed. It was determined that the utilisation of these instruments and methods was pertinent to the study's stated objectives. Cronbach alpha, the Simple Percentage Method, the Mean and Standard Deviation, the Correlation Coefficient, and the Chi-Square test were used to analyse the collected data. In addition, one-way ANOVA was employed to determine the significance of the variables considered. SPSS 17.0 (Statistical Packages for the Social Sciences) software was utilised to generate tables and statistical results

3. RESULT AND DISCUSSION

3.1 Acute toxicity Study

Animals were observed at regular time intervals. In all the cases no death was observed within first 24 hours. Additional observations like behavioural changes in skin, fur, eyes, mucous membranes, respiratory, autonomous and central nervous systems and somatic motor activity and behavioural pattern. Attention was also given to observation of tremors and convulsions. Studies of toxicity were conducted in accordance with the OECD rules. No death or negative events of up to 5000 mg /kg were found in the herbal formulation.

S/No.	No. of Animals	Extract Dose (mg/kg)	Causality
1	4	0	0
2	4	5	0
3	4	50	0
4	4	300	0
5	4	2000	0
6	4	5000	0

Table 2. Acute toxicity of Polyherbal formulation fruit

3.2 Effect of polyherbal formulation on Oral Glucose Tolerance Test

The average OGTT levels within the group under control was 234 ± 2.7 mg-h/dL. In the diabetes control group, the levels were dramatically increased to 588 ± 2.7 mg- h/dL. All these findings are significant. These elevated levels were dramatically reduced by the administration of polyherbal formulation, as indicated by statistical importance.

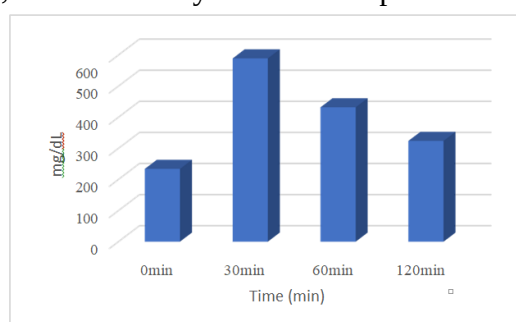


Figure 1. Effect of polyherbal formulation on AUC

3.3 Effect of polyherbal formulation on Serum Glucose Levels

During the investigation's progression, levels of glucose in the serum of the diabetic control group was found to have significantly increased. Group was treated with polyherbal formulation experienced a considerable reduction in their elevated serum glucose levels.

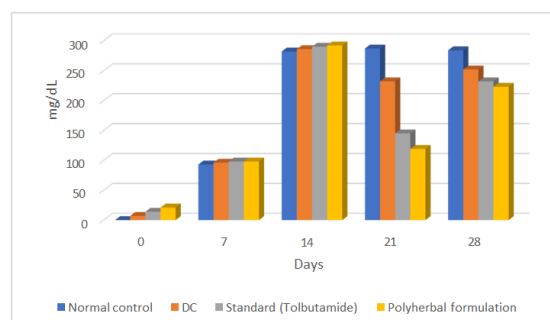


Figure 2. Effect of PF on serum glucose levels

3.4 Effect of Polyherbal formulation on Lipid Parameters

3.4.1 Triglyceride levels

In the typical control cohort, the average serum triglyceride level was 72 ± 3.0 mg/dL. However, in the diabetic control group, the triglyceride level considerably increased to 121 ± 2.9 mg/dL of total cholesterol. The increased serum triglyceride level was low as a result of the therapy with polyherbal formulation.

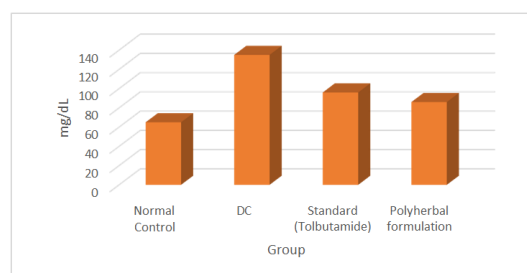


Figure 3. Effect of PF on TG levels

3.4.2 Cholesterol Levels

The standard control group had an average serum total cholesterol level of 77 ± 4.0 mg/dL, whereas the diabetes control group had a much higher level of 156 ± 5.6 mg/dL. This indicates showed the control group of diabetics had substantially increased concentrations of total cholesterol.

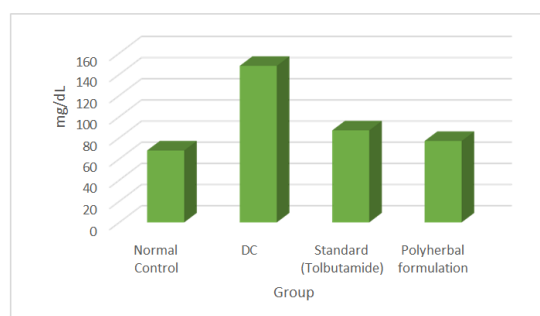


Figure 4. Effect of PF on cholesterol levels

3.4.3 HDL-c Levels

Induction of diabetes produced a noteworthy reduction in serum HDL-cholesterol levels, which dropped from 28 ± 1.1 mg/dL to 16 ± 1.1 mg/dL in contrast to the group under control, which was under normal conditions.

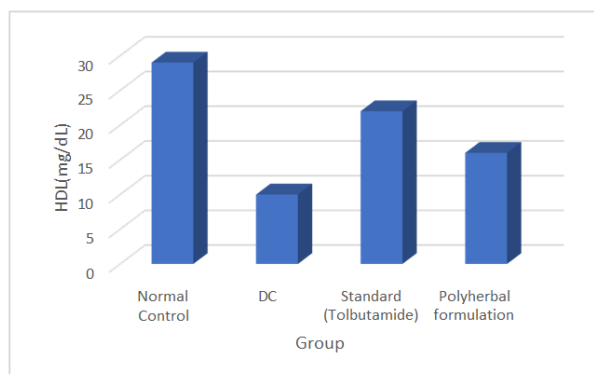


Figure 5. Effect of PF on HDL levels

3.5 Effect of poly herbal formulation on pancreases

3.5.1 LDH Levels

In contrast to the normal control group, the development of diabetes resulting in a notable rise in liver LDH levels, which went from 215 ± 6.4 U/mg protein to 558 ± 9.7 U/mg. The administration of Polyherbal formulation had the effect of lowering the elevated levels of LDH.

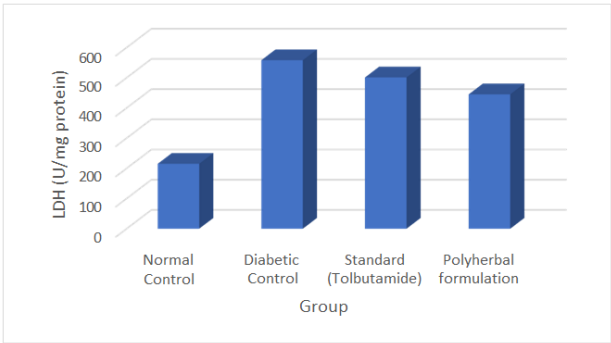


Figure 6. Effect of PF on LDH levels

3.5.2 SGOT Levels

Diabetes induction resulted comparatively speaking to the typical safeguard group. in a substantial rise within the ranges of SGOT in the liver, which went from 43 ± 3.5 U/L to 167 ± 6.9 U/L. Treatment with polyherbal formulation prompted a decrease in the elevated levels of SGOT after treatment.

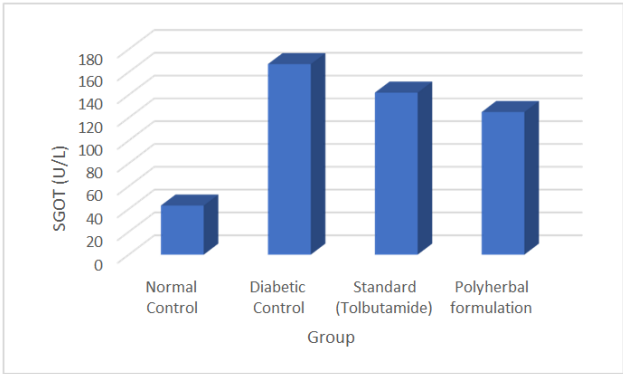


Figure 7. Effect of PF on SGOT levels

3.5.3 SGPT Levels

Inducing diabetes led to a difference when in contrast to the standard control group considerably elevated level of SGPT in the liver, which increased from 21 ± 2.9 U/L to 166 ± 5.0 U/L. therapy with PF resulted in a reduction of the elevated SGPT levels.

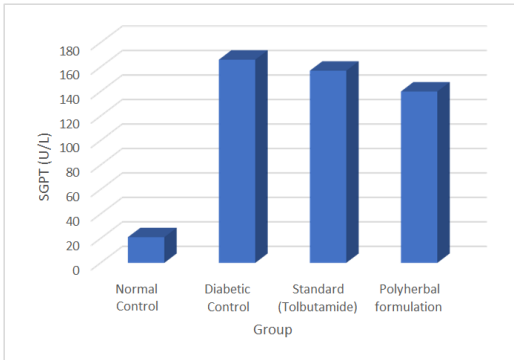


Figure 8. Effect of PF on SGPT levels

3.5.4 ALP Levels

Diabetes production led to a notable rise in the Alkaline phosphatase (ALP) levels inside the liver, which went from 88 ± 7.3 U/L to 135 ± 3.4 U/L in contrast to the group under control, which was considered normal. Conversely, though, the treatment with PF and standard

brought about a decline in the elevated levels of ALP.

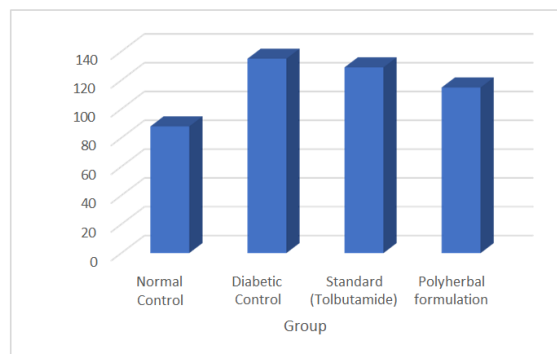


Figure 9. Effect of PF on ALP levels

3.5.5 Total Bilirubin Levels

Diabetes induction led to a significant increase relative to the standard control group, the levels of total bilirubin in the liver, which increased from $0.53 \pm 0.067 \mu\text{mol/L}$ to $1.1 \pm 0.065 \mu\text{mol/L}$. The administration of PF prompted a decrease of the elevated total bilirubin levels.

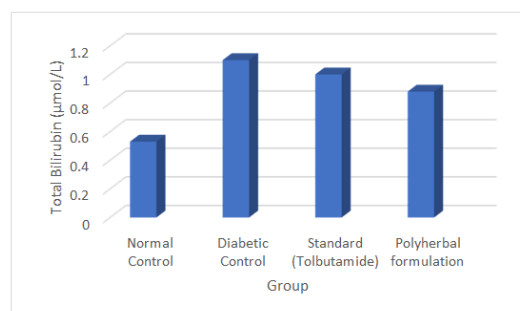


Figure 10. Effect of PF on Total bilirubin levels

3.5.6 γ Glutamyl Transferase Levels

Diabetes caused a notable rise in γ glutamyl transferase quantities in the hepatic, rising from $2.9 \pm 0.16 \text{ U/L}$ to $7.2 \pm 0.38 \text{ U/L}$ compared to the control group. The therapy with polyherbal formulation effectively toward the increased levels of γ glutamyl transferase compared to the control group of diabetics.

3.6 Effect of polyherbal formulation on Anti-oxidant Levels

3.6.1 Effect of polyherbal formulation on Catalase Level

In the group under normal management, the average serum catalase level was $8.8 \pm 0.41 \text{ nmol/min/mL}$, while the control group with diabetes had a noticeably lower level of $3.4 \pm 0.33 \text{ kU}$ in contrast to the standard control group. Catalase levels decreased in the PF and standard group members.

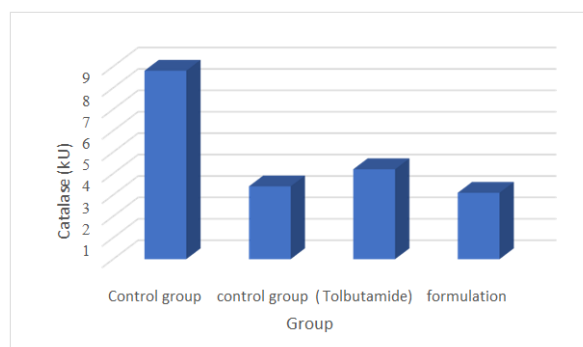


Figure 11. Effect of compounds PF on serum catalase level

3.6.2 Effect on GSH Level

The group under authority of diabetics had a far lower serum GSH level of 19 ± 2.6 mg/dL after the intervention in contrast to the standard control group, which had a mean level of 38 ± 1.7 mg/dL. The PF and standard groups exhibited considerably elevated levels of GSH in contrast to the diabetic control group, which had experienced a notable decrease.

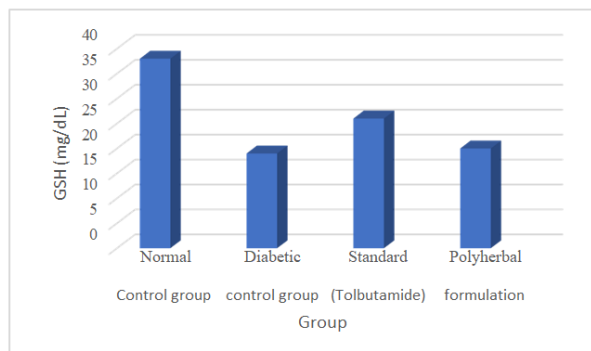


Figure 12. Effect of compounds PF on GSH level

3.7 Histopathology Studies: - It is evident from the histopathological tests that in control group, the pancreases show small amount of hemorrhages which are shown by the arrow. In DC group, there are mild changes are seen in acinar cells which validate minor degenerative changes. In group III, administration of Standard (Tolbutamide) shows moderate hypertrophy in islets of Langerhans cells of pancreas.

In group IV, administration of Polyherbal formulation (FMST-1) show mild hypertrophy in pancreases.

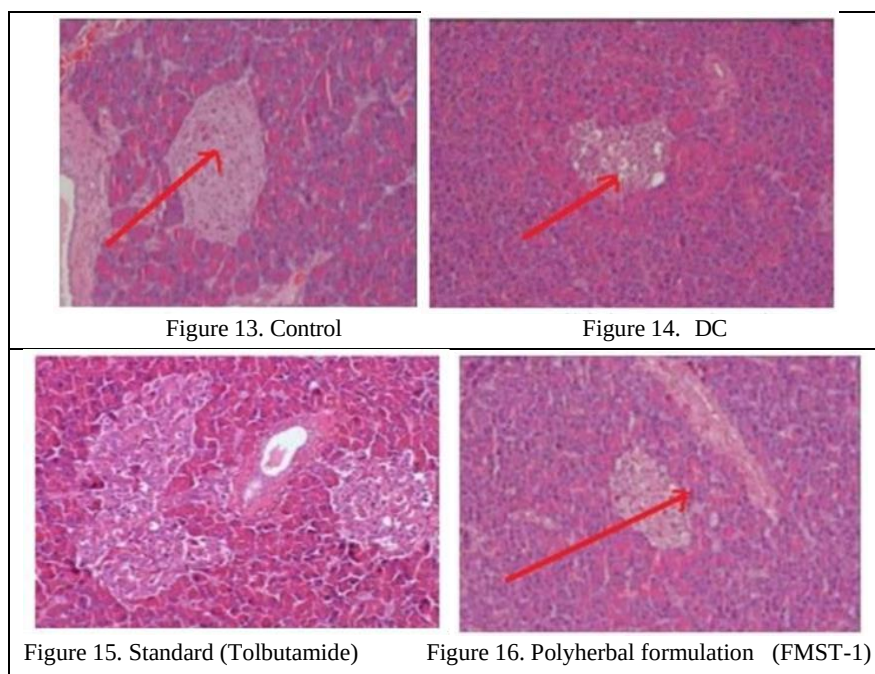


Figure 13. Control

Figure 14. DC

Figure 15. Standard (Tolbutamide)

Figure 16. Polyherbal formulation (FMST-1)

4. CONCLUSION

Diabetes is one of the most prevalent chronic diseases of the world. Diabetes currently affects 246 million people worldwide and is expected to affect 380 million by 2025. Each year of further 7 million people develop diabetes. Acute oral toxicity study indicated the non-toxic

effect of plant parts (*Trigonella foenum-graecum*, *Ficus bengalensis*, *Syzygium cumini* and *Momordica charantia*). No death of animals was reported for any doses at the entire period of study. The administration of PF for OGTT which resulted in a considerable decrease in these elevated levels within the body. The polyherbal formulation shows that significant regain of lipid parameters of experimental animal during study period. The increased serum triglyceride level was low as a result of the therapy with PF. The course of treatment resulted in a reduction of these elevated levels with PF Treatment with PF caused significant increase in serum HDL-c levels, when compared to the control group of diabetics. In Biochemical studies, The administration of PF had the effect of lowering the elevated levels of LDH.

Treatment with PF prompted a decrease in the elevated levels of SGOT. therapy with PF resulted in a reduction of the elevated SGPT levels. In contrast, the PF treatments did not demonstrate any significant rise in ALP levels in contrast to the diabetic control group. The administration of PF resulted in a reduction of the elevated total bilirubin levels. When compared to the diabetic control group, the therapy with PF effectively reduced the elevated levels of γ glutamyl transferase. PF group didn't demonstrate any appreciable rise in catalase levels in comparison to the diabetic control group, the low as catalase levels. Compared to the diabetic control group, the PF and standard groups all had a much higher amount of GSH, which had been significantly low.

The histological examination of diabetic pancreatic that had been treated with PF in ethanol from prevealed that the pancreas had improved and that the normal cellular population size had been restored, which was accompanied by minor pathological alterations.

According to the findings of this research, the plant parts (*Trigonella foenum-graecum*, *Ficus bengalensis*, *Syzygium cumini* and *Momordica charantia*) possess the capacity to decrease blood sugar levels, and they also assist in improving a variety of issues that are associated with diabetes mellitus.

5. REFERENCES

1. Glovaci D, Fan W, Wong ND. Epidemiology of diabetes mellitus and cardiovascular disease. *Curr Cardiol Rep.* 2019; 21(4):1-8.
2. Wasana KGP, Attanayake AP, Jayatilaka KAPW, Weerarathna TP. Antidiabetic activity of widely used medicinal plants in the Sri Lankan traditional healthcare system: New insight to medicinal flora in Sri Lanka. *EvidBased Compl Altern Med.* 2021; Vol. 2021, Article ID 6644004, 12 pages.
3. Ramadan BK, Schaalán MF, Tolba AM. Hypoglycaemic and pancreatic protective effects of *Portulaca oleracea* extract in alloxan induced diabetic rats. *BMC Compl Altern Med.* 2017; 17:37.
4. Diriba DC, Leung DYP, Seun LKP. The effects of diabetes self-management interventions on physiological outcomes in people living with diabetes in Africa: A systematic review and meta-analysis. *Diabet Med.* 2020; 38(5): e14501.
5. Mbanya JC, Ramiaya K. Diabetes Mellitus. In: Jamison DT, Feachem RG, Makgoba MW, Bos ER, Baingana FK, Hofman KJ, Rogo KO. *Diseases and mortality in subSaharan Africa.* 2nd ed. Washington D.C. USA: The World Bank. 2006; Chapter 19: 267-287.

6. Dall'Asta M, Bayle M, Neasta J, Scazzina F, Bruni R, Cros G, Del Rio D, Oiry C. Protection of pancreatic β -cell function by dietary polyphenols. *Phytochem Rev*. 2015; 14(6): 933-959.
7. Indah MA. Differential expression analysis of diabetic related genes in streptozotocin- induced diabetic rats in response to *Abelmoschus esculentus* treatment: International Conference on Bioscience, Biochemistry and Bioinformatics. IPCBEE 5: IACSIT Press, Singapore. 2011; 5: 151-155
8. Savi M, Bocchi L, Pedro M, Dall'Asta M, Crozier A, Brighenti F, Stilli D, Del Rio D. *In vivo* administration of urolithin A and B prevents the occurrence of cardiac dysfunction in streptozotocin-induced diabetic rats. *Cardiovasc Diabetol*. 2017; 16(80): 1-13
9. Zhang J, Wang Y, Li L, Zhang R, Guo R, Li H, Han Q, Teng G, Liu F. Diabetic retinopathy may predict the renal outcomes of patients with diabetic nephropathy. *Renal Failure*. 2018; 40(1):243-251.
10. Coffey L, Mahon C, Gallagher P. Perceptions and experiences of diabetic foot ulceration and foot care in people with diabetes: A qualitative meta-synthesis. *Int Wound J*. 2019; 16(1): 183-210.
11. Rosenberger DC, Blechschmidt V, Timmerman H, Wolff A, Treede R. Challenges of neuropathic pain: Focus on diabetic neuropathy. *J Neural Transm*. 2020; 12(4): 589-624.
12. Pasquel FJ, Tsegka K, Wang H, Cardona S, Galindo RJ, Fayfman M, Davis G, Vellanki P, Migdal A, Gujral U, Narayan KMV, Umpierrez GE. Clinical outcomes in patients with isolated or combined diabetic ketoacidosis and hyperosmolar hyperglycaemic state: A retrospective, hospital-based cohort study. *Diabetes Care*. 2020; 43(2): 349-357.
13. Reddy PK, Kuchay MS, Mehta Y, Mishra SK. Diabetic ketoacidosis precipitated by COVID-19: A report of two cases and review of literature. *Diabetes Metab Syndr*. 2020; 14(5): 1459-1462.
14. Zhang L, Liu Y, Ke Y, Liu Y, Luo X, Li C, Zhang Z, Liu A, Shen L, Chen H, Hu B, Wu H, Wu W, Lin D, Li S. Antidiabetic activity of polysaccharides from *Suillelus luridus* in streptozotocin-induced diabetic mice. *Int J Biol Macromol*. 2018; 119: 134-140.
15. Moradi B, Abbaszadeh S, Shahsavari S, Alizadeh M, Beyranvand F. The most useful medicinal herbs to treat diabetes. *Biomed Res Therapy*. 2018; 5(8): 2538-2551
16. Jafer D. The role of tomato products for human health (*Solanum lycopersicum*): A review. *J Health Med Nurs*. 2016; 33(2015): 66-76.
17. Carvalho GC, Furquim de Camargo BA, Cavalcante de Araujo JT, Chorilli M. Lycopene: From tomato to its nutraceutical use and its association with nanotechnology. *Trends Food Sci Technol*. 2021; 118(A):447-458.
18. Friesen N, Fritsch RM, Blattner FR. Phylogeny and new intrageneric classification of *Allium sativum* L. (Alliaceae) based on nuclear ribosomal DNA ITS sequences. *Aliso*. 2006; 22(1): 372-395
19. Bradley PR. British herbal compendium: a handbook of scientific information on widely used plant drugs/published by the British Herbal Medicine Association and produced by its Scientific Committee. Bournemouth, Dorset. 1992. 105-108 p.

20. Jaber MA and Al-Mossawi A. Susceptibility of some multiple resistant bacteria to garlic extracts. *Afr J Biotechnol.* 2007; 6(6):771-776
21. Karole S, Shrivastava S, Thomas S, Soni B, Khan S, Dubey J, Dubey SP, Khan N, Jain DK. Polyherbal formulation concept for synergic action: A review. *J Drug Deliv Ther.* 2019; 9(1-s):453-466.
22. Varghese S, Kannappan P, Kanakasabapathi D, Madathil S, Perumalsamy M. Antidiabetic and antilipidaemic effect of *Clerodendrum paniculatum* flower ethanolic extract. An *in vivo* investigation in Albino Wistar rats. *Biocatal Agric Biotechnol.* 2021; 36:102095.
23. Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. The 1996 guide for the care and use of laboratory animals. *ILAR Journal.* 1997; 38(1): 41-48.
24. AOAC. Minerals: In Official Methods of analysis, Washington, DC: Association of Official Analytical Chemists. 2010; 16(3): 99-103.
25. Achikanu CE, Eze-Stephen PE, Ude CM, Ugwuokolie OC. Determination of the vitamin and mineral composition of commonly leafy vegetables in South-Eastern Nigeria. *Int J Curr Microbiol Appl Sci.* 2013; 2(11): 347-353