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FORMULATION DEVELOPMENT AND EVALUATION OF ANTI-DIABETIC ACTIVITY OF POLYHERBAL TABLET IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT
Objective: The current research work emphasized on the formulation development and evaluation of polyherbal antidiabetic tablet containing *Terminalia catappa*, *Costus igneus*, and *Cassia fistula* in streptozotocin-induced diabetic rats.
Method: The study evaluated a polyherbal blend in Albino Wistar rats (150–200 g) through acute toxicity, OGTT, and antidiabetic activity, with a focus on formulating a polyherbal tablet. Diabetes was induced using streptozotocin (60 mg/kg). Over 28 days, 13 groups were observed: normal, diabetic control, standard treatment (Glibenclamide 5 mg/kg), and various doses of the polyherbal blend at 200 mg/kg and 400 mg/kg. Blood samples were collected via retro orbital puncture on days 7, 14, 21, and 28 to assess blood glucose levels, body weight changes, and profiles of liver, lipids, and kidneys, along with histopathological improvements post-treatment.
Results: The polyherbal blend PHB-2B (TC: CI: CF: 2: 1: 1) exhibited optimal blood glucose levels and enhanced body weight in diabetic rats. It also significantly reduced total cholesterol, improved lipid profiles (increased HDL, decreased LDL and VLDL), and enhanced liver and kidney functions. For tablet formulation optimization, PHB 2B was selected, with formulation PHT7 demonstrating the best pre and post-evaluation outcomes among the nine formulations (PHT1-PHT9).
Conclusion: Formulation PHT 7 demonstrates significant antidiabetic benefits and safeguards biochemical and histological markers, indicating its safety and effectiveness in vivo. It presents a secure and practical alternative for diabetes management, leveraging Ayurvedic components to offer new potential in treating lifestyle diseases.

KEYWORDS: IDDM, Polyherbal blend, Streptozotocin, Antidiabetic activity, BGL.

INTRODUCTION: Diabetes mellitus (DM) is a severe, long-lasting illness that arises when the body fails to effectively utilize the hormone insulin, which controls blood glucose levels, or when the pancreas is unable to manufacture adequate amounts of insulin. It is a significant public health concern that health-care professionals have identified as a priority. Early diagnosis is a prerequisite to living well with an illness since the longer a person is untreated and undiagnosed, the more probable it is that the consequences will worsen. In India, the etiology of diabetes is multifaceted, integrating genetic variables along with obesity, sedentary lifestyle, and elevated urban migration [1,2].Access to essential facilities such as blood glucose and HbA1c testing is crucial in healthcare organizations. Efficient referral systems are necessary to ensure timely evaluation and specialist care for diabetes-related issues. By Improved diabetes diagnosis and management can greatly improve patient outcomes. Despite the availability of numerous antidiabetic drugs, prolonged use may culminate severe side effects such as excessively low blood sugar, lactic acidosis, and stomach sickness and a lot more.To overcome the drawbacks and side effects of oral hypoglycemics, Plant-based substitutes are gaining popularity. Increase efficacy and reduce problems, the study integrates different phytoconstituents based on ethno botanical ideas.It evaluates this polyherbal blend's antidiabetic qualities in animal models, emphasizing Ayurveda's approval of its effectiveness, safety, affordability, and accessibility.As per the principle of Ayurveda the drugs formulation is based on[3]
1. To achieve greater therapeutic efficacy, a mixture of herbs is used rather than single herb, the concept known as polyherbalism which promotes synergism.
2. Polyherbal (pH) has a larger therapeutic index than allopathic hypoglycemia medicines, making it both safer at high dosages and efficacious at low levels. It is appropriate for therapeutic usage because of its efficacy, safety, affordability, and acceptability[4]. Proper application might increase its health advantages. Patients and healthcare systems are under tremendous financial strain due to a higher prevalence of diabetes. Thus the present research focused on the polyherbal mechanism of crude drugs *Terminaliacatappa* (Indian almond), *Costusigneus* (insulin plant), and *Cassia fistula* (golden shower) for the effective and safe treatment of diabetes. All the used herbs in polyherbal formulation possess antidiabetic activity individually as per the following scientific reports available in the database:

- *Cassia fistula* Linn (Leaf) [5]
- *CostusIgneus* (Leaf) [6]

- *Terminaliacatappa*(Leaf) [7,8]

MATERIAL AND METHOD

Collection and Authentication of the plant:

Taxonomically identified leaves of *T. catappa, C. igneus*and *C. fistula* will be collected from local nursery and wild area and authenticated from ICAR-Indian Agricultural Research Institute, Regional station, Indore (M.P.) India. The voucher specimen submitted to the ICAR-IARI, Regional station, Indore (M.P.) India for further reference.

Preparation of Extract The shadow, air-dried leaves of *T. catappa*, *C. igneus*, and *C. fistula* plants were powdered and extracted with ethanol and hydro alcoholic extract, methanol, n-hexane and petroleum ether separately using a soxhlet apparatus for 10 hours at temperature 60-70ºC. The extractions continue until a clear solution is achieved in the siphon tube. The extract is then cooled in RBF and filtered to get rid of any leftovers. In a rotary evaporator, all extracts were concentrated under low pressure to create powder. They were put in an amber glass container and refrigerated once the percentage yield was determined. Further research is conducted using the solvent with the higher yield.This extracts was acts as the medicinal ingredient to the tablet. Preliminary phytochemical screening was performed on mother solvent of each extract to identify the secondary metabolite present in the drug[9,10].

Pharmacological Evaluation: To determine the antidiabetic activity of all the three extracts, combine each extract in different proportion to create polyherbal blend (PHB1-PHB5). The ratios of blends are as following in table 1-

Table 1: Polyherbal blend composition table

S.No.	Code.	Formulation	Ratio
1	PHB1	TC: CI: CF	1:1:1
2	PHB 2	TC: CI: CF	2:1:1
3	PHB 3	TC: CI: CF	1:2:1
4	PHB 4	TC: CI: CF	1:1:2
5	PHB 5	TC: CI: CF	2:2:1

TC-Terminaliacatappa, CI-*Costusigneus*, CF-*Cassia fistula,* PHB- Polyherbal Blend

Procurement and Acclimatization of Animal: Adult Albino Wistar rats in good health, weighing between 180 and 200 grams, were obtained from Animal House. Acute toxicity and antidiabetic efficacy were determined using the animals. All rats were kept in polypropylene cages with typical laboratory settings (ambient temperature, ~25 ± 2 °C; relative humidity, ~60%; 12 h light/dark cycle) for two weeks prior to the start of the investigation. A typical pellet diet and free access to water were given to them. The Institutional Animal Ethics Committee of ADINA Institute of Pharmaceutical Science, Sagar (IAEC Registration no. 1546/PO/E/S/11/CPCSEA) examined and approved the experimental protocol. The experiment was carried out in accordance with the guidelines established by the CPCSEA, Government of India [11].

Acute Oral Toxicity Study: The acute oral toxicity investigation in adult male albino rats was carried out in according to OECD Guideline No. 42311. The fixed-dose protocol described in Annex 2d was used, with a starting dose of 300–2000 mg/kg body weight. All five polyherbal blends (PHB 1 to PHB 5) were dispersed in a 0.5% w/v sodium CMC suspension and fed orally to fasted animals separated into groups of five the following morning, whereas the control group received solely water. The animals were continuously monitored for neurological, autonomic, and general behavioral abnormalities.Initially, all observations were made continuously for three hours, then every thirty minutes for the next three hours. After that, mortality and delayed toxic effects were monitored for up to fourteen days [11]**.**

Dose Selection for Anti diabetic Study: For the study of antidiabetic activity, two dose levels were chosen: one dose was roughly a tenth of the maximum dose (200mg/kg) during acute toxicity tests, and the high dose was twice that of the tenth dose (400mg/kg)[12].

Oral Glucose tolerance test (OGTT): The effective hypoglycemic dose was also determined through initial screening using the Oral Glucose Tolerance Test (OGTT) [12,13].

Experimental setup:

All 24 diabetic rats, divided into four groups of six, were fasted overnight before the OGTT procedure, and Blood samples were drawn from the retro-orbital sinus under ether inhalation anesthesia to determine fasting blood glucose (FBG) levels.All the animals divided in groups fed by oral administration of different vehicle, standard and PHB 1 was given as per the dose selection.

Group1: Normal control (Neither treated with extract nor standard drug)

Group 2: Standard treatment- Glibenclamide (5mg/kg)

Group 3: Polyherbal Blend PHB 1- 200mg/kg
Group 4: Polyherbal Blend PHB 1- 400mg/kg

Determination of OGTT (Oral Glucose Tolerance Test)
Rats were given water, glibenclamide (Standard) and PHB after 30 minutes. A gavage needle was then used to provide 2g/kg b.w. of glucose orally. The blood glucose levels of the rats were measured in every 30 till 180 minutes after the glucose injection, as well as at 0 minutes before the injection [13]. The data were displayed as value ± SEM, n = 6. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significant variations in group means are indicated by $p<0.05$ [14].
Induction of Diabetes: Diabetes was induced in rats using a 60 mg/kg streptozotocin injection after an acclimatization period. Following the injection, the rats were given food, water, and a 5% glucose solution to prevent hypoglycemic shock. Symptoms like moderate polydipsia and polyuria were confirmed by blood glucose measurements after three days, classifying rats with levels around 250 mg/dl after seven days as diabetic. Glibenclamide was administered orally once daily for 30 days, and glycated hemoglobin (HbA1c) levels were assessed after 90 days to evaluate long-term glycemic control [15,16].

Table 2: Animal Grouping and Treatment Schedule for STZ-Induced Diabetic Rats

S.No.	Group	Treatment	PHB Ratio (TC:CI:CS)	Dose
1.	Group I	Normal saline	—	1 ml/kg
2.	Group II	STZ + vehicle	—	—
3.	Group III	STZ + Glibenclamide	—	150 mg/kg
4.	Group IV	STZ + PHB-1A	**1 : 1 : 1**	200 mg/kg
5.	Group V	STZ + PHB-1B	**1 : 1 : 1**	400 mg/kg
6.	Group VI	STZ + PHB-2A	**2 : 1 : 1** (TC dominant)	200 mg/kg
7.	Group VII	STZ + PHB-2B	**2 : 1 : 1**	400 mg/kg
8.	Group VIII	STZ + PHB-3A	**1 : 2 : 1** (CI dominant)	200 mg/kg
9.	Group IX	STZ + PHB-3B	**1 : 2 : 1**	400 mg/kg
10.	Group X	STZ + PHB-4A	**1 : 1 : 2** (CF dominant)	200 mg/kg
11.	Group XI	STZ + PHB-4B	**1 : 1 : 2**	400 mg/kg
12.	Group XII	STZ + PHB-5A	**2 : 1 : 2** (TC+CF high)	200 mg/kg
13.	Group XIII	STZ + PHB-5B	**2 : 1 : 2** (TC+ CF high)	400 mg/kg

*A denotes for 200mg/kg and B denotes for 400 mg/kg dose of each ratio of PHB (Poly herbal blend)

Antidiabetic study: An antidiabetic study was conducted on diabetic rats using a polyherbal blend over 28 days, divided into thirteen groups were administered doses of 200 mg/kg and 400 mg/kg according to an oral glucose tolerance test (OGTT). A 60 mg/kg intraperitoneal injection of streptozotocin (STZ) was used to induce diabetes, and diabetes was confirmed by fasting blood glucose levels > 250 mg/dl. Glibenclamide was given for 21 days as standard. Blood glucose levels were measured on days 1, 7, 14, 21, and 28.while body weight was monitored weekly. Post 28 days euthanasia was performed, blood was taken viaretro-orbital puncture [17].

Biochemical Analysis: Biochemical markers such asblood glucose level[12],change in body weight[17], liver glycogen [18],serum glutamic oxaloacetic transaminase (SGOT)[19], and serum glutamic pyruvic transaminase (SGPT) [19]**,** Alkaline phosphate test-ALP[20], serum bilirubin [21]**,** total cholesterol[22],HDL[22], Serumtriglycerides[23], total protein[24]**,** urea[25]and creatinine[26], was determined usingdiagnostic kits for several parameters**.**

Histological evaluation
Each rat's pancreas was removed and washed with ice-cold saline.Some liver tissues were preserved in a 10% formalin solution for histological analysis. The stained paraffin section of 5-micron thick Hematoxylin and Eosin (H and E) was used in histopathological tests on albino rats seen at $400\times^{23}$. A 10% potassium chloride solution that had been chilled on ice before being centrifuged at 2000 rpm for 10 minutes was used to homogenize the pancreas of certain animals [27]**.**

Formulation of Polyherbal Tablet

Table 3: Polyherbal Tablet formulation

Ingredients	Function	PHT 1	PHT 2	PHT 3	PHT 4	PHT 5	PHT 6	PHT 7	PHT 8	PHT 9
PHB-2B (Optimized)	API	300	300	300	300	300	300	300	300	300

Microcrystalline cellulose (MCC PH102)	DC filler	155	150	145	140	135	130	125	120	115
PVPK30	Binder	10	15	20	10	15	20	12	18	22
Colloidal Silicon dioxide	Glidant	30	30	30	35	35	35	40	40	40
Croscarmellose Sodium	Superdisinte--grant	2	2	2	2	2	2	3	3	3
Magnesium stearate	Lubricant	3	3	3	3	3	3	2	2	2
	Total weight	**500**	**500**	**500**	**500**	**500**	**500**	**500**	**500**	**500**

Preparation of Formulation (Tablet) by direct compression Method: The polyherbal tablet was prepared by direct compression process for its cost-effectiveness and convenience, with reduced hygroscopicity. The procedure was as follows:

1. The dried extracts of *T.catappa, C.igneus*, and *C.fistula* were accurately weighed and pulverized to a uniform size using #60 sieves.
2. Nine formulations (PHB 1-9) were created for optimization, using API: excipients ratios as per given table.
3. Most materials were passed through sieve no. 40, althoughcolloidal silicon dioxide and magnesium stearate were passed through sieve no. 60.
4. The extracts were blended with PVP K30 and MCC PH102 for 10-15 minutes.
5. Croscarmellose sodium was added and mixed for an additional 5-10 minutes, followed by colloidal silicon dioxide for 3 minutes.
6. Magnesium stearate was added last, and blending continued gently for 2-3 minutes to avoid over-lubrication.
7. Pre-compression tests were conducted before compressing the mixtures into 500 mg tablets using a tablet compression machine with a 14 mm die punch.
8. Processing conditions maintained at 25 ± 2°C and 30 ± 2% humidity [28, 29].

Preformulation Studies:

Preformulation studies are essential for developing dosage forms and require a thorough understanding of a drug's physical and chemical properties. Key preformulation parameters, including angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio, were assessed for the polyherbal blend according to USP standards [29].

Post-Compression Evaluation:

The study involved several tests on tablets to assess their physical properties. Measurements of diameter and thickness were performed using a Vernier caliper on six randomly selected tablets. The hardness test, conducted with a Monsanto hardness tester, evaluated the tablets' resistance to breaking under pressure, displaying results as mean ± standard deviation. Friability, indicating abrasion resistance, was assessed using a Roche Friabilator, with results calculated based on the weight loss percentage. Weight variation was analyzed by weighing 20 tablets individually to ensure compliance with pharmacopeial limits. The disintegration test measured the time taken for tablets to break down in water at 37 ± 2 °C. Additionally, a stability study followed ICH guidelines, storing tablets under controlled conditions to monitor physical appearance and drug release over three months [29, 30].

RESULTS ANDDISCUSSION:

Extraction of crude drug: All the crude drugs were extracted and concentrated under reduced pressure and extracts selected as per the highest yield obtained. The results of extraction and their crude drug authentication no enumerated in the following table.

Table 4: Constituents of Polyherbal formulation

S.No.	Crude drug Extract	Authentication No.	Color of dried extract	Consistency of dried extract	% yield (W/W)
1	Ethanol extract of *T.catappa*	TECA/LF/RP/ 24 /104	Dark Brown	Sticky	24.96%
2	Ethanol extract of*C.igneus*	COIG/LF/RP/24/ 103	Dark green	Sticky	25.80%
3	Hydroalcoholic extract of *C. fistula*	CAFI/LF/RP/24/ 114	Mustard brown	Sticky	16.34%

Pharmacological screening

Acute toxicity studies : The acute toxicity study of five polyherbal blend (PHB1 – PHB5) was determined according to OECD guidelines 423.The studies did not show any sign of toxicity reaction and any death at 2000

mg/kg body weight as single oral administration of polyherbal blend in Wistar rats, hence the 1/10th of highest dose and twice of 1/10th i.e. 200 mg/kg and 400mg/kg respectively was selected as low dose and high dose.

Oral glucose tolerance test (OGTT): The OGTT of PHB-1 (equal quantities of all extracts) at 200 and 400 mg/kg effectively reduced blood glucose levels 30 minutes after glucose administration. The blood glucose level was lowered to (P<0.01) 66.23±2.65 mg/dl at 400 mg/kg as opposed to (P<0.01) 69.52±1.32 mg/dl at 200 mg/kg, demonstrating the dose-dependent effect. Glibenclamide action was consistent throughout all time intervals evaluated.

Table 5: Blood Glucose Levels at Different Time Intervals during OGTT

Group	Blood Glucose Level (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Normal	90.20±2.45	110.35±1.25	107.45±1.16	105.38±1.28	94.35±1.44
Standard Treated (Glibenclamide) (5mg/kg)	89.43±2.65	98.27±2.19[a]	76.63±1.47[b]	64.39±0.52[b]	59.45±2.53[b]
Polyherbal Blend PHB 1- 200mg/kg	90.65±2.72	109.15±2.31[a]	77±1.46[b]	70.53±2.39[b]	69.52±1.32[b]
Polyherbal Blend PHB 1- 400mg/kg	90.52±1.85	93.45±3.24[a]	83.28±1.27[b]	68.72±1.36[b]	66.23±2.65[b]

*Data shown as Mean±SD (n=6); a-P <0.05; b-P <0.01 compared to normal animals (ANOVA next to Dunnett's test).

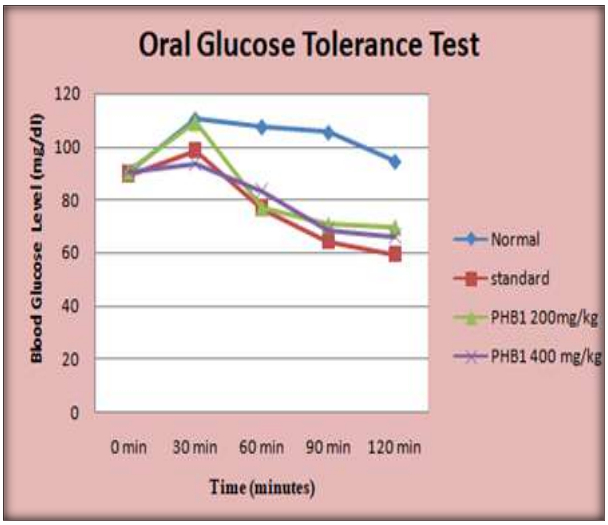

Figure 1 illustrates the graph of OGTT results

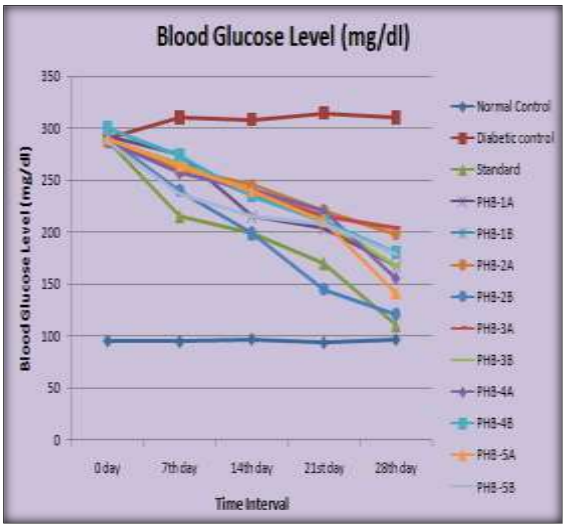

Figure 2 illustrates the graph of blood glucose Level

Antidiabetic activity: The anti-diabetic study involved healthy male Wistar rats (n = 78), randomized into thirteen groups. Animals were acclimatized with free access to water. The research adhered to ethical guidelines (IAEC Reg. no. 1546/PO/E/S/11/CPCSEA). Oral administration of PHB formulations led to a gradual decrease in blood glucose levels over 28 days, with significant results ($p < 0.05$) compared to a diabetic control group. A dosage-dependent effect was noted, with the highest dose (400 mg/kg) showing the most pronounced antihyperglycemic impact.

Effect of PHBs on body weight: Body weights of normal control, diabetic control, standard, and PHB-treated groups were analyzed over 28 days. Significant differences across groups were shown by ANOVA ($p < 0.05$). While the weight of the normal control group increased, that of the diabetic control group declined. Table 7 explained improvement in body weight in PHB-treated animals showed weight stabilization from day 14, with groups PHB-2B and PHB-5A nearing normal weight by day 28. The weight increase in PHB groups was significant compared to the diabetic control but not the normal control. Overall, PHB treatments effectively prevented weight loss in diabetic rats, suggesting potential therapeutic benefits.

Table 6: Effect of PHBs on Blood Glucose Level (mg/dl) in STZ induced diabetic rat

Group	Treatment	Blood Glucose Level (mg/dl)				
		0 day	7th day	14th day	21st day	28th day
Normal Control	Vehicle	95.15±2.35	94.35±2.36	96.56±1.23	93.42±1.26	96.26±2.56
Diabetic control	STZ treated 40mg/kg	290.12±1.24[a]	310.26±2.20[a]	308.25±1.39[a]	314.36±1.37[a]	310.56±1.28[a]
Standard	STZ 40 mg/kg +Glibenclamide 5mg/kg	289.23±1.63[a]	215.34±2.41[b]	198.51±1.35[b]	170.12±1.49[b]	110.24±1.53[b]
PHB-1A	STZ+PHB-1B 200mg/kg	292.36±2.53[a]	275.12±2.3[b]	215.2±1.21[b]	203.41±2.36[b]	167.26±2.37[b]
PHB-1B	STZ+PHB-1A 400 mg/kg	288.45±1.32[a]	264.35±1.26[b]	235.43±2.65[b]	210.82±2.49[b]	179.32±1.27[b]
PHB-2A	STZ+PHB-2A 200mg/kg	286.96±1.26[a]	260.5±2.34[b]	245.21±2.39[b]	220.35±1.89[b]	198.26±2.46[b]
PHB-2B	STZ+PHB-2B 400mg/kg	291.24±1.38[a]	239.27±1.35[b]	198.76±2.95[b]	145.24±2.35[b]	121.16±2.46[b]
PHB-3A	STZ+PHB-3A 200mg/kg	284.35±1.85[a]	264.56±1.56[b]	238.39±2.64[b]	214.65±1.24[b]	203.5±1.52[b]
PHB-3B	STZ+PHB-3B 400mg/kg	289.32±2.25[a]	266.12±1.63[b]	240.13±1.76[b]	210.28±2.38[b]	167.23±2.43[b]
PHB-4A	STZ+PHB-4A 200 mg/kg	287.23±1.64[a]	256.65±1.64[b]	241.26±2.64[b]	220.37±1.62[b]	155.28±1.25[b]
PHB-4B	STZ+PHB-4B 400mg/kg	300.24±1.26[a]	274.21±2.78[b]	235.11±2.81[b]	210.84±1.36[b]	180.56±2.61[b]
PHB -5A	STZ+PHB -5A 200mg/kg	290.41±2.41[a]	264.35±2.34[b]	240.24±2.49[b]	210.86±2.58[b]	141.43±1.43[b]
PHB -5B	STZ+PHB -5B 400 mg/kg	287.23±1.23[a]	236.24±1.38[b]	215.36±1.86[b]	207.56±2.69[b]	178.26±1.65[b]

*Data shown as Mean±SD (n=6); a-P <0.05; b-P <0.01 compared to normal animals (ANOVA next to Dunnett's test).

Table 7: Effect of PHB on Changes of body weight in STZ induced diabetic rats

Group	Treatment	Body Weight (gms)				
		0 day	7th day	14th day	21st day	28th day
Normal Control	Vehicle	163.23±2.35	165.42±1.26	166.25±2.56	167.29±2.36	169.65±1.23
Diabetic control	STZ treated 60mg/kg	162.32±1.24[a]	130.25±1.37[a]	126.21±1.28[a]	118.27±2.20[a]	107.15±1.39[a]
Standard	STZ 60 mg/kg + Glibenclamide 5mg/kg	155.25±1.63[a]	138.24±1.49[b]	142.39±1.53[b]	148.26±2.41[b]	153.56±1.35[b]
PHB-1A	STZ+PHB-1B 200mg/kg	157.23±2.53[a]	139.86±2.36[b]	143.26±2.37[b]	148.26±2.3[b]	150.12±1.21[b]
PHB-1B	STZ+PHB-1A400 mg/kg	162.45±1.32[a]	148.28±2.49[b]	151.43±1.27[b]	153.45±1.26[b]	158.23±2.65[b]
PHB-2A	STZ+PHB-2A 200mg/kg	163.41±1.26[a]	140.29±1.89[b]	145.26±2.46[b]	151.23±2.34[b]	156.27±2.39[b]
PHB-2B	STZ+PHB-2B 400mg/kg	165.23±1.38[a]	139.56±2.35[b]	151.48±2.46[b]	155.23±1.35[b]	161.25±2.95[b]
PHB-3A	STZ+PHB-3A 200mg/kg	159.27±1.85[a]	147.43±1.24[b]	143.23±1.52[b]	149.50±1.56[b]	152.86±2.64[b]
PHB-3B	STZ+PHB-3B 400mg/kg	167.37±2.25[a]	148.62±2.38[b]	150.5±2.43[b]	155.23±1.63[b]	157.23±1.76[b]
PHB-4A	STZ+PHB-4A200 mg/kg	161.23±1.64[a]	145.41±1.62[b]	148.23±1.25[b]	151.27±1.64[b]	155.76±2.64[b]
PHB-4B	STZ+PHB-4B 400mg/kg	162.38±1.26[a]	148.27±1.36[b]	152.34±2.61[b]	155.6±2.78[b]	156.5±2.81[b]
PHB -5A	STZ+PHB-5A 200mg/kg	164.35±2.41[a]	148.55±2.58[b]	153.43±1.43[b]	156.3±2.34[b]	158.23±2.49[b]
PHB -5B	STZ+PHB-5B400 mg/kg	161.26±1.23[a]	143.51±2.69[b]	149.24±1.65[b]	151.42±1.38[b]	154.24±1.86[b]

*Data shown as Mean±SD (n=6); a-P <0.05; b-P <0.01 compared to normal animals (ANOVA next to Dunnett's test).

Fig3: Graph illustrate effect of PHB on Body Weight changes

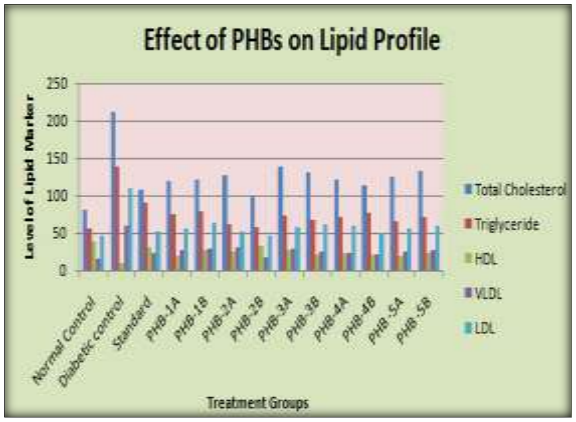

Fig 4: Graph illustrate the effect of PHB on Lipid Level Marker

Effect of PHBs Lipid Profile: There were notable differences in serum triglycerides, total cholesterol, HDL-C, LDL-C, and VLDL-C between groups and weeks. HDL-C significantly lowered ($P < 0.001$) while TG, TC, LDL-C, and VLDL-C substantially elevated ($P < 0.01$) in the positive control group. compared to the normal group, with no week-to-week fluctuations. Treatment with polyherbal blend (PHBs) notably improved lipid profiles. PHBs at 200 mg/kg significantly reduced lipid levels when compared to the positive control, with marked changes in TC and HDL-C. A higher dose of 400 mg/kg resulted in even greater improvements, particularly with PHB-2B, which demonstrated the highest HDL-C levels and lowest TG, LDL-C, and VLDL-C compared to other groups. The results are further illustrated in the accompanying table 8 and figure 4.

Effect of PHBs on Hepatic Markers: Hepatic glycogen and liver enzyme levels (SGOT, SGPT, ALP, and serum bilirubin) were significantly affected by treatment and duration, as shown by ANOVA. The untreated diabetic control group had higher liver enzyme levels than the normal group, indicating hepatic impairment. Treatment with ($P < 0.01$)PHBs at 200 and 400 mg/kg improved these biomarkers, yet values remained significantly different from the normal control group. Notably, PHB-2B at 400 mg/kg($P < 0.001$) demonstrated the most substantial hepatoprotective effect, reducing liver injury markers and restoring hepatic glycogen significantly. Overall, PHB-2B at this dosage proved more effective in alleviating hepatic dysfunction and boosting glycogen storage in diabetic patients.

Effect of PHBs on Renal Marker: Diabetic animals had lower level of total protein and higher serum urea and creatinine levels compared to control animals, while PHBs treated and glibenclamide treated animals had normal total protein and total serum urea and creatinine levels. The liver tissue's glycogen may not have been depleted due to the stimulation of insulin release from the β cells, which activates the glycogen synthase machinery. The effect of Polyherbal blend and standard on kidney function paramerters are dipicted in table 9.

Table 8: Effect of PHBs on lipid profile in STZ induced diabetic rats

Group	Treatment	Lipid Profile (mg/dl)				
		Total Cholesterol	Triglyceride	HDL	VLDL	LDL
Normal Control	Vehicle	80.21±3.35	55.34±2.36	37.98±1.23	15.32±3.26	45.21±2.56
Diabetic control	STZ treated 40mg/kg	212.34±3.24	138.26±2.20	9.03±1.39	60.25±1.37	110.56±3.28
Standard	STZ 40 mg/kg +Glibenclamide 5mg/kg	108.25±3.63[c]	89.56±2.41[c]	31.25±1.35[c]	23.54±3.49[c]	52.45±3.53[c]
PHB-1A	STZ+PHB-1B 200mg/kg	119.26±2.53[c]	75.45±2.3[c]	19.62±1.21[c]	27.23±2.36[c]	56.53±2.37[c]
PHB-1B	STZ+PHB-1A 400 mg/kg	120.28±2.32[c]	78.64±3.26[c]	26.23±2.65[c]	28.36±2.49[c]	63.23±3.27[c]
PHB-2A	STZ+PHB-2A 200mg/kg	127.37±3.26[c]	62.47±2.34[c]	24.35±2.39[c]	31.23±3.89[c]	51.24±2.46[c]
PHB-2B	STZ+PHB-2B 400mg/kg	99.26±2.38[c]	58.23±3.35[c]	33.21±2.95[c]	16.45±2.35[c]	46.25±1.46[c]
PHB-3A	STZ+PHB-3A 200mg/kg	138.71±3.85[c]	73.47±1.56[c]	27.26±2.64[c]	29.56±3.24[c]	57.85±3.52[c]
PHB-3B	STZ+PHB-3B 400mg/kg	130.64±2.25[c]	68.24±3.63[c]	21.56±1.76[c]	24.23±2.38[c]	62.35±2.43[c]
PHB-4A	STZ+PHB-4A 200 mg/kg	121.28±3.64[c]	71.23±3.64[c]	23.12±2.64[c]	23.41±3.62[c]	59.54±3.25[c]
PHB-4B	STZ+PHB-4B 400mg/kg	114.23±3.26[c]	77.29±2.78[c]	20.41±2.81[c]	21.25±3.36[c]	49.56±2.61[c]
PHB -5A	STZ+PHB -5A 200mg/kg	124.65±1.41[c]	64.89±3.34[c]	19.89±2.49[c]	25.12±2.58[c]	55.24±1.43[c]
PHB -5B	STZ+PHB -5B 400mg/kg	132.32±3.23[c]	70.25±2.38[c]	22.35±1.86[c]	26.41±3.69[c]	60.23±3.65[c]

*All data presented in Mean±SD (n=6); Values are expressed as Mean±SEM (n=6); a- $p<0.05$, b- $p<0.01$, c- $p<0.001$ compared to diabetic control (one-way ANOVA followed by a Dunnett's t test)

Table 9: Effect of PHBs on Hepatic & Renal Marker in STZ induced diabetic rats

		Hepatic Glycogen (g/100ml tissue)	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	Serum Bilirubin (mg/dl)	Total Protein	Serum Urea
Normal Control	Vehicle	5.71±0.35	102.52±2.36	80.91±1.23	80.42±0.26	0.683±0.06	8.68±0.35	37.2±2.36
Diabetic control	STZ treated 40mg/kg	2.89±1.24	160.33±2.20	160.42±1.39	212.27±1.37	0.942±0.08	3.84±1.24	84.26±2.20
Standard	STZ 40 mg/kg +Glibenclamide 5mg/kg	5.82±1.63^{c}	115.24±2.41^{c}	106.25±1.35^{c}	100.42±0.49^{c}	0.583±0.03^{c}	8.07±1.63^{c}	44.32±2.41^{c}
PHB-1A	STZ+PHB-1B 200mg/kg	3.59±0.53^{b}	135.26±2.34^{a}	130.23±1.21^{c}	165.23±0.36^{a}	0.712±0.07^{c}	7.26±0.53^{b}	50.23±2.34^{a}
PHB-1B	STZ+PHB-1A 400 mg/kg	3.52±1.32^{a}	136.56±3.26^{a}	126.56±2.65^{c}	149.25±0.49^{a}	0.741±0.07^{c}	8.34±1.32^{a}	65.21±3.26^{a}
PHB-2A	STZ+PHB-2A 200mg/kg	4.23±0.26^{c}	128.26±2.34^{b}	118.46±2.39^{c}	127.42±1.89^{a}	0.612±0.06^{c}	7.02±0.26^{c}	54.28±2.34^{b}
PHB-2B	STZ+PHB-2B 400mg/kg	5.73±1.38^{c}	118.56±3.35^{a}	102.23±2.95^{c}	101.48±0.35^{c}	0.589±0.06^{c}	8.66±1.38^{c}	36.42±3.35^{a}
PHB-3A	STZ+PHB-3A 200mg/kg	4.04±0.85^{b}	123.48±1.56^{a}	128.46±2.64^{c}	115.26±0.24^{c}	0.632±0.02^{c}	7.53±0.85^{b}	53.14±1.56^{a}
PHB-3B	STZ+PHB-3B 400mg/kg	3.75±0.25^{c}	131.42±3.63^{a}	130.24±1.76^{c}	120.53±0.38^{c}	0.692±0.03^{c}	7.15±0.25^{c}	44.56±3.63^{a}
PHB-4A	STZ+PHB-4A 200 mg/kg	4.19±1.64^{b}	120.23±3.64^{b}	127.56±2.64^{c}	130.54±1.62^{c}	0.841±0.05^{c}	8.42±1.64^{b}	39.51±3.64^{b}
PHB-4B	STZ+PHB-4B 400mg/kg	3.75±0.26^{c}	134.56±2.78^{c}	131.25±2.81^{c}	145.28±0.36^{c}	0.764±0.01^{c}	7.63±0.26^{c}	61.23±2.78^{c}
PHB -5A	STZ+PHB -5A 200mg/kg	4.67±1.41^{c}	130.56±3.34^{b}	132.42±2.49^{c}	136.12±1.58^{c}	0.684±0.03^{c}	8.25±1.41^{c}	55.24±3.34^{b}
PHB -5B	STZ+PHB -5B 400mg/kg	5.14±1.23^{a}	126.53±2.38^{a}	121.27±1.86^{c}	124.89±0.69^{c}	0.607±0.05^{c}	7.28±1.23^{a}	45.26±2.38^{a}

*All data presented in Mean±SD (n=6); Values are expressed as Mean±SEM (n=6); a- $p<0.05$, b- $p<0.01$, c- $p<0.001$ compared to diabetic control (one-way ANOVA followed by a Dunnett's t test)

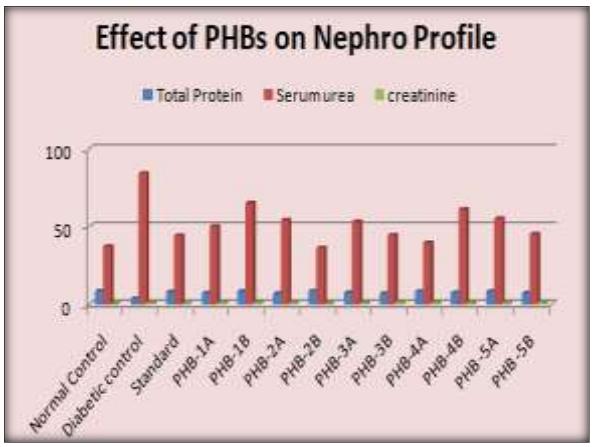

Fig 5: Graph illustrate effect of PHB on Nephro Profile

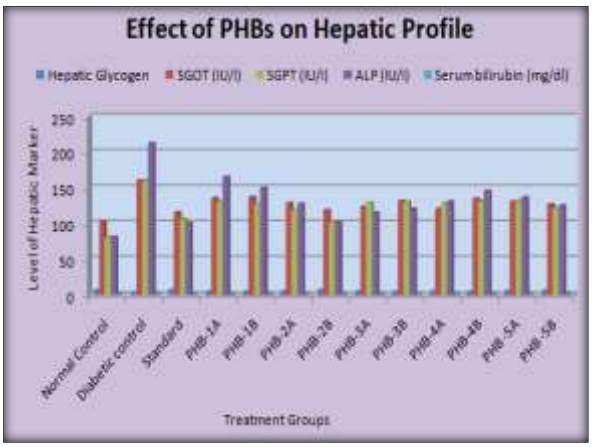

Fig 6: Graph illustrate the effect of PHB on Hepatic Marker

Histopathological Studies: Histopathological analysis of pancreatic tissues from diabetic rats revealed significant damage in the streptozotocin (STZ)-induced group, characterized by bleeding, vascular congestion, cellular necrosis, and inflammatory cell infiltration. In contrast, the normal control group exhibited intact pancreatic architecture and normal β-cell morphology. Treatment with PHB extracts preserved pancreatic tissue, with most sections showing reduced abnormalities and maintained β-cell integrity, particularly after PHB-2B therapy, which restored normal islet size and number, and ensured intact pancreatic ducts and exocrine components. These findings indicate that PHB formulations effectively protect and restore pancreatic tissue in STZ-induced diabetic rats.

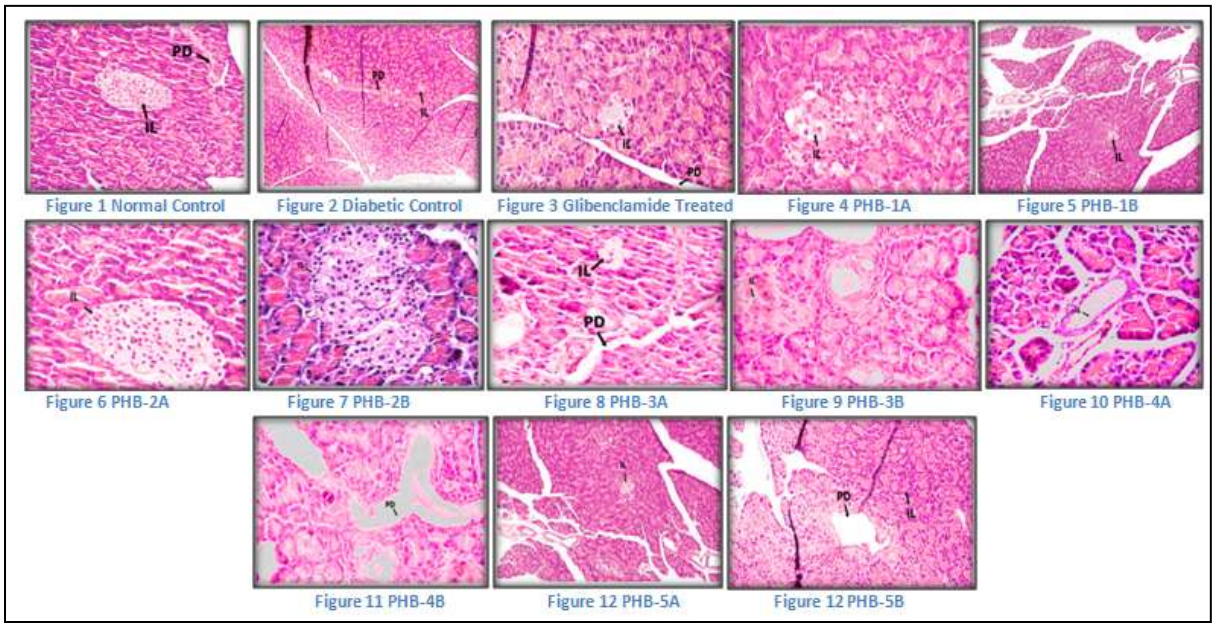

Fig 7: Histopathology of a 5 micron thick H and E stained paraffin sections of Albino rats

Evaluation of Polyherbal tablet:

Preformulation studies:

The direct compression method was used to create formulations F1 through F9 following all powder mixes undergone preformulation assessment. All blends were examined for bulk density, tapped density, angle of repose, compressibility index, and Hausner's ratio in order to analyze a flow behavior.Polyherbal tablet PHT 7 had excellent flow characters among all the formulation.The data in Table 10 support this.

Table 10: Pre-compression evaluation of powder blend of polyherbal tablet

Code	Angle of repose (degree°)	LBD (gm/cm^2)	TBD (gm/cm^2)	Compressibility index %	Hausner's ratio	Flow
PHT 1	29.7	0.31	0.51	13.25	1.19	Good
PHT 2	32.2	0.32	0.55	14.26	1.18	Good
PHT 3	33.5	0.33	0.46	15.63	1.16	Good
PHT 4	34.3	0.29	0.48	19.24	1.17	Fair
PHT 5	32.8	0.31	0.47	18.25	1.13	Fair
PHT 6	27.8	0.28	0.49	11.52	1.14	Passable
PHT 7	30.5	0.30	0.45	10.08	1.10	Excellent
PHT 8	31.7	0.35	0.48	17.63	1.13	Passable
PHT 9	28.5	0.34	0.43	18.04	1.15	Passable

LBD-Loose Bulk Density, TBD-Tapped Bulk Density

Table No 11: Post-compression evaluation parameter of powder blend of polyherbal tablet

Code	Thickness (mm)	Hardness (kg/cm2)	Friability (%)	Average weight variation	Disintegration Time (min)
PHT 1	4.10	5.18±0.126	0.14	500.59±1.304	10±0.68
PHT 2	4.09	4.82±0.135	0.36	500.68±1.285	09±0.52
PHT 3	4.10	4.68±0.145	0.84	500.78±0.907	11±0.80
PHT 4	4.11	12.5±0.235	1.34	498.80±0.125	08±0.54
PHT 5	4.12	5.08±0.452	1.28	499.55±1.374	05±0.40
PHT 6	4.08	4.65±0.125	0.89	500.20±0.752	09±0.60
PHT 7	4.10	5.38±0.257	0.23	500.25±1.305	04±0.68
PHT 8	4.13	4.45±0.652	0.29	500.52±0.145	03±0.52
PHT 9	4.08	5.42±0.234	0.54	500.75±1.851	04±0.30

Post compression evaluation: The prepared polyherbal tablets were spherical, light brown in color, and had a distinctive odor with a bitter flavor. After evaluation, it was discovered that the formulation's physicochemical properties remained within acceptable pharmacopeial bounds. The pH of the 1% aqueous solution was determined to be 7.21 ± 0.21, suggesting that the formulation was almost neutral. With moisture content of 4.05 ± 0.5% w/w; there was a lower chance of microbiological contamination and sufficient stability. Tablet compression and die

filling were determined to be uniform; with an average weight of 500.3 ± 3.4 mg.For the polyherbal tablets, post-compression characteristics including hardness, friability, thickness, weight fluctuation, and disintegration time were measured.Every formulation surpassed pharmacopoeial requirements. Among the manufactured batches, PHT 7 produced the best results, with acceptable hardness, low friability, homogeneous tablet weight, and quick dissolution. In comparison to the other formulations, the optimum concentration of binder and superdisintegrant demonstrated higher overall performance by providing adequate mechanical strength and effective tablet disintegration.

Stability Studies: The short term accelerated studies were performed on PHT 7 by applying ICH guidelines. The tablets were kept on 40±2ºC and 75±5% RH for 3 month time period for assessing color, appearance, average weight, thickness, % friability, disintegration time. The accelerated stability testing didn't show any considerable change in physical properties, average weight, friability, hardness and dissolution for the six month. The polyherbal formulation PHT 7 was assuring the quality, safety and efficacy as a representative of traditional knowledge. Therefore the well developed polyherbal formulation will generate a trait to cure diabetes mellitus more effectively and ensures minimum side effects.

Table 12: Stability testing of Polyherbal tablet of PHT 7

Physical Parameters	**Accelerated Stability Study**				**Long term Stability testing**
	Initial	**1 month**	**2 month**	**3 month**	**6 month**
Colour	Light brown	Light brown	Light brown	Light brown	Light brown
Appearance	Smooth	Smooth	Smooth	Smooth	Smooth
Average weight	500.50	499.68	499.35	499.02	498.80
Thickness	4.10	4.10	4.10	4.10	4.10
% friability	0.28	0.28	0.28	0.23	0.23
Disintegration time (min)	4.10	4.10	4.15	4.20	4.20

CONCLUSION

The study focuses on the strong antidiabetic potential of apolyherbal formulation containing *Terminaliacatappa*, *Costusigneus* and *Cassia fistula*, which significantly restored blood glucose levels in treated groups. In addition to glycemic management, biochemical measures such as serum SGPT, SGOT, urea, creatinine, and lipid profile improved, indicating protection against diabetes-related hepatic, renal, and metabolic problems. All formulations' precompression properties satisfied pharmacopoeialrequirements, exhibiting acceptable compressibility and flowability. Adequate hardness, low friability, uniform weight variation, and quick disintegration time were among the superior post-compression features of formulation PHT 7, which proved to be the most suitable variant. The balanced concentrations of binder and superdisintegrant improved tablet integrity and disintegration efficiency.Moreover, stability experiments indicated that formulation PHT 7 remained physically stable throughout the study period, with no significant changes in friability or disintegration characteristics, confirming its suitability for pharmaceutical use and storage stability.

Since diabetes mellitus is a chronic metabolic disease that requires lifelong treatment, it is still crucial to create safer and more effective alternative therapeutic strategies. The current study's findings provide scientific evidence supporting the therapeutic efficacy of combined plant extracts as a polyherbal formulation, which showed increased antihyperglycemic action when compared to individual plant extracts. The synergistic activity of the chosen medicinal herbs may minimize the side effects typically associated with long-term synthetic antidiabetic medication while improving glycemic control and reducing diabetes-related problems.

Conflict of interest- We declare that there is no conflict of interest**.**

Funding source- None

Author Contributions-ArchanaPatidar created the methodology, carried out the data collection, came up with the original idea, and wrote the paper. Prof Dr L N Patidar helped interpret the findings, gave significant edits to the manuscript, and supervised the project. The final draft of the work was accepted by both writers.

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