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Ameliorative Effects of a Polyherbal Formulation on Hepatic Function and Pancreatic Histomorphology in Streptozotocin-Induced Diabetic Male Wistar Rats

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ABSTRACT: This study evaluated the ameliorative effects of a polyherbal formulation comprising *Garcinia kola*, *Buchholzia coriacea*, and *Picralima nitida* on hepatic function and pancreatic histomorphology in streptozotocin (STZ)-induced diabetic male Wistar rats. Thirty-five Wistar rats were randomly divided into seven groups: normal control, diabetic control, four groups treated with the polyherbal extract (100, 150, 200, and 250 mg/kg), and a positive control treated with metformin (50 mg/kg). Diabetes was confirmed 48 hours post-STZ injection (60 mg/kg). Treatment lasted 15 days, with blood glucose monitored at three-day intervals. Liver function (total bilirubin), organ weights, and histopathological changes in hepatic and pancreatic tissues were assessed at the end of the experiment. The polyherbal formulation significantly ($p < 0.05$) reduced blood glucose levels, particularly at 250 mg/kg, where near-normal levels (125.6 ± 11.1 mg/dL) were achieved by day 15. Total bilirubin levels were markedly reduced at 150 mg/kg (0.06 ± 0.06 mg/dL) and 250 mg/kg (0.20 ± 0.11 mg/dL). GC-MS analysis identified key bioactive compounds, including phytol (20.81%), neophytadiene (20.21%), and palmitic acid (12.15%). Histological findings showed preserved hepatic architecture and pancreatic islet hyperplasia. The polyherbal formulation demonstrated significant antihyperglycemic and hepatoprotective effects.

Keywords: Polyherbal, Hepatoprotective, Pancreatic histomorphology, Medicinal plants, Bioactive compounds

Introduction

Diabetes mellitus is a metabolic disorder characterised by persistent hyperglycemia caused by a lack of or low insulin secretion, insulin action, or both. In type 2 diabetes, insulin resistance in the liver, muscles, and fat tissue often leads to obesity and chronic inflammation (DeFronzo *et al*., 2015). At the molecular level, insulin resistance impairs glucose uptake in peripheral tissues and reduces insulin's ability to suppress hepatic glucose production, leading to elevated blood glucose levels. Pancreatic β-cells first try to keep up by making more insulin. Still, long-term damage from high blood sugar, fats, oxidative stress, and amyloid buildup eventually leads to failure, resulting in low insulin levels (Halban *et al*., 2014). High blood sugar over a long period damages cells and blood vessels through multiple pathways, leading to complications such as retinopathy, nephropathy, and neuropathy, as well as large-vessel diseases such as heart and brain disorders (Forbes and Cooper, 2013; Paneni *et al*., 2013).

The relationship between diabetes and hepatic dysfunction is complex. Individuals with diabetes are at significantly increased risk for a range of liver disorders collectively termed diabetic hepatopathy, such as non-

alcoholic fatty liver disease, glycogen hepatopathy, hepatic fibrosis, and cirrhosis (Elkrief *et al*., 2016). Current epidemiological studies have shown that metabolic dysfunction-associated steatotic liver disease (MASLD) affects 15-70% of individuals with type 1 diabetes, with prevalence varying based on diagnostic methods and population characteristics (de Vries *et al*., 2020). Liver damage caused by diabetes occurs through several related processes, including the buildup of fatty acids in the liver, increased oxidative stress due to excess reactive oxygen species, formation of advanced glycation end-products, overactivation of the polyol pathway, and increased release of pro-inflammatory cytokines (Mohamed *et al*., 2016). Total bilirubin is an important marker of liver function in diabetes. Studies show that about 37.2% of patients with type 2 diabetes have reduced serum total bilirubin levels, and lower levels are linked to poor glycemic control, as indicated by HbA1c (Dey *et al*., 2024). Changes in liver enzymes and bilirubin levels are also associated with hepatic insulin resistance. In particular, direct bilirubin may indicate liver cell damage even when total bilirubin appears normal. In addition, Seko *et al*. (2015) reported that serum alanine aminotransferase and total bilirubin are independently associated with serum lactate levels in patients with type 2 diabetes, further highlighting the close relationship between liver function and metabolic imbalance.

Streptozotocin (STZ)-induced diabetes in animal models has become an important tool for investigating diabetic complications and assessing potential therapeutic interventions. STZ is a glucosamine-nitrosourea compound that selectively destroys pancreatic β-cells through DNA alkylation and free radical generation, producing a diabetic state that closely mimics human type 1 diabetes (Furman, 2021). Histopathological examination of STZ-induced diabetic rats reveals characteristic changes, including decreased pancreatic islet numbers, β-cell loss, inflammatory cell infiltration, and hepatic architectural disturbances, such as hydropic degeneration and fibrosis (Szkudelski, 2001). These morphological alterations correlate with biochemical abnormalities, including hyperglycemia, elevated liver enzymes, and disrupted glycogen metabolism (Ghasemi *et al*., 2014).

In recent years, polyherbal formulations have garnered increasing scientific attention as potential therapeutic approaches for diabetes and its complications (Parveen *et al*., 2018). The rationale for polyherbal combinations stems from the synergistic interactions between multiple phytoconstituents, which may simultaneously target different pathophysiological pathways (Caesar and Cech, 2019). Phytochemicals, particularly phenolic compounds including flavonoids and phenolic acids, exert antihyperglycemic effects through diverse mechanisms such as improving glucose metabolism, enhancing antioxidant defence, reducing inflammation, and modulating key signalling pathways including phosphoinositide 3-kinase (PI3K), adenosine monophosphate-activated protein kinase (AMPK), and mitogen-activated protein kinase (MAPK) (Alkhalidy *et al*., 2018).

Previous investigations have demonstrated the hepatoprotective and pancreas-protective potential of various polyherbal formulations in STZ-induced diabetic models. Mitra *et al*. (1996) reported that D-400, a herbomineral formulation, significantly increased liver glycogen content and restored pancreatic islet cell superoxide dismutase activity in STZ-diabetic rats, with partial reversal of histopathological changes in both organs. A similar study was conducted by Odita *et al*. (2025), and it showed that a polyherbal combination of *Acanthus montanus* and *Moringa oleifera* significantly reduced fasting blood glucose and restored serum liver enzymes (ALT, AST, ALP) toward normal levels in alloxan-induced diabetic rats. Mobasheri *et al*. (2023) systematically reviewed multiple herbal agents, including Moringa oleifera, Morus alba, Silybum marianum, and Portulaca oleracea, that showed beneficial effects on diabetic hepatopathy through antioxidant and anti-inflammatory mechanisms.

Although individual herbs have shown hepatoprotective benefits, limited research exists on polyherbal formulations specifically designed to protect both the liver and pancreas in streptozotocin (STZ)-induced diabetes. In diabetes, the pancreatic–hepatic axis is a key therapeutic target because β-cell dysfunction and hepatic damage mutually exacerbate and accelerate disease progression (Taylor, 2021). Persistent hyperglycemia increases oxidative stress, which damages pancreatic islets and hepatocytes, while hepatic insulin resistance further disrupts metabolic balance (Tangvarasittichai, 2015). Therefore, treatments that protect both organs simultaneously may yield better outcomes. This study was conducted to evaluate the ameliorative effects of a novel polyherbal formulation on liver function and pancreatic histomorphology in STZ-induced diabetic male Wistar rats, using biochemical and histopathological assessments to determine its multi-organ protective potential.

Materials and methods

Collection and identification of plant materials: The plant materials (*Garcinia kola*, *Buchholzia coriacea*, and *Picralima nitida*) were collected in Warri, Delta State, Nigeria, and identified. They were identified and authenticated at the Herbarium Unit, Department of Plant Biology and Biotechnology, University of Benin, with

voucher numbers UBH-G365 (*Garcinia kola*), UBH-B271 (*Buchholzia coriacea*), and UBH-P424 (*Picralima nitida*), respectively.

Preparation of plant extract and Polyherbal formulation: The plant materials were ground into fine powder using electric and mechanical grinders. The three powdered samples were combined in a 4:3:3 ratio: *Buchholzia coriacea* **(4)**, *Garcinia kola* **(3)**, and *Picralima nitida* **(3)**. Following Lenka *et al*. (2016), 500 g of the combined powder was soaked in 1500 ml of distilled water for 72 hours with occasional shaking. The mixture was then stirred, filtered through Whatman No. 1 paper, and concentrated on a steam bath at 60 ^{o}C to a semi-liquid form. The extract was stored in a plastic container in the refrigerator until use.

Procurement and housing of experimental animals: Thirty-five (35) adult male Wistar rats weighing 100–200 g were used in the study. They were purchased from the Faculty of Basic Medical Sciences Animal Farm, Delta State University, Abraka, Nigeria, and housed in clean plastic cages. The rats were fed a regular mash diet containing 17% protein, 4.5% fat, 0.96% calcium, 3.92% phosphorus, and 2450 kcal of energy, with free access to water.

Ethical approval: The experimental protocol received ethical approval from the Faculty of Life Sciences Ethical Committee at the University of Benin, Nigeria. All procedures strictly adhered to the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. Special care was taken to minimise animal suffering throughout the study duration, including proper housing conditions, humane handling techniques, and euthanasia methods approved by the committee.

Experimental design: A complete randomised design was used for this study with 35 male Wistar rats. The 35 male Wistar rats were randomly allocated to 7 experimental groups of 5 rats each. Group one served as the normal control and consisted of non-diabetic rats that were neither induced with streptozotocin (STZ) nor subjected to any treatment. Group two functioned as the diabetic control and included rats induced with STZ but left untreated throughout the experimental period. Groups three to six comprised STZ-induced diabetic rats treated with graded doses of the polyherbal formulation. Specifically, group three received 100 mg/kg body weight of the extract, group four received 150 mg/kg body weight, group five received 200 mg/kg body weight, and group six received 250 mg/kg body weight. Group seven served as the positive control and consisted of STZ-induced diabetic rats treated with 50 mg/kg body weight of metformin.

Induction of diabetes and sample collection: Diabetes was induced in the rats as described by Ossai *et al*. (2021) and Idu *et al*. (2024), with slight modifications. The animals were fasted for 12 hours before induction and received a single intraperitoneal injection of streptozotocin (STZ) at 60 mg/kg. After 48 hours, blood glucose levels were measured using a New Spring glucometer (KF-B12, Changsha, Hunan, China), and rats with levels above 250 mg/dL were confirmed as diabetic. On the 15th day of the experiment, the animals were fasted for 12 hours before sample collection. Each rat was euthanised by cervical dislocation, and a laparotomy was performed to expose the internal organs. Blood was collected via cardiac puncture using a 2 ml syringe with a 23G needle, and the liver and pancreas were carefully excised for subsequent biochemical and histological analyses.

Determination of blood glucose levels: At 3-day intervals, blood samples were collected from the tail arteries of the rats, and blood glucose levels were determined using the glucose oxidase principle with a glucometer (New Spring, KF-B12, Changsha, Hunan, China). The results were expressed as mg/dl.

Serum Preparation for the Liver Function Test (Total Bilirubin): Blood samples were allowed to clot at room temperature, then centrifuged at 1500 rpm to separate the serum from cellular components (WHO, 2012). The clear serum supernatant was carefully collected using a pipette and transferred into clean, labelled sample bottles. The serum samples were stored in a refrigerator at approximately 4°C until analysis to preserve their integrity and prevent bilirubin degradation (Flores *et al*., 2020).

Determination of total bilirubin

Principle: The total bilirubin is determined in the presence of caffeine by the reaction with diazotised Sulphanilic acid to produce an extremely coloured diazo dye (560-600nm). The intensity of the colour of the dye formed is proportional to the concentration of total bilirubin.

$$\text{Sulfanilic acid} + NaNO_2 \xrightarrow{HCl} \text{Diazotized sulfanilic acid}$$

Procedure: To 200 µL of the sample, add 200µL of Sulfanilic acid to both samples (serum) and the blank. Add one drop of Sodium nitrite to all samples except the blank. One (1) ml of R3 was added to all samples and the blank, after which 200 µL was added to both samples and the blank. The samples were mixed and allowed to sit for 10 minutes at 20-25 °C. The spectrophotometer was zeroed, and the absorbance of the sample was measured against the blank sample at 578nm.

Calculation**:** Direct bilirubin (mg/dL) = ^{A}Sample × 10.8

*Histological analysis***:** The liver and pancreas were carefully removed from the experimental rats and fixed in neutral buffered formalin. The attached liver and pancreas were thoroughly dehydrated in 96% and 70% ethanol,

then washed with distilled water. Four (4) µm sections were prepared with haematoxylin-eosin dye as stain. The stained tissues were observed at ×10 and ×100 magnifications.

Statistical analysis: The data obtained from the experiment were subjected to descriptive and inferential statistics. They were presented in tables and charts as Mean ±Standard deviation, and the means were separated using Duncan's Multiple Range Test (DMRT). The statistics were carried out using Microsoft Excel (2016) and GraphPad Prism 9.

Results

Effect of the polyherbal on the blood glucose (mg/dL) level in male Wistar rats: Table 1 shows the significant effects of polyherbal treatment on blood glucose levels in different treatment groups over 15 days. The Normal Control group maintained stable glucose levels, while the Positive Control (untreated diabetic group) showed consistently high glucose levels, indicating persistent hyperglycemia. Among the polyherbal-treated groups, higher doses (150–250 mg/kg) progressively reduced blood glucose levels, with the 250 mg/kg dose showing the most pronounced effect compared to Metformin (50 mg/kg). The 200 and 250 mg/kg doses exhibited a dose-dependent reduction, attaining near-normal glucose levels by Day 15. In contrast, the 100 mg/kg dose had minimal impact.

Table 1: Effect of the polyherbal on blood glucose (mg/dL) level in male Wistar rats.

Groups	Base	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Normal control	98.4 ± 27.2 ^{b}	99.6 ± 15.7 ^{d}	99.2 ± 13.8 ^{e}	100.6 ± 17.1 ^{e}	101.2 ± 18.2 ^{e}	94.2 ± 8.8 ^{e}	96.0 ± 6.9 ^{d}
Diabetic control	84.6 ± 19.6 ^{c}	318.0 ± 36.6 ^{c}	332.0 ± 33.5 ^{c}	317.2 ± 33.1 ^{c}	325.0 ± 50.5 ^{c}	330.8 ± 39.2 ^{c}	346.6 ± 46.3 ^{a}
100 mg/kg	90.0 ± 8.4 ^{b}	444.2 ± 87.4 ^{a}	425.6 ± 98.5 ^{a}	396.2 ± 100.4 ^{a}	381.2 ± 83.8 ^{a}	377.2 ± 85.6 ^{a}	357.0 ± 94.0 ^{a}
150 mg/kg	91.0 ± 13.1 ^{b}	363.2 ± 73.6 ^{b}	332.0 ± 75.1 ^{c}	311.8 ± 79.1 ^{c}	262.4 ± 56.2 ^{b}	216.6 ± 54.5 ^{c}	194.8 ± 46.5 ^{bc}
200 mg/kg	88.2 ± 25.7 ^{b}	449.2 ± 97.4 ^{a}	357.0 ± 54.2 ^{b}	262.4 ± 72.9 ^{b}	243.2 ± 73.7 ^{b}	214.0 ± 61.8 ^{c}	168.4 ± 28.4 ^{bc}
250 mg/kg	90.8 ± 11.8 ^{b}	443.4 ± 84.0 ^{a}	346.8 ± 70.1 ^{b}	257.4 ± 58.0 ^{b}	196.2 ± 36.3 ^{d}	155.0 ± 14.0 ^{d}	125.6 ± 11.1 ^{c}
Positive control (50 mg/kg)	90.0 ± 6.3 ^{b}	299.6 ± 37.7 ^{c}	277.6 ± 28.4 ^{d}	247.2 ± 6.2 ^{b}	222.4 ± 7.3 ^{c}	188.4 ± 4.3 ^{b}	178.2 ± 8.3 ^{bc}

*Values are expressed as Mean ± Standard deviation of five (5) replicates and separated using Duncan's Multiple Range Test DMRT. Values with the same letters indicate no significant difference ($p>0.05$). Normal control-distilled water; Positive control-metformin.

*Effect of the polyherbal formulation on the organ weight of male Wistar rats***:** Table 2 shows the effect of the polyherbal formulation on the weight of the liver and pancreas of male Wistar rats. The diabetic control group showed a slight reduction in liver weight and an increase in pancreatic weight compared to the normal control. Treatment with the polyherbal extract, particularly at 150 mg/kg, 200 mg/kg, and 250 mg/kg, normalised liver and pancreatic weights. The 150 mg/kg dose showed the highest liver weight (8.33 ± 2.08 g). Pancreatic weights were significantly reduced at 100 mg/kg and with metformin, whereas the 200 mg/kg dose appeared to maintain near-normal pancreatic weight.

Table 2: Effect of the polyherbal formulation on the weight of the liver and pancreas in male Wistar rats

S/N	Groups	Liver (g)	Pancreas (g)
1	Normal Control	7.00 ± 1.41	1.50 ± 1.00
2	Diabetic control	6.00 ± 1.00	1.67 ± 0.58
3	100mg/kg	6.67 ± 1.15	0.57 ± 0.38
4	150mg/kg	8.33 ± 2.08	0.99 ± 0.02
5	200mg/kg	7.33 ± 1.53	1.00 ± 0.00
6	250mg/kg	7.67 ± 1.15	0.85 ± 0.26
7	Positive control (50mg/kg)	5.67 ± 0.58	0.42 ± 0.10

Values are expressed as Mean ±Standard deviation of five (5) replicates.

*Effects of the polyherbal formulation on total bilirubin in Streptozotocin-induced diabetic male Wistar rats***:** Table 3 shows the effects of the polyherbal formulation on a liver function parameter (Total bilirubin) in male Wistar rats. The diabetic (positive control) group showed elevated total bilirubin levels. Total bilirubin was also markedly reduced at 150 mg/kg (0.06 ± 0.06 mg/dL).

Table 3: Effect of the Polyherbal formulation on biochemical parameters in male Wistar rats

S/N	Groups	Total Bilirubin (mg/dL)
	Normal control	0.48 ± 0.56^a
	Diabetic control	0.94 ± 0.33^a
	100mg/kg	0.53 ± 0.67^a
	150mg/kg	0.06 ± 0.06^a
	200mg/kg	0.94 ± 0.72^a
	250mg/kg	0.20 ± 0.11^a
	Positive Control (Metformin)	0.52 ± 0.40^a

Values are expressed as Mean ± Standard deviation of five (5) replicates. Values with the same letters in the same column indicate no significant difference ($p>0.05$).

GC-MS analysis of the polyherbal formulation: Table 4 lists complex bioactive compounds with potential therapeutic properties. Major constituents included fatty acids and their esters, such as palmitic acid (12.15%) and phytol (20.81%), as well as terpenoids, including neophytadiene (20.21%) and α-pinene (1.69%). Phenolic compounds such as 2,4-di-tert-butylphenol (1.11%) and glycerol diacetate (1.91%) were also identified.

Table 4: GC-MS analysis of the polyherbal formulation.

S/N	RT (min)	Compound Name	Concentration (%)
1	4.420	2-Butoxyethyl acetate	0.64%
2	4.727	1,2,3-Propanetriol, 1-acetate [monoacetin]	4.97%
3	4.727	Butanoic acid, 4-(methylthio)-	4.97%
4	4.727	p-Dioxane-2,3-diol [Glyoxal mono-ethylene acetal]	4.97%
5	5.910	Methyl salicylate [Wintergreen oil]	0.71%
6	6.672	Octadecanoic acid, 3-hydroxy-, methyl ester [methyl 3-hydroxy stearate]	3.27%
7	6.672	Hexadecanoic acid, 3-hydroxy-, methyl ester [methyl 3-hydroxypalmitate]	3.27%
8	6.672	1,3-Oxathiolane,2-[[(2-chloroethyl) thio]methyl]-2-methyl-	3.27%
9	6.729	Glycerol 1,2-diacetate [Diacetin]	1.91%
10	6.729	Butyl 2-methylbutanoate	1.91%
11	8.244	6,11-Dimethyl-2,6,10-dodecatrien-1-ol [farnesol]	1.55%
12	8.244	Cyclooctanemethanol	1.55%
13	8.244	Geranic acid	1.55%
14	8.364	2-Cyclobutyl-2-propanol	2.14%
15	8.364	Hexane, 2,5-dimethyl-	2.14%
16	8.364	2-Pentenoic acid, 4-methyl-	2.14%
17	10.097	2,4-Di-tert-butylphenol	1.11%
18	10.097	Phenol, 3,5-bis(1,1-dimethylethyl)	1.11%
19	10.398	Ethanamine, 2-phenoxy-	0.65%
20	10.398	3-Cyclopentyl-1-propyne	0.65%
21	10.398	1,7-Octadiyne	0.65%
22	10.756	Dodecanoic acid [Lauric acid]	0.68%
23	10.756	(4Z)-5-Chloro-3,4-dimethyl-2,4-heptadiene	0.68%
24	10.756	Benzenesulfonamide,N-[2-(4-methyl-4H-[1,2,4]triazol-3 ylsulfanyl)ethyl]-	0.68%
25	13.106	6-Nonynoic acid	0.69%
26	13.106	1,5-Heptadiyne	0.69%
27	13.687	Bicyclo[3.1.1]heptane2,6,6-trimethyl-,[1R (1.alpha.,2.beta.,5.alpha.)]- [Pinene]	1.69%
28	13.687	3-Nonen-1-ol, (Z)- [(Z)3-nonenol]	1.69%
29	13.760	Cyclododecanol, 1-ethenyl- [vinylcyclododecanol]	1.42%
30	13.760	1-Dodecanol, 3,7,11-trimethyl- [Tetrahydofarnesol]	1.42%
31	13.760	Oxirane, tridecyl-	1.42%
32	13.942	Pentaleno[1,2-b]oxirene	0.71%
33	13.942	9,12-Tetradecadien-1-ol, acetate, (Z, E)-	0.71%
34	13.942	2(3H)-Benzofuranone, hexahydro-3-methylene-	0.71%
35	14.123	3,4-Octadiene, 7-methyl-	0.66%
36	14.123	3,8,11-Trioxatetracyclo[4.4.1.0(2,4).0(7,9)] undecane, (1.alpha.,2.alpha.,4.alpha.,6.alpha.,7.beta.,9.beta.)-	0.66%
37	14.123	Cyclobutane, 1,2-diethenyl-3,4-dimethyl-	0.66%
38	14.985	Di-sec-butyl phthalate	2.63%
39	14.985	1,2-Benzenedicarboxylic acid, monobutyl ester [monobutyl phthalate]	2.63%

Table 4: GC-MS analysis of the polyherbal formulation (contd.)

S/N	RT (min)	Compound Name	Concentration (%)
40	14.985	Didodecyl phthalate	2.63%
41	15.099	n-Hexadecanoic acid [palmitic acid]	12.15%
42	15.239	Undecanoic acid, ethyl ester	1.05%
43	15.239	Docosanoic acid, ethyl ester	1.05%
44	15.239	Hexanoic acid, ethyl ester	1.05%
45	16.385	Phytol	20.81%
46	16.385	Cyclohexanol,5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.beta.)-	20.81%
47	16.728	9,12,15-Octadecatrien-1-ol [linolenyl alcohol]	8.45%
48	16.728	9,12,15-Octadecatrienal [linolenal]	8.45%
49	16.728	(Z)6,(Z)9-Pentadecadien-1-ol	8.45%
50	16.873	Cyclododecyne	2.18%
51	16.873	Cyclooctene, 3-ethenyl-	2.18%
52	16.873	Cyclopentaneundecanoic acid	2.18%
53	17.325	Neophytadiene	20.21%
54	17.325	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	20.21%
55	20.770	Di-n-octyl phthalate	9.71%
56	20.770	Bis(2-ethylhexyl) phthalate	9.71%

*Effect of the polyherbal extract on liver histology in streptozotocin-induced diabetic male Wistar rat***:** The induction of Streptozotocin and administration of the polyherbal formulation at different doses had no effects on hepatic tissues, as shown in Plate 1

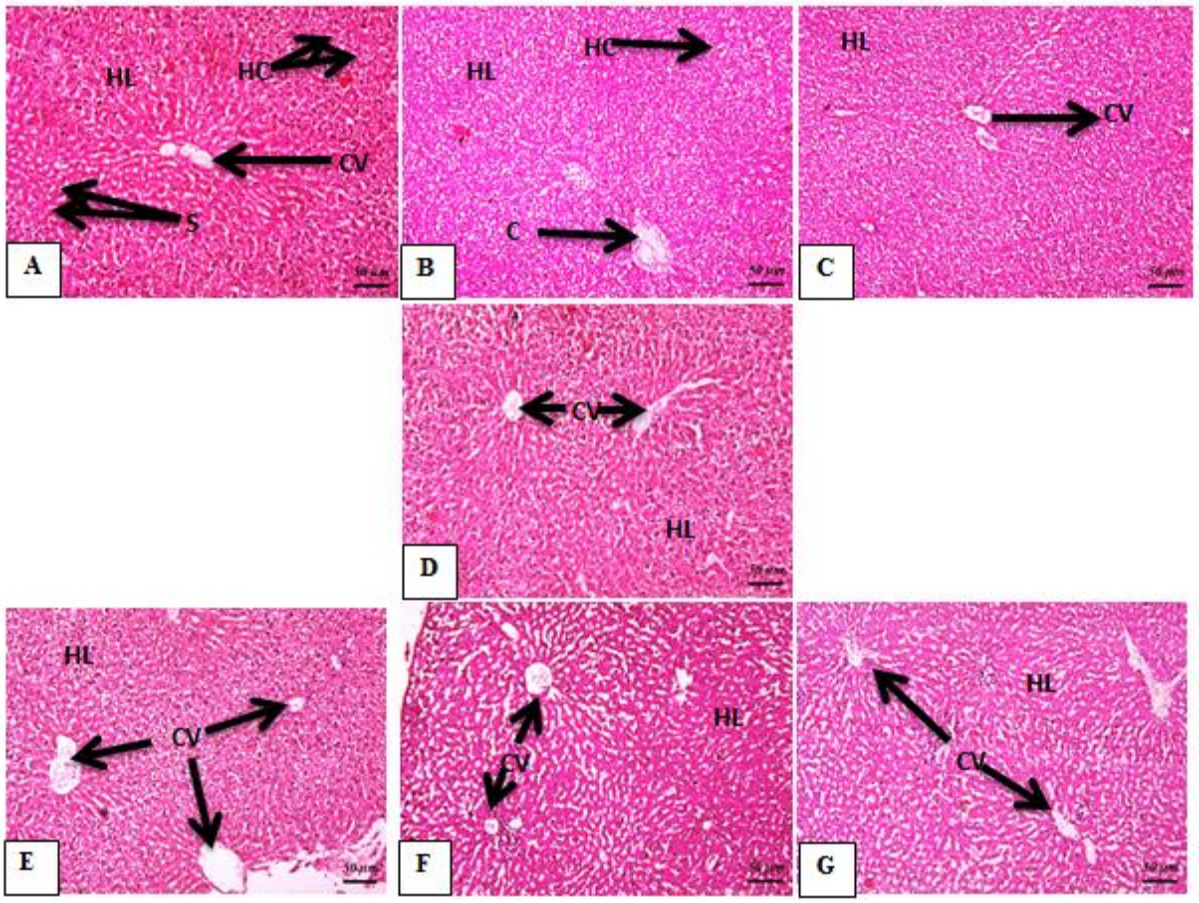

Plate 1: Effects of a polyherbal formulation on hepatic tissues of streptozotocin-induced diabetic male Wistar rats. **Keys:** CV – central vein, HL – hepatic lobule, HC – Hepatocytes, S – Sinusoids. **A-Normal control:** The section of the Liver shows features of normal liver tissue; **B-Diabetic control:** The section of the Liver shows features of normal liver tissue; **C-100 mg/kg:** The section of the Liver shows features of normal liver tissue; **D-150mg/kg:** The section of the Liver shows features of a normal liver tissue; **E-200mg/kg:** The section of the

Liver shows features of a normal liver tissue; **F-250mg/kg:** The section of the Liver shows features of a normal liver tissue and **G-Positive control:** The section of the Liver shows features of a normal liver tissue

*Effect of the polyherbal extract on histology of the pancreas in streptozotocin-induced diabetic male Wistar rats***:** The induction of Streptozotocin and administration of the polyherbal formulation at different doses caused pancreatic islet hyperplasia in pancreatic tissues, as shown in Plate 2

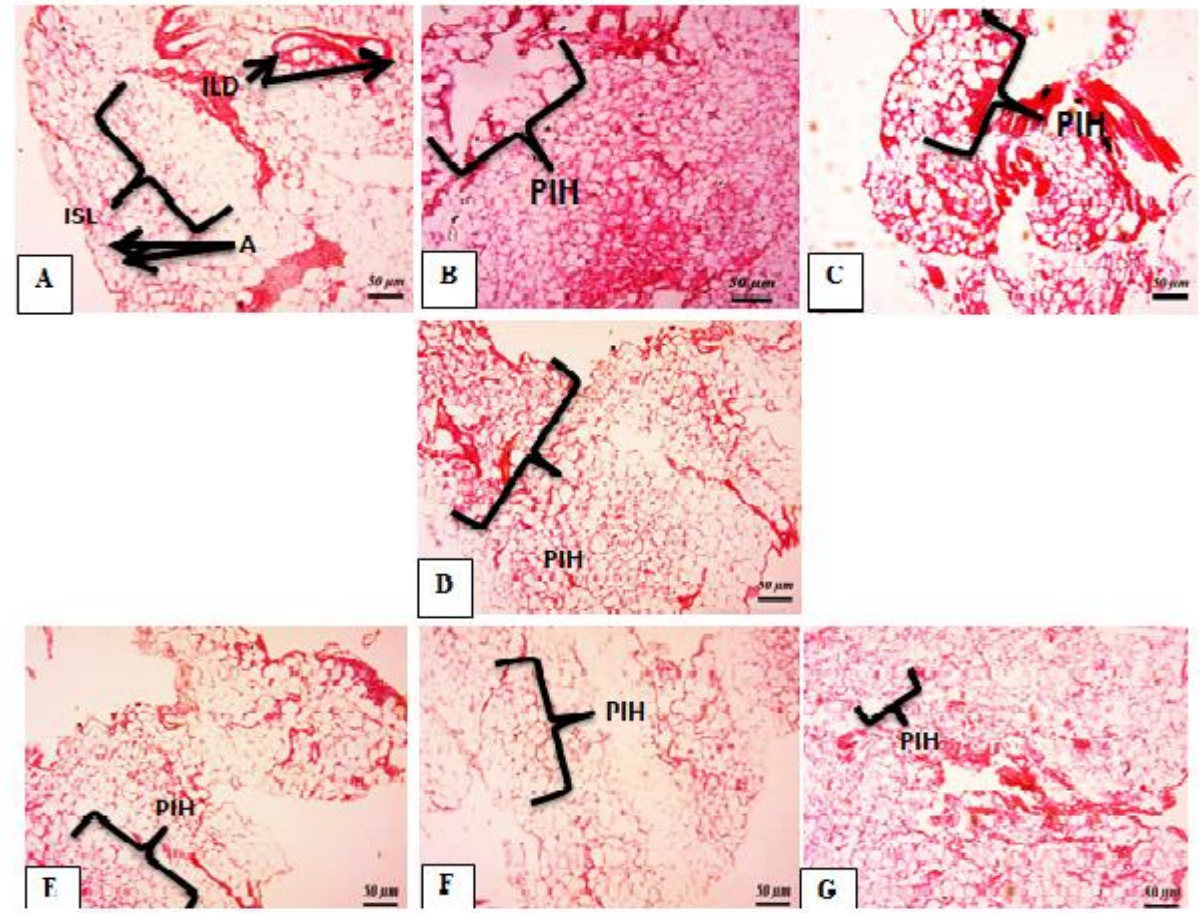

Plate 2: Effects of a polyherbal formulation on pancreatic tissues of streptozotocin-induced diabetic male Wistar rats. **Keys:** A – acini, ILD – interlobular duct, ISL – Islet of Langerhans. **A-Normal control:** The section of the pancreas shows features of normal pancreatic tissue; **B-Diabetic control:** The section of the pancreas shows features of Pancreatic Islet Hyperplasia; **C- 100mg/kg:** The section of the pancreas shows features of Pancreatic Islet Hyperplasia; **D- 150mg/kg:** The section of the pancreas shows features of Pancreatic Islet Hyperplasia; **E-200mg/kg:** The section of the pancreas shows features of Pancreatic Islet Hyperplasia; **F-250mg/kg:** The section of the pancreas shows features of Pancreatic Islet Hyperplasia and **G-Positive control:** The section of the pancreas shows features of Pancreatic Islet Hyperplasia

Discussion

The persistent hyperglycemia observed in the diabetic control group confirms the successful induction of diabetes through the destruction of pancreatic β-cells, aligning with established streptozotocin models (Szkudelski, 2001). The ability of higher doses of the polyherbal extract to significantly reduce blood glucose levels suggests potential mechanisms such as improved utilisation of peripheral glucose, improved insulin sensitivity, or possible regeneration of residual pancreatic β-cells. These findings align with previous reports on the potential of herbal formulations to significantly reduce blood glucose (Patel *et al*., 2012; Kooti *et al*., 2016).

Alterations in the weights of internal organs are widely regarded as sensitive and reliable indices for assessing systemic toxicity following exposure to xenobiotic substances (Tofovic and Jackson, 1999; Teo *et al*., 2002). Variations in organ weight serve as important morphometric indicators of pathological changes. Also, an increase in relative organ weight may reflect inflammatory processes, hypertrophy, or congestion, whereas a

decrease may suggest cellular constriction, atrophy, or degenerative changes within the affected tissue (Moore and Dalley, 1999; Erhabor *et al*., 2023). Analysis of organ weights showed significant treatment-related effects on the liver and pancreas (Table 2). The diabetic control group showed alterations in both liver and pancreatic weights, reflecting metabolic disturbances associated with chronic hyperglycemia. Treatment with the polyherbal formulation, particularly at doses of 150–250 mg/kg, restored these weights toward normal values, suggesting protective effects on both organs. The increase in liver weight observed at 150 mg/kg may indicate enhanced hepatic glycogen storage, while the preservation of pancreatic weight at 200 mg/kg could reflect protection against diabetes-induced pancreatic damage. The differences observed between the polyherbal formulation and Metformin in their effects on liver and pancreatic weights may point to distinct mechanisms of action. While Metformin primarily reduces hepatic glucose production (Foretz *et al*., 2014), the polyherbal formulation appears to exert broader metabolic effects, including antioxidant and anti-inflammatory activities that protect hepatic and pancreatic tissues. This multifactorial action may explain the superior glucose-lowering effect observed at the 250 mg/kg dose compared to Metformin in this study.

The biochemical assay showed significant effects of the polyherbal formulation on a liver function parameter in diabetic rats (Table 3). The effects of the polyherbal formulation on total bilirubin levels were also dose-dependent. Although the diabetic control showed elevated bilirubin (0.94 ± 0.33 mg/dL), which may indicate hepatic stress, the 150 mg/kg and 250 mg/kg doses reached levels below or near the normal range (0.06 ± 0.06 and 0.20 ± 0.11 mg/dL, respectively). This suggests potential hepatoprotective effects at these doses. This correlates with the observed normalisation of liver weights and supports previous studies on the hepato-protective properties of herbal preparations rich in flavonoids (Modak *et al*., 2007).

GC–MS analysis showed that the formulation contains a diverse range of bioactive compounds with strong therapeutic potential (Table 4). Major constituents included terpenoids such as phytol (20.81%) and neophytadiene (20.21%), which are known for their antioxidant and anti-inflammatory activities and may reduce oxidative stress (Gutiérrez-Grijalva *et al*., 2020; Rahman *et al*., 2022; Câmara *et al*., 2024; Rajeswaran and Rajan, 2025). The presence of palmitic acid (12.15%) suggests a possible role in lipid metabolism. Although high levels of this fatty acid have been linked to metabolic dysfunction, its effects here may be moderated by other bioactive compounds in the formulation (Palmieri *et al*., 2021; Yang *et al*., 2021). Phenolic compounds, such as 2,4-ditert-butylphenol (1.11%), further contribute to antioxidant and protective effects (Kumar and Pandey, 2013). The combination of multiple terpenoids, including farnesol (1.55%), which has insulin-sensitising properties, suggests synergistic interactions that may explain the optimal therapeutic effects observed at moderate doses (150–250 mg/kg) without toxicity, supporting the advantage of polyherbal formulations over single-plant extracts (Efferth and Koch, 2011; Moon *et al*., 2020).

The histological evaluation in this study revealed that the liver exhibited normal histology in all groups, including diabetic controls and treated rats, suggesting that STZ-induced diabetes and the polyherbal treatment did not induce hepatotoxicity. This aligns with studies indicating that STZ primarily affects pancreatic β-cells without significant hepatic damage (Eleazu *et al*., 2013). The absence of liver pathology in the treated groups further supports the safety of the polyherbal formulation at the administered doses with respect to hepatic function. The pancreatic histology across all treatment groups showed persistent islet cell hyperplasia, indicating a compensatory response to insulin deficiency. This finding contrasts with studies where certain herbal treatments promoted β-cell regeneration (Eidi *et al*., 2016). The inability of β-cells to recover in this study suggests that the polyherbal formulation may not sufficiently counteract STZ-induced β-cell destruction, unlike some documented antidiabetic herbs such as *Gymnema sylvestre*, which has been shown to enhance β-cell proliferation (Pothuraju *et al*., 2014). However, this may also be caused by the short duration of the experiment.

Conclusion

This study showed that a polyherbal formulation comprising *Garcinia kola*, *Buchholzia coriacea*, and *Picralima nitida* has significant antihyperglycemic and hepatoprotective effects in streptozotocin-induced diabetic male Wistar rats. It is recommended that further studies be conducted to determine the long-term effects of administering the tri-herbal extract across various models.

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