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Research Article

Polyherbal Formulation as A Potential Treatment for Type 2 Diabetes: An Experimental Study in Streptozotocin - Nicotinamide Induced Diabetic in Rats

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ABSTRACT

Herbal medicines have been used traditionally to manage various diseases, including diabetes. Herbs such as *Camellia sinensis, Cinnamomum verum, Syzygium aromaticum, Curcuma longa, and Elettaria cardamomum* have been observed to have pharmacological properties. The main objective was to see if this polyherb composite can be an adjuvant therapy for the diabetic patient. We created a formulation that included multiple herbal components by combining individual ethanolic extracts of traditional medicinal herbs in a predefined ratio. HPTLC was carried out to identify the bioactive constituents present in the formulation. Type 2 diabetes mellitus (T2DM) was experimentally induced in male *Wistar* rats by administering STZ, 50 mg/kg, i.p., followed by nicotinamide 120 mg/kg, p.o. After confirmation of hyperglycemia, rats were treated orally with the polyherbal formulation (250 and 500 mg/kg) and glibenclamide (0.25 mg/kg) for 21 days. At the end of the treatment, body weight, serum insulin, and pancreatic tissue were obtained for analysis of antioxidants, inflammatory markers, and histopathological examination. HPTLC indicates the presence of stable and the most dominant bioactive constituents in the polyherbal extract. The post-treatment body weight was statistically not changed within the groups, except in the STZ control (p <0.05) group. There was a significant change in blood glucose and serum insulin level in the STZ control group (p <0.01) and a reduction in the level of glucose and a rise in insulin levels after administration of the test formulation and glibenclamide (p <0.05, p <0.01). The concentration linked to the antioxidant enzyme GPx showed a marked elevation in the test formulation (p <0.05) and lower LPO levels. Increased levels of TNF-α and IL-6 were reported in the STZ control group (p <0.001) but were significantly lower after administration of the formulation (p <0.05, p <0.01). Pancreatic tissue showed well-organized, significant restoration of pancreatic structure, and islet integrity was observed. The polyherbal formulation showed a significant reduction in hyperglycemia and antioxidant and anti-inflammatory effects, supporting its potential as a complementary therapy for diabetes management.

Introduction

Diabetes mellitus is a metabolic disease marked by persistent hyperglycemia, resulting from abnormal glucose regulation. These elevated levels may be due to failure by the body to produce adequate or even no insulin, tissue insensitivity to insulin, or dysfunction in the β-cells of the pancreas. This disorder may result in various complications, such as CVS disorders, neuropathy, and renal impairment. Effective management typically includes lifestyle modifications, pharmacological interventions, and routine monitoring of blood glucose to maintain optimal health.[1] The global percentage of people having diabetes has been projected as The global prevalence of diabetes was estimated at 9.3% (463 million people) in 2019, projected to rise to 10.2% (578 million) by 2030, and 10.9% (700 million) by 2045. The condition is more common in urban populations compared to rural ones (10.8 vs. 7.2%) and in high-income countries relative

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to low-income countries (10.4 vs. 4.0%). Unfortunately, half of the T2DM patients do not realize that they are the casualties of the disease. [2] The complications of Type 2 diabetes mellitus include the dysfunction of various crucial organs that are found throughout the entire body, essentially the kidneys, cardiovascular system, and retina. Other new developments that take place as peripheral diseases due to diabetes include liver fibrosis and lung fibrosis with cognitive dysfunction.[3] There are changes in lifestyle that are associated with poor lifestyles in terms of consumption of poor diets and inactivity, which result in high BMI and fasting. Specifically, individuals with high BMI are also susceptible to developing type 2 diabetes. [4] Diabetes Mellitus is a leading cause of morbidity, disability, and mortality across the globe. The disorder also results in considerable economic impact, with high direct medical expenses and indirect costs such as lost productivity. [5]

Polyherbal Formulations in Type 2 Diabetes Mellitus

The polyherbal formulation, *Camellia sinesis* (green tea), belongs to the Theaceae family and has the phytoconstituents catechins, flavonoids, and phenolic acid, and it has antidiabetic, antioxidant, and anticancer effects.[6] *Cinnamomum zeylanicum* (Ceylon cinnamon, Lauraceae family), cinnamon, a natural product, is rich in polyphenolic components that have been shown to improve antioxidant activities.[7] Its main components are cinnamaldehyde, cinnamic acid, and tannin. It possesses a significant impact in antidiabetic, anti-allergic, antipyretic, and antioxidant properties.[8] *Syzygium aromaticum* is a rich source of phenolic compounds, that is, flavonoids and terpenoids, and the pharmacological properties of it have been documented to possess antiseptic, antioxidant, anti-inflammatory, antifungal, antiviral, and antiparasitic activities. [9] *Curcuma longa* rhizomes of turmeric (Zingiberaceae family), mentioned in Ayurvedic and Chinese traditional medicine for thousands of years in the treatment of diabetes. Its primary bioactive compound, curcumin, is well recognized for its health-promoting properties. Rhizome curcumin extract of turmeric has been reported to have anticancer, anti-inflammatory, and antidiabetic effects.[10] *Elettaria cardamomum,* a plant of the Zingiberaceae family, has long been utilized in traditional medicine systems, particularly Ayurveda. The therapeutic action is antioxidant, anticancer, and antidiabetic. Cardamom reduced blood pressure, glycemic indices, and serum cholesterol levels in a different investigation.[11]

Herbal remedies offer a potential approach to mitigate the adverse effects associated with synthetic drugs. Several medicinal plants have been traditionally employed in managing diabetes. Bioactive compounds form the fundamental core of modern medications, especially in the rural setting, as they are easily accessible, have minimal side effects, and are cost-effective.[12] Due to the rising trends in the worldwide incidence of diabetes mellitus, there has been an increase in demand for safer and more effective alternatives to synthetic medication, with many of the services resulting in unwanted side effects. The potential solution with medicinal plants is possible because of the antidiabetic, antioxidant, and anti-inflammatory activity of their bioactive compounds. Despite extensive documentation of the individual antidiabetic, antioxidant, and anti-inflammatory properties of *C. sinensis, C. verum, S. aromaticum, C. longa, and E. cardamomum*, limited evidence exists regarding their combined efficacy, standardized formulation, and mechanistic evaluation in diabetes management. This study addresses this gap by investigating a polyherbal combination, with emphasis on its synergistic effects, phytochemical standardization (HPTLC), and mechanistic insights, thereby contributing novel evidence to the field of herbal therapeutics. The strategy is consistent with the pharmacotherapeutic objectives of both safety and efficacy, but is also industrially attractive as a source of scalable, natural, and marketable diabetes therapies.

Materials And Methods

Procurement and Authentication of Plant Specimens

The materials of herbs such as *C. sinensis (leaves)*, bark of *C. verum (bark)*, *S. aromaticum* (clove buds), *C. longa* (rhizomes), and *E. cardamomum* (seeds) were procured in March 2025 from Bangalore, Karnataka, India. The central Ayurveda research institute of Bangalore authenticated and identified the material of the herb. Reg. no. Authentication/SMPU/CARI/BAG/2025-26/350.

Extraction and Preparation of Herbs

The herbs were dried in the shade under warm room conditions to maintain their phytoconstituents. A mixing grinder was used to coarsely powder the dried materials. The 99% ethanol was used in the Soxhlet extraction of 3 successive samples at 2 hours each. The extracts obtained were subsequently concentrated and dried under room temperature conditions to be prepared into a formulation.

Polyherbal Mixture Formulation

The ethanolic extract of the five herbs was mixed in a certain proportion of a 2:2:1:1:1 ratio of *C. sinensis, C. verum, S. aromaticum, C. longa, and E. cardamomum*, respectively, according to their historical usage of these five herbs as well as the reported antidiabetic effects.

Chromatographic Fingerprint by HPTLC

A 3.0 μL sample of the methanolic extract was spotted on HPTLC pre-coated plates with a stationary phase of silica gel 60 F 254. Toluene, ethyl acetate, and methanol were used as the mobile phase in a proportional ratio of 4:4:1 (v/v/v) for the development. The pattern of development of the plate was in an ascending manner, employing a twin-trough glass chamber, which was pre-saturated with the mobile phase. The plates were allowed to dry after development and thereafter placed under a UV light of 254 nm to see whether there was any chromatographic peak. It was detected in absorbance mode, after which a chromatographic fingerprint map was recorded and documented to determine the occurrence of various phytoconstituents.[13]

Dose Selection and Acute Toxicity

The acute oral safety profile of the polyherbal formulation was assessed according to OECD Guideline No. 425 (Up-and-Down Procedure). No mortality or signs of toxicity were observed at a Maximum Tolerated Dose (MTD) of 5000 mg/kg body weight, indicating a high safety margin. Based on these findings, two doses: one-tenth (500 mg/kg, b.w.) and one-twentieth (250 mg/kg, b.w.) of the MTD were selected for subsequent oral administration in the efficacy study.

T2DM induction in experimental animals

Rats were induced to develop T2DM in accordance with the procedure published by *Aboonabi et al*. (2014) with slight modifications. Nicotinamide (120 mg/kg, p.o.), diluted in normal saline, was used once daily seven days before and three days after the administration of STZ, 50 mg/kg, i.p., which was prepared just prior to use in 0.05M citrate buffer (pH 4.5).[14] Hyperglycemia was confirmed through the measurement of fasting blood glucose values prior to and 72 hours after STZ injection, whereby significantly high levels of glucose were noted as indicative of the development of T2DM.

Experimental Design and Grouping

In this research, male Wistar rats (150–200 g, 8–10 weeks) were employed. Before the experiment took place, the animals were held in adaptations and adjustments. All the experimentation activities were conducted according to the CPCSEA, Government of India guidelines. All due administrative procedures were cleared in the IAEC of Karnataka College of Pharmacy, in reference number KCP-IAEC/16/24-25/02/10/03/25.

Group 1 Normal control: Received 2 mL/kg, p.o., normal saline every day over 21 days

Group 2 diabetic Control: Induced with Nicotinamide (120 mg/kg, orally), 7 days prior and 3 days consecutive with STZ, 50 mg/kg, i.p.

Group 3 Standard Drug Group: Received orally glibenclamide (0.25 mg/kg) daily over a period of 21 days upon the confirmation of T2DM.

Group 4 Polyherbal formulation (250 mg/kg, orally) daily over a period of 21 days.

Group 5 Polyherbal formulation (500 mg/kg, orally) daily over a period of 21 days.

At study completion, the animals were recorded for body weight and fasting glucose levels. Subsequently, the animals were sacrificed to collect blood samples for serum insulin estimation using an ELISA kit.[15] The pancreas was dissected out and divided into portions: one part was homogenized for the biochemical analysis of oxidative stress markers (GPx, LPO)[16,17] and inflammatory cytokines (IL-6, TNF-α)[18], while the other part was fixed in 10% formalin for histopathological examination using H&E staining.[19]

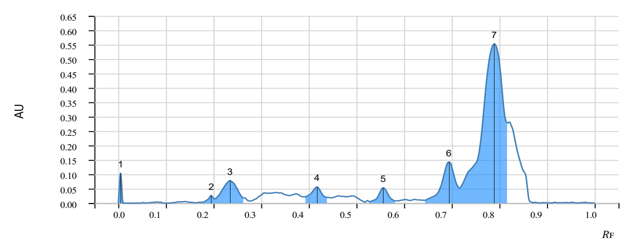
Data Analysis

The results were given in Mean +/- S.E.M. with n = 8 rats per group. All experimental data were subjected to statistical evaluation using GraphPad Prism software version 10.1.1. The importance of each variation among the groups was determined using ANOVA with the addition of the Tukey post hoc test. The differences between the normal control (untreated) group and all other groups were tested, and $p < 0.05$ was determined to be significant.

Results

Chromatographic Fingerprint Map

Several distinct peaks were consistently observed at specific Rf values in the TLC chromatogram under 254 nm, indicating the presence of stable phytochemical constituents across the samples. Remarkably, there were main peaks at the Rf range 0.60–0.70, and they had a higher %area (Figs 1 and 2), indicating that they are the



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.005	0.1037	10.23	0.010	0.0000	0.00055	1.34
2	0.167	0.0019	0.195	0.0261	2.58	0.202	0.0152	0.00039	0.95
3	0.202	0.0152	0.234	0.0780	7.70	0.263	0.0178	0.00292	7.18
4	0.393	0.0214	0.417	0.0561	5.53	0.438	0.0198	0.00162	3.97
5	0.527	0.0047	0.556	0.0534	5.27	0.580	0.0076	0.00133	3.27
6	0.644	0.0097	0.694	0.1429	14.09	0.716	0.0528	0.00477	11.71
7	0.716	0.0528	0.789	0.5536	54.61	0.817	0.2781	0.02912	71.57

Fig. 1: Integration parameters and Area calibration for Polyherbal Substance

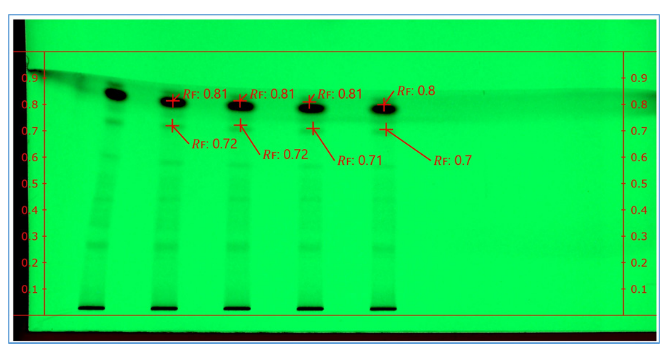


Fig. 2: TLC Visualize for Polyherbal Substance.

most dominant bioactive constituents in the extract. A very intense and strongly fluorescing peak near Rf 0.70 was noted, probably representing a main compound with a high degree of conjugation, e.g., flavonoids, coumarins, and other phenolic constituents. Also, peaks in the Rf range of 0.60–0.65 and out of the range of Rf 0.80 are probably secondary metabolites or possible degradation products due to extraction or processing. The correlation Table 01 HPTLC spectrum vs. phytochemicals present in the formulation of polyherbal reflects Gallic acid, EGCG, Curcumin, Flavonoids, and many more.

Primary Endpoints

In terms of body weight, the statistical analysis revealed no significant difference (^{ns}p >0.05) among the groups prior to treatment, as expected. Following treatment, A notable change was observed only in the disease (STZ+NA) control group ($^{*}p$ <0.05), while the remaining test groups, although showing some variations, did not exhibit statistically significant differences (^{ns}p >0.05). Fig. 3. For blood glucose levels, a highly significant difference was noted between the disease (STZ+NA) group and the normal saline (Healthy animals) group ($^{\#\#}p$ <0.01). Treatment with various test drugs, including the standard drug glibenclamide, resulted in significant improvements compared to the disease (STZ+NA) control group, as indicated by p <0.05 and *p <0.01 at dose 500 mg/kg, the polyherbal formulation in Figure 4. Regarding serum insulin levels, a significant reduction was recognized in the disease (STZ+NA) control group with respect to the normal control, saline (p <0.01). Test groups, including those treated with glibenclamide, led to a marked restoration of circulating insulin concentrations (*p <0.05, **p <0.01 polyherbal formulation at a dose of 500 mg/kg) with respect to the disease (STZ+NA) control group (Figure 5).

Secondary Endpoint

For oxidative stress markers, glutathione peroxidase (GPx) levels were reduced in the disease (STZ+NA) control group compared to the normal healthy control group (p <0.05). No statistically significant changes (^{ns}p >0.05) were observed in the 250 mg/kg polyherbal composite and the standard drug group, while the 500 mg/kg dose test group exhibited a significant improvement (*p <0.05) compared to the disease (STZ+NA) control group. Figure 6. Lipid peroxidation (LPO) levels were increased in the

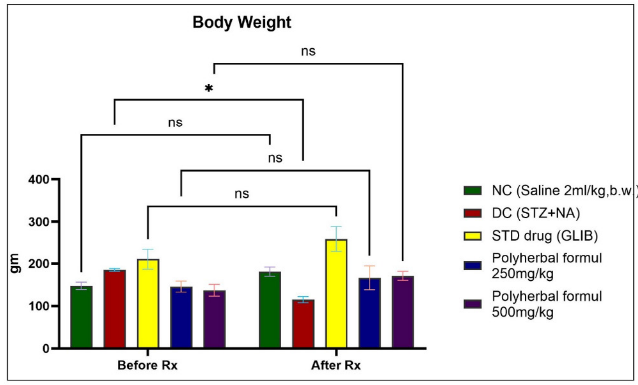


Fig. 3: Graphical representation of body weight changes in STZ+NA-induced T2DM in rats, measured before and after treatment. Data are presented as mean ± SEM (n = 8 rats per group). ^{ns}p >0.05 indicates a statistically ns (not significant) difference within the groups, except *p <0.05 (Post Rx, STZ + NA).

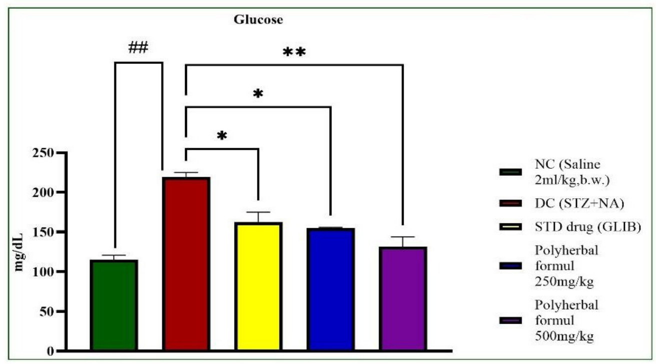


Fig. 4: Graphical representation of blood glucose levels in STZ+NA-induced T2DM in rats. Data are presented as mean ± SEM (n = 8 rats per group). ##p <0.01 compared to normal control. *p <0.05, **p <0.01 compared to the disease (STZ+NA) control vs different test drugs including standard drug.

disease (STZ+NA) control group when compared to the normal healthy animals (p >0.05); treatment with test drugs and the standard drug significantly reduced LPO levels (*p <0.05, **p <0.01 at a dose of 500 mg/kg of the polyherbal formulation) (Figure 7).

Inflammatory Markers

Increased TNF-α was observed in the disease (STZ+NA) control group with respect to the normal control ($^{\#\#\#}p$ <0.001), indicating inflammation. Treatment with test

Table 1: Correlation Table: HPTLC wPeaks vs. Phytochemicals in Polyherbal Formulation

Rf Value (approx.)	*Probable Phytochemical(s)*	*Plant Source(s)*
0.20–0.25	Gallic acid	*Camellia sinensis*, *Syzygium aromaticum*
0.39–0.45	Eugenol, EGCG	*Syzygium aromaticum*, *Camellia sinensis*
0.60–0.63	Cinnamaldehyde, quercetin	*Cinnamomum verum*, *Elettaria cardamomum*
0.68–0.75	Curcumin, Demethoxycurcumin	*Curcuma longa*
0.80–0.83	Flavonoids (e.g., kaempferol, quercetin)	*Camellia sinensis*, *Elettaria cardamomum*

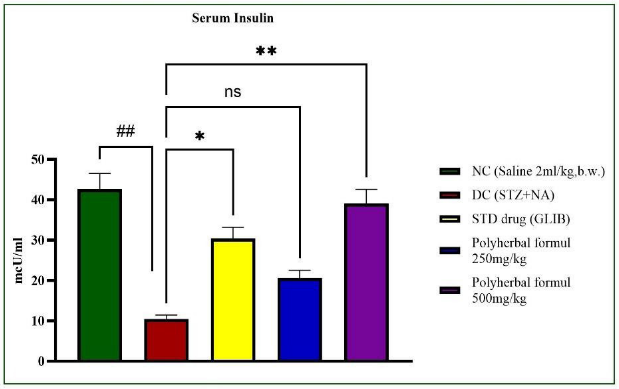


Figure 5: Graphical representation of Serum Insulin levels in STZ+NA-induced T2DM in rats. Data are presented as mean ± SEM (n = 8 rats per group). ##*p* >0.01 compared to the normal healthy animals. ns*p* >0.05, **p* <0.05, and ***p* <0.01 compared to the disease (STZ+NA) control group

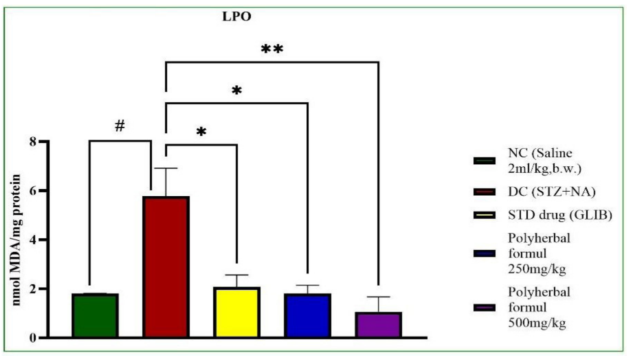


Figure 6: Graphical representation of GPx levels in STZ+NA-induced T2DM in rats. Data are presented as mean ± SEM (n = 8 rats per group). #*p* <0.05 compared to the normal healthy animals. ns*p* >0.05 and **p* <0.05 compared to the disease (STZ+NA) control.

drugs and the standard drug significantly reduced TNF-α levels (*p <0.05, **p <0.01), Figure 8. Similarly, interleukin-6 (IL-6) levels showed a significant elevation in the disease (STZ+NA) control group compared to the normal healthy animals (###p <0.001), which was significantly attenuated in the polyherbal tested groups (*p <0.05, **p <0.01), indicating the anti-inflammatory potential of the test drugs Figure 9.

Histopathological analysis of pancreatic tissue

The normal tissue (Figure 10A) shows acinar cells. Clearly visible around the islet, showing the typical pyramidal shape and apical acidophilia.

Islet of langerhans

Central, pale-stained cluster indicating the endocrine portion. Beta cells (~70% of islet cells), alpha cells (~20% of islet cells). In diseased tissue (STZ+NA, without treatment) (Figure 10B), acinar cells are present, arranged in clusters, with some disorganization.

Apical acidophilic cytoplasm

Less prominent due to enzyme loss and cell stress.

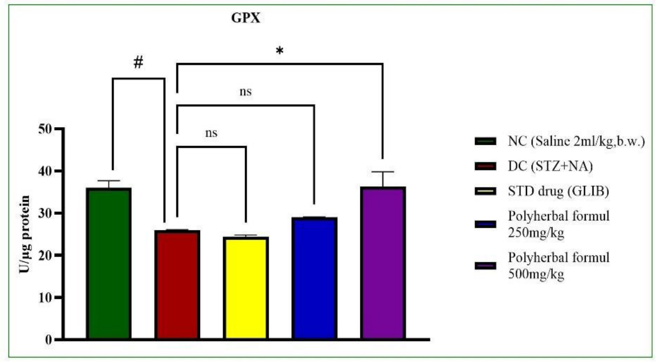


Fig. 7: Graphical representation of LPO levels in STZ+NA-induced T2DM in rats. Data are presented as mean ± SEM (n = 8 rats per group). #*p* >0.05 compared to the normal healthy animals. **p* <0.05 and ***p* <0.01 compared to the disease (STZ+NA) control group

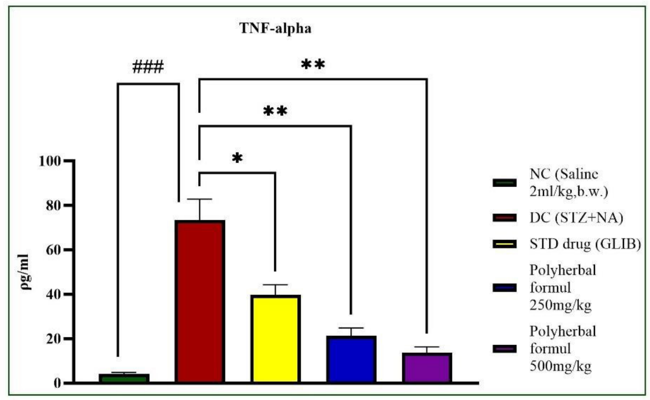


Fig. 8: Graphical representation of TNF-alpha in STZ+NA-induced T2DM in rats. Data are presented as mean ± SEM (n = 8 rats per group). ###*p* <0.001 compared to the normal healthy animals. **p* <0.05 and ***p* <0.01 compared to the disease (STZ+NA) control

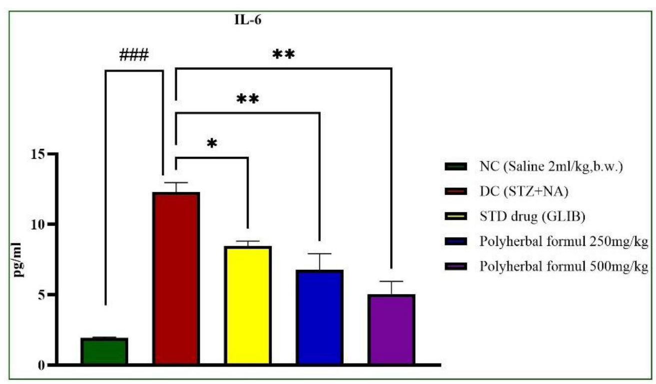


Fig. 9: Graphical representation of IL-6 in STZ+NA-induced T2DM in rats. Data are presented as mean ± SEM (n = 8 rats per group). ###*p* >0.001 compared to the normal healthy animals. **p* <0.05 and ***p* <0.01 compared to the disease (STZ+NA) control group

Acinar arrangement

Still visible but mildly disrupted. Islets of Langerhans are shrunken and marked by a reduction.

In the standard drug (Figure 10C), acinar cells are better preserved. Columnar epithelial arrangement and apical acidophilic cytoplasm are more evident.

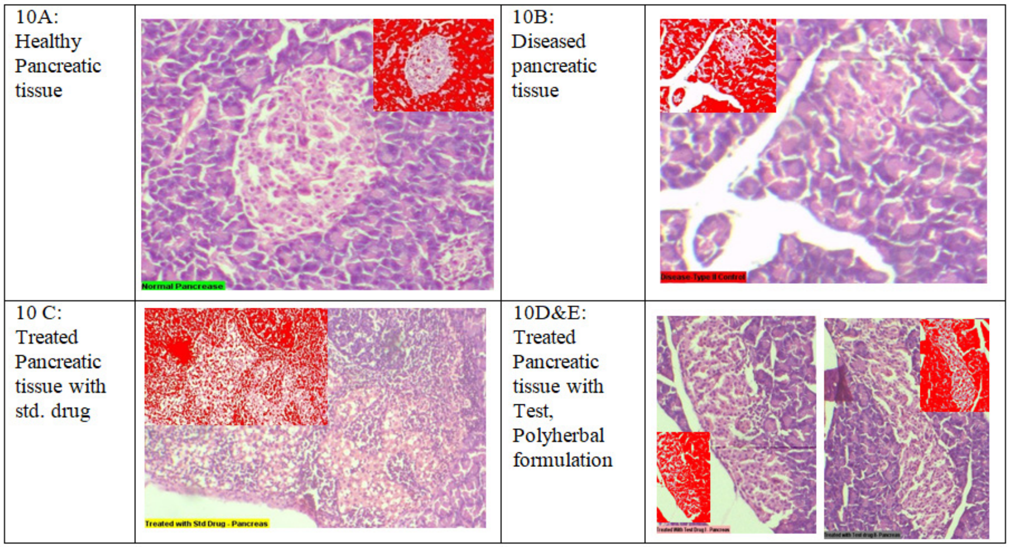


Fig. 10: Pancreatic tissue histological assessment. 10A: Normal control, 10B: Disease control (STZ+NA), 10C: Std. Drug treatment, and 10D&E: Polyherbal formulation 250 and 500 mg/kg

Acinar arrangement

Well-organized clusters indicating preserved enzyme-secreting structures. Islets are more prominent, suggesting beta cells appear repopulated (central), implying restored insulin production. At a dose of 250 mg/kg of polyherbal formulation (Figure 10D), acinar cells are well-formed with columnar epithelial lining and distinct apical acidophilic cytoplasm.

Acinar arrangement

Preserved and neatly packed, suggesting minimal tissue damage. Islets are clearly visible, centrally located with a dense beta cell population. Alpha cells are likely at the islet periphery. Overall architecture resembles near-normal pancreatic histology, suggesting effective protection. At a dose of 500 mg/kg of polyherbal formulation (Figure 10E), well-organized clusters as shown in the image, show significant restoration.

Discussion

HPTLC indicates the presence of stable phytochemical constituents across the samples. Remarkably, there were main peaks at the Rf range 0.60–0.70, and they had a higher % area, indicating that they are the most dominant bioactive constituents in the extract, such as flavonoids, coumarins, and phenolic constituents. The study found that the weight of the body was measured before and after treatment. In the pre-treatment stage, there was no difference (ns); however, between the groups after treatment, which means that the treatments, i.e., the standard drug and two doses of the polyherbal formulation, prevented further gain of weight but statistically not significant changes. This is true with the decrease in weight gain, which has been attributed to a decrease in adipose tissue and food intake, together with an increase in lipolysis. Based on the explanation in the study, the diabetic control rat group exhibited extremely high blood glucose levels compared to those of the normal control rats, thus confirming hyperglycemia resulting in diabetes induction in the test group. The most significant level of glucose was seen in the DC (STZ+NA) group (which is impressively higher than the value demonstrated by the NC group, which does not exceed 150 mg/dL). Administering the normal drug and both doses of the polyherbal drug (250 and 500 mg/kg) significantly decreased the level of blood glucose. The higher dose of polyherbal product (500 mg/kg) appears to reduce glucose levels reasonably near to the NC group, unlike the comparable standard drug. Therefore, the polysaccharide superperfect extract presented in the paper (Liu *et al.,* 2022) has not resulted in significant amelioration of diabetes, but the polyherbal formulation portrayed in the graph works efficiently in decreasing levels of glucose in diabetic rats, exhibiting efficacy against diabetes[20] The DC (STZ+NA) group showed a significant reduction in the level of insulin in serum, thus confirming the beta-cell reduction or resistance to producing enough insulin commonly reported in type 2 diabetes. There was a significant increase in insulin level after administration of the standard drug glibenclamide as compared to the DC (STZ+NA), in line with the insulinotropic effects of this standard drug. The 500 mg/kg dose of polyherbal-treated groups exhibited a significant improvement in insulin level bordering on the non-diabetic control group, whereas the 250 mg/kg dose showed a moderate improvement. Such observations confirm the previous discoveries by Sayeli and Shenoy (2021) that bio-enhanced turmeric extract beneficially affected serum insulin and beta-cell functions better than non-enhanced forms. In general, the conclusion is that polyherbal formulations used in higher doses can help to improve the beta-cell stimulation of the pancreatic tissue and production of insulin, which can be of therapeutic help to diabetes management as compared to standard medicines. [21] The diabetic control group showed significantly lower activity of GPX as compared to the normal control, which depicts the high level of oxidative stress normally seen with diabetes-associated co-morbidities. The inability to reverse GPX activity to normal was observed in the standard drug, such as glibenclamide, with no significant differences compared to the DC (STZ+NA). On the other hand, a polyherbal formulation dosage of 500 mg/kg led to a remarkable rise in GPX activity, which was very near normal controls, as compared to a moderate but insignificant rise (500 mg/kg) dosage of 250 mg/kg. These results are in confirmation with what has been discussed above concerning Arabic gum treatment reported by Ahmed *et al. (*2024), in which the levels of antioxidant enzymes, including GPX, were highly renewed in the diabetic model, indicative of protection against oxidation. [22] As is known, hyperglycemia leads to overproduction of free radicals, which is accompanied by a significant increase in the concentrations of malondialdehyde (MDA) or LPO in diabetic (STZ+NA) control rats, which was reflected in the study. The DC (STZ+NA) group displays an abrupt increase in the MDA, whereas that of the normal control shows a baseline of anti-oxidative enzyme. The treatment with the standard drug GLIB and both doses of the polyherbal formulation (250 and 500 mg/kg) led to a significant level of reduction in MDA, which shows better antioxidant defense. It is observed that the elevated concentration of the polyherbal formulation (500 mg/kg) diluted MDA levels to the normal control, as mentioned in the paper (Aboonabi *et al.,* 2014), where pomegranate seed and pomegranate juice were used to diminish MDA levels. This implies that pomegranate and the polyherbal formulas have antioxidant effects on diabetic rats since they suppress lipid peroxidation. [23] The TNF-alpha levels in the diabetic group (STZ + NA) were significantly high, which indicates a high degree of inflammatory processes, characteristic of type 2 diabetes. Standard drug glibenclamide treatment was noted to have a significant decrease in the level of TNF-α, which showed that there was an anti-inflammatory and glycemic regulatory action. It was notwithstanding that both polyherbal doses also significantly lowered TNF-alpha, with the 500 mg/kg dose doing so the most significantly, equivalent to the standard drug and possibly better, indicating a dose-dependent anti-inflammatory action of the polyherbal formulation. The results obtained correspond with the preceding findings by Zulaikhah *et al.* (2021) concerning natural products of tender coconut water that also lowered the TNF-alpha expression in diabetic rats. Collectively, the evidence supports the contribution of inflammation to diabetes and the possibility of plant-endorsed therapies in altering TNF-alpha to provide treatment-related advantages in the treatment of T2DM inflammation. IL-6 is highly increased in diabetic control rats (using STZ+NA), and this is a confirmation of the systemic inflammation that is related to type 2 diabetes mellitus. This is in line with the discussion that which pointed out IL-6 as a major proinflammatory cytokine that causes insulin resistance and predisposes individuals to diabetes. The standard drug glibenclamide reflects a moderately decreasing IL-6 level, and both doses of the polyherbal formulation (250 and 500 mg/kg) observed a considerable decline in IL-6 concentrations, with the larger dose approximating normal values as found in the normal control group. The study results support the anti-inflammatory effect of polyherbal therapy in controlling diabetes and are effective in repressing the expression of IL-6.[24] Further, Normal pancreatic tissue exhibited well-structured acinar cells with a large number of islets of Langerhans. In the disease control group, acinar disorganization and shrunken islets were present. Standard drug treatment maintained acinar structure and enhanced the appearance of islets. With a 250 mg/kg polyherbal dose, the architecture of acinar tissue and islets was maintained, being very close to normal tissue. Potent protection was shown by significant recovery of pancreatic structure and islet integrity at 500 mg/kg of polyherbal formulation.

Although the antidiabetic potential of each herb is well established, the scientific rationale for their combination lies in the complementary pharmacological actions of their major bioactive constituents, such as catechins, cinnamaldehyde, eugenol, curcuminoids, and terpenes, which collectively target oxidative stress, inflammation, and insulin resistance. The specific ratio employed was designed to maximize these complementary effects, thereby offering potential synergistic activity rather than a mere additive outcome. Beyond the established effects, this formulation appears to modulate key biomarker profiling, including insulin sensitivity indices, biomarkers related to oxidative stress and proinflammatory signaling molecules, providing mechanistic insights that substantiate the enhanced efficacy of the polyherbal approach. The formulation demonstrated superior efficacy compared to single components, confirming a synergistic advantage. Such evidence underscores the importance of evaluating polyherbal combinations systematically, rather than extrapolating from individual herb effects. This quality control strategy enhances its translational potential for further preclinical and clinical development. Taken together, the study bridges a critical research gap by moving beyond the known antidiabetic activity of individual herbs and providing mechanistic, comparative, and standardization-based evidence for a novel polyherbal formulation.

Conclusion

The polyherbal formulation, administered at doses of 250 and 500 mg/kg body weight, exhibited pronounced antidiabetic activity in STZ- and nicotinamide-induced type 2 diabetic rats. Treatment effectively regulated body weight, lowered blood glucose levels, and increased circulating insulin concentrations. Moreover, the formulation demonstrated antioxidant and anti-inflammatory properties, as indicated by reductions in TNF-α and IL-6 levels. Histopathological evaluation showed preservation of pancreatic β-cells along with enhanced insulin production, corroborating the antidiabetic potential of the formulation. High-performance thin-layer chromatography (HPTLC) analysis confirmed the presence of bioactive constituents, which may underlie the observed therapeutic effects.

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Conflict of Interest

None.

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