FORMULATION AND IN- VIVO EVALUATION OF ANTI-DIABETIC ACTIVITY OF CORIANDRUM SATIVUM AND PUNICA GRANATUM EXTRACT IN STREPTOZOTOCIN INDUCED ALBINO WISTAR RATS

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Abstract

Diabetes mellitus is indeed a common endocrine disorder characterized by high blood sugar levels (hyperglycemia) due to problems with insulin production, insulin action, or both. It can lead to various complications and is a significant cause of morbidity and mortality worldwide. While traditional medicines from various cultures often suggest natural remedies for preventing and managing diabetes, it's important to approach these remedies with caution and in conjunction with evidence-based medical care. The present study aims to demonstrate the potential anti-diabetic effects of ethanolic extracts of *Punica granatum* and *Coriandrum sativum* in a rat model of STZ-induced diabetes and analyze the new polyherbal formulation. This type of research is often conducted to evaluate the potential therapeutic benefits of natural compounds on diabetes, and it typically involves various molecular and biochemical analyses. In this Group 4, treated with a specific ratio of the extracts, showed the most promising results, although glibenclamide remains the most effective treatment among all the groups tested. The therapeutic effectiveness in the test groups seems to be dosage-dependent. These findings provide valuable insights into the potential use of these plant extracts for managing diabetes.

Keywords antidiabetic, albino wistar rats, *Coriandrum sativum*, *Punica granatum*, streptozotocin, treatment

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Introduction

Diabetes mellitus (DM) is indeed a chronic metabolic disease characterized by elevated blood sugar levels, also known as hyperglycemia, in both postprandial (after eating) and fasting states. This condition results from defects in insulin secretion, insulin action, or a combination of both. Insulin is a hormone produced by the pancreas that plays a crucial role in regulating blood sugar levels by facilitating the uptake of glucose into cells for energy or storage. The prevalence of diabetes has been on the rise globally total number of diabetic patients worldwide was estimated to be 171 million in the year 2000 which indicates that by 2030, the number of people with diabetes is expected to increase significantly, reaching 366 million individuals. This increase in the number of individuals affected by diabetes is a major public health concern. It is primarily driven by factors such as lifestyle changes, including unhealthy diets and reduced physical activity, as well as genetic predisposition. Managing diabetes is essential to prevent complications and improve the quality of life for those affected by the condition. This typically involves medication, dietary modifications, regular physical activity, and monitoring blood sugar levels. Additionally, early diagnosis and education about diabetes prevention are crucial in addressing this global health challenge [1-6]. In the ancient Ayurveda system of medicine, which originated in India over 5,000 years ago, the pomegranate (*Punica granatum*) has been used extensively as a source of traditional remedies for various health purposes. Pomegranates are highly regarded in Ayurveda for their medicinal properties and have been incorporated into many Ayurvedic formulations and practices. *Punica granatum*, (family Puniacaceae) commonly known as pomegranate or anar, is indeed a plant that has a long history of traditional use for various medicinal purposes. Many of its parts, including the fruit, flowers, and seeds, have been utilized in traditional medicine for their potential health benefits against kidney-related issues, urinary problems, diarrhea, dysentery, cardio-related, digestive disorders, anemia, piles, and cough relief. Regarding the phytochemicals found in *P. granatum*, it is rich in various compounds, including hydrolyzable tannins (punicalagins and punicalins), condensed tannins, anthocyanins, phenolic compounds (gallic acid and ellagic acid), and organic acids (malic acid). These compounds are known for their antioxidant and anti-inflammatory properties, which have been associated with potential health benefits. However, it's important to note that while there is some scientific evidence supporting certain health-promoting effects of pomegranate, more research is needed to fully understand its mechanisms of action and to confirm its efficacy for specific medical conditions. As mentioned, pomegranate has gained popularity for its antioxidant properties, but consumers should be cautious about exaggerated health claims made by manufacturers and marketers of pomegranate products and seek advice from healthcare professionals for specific health concerns.[7-16]

Coriander (*Coriandrum sativum* L.) is indeed a versatile herb that belongs to the family Apiaceae (formerly known as Umbelliferae). It is widely used in culinary and medicinal applications due to its aromatic leaves and seeds. It is an annual herb that typically grows up to 50 centimeters (20 inches) in height, known for its rich aroma and essential oil content. including linalool and geranyl acetate, which contribute to its characteristic fragrance used in the fragrance industry as a cooking ingredient with biologically active components viz antioxidants, antibacterial, and antifungal compounds [17-22]. This type of research is often conducted to evaluate the potential therapeutic benefits of natural compounds on diabetes, and it typically involves various molecular and biochemical analyses. The primary goal of the study is likely to determine whether the alcoholic extracts of *Punica granatum* and *Coriandrum sativum* have any beneficial effects on diabetic rats at the molecular and biochemical levels.

Methodology

The yield of powdered whole-plant of *Punica granatum* and *Coriandrum sativum*, extracted using the Soxhlet technique by using ethanol as the solvent and the dark greyish brown ethanolic extract yielded 6.80% and 6.50%. From this yeild further study was done.

Punica granatum and *Coriandrum sativum*: A Toxicity Analysis

I. Acute oral toxicity study: According to OECD 423 ANNEX 2c guidelines, we tested for acute oral toxicity.

Study design: Three animals were selected for each group. Three polyherbal formulations is prepared by the ethanolic extract of *Punica granatum* and *Coriandrum sativum.*

Test sample A: (50:50) ethanolic extract of *Punica granatum* & *Coriandrum sativum*

Test sample B: (25:75) ethanolic extract of *Punica granatum* & *Coriandrum Sativum*
Test sample C: (75:25) ethanolic extract of *Punica granatum* & *Coriandrum sativum*
Group 1: 200 mg/kg (50:50) ethanolic extract of *P.granatum* & *C. sativum*
Group 2: 2000 mg/kg (50:50) ethanolic extract of *P.granatum* & *C. sativum.*
Group 3: 200 mg/kg (25:75) ethanolic extract of *P.granatum* & *C. sativum.*
Group 4: 2000 mg/kg (25:75) ethanolic extract of *P.granatum* & *C. sativum.*
Group 5: 200 mg/kg (75:25) ethanolic extract of *P.granatum* & *C. sativum*
Group 6: 2000 mg/kg (75:25) ethanolic extract of *P,granatum* and *C. sativum.*

II. Experimental Design for screening model *Streptozotacin*:

In this study, 48 mature albino wistar rats were divided into 12 groups of 6. The following are the ways in which these subsets were treated differently:
Group 1: Rats treated with Streptozotacin (60mg/kg) + 200 mg/kg (50:50) Ethanolic extract of *Punica granatum* & *Coriandrum sativum.*
Group 2: Rats treated with Streptozotacin (60mg/kg)+ 400 mg/kg (50:50) Ethanolic extract of *Punica granatum* & *Coriandrum sativum.*
Group 3: Rats treated with Streptozotacin (60mg/kg)+ 200 mg/kg (25:75) Ethanolic extract of *Punica granatum* & *Coriandrum sativum.*
Group 4: Rats treated with Streptozotacin (60mg/kg)+ 400 mg/kg (25:75) Ethanolic extract of *Punica granatum* and *Coriandrum sativum.*
Group 5: Rats treated with Streptozotacin (60mg/kg)+ 200 mg/kg (75:25) Ethanolic extract of *Punica granatum* and *Coriandrum sativum.*
Group 6: Rats treated with Streptozotacin (60mg/kg) + 400 mg/kg (75:25) Ethanolic extract of *Punica granatum* and *Coriandrum sativum.*
Group 7: Rats treated with Streptozotacin (60mg/kg) + Standard Drug Glibenclamide (10 mg/kg b.w)
Group 8: Rats treated with Streptozotacin (60mg/kg) + Normal Control (Normal saline 5ml/kg)

III. Experimental procedure

In 48 mature albino Wistar rats, diabetes was produced. Cages with a 22-degree Celsius temperature and 12-hour light/dark cycle housed the animals. After fasting for 12 hours, rats were administered with 60 mg/kg STZ intraperitoneally to establish diabetes. A 0.05 M citrate buffer at pH 4.5 dissolved fresh STZ. One hand held the rat dorsally, the injection site was swabbed with povidone-iodine solution, and a sterile needle administered the specified amount of STZ into the caudal abdominal cavity. On day 6th, day blood sugar was tested. Glucometers measure blood sugar. Diabetic animals had blood glucose exceeding 200 mg/dl. The experiment requires them.

Route of Administration - Oral route
Observation parameters for anti diabetic activity-
Different indicators of anti-diabetic action include:
1. Blood Glucose 2. Oral Glucose tolerance test 3. Histopathology

Detail of experiment:
Method : Diabetes Caused by Streptozotocin (60 mg/kg, i.p.)
Animal utilized : Albino wistar rats Weight : 150-200 gms
No. of group : Eight
Administration route : P.O.
The standard medication : Glibenclamide (10 mg/kg body weight, orally)

Developing diabetes in rats

Streptozotocin was used to cause diabetes in albino wistar rats after they had fasted for 16 hours (without food but with unrestricted access to water). Intraperitoneal (i.p.) streptozotocin (STZ) was 60 mg/kg body weight in 0.1M sodium citrate buffer (pH 4.5). To treat medication-induced hypoglycemia, rats drank 5% glucose overnight. Experiment employed diabetic rats with blood glucose levels of 200 mg/dl.

IV. Drug Profile

Sr. No.	Drugs	Dose
1	Streptozoticin (STZ)	60 mg/kg
2	Glibenclamide (GBC)	10 mg/kg

Result & Discussion

Table 1: Effect of *Punica granatum* and *Coriandrum sativum* extracts on body weight

Groups	BeforeSTZ	Body weight after streptozotocin (gm)			
		0th	7th	14th	21st
Group 1	164.5±5.6	160.1±4.472	159.7±5.587	160.7±6.382	161.4±7.729
Group 2	165.3±7.657	159.1±7.158	158.8±6.725	159.6±5.725	160.1±5.329
Group 3	161.3±7.657	157.8±7.158	157.1±6.725	159.8±5.725	160.9±5.329
Group 4	162.8±8.496	160.7±8.369	159.4±7.441	159.8.1±6.158	161.3±5.284
Group 5	163.6±8.124	157.6±8.441	157.9±8.408	158.4±7.462	159.1±6.367
Group 6	162.5±7.614	157.2±7.146	157.4±6.689	158.1±5.729	158.6±5.073
Group 7	156.9±7.232	153.2±7.441	153.6±6.146	154.2±5.689	156.2±5.146
Group 8	159.3±8.124	154.6±8.441	155.2±8.408	155.8±7.462	155.9±6.367

Results of the statistical analysis, which was done using Graph Pad PRISM (version 4.03), are shown as the mean SEM. Each set had a total of six different monsters. For the study, a one-way analysis of variance was used.

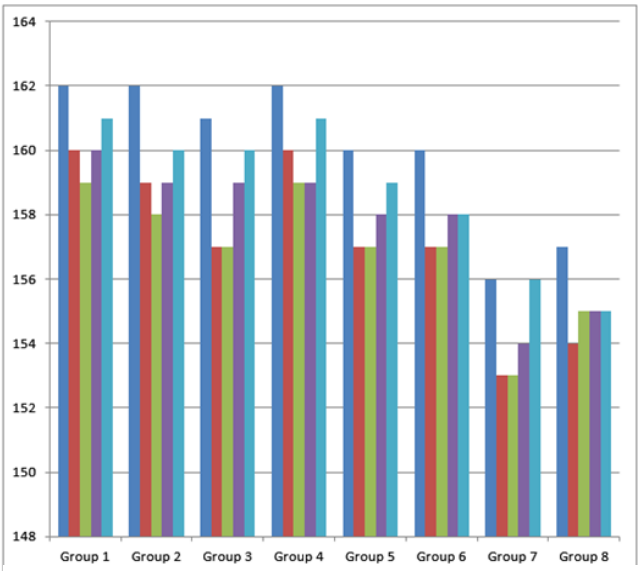

Figure 1: Effect of *Punica granatum* and *Coriandrum sativum* extracts on body weight

V. Experimental group design-

48 mature albino wistar rats were divided into six-rat groups. Here's how various subgroups were treated differently:

Group 1: Rats treated with Streptozotacin (60mg/kg) + 200 mg/kg (50:50) ethanolic extract of *Punica granatum* and *Coriandrum sativum.*

Group 2: Rats treated with Streptozotacin (60mg/kg)+ 400 mg/kg (50:50) ethanolic extract of *Punica granatum* and *Coriandrum sativum.*

Group 3: Rats treated with Streptozotacin (60mg/kg)+ 200 mg/kg (25:75) ethanolic extract of *Punica granatum* and *Coriandrum sativum.*

Group 4: Rats treated with Streptozotacin (60mg/kg)+ 400 mg/kg (25:75) thanolic extract of *Punica granatum* and *Coriandrum sativum.*

Group 5: Rats treated with Streptozotacin (60mg/kg)+ 200 mg/kg (75:25) ethanolic extract of *Punica granatum* and *Coriandrum sativum.*

Group 6: Rats treated with Streptozotacin (60mg/kg)+ 400 mg/kg (75:25) ethanolic extract of *Punica granatum* and *Coriandrum sativum.*

Group 7: Rats treated with Streptozotacin (60mg/kg)+ Standard Drug (Glibenclamide 10 mg/kg)

Group 8: Rats given Streptozotacin (60mg/kg) + Normal Control (Normal saline 5ml/kg)

Route of Administration - Oral route

Observation parameters for anti-diabetic activity: The parameters listed below were seen during anti-diabetic activity:

- Blood Glucose
- Oral Glucose tolerance test
- Histopathology

VI. Blood Glucose level

Table 2 : Blood Glucose level

Blood Glucose	H	B	T	HB	BT	HT
Group 1	144	146	142	148	145	149
Group 2	130	128	134	127	135	132
Group 3	122	126	128	124	127	126
Group 4	113	115	109	110	114	118
Group 5	164	166	162	168	164	167
Group 6	152	158	156	150	153	157
Group 7	102	105	108	101	106	103
Group 8	210	195	203	204	198	196

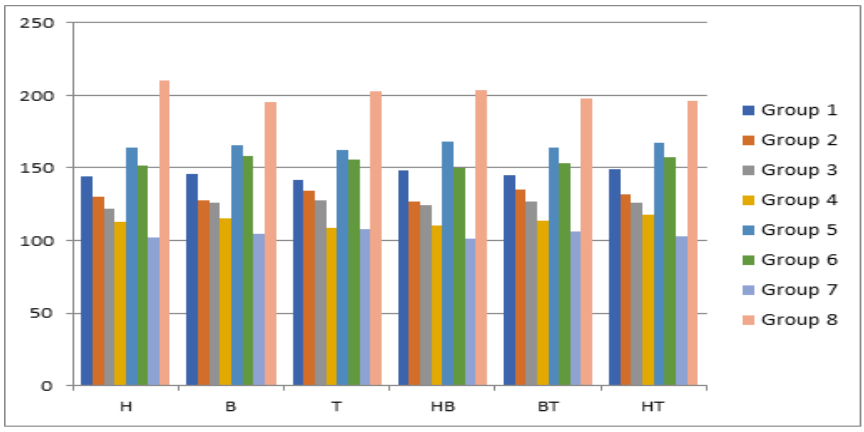

Figure 2: Effect of *Punica granatum* and *Coriandrum sativum* extracts on Blood glucose level

Table 3: Blood glucose level (Mean ± SD)

Group	Blood Glucose (Mean ± SD)
Group 1	145.66 ± 2.58
Group 2	131 ± 3.22
Group 3	125.5 ± 2.16**
Group 4	113.16 ± 3.31***
Group 5	165.16 ± 2.22
Group 6	154.33 ± 3.14
Group 7	104.16 ± 2.63***
Group 8	201 ± 5.72

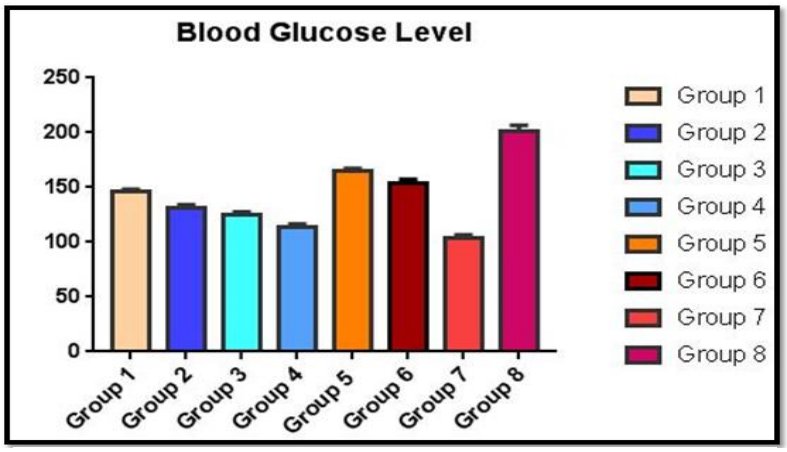

Figure 3: Effect of *Punica granatum* and *Coriandrum sativum* extracts on Blood glucose level (Mean ± SD)

VII. Histopathology

Histopathological observation

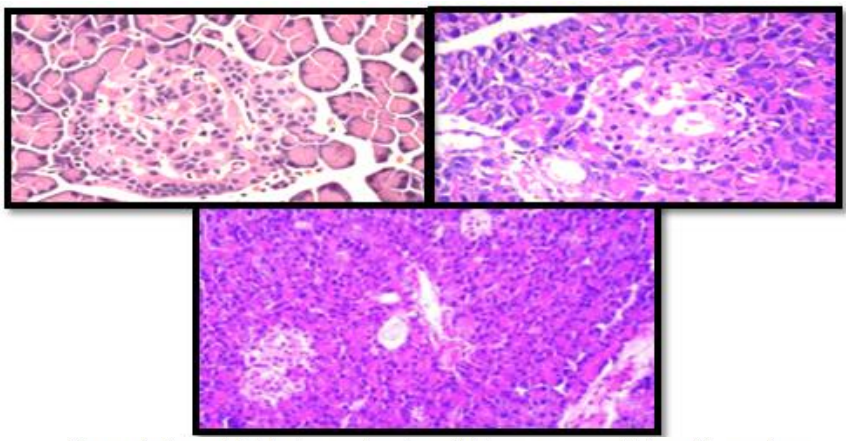

Figure 4: Group 1 Diabetic rats showing mild improvement of Islets of Langerhans

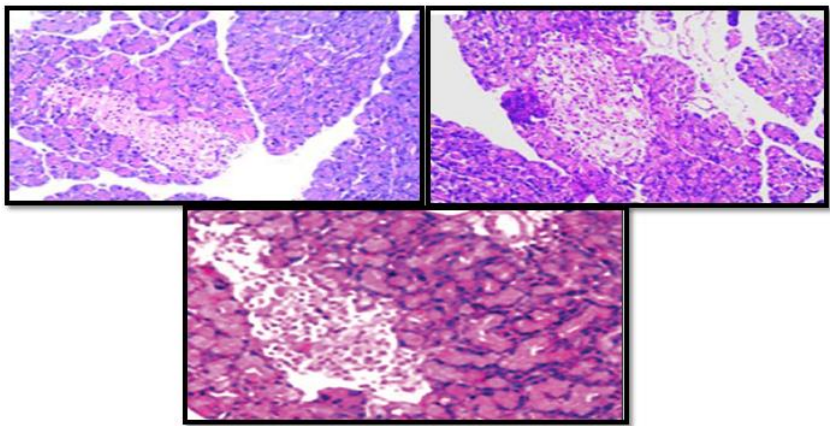

Figure 5: Group 2 Diabetic rats showing mild improvement of Islets of Langerhans

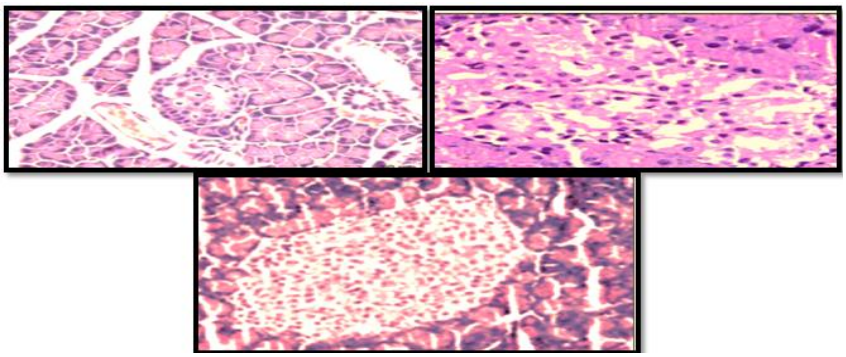

Figure 6: Group 3 Diabetic rats showing marked improvement of Islets of Langerhans

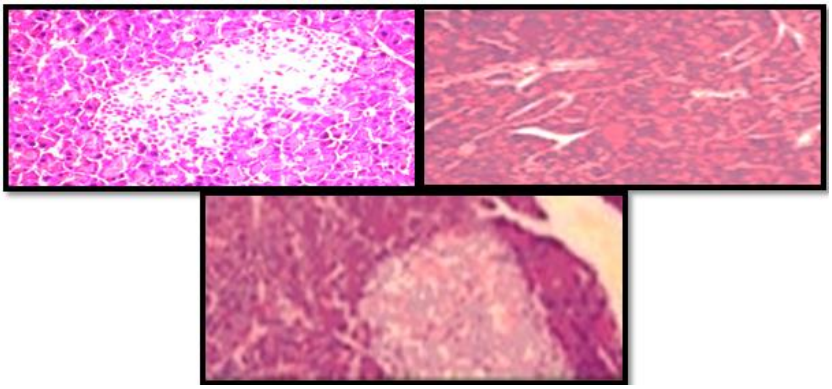

Figure7: Group 4 The Islets of Langerhans of diabetic rats have shown dramatic recovery

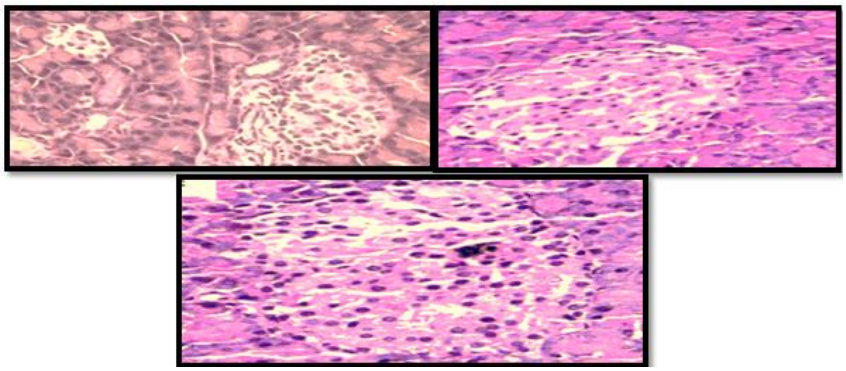

Figure 8: Group 5 Islets of Langerhans function somewhat better in diabetic rats

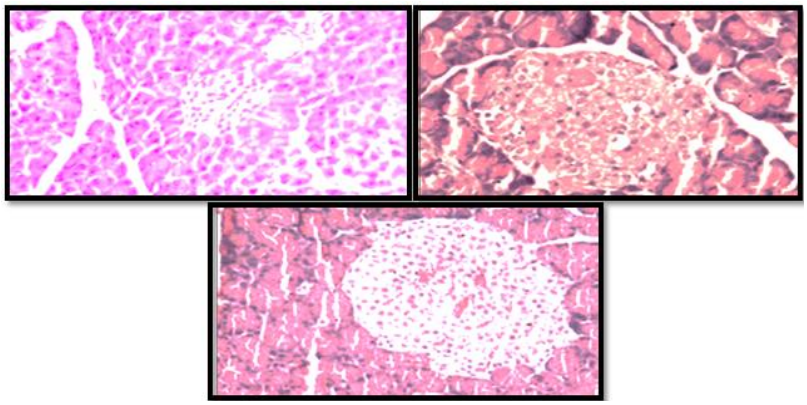

Figure:9 Group 6 Islets of Langerhans are somewhat more functional in diabetic rats

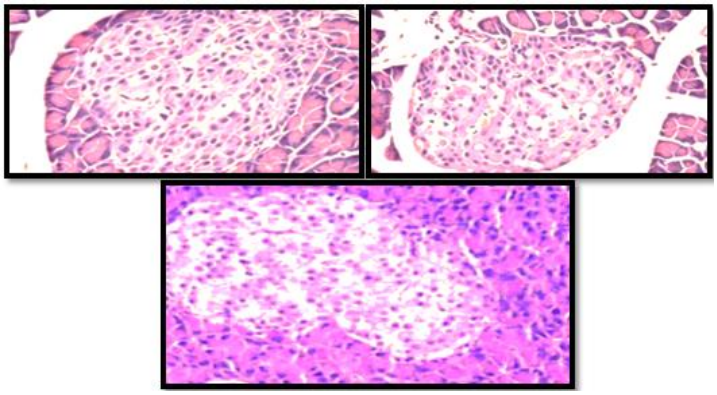

Figure 10: Group 7 Histopathological observations in diabetic rats reveal dramatic enhancement of islet function

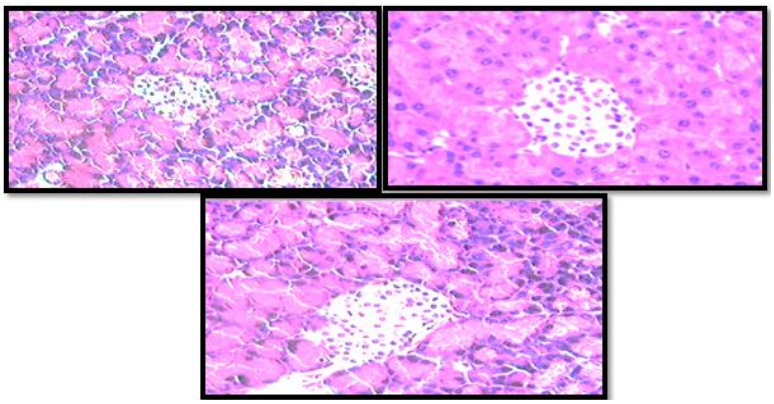

Figure 11: Group 8 Degenerative and necrotic alterations in the pancreas and shrunken Islets of Langerhans in diabetic rats

Streptozotocin (STZ)-induced diabetes in rats and its evaluation of the anti-diabetic effect of ethanolic extracts of *Punica granatum* and *Coriandrum sativum*. The alcoholic extracts of *Punica granatum* and *Coriandrum sativum* were prepared with the yield percentages of 6.80% and 6.50%, respectively. Toxicity testing was performed on albino Wistar rats, divided into three groups for different dose levels (200 mg and 2000 mg/kg). No significant toxicity or behavioral changes were observed, with LD_{50} values above 2000 mg/kg, meeting OECD standards. The induction of Diabetes was induced in rats by administering streptozotocin (STZ) at 60 mg/kg via the intraperitoneal (i.p.) route. After inducing diabetes, the rats were divided into eight groups, each containing six animals and their blood sugar levels were monitored using a glucometer during the study to assess the diabetic condition. Hematological analysis was conducted on different groups of rats to evaluate the impact of the extracts on blood parameters. Hematological analysis was conducted on different groups of rats to evaluate the impact of the alcoholic extracts on blood parameters. Weight loss was observed in the rats following STZ administration, with gradual recovery noted on the 14th and 21st days.

Group 4, treated with 400 mg/kg (25:75) ethanolic extract, showed the most promising results against the diabetes. All the groups treated with plant extracts exhibited anti-diabetic effects, whereas the Group 8 didn't showed any significant improvement in diabetes symptoms as it did not receive any anti-diabetic drug or plant extract, although glibenclamide remains the most effective treatment among all the groups tested.

Conclusion

The purpose of this research was to analyze the new polyherbal formulation of *Punica granatum and Coriandrum sativum* for its anti-diabetic efficacy. Both plants were used to create polyherbal preparations, which were then tested for their anti-diabetic efficacy. It was shown that the ethanolic extract of *Punica granatum and Coriandrum sativum* (25:75). in Test Group fourth produced the most results that were comparable to those produced by the gold standard medication glibenclamide. The research investigation demonstrates the potential anti-diabetic effects of ethanolic extracts of *Punica granatum* and *Coriandrum sativum* in a rat model of STZ-induced diabetes. Group 4, treated with a specific ratio of the extracts, showed the most promising results, although glibenclamide remains the most effective treatment among all the groups tested. These findings provide valuable insights into the potential use of these plant extracts for managing diabetes.

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