# PHARMACOLOGICAL EVALUATION OF THE ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF A POLYHERBAL EXTRACT (AEGLE MARMELOS AND PIPER NIGRUM) IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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# PHARMACOLOGICAL EVALUATION OF THE ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF A POLYHERBAL EXTRACT (AEGLE MARMELOS AND PIPER NIGRUM) IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

**ABSTRACT-**

**Background:** Diabetes mellitus is a chronic metabolic disorder with rising global prevalence, characterized by persistent hyperglycemia and oxidative stress. Conventional antidiabetic therapies often present limitations, prompting exploration of plant-based alternatives. *Aegle marmelos* (Bael) and *Piper nigrum* (Black Pepper) are traditional medicinal plants with reported antidiabetic and antioxidant properties.

**Objective:** This study evaluated the antidiabetic and antioxidant potential of a polyherbal extract containing *Aegle marmelos* and *Piper nigrum*, alone and in combination with metformin, in streptozotocin (STZ)-induced diabetic Wistar rats.

To prepare and characterize the selected herbal extract.

To evaluate the acute toxicity profile of the herbal extract.

To induce diabetes using streptozotocin in experimental rats.

To assess the effect of the herbal extract on blood glucose levels.

To evaluate biochemical and antioxidant parameters.

To compare the activity of the herbal extract with a standard antidiabetic drug.

To study histopathological changes in pancreatic tissue.

**Keywords:** *Aegle marmelos*, *Piper nigrum*, polyherbal formulation, streptozotocin, diabetes mellitus, antioxidant, metformin, oxidative stress, Wistar rats

## 1. INTRODUCTION

### 1.1 Diabetes Mellitus: A Global Health Challenge

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent elevation of blood glucose levels resulting from defects in insulin secretion, insulin action, or both. Insulin, a peptide hormone produced by pancreatic β-cells, plays a crucial role in regulating carbohydrate, fat, and protein metabolism. When insulin production is inadequate or when body tissues become resistant to insulin, glucose accumulates in the bloodstream, leading to hyperglycemia (Porwal et al., 2025).

The global prevalence of diabetes has increased dramatically over recent decades due to rapid urbanization, population growth, aging, sedentary lifestyles, unhealthy dietary habits, and rising obesity rates. The disease affects individuals of all age groups and socioeconomic backgrounds, with a particularly high burden in low- and middle-income countries. India bears a substantial proportion of the global diabetes burden and is recognized as one of the countries with the largest diabetic populations (Fahrurroji et al., 2024; Herman et al., 2023).

Diabetes mellitus is broadly classified into Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes mellitus, and other specific types (Zhang et al., 2025). T1DM is an autoimmune disorder characterized by destruction of insulin-producing pancreatic β-cells, resulting in absolute insulin deficiency and accounting for approximately 5-10% of all diabetes cases (Guo et al., 2025). T2DM, the most common form accounting for 90-95% of cases worldwide, is characterized by insulin resistance combined with progressive pancreatic β-cell dysfunction (Wassif et al., 2025).

### 1.2 Pathophysiology and Complications

The pathophysiology of diabetes involves complex metabolic disturbances resulting from impaired insulin secretion, impaired insulin action, or both. Chronic hyperglycemia triggers several biochemical changes contributing to tissue damage, including increased production of reactive oxygen species (ROS), oxidative stress, and formation of advanced glycation end products (AGEs) (Liu et al., 2023; Rani et al., 2023).

Diabetes is associated with a wide range of complications that develop as a result of prolonged hyperglycemia. Microvascular complications include diabetic retinopathy, nephropathy, and neuropathy (Vitale et al., 2022; Sharma et al., 2022). Macrovascular complications involve accelerated atherosclerosis, leading to coronary artery disease, cerebrovascular disease, and peripheral arterial disease (Patel et al., 2022). Additionally, diabetes significantly impairs wound healing mechanisms, making patients more susceptible to chronic wounds and infections (Patel et al., 2024).

### 1.3 Oxidative Stress in Diabetes

Oxidative stress plays a central role in the pathogenesis and progression of diabetes and its complications. Hyperglycemia promotes the generation of ROS through multiple pathways, including glucose autoxidation, polyol pathway activation, advanced glycation, and

mitochondrial dysfunction. The imbalance between ROS production and antioxidant defense mechanisms results in oxidative damage to cellular proteins, lipids, and DNA.

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) constitute the primary defense against oxidative stress. SOD catalyzes the dismutation of superoxide radicals to hydrogen peroxide, which is subsequently detoxified by CAT and glutathione peroxidase. Depletion of these enzymatic antioxidants is a hallmark of diabetes-induced oxidative stress (Dubey et al., 2023; Ahmed et al., 2023).

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### 1.4 Herbal Medicine and Diabetes Management

Medicinal plants have gained significant attention in diabetes management due to their rich content of bioactive phytoconstituents and their ability to address multiple pathological pathways simultaneously. *Aegle marmelos* (L.) Corrêa, commonly known as Bael, is a medicinal plant extensively used in the Indian system of medicine for treating various disorders including diabetes (Sharma et al., 2023; Haimed et al., 2022). Phytochemical analysis has revealed the presence of alkaloids, flavonoids, coumarins, terpenoids, and phenolic compounds in different parts of the plant (Bavage et al., 2023).

Previous studies have demonstrated that *A. marmelos* extracts possess significant hypoglycemic, antihyperlipidemic, and antioxidant properties (Chandra et al., 2024; Kamalakkannan et al., 2008; Kamalakkannan et al., 2005; Kamalakkannan et al., 2003). The methanolic extract of *A. marmelos* has been shown to significantly reduce hyperglycemia, improve lipid profiles, and enhance insulin secretion in STZ-induced diabetic models. Histopathological analysis has revealed restoration of β-cell density and pancreatic architecture following treatment (Haimed et al., 2022).

*Piper nigrum* L. (Black Pepper) is another medicinal plant with well-documented pharmacological properties. Piperine, the principal bioactive alkaloid in *P. nigrum*, has demonstrated significant antidiabetic, antioxidant, and anti-inflammatory activities (Bandigari et al., 2018). Studies have shown that piperine (50 mg/kg) possesses significant antihyperglycemic activity in STZ-induced diabetic rats.

### 1.5 Polyherbal Formulations: Rationale and Advantages

Polyherbalism is a therapeutic approach involving the use of two or more medicinal plants in a single formulation to achieve enhanced therapeutic efficacy. The scientific basis lies in the principle of synergism, where the combined effect of different herbal components is greater than the sum of their individual effects. Each herb contributes specific bioactive compounds that act through different mechanisms, resulting in improved therapeutic outcomes (Kumar et al., 2015; Sharma et al., 2015).

Polyherbal formulations offer several advantages over single-herb preparations: synergistic therapeutic activity, multi-target therapeutic action, improved bioavailability, sustained therapeutic effect, and cost-effectiveness (Mohanty et al., 2014; Agyare et al., 2014; Patel et al., 2014). The combination of *A. marmelos* and *P. nigrum* in a polyherbal formulation may provide

complementary therapeutic benefits by simultaneously addressing hyperglycemia, oxidative stress, and metabolic disturbances.

### 1.6 Study Rationale and Objectives

Despite the individual antidiabetic potential of *A. marmelos* and *P. nigrum*, limited research has investigated their combined effects, particularly in combination with conventional antidiabetic agents. The present study was therefore undertaken to:

1. Evaluate the antidiabetic activity of a polyherbal extract containing *A. marmelos* and *P. nigrum* in STZ-induced diabetic Wistar rats
2. Assess the antioxidant potential of the polyherbal extract
3. Investigate the synergistic effects of the polyherbal extract in combination with metformin
4. Evaluate histopathological changes in pancreatic, hepatic, and renal tissues
5. Compare the efficacy of the polyherbal formulation with the standard antidiabetic drug metformin

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

Streptozotocin (STZ), metformin hydrochloride, and all other analytical grade chemicals were procured from Merck Specialities Pvt. Ltd., Mumbai, India. Biochemical assay kits were obtained from Pathozyme and Spinreact Diagnostics, India. All chemicals and reagents were of analytical grade and used as received without further purification. The details of chemicals, instruments, and kits used are provided in supplementary Tables S1-S3.

### 2.2 Plant Material and Extract Preparation

Dried powdered materials of *Aegle marmelos* (leaves) and *Piper nigrum* (fruits) were obtained from a certified supplier. The plant materials were authenticated by a qualified botanist. The polyherbal extract was prepared by mixing equal proportions of *A. marmelos* and *P. nigrum* powders and extracting with distilled water using a maceration technique. The extract was filtered, concentrated under reduced pressure, and stored in airtight amber-colored bottles at room temperature until use. A fresh drug solution was prepared daily using distilled water as a vehicle.

**2.3 Preliminary Phytochemical Screening**The polyherbal extract was subjected to preliminary phytochemical screening using standard qualitative tests to detect the presence of various phytoconstituents including alkaloids (Dragendorff's reagent test), flavonoids ($H_2SO_4$ test),

tannins ($FeCl_3$ test), saponins (foam test), terpenoids (chloroform test), steroids (Salkowski test), glycosides, carbohydrates, proteins, and amino acids using standard protocols (Kumar et al., 2010; Das et al., 2010).

**2.4 Experimental Animals**

Adult Wistar rats of either sex (180-220 g) were obtained from the institutional animal house. Animals were housed in polypropylene cages under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 55 ± 5%, 12-hour light/dark cycle) with free access to standard pellet diet and water ad libitum. All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethics Committee.

**2.5 Acute Toxicity Study**

Acute oral toxicity was evaluated according to OECD Guidelines 423. Rats were fasted overnight and administered the polyherbal extract at increasing doses (5, 50, 300, 500, 1000, and 2000 mg/kg) orally. Animals were observed for 14 days for signs of toxicity, mortality, and behavioral changes. The maximum non-toxic dose was determined for subsequent experiments.

**2.6 Induction of Diabetes**

Diabetes was induced in overnight-fasted rats by a single intraperitoneal injection of streptozotocin (STZ, 65 mg/kg body weight) dissolved in 0.1 M citrate buffer (pH 4.5). Control animals received an equivalent volume of citrate buffer. After 72 hours, blood glucose levels were measured using a digital glucometer using blood collected from the tail vein. Rats with fasting blood glucose levels >250 mg/dL were considered diabetic and included in the study (Ghasemi & Jeddi, 2023; Furman, 2015).

**2.7 Experimental Design**

Sixty rats were randomly divided into six groups (n=6 per group):

**Group I (Vehicle Control):** Non-diabetic rats received distilled water (1 mL/day, p.o.) for 28 days.

**Group II (Diabetic Control):** Diabetic rats received distilled water (1 mL/day, p.o.) for 28 days.

**Group III (Polyherbal Extract):** Diabetic rats received polyherbal extract (*A. marmelos* + *P. nigrum*, 1 mL/day, p.o.) for 28 days.

**Group IV (Metformin):** Diabetic rats received metformin (100 mg/kg/day, p.o.) for 28 days.

**Group V (Low-Dose Combination):** Diabetic rats received polyherbal extract (1 mL/day) + metformin (100 mg/kg/day, p.o.) for 28 days.

**Group VI (High-Dose Combination):** Diabetic rats received polyherbal extract (2 mL/day) + metformin (100 mg/kg/day, p.o.) for 28 days.

All treatments were administered once daily by oral gavage.

**2.8 Blood Collection and Glucose Measurement**-Blood samples were collected from the retro-orbital plexus under light diethyl ether anesthesia on days 0, 7, 14, 21, and 28 of treatment.

Blood glucose levels were immediately measured using a digital glucometer. For biochemical analysis, blood samples were collected into plain tubes, allowed to clot at room temperature for 30 minutes, and centrifuged at 2,500 rpm for 10 minutes to separate serum. Serum samples were stored at -40°C until analysis (Za'abi et al., 2021).

**2.9 Biochemical Parameters**

Serum biochemical parameters were estimated using commercially available diagnostic kits following the manufacturer's protocols:

- **Total cholesterol (TC):** Enzymatic colorimetric method
- **Triglycerides (TG):** Enzymatic colorimetric method
- **High-density lipoprotein (HDL):** Direct enzymatic method
- **Low-density lipoprotein (LDL):** Calculated using Friedewald formula
- **Serum glutamic-oxaloacetic transaminase (SGOT/AST):** IFCC kinetic method
- **Serum glutamic-pyruvic transaminase (SGPT/ALT):** IFCC kinetic method
- **Serum insulin:** ELISA method using rat insulin ELISA kit

**2.10 Oxidative Stress Markers**

**1 Tissue Homogenate Preparation**

**2 Lipid Peroxidation (MDA)**

**3 Superoxide Dismutase (SOD)**

**4 Reduced Glutathione (GSH)**

**5 Catalase (CAT)**

**6 Nitric Oxide (Nitrite) Estimation**

**7 Protein Estimation**

**2.11 Histopathological Analysis**

On day 29, all animals were sacrificed under deep anesthesia, and pancreas, liver, and kidney tissues were collected. Tissues were fixed in 10% formalin, processed through graded alcohol (isopropyl alcohol), cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a microtome, stained with hematoxylin and eosin (H&E), and examined under a light microscope (Motic BA) for histopathological changes. Tissue sections were analyzed qualitatively at 10X and 40X magnification for inflammatory influx, congestion, edema, necrosis, and other pathological changes (Yukari et al., 2004).

**2.12 Statistical Analysis**

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using GraphPad Prism 10.0 software (GraphPad, San Diego, USA). A p-value $< 0.05$ was considered statistically

significant. Statistical comparisons were made between drug-treated groups and disease control animals (vehicle control). ns = $p>0.05$ (non-significant), *$p<0.05$, **$p<0.01$ when compared with positive control group.

## 3. RESULTS

### 3.1 Phytochemical Screening

Preliminary phytochemical screening of the polyherbal extract revealed the presence of flavonoids, terpenoids, alkaloids, steroids, and tannins. The results are summarized in Table 1 and shown in Figures 1a and 1b.

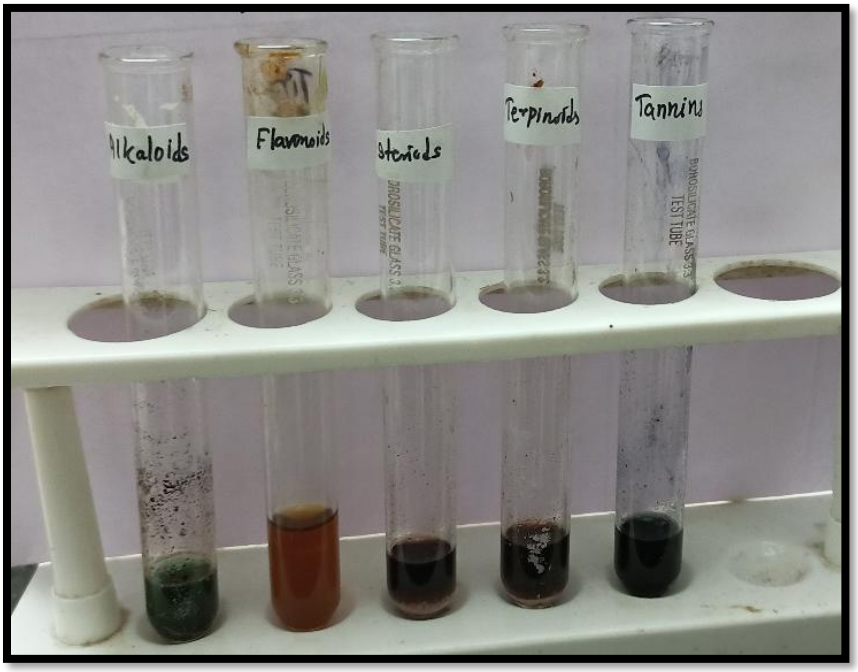
**Table 1: Phytochemical Constituents of the Polyherbal Extract**

| Phytochemical Test | *A. marmelos* | *P. nigrum* | Polyherbal Extract |
|---|---|---|---|
| Flavonoids ($H_2SO_4$ Test) | + | + | + |
| Terpenoids (Chloroform Test) | + | + | + |
| Alkaloids (Dragendorff's Reagent) | + | + | + |
| Steroids (Salkowski Test) | + | + | + |
| Tannins ($FeCl_3$ Test) | + | + | + |
| Saponins | - | + | + |
| Carbohydrates | + | + | + |
| Proteins | + | + | + |

| Phytochemical Test | *A. marmelos* | *P. nigrum* | Polyherbal Extract |
|---|---|---|---|
| **Free Amino Acids** | + | + | + |
| **Vitamin C** | + | + | + |

*(+) = Present, (-) = Absent*

**Figure 1: Phytochemical Test Results - *Aegle marmelos* Extract**




**Histopathological Analysis**

**1 Pancreas**

Histopathological examination of pancreatic tissue (Table 6, Figure 6) revealed normal architecture in the vehicle control group, with well-defined Islets of Langerhans containing cells with normal cytoplasm. In contrast, the diabetic control group showed shrunken and distorted islets with degenerative and necrotic changes in the islet cells.

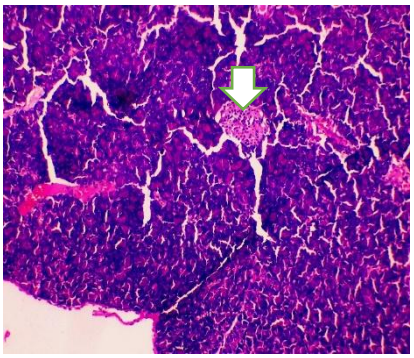
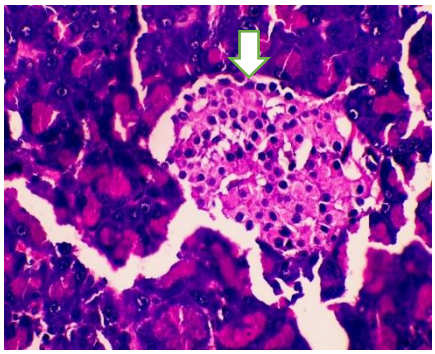
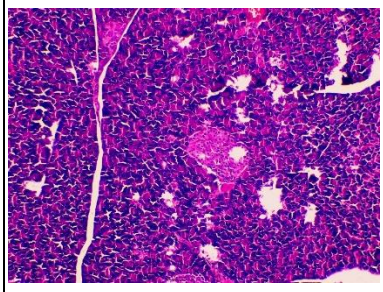
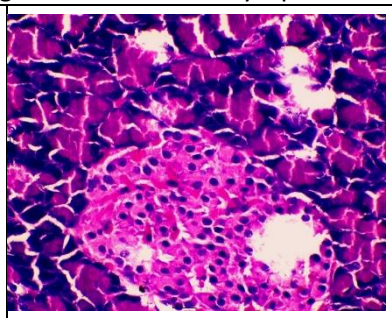
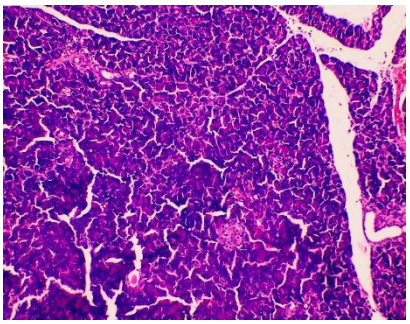
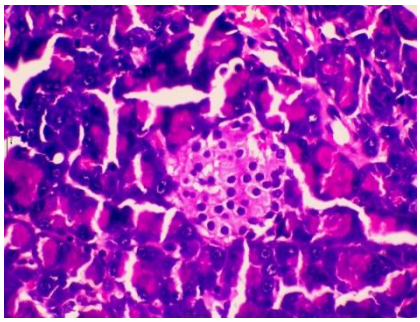
The polyherbal extract-treated group showed significant improvement, with restoration of islet architecture and normal cytoplasm. The metformin and combination therapy groups also demonstrated normal islet morphology with minimal degenerative changes. The high-dose

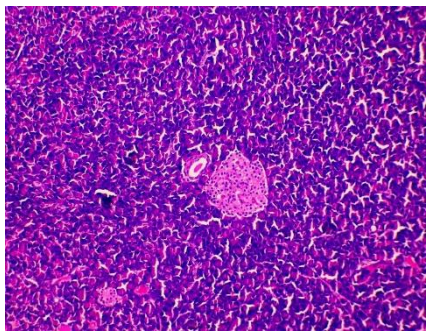
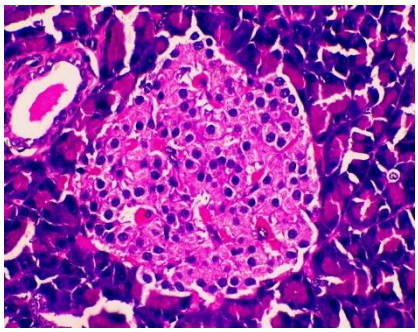
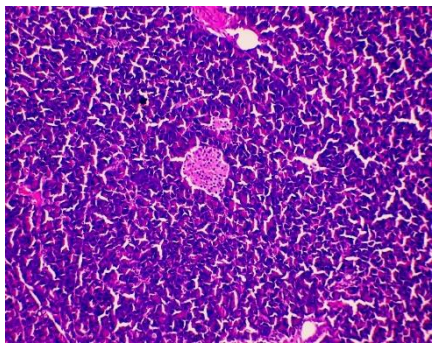
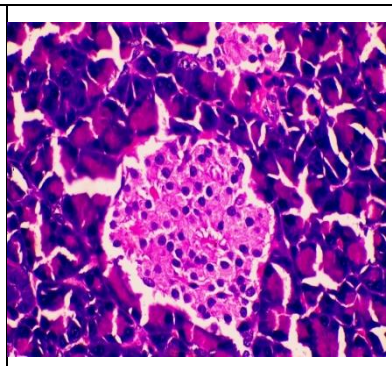
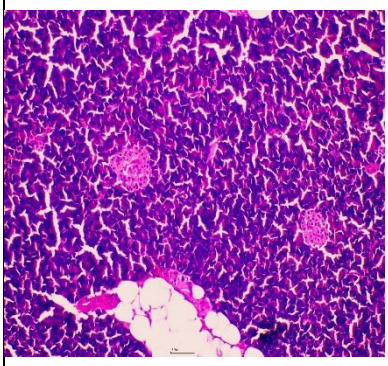
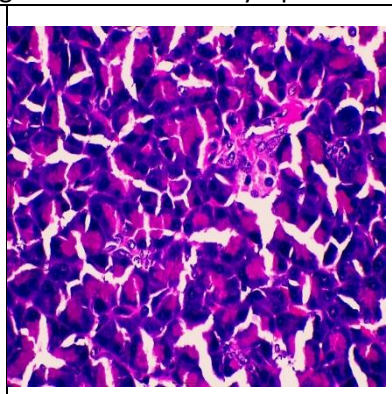
combination group showed the most remarkable recovery, with islet architecture closely resembling that of the vehicle control group.

**Table 6: Histopathological Findings in Pancreatic Tissue**

| Group | 10X Magnification | 40X Magnification |
|---|---|---|
| **I - Vehicle Control** | Normal Islets of Langerhans with normal cytoplasm | Normal Islets of Langerhans with normal cytoplasm |
| **II - Diabetic Control** | Shrunken and distorted islets with degenerative and necrotic changes | Shrunken and distorted islets with degenerative and necrotic changes |
| **III - Polyherbal Extract** | Normal Islets of Langerhans with normal cytoplasm | Normal Islets of Langerhans with normal cytoplasm |
| **IV - Metformin** | Normal Islets of Langerhans with normal cytoplasm | Normal Islets of Langerhans with normal cytoplasm |
| **V - Low-Dose Combination** | Normal Islets of Langerhans with normal cytoplasm | Normal Islets of Langerhans with normal cytoplasm |
| **VI - High-Dose Combination** | Normal Islets of Langerhans with normal cytoplasm | Normal Islets of Langerhans with normal cytoplasm |

**Figure 6: Histopathology of Pancreas**

| Group | Pancreas (10X) | Pancreas (40X) |
|---|---|---|
| **I-Vehicle** |  |  |
| | Normal Islet of Langerhans displaying cells with normal cytoplasm | |
| **II-Diabetes induced control** |  |  |
| | Showing shrunken and distorted islets of Langerhans contained cells with degenerative and necrotic changes. Presence of normal pancreatic islet cells | |
| **III-Agel marmaloes+ piprum nigrum +diabetic group** |  |  |
| | Normal Islet of Langerhans displaying cells with normal cytoplasm | |

| | | |
|---|---|---|
| **IV-Metformin+Administered diabetic group** |  |  |
| | Normal Islet of Langerhans displaying cells with normal cytoplasm. Presence of normal pancreatic islet cells. | |
| **V-Agel marmaloes piprum nigrum+ metformin treated diabetic group** |  |  |
| | Normal Islet of Langerhans displaying cells with normal cytoplasm | |
| **VI-Agel marmaloes and piprum nigrum metformin treated diabetic group** |  |  |
| | Normal Islet of Langerhans displaying cells with normal cytoplasm. | |

**2 Liver**

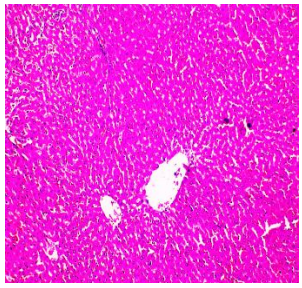
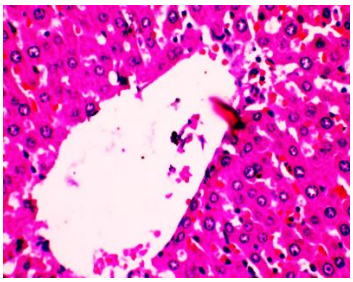
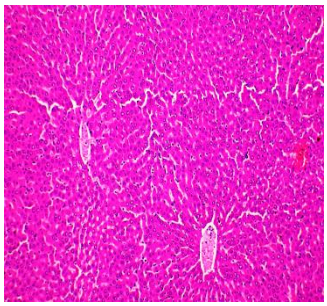
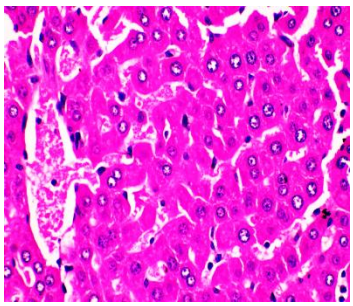
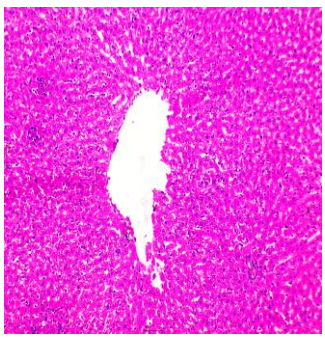
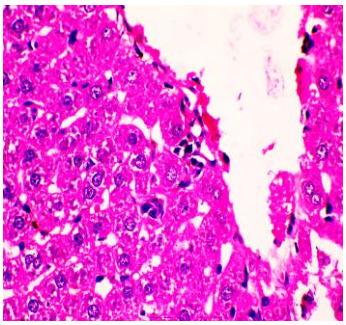
Histopathological examination of liver tissue (Table 7, Figure 7) showed normal hepatocytes arranged in cords with distinct sinusoids and central vein in the vehicle control group. The diabetic control group exhibited mild abnormality with some hemorrhage and ballooning degeneration.

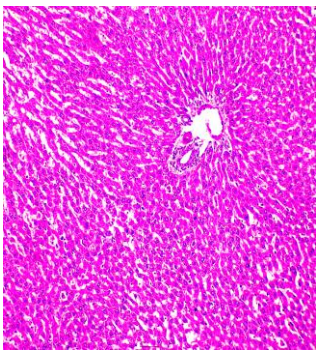
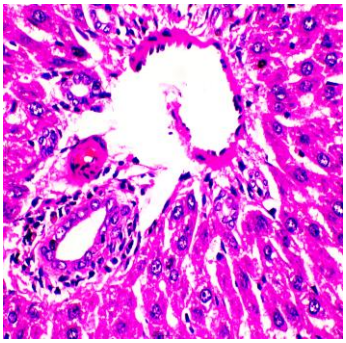
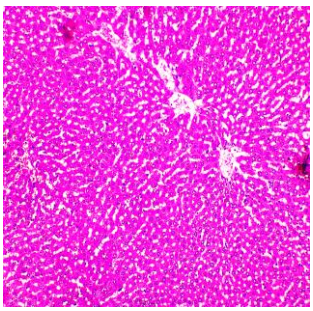
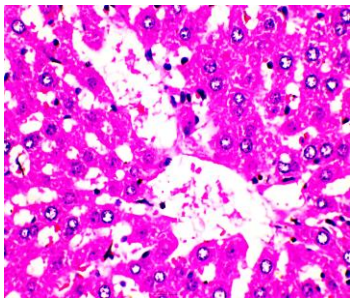
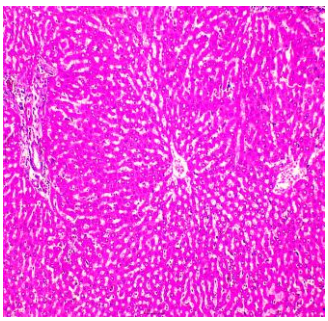
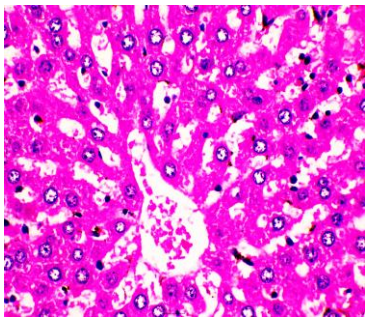
Treatment with the polyherbal extract, metformin, and combination therapies resulted in restoration of normal hepatic architecture, with well-arranged hepatocytes, obvious sinusoids, and central vein. The combination therapy groups showed the most significant improvement.

**Table 7: Histopathological Findings in Liver Tissue**

| Group | 10X Magnification | 40X Magnification |
|---|---|---|
| **I - Vehicle Control** | Normal hepatocytes arranged in cords with obvious sinusoids and central vein | Normal hepatocytes arranged in cords with obvious sinusoids and central vein |
| **II - Diabetic Control** | Normal hepatocytes arranged in cords with obvious sinusoids; mild abnormality and mild hemorrhage | Normal hepatocytes arranged in cords with obvious sinusoids; mild abnormality |
| **III - Polyherbal Extract** | Normal hepatocytes arranged in cords with obvious sinusoids and central vein | Normal hepatocytes arranged in cords with obvious sinusoids and central vein |
| **IV - Metformin** | Normal hepatocytes arranged in cords with obvious sinusoids and central vein | Normal hepatocytes arranged in cords with obvious sinusoids and central vein |
| **V - Low-Dose Combination** | Normal hepatocytes arranged in cords with obvious sinusoids and central vein | Normal hepatocytes arranged in cords with obvious sinusoids and central vein |
| **VI - High-Dose Combination** | Normal hepatocytes arranged in cords with obvious sinusoids and central vein | Normal hepatocytes arranged in cords with obvious sinusoids and central vein |

**Figure 7: Histopathology of Liver**

| Group | Liver (10X) | Liver (40X) |
|---|---|---|
| **I-Vehicle** |  |  |
| | Normal hepatocytes arranged, with obvious sinusoids, and central vein. | |
| **II-Diabetes induced control** |  |  |
| | Exhibited a normal hepatocyte arranged in cords, obvious sinusoids. Mild abnormality and Mild hemorrhage detected. | |
| **III-Agel marmaloes+ piper nigrum +diabetic group** |  |  |
| | Normal hepatocytes arranged, with obvious sinusoids, and central vein | |

| | | |
|---|---|---|
| **IV-Metformin+Administered diabetic group** |  |  |
| | Exhibited a normal hepatocyte arranged in cords, obvious sinusoids. No abnormality detected. | |
| **V-Agel marmaloes piper nigrum+ metformin treated diabetic group** |  |  |
| | Exhibited a normal hepatocytes arranged in cords, obvious sinusoids. No abnormality detected. | |
| **VI-Agel marmaloes and piper nigrum metformin treated diabetic group** |  |  |
| | Exhibited a normal hepatocyte arranged in cords, obvious sinusoids. No abnormality detected. | |

### 3.6.3 Kidney

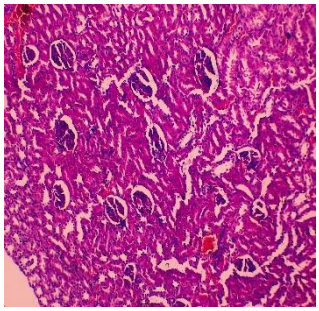
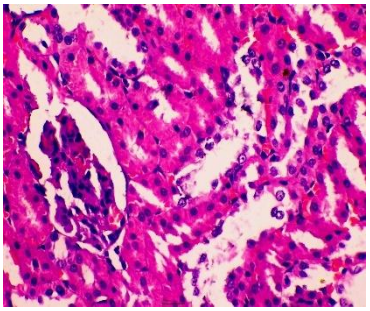
Histopathological examination of kidney tissue (Table 8, Figure 8) showed normal glomerular structure with well-defined proximal and distal convoluted tubules in the vehicle control group. The diabetic control group exhibited mild abnormality with some changes in proximal and distal convoluted tubules.

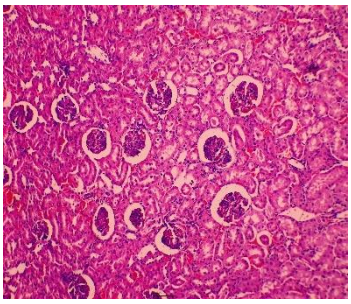
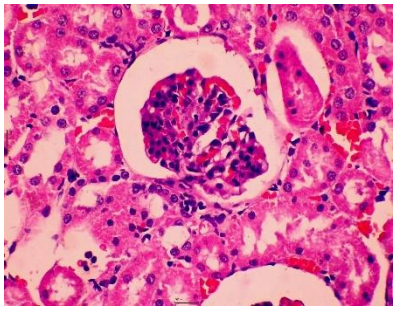
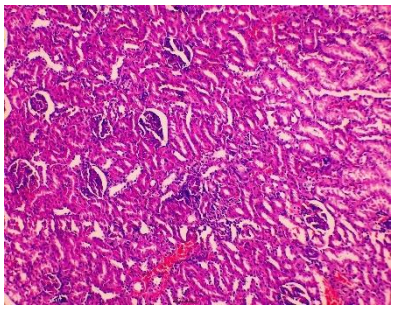
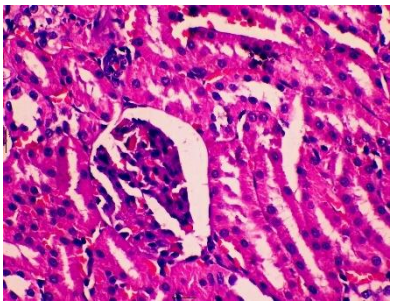
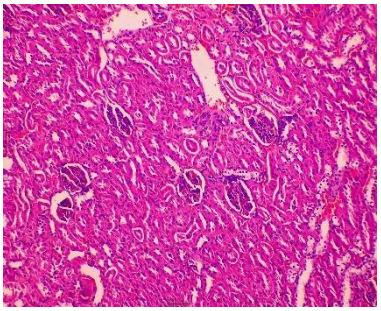
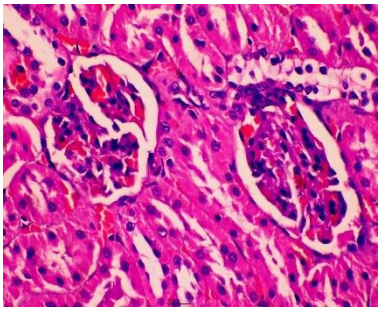
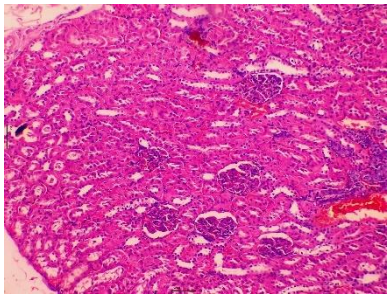
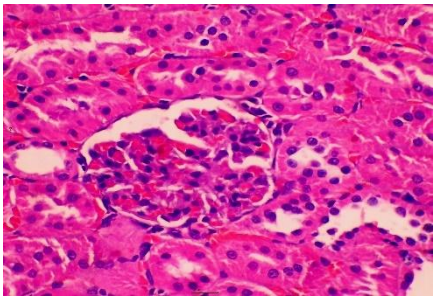
Treatment groups showed restoration of normal renal architecture with well-defined glomeruli and tubules. The polyherbal extract and combination therapy groups demonstrated the most significant improvement.

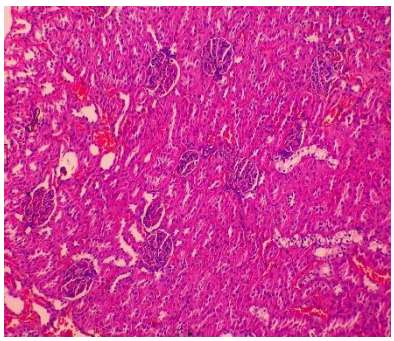
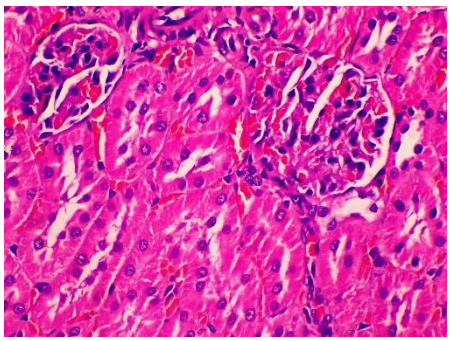
**Table 8: Histopathological Findings in Kidney Tissue**

| Group | 10X Magnification | 40X Magnification |
|---|---|---|
| **I - Vehicle Control** | Normal histological structure with glomerulus | Normal histological structure with glomerulus |
| **II - Diabetic Control** | Normal structure with mild abnormality; proximal and distal convoluted tubules | Normal structure with mild abnormality; proximal and distal convoluted tubules |
| **III - Polyherbal Extract** | Normal histological structure with glomerulus | Normal histological structure with glomerulus |
| **IV - Metformin** | Normal histological structure with glomerulus | Normal histological structure with glomerulus |
| **V - Low-Dose Combination** | Normal histological structure with glomerulus | Normal histological structure with glomerulus |
| **VI - High-Dose Combination** | Normal histological structure with glomerulus | Normal histological structure with glomerulus |

**Figure 8: Histopathology of Kidney**

| Group | Kidney (10X) | Kidney (40X) |
|---|---|---|
| I-Vehicle |  |  |

| | | |
|---|---|---|
| | Showing normal histological structure with glomerulus. | |
| **II-Diabetes induced control** |  |  |
| | Normal structure with mild abnormality and proximal co convoluted tubules and distal convoluted. | |
| **III-Agel marmaloes+ piprum nigrum +diabetic group** |  |  |
| | Showing normal histological structure with glomerulus. | |
| **IV-Metformin+Administered diabetic group** |  |  |
| | Showing normal histological structure with glomerulus. | |
| **V-Agel marmaloes piprum nigrum+ metformin treated diabetic group** |  |  |
| | Showing normal histological structure with glomerulus with mild | |

| | | |
|---|---|---|
| | abnormality. | |
| **VI-Agel marmaloes and piprum nigrum metformin treated diabetic group** |  |  |
| | Showing normal histological structure with glomerulus. | |

## 5. CONCLUSION-

The present study demonstrates that the polyherbal extract containing *Aegle marmelos* and *Piper nigrum* possesses significant antidiabetic and antioxidant activities in STZ-induced diabetic Wistar rats. The key findings are summarized as follows:

1. **Antidiabetic Activity:** The polyherbal extract significantly reduced blood glucose levels in diabetic rats, with effects comparable to the standard antidiabetic drug metformin. The progressive reduction over 28 days suggests both acute and chronic hypoglycemic effects.
2. **Antioxidant Effects:** The extract significantly improved oxidative stress markers (SOD, MDA, GSH, CAT), indicating potent antioxidant activity. The combination therapy showed enhanced effects, with the high-dose combination achieving near-normalization of all oxidative stress parameters.
3. **Metabolic Improvement:** The extract improved lipid profile (reduced cholesterol, triglycerides, LDL; increased HDL) and restored serum insulin levels, indicating comprehensive metabolic benefits.
4. **Organ Protection:** Histopathological examination revealed restoration of pancreatic islet architecture, normalization of hepatic structure, and preservation of renal integrity, confirming the organ-protective effects of the polyherbal formulation.
5. **Synergistic Effects:** The combination of the polyherbal extract with metformin showed enhanced efficacy, suggesting a synergistic interaction. The high-dose combination achieved the most significant improvements across all parameters.
6. **Safety:** The extract was found to be safe at the tested doses, with no signs of acute toxicity, supporting its potential for therapeutic use.

These findings support the traditional use of *A. marmelos* and *P. nigrum* in diabetes management and suggest that this polyherbal formulation may serve as an effective adjunct therapy to conventional antidiabetic agents. The synergistic effects with metformin indicate potential for dose reduction and improved therapeutic outcomes.

Future research should focus on:

- Identification and characterization of the active compounds responsible for the observed effects using advanced analytical techniques
- Elucidation of the molecular mechanisms of action, including signaling pathways
- Long-term safety and efficacy studies in animal models
- Clinical trials to validate the findings in human subjects
- Development of standardized dosage forms for clinical use

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