



Original Research Paper

SYNERGISTIC ANTIDIABETIC EFFECTS OF BERBERIS ARISTATA AND HAMELIA PATENS EXTRACTS IN HIGH-FAT DIET/STREPTOZOTOCIN-INDUCED TYPE 2 DIABETIC RATS: BIOCHEMICAL, HISTOPATHOLOGICAL, AND PHYTOCHEMICAL INVESTIGATIONS

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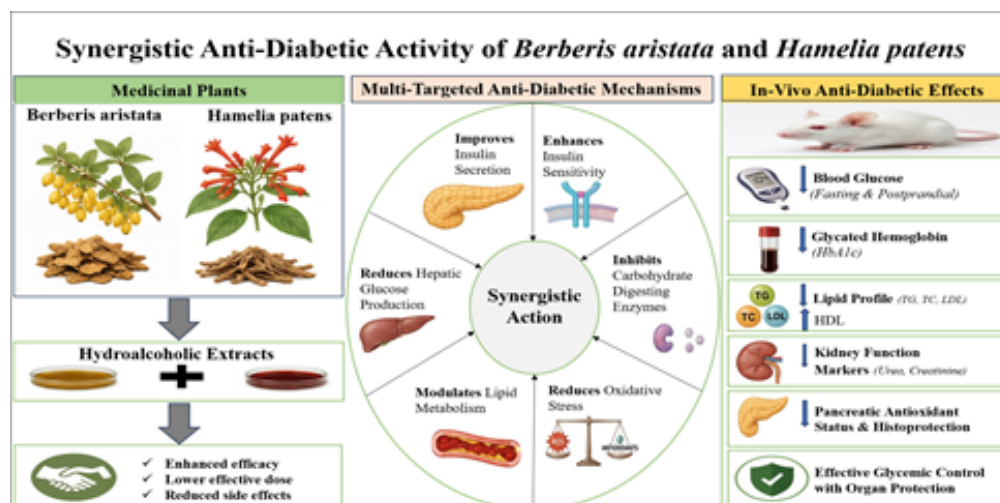
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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder marked by chronic hyperglycemia, insulin resistance, oxidative stress, and pancreatic β -cell dysfunction. Although conventional antidiabetic drugs improve glycemic control, long-term use can cause adverse effects, underscoring the need for safer, multitarget therapies. *Berberis aristata* and *Hamelia patens* have antihyperglycemic and antioxidant properties; however, their combined efficacy remains underexplored. This study investigated the synergistic antidiabetic effects of combined extracts of *B. aristata* and *H. patens* in an HFD/STZ-induced diabetic rat model. Thirty-six Wistar rats were divided into six groups ($n = 6$). Diabetes was induced with HFD followed by streptozotocin (50 mg/kg, i.p.). Diabetic rats received gliclazide (10 mg/kg), *B. aristata* (100 mg/kg), *H. patens* (100 mg/kg), or their combination (200 mg/kg) orally for six weeks. Body weight, fasting blood glucose, HbA1c, lipid profile, renal biomarkers, urinary parameters, phytochemicals, and pancreatic histopathology were evaluated. The combined extract showed superior antidiabetic activity compared with the individual extracts, significantly improving glycemic control, body weight, lipid profile, and renal function. Fasting blood glucose decreased from 339.0 ± 1.63 to 267.8 ± 2.27 mg/dL, whereas diabetic controls remained hyperglycemic (357.0 ± 1.73 mg/dL). The combination normalized fasting glucose (91.6 mg/dL) and HbA1c (4.6%), improved blood urea (20.36 mg/dL) and creatinine (0.66 mg/dL), eliminated urinary glucose, and restored pancreatic islet architecture with marked β -cell preservation. Overall, the combination exhibited significant synergistic antidiabetic effects and represents a promising polyherbal therapeutic candidate for T2DM management.

Keywords: *Berberis aristata*; *Hamelia patens*; Diabetes mellitus; Streptozotocin; Polyherbal therapy; Synergistic effect; Histopathology; Hyperglycemia.



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1. INTRODUCTION

Diabetes Mellitus (DM) is among the fastest-growing global health problems and a major contributor to morbidity, mortality, and healthcare costs [1]. Chronic hyperglycemia arising from impaired insulin secretion, insulin resistance, or both progressively disrupts carbohydrate, lipid, and protein metabolism, leading to microvascular and macrovascular complications such as nephropathy, neuropathy, retinopathy, and cardiovascular disease. Type 2 Diabetes Mellitus (T2DM) accounts for the majority of cases and is driven by rising obesity, sedentary lifestyles, unhealthy diets, and population aging [2]. Over 530 million adults currently live with diabetes, a figure projected to exceed 780 million by 2045. Pharmacological management relies largely on oral hypoglycemic agents (sulfonylureas, biguanides, DPP-4 inhibitors, SGLT2 inhibitors, GLP-1 receptor agonists). While effective at lowering blood glucose, long-term use can produce hypoglycemia, gastrointestinal disturbances, weight gain, hepatic or cardiovascular risks, and progressive loss of efficacy; none fully arrest pancreatic β -cell decline. Consequently, multi-target agents with fewer adverse effects are urgently needed [3].

Medicinal plants remain a rich source of such agents. Their diverse phytoconstituents—polyphenols, flavonoids, alkaloids, terpenoids, tannins, and glycosides—often act synergistically to improve glucose homeostasis, reduce oxidative stress, preserve β -cell function, dampen inflammation, and enhance insulin sensitivity [4]. *Berberis aristata* DC. (Indian barberry/Daruharidra), widely used in Ayurveda, is rich in isoquinoline alkaloids, principally berberine. Berberine activates AMPK, improves insulin-receptor signaling, promotes GLUT4 translocation, inhibits hepatic gluconeogenesis, modulates lipid metabolism, and exerts antioxidant, anti-inflammatory, hepatoprotective, nephroprotective, and cardioprotective effects [5]. *Hamelia patens* Jacq. (firebush) contains flavonoids, phenolic acids, tannins, alkaloids, and iridoids that confer antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and antihyperglycemic activities, protecting β -cells from oxidative damage and supporting insulin secretion [6].

Oxidative stress is central to T2DM pathogenesis: chronic hyperglycemia generates excess reactive oxygen species that damage β -cells, promote insulin resistance, endothelial dysfunction, and diabetic complications [7]. Agents combining antihyperglycemic and antioxidant actions therefore offer therapeutic advantages. Herbal synergism—where phytochemicals from different plants enhance efficacy, bioavailability, or safety while modulating multiple pathways—is increasingly recognized as a rational strategy for complex metabolic diseases [8]. Although both plants show individual promise, systematic evaluation of their combined synergistic potential in validated T2DM models remains limited [9]. The high-fat diet plus low-dose streptozotocin

(HFD-STZ) rat model closely recapitulates human T2DM by inducing insulin resistance together with partial β -cell destruction, chronic hyperglycemia, oxidative stress, and dyslipidemia. The present study therefore investigates the synergistic antidiabetic activity of *Berberis aristata* and *Hamelia patens* extracts, alone and in combination, in this model [10]. Outcomes assessed include body weight, fasting blood glucose, urine and kidney-function markers, lipid profile, glycated hemoglobin (HbA1c), phytochemical composition, and pancreatic histopathology. The findings aim to supply experimental support for a standardized polyherbal approach that may improve glycemic control and mitigate metabolic complications of T2DM with reduced reliance on synthetic drugs [11].

The present study was thus conducted to explore the synergistic antidiabetic activity of the mixture of *Berberis aristata* and *Hamelia patens* extracts in a diabetic model induced by high fat diet and STZ in rats [12]. The findings of the study indicate that changes in body weight, fasting blood glucose, urine parameters, kidney function markers, lipid profile, glycated hemoglobin (HbA1c) [13], phytochemical profile and pancreatic histopathology were evaluated after treatment with the individual/herbal and combined herbal extracts comprehensively [14]. The results will be useful to provide experimental support for the development of an effective polyherbal therapeutic approach in the management of type 2 diabetes mellitus (T2DM) and associated metabolic complications [15, 29].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The experimental diabetes was induced by streptozotocin (STZ) treatment. Gliclazide (Abbott Healthcare Pvt. Ltd., India) was used as the reference antidiabetic drug. Commercial diagnostic kits for blood glucose level, triglyceride level, total cholesterol level and biochemical parameters were purchased from Span Diagnostics, Surat, India. Reduced glutathione (GSH), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), N-(1-naphthyl) ethylenediamine dihydrochloride and formaldehyde were purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Hematoxylin and eosin (H&E) stains were purchased from V.K. Chemicals (Ambala, India). All chemicals and reagents were used throughout the study except laboratory grade and freshly prepared before use [16].

2.2 Collection of Plant

Fresh bark of *Berberis aristata* DC. (Family: Berberidaceae) were gathered in Dehradun, Uttarakhand, India. Stems of *Hamelia patens* Jacq. (Family: Rubiaceae) were collected. The plants of these species (Family: Rubiaceae) were collected in Meerut district, Uttar Pradesh, India in February-April 2023. Botanical identification and authentication were done by the Department of Pharmacognosy, Chaudhary Charan Singh University, Meerut, Uttar Pradesh. The voucher

specimens are lodged in the departmental herbarium with reference number Bot/PB/294.

2.3 Preparation of extract

The collected plant materials were washed using distilled water, shade dried (at ambient temperature) and then ground into coarse powder using mechanical grinding method [37]. *B. aristata* was extracted as powdered bark while *H. patens* were extracted as stem using Soxhlet extractor with distilled water as the solvent. The extracts were concentrated by using rotary evaporator under reduced pressure to get rid of all the solvent and then dried to get residues in solid form. The dried extracts were kept for pharmacological evaluation in air-tight containers at 4°C. The two extracts in equal amount was made into polyherbal combination extract for the experimental study [17]. The percentage yield was calculated, followed by preliminary phytochemical screening of the extract. Petroleum ether, chloroform, ethanol and aqueous extracts were extracted and the percentage yield calculated from the dried extract weight to the dried plant material [18]. Qualitative preliminary phytochemical screening was carried out to identify alkaloids, carbohydrates, flavonoids, proteins, amino acids, steroids and saponins, using standard pharmacognostic methods. Phytochemical profile of the combined extract was used to correlate the observed pharmacological activities with the presence of bioactive secondary metabolites [19].

2.4 Physicochemical Evaluation

The standard physicochemical parameters such as the total ash, acid-insoluble ash and loss on drying were determined as per the standard procedures laid down in Indian Pharmacopeia and WHO. The analyses were performed to assess the quality, purity and authenticity of the crude plant materials prior the pharmacological investigations [20].

2.5 Experimental Animals

Wistar Albino rats of adult age (200-250 g) were purchased from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India (Receipt No. 3099). Animals were kept in polypropylene cages in a controlled laboratory environment ($24 \pm 1^\circ\text{C}$, relative humidity $50 \pm 10\%$, and a 12 h light-dark cycle). The standard laboratory pellet diet and filtered drinking water were fed to them ad libitum throughout the study. Acclimatization was carried out for one week prior to experiments [21].

2.6 Ethical Approval

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of IIMT College of Medical Sciences, IIMT University, Meerut, India (Approval No. IIMTU/CMS/IAEC/2021-22/007). All experimental approaches were carried out according to the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules of Gov. of India [43].

2.7 Induction of Type 2 Diabetes.

Diabetes mellitus type 2 was induced by a high-fat diet (HFD) and streptozotocin (STZ). Diabetes was induced in rats fed a high-fat diet that provided about 60% of their calories from fat and 20% of their calories from carbohydrate[47]. Streptozotocin was freshly prepared in citrate buffer and injected intraperitoneally (i.p.) in a dose of 50 mg/kg body weight. Blood glucose was measured by a glucometer on the 7th day after injection. Fasting blood glucose (FBG) greater than 200 mg/dL was considered diabetic, and the animals were included in the experimental study[23].

2.8 Experimental Design

Group I: (Normal control) rats received normal saline water was administered orally.

Group II: (Diabetic control) rats received streptozotocin (50mg/kg) was injected intraperitoneally as a single dose.

Group III: (Standard) Diabetic rats received gliclazide (10 mg/kg) once daily, orally after 15 days of streptozotocin treatment.

Group IV: Plant extract of *Barberis aristata* (100 mg/kg of body wt.) once daily, orally after 15 days of streptozotocin treatment.

Group V: Plant extract of *Hamelia patens* (100 mg/kg of body wt.) once daily, orally after 15 days of streptozotocin treatment to study the diabetic nature of rats.

Group VI: Combination of Plant extract of *Barberis aristata* and *Hamelia patens* (200 mg/kg of body wt.) once daily, orally after 15 days of streptozotocin treatment.

The combination of *B. aristata* 200 mg/kg (p.o.) and *H. patens* 200 mg/kg (p.o.) was administered to group VI. Group VI received combination of *B. aristata* 200 mg/kg (p.o.) and *H. patens* 200 mg/kg (p.o.). For six consecutive weeks treatments were given orally every day starting 15 days after STZ administration.

2.9 Determine body weight.

All experimental animals were weighed with a calibrated digital balance machine before treatment and then weekly until the end of the 6-week treatment. Metabolic recovery after treatment was measured by weight gain in the body[24].

2.10 Determine the fasting blood glucose.

Fasting blood sugar was taken every week after 8 hours fasting. Blood samples were taken from the tail vein using sterile lancets and glucose levels were analyzed using a validated glucometer and compatible glucose test strip. The following measurements were carried out under aseptic conditions during the experimental period[25].

2.11 Urine Analysis

Urine samples were collected by gentle abdominal massage at specific times after birth. Physical parameters such as volume, colour and appearance were noted and subsequently biochemical parameters such as glucose,

albumin, and presence of ketone bodies, pus cells, epithelial cells, red blood cells, crystals and mucus threads were examined by routine clinical laboratory tests[26].

2.12 Biochemical Investigations

Overnight fasted animal were sacrificed at the end of experimental period under anesthesia and blood was drawn from lateral tail vein[53]. Serum was collected after centrifugation at 10,000 rpm for 10 minutes and biochemical parameters such as fasting blood glucose, glycated hemoglobin (HbA1c), total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, blood urea, creatinine, uric acid, albumin, and globulin were measured using commercially available diagnostic kits according to the manufacturer's instructions[27].

2.13 Histopathological Evaluation

The animals were killed using cervical dislocation method which was recommended by CPCSEA after they were drawn for blood. The pancreatic tissues were then removed immediately and fixed in 10% neutral buffered formalin, processed in a series of graded ethanol and xylene, embedded in paraffin wax and cut into 5 μ m sections. All tissue sections were stained with hematoxylin and eosin (H&E) and evaluated using a binocular light microscope for the following parameters: pancreatic architecture, islets of Langerhans, β -cell morphology, inflammatory infiltration, vacuolation, and cellular degeneration. Histopathological comparisons were made among the treatment groups by representative

Table 1. Percentage yield of different solvent extracts of the combined *Berberis aristata* bark and *Hamelia patens* stem:

S. No	Solvent	Percentage yield (%)
1	Petroleum ether	4.46
2	Chloroform	3.68
3	Ethanol	14.30
4	Distilled water	20.07

Percentage yield obtained from successive solvent extraction of the combined herbal material using solvents of increasing polarity.

3.2 Preliminary Phytochemical Screening

Qualitative phytochemical screening demonstrated that solvent polarity substantially influenced the extraction of secondary metabolites (Table 2). The aqueous extract showed the richest phytochemical profile, exhibiting abundant alkaloids (+++), carbohydrates (+++), flavonoids (++), proteins/amino acids (++), and detectable steroids (+), whereas saponins were absent. Ethanolic extracts contained moderate quantities of alkaloids,

Table 2. Preliminary phytochemical screening of the combined extracts of *Berberis aristata* bark and *Hamelia patens* stem.

S. NO	Phytoconstituent	Petroleum Ether	Chloroform	Ethanol	Aqueous
1	Alkaloids	++	+	++	+++
2	Carbohydrates	+	+	++	+++
3	Flavonoids	—	—	+	++
4	Proteins/Amino acids	—	—	+	++
5	Steroids	—	—	—	+
6	Saponins	—	—	—	—

photomicrographs[28].

2.14 Statistical Analysis

Experimental data are presented as mean $\pm$ standard error of the mean (SEM). GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, CA, USA) was used for statistical analyses. The one-way analysis of variance (ANOVA) test was used in inter group comparisons, fitted with a proper multiple comparison test. The differences were deemed to be statistically significant when $p < 0.05$.

3. RESULTS

3.1 Percentage Yield of Different Solvent Extracts

The extraction efficiency of the combined *Berberis aristata* bark and *Hamelia patens* stem was significantly different depending upon the polarity of the solvent used (Table 1). The percentage obtained from various solvents used investigated was highest in aqueous extract (20.07%), followed by ethanolic extract (14.30%), petroleum ether extract (4.46%) and chloroform extract (3.68%). This suggests that the plant materials mainly contain polar or moderately polar phytoconstituents, as confirmed by the higher recovery amounts obtained with the aqueous and ethanolic solvents. These results led to the choice of aqueous extract for further pharmacological investigation as it was found to be the most effective extractant and able to isolate various bioactive components.

carbohydrates, flavonoids, and proteins, while petroleum ether and chloroform extracts yielded only limited amounts of alkaloids and carbohydrates. The predominance of alkaloids and flavonoids in the aqueous extract is particularly important because these compounds are recognized for their antihyperglycemic, antioxidant, anti-inflammatory, and β -cell protective activities. The presence of multiple bioactive phytoconstituents suggests that the observed pharmacological effects of the polyherbal formulation are likely mediated through complementary and synergistic mechanisms rather than by a single active constituent

Symbols: (+) Present; (++) Moderately present; (+++) Abundantly present; (–) Absent

Qualitative phytochemical constituents detected in different solvent extracts of the combined herbal formulation.

3.3 Physicochemical Evaluation

The physicochemical characteristics of the combined herbal formulation complied with standard pharmacognostic quality requirements (Table 3). Parameters including loss on drying, total ash, and acid-insoluble ash were determined to evaluate purity, moisture content, and inorganic contamination of the crude drug.

Table 3. Physicochemical parameters of the combined *Berberis aristata*–*Hamelia patens* formulation.

S. NO	Parameter	<i>Berberis aristata</i> bark	<i>Hamelia patens</i> stem
1	Loss on drying	5.45	4.9
2	Total ash	1.21	1.02
3	Acid-insoluble ash	7.11	5.86

Physicochemical quality control parameters of the combined herbal formulation.

3.4 Effect of Herbal Treatment on Body Weight

Body weight changes observed during the six-week treatment period are summarized in Table 4 and Figure 1. Streptozotocin administration produced progressive body weight loss in the diabetic control group, reflecting impaired glucose utilization, enhanced protein catabolism, dehydration, and increased lipid mobilization associated with uncontrolled diabetes. In contrast, rats treated with gliclazide, *Berberis aristata*, *Hamelia patens*, and particularly the combined extract exhibited progressive recovery of body weight throughout the experimental period. The greatest improvement was observed in the combination-treated group, whose body weight increased from 209.0 ± 1.15 g at treatment initiation to 248.3 ± 1.60

g after six weeks, whereas diabetic control animals declined from 209.2 ± 1.34 g to 187.2 ± 1.46 g during the same period. The magnitude of weight recovery produced by the combination therapy was comparable to that observed in the gliclazide-treated group and exceeded the improvements produced by either individual extract.

The restoration of body weight suggests improved glycemic control, enhanced nutrient utilization, reduced protein degradation, and recovery of metabolic homeostasis after treatment. These findings indicate that the combined extract effectively attenuated diabetes-induced cachexia and provided superior metabolic protection compared with monotherapy, supporting a synergistic interaction among the phytoconstituents of *Berberis aristata* and *Hamelia patens*.

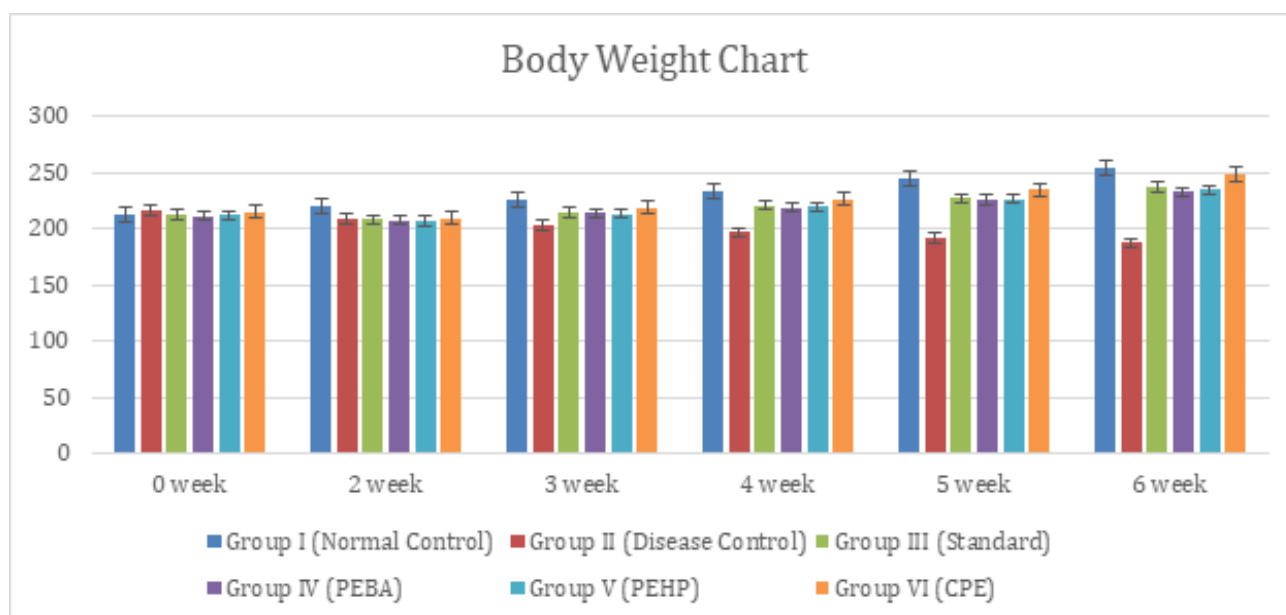


Figure 1. Effect of *Berberis aristata* and *Hamelia patens* extracts on body weight in streptozotocin-induced diabetic rats during the 6-week treatment period.

Figure 2 shows body-weight changes over six weeks. Diabetic rats declined from 216.83 ± 2.03 to 187.17 ± 1.46 g ($p < 0.001$), indicating metabolic impairment. CPE treatment increased body weight from 214.83 ± 1.86 to 248.33 ± 1.60 g ($p < 0.001$ vs. diabetic control), approaching normal (254.33 ± 2.11 g). Individual extracts and standard drug also improved body weight, demonstrating restoration of nutrient utilization and metabolic homeostasis.

3.5 Effect of the Combined Extract on Urine Output

Polyuria is a characteristic manifestation of uncontrolled diabetes mellitus resulting from osmotic diuresis caused by persistent hyperglycemia. As shown in Table 5 and Figure 2, streptozotocin-induced diabetic rats exhibited a marked increase in urine output throughout the experimental period compared with the normal control group. Urine volume in the diabetic control group progressively increased from 7.25 ± 0.51 mL at baseline to 14.20 ± 0.52 mL after six weeks, indicating severe impairment of glucose homeostasis and renal water

conservation.

Administration of gliclazide, *Berberis aristata*, *Hamelia patens*, and particularly their combined extract significantly attenuated diabetes-induced polyuria. The combination-treated group demonstrated the greatest reduction in urine output, decreasing from 11.42 ± 0.31 mL at Week 2 to 8.98 ± 0.18 mL at Week 6, approaching values observed in the normal control group (9.60 ± 0.32 mL). Although individual plant extracts also reduced urine volume, their effects were comparatively less pronounced than those produced by the combined formulation. The observed reduction in urine output is consistent with improved glycemic regulation and reduced glucosuria, suggesting restoration of renal tubular function and overall metabolic homeostasis. These findings indicate that the combined herbal formulation effectively alleviated one of the major clinical manifestations of diabetes and exhibited therapeutic efficacy comparable with the standard drug gliclazide.

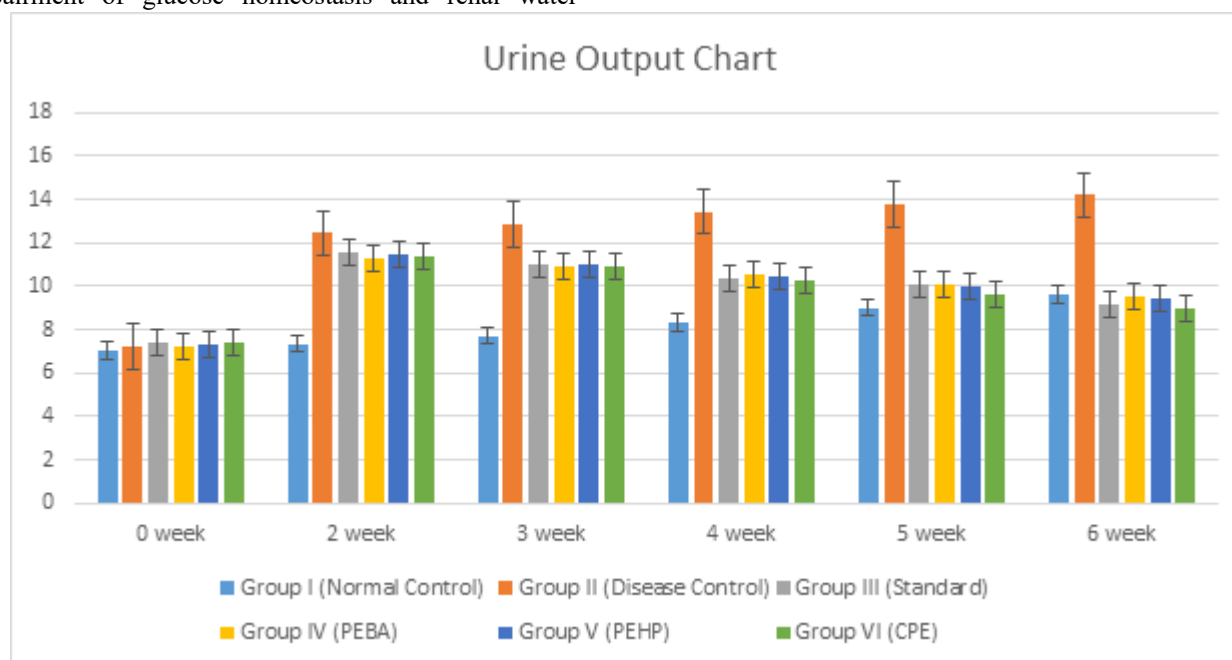


Figure 2. Effect of *Berberis aristata* and *Hamelia patens* extracts on urine output in streptozotocin-induced diabetic rats during the 6-week treatment period.

Figure 2 depicts urine output over six weeks. Diabetic rats showed progressive polyuria, increasing from 7.65 ± 0.39 to 14.20 ± 0.52 mL ($p < 0.001$). CPE treatment reduced urine output from 7.42 ± 0.31 to 8.98 ± 0.18 mL ($p < 0.001$ vs. diabetic control), approaching normal (9.60 ± 0.26 mL). Individual extracts and standard drug also reduced polyuria, indicating improved glycemic control and renal function.

3.6 Effect of the Combined Extract on Fasting Blood Glucose

The antihyperglycemic activity of the herbal extracts was evaluated by monitoring fasting blood glucose levels over six weeks (Table 6 and Figure 3). Following streptozotocin administration, diabetic rats developed

marked hyperglycemia, with fasting blood glucose increasing from 117.17 ± 2.79 mg/dL to 357.00 ± 1.73 mg/dL after six weeks. In contrast, blood glucose levels remained within the normal physiological range in untreated healthy animals (129.67 ± 1.70 mg/dL).

Oral administration of gliclazide and the herbal extracts significantly reduced fasting blood glucose compared with diabetic controls. The greatest antihyperglycemic response was observed in the combination-treated group, where blood glucose progressively declined from 339.00 ± 1.63 mg/dL at Week 2 to 267.83 ± 2.27 mg/dL at Week 6. This reduction exceeded that achieved by gliclazide (290.17 ± 2.34 mg/dL) and by either *Berberis aristata* (299.67 ± 2.98 mg/dL) or *Hamelia patens* (294.83 ± 2.11

mg/dL) administered individually.

The progressive decline in blood glucose following combination therapy suggests synergistic enhancement of glycemic regulation, potentially through improved insulin secretion, increased peripheral glucose utilization,

inhibition of hepatic gluconeogenesis, and attenuation of oxidative stress-induced β -cell damage. Overall, the combined extract exhibited superior antihyperglycemic efficacy among all treatment groups and demonstrated efficacy comparable to, or exceeding, the standard drug.

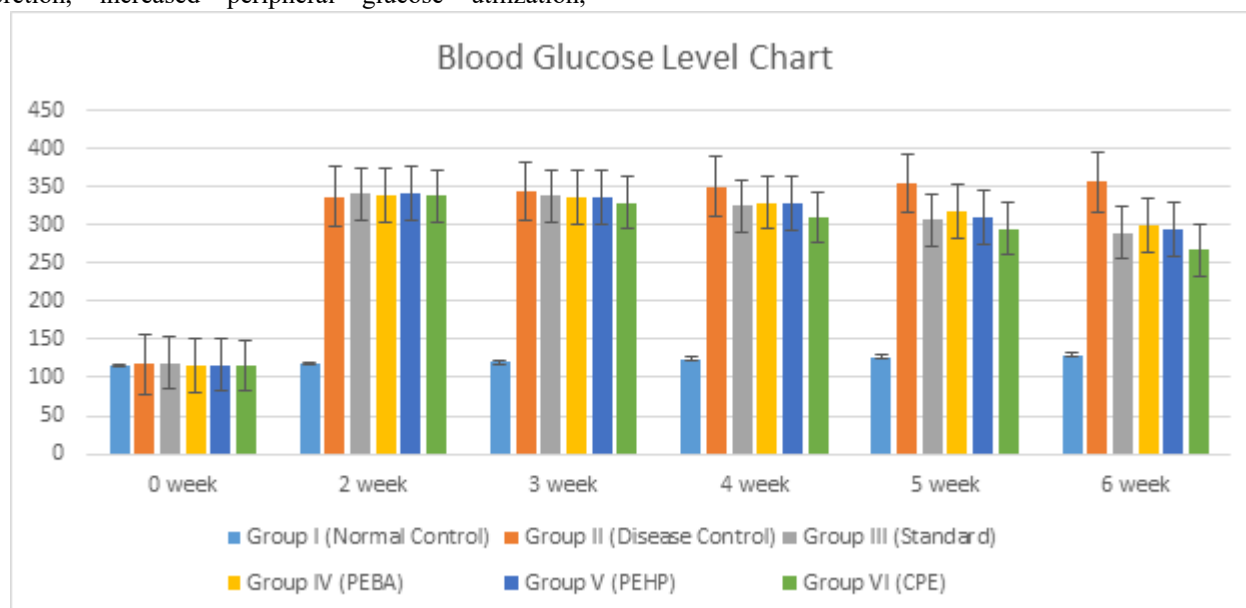


Figure 3. Effect of *Berberis aristata* and *Hamelia patens* extracts on blood glucose levels in streptozotocin-induced diabetic rats during the 6-week treatment period

. Figure 3 shows blood glucose changes over six weeks. Diabetic rats exhibited persistent hyperglycemia, increasing from 339.00 ± 1.63 to 357.00 ± 1.73 mg/dL ($p < 0.001$). CPE produced the greatest reduction, decreasing glucose from 339.00 ± 1.73 to 267.83 ± 2.27 mg/dL ($p < 0.001$ vs. diabetic control), compared with 290.17 ± 2.09 mg/dL for the standard drug. Individual extracts also significantly improved glycemic control, but less effectively

3.7 Effect of the Combined Extract on Kidney Function Biomarkers

The effects of different treatments on renal biochemical markers are summarized in Table 7 and Figure 4. Streptozotocin-induced diabetes markedly altered renal function, as evidenced by elevated blood urea (48.22 mg/dL), uric acid (8.40 mg/dL), albumin (5.20 g/dL), and globulin (3.92 g/dL) compared with the normal control

group, indicating diabetes-associated renal dysfunction. Treatment with gliclazide and the herbal extracts significantly improved renal biochemical parameters. Among all treatment groups, the combined extract produced the greatest normalization of renal function markers, reducing blood urea to 20.36 mg/dL, creatinine to 0.66 mg/dL, and uric acid to 3.89 mg/dL. Albumin levels returned to values comparable with normal controls (4.21 g/dL), while globulin concentrations were also reduced. These improvements indicate that the combined extract effectively protected renal tissues from hyperglycemia-induced oxidative and inflammatory injury. The nephroprotective activity observed may be attributed to the antioxidant phytochemicals present in *Berberis aristata* and *Hamelia patens*, which reduce oxidative stress, preserve glomerular filtration, and improve renal metabolic function.

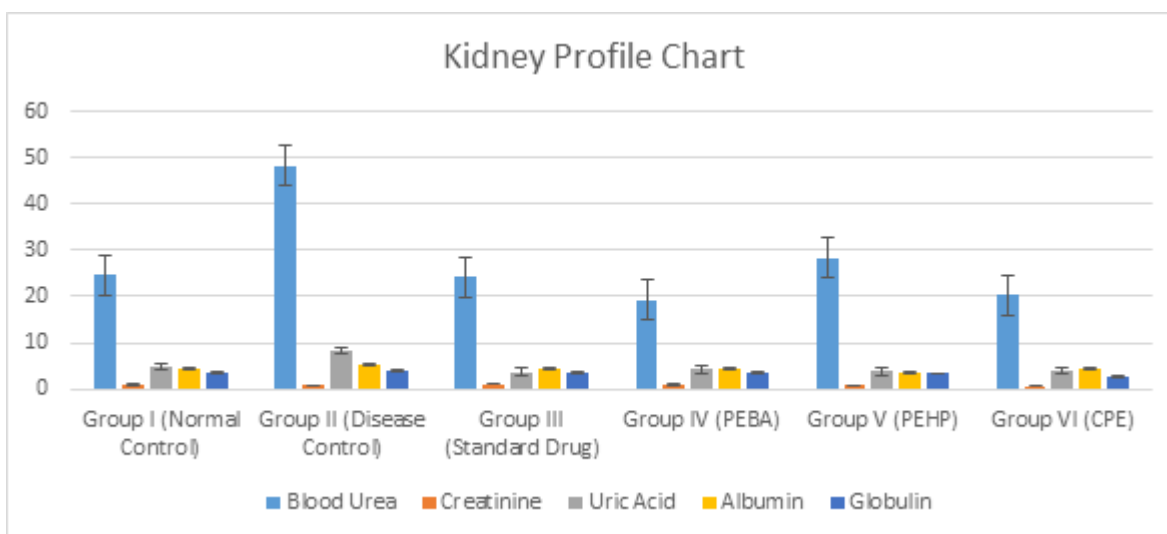


Figure 4 Effect of *Berberis aristata* and *Hamelia patens* extracts on kidney biochemical parameters in streptozotocin-induced diabetic rats

. Figure 4 shows renal biochemical changes in diabetic rats. Diabetic controls exhibited elevated blood urea (48.22 ± 3.12 mg/dL), creatinine (1.20 ± 0.12 mg/dL), uric acid (8.40 ± 0.62 mg/dL), albumin (5.20 ± 0.31 g/dL), and globulin (3.92 ± 0.24 g/dL). CPE significantly improved these parameters, reducing them to 20.36 ± 4.62 , 0.66 ± 0.11 , 4.02 ± 0.33 , 4.50 ± 0.31 , and 2.30 ± 0.12 , respectively ($p < 0.001$).

3.8 Effect of the Combined Extract on Lipid Profile

Diabetes-induced dyslipidemia was assessed by measuring serum triglycerides, HDL-cholesterol, and LDL-cholesterol (Table 8 and Figure 5). The diabetic control group exhibited marked disturbances in lipid metabolism, characterized by elevated triglyceride levels (182.3 mg/dL) and abnormal lipoprotein concentrations, confirming the development of diabetic dyslipidemia. Treatment with gliclazide and the herbal extracts improved lipid metabolism to varying degrees. The standard drug produced the greatest reduction in

triglycerides (94.36 mg/dL), whereas the combined extract also markedly reduced triglyceride concentration to 125.3 mg/dL, representing a substantial improvement compared with diabetic controls. The combined formulation additionally improved HDL- and LDL-cholesterol profiles, reflecting partial restoration of lipid homeostasis. Individual administration of *Berberis aristata* or *Hamelia patens* also exerted beneficial effects, although these were less pronounced than those achieved by combination therapy. The improvement in serum lipid profile following combined treatment may be attributed to enhanced insulin sensitivity, reduced hepatic lipogenesis, increased lipid utilization, and potent antioxidant activity of the phytoconstituents. Collectively, these findings demonstrate that the polyherbal formulation not only improved glycemic control but also effectively attenuated diabetes-associated dyslipidemia, thereby potentially reducing the risk of long-term cardiovascular complications.

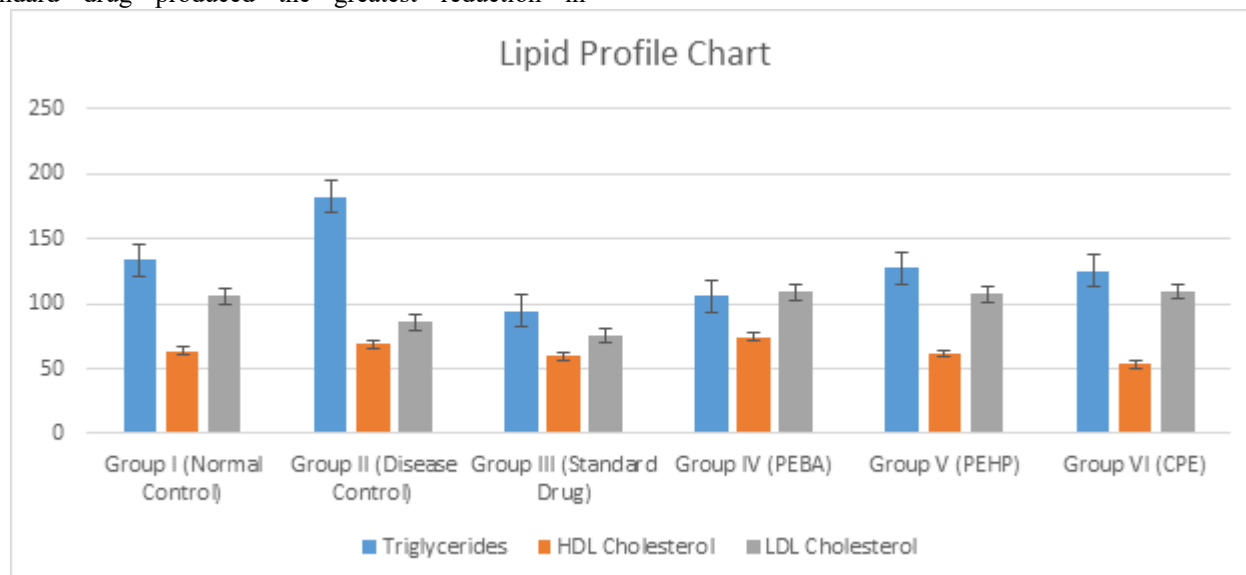


Figure 5. Effect of *Berberis aristata* and *Hamelia patens* extracts on lipid profile in streptozotocin-induced diabetic rats

Figure 5 shows serum lipid profiles in diabetic rats. Diabetic controls exhibited elevated triglycerides (182.30 ± 11.65 mg/dL) and LDL-cholesterol (84.26 ± 6.43 mg/dL) with reduced HDL-cholesterol (67.96 ± 2.94 mg/dL) ($p < 0.001$). CPE significantly improved lipid parameters, reducing triglycerides to 124.90 ± 13.55 mg/dL and LDL-cholesterol to 76.32 ± 6.08 mg/dL, while improving HDL-cholesterol to 53.14 ± 4.95 mg/dL. These findings demonstrate marked antihyperlipidemic activity.

3.9 Effect of the Combined Extract on Fasting Blood Glucose and Glycated Hemoglobin (HbA1c)

Long-term glycemic control was evaluated by measuring fasting blood glucose and glycated hemoglobin (HbA1c) at the end of the treatment period (Table 9 and Figure 6). Streptozotocin-induced diabetic rats exhibited marked elevations in both fasting blood glucose (159.1 mg/dL) and HbA1c (8.2%) compared with the normal control group (94.23 mg/dL and 4.6%, respectively), confirming persistent hyperglycemia and impaired glucose

metabolism. Treatment with gliclazide and the herbal extracts significantly improved both parameters. Among all treatment groups, the combined extract (CPE) produced the greatest improvement, reducing fasting blood glucose to 91.6 mg/dL and HbA1c to 4.6%, values that were essentially comparable with those of the normal control group. Individual treatment with *Berberis aristata* and *Hamelia patens* also reduced fasting blood glucose and HbA1c; however, the combination therapy demonstrated superior efficacy, suggesting a synergistic interaction between the two medicinal plants. Normalization of HbA1c indicates sustained improvement in glycemic control throughout the treatment period rather than a transient reduction in circulating glucose. The simultaneous reduction in fasting blood glucose and HbA1c further confirms that the combined extract effectively restored carbohydrate metabolism and may protect against chronic diabetic complications associated with prolonged hyperglycemia.

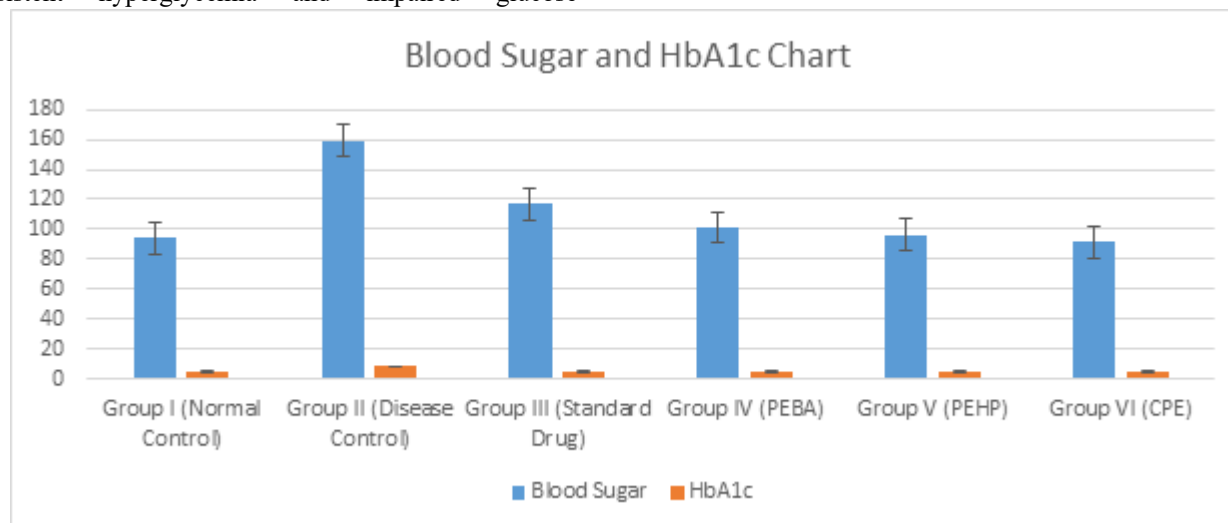
**Figure 6.** Effect of *Berberis aristata* and *Hamelia patens* extracts on fasting blood glucose and glycated hemoglobin (HbA1c) levels in streptozotocin-induced diabetic rats.

Figure 6 shows fasting blood glucose and HbA1c levels in diabetic rats. Diabetic controls exhibited elevated glucose (159.1 ± 9.80 mg/dL) and HbA1c ($8.2 \pm 0.61\%$) ($p < 0.001$). CPE significantly reduced fasting glucose to 91.6 ± 11.71 mg/dL and normalized HbA1c to $4.6 \pm 0.20\%$, comparable to normal controls (94.23 ± 11.92 mg/dL; $4.6 \pm 0.10\%$). These findings demonstrate improved sustained glycemic control and pancreatic β -cell function.

3.10 Urine Analysis and Microscopic Examination

Urinalysis was performed to assess diabetes-associated renal abnormalities and the therapeutic effects of the herbal treatments. As summarized in Tables 10 and 11, the diabetic control group demonstrated persistent glucosuria (24 mg/dL glucose) while maintaining acidic urine without detectable albumin or ketone bodies. In contrast, glucose was absent in the urine of all treatment groups, including animals receiving gliclazide, *Berberis aristata*,

Hamelia patens, and the combined extract, indicating effective correction of hyperglycemia-induced glucosuria.

Microscopic examination further demonstrated increased inflammatory changes in diabetic animals. The diabetic control group exhibited 4 pus cells/high-power field (HPF), whereas the normal control and gliclazide-treated groups showed only 1 cell/HPF. Animals treated with *Berberis aristata* also exhibited 1 cell/HPF, while *Hamelia patens* and the combined extract demonstrated only 2 cells/HPF, indicating a marked reduction in inflammatory cellular infiltration compared with untreated diabetic rats. Importantly, epithelial cells, red blood cells, crystals, and mucus threads were absent in all experimental groups. These findings indicate that the combined herbal formulation effectively prevented glucosuria and substantially improved urinary microscopic characteristics, reflecting improved glycemic regulation.

and preservation of renal integrity. The absence of albuminuria and ketonuria further suggests that treatment

protected against diabetes-associated renal dysfunction and metabolic decompensation.

Table 4. Macroscopic urine analysis following six weeks of treatment.

Groups	Volume	Appearance	Colour	Albumin	Sugar	Ketone
Group I (Normal Control)	10 ml	Clear	Pale Yellow	Nil	Nil	Nil
Group II (Disease Control)	10 ml	Clear	Dark Yellow	Nil	24 mg/dl	Nil
Group III (Standard Drug)	10 ml	Clear	Pale Yellow	Nil	Nil	Nil
Group IV (PEBA)	15 ml	Clear	Pale Yellow	Nil	Nil	Nil
Group V (PEHP)	15 ml	Clear	Pale Yellow	Nil	Nil	Nil
Group VI (CPE)	15 ml	Clear	Pale Yellow	Nil	Nil	Nil

Physical and biochemical characteristics of urine in experimental groups showing complete elimination of glucosuria following herbal treatment.

Table 5. Microscopic examination of urine sediment in experimental groups.

Groups	Reaction	Pus Cells	Epithelial Cell	RBC	Crystals	Mucus Threads
Group I (Normal Control)	Acidic	1/HPF	Nil	Nil	Nil	Nil
Group II (Disease Control)	Acidic	4/HPF	Nil	Nil	Nil	Nil
Group III (Standard Drug)	Acidic	1/HPF	Nil	Nil	Nil	Nil
Group IV (PEBA)	Acidic	1/HPF	Nil	Nil	Nil	Nil
Group V (PEHP)	Acidic	2/HPF	Nil	Nil	Nil	Nil
Group VI (CPE)	Acidic	2/HPF	Nil	Nil	Nil	Nil

Microscopic urine findings demonstrating reduced inflammatory cells following treatment and absence of epithelial cells, red blood cells, crystals, and mucus threads.

3.11 Histopathological Evaluation of Pancreatic Tissue

Histopathological examination of pancreatic tissue (Figure 7) provided morphological evidence supporting the biochemical findings. The pancreatic sections of the normal control group (Group I) exhibited well-preserved architecture characterized by compact, uniformly distributed islets of Langerhans surrounded by normally arranged acinar cells with granular eosinophilic cytoplasm. The endocrine and exocrine components remained intact, with negligible intercellular spaces and no evidence of inflammatory infiltration, necrosis, or fibrosis, indicating normal pancreatic morphology. In contrast, the diabetic control group (Group II) displayed severe pancreatic damage following streptozotocin administration. Histological alterations included marked architectural disorganization of the islets, cellular shrinkage, increased eosinophilia, cytoplasmic vacuolization, widening of the intercellular spaces, disruption of acinar tissue, and inflammatory cell infiltration. The islets of Langerhans appeared markedly reduced and poorly defined, indicating extensive β -cell destruction consistent with persistent hyperglycemia.

Treatment with gliclazide (Group III) produced substantial restoration of pancreatic morphology. The islets appeared larger and more organized than those observed in diabetic controls, with reduced inflammatory

changes and improved cellular arrangement. Residual architectural alterations were present but considerably less severe, indicating partial regeneration and protection of pancreatic β -cells. Administration of *Berberis aristata* (Group IV) and *Hamelia patens* (Group V) also improved pancreatic histology, demonstrating partial restoration of islet architecture, reduced vacuolar degeneration, and decreased intercellular widening. Nevertheless, minor structural abnormalities remained evident compared with the normal control group.

The combined extract (Group VI) produced the most pronounced histological recovery among all treatment groups. Pancreatic sections showed nearly normal islet morphology with well-defined endocrine tissue, closely arranged acinar cells, minimal inflammatory infiltration, and marked reduction of vacuolization and intercellular widening. The restoration of pancreatic architecture closely paralleled the improvements observed in fasting blood glucose, HbA1c, body weight, and other biochemical parameters, indicating effective preservation and regeneration of β -cell structure. Overall, histopathological findings confirmed that the combined extract exerted superior pancreatic protective activity compared with either individual extract and produced tissue recovery approaching that observed with the standard antidiabetic drug. The structural preservation of the islets of Langerhans provides morphological evidence supporting the synergistic antidiabetic potential of the *Berberis aristata*–*Hamelia patens* combination.

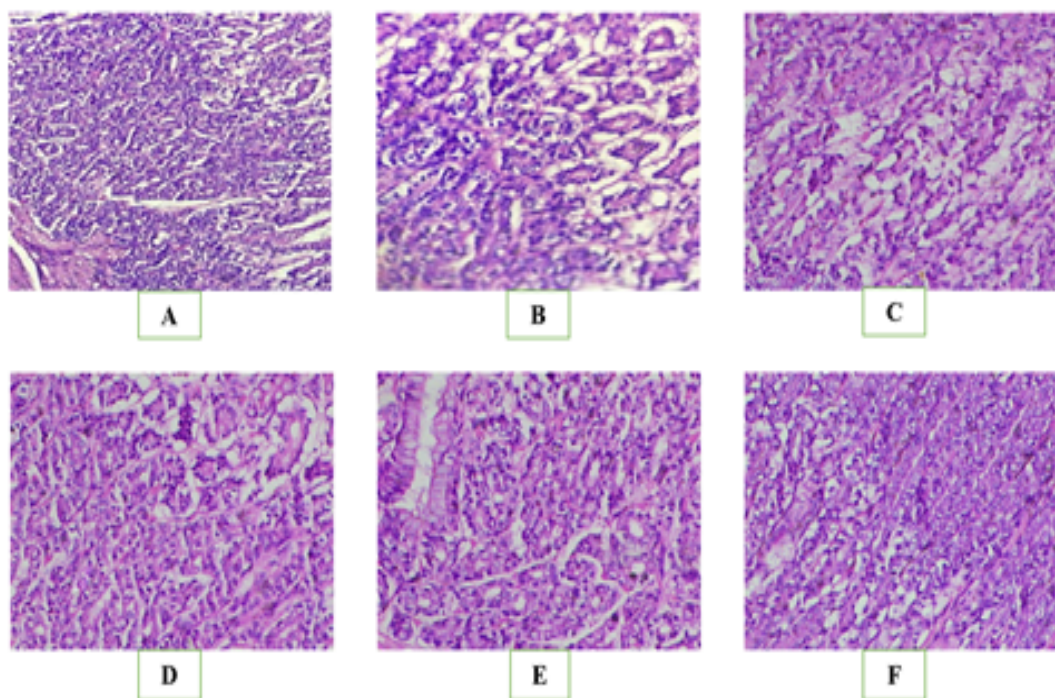


Figure 7. Representative photomicrographs of H&E-stained pancreatic sections from experimental groups.

(A) Normal control showing intact islets of Langerhans and normal acinar architecture; (B) diabetic control showing marked β -cell degeneration, islet shrinkage, vacuolization, widened intercellular spaces, and inflammatory infiltration; (C) gliclazide-treated group demonstrating partial restoration of pancreatic morphology; (D) *Berberis aristata*-treated group showing moderate improvement; (E) *Hamelia patens*-treated group exhibiting partial regeneration of islet architecture; and (F) combination-treated group showing near-normal pancreatic architecture with preserved islets of Langerhans, minimal inflammatory changes, and marked β -cell protection.

4. DISCUSSION

The combined aqueous extracts of *Berberis aristata* bark and *Hamelia patens* stem demonstrated significant antihyperglycemic, antihyperlipidemic, nephroprotective, and pancreatic protective effects in streptozotocin-induced diabetic rats, with greater efficacy than either individual extract. Extraction yielded 20.07% aqueous extract, 14.30% ethanolic extract, 4.46% petroleum ether extract, and 3.68% chloroform extract. Phytochemical screening of the aqueous extract revealed abundant alkaloids, carbohydrates, flavonoids, proteins, amino acids, and detectable steroids, supporting its multitarget pharmacological activity. Diabetic controls developed marked metabolic abnormalities, with blood glucose increasing to 357.0 ± 1.73 mg/dL, fasting blood glucose reaching 159.1 mg/dL, and HbA1c increasing to 8.2%. The combined treatment produced the strongest glycemic

improvement, reducing blood glucose from 339.0 ± 1.63 to 267.83 ± 2.27 mg/dL and normalizing fasting blood glucose to 91.6 mg/dL. HbA1c decreased to 4.6%, comparable to normal controls and indicating sustained glycemic control. Diabetes caused progressive body-weight loss from 216.83 ± 2.03 to 187.17 ± 1.46 g, whereas combination treatment increased body weight to 248.33 ± 1.60 g after six weeks, indicating improved energy and glucose utilization. Polyuria was also markedly reduced: urine volume decreased from 14.20 ± 0.52 mL in diabetic controls to 8.98 ± 0.18 mL following combination treatment. Urinary glucose disappeared in treated groups, while microscopic examination showed only minimal pus cells and no epithelial cells, erythrocytes, crystals, ketone bodies, or mucus threads. Renal protection was evident from reductions in blood urea from 48.22 to 20.36 mg/dL, creatinine to 0.66 mg/dL, and uric acid from 8.40 to 3.89 mg/dL. Diabetes-associated dyslipidemia was also improved, with triglycerides decreasing from 182.3 to 125.3 mg/dL; gliclazide produced a slightly greater triglyceride reduction to 94.36 mg/dL. HDL- and LDL-cholesterol profiles were also improved following combination treatment. Histopathological examination revealed severe β -cell degeneration, vacuolization, inflammatory infiltration, widened intercellular spaces, and disrupted islet architecture in diabetic controls. In contrast, combination-treated animals showed well-preserved islets of Langerhans, reduced vacuolization and inflammation, closely arranged acinar tissue, and marked β -cell preservation. Collectively, these findings indicate

synergistic antioxidant, antihyperglycemic, insulin-sensitizing, antihyperlipidemic, nephroprotective, and β -cell protective effects of the *B. aristata*–*H. patens* combination.

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