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Investigation of the Antidiabetic and Antioxidant Potential of Artabotrys hexapetalus Extract: In Vitro and In Vivo Evaluation with Phytochemical Profiling

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ABSTRACT

Background: Artabotrys hexapetalus (AH) is a medicinal plant reported to possess diverse phytoconstituents and pharmacological activities. However, integrated evidence regarding its antioxidant and antidiabetic potential, particularly using in vivo experimental models, remains limited. The present study investigated the phytochemical profile, antioxidant activity, and antidiabetic potential of a 70% hydroalcoholic extract of AH using complementary in vitro and in vivo approaches.

Methods: The 70% hydroalcoholic extract prepared from the leaves and bark of AH was subjected to HPTLC profiling using rutin, gallic acid, quercetin, and kaempferol as reference standards. Antioxidant activity was evaluated by DPPH and nitric oxide radical-scavenging assays, while α-amylase and α-glucosidase inhibition assays were used to assess in vitro antidiabetic activity. Acute oral toxicity was evaluated according to OECD guideline 423. In vivo antidiabetic activity was investigated in high-fat diet/streptozotocin-induced diabetic rats treated with 100, 200, and 400 mg/kg extract for four weeks. Fasting blood glucose, glucose tolerance, insulin tolerance, serum lipid profile, total protein, insulin, and pancreatic histopathology were evaluated.

Results: HPTLC fingerprinting revealed multiple phytoconstituents, with peaks corresponding to rutin, gallic acid, and quercetin, along with several additional chromatographic peaks. The extract demonstrated concentration-dependent antioxidant activity, with IC_{50} values of 30.813 µg/mL for DPPH and 26.923 µg/mL for nitric oxide scavenging. No mortality or treatment-related toxicity was observed up to 2000 mg/kg. In diabetic rats, Artabotrys hexapetalus treatment produced a dose-related reduction in fasting blood glucose, improved glucose tolerance, and enhanced glucose lowering following insulin challenge, with the 200 and 400 mg/kg doses showing greater effects. The 400 mg/kg dose also improved the diabetes-associated lipid abnormalities, increasing HDL and reducing LDL and triglycerides, while partially restoring serum total protein and insulin levels. Histopathological examination demonstrated improved pancreatic architecture and preservation of islet morphology at 400 mg/kg.

Conclusion: The findings indicate that the 70% hydroalcoholic extract of AH possesses appreciable antioxidant and antidiabetic potential in vitro and in vivo. The higher extract doses, particularly 400 mg/kg, produced consistent improvements in glycemic, lipid, insulin-related, and pancreatic histological parameters. These findings support further investigation of AH and its bioactive constituents as potential candidates for antidiabetic drug development.

KEYWORDS: Artabotrys hexapetalus, antidiabetic activity, antioxidant activity, type 2 diabetes mellitus, hydroalcoholic extract, α-amylase, α-glucosidase.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia arising from abnormalities in insulin secretion, insulin action, or both. The global burden of diabetes continues to increase; the International Diabetes Federation estimated that 589 million adults aged 20–79 years were living with diabetes in 2024, with the number projected to reach approximately 853 million by 2050 (International Diabetes Federation, 2025). Type 2 diabetes mellitus (T2DM) represents the predominant form and is characterized by a complex interaction between insulin resistance, progressive β-cell dysfunction, altered hepatic glucose metabolism, and other metabolic abnormalities (DeFronzo et al., 2015; American Diabetes Association Professional Practice Committee, 2026; Shaw et al., 2010; Wild et al., 2004; Guilherme et al.,

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2008; Yoon et al., 2006).

Chronic hyperglycemia is not only a consequence of impaired metabolic regulation but also an important contributor to progressive tissue injury. Excess glucose availability can increase the generation of reactive oxygen species (ROS), disturb cellular redox homeostasis, and promote molecular pathways associated with inflammation and vascular dysfunction. Oxidative stress has therefore been implicated in the pathogenesis and progression of several diabetic complications, including nephropathy, retinopathy, neuropathy, and cardiovascular disease (Brownlee, 2001). Consequently, therapeutic strategies capable of improving glycemic regulation while simultaneously attenuating oxidative stress may have value as complementary approaches in the management of diabetes (Evans et al., 2002).

Although several pharmacological classes are available for glycemic management, treatment of T2DM remains challenging because of the progressive and multifactorial nature of the disease. The limitations associated with long-term therapy, including adverse effects, treatment cost, polypharmacy, and variable therapeutic response, have sustained interest in the identification of safer and pharmacologically active natural products. Medicinal plants are particularly relevant in this context because they contain chemically diverse secondary metabolites that may act on multiple molecular targets. Plant-derived polyphenols, flavonoids, alkaloids, terpenoids, and related constituents have been investigated for antioxidant, anti-inflammatory, enzyme-inhibitory, and metabolic effects (Tiwari & Rao, 2002; Hanhineva et al., 2010).

The family Annonaceae is a chemically diverse group of predominantly tropical and subtropical flowering plants. Molecular and taxonomic studies recognize Artabotrys as a distinct and species-rich genus within the family, with approximately 100 species reported in the genus (Chatrou et al., 2012). Members of Artabotrys have a long history of ethnomedicinal use, and phytochemical investigations have identified a wide range of metabolites, including alkaloids, terpenoids, sterols, flavonoids, acetogenins, and other specialized natural products. A comprehensive review of the genus has documented antioxidant and antidiabetic activities among the reported pharmacological properties of Artabotrys species (Hop & Son, 2022).

Artabotrys hexapetalus, commonly known as Hari Champa, is a medicinal species of the Annonaceae family distributed in parts of the Indian subcontinent and other regions of South and Southeast Asia. The plant has been used in traditional medicine for several ailments, and different parts of the plant have been investigated for biologically active constituents. Reviews of AH describe a diverse phytochemical profile and report pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, antileishmanial, and other biological effects (Puri, 2020; Kousalya & Doss, 2021). Chemical investigations have identified several alkaloids and other secondary metabolites from the species, supporting its potential as a source of pharmacologically relevant natural products (Li & Yu, 1998).

The antioxidant potential of AH has received increasing experimental attention. Rahman et al. (2023) reported that leaf extracts of AH contained appreciable phenolic, flavonoid, and tannin contents and demonstrated free-radical-scavenging and reducing activities in a range of in vitro assays. These findings are relevant to diabetes research because oxidative stress is closely associated with chronic hyperglycemia and may contribute to tissue injury. In addition, Bajaj et al. (2018) developed and validated an HPTLC method for simultaneous analysis of quercetin and apigenin in AH extracts, demonstrating the value of chromatographic standardization for characterization of this medicinal species (Singleton & Rossi, 1965; Zhishen et al., 1999).

More recently, evidence has emerged specifically regarding the antihyperglycemic potential of A. hexapetalus. Edmond et al. (2026) evaluated AH and A. siamensis leaf extracts and reported inhibition of key carbohydrate-metabolizing enzymes, including α-amylase and α-glucosidase, together with identification of major metabolites in A. hexapetalus. While these findings strengthen the rationale for investigating the species in the context of glucose metabolism, they remain focused on in vitro and in silico mechanisms. Evidence integrating antioxidant activity, carbohydrate-digesting enzyme inhibition, phytochemical profiling, and sustained in vivo evaluation of diabetes-related metabolic outcomes remains comparatively limited.

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Therefore, a systematic evaluation of AH using an integrated in vitro and in vivo experimental framework is warranted. In the present study, the hydroalcoholic extract of AH was investigated for its antioxidant and antidiabetic potential using complementary in vitro assays, followed by evaluation in an experimental model of type 2 diabetes. Phytochemical profiling was incorporated to identify characteristic bioactive constituents and to provide a chemical basis for the observed biological effects. The study was designed to examine the relationship between the phytochemical composition of AH extract and its potential effects on glycemic regulation and oxidative stress, thereby providing experimental evidence to support further investigation of the extract as a potential plant-derived antidiabetic candidate.

MATERIALS AND METHODS

Materials

Collection and Authentication of Plant Material

Mature and healthy leaves and bark of AH were collected from a home garden during November to February. Only mature and healthy trees without visible pest infestation, disease, or physical damage were selected. Approximately 1–2 kg of leaves and bark were collected collectively and placed in clean, labelled cotton bags. The material was authenticated by Dr. D. S. Dongarwar, Professor and Head, Department of Botany, Rashtra Sant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India. The authenticated specimen was assigned authentication number 1032. Representative specimens were prepared as herbarium sheets and deposited in the Herbarium of the Department of Botany, R.T.M. Nagpur University, Nagpur.

Chemicals and Kits

Streptozotocin (STZ; Catalogue No. 18883–66–4) was procured from Sigma-Aldrich. Commercial diagnostic kits supplied by Coral Clinical System were used for estimation of serum glucose (Catalogue No. 1102113150), low-density lipoprotein (LDL; Catalogue No. 1102170040), high-density lipoprotein (HDL; Catalogue No. 1102150040), total cholesterol (TC; Catalogue No. 1102050075), triglycerides (TG; Catalogue No. 1102230020), and total protein (Catalogue No. 1101201150). Rat insulin was estimated using an ELISA kit from Elabscience (Catalogue No. E-EL-R2466).

Instruments

Biochemical parameters were analyzed using a CoralLab autoanalyzer. Blood glucose concentrations were measured using an OneTouch glucometer. Insulin concentrations were determined using a Bio-Rad iMark ELISA plate reader. Tissue sections were examined using a Nikon E1 microscope.

Experimental Animals

Adult male Sprague-Dawley rats weighing 200–220 g were used for the type 2 diabetes experiment. Animals were obtained from a CPCSEA-registered breeder and acclimatised for one week before experimentation. The animals were housed individually in polypropylene cages at 22 ± 2 °C temperature and 70 ± 5% relative humidity under a 12 h light/dark cycle, with standard feed and drinking water provided ad libitum. All experimental procedures were approved by the Institutional Animal Ethics Committee and conducted according to CCSEA guidelines. Protocol approval number (IAEC/CPD/415/13).

METHODS

Preparation of the Hydroalcoholic Extract

The collected leaves and bark of AH were cleaned to remove surface debris and dust, shade-dried at room temperature (25–30 °C) for 10–12 days until constant weight was obtained, and coarsely powdered using a mechanical grinder. The powdered material was defatted with n-hexane at a ratio of 1:3 (50 g:150 mL) at room temperature for 24 h with occasional stirring. After filtration, the defatted residue was air-dried completely. The dried defatted powder was extracted in a Soxhlet apparatus using 70% ethanol (ethanol:water, 70:30, v/v) at a solvent-to-solid ratio of 10:1 (50 g:500 mL). For the combined bark and leaf material, extraction was continued for 12–18 h at 60–65 °C, with completion indicated by a colourless or very pale siphon extract. The solvent was removed under reduced pressure using a rotary evaporator at a temperature not exceeding 45 °C.

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The crude extract was stored in an airtight, light-resistant container under refrigeration (Harborne, 1998).

HPTLC-Based Phytochemical Profiling

The 70% hydroalcoholic extract of AH was subjected to high-performance thin-layer chromatography (HPTLC) for preliminary phytochemical profiling following the methodology described by Wagner and Bladt (1996). Chromatographic separation was performed on pre-coated silica gel 60 F254 plates measuring 10 × 10 cm. The mobile phase consisted of toluene, ethyl acetate, and formic acid in a ratio of 5.4:3.8:0.8 (v/v/v). Quercetin, gallic acid, kaempferol, and rutin were employed as reference standards. Densitometric analysis was carried out using a CAMAG TLC Scanner at 254 nm in absorption/remission mode. The constituents present in the extract were tentatively assigned by comparing the Rf values of sample peaks with those obtained from the corresponding reference standards.

In Vitro Antioxidant Activity

The antioxidant potential of the AH tincture was evaluated using DPPH radical-scavenging and nitric oxide (NO) scavenging assays. For the DPPH assay, different concentrations of the extract ranging from 20–100 µg/mL were evaluated, with ascorbic acid serving as the reference antioxidant. Absorbance was measured at 517 nm. The concentration required to produce 50% inhibition (IC_{50}) was calculated from the respective concentration–response relationship. Nitric oxide scavenging activity was assessed using sodium nitroprusside followed by reaction with Griess reagent. Absorbance was recorded at 546 nm, and the IC_{50} value was determined from the concentration–response curve (Apak et al., 2013).

In Vitro Antidiabetic Activity

The in vitro antidiabetic potential of the AH tincture was investigated through α-amylase and α-glucosidase inhibition assays. α-Amylase inhibitory activity was determined at extract concentrations of 100–500 µg/mL using the dinitrosalicylic acid (DNSA) method, with absorbance measured at 540 nm. α-Glucosidase inhibitory activity was evaluated using extract concentrations ranging from 1–1000 µg/mL and p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate. Absorbance was measured at 405 nm. Acarbose was used as the positive control in both enzyme inhibition assays (Kwon et al., 2008; Kumar et al., 2011; Holman et al., 1999).

Acute Oral Toxicity Study

The acute oral toxicity of the AH tincture was assessed in female Sprague-Dawley rats weighing 180–220 g in accordance with OECD Guideline 423, Acute Oral Toxicity-Acute Toxic Class Method. Three animals were assigned to each of the 300 and 2000 mg/kg dose groups for the AH study. Animals were acclimatised to laboratory conditions for five days before dosing and were observed at least once during the first 30 min, periodically during the first 24 h, and daily thereafter for 14 days for mortality, treatment-related clinical signs, and changes in body weight. At the end of the observation period, animals receiving the 2000 mg/kg limit dose were subjected to terminal histopathological examination as specified in the experimental protocol.

Preparation of Extract for Oral Administration

The concentrated 70% hydroalcoholic extract was maintained as a tincture at 4 °C. Immediately before administration, the tincture was appropriately diluted with 0.5% carboxymethylcellulose (CMC) to obtain the required doses of 100, 200, and 400 mg/kg body weight. The freshly prepared formulations were administered orally by gastric gavage, with the administration volume maintained between 0.5 and 1 mL per animal.

Induction of Type 2 Diabetes Mellitus

Type 2 diabetes mellitus was induced by combining high-fat diet feeding with low-dose streptozotocin (STZ). Following acclimatization, the diabetic groups were maintained on the high-fat diet for a total duration of 9 weeks. The diet contained cholesterol (1%), lard (10%), egg yolk powder (10%), milk powder (6%), carbohydrate (21%), fat (40%), and protein (12%). Body weight and fasting blood glucose were monitored weekly. At the end of the third week of high-fat diet feeding, animals were fasted overnight for approximately 16 h with free access to water and administered a single intraperitoneal injection of STZ at 35 mg/kg body weight. STZ was freshly prepared in ice-cold 0.1 M citrate buffer (pH 4.5). A 5% glucose solution was

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provided for 24 h after STZ administration to reduce hypoglycaemia-associated mortality. Seventy-two hours after STZ administration, fasting blood glucose was measured from tail-vein blood. Animals with fasting blood glucose >=250 mg/dL were considered diabetic and included in the treatment protocol. The diabetic animals continued to receive the high-fat diet throughout the remaining experimental period (Like & Rossini, 1976; Srinivasan et al., 2005; Skovsø, 2014).

Experimental Grouping and Treatment

Following confirmation of diabetes, the animals were randomly allocated into six experimental groups, with five animals in each group (n = 5): Group I, normal control + vehicle; Group II, diabetic control + vehicle; Group III, AH tincture, 100 mg/kg; Group IV, AH tincture, 200 mg/kg; Group V, AH tincture, 400 mg/kg; and Group VI, glibenclamide, 5 mg/kg body weight. Treatment commenced at week 6 and continued through week 9. The plant extract and glibenclamide were administered orally once daily by gastric gavage. Glibenclamide was suspended in 0.5% CMC, and the dosing volume was maintained between 0.5 and 1 mL per animal.

Monitoring of Body Weight and Fasting Blood Glucose

Body weight and fasting blood glucose levels were monitored at regular weekly intervals throughout the experimental period. Before FBG determination, animals were subjected to a 5 h fasting period. Blood samples were obtained from the tail vein, and glucose concentrations were measured using a commercial glucose-testing system.

Intraperitoneal Glucose Tolerance Test (IPGTT)

In the second week of the treatment period, an intraperitoneal glucose tolerance test (IPGTT) was performed. Animals were fasted for 5 h with free access to water, and baseline blood glucose was measured from tail-vein blood. Glucose was administered intraperitoneally at a dose of 2 g/kg body weight. Blood glucose concentrations were subsequently measured at 15, 30, 60, and 120 min after glucose administration. The glucose tolerance profiles were compared among the experimental groups based on the changes in blood glucose concentrations following glucose administration (Ayala et al., 2010).

Intraperitoneal Insulin Tolerance Test (IPITT)

In the second week of the treatment period, an intraperitoneal insulin tolerance test (IPITT) was performed. Animals were fasted for approximately 5 h before the test, and baseline blood glucose was recorded before insulin administration. Insulin was administered intraperitoneally at a dose of 0.75 U/kg body weight. Blood glucose concentrations were measured from tail-vein samples at 0, 15, 30, 60, and 120 min after insulin administration. The decline in blood glucose following insulin administration was used as an indicator of insulin sensitivity.

Blood Collection and Biochemical Analysis

At the end of the experimental period, blood samples were collected from the retro-orbital plexus under appropriate anaesthesia. The samples were allowed to clot and were centrifuged at 2500 rpm for 15 min. The separated serum was stored at −20 °C until biochemical analysis. Serum glucose, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, total protein, and insulin concentrations were determined using the respective commercial assay kits. Glucose was estimated by the glucose oxidase-peroxidase (GOD-POD) method; total cholesterol by the cholesterol oxidase-peroxidase (CHOD-PAP) method; triglycerides by the glycerol-3-phosphate oxidase-peroxidase (GPO-PAP) method; and total protein by the Biuret method. Insulin concentration was determined using a sandwich ELISA kit (Elabscience, Catalogue No. E-EL-R2466) according to the manufacturer's instructions (Allain et al., 1974; Fossati & Prencipe, 1982).

Histopathological Examination

Following blood collection, animals were humanely euthanised using an anaesthetic overdose. The pancreas was excised immediately and washed with ice-cold normal saline. The tissue was fixed in 10% neutral buffered formalin for 24–48 h, dehydrated through ascending grades of alcohol, cleared in xylene, and embedded in

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paraffin wax. Sections approximately 5 µm thick were prepared using a rotary microtome, stained with hematoxylin and eosin, and examined under a light microscope for histopathological alterations associated with diabetes and treatment (Suvarna et al., 2013).

Statistical Analysis

Experimental data were expressed as mean ± standard error of the mean (SEM). Longitudinal data for body weight, fasting blood glucose, IPGTT, and IPITT were analysed using two-way repeated-measures analysis of variance with group and time as factors, followed by Bonferroni's multiple-comparison test. Terminal biochemical parameters were analysed using one-way ANOVA followed by Bonferroni's multiple-comparison test. A probability value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

HPTLC-Based Phytochemical Profiling

HPTLC fingerprinting of the 70% hydroalcoholic extract of AH revealed multiple phytoconstituents, with distinct chromatographic peaks observed at Rf values of 0.02, 0.15, 0.32, 0.41, 0.48, 0.65, 0.71, 0.74, and 0.77 at 254 nm. Comparison of the chromatographic profile of the extract with the reference standards indicated peaks corresponding to rutin (Rf 0.02), gallic acid (Rf 0.13–0.15), and quercetin (Rf 0.65–0.72). Several additional peaks were observed at Rf values of 0.32, 0.41, 0.48, 0.74, and 0.77, suggesting the presence of other phytoconstituents in the extract. Although kaempferol was included as a reference standard, a corresponding extract peak within the reported kaempferol Rf range (0.83–0.88) was not observed in the present chromatographic profile. The resulting chromatographic pattern provides a characteristic HPTLC fingerprint of the 70% hydroalcoholic extract of A. hexapetalus.

Acute Oral Toxicity Study

No mortality or treatment-related overt toxicity was observed in female rats administered AH tincture at 300 or 2000 mg/kg during the 14-day observation period. Body weight increased from 189.00 ± 12.17 to 211.33 ± 14.19 g at 300 mg/kg and from 217.00 ± 3.61 to 233.33 ± 2.08 g at 2000 mg/kg. The recorded clinical observations remained within normal limits. Histopathological examination at the 2000 mg/kg dose did not reveal treatment-related microscopic abnormalities in the examined tissues. These findings support the absence of detectable acute toxicity at the tested limit dose and are consistent with OECD 423 (OECD, 2002; Parasuraman, 2011).

In Vitro Antioxidant Activity

DPPH Radical Scavenging Activity

The AH tincture showed concentration-dependent DPPH radical-scavenging activity. Inhibition increased from 49.147% at 20 µg/mL to 79.907% at 100 µg/mL, with an IC_{50} of 30.813 µg/mL. Ascorbic acid was more potent, with an IC_{50} of 8.500 µg/mL. The concentration–response pattern supports the presence of constituents capable of reducing the DPPH radical (Brand-Williams et al., 1995; Rahman et al., 2023).

Nitric Oxide Scavenging Activity

Nitric oxide scavenging also increased with extract concentration, from 51.372% inhibition at 20 µg/mL to 82.394% at 100 µg/mL. The IC_{50} was 26.923 µg/mL compared with 5.986 µg/mL for ascorbic acid. The response indicates appreciable nitric oxide-scavenging activity (Sreejayan & Rao, 1997).

In Vitro Antidiabetic Activity

The 70% hydroalcoholic extract of AH demonstrated concentration-dependent inhibition of both α-amylase and α-glucosidase activities. In the α-amylase assay, inhibition increased from 45.141% at 100 µg/mL to 75.404% at 500 µg/mL, with an IC_{50} value of 138.888 µg/mL. In the α-glucosidase assay, inhibition increased from 44.433% at 100 µg/mL to 68.652% at 500 µg/mL, with an IC_{50} value of 177.627 µg/mL.

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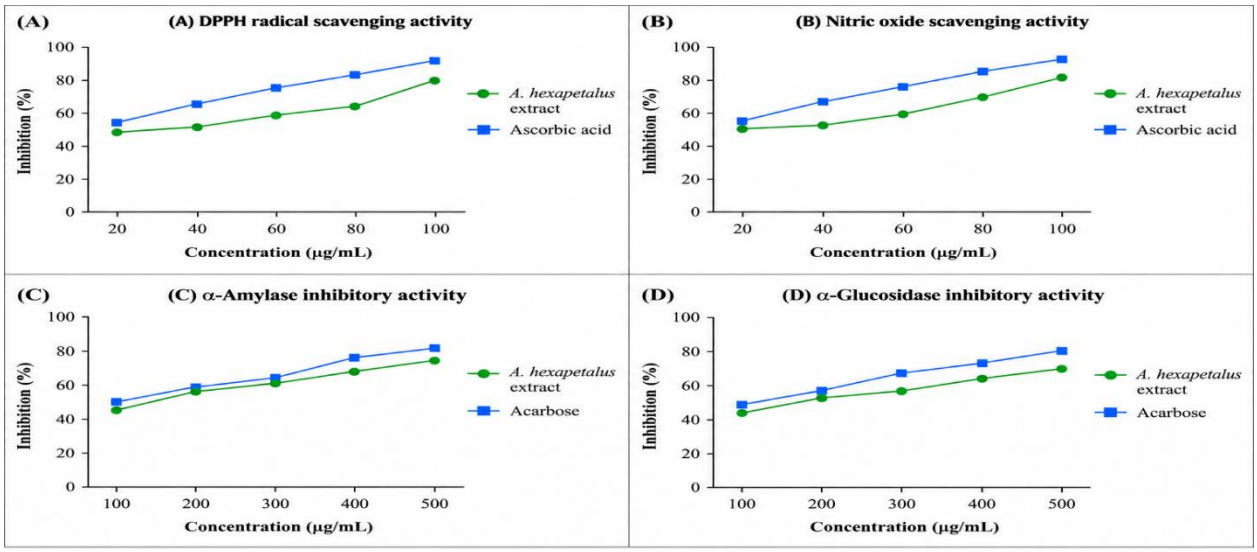

Figure 1. In vitro antioxidant and antidiabetic enzyme inhibitory activities of AH tincture. (A) DPPH radical scavenging activity; (B) nitric oxide scavenging activity; (C) α-amylase inhibition; (D) α-glucosidase inhibition

In Vivo Antidiabetic Activity

Body Weight

At week 10, body weights were 248.4 ± 3.85 g in the normal control, 298.0 ± 4.47 g in the diabetic control, 241.0 ± 4.47 g in the 100 mg/kg group, 237.0 ± 4.47 g in the 200 mg/kg group, 239.0 ± 4.47 g in the 400 mg/kg group, and 241.8 ± 3.70 g in the glibenclamide group. The treated groups therefore showed a body-weight profile closer to the normal-control and glibenclamide groups than the diabetic control at the end of the study. Body weight was interpreted as a metabolic monitoring parameter rather than as an independent efficacy endpoint.

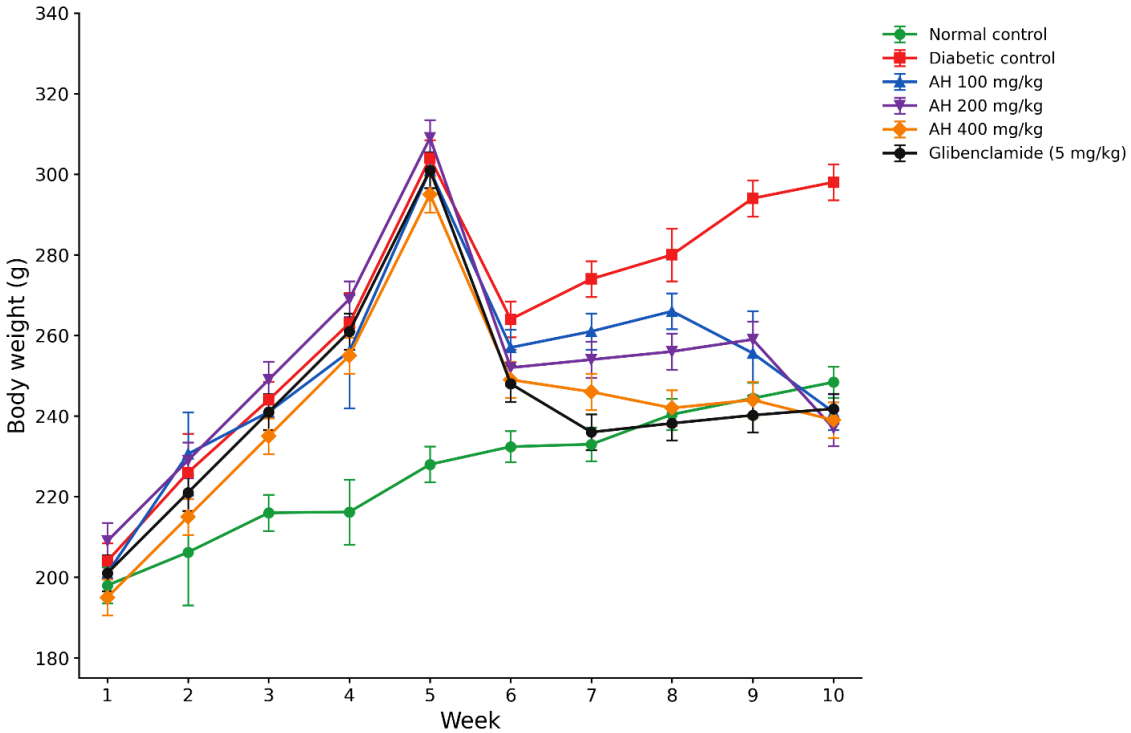

Figure 2. Effect of AH tincture on body weight in STZ-induced diabetic rats during the experimental period. Body weight was recorded weekly in normal control, diabetic control, AH (100, 200, and 400 mg/kg), and glibenclamide (5 mg/kg) treated groups. Values are expressed as mean ± SEM (n = 5)

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Fasting Blood Glucose

The diabetic control showed persistent hyperglycemia, reaching 401.6 ± 9.24 mg/dL at week 10. AH treatment reduced week-10 FBG to 347.2 ± 26.62, 277.0 ± 5.83, and 268.8 ± 20.76 mg/dL at 100, 200, and 400 mg/kg, respectively, while glibenclamide produced 247.4 ± 10.21 mg/dL. The reduction was more marked at 200 and 400 mg/kg, indicating a dose-related antihyperglycemic pattern. The HFD/STZ model is widely used to reproduce key features of insulin resistance and β-cell dysfunction relevant to experimental T2DM (Reed et al., 2000; Gheibi et al., 2017; Furman, 2015).

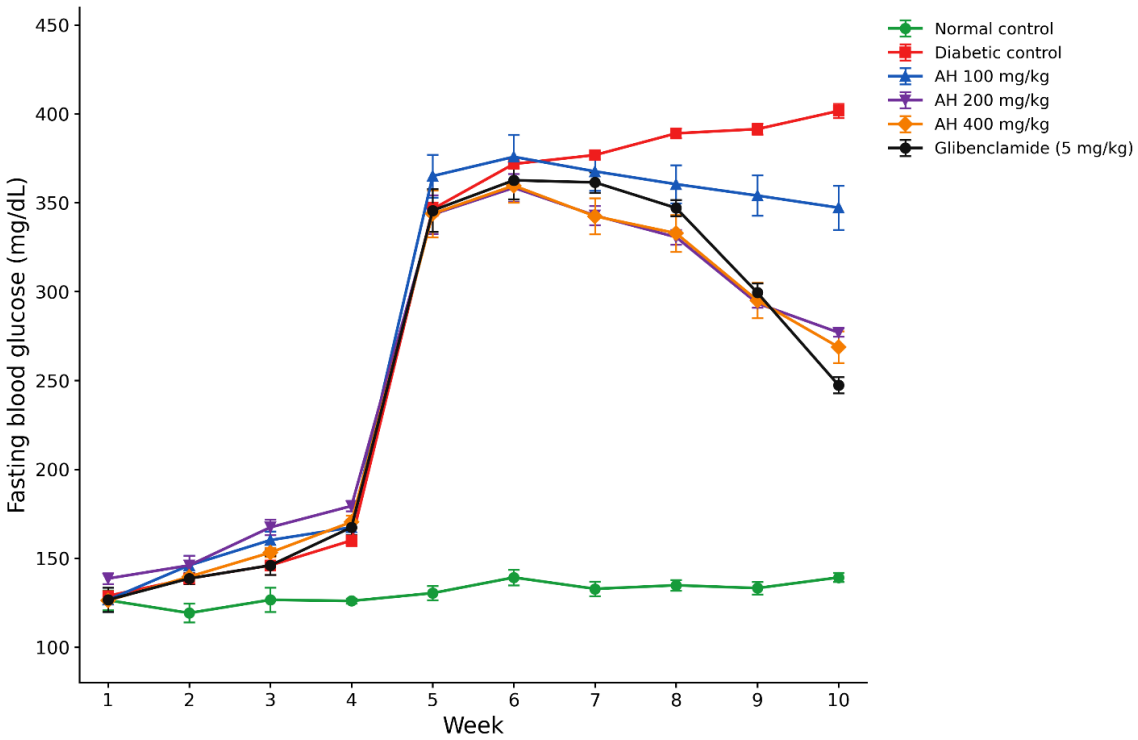

Figure 3. **Effect of** AH **tincture on fasting blood glucose (FBG) levels in STZ-induced diabetic rats over the 10-week experimental period.** Data are presented as mean ± SEM (n = 5)

Intraperitoneal Glucose Tolerance Test

The diabetic control maintained markedly elevated glucose concentrations throughout the IPGTT, with values of 401.60 ± 8.96, 427.20 ± 12.48, 460.40 ± 14.77, 432.60 ± 10.33, and 413.20 ± 15.02 mg/dL at 0, 15, 30, 60, and 120 min, respectively. AH reduced glucose excursions, with the 400 mg/kg group showing 268.80 ± 19.97, 359.60 ± 21.37, 332.80 ± 23.13, 319.20 ± 21.99, and 295.00 ± 22.25 mg/dL at the corresponding time points. The 200 mg/kg group also showed a clear reduction, while glibenclamide produced the lowest post-load profile among the treated groups.

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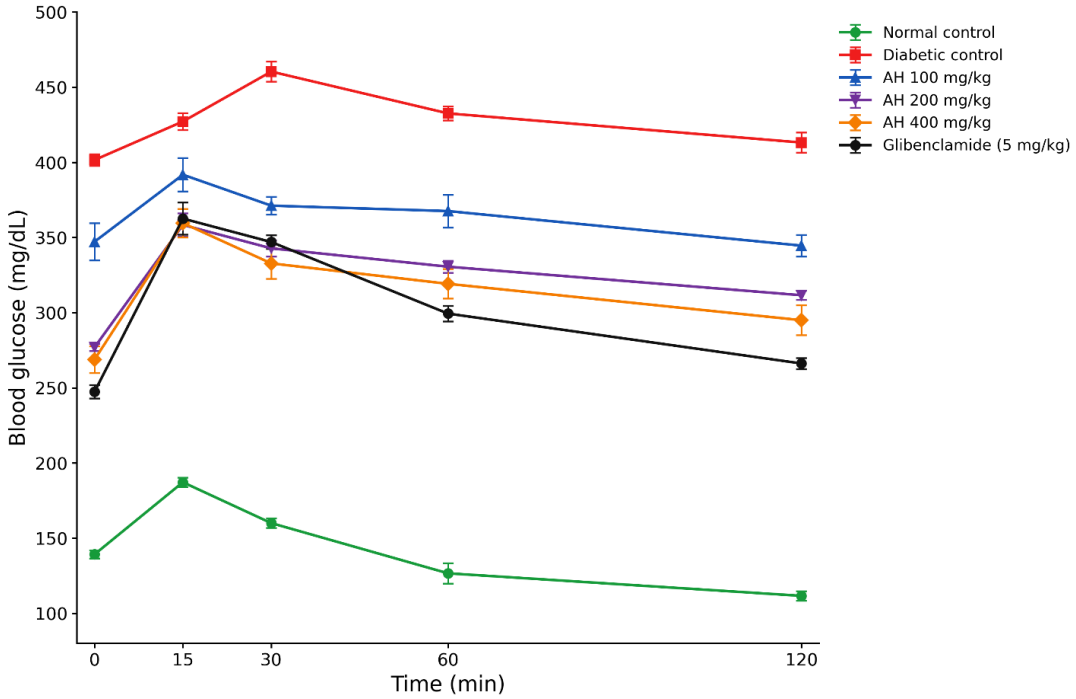

Figure 4. **Effect of** AH **tincture on blood glucose levels during the intraperitoneal glucose tolerance test (IPGTT) in STZ-induced diabetic rats.** Values are expressed as mean ± SEM (n = 5)

Intraperitoneal Insulin Tolerance Test

Following insulin administration, diabetic control animals showed a relatively limited decline in glucose compared with the treated groups. At 120 min, glucose concentrations were 146.00 ± 11.20 mg/dL in the diabetic control, 146.00 ± 11.20, 120.80 ± 4.66, and 99.40 ± 3.78 mg/dL in the 100, 200, and 400 mg/kg groups, respectively; glibenclamide produced 87.20 ± 4.02 mg/dL. The progressive decline in the treated groups suggests improved insulin responsiveness, particularly at 200 and 400 mg/kg.

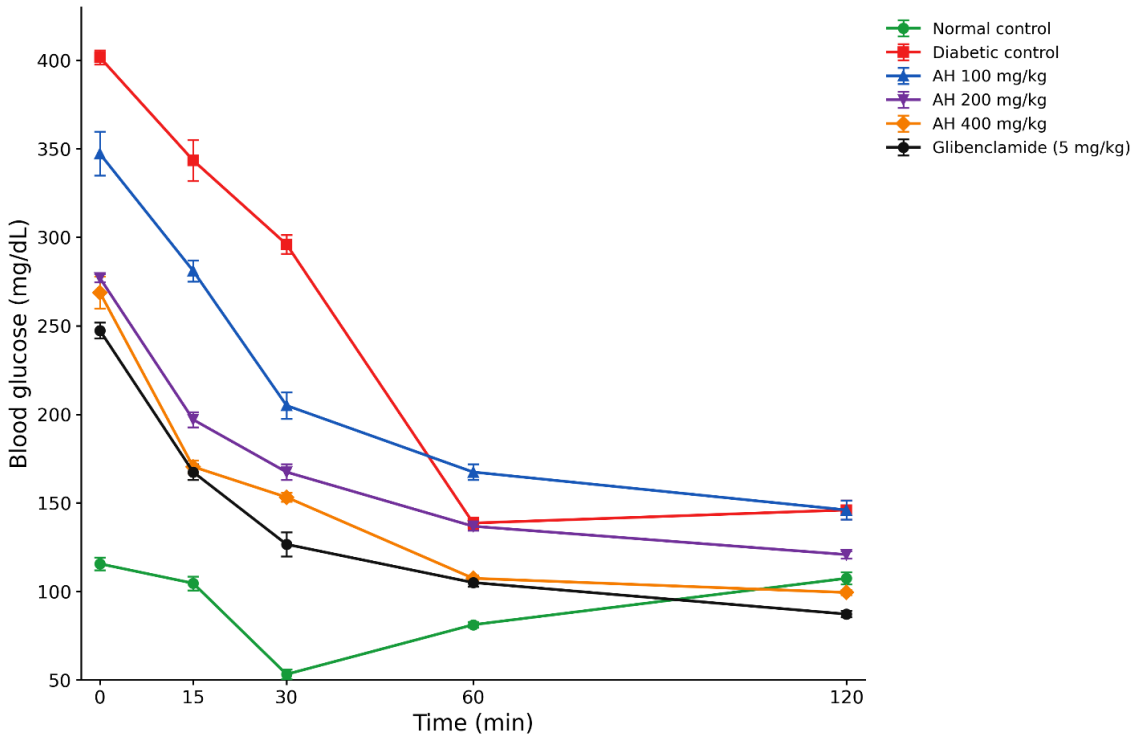

Figure 5. **Effect of** AH **tincture on blood glucose levels during the insulin tolerance test (IPITT) in STZ-induced diabetic rats.** Values are expressed as mean ± SEM (n = 5)

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Serum Lipid Profile

Diabetes was associated with a marked deterioration in the serum lipid profile. Serum total cholesterol increased from 70.20 ± 3.40 mg/dL in the control group to 475.58 ± 13.77 mg/dL in the diabetic control group. Treatment with AH tincture produced a dose-dependent reduction in total cholesterol, with values decreasing to 383.30 ± 8.40, 269.88 ± 10.12, and 160.70 ± 9.88 mg/dL at doses of 100, 200, and 400 mg/kg, respectively. LDL cholesterol similarly increased from 22.38 ± 1.50 mg/dL in the control group to 67.92 ± 2.54 mg/dL in diabetic controls, while treatment with AH reduced LDL cholesterol to 53.13 ± 2.04, 41.30 ± 2.10, and 32.38 ± 2.03 mg/dL at 100, 200, and 400 mg/kg, respectively. In contrast, HDL cholesterol decreased from 42.75 ± 3.51 mg/dL in controls to 23.57 ± 1.27 mg/dL in diabetic controls, whereas AH treatment increased HDL cholesterol to 28.48 ± 1.16, 38.48 ± 1.65, and 43.27 ± 1.59 mg/dL at 100, 200, and 400 mg/kg, respectively. Serum triglycerides were also elevated in diabetic controls compared with normal controls and were progressively reduced following AH treatment, with the greatest improvement observed at 400 mg/kg. Overall, these findings indicate that AH treatment improved multiple components of the diabetes-associated dyslipidemic profile, with the 400 mg/kg dose producing the most pronounced improvement (Gheibi et al., 2017; DeFronzo et al., 2015).

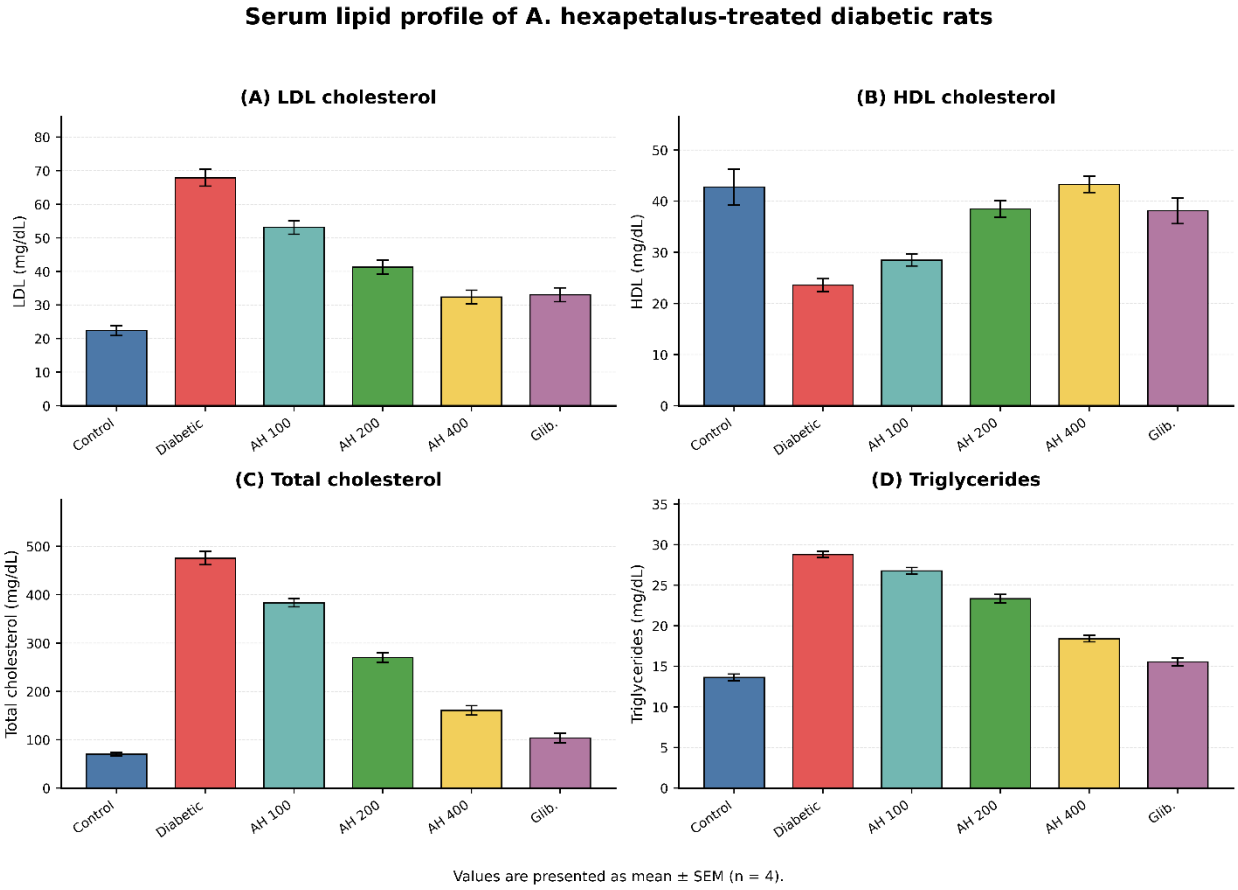

Figure 6. **Effect of AH tincture on serum lipid parameters in diabetic rats. (A) LDL cholesterol, (B) HDL cholesterol, (C) total cholesterol, and (D) triglycerides. Values are presented as mean ± SEM (n = 4)**

Serum Total Protein and Insulin

Serum total protein levels were reduced in diabetic control animals compared with the normal control group, decreasing from 7.22 ± 0.17 g/dL to 5.38 ± 0.20 g/dL. Treatment with AH tincture increased serum total protein levels to 5.75 ± 0.14, 6.28 ± 0.20, and 7.12 ± 0.22 g/dL at doses of 100, 200, and 400 mg/kg, respectively, while glibenclamide produced a value of 7.08 ± 0.14 g/dL. Serum insulin concentration was also reduced in diabetic control animals, from 1575.75 ± 15.72 pg/mL in the normal control group to 1317.25 ± 34.78 pg/mL. Treatment with AH produced insulin concentrations of 1243.50 ± 15.68, 1372.75 ± 15.56, and

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1429.00 ± 16.41 pg/mL at 100, 200, and 400 mg/kg, respectively, whereas glibenclamide produced 1500.00 ± 11.53 pg/mL. The progressive increase in serum total protein and insulin at the higher doses of AH indicates partial restoration of diabetes-associated alterations in protein and insulin-related metabolic parameters, with the 400 mg/kg dose producing the greatest improvement among the extract-treated groups (Matthews et al., 1985).

Table 1. Effect of AH tincture on serum total protein and insulin levels

Group	Total protein (g/dL)	Insulin (pg/mL)
Normal control	7.22 ± 0.17	1575.75 ± 15.72
Diabetic control	5.38 ± 0.20	1317.25 ± 34.78
AH 100 mg/kg	5.75 ± 0.14	1243.50 ± 15.68
AH 200 mg/kg	6.28 ± 0.20	1372.75 ± 15.56
AH 400 mg/kg	7.12 ± 0.22	1429.00 ± 16.41
Glibenclamide	7.08 ± 0.14	1500.00 ± 11.53

Values are **mean ± SEM (n = 4)**

Statistical Analysis

Two-way repeated-measures ANOVA demonstrated significant effects of group, time, and group × time interaction for body weight, fasting blood glucose, IPGTT, and IPITT (all $p < 0.001$). For terminal biochemical parameters, Bonferroni-adjusted comparisons versus the diabetic control showed significant differences for LDL at 100 mg/kg ($p = 0.01966$), 200 mg/kg ($p = 0.000974$), 400 mg/kg ($p = 0.000176$), and glibenclamide ($p = 0.000203$); for HDL at 200 mg/kg ($p = 0.001852$), 400 mg/kg ($p = 0.000344$), and glibenclamide ($p = 0.01036$); for total protein at 400 mg/kg ($p = 0.005257$) and glibenclamide ($p = 0.002177$); for total cholesterol at 100 mg/kg ($p = 0.006189$), 200 mg/kg ($p = 0.00009993$), 400 mg/kg ($p = 0.000007856$), and glibenclamide ($p = 0.000002887$); and for triglycerides at 200 mg/kg ($p = 0.0007299$), 400 mg/kg ($p = 0.000006895$), and glibenclamide ($p = 0.000003469$). Changes in insulin were not statistically significant versus diabetic control at 100 mg/kg ($p = 0.50694$), 200 mg/kg ($p = 0.97742$), or 400 mg/kg ($p = 0.13560$), whereas glibenclamide showed a significant difference ($p = 0.01241$). One-way ANOVA showed significant overall differences in LDL, HDL, total cholesterol, triglycerides, total protein, and insulin (all $p < 0.001$). Acute toxicity and in vitro assays were evaluated descriptively because replicate-level data were unavailable for inferential analysis.

Histopathological Evaluation

Histopathological examination of pancreatic tissue revealed normal pancreatic architecture in the normal control group, characterized by intact islets of Langerhans and well-organized acinar cells. In contrast, the diabetic control group exhibited marked pathological alterations, including β-cell degeneration, inflammatory-cell infiltration, acinar-cell damage, and distortion of the pancreatic architecture. Treatment with AH tincture at 400 mg/kg ameliorated these diabetes-associated histopathological alterations, as evidenced by improved cellular organization, reduced necrosis, and better preservation of islet morphology compared with the diabetic control group. These findings provide morphological support for the beneficial effects of AH treatment on pancreatic tissue in diabetic animals.

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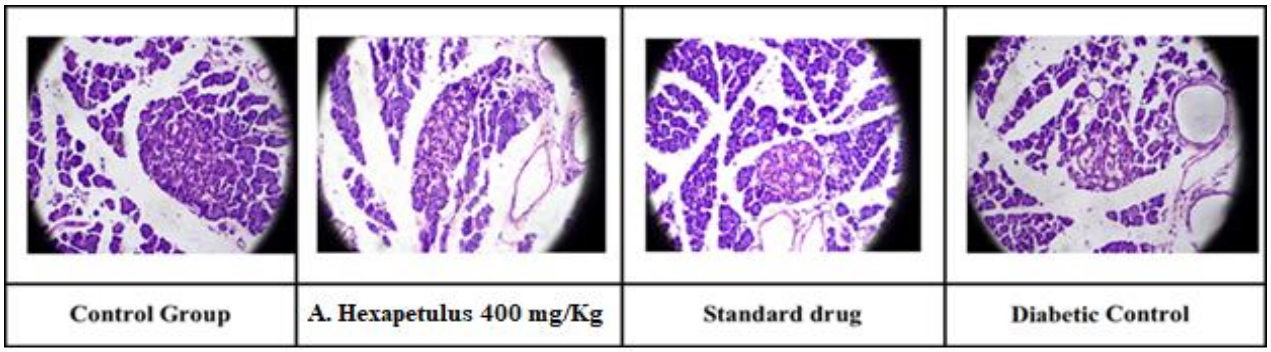

Figure 7. Histopathological evaluation of pancreatic tissues in different experimental groups. (H&E staining, 400× magnification)

DISCUSSION

The present study provides an integrated preliminary evaluation of the phytochemical profile, antioxidant activity, carbohydrate-digesting enzyme inhibitory activity, acute oral safety, and antidiabetic effects of the 70% hydroalcoholic extract of *AH*. HPTLC fingerprinting demonstrated a characteristic chromatographic profile, with tentative identification of rutin, gallic acid, and quercetin based on comparison with reference standards. The extract exhibited concentration-dependent DPPH and nitric oxide radical-scavenging activity and inhibited both α-amylase and α-glucosidase in vitro. In the HFD/STZ-induced experimental model of diabetes, treatment with AH tincture was associated with improvements in fasting blood glucose, glucose tolerance, insulin responsiveness, and serum lipid abnormalities, particularly at the higher treatment doses. Pancreatic histopathological findings further supported the observed biochemical improvements. These findings are relevant to the multifactorial pathophysiology of type 2 diabetes mellitus, in which hyperglycemia, insulin resistance, oxidative stress, and dyslipidemia interact and contribute to disease progression (Brownlee, 2001; Saltiel & Kahn, 2001; DeFronzo et al., 2015).

The higher activity observed at 200 and 400 mg/kg across several metabolic endpoints suggests a dose-related response, with the 400 mg/kg dose generally approaching the glibenclamide group for several parameters. Direct equivalence with glibenclamide should not be claimed because the extract is a chemically complex preparation and the study was not designed as a non-inferiority comparison. The antioxidant results also remain assay-specific; IC_{50} values should be interpreted comparatively within each assay rather than as absolute measures of in vivo antioxidant efficacy (Brand-Williams et al., 1995; Sreejayan & Rao, 1997).

The study should therefore be interpreted as evidence supporting further pharmacological and phytochemical investigation of A. hexapetalus.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Atreyee Mamidwar: investigation, methodology, data collection, formal analysis, and preparation of the original manuscript. Dr. Md. Rageeb Md Usman: conceptualization, supervision, validation, interpretation of the findings, and critical review and revision of the manuscript. Both authors approved the final version of the manuscript.

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