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Protective and Antidiabetic Effects of Polygonum plebeium R.Br. in STZ-Induced Diabetic Rats

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Abstract

Polygonum plebeium R. Br. (Polygonaceae), commonly known as small knotweed, is traditionally used in South Asia to treat intestinal disorders, pneumonia, and menstrual ailments. The present study systematically evaluated its antidiabetic potential through integrated phytochemical, in vitro, in vivo, and in silico approaches. Aerial parts were sequentially extracted using solvents of increasing polarity, and the ethanol extract was analyzed by GC–MS, identifying 11 phytoconstituents. Oleic acid (24.06%) and pentadecanoic acid ethyl ester (21.99%) were major compounds. In vitro antidiabetic activity demonstrated significant enzyme inhibition, with IC_{50} values of 95.06 ± 0.35 µg/ml for α-amylase and 90.32 ± 0.64 µg/ml for α-glucosidase. In vivo studies included oral glucose tolerance test (OGTT), acute and chronic antihyperglycemic evaluations, and streptozotocin-induced diabetic rat models. At 400 mg/kg, the extract reduced blood glucose by 30.52% after 3 h in OGTT. Acute treatment produced a 55.74 ± 3.47% reduction at 8 h, while 30-day administration resulted in a 59.79 ± 1.07% decrease. Significant improvements were observed in liver enzymes, HbA1c, total protein, and lipid profile. Histopathological analysis revealed regeneration of pancreatic islets and restoration of liver and kidney architecture. Molecular docking studies against α-amylase, α-glucosidase, PPAR-γ, and GLUT-2 showed strong binding affinity of stevioside with key targets, supporting the experimental findings. In conclusion, highlight the promising antidiabetic potential of *P. plebeium* and justify further investigation for therapeutic development.

INTRODUCTION

Diabetes has emerged as a global epidemic in human history because of its rapidly rising prevalence and impact. It is now counted among the main causes of death in adults across the globe; roughly one in nine adults is currently affected by the disease [1]. In 2019, 452 million people globally had diabetes, a condition linked to nearly 5 million deaths annually. This increasing health challenge is expected to affect 693 million people by 2045, highlighting the urgent need for vigilant monitoring and management of diabetic complications worldwide [2]. Diabetes mellitus is a long-term, complex metabolic disease in which blood sugar remains high because the body does not produce enough insulin or does not respond to it properly. Over time, this persistent hyperglycaemia disturbs normal carbohydrate, fat, and protein metabolism and damages blood vessels, leading to severe complications, increased risk, and death [3]. Overweight is a major risk factor for diabetes, contributing to severe complications including stroke, heart attack, kidney failure, vision loss, and nerve damage. For pregnant women, uncontrolled diabetes can increase the risk of fatal death and other health problems [4]. Oxidative stress plays a key role in many diseases and aging, making antioxidant therapy a promising approach to improve disease outcomes. In hyperglycaemia, a metabolic pathway involving diacylglycerol (DAG), protein kinase C (PKC), and NADPH-oxidase triggers the production of reactive oxygen species (ROS) [5]. In diabetic patients, high blood sugar triggers the excessive production of ROS, and harmful elements that can damage the cells by activating the various enzymes, including mitochondrial enzymes, xanthine oxidase, cyclooxygenase (COX), lipoxygenase (LOX), nitric oxide synthases, and peroxidases, leading to increased oxidative stress [6–8].

Globally, medicinal plants are recognized as sources of phytochemicals, with 80–85% of the population relying on them. Currently, people use plant extracts or their active components as traditional medicine to address their primary health-care needs [9]. Recently, herbal medicines have occupied prime importance due to their high standard of safety and therapeutic efficacy. Plants exhibit secondary metabolites with high therapeutic value, including phenolics, flavonoids, glycosides, alkaloids, terpenoids, coumarins, saponins, and others. They also contain antioxidants that contribute to treating various disorders by activating endogenous antioxidants to counter oxidative damage [10].

Type 2 diabetes is a complex metabolic disorder marked by insulin resistance and beta-cell dysfunction, leading to chronic hyperglycemia and disrupted glucose homeostasis [11]. Type 2 diabetes mellitus pathophysiology arises from genetic, environmental, and lifestyle factors that drive disease onset and progression. A hallmark is insulin resistance, primarily in skeletal muscle, intestines, and adipose tissue [12]. Recent studies implicate gut microbiota dysbiosis in the pathogenesis of T2DM. This alters gut barrier function, permitting endotoxin-like lipopolysaccharides to leak into circulation, which drives chronic inflammation and exacerbates insulin resistance [13].

Several medicinal plants are well-recognized for their use in treating diabetes mellitus in various types of traditional medicine systems across the globe. However, some of them have been studied systematically and scientifically for their antidiabetic efficacy [14]. Several medicinal plants, *Morus alba* L., *Cinnamomum zeylanicum* J. Presl, *Trigonella foenum-graecum* L., *Phaseolus vulgaris* L., *Zingiber officinale* Rosc., and *Panax ginseng* C.A. Meyer, have been scientifically validated for antidiabetic activity. Their efficacy is largely attributed to bioactive phytochemicals with glucose-lowering and antioxidant properties, which help counter oxidative stress associated with diabetes and its complications [15].

Polygonum plebeium R. Br. (Polygonaceae), commonly known as small knotweed, is a traditional medicinal plant widely distributed in South Asia. It is used in rural communities to treat intestinal disorders, pneumonia, and menstrual problems. The plant contains bioactive compounds such as alkaloids, flavonoids, phenolics, tannins, and essential oils, which contribute to its reported antioxidant, anti-inflammatory, anticancer, antinociceptive, cytoprotective, and neuroprotective activities, supporting its use in the management of diarrhea, liver disorders, eczema, and fungal infections [16]. The species is native to Madagascar, Pakistan, Sri Lanka, and many regions of India, including Andhra Pradesh, Assam, Daman, Goa, Gujarat, Himachal Pradesh, Maharashtra, Orissa, Tamil Nadu, and West Bengal. Whole portions used in Orissa for the treatment of pneumonia among ethnic populations, as well as among tribal communities in Bihar, Jharkhand, Uttar Pradesh, and Orissa, are employed as famine food [17]. The rural community of Sivagangai, Tamil Nadu, India, utilises a paste derived from the roots of P. plebeium, applied twice per day to reduce inflammation [18]. The whole plant extract is traditionally applied for its analgesic, anthelmintic, astringent, and purgative properties. The plant's aqueous extract serves as a tonic for treating respiratory diseases, such as pneumonia, and digestive issues, including diarrhoea. Whole plant juice is preferred due to its expectorant, diuretic, and vasoconstrictive characteristics [19, 20]. In Pakistan, communities residing in rural regions have historically employed specific treatments to manage various health issues, including liver disease, inflammation, dysentery, eczema, and ringworm [21].

Despite extensive traditional use and promising *in-vitro* findings, the safety and efficacy of plant extracts cannot be reliably predicted without evaluation in a whole-organism system. Plant extracts comprise complex mixtures of bioactive constituents that may exert synergistic, antagonistic, or toxic effects when

metabolized *in vivo*. Animal studies are therefore essential to assess systemic pharmacological activity, dose–response relationships, bioavailability, and potential organ-specific toxicity. Such models allow investigation of pharmacokinetic and pharmacodynamic parameters that cannot be adequately replicated by *in-vitro* or *in-silico* methods. Furthermore, animal experimentation is required to meet ethical and regulatory standards aimed at minimizing human risk prior to clinical exposure. Conducting these studies in accordance with the principles of Replacement, Reduction, and Refinement ensures ethical use of animals while generating scientifically robust data necessary for the safe translation of plant-based therapeutics to human applications.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) was acquired from Himedia Laboratory Pvt. Ltd in Mumbai to induce diabetes in a rat model. The process uses analytical-grade chemicals and solvents, Acarbose (Alkem Laboratories Ltd.), Metformin (Lupin Ltd.), alpha-amylase (Sigma-Aldrich), alpha-glucosidase (Sigma-Aldrich), sodium carbonate (Sigma-Aldrich), sodium chloride (Merck), dinitro salicylic acid (Sigma-Aldrich), Tween 80 (Sigma-Aldrich)

Collection and preparation of plant material

The aerial parts of *P. plebeium* were collected from Mulugaon village, Jagatsinghpur, Odisha, India. The plant specimen was authenticated by Dr. K. Karthigeyan, Scientist-E, BSI, Kolkata, West Bengal (CNH//2316Tech.II/2023/94). The voucher specimen was deposited in the Department of Pharmacology, Faculty of Pharmacy, SOA University, Bhubaneswar, for future reference. The collected plant material was thoroughly washed under running tap water to remove adhering impurities and subsequently shade-dried at room temperature. The dried material was ground in a mechanical grinder and passed through a 60# sieve. The powdered plant material was then stored in an airtight container until further use.

Extraction of P. plebeium

Approximately 100 gm of *P. plebeium* powder was initially defatted using petroleum ether (40–60°C) in the Soxhlet apparatus. The defatted marc was then subjected to successive solvent extraction based on increasing polarity using chloroform, ethyl acetate, ethanol, and water. The resulting extracts were concentrated using a rotary evaporator, stored in a refrigerator, and the % yield of each extract was determined [22].

GC-MS profiling of P. plebeium extracts

A GC-MS analysis equipped with the GC-MS-QP2010S type was used for this purpose. The GC-MS system uses a special glass tube and helium gas that moves at a steady speed of 1 ml/min to separate plant chemicals from leftover plant material. The instrument was given 1 µl of the sample residue. The initial temperature was sustained at 100°C, while the injector temperature was adjusted to 250°C. The temperature was maintained at 10°C throughout the operation. After 5 min of running at a final temperature of 280°C, the separation became visible after 24 min. The spectrum of phytochemicals was identified by comparison with the National Institute of Standards and Technology (NIST) collection. The NIST library consists of an array of unknown and known component spectra. The molecular weights of phytochemical compounds made it possible to identify the test materials' component structures. The biological effect of the detected phytocompounds was assessed by comparing them to Dr Duke's phytochemical and ethnobotanical databases, supplemented by a literature review [23].

Assessment of antidiabetic efficacy by in vitro tests

α-amylase inhibition assay

Petroleum ether, chloroform, ethyl acetate, ethanol, and aqueous solvent extracts of the aerial parts of *P. plebeium* were examined for their α-amylase enzyme inhibitory activity, following the method outlined by Ranilla et al. (2008), with slight modification. A volume of 0.5 ml from each solvent extract was mixed with 0.5 ml of α-amylase solution, which contained a sodium phosphate buffer at a pH of 6.9 and a sodium chloride concentration of 0.006 M. The mixture was maintained at ambient temperature for a duration of 10 min. afterwards, a volume of 0.5 ml of a 1% starch solution was added to a 0.02 M sodium phosphate buffer with a pH of 6.9 and a sodium chloride concentration of 0.006 M. In this mixture, 1 mL of dinitro salicylic acid colour reagent was added, and the solution was left at room temperature for 10 min to allow the reaction to complete. The mixture was then placed in a water bath set to a consistent temperature of 100°C for a duration of 5 min. Following this, it was allowed to cool to room temperature. To dilute the mixture, an additional 10 ml of deionized water was incorporated. The absorbance of this resulting mixture was measured at 540 nm. The blank solution was prepared by using the solvent extract without α-amylase solution, and a sodium phosphate buffer of 0.02 M, pH 6.9, was used as a control sample. Acarbose is used as a standard drug. The absorbance of the blank, control, and acarbose solution was measured at 540nm [24]. The results were expressed as percentage inhibition, calculated using the following formula.

$$\text{Inhibitory activity (\%)} = \frac{\mathrm{Abs}_{\mathrm{Control}} - \mathrm{Abs}_{\mathrm{Sample}}}{\mathrm{Abs}_{\mathrm{Control}}} \times 100$$

Where, $\mathrm{Abs}_{\mathrm{Control}}$ corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α-amylase solution, and $\mathrm{Abs}_{\mathrm{Sample}}$ Corresponds to the solution with the extract and α-amylase solution.

α-glucosidase inhibition assay

The α-glucosidase inhibition assay was performed by adopting the method of Sagbo et al. (2018), with minor alterations. Briefly, a 50 µg/ml α-glucosidase solution was put onto a 96-well plate in 20 µl amounts. Afterwards, 5 µl of the crude extract, prepared in various dosage forms, such as 31.2, 62.5, 125, 250, and 500 µg/mL, was added to it. To it was added 60 µl of 67 mM potassium phosphate buffer (pH 6.8). The mixture was incubated for a duration of 5 min. Afterwards, to the mixture was added 10 µl of a 10 mM solution of p-nitrophenyl-α-D-glucoside (PNPGLUC). Thereafter, the mixture was heated to 37°C for 20 min. Following this, the mixture was incubated a few times so as to maintain the consistency of the solution. To it, add 25µl of a 100 mM sodium carbonate (Na_2CO_3) solution, and the absorbance of the mixture was measured at 405 nm using a UV-visible spectrophotometer. Likewise, 20 µL of deionized water is added in place of the enzyme, along with 5 µL of plant extract, to produce an enzyme blank and a sample blank. The UV-visible spectrophotometer reading was zeroed using the blank sample. In this experiment, acarbose was used as the positive control [24]. The results were expressed as percentage inhibition, calculated using the following formula.

Inhibitory activity (%) = $\frac{\mathrm{Abs_{Control}} - \mathrm{Abs_{Sample}}}{\mathrm{Abs_{Control}}} \times 100$

Where, $Abs_{Control}$ corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α- glucosidase solution, and $\mathrm{Abs_{Sample}}$ Corresponds to the solution with the extract and α-glucosidase solution.

Experimental animals

Healthy male Wistar rats, weighing around 180–220 g and aged 4–5 months, were acquired from M/S Chakraborty Enterprises, Narkeldanga, Kolkata–700011, Registration No. 1443/PO/BT/s/11/CPCSEA, and housed in polycarbonate cages. They were granted free access to water and provided with a conventional pellet diet. Animals were required to undergo a fasting period of 10–12 hrs. and acclimatize to the circumstances before any experimentation. The room temperature typically ranged from 25 to 30°C, with relative humidity between 45 and 55%. This study examined rats with blood glucose levels ranging from 66 to 110 mg/dl, indicative of the usual range.

Approval from the animal ethical committee

Approval for animal testing was taken from the Institutional Animal Ethical Committee (IAEC). The letter-number from the ethical committee was KMIPS/IAEC/1/2024 and KMIPS/IAEC/2/2024 on dated 6th December 2024.

Acute toxicity study

The Organization for Economic Co-operation and Development (OECD) used Wistar rats to evaluate the toxicity of the extracts before the comprehensive study. Extracts were administered with the test drug (2000 mg/kg body weight), whereas the control group received only the vehicle (distilled water + Tween 80). The test and control groups of animals were accurately observed for behavioral alterations and acute toxicity symptoms, beginning 30 min. post-extract delivery and persisting for 4 hrs. The rats were watched for significant toxic effects for up to 72 hrs., with infrequent observations for any mortality until 30 days [25].

Oral glucose tolerance test in normal rats

The rats were required to undergo a 12-hrs fast, drinking only water, before the oral glucose tolerance test. They were divided into five groups, each comprising six rats. After a 30-min administration of the extract, glucose was delivered at a dosage of 2 g/kg of body weight. Blood glucose levels in healthy rats were assessed before and at 0.5, 1, 2, and 3 hrs. into the assigned time frame using a glucometer with a photometric endpoint.

Induction of diabetes

Diabetes was induced in fasting rats using a single intraperitoneal injection of streptozotocin at a dosage of 40 mg/kg body weight, accompanied by 0.1 M citrate buffer (pH 4.5), prepared shortly before administration. After 12 to 14 days of streptozotocin (STZ) induction, animals with blood glucose levels beyond 250 mg/dl were considered stable and included in our experiment as diabetic rats [26, 27].

Design of experimental study

Wistar rats were used for all animal models, including acute toxicity studies, oral glucose tolerance tests, and streptozotocin-induced diabetes models. A total of 30 rats were used in the research investigation, divided into 5 groups, each including 6 rats [26].

Group I: Solvent control (Tween 80 in distilled water, 10 ml/kg)

Group II: Diabetic control

Group III: Standard group; metformin (250 mg/kg)

Group IV: Ethanol extracts of *P. plebeium* (200 mg/kg)

Group V: Ethanol extracts of *P. plebeium* (400 mg/kg)

The solvent control groups received oral administration of distilled water and two drops of Tween 80. All other groups, with the exception of that one, were administered metformin and extracts (both lower and higher doses) diluted in distilled water via an oral gavage tube.

Acute effect of Polygonum plebeium on blood glucose in normal and diabetic rats

Animals showing stable blood glucose levels on day 15 post-STZ induction were considered appropriate for the investigation. The rats had a 12-hour overnight fast, after which the drug was administered according to the assigned experimental group. Blood glucose levels were assessed at 0, 1, 2, 3, 4, 6, and 8 hrs following administration. Blood was obtained via the pricking method from the tail vein to test blood glucose levels.

Long-term effect of daily administration of Polygonum plebeium in diabetic rats

Test drugs were administered daily to the study subjects for a period of 30 days. Blood glucose levels and body weights were evaluated at intervals of 0, 5, 10, 15, 20, 25, and 30 days. On the 30th day, all experimental rats were anesthetized with ketamine and euthanized by cervical decapitation, and blood samples were collected via heart puncture and retro-orbital for the evaluation of serum biochemical parameters and lipid profiles. The liver, kidney, and pancreas were excised and kept in a sterile container with a diluted formalin solution for histological analysis [28].

Serum biochemical parameters

After the final treatment, animals were anesthetized by using ketamine, and blood was collected from the retro-orbital sinus. Blood samples were placed in citrate buffer tubes and centrifuged at 3000 rpm for 10 minutes at 4°C to isolate serum, which was then stored in labelled Eppendorf tubes at − 20°C. The serum was analyzed for biochemical parameters, including AST, ALT, ALP, albumin, globulin, bilirubin, and total protein, using BIO-LA-TEST (USA) diagnostic kits [23].

Tissue homogenate preparation

The pancreas, liver, and kidneys of the sacrificed rats were carefully dissected, chopped into small pieces, and homogenized in phosphate-buffered saline. The homogenates were centrifuged at 800 rpm for 10 min at 4°C, and the resulting supernatants were collected for enzymatic and biochemical analyses [23, 29].

Molecular docking

The process of molecule docking was investigated using the molecular modelling software Autodock-Vina. The enzymes α-glucosidase, α-amylase, PPAR-γ, and GLUT-2 proteins were chosen, and their respective proteins were extracted from the protein data bank. Specifically, proteins 3WY2, 3BAJ, 3SZ1 and 4ZWC were selected for molecular modeling. The ChemDraw software was used to depict the 2D structures of phytomolecules, such as Phytol, Linoleic acid ethyl ester, α-tocopherol-β-D mannoside, Ethyl tridecanoate, Stevioside, Oleyl alcohol, and tetratetracontane, which were subsequently converted into 3D structures. The MM2 Interface Programme on ChemBio3D Ultra 12.0 was used to minimize the energy of the phytomolecules. The phytomolecules that were developed were saved in pdb format. In order to determine the most active molecule, the internal ligand was first removed, and docking was performed using the routine method.

The protein structure was prepared using AutoDock Tools version 1.5.7, and chain A of the crystallographic structure was selected for the docking study [30]. Water molecules and co-crystallized ligands were removed to identify the active site, followed by the addition of polar hydrogens and application of Kollman charges. Ligands were prepared using Open Babel software (version 2.4.1) [31]. The active site was defined based on the amino acid residues reported to interact with ligands in the protein data files. The grid box dimensions were set to 60 × 58 × 60 points for α-glucosidase and 60 × 60 × 60 points for α-amylase, PPAR-γ, and GLUT-2 along the x, y, and z axes. The grid centers for α-glucosidase, α-amylase, PPAR-γ, and GLUT-2 were positioned at (− 40.844, 12.284, − 17.854), (8.330, 28.629, 50.430), (13.187, − 0.527, 16.862), and (− 47.947, 5.589, 13.063), respectively. Molecular docking was performed using AutoDock Vina to obtain docking scores, and 3D visualization of the interactions was carried out using BIOVIA Discovery Studio 2021 Client [32].

Statistical analysis

Statistical analysis was performed utilising the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS, Inc., Chicago, USA), licensed by the university. Summary statistics (mean, standard error of the mean) were employed to show the data, and mean comparisons among groups were performed using analysis of variance (ANOVA), followed by post-hoc analysis (Tukey's test). The results were considered statistically significant, with a p-value below 0.05.

RESULTS

Gas Chromatography Mass Spectroscopy (GC-MS) Analysis

The aerial parts of *P. plebeium* had 11 distinct plant chemicals in the ethanol extracts. Each chemical was identified by analysing its peak area, duration of presence in the system, and mass spectrum fragmentation pattern, and comparing these characteristics to the established database of compounds in the National Institute of Standards and Technology's (NIST) repository. Among the eleven identified compounds, oleic acid (24.06%) and pentadecanoic acid, ethyl ester (21.99%) exhibited the highest peak area percentages. Notably, a majority of the detected constituents have been previously reported to possess antidiabetic potential. The identified compounds, along with their molecular formula, retention time, peak area percentage (optimal region %), chemical nature, and reported biological activities, are represented in Table 1, and the chromatogram is depicted in Fig. 1.

In vitro antidiabetic activity

The crude extracts of *P. plebeium* demonstrated a concentration-dependent inhibition of both α-amylase and α-glucosidase enzymes when compared with the standard drug, acarbose. In the α-amylase inhibitory assay, all extracts showed progressive increases in percentage inhibition with rising concentrations (31.2–500 µg/ml). Among the tested fractions, PPEA and PPE exhibited comparatively stronger activity at higher concentrations, achieving notable inhibition values close to the standard at 250 and 500 µg/ml. The IC_{50} of PPE (95.06 ± 0.35 µg/ml) and PPEA (115.1 ± 0.24 µg/ml) displayed significantly greater inhibitory potency than PPP and PPC. In the α-glucosidase inhibition assay, PPE demonstrated the strongest activity among the extracts, reaching 68.77 ± 0.88% inhibition at 500 µg/ml with an IC_{50} value of 90.32 ± 0.64 µg/ml (Table 2, 3, and Fig. 2).

Acute toxicity study

No indications of acute toxicity or mortality were observed at the maximum dosage (2000 mg/kg body weight) following 72 hrs of observation. Established our dosage to be one-tenth of the lethal amount, specifically 200 mg/kg of body weight, which is referred to as the cut-off dose. Body weights were determined to be normal in the test groups. Consequently, the extracts were determined to be safe for prolonged administration.

Oral glucose tolerance test

The oral glucose tolerance test demonstrated that *P. plebeium* extracts produced a clear, time-dependent antihyperglycemic effect in both normal and diabetic rats when compared with the solvent control and metformin. In normal rats, ethyl acetate, ethanol, and aqueous extracts of PP at 400 mg/kg progressively lowered blood glucose from 0.5 to 3 hours. Among the tested extracts, the PPE at 400 mg/kg p.o. showed the most significant decrease in blood glucose, i.e., 14.97 ± 4.59% at 3 hours post-glucose load in diabetic rats (Table 4).

The oral glucose tolerance test (OGTT) results showed a significant reduction in blood glucose levels in streptozotocin-induced diabetic rats treated with PPE compared with the standard group (Table 5). Metformin (250 mg/kg) produced a significant reduction in blood glucose levels from 1 h onward, decreasing from 277.33 ± 7.49 mg/dl at baseline to 163.83 ± 5.84 mg/dl at 3 h, corresponding to a 40.43 ± 3.74% reduction ($p < 0.001$). Among the test groups, the PP ethanol extract at 200 mg/kg produced a moderate effect, with a maximum reduction of 16.75 ± 2.90% at 3 h (263.33 ± 3.26 mg/dl). In contrast, the higher dose of PP ethanol extract (400 mg/kg) demonstrated a pronounced, dose-dependent effect, significantly reducing blood glucose levels from 310.66 ± 7.49 mg/dl to 214.17 ± 3.67 mg/dl at 3 h, representing a 30.52 ± 2.77% reduction ($p < 0.001$).

Acute effect of P. plebeium on blood glucose in rats

Acute administration of *P. plebeium* ethanolic extract produced a significant, dose-dependent reduction in blood glucose levels in streptozotocin-induced diabetic rats (Table 6). The solvent control group showed progressive hyperglycemia, whereas metformin (250 mg/kg) elicited a rapid and marked antihyperglycemic effect, reducing blood glucose by 68.02 ± 3.51% at 8 h ($p < 0.001$). Among the test doses, PPE extract at 200 mg/kg produced a moderate glucose-lowering effect (41.04 ± 3.07%), while the 400 mg/kg dose demonstrated a pronounced and sustained reduction, achieving a 55.74 ± 3.47% decrease at 8 h ($p < 0.001$).

Effect of prolonged daily administration of P. plebeium in diabetic rats

Chronic oral administration of PP ethanolic extract for 30 days resulted in a progressive and dose-dependent improvement in glycaemic control (Table 7). Metformin reduced blood glucose by 64.54 ± 1.23% at day 30 ($p < 0.001$). PPE at 200 mg/kg showed a modest effect (23.53 ± 1.58%), whereas PPE at 400 mg/kg significantly reduced blood glucose levels by 59.79 ± 1.07% by day 30 ($p < 0.001$).

Effect of P. plebeium ethanol extracts on body weight

Diabetic rats in the solvent control group exhibited a progressive loss in body weight over the 30-day study period (Table 8). Metformin treatment partially attenuated weight loss, whereas PP ethanolic extract produced dose-dependent effects. PP extract at 200 mg/kg showed the greatest percentage body weight loss across all time points, while the 400 mg/kg dose significantly reduced weight loss compared with the control, particularly by day 30 (10.5 ± 1.04%; $p < 0.01$).

Effect of serum biochemical parameters

Diabetic control rats showed significant elevations in serum AST, ALP, and HbA1c levels, along with reductions in total protein, compared with normal controls ($p < 0.001$), confirming hepatic dysfunction and poor glycaemic control. Metformin treatment significantly normalized AST, ALT, ALP, albumin, total protein, and HbA1c levels ($p < 0.001$). Treatment with PPE produced dose-dependent improvements in serum biochemical parameters. PPE at 200 and 400 mg/kg significantly reduced ALT, ALP, and HbA1c levels while restoring albumin and total protein levels ($p < 0.001$), with the 400 mg/kg dose showing effects comparable to metformin. Bilirubin parameters remained unchanged across groups, indicating preserved hepatic excretory function (Table 9).

Effect of lipid profile

Diabetic control rats exhibited marked dyslipidaemia, characterized by significant elevations in total cholesterol (TC), triglycerides (TG), LDL, and LDL/HDL ratio, along with a reduction in HDL levels compared with normal controls ($p < 0.001$). Metformin treatment significantly normalized all lipid parameters, restoring TC, TG, LDL, HDL, and lipid ratios toward normal values ($p < 0.001$). Treatment with *Polygonum plebeium* ethanolic extract (PPE) produced dose-dependent hypolipidemic effects. PPE at 200 mg/kg significantly reduced TC, TG, and LDL levels, while the 400 mg/kg dose produced a more pronounced improvement, significantly lowering TC, TG, LDL, VLDL, and LDL/HDL ratio ($p < 0.001$), with partial restoration of HDL levels. These findings indicate a strong lipid-modulatory effect of PPE, particularly at the higher dose (Table 10).

Histopathological studies

Microscopic examination of pancreatic tissue from the normal control group Fig. 3 (1A and 1B) demonstrated normal pancreatic architecture. In contrast, pancreatic sections from the STZ-induced diabetic group Fig. 3 (2A and 2B) showed extensive pathological alterations, including severe degeneration and necrosis affecting both exocrine and endocrine regions. These changes were accompanied by marked vacuolar degeneration and vascular congestion, reflecting substantial pancreatic damage. Treatment with metformin at 250 mg/kg Fig. 3 (3A and 3B) markedly improved pancreatic morphology with noticeable restoration of acinar cells. Treatment with PPE at 400 mg/kg Fig. 3 (4A and 4B) revealed significant improvement in pancreatic histoarchitecture and restoration of Langerhans.

Histological evaluation of liver tissue from the normal group Fig. 4 (1A and 1B) showed intact hepatic lobular organization, characterized by well-arranged hepatocyte cords, a normal central vein, and uniformly distributed sinusoidal spaces. Conversely, the STZ-induced diabetic group Fig. 4 (2A and 2B) exhibited severe histopathological damage, including hepatocellular degeneration, centrilobular necrosis, sinusoidal dilation, cytoplasmic vacuolization, and inflammatory cell infiltration, confirming significant STZ-mediated liver injury. Metformin at 250 mg/kg Fig. 4 (3A and 3B) resulted in substantial hepatoprotection, as evidenced by restoration of hepatic architecture. Similarly, administration of PPE 400 mg/kg Fig. 4 (4A and 4B) resulted in a marked improvement in liver histology, characterized by regenerated hepatocytes, minimal vacuolization, reduced sinusoidal dilation, and normal lobular arrangement.

Histopathological examination of the kidney from the normal control group Fig. 5 (1A and 1B) revealed normal renal architecture. The diabetic control group Fig. 5 (2A, and 2B) showed tubular dilation and disorganization of endocytic vacuoles within tubular epithelial cells. Treatment with metformin 250 mg/kg Fig. 5 (3A, and 3B) improves renal histology, showing restoration of nephron organization, improved glomerular structure, and reduced vacuolar changes. Similarly, administration of PPE 400 mg/kg Fig. 5 (4A and 4B) resulted normalize renal histology, characterized by well-preserved glomeruli, normalized tubular arrangement, and minimal vacuolization.

Molecular Docking studies

Molecular docking analysis was performed to evaluate the binding affinity and interaction patterns of selected compounds from GC-MS analysis of PP against key antidiabetic targets, namely α-glucosidase, α-amylase, GLUT-2, and PPAR-γ, and the results are summarized in Table 11, and the binding interactions are represented in Figs. 6 and 7.

Metformin, used as the reference standard, exhibited strong binding affinity across all targets, with docking scores of − 7.6 kcal/mol for α-glucosidase, − 7.2 kcal/mol for α-amylase, − 7.7 kcal/mol for GLUT-2, and − 8.6 kcal/mol for PPAR-γ. Among the phytoconstituents, stevioside demonstrated the most prominent binding affinity across all targets, surpassing or closely matching the reference drug. Stevioside showed docking scores of − 7.9 kcal/mol against α-glucosidase and − 8.2 kcal/mol against α-amylase, forming stable hydrogen bond interactions with critical residues such as VAL335, GLU377, GLY399, LEU300, TYR151, HIS305, and GLU233. Notably, stevioside exhibited strong binding to GLUT-2 (− 8.5 kcal/mol) through hydrogen bonding with ASN413, THR28, SER71, and SER64, suggesting potential involvement in glucose transport regulation. Furthermore, stevioside showed the highest affinity toward PPAR-γ (− 10.3 kcal/mol), interacting with ARG234, ASN375, and GLU378. Phytol also demonstrated moderate binding affinity toward α-glucosidase (− 7.0 kcal/mol), α-amylase (− 6.6 kcal/mol), GLUT-2 (− 5.5 kcal/mol), and PPAR-γ (− 5.3 kcal/mol), with limited hydrogen bond interactions, suggesting a supportive but less dominant role in enzyme inhibition. Similarly, agaric acid exhibited consistent binding across all targets, particularly with GLUT-2 (− 6.9 kcal/mol) and PPAR-γ (− 6.2 kcal/mol), indicating potential multi-target activity.

Fatty acid derivatives such as ethyl tridecanoate, pentadecanoic acid ethyl ester, linoleic acid ethyl ester, and oleic acid showed comparatively lower docking scores and fewer hydrogen bond interactions, suggesting weaker binding affinity toward the selected targets. However, selective interactions were observed, such as pentadecanoic acid ethyl ester forming a hydrogen bond with CYS285 of PPAR-γ and linoleic acid ethyl ester interacting with ARG234, indicating possible auxiliary contributions to overall biological activity. The docking results highlight stevioside as the most potent bioactive compound, exhibiting strong and stable interactions with all four antidiabetic targets.

Table 1
Phytochemical components detected in the Ethanol extract of Polygonum plebeium were analysed using GCMS

Sl. No.	Name of the compound	M.F.	M.W.	RT	Optimal region %	Nature of Compound	Biological activity	Ref.
1	Ethyl tridecanoate	$C_{15}H_{30}O_2$	242	11.84	2.17	Fatty acid ester	Anti-diabetic activity.	[33]
2	Stevioside	$C_{38}H_{60}O_{18}$	804	12.76	4.64	Diterpenoid glycoside	Antitumor, Antibacterial, Antihyperglycemic, Anti-inflammatory activities.	[34]
3	Phytol	$C_{20}H_{40}O$	296	20.32	5.44	Acyclic diterpene alcohol	Lipid-lowering, Antidiabetic, cytotoxic, Antitumoral, antimutagenic, Anti-atherogenic, and Antimicrobial Inhibitors of spasmodic and epileptic, Anti-inflammatory, Antioxidant, and Antinociceptive Immunoadjuvant, Antidepressant, and anxiolytic.	[35]
4	Agaricic acid	$C_{22}H_{40}O_7$	416	20.47	0.40	Carbonyl compound	Not reported	
5	1-Tetradecyne	$C_{14}H_{26}$	194	20.73	2.04	-	Not reported	
6	1,2-Benzene dicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$	334	22.84	3.64	-	Antifungal.	[36]
7	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	270	23.34	21.99	-	Not reported	
8	Tetratetracontane	$C_{44}H_{90}$	618	25.60	2.68	Long-chain alkane	Hypoglycaemic, Antioxidant, Antibacterial	[37]
9	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	26.37	11.37	Long-chain fatty acid	Anti-diabetics, Analgesics, Antioxidants, Anti-inflammatory, Antibacterial activity	[38]
10	Oleic Acid	$C_{18}H_{34}O_2$	282	26.48	24.06	Monounsaturated omega-9 fatty acid	Anti-inflammatory	[39]
11	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	228	26.83	4.23	-	Not reported	

Molecular formula (M.F.), Molecular weight (M.W.), Retention time (RT)

In vitro antidiabetic assay

Table 2
Estimation of α-amylase inhibitory activities of the crude extract of *P. plebeium* and standard in percentage (%) inhibition

Concentration (µg/ml)	Acarbose	PPP	PPC	PPEA	PPE	PPA
31.2	33.05 ± 0.52*	14.63 ± 0.26*	9.53 ± 0.12*	19.53 ± 0.28*	18.22 ± 0.11*	16.64 ± 0.76*
62.5	45.78 ± 0.64*	24.42 ± 0.92*	19.69 ± 0.53*	31.55 ± 0.42*	34.28 ± o.86*	30.55 ± 0.93*
125	61.12 ± 0.86*	37.21 ± 0.59*	32.48 ± 0.02*	44.25 ± 0.77*	44.42 ± 0.96*	42.32 ± 0.18*
250	80.45 ± 0.42*	51.26 ± 0.84*	46.98 ± 0.92*	60.05 ± 0.19*	59.12 ± 0.28*	55.29 ± 0.25*
500	83.23 ± 0.79*	61.82 ± 0.18*	56.88 ± 0.76*	69.25 ± 0.61*	69.53 ± 0.56*	66.59 ± 0.39*
IC_{50}	77.10 ± 0.91	140.7 ± 0.74	132.1 ± 0.05	115.1 ± 0.24	95.06 ± 0.35	105.6 ± 0.63*

The data are shown as the mean ± SEM for n = 3. * Significant at $p < 0.05$

Table 3
Estimation of α-Glucosidase inhibitory activities of the crude extract of *P. plebeium* and standard in percentage (%) inhibition

Concentration (µg/ml)	Acarbose	PPP	PPC	PPEA	PPE	PPA
31.2	41.24 ± 0.23*	12.45 ± 0.13*	11.95 ± 0.65*	18.88 ± 0.43*	23.65 ± 0.28*	13.52 ± 0.82*
62.5	56.45 ± 0.61*	25.62 ± 0.41*	20.56 ± 0.34*	28.59 ± 0.67*	35.49 ± 0.30*	26.71 ± 0.57*
125	71.02 ± 0.05*	29.52 ± 0.51*	27.12 ± 0.16*	36.67 ± 0.91*	48.67 ± 0.66*	33.96 ± 0.75*
250	83.25 ± 0.86*	36.84 ± 0.17*	33.19 ± 0.12*	47.59 ± 0.19*	58.79 ± 0.28*	39.26 ± 0.33*
500	89.35 ± 0.57*	49.88 ± 0.74*	43.45 ± 0.49*	54.99 ± 0.38*	68.77 ± 0.88*	52.12 ± 0.23*
IC_{50}	53.83 ± 0.32	175.8 ± 0.25	158.9 ± 0.44	115.0 ± 0.59	90.32 ± 0.64	98.07 ± 0.71
The data are shown as the mean ± SEM for n = 3. * Significant at $p < 0.05$						

Table 4
OGTT of PP ethyl acetate, ethanol, and aqueous extracts on blood glucose levels(mg/dl) in normal rats

Treatment	0hr	0.5hr [b]	1hr	2hr	3hr	% decrease at the end of 3 hours.
Solvent control(10ml/kg)	94.83 ± 2.74	125.33 ± 4.21	122.67 ± 2.47 [c]	121.00 ± 5.03 [d]	115.33 ± 4.94 [d]	-
Metformin(250mg/kg)	85.67 ± 4.36	130.33 ± 1.61	98.33 ± 2.04 [d]	110.50 ± 3.69 [d]	68.17 ± 2.82 [c]	19.71 ± 4.40
PP Ethyl Acetate Extract(400mg/kg)	103.83 ± 5.76	126.33 ± 7.39	117.17 ± 5.88 [c]	110.17 ± 2.88 [c]	100.83 ± 5.74 [c]	2.27 ± 5.39
PP Ethanol Extract(400mg/kg)	107.17 ± 5.99	149.33 ± 6.45	146.83 ± 10.39 [d]	133.33 ± 7.97 [d]	91.00 ± 3.70 [c]	14.97 ± 4.59
PP Water Extract (400mg/kg)	102.00 ± 7.54	112.83 ± 7.74	107.00 ± 4.51 [d]	101.33 ± 3.04 [c]	92.83 ± 6.30 [c]	8.17 ± 4.44
F-statistic for decrease in blood glucose	-	-	9.83	6.27	18.8	13.54
P Value	-	-	0.001***	0.001**	0.001***	0.001***

Value expressed as mean ± SEM (n = 6) in each group.

** significant difference at α = 0.01, *** significant difference at α = 0.001, [b] base line values for comparison in paired t-test. [c] significant decrease at α = 0.05 level, [d] significant decrease at α = 0.01 level

Table 5
OGTT of PP ethanol extracts on blood glucose levels(mg/dl) in Diabetic rats

Treatment	0hr	0.5hr [b]	1hr	2hr	3hr	% decrease at the end of 3 hours
Solvent control(10ml/kg)	306.16 ± 6.75	343.17 ± 5.34	342.00 ± 0.58 [d]	330.50 ± 0.76 [d]	365.17 ± 0.60 [d]	-
Metformin(250mg/kg)	277.33 ± 7.49	317.33 ± 5.31	244.33 ± 6.60 [c]	191.50 ± 6.98 [c]	163.83 ± 5.84 [c]	40.43 ± 3.74
PP Ethanol Extract(200mg/kg)	317.66 ± 7.49	364.00 ± 5.05	316.83 ± 6.17 [c]	272.83 ± 1.03 [d]	263.33 ± 3.26 [c]	16.75 ± 2.90
PP Ethanol Extract(400mg/kg)	310.66 ± 7.49	343.83 ± 7.84	275.67 ± 16.89	234.83 ± 3.27 [c]	214.17 ± 3.67 [c]	30.52 ± 2.77
F statistics for decrease in blood glucose	-	-	20.42	219.35	503.78	73.62
P Value	-	-	0.001***	0.001***	0.001***	0.001***

Value expressed as mean ± SEM (n = 6) in each group.

*** significant difference at α = 0.001, [b] base line values for comparison in paired t-test. [c] significant decrease at α = 0.05 level, [d] significant decrease at α = 0.01 level

Table 6
Acute effect of PP Ethanolic extracts on blood glucose level (mg/dl) in Diabetic rats

Treatment	0hr [b]	1hr	2hr	3hr	4hr	6hr	8hr	% decrease at the end of 8 hours
Solvent control(10ml/kg)	264.00 ± 9.07	282.66 ± 9.69 [c]	295.00 ± 13.48 [d]	319.66 ± 6.54 [c]	324.00 ± 7.63 [d]	334.16 ± 8.18 [d]	338.66 ± 6.92 [c]	
Metformin(250mg/kg)	295.16 ± 4.09	264.16 ± 9.31 [c]	235.66 ± 3.88 [d]	203.83 ± 6.08 [d]	164.33 ± 5.85 [d]	135.5 ± 5.19 [d]	94.66 ± 11.12 [c]	68.02 ± 3.51 [d]
PP Ethanol Extract(200mg/kg)	361.33 ± 4.95	333.33 ± 12.00	304.83 ± 8.28 [c]	265.5 ± 9.17 [c]	243.5 ± 10.06 [c]	227.83 ± 7.49 [c]	212.33 ± 8.58	41.04 ± 3.07 [c]
PP Ethanol Extract(400mg/kg)	285.33 ± 6.46	274.5 ± 9.05 [c]	234.16 ± 10.21 [d]	213.66 ± 9.84 [c]	194.66 ± 11.11 [c]	164.5 ± 9.85 [d]	125.16 ± 6.99 [c]	55.74 ± 3.47 [d]
F-statistic for decrease in blood glucose		9.28	15.408	43.639	61.184	125.21	162.518	103.368
P Value		0.001***	0.001***	0.001***	0.001***	0.001***	0.001***	0.001***

Values are expressed as Mean ± SEM (n = 6) in each group

*** Significant difference at α = 0.001, [b] base line values for comparison in paired t-test. [c] significant decrease at α = 0.05 level, [d] significant decrease at α = 0.01 level

Table 7
Long-term effects of daily administration of PP extract on blood glucose levels (mg/dl) in diabetic rats.

Treatment	Day 0 [b]	5th Day	10th Day	15th Day	20th Day	25th Day	30th Day	% decrease at the end of 30 days.
Solvent control(10ml/kg)	279.83 ± 9.08	307.16 ± 10.46 [d]	316.83 ± 9.07 [c]	315.16 ± 11.12 [d]	319.66 ± 11.83 [d]	325.83 ± 13.23 [d]	340.66 ± 11.51 [c]	-
Metformin(250mg/kg)	294.5 ± 9.33	266.83 ± 12.14 [c]	242.5 ± 11.38 [c]	193.5 ± 11.64 [d]	162 ± 8.8 [c]	132 ± 8.55 [c]	104.33 ± 4.69 [c]	64.54 ± 1.23 [d]
PP Ethanol Extract(200mg/kg)	302.5 ± 10.61	294.66 ± 9.6 [d]	281.66 ± 12.69 [d]	264.16 ± 11.66	255 ± 11.37 [d]	244.33 ± 14.6 [c]	232 ± 11.97 [d]	23.53 ± 1.58 [c]
PP Ethanol Extract(400mg/kg)	285.66 ± 14.99	263.5 ± 12.26 [c]	242.66 ± 12.18 [d]	212.5 ± 9.39 [c]	173.5 ± 12.98 [d]	157.16 ± 13.01 [d]	115.33 ± 7.95 [d]	59.79 ± 1.07 [d]
F-statistic for decrease in blood glucose	-	3.63	9.79	24.77	42.56	49.4	136.93	291.13
P Value	-	0.031*	0.001***	0.001***	0.001***	0.001***	0.001***	0.001***

Values are expressed as Mean ± SEM (n = 6) in each group

* Significant difference at α = 0.05, *** significant difference at α = 0.001, [b] base line values for comparison in paired t-test. [c] significant decrease at α = 0.05 level, [d] significant decrease at α = 0.01 level

Table 8
Effect of PP on percentage loss in body weights of diabetic rats.

Treatment	Tenth day	Twentieth day	Thirtieth day
Solvent control(10ml/kg)	3.5 ± 1.04	6.5 ± 1.04	8.5 ± 1.87
Metformin(250mg/kg)	2.66 ± 0.81	5.5 ± 1.87	6.5 ± 1.87
PPE 200	6.5 ± 1.37	11.16 ± 1.16	17 ± 4.56
PPE 400	4.33 ± 1.03 #	6.83 ± 1.47	10.5 ± 1.04
F value	13.75	18.57	17.21
p value	0.001**	0.001**	0.001**

Values are expressed as Mean ± SEM (n = 6) in each group

** significant difference at α = 0.01, # significant change in body weight at α = 0.05 using paired t-test.

Table 9
Serum biochemical parameters of Polygonum plebeium

Treatment	AST	ALT	ALP	BT	BD	Albumin	TP	HbA1c
Group-I Normal control	85.7 ± 9.72	56.8 ± 1.40	235 ± 10.7	0.82 ± 0.13	0.25 ± 0.02	2.92 ± 0.01	6.12 ± 0.16	6.88 ± 0.12
Group-II Diabetic control	124 ± 5.88 ##	61.8 ± 6.01 ns	71.0 ± 4.62 ###	0.34 ± 0.12 ns	0.27 ± 0.03 ns	2.90 ± 0.02 ns	5.62 ± 0.2 ns	10.8 ± 0.28 ###
Group-III Metformin (250 mg/kg)	30.7 ± 6.12 ***	25.8 ± 1.30 ***	144 ± 9.55 ***	0.75 ± 0.12 ns	0.24 ± 0.01 ns	2.72 ± 0.02 ***	6.91 ± 0.29 **	4.70 ± 0.16 ***
Group-IV PPE 200 mg/kg	70.3 ± 1.74 ***	35.2 ± 2.20 ***	176 ± 5.46 ***	0.91 ± 0.17 ns	0.29 ± 0.03 ns	3.15 ± 0.03 ***	6.78 ± 0.13 **	7.40 ± 0.12 ***
Group-V PPE 400 mg/kg	64.8 ± 2.85 ***	30.8 ± 1.49 ***	170 ± 14.8 ***	0.88 ± 0.17 ns	0.24 ± 0.01 ns	3.05 ± 0.02 ***	6.42 ± 0.25 ns	5.52 ± 0.29 ***

The values were expressed as mean ± SEM (n = 6). The data were carried out by one-way ANOVA (Tukey's test), # < 0.05, ## < 0.01, ### < 0.001 compared between normal control and diabetic control groups. * < 0.05, ** < 0.01, *** < 0.001compared to Group-II VS Group III, IV, and V.

Table 10
Lipid profile parameters of Polygonum plebeium

Treatment	TC	TG	HDL	LDL	VLDL	CHOL/HDL	LDL/HDL Ratio
Group-I Normal control	70.3 ± 2.23	124 ± 9.63	48.3 ± 2.11	39.3 ± 6.21	24.9 ± 2.14	1.47 ± 0.05	0.82 ± 0.12
Group-II Diabetic control	129 ± 1.38 ###	164 ± 5.89 ##	30.7 ± 3.17 ###	63.5 ± 5.15 ##	32.5 ± 1.38 ns	2.42 ± 0.26 ns	2.14 ± 0.19 ###
Group-III Metformin (250 mg/kg)	51.7 ± 3.74 ***	63.7 ± 3.54 ***	47.7 ± 1.54 ***	21.3 ± 2.98 ***	21.4 ± 2.42 **	1.48 ± 0.04 ns	0.44 ± 0.05 ***
Group-IV PPE 200 mg/kg	81.2 ± 3.66 ***	116 ± 9.68 ***	24.1 ± 1.73 ns	23.4 ± 3.63 ***	33.8 ± 1.90 ns	3.0 ± 0.25 ns	0.99 ± 0.16 ***
Group-V PPE 400 mg/kg	60.8 ± 4.35 ***	70.8 ± 3.09 ***	20.2 ± 2.0 *	21.4 ± 2.16 ***	18.6 ± 2.17 ***	3.69 ± 0.46 *	1.10±.15 ***

The values were expressed as mean ± SEM (n = 6). The data were carried out by one-way ANOVA (Tukey's test), # < 0.05, ## < 0.01, ### < 0.001 compared between normal control and diabetic control groups. * < 0.05, ** < 0.01, *** < 0.001compared to Group-II VS Group III, IV, and V.

Table 11
Binding affinity of compounds from GC-MS analysis of PPE.

Sl. No.	Name of compound	Alpha glucosidase (3wy2)			Alpha-Amylase (3baj)			GLUT-2 (3sz1)			PPAR-γ (4
		Score (kcal/mol)	Hydrogen bond	Distance (Å)	Score (kcal/mol)	Hydrogen bond	Distance (Å)	Score (kcal/mol)	Hydrogen bond	Distance (Å)	Score (kcal/mol)
1	Metformin	-7.6	HIS332, ARG400, ARG200, ASP202, HIS332, GLU271, ASP333	2.58, 2.3, 2.43, 2.1, 2.36, 3.46, 3.43	-7.2	THR314, GLN302, ILE312, THR314, ASP317	2.28, 2.52, 2.30, 2.43, 2.42	-7.7	GLN280, GLU378	2.14, 3.57	-8.6
2	Ethyl tridecanoate	-5.7	-	-	-4.8	-	-	-5.0	-	-	-4.4
3	Stevioside	-7.9	VAL335, GLU377, GLY399, LEU300	2.23, 2.16, 2.79, 3.79	-8.2	TYR151, HIS305, GLU233, ASP300, GLU233	3.13, 2.37, 2.97, 2.63, 2.98	-8.5	ASN413, SER64, THR28, ASN413, SER71	3.15, 2.92, 2.10, 2.03, 3.06	-10.3
4	Phytol	-7.0	ASN301	2.95	-6.6	GLN63	2.95	-5.5	-	-	-5.3
5	Agaricic acid	-7.0	-	-	-6.2	-	-	-6.9	-	-	-6.2
6	1-Tetradecyne	-5.6	-	-	-4.7	-	-	-4.9	-	-	-4.0
7	1,2-Benzene dicarboxylic acid, butyl 2-ethylhexyl ester	-7.3	-	-	-7.0	-	-	-7.2	-	-	-6.9
8	Pentadecanoic acid, ethyl ester	-5.4	-	-	-4.9	-	-	-5.0	-	-	-5.1
9	Tetratetracontane	-5.5	-	-	-5.4	-	-	-4.2	-	-	-5.2
10	Linoleic acid ethyl ester	-5.2	-	-	-5.5	-	-	-5.5	-	-	-4.9
11	Oleic Acid	-6.4	-	-	-5.4	-	-	-5.3	SER412	3.59	-4.8
12	Dodecanoic acid, ethyl ester	-4.3	-	-	-5.2			-4.5			-4.4

DISCUSSION

Type 2 diabetes is rising rapidly worldwide, driven by ageing populations, increasing obesity and physical inactivity, and longer survival due to improved care. Despite better management, vascular complications remain the leading cause of disability, death, and the overall social and economic burden of the disease [40]. Medicinal plants represent an important therapeutic resource in the management of diabetes mellitus. Numerous traditionally used plants exhibit significant antidiabetic potential with minimal adverse effects, largely attributed to their rich content of bioactive phytochemicals such as flavonoids, alkaloids, phenolics, and tannins. These compounds modulate glucose homeostasis through multiple mechanisms, including enhancement of pancreatic β-cell function, stimulation of insulin secretion, and attenuation of intestinal glucose absorption, highlighting their relevance as complementary or alternative strategies for diabetes management [41].

Gas chromatography – mass spectrometry (GC–MS) analysis was employed to identify the phytoconstituents present in the extract, with mass spectra of unknown compounds compared against the (NIST) mass spectral library [42]. The ethanolic extract of *P. plebeium* revealed the presence of 11 phytoconstituents, among which oleic acid was detected in the highest proportion, a compound previously reported for its anti-inflammatory activity [39]. In addition, stevioside, known for its promising antidiabetic potential, was also identified. The presence of these bioactive constituents provides a plausible phytochemical basis for the antidiabetic and associated pharmacological effects observed with the ethanolic extract [34].

In vitro inhibition of α-amylase and α-glucosidase is widely used as an initial screening approach in antidiabetic research, as these enzymes play a central role in carbohydrate digestion and glucose release. α-Amylase initiates starch digestion, while α-glucosidase completes the process by releasing absorbable glucose. Inhibiting these enzymes slows glucose absorption and helps reduce postprandial blood glucose spikes, a key goal in type 2 diabetes management. This mechanism is similar to that of standard drugs like acarbose, making these assays reliable indicators of antihyperglycemic potential. Inhibiting both enzymes offers better glycemic control, while IC_{50} values allow straightforward comparison of inhibitory strength among different extracts and compounds [43]. The α-amylase and α-glucosidase inhibitory activities of *P. plebeium* extracts (PPP, PPC, PPEA, PPE, and PPA) showed notable effects. Among these, the ethanolic extract (PPE) exhibited the highest percentage inhibition and a favorable inhibitory concentration, comparable to the standard drug acarbose.

The oral glucose tolerance test (OGTT) is a key tool in both diabetes research and diagnosis. Unlike a simple fasting glucose test, it shows how the body handles sugar after a standard glucose load, giving a clearer picture of glucose regulation [44]. The OGTT results indicate that *P. plebeium* extracts modulate glucose homeostasis in both normal and diabetic rats. In normal animals, PP extracts attenuated the postprandial rise in blood glucose, with the ethanolic

extract (PPE, 400 mg/kg) showing the most pronounced reduction at later time points, comparable to metformin. In diabetic rats, marked glucose intolerance in the control group was significantly improved by PPE in a dose-dependent manner. The higher dose (400 mg/kg) produced a sustained reduction in blood glucose levels, though less potent than metformin, demonstrating meaningful antihyperglycemic activity.

Acute and long-term effects on blood glucose levels in diabetic rats are crucial for validating antidiabetic activity. The acute antihyperglycemic response indicates immediate pharmacological action, suggesting mechanisms such as insulin secretagogue activity, enhanced peripheral glucose utilization, inhibition of intestinal glucose absorption, or suppression of hepatic gluconeogenesis. Conversely, sustained glucose reduction following repeated administration reflects long-term improvement in glycemic control, associated with enhanced insulin sensitivity, protection or regeneration of pancreatic β-cells, and regulation of carbohydrate-metabolizing enzymes [45–47]. The present study demonstrated that the PPE exerts significant antihyperglycemic effects in diabetic rats under both acute and chronic treatment conditions. In the acute study, diabetic control animals showed a progressive rise in blood glucose levels, indicating impaired glucose utilization. In contrast, the PPE at 400 mg/kg produced a reduction in blood glucose and showed a marked and sustained decline from 2 hrs onward, comparable to the standard drug metformin. The significant reduction in 2 to 8 hrs post-treatment glucose levels suggest improved glucose clearance and enhanced peripheral glucose uptake. Long-term administration of the PPE further confirmed its antihyperglycemic activity. Daily treatment for 30 days resulted in a significant and progressive reduction in fasting blood glucose levels. PPE at the dose of 400 mg/kg achieved a significant reduction in blood glucose level comparable to metformin.

In diabetic rats, prolonged high blood glucose levels cause increased breakdown of muscle and fat, leading to a gradual loss of body weight. Effective antidiabetic treatment can prevent or reverse this weight loss by improving blood glucose control and restoring insulin function, which helps normalize metabolism [48, 49]. In this study, 400 mg/kg of PPE effectively preserved body weight and showed an effect comparable to metformin by day 30. This improvement suggests enhanced metabolic control, likely due to improved glycemic regulation and nutrient utilization.

Diabetes causes marked changes in serum biochemical and lipid profile parameters due to impaired insulin action and persistent hyperglycemia. Diabetic conditions are commonly associated with elevated blood glucose and metabolic stress markers, along with dyslipidemia characterized by increased total cholesterol, triglycerides, LDL, and VLDL, and reduced HDL levels, indicating disturbed lipid metabolism and increased cardiovascular risk. These abnormalities result from enhanced lipolysis, increased hepatic lipid synthesis, and reduced lipid clearance. Effective antidiabetic treatment helps restore these altered parameters toward normal levels by improving insulin sensitivity and metabolic regulation [50–52]. The present study indicates that administration of PPE at 400 mg/kg showed remarkable alteration of serum biochemical and lipid profiles.

Histopathological analysis of the pancreas, liver, and kidney revealed significant tissue damage in diabetic rats, which was notably improved with treatment. In the diabetic group, the pancreas showed severe degeneration and necrosis in both exocrine and endocrine regions, with vacuolar changes and vascular congestion; the liver exhibited distorted architecture and centrilobular necrosis; and the kidneys displayed tubular dilation, disorganized nephron structure, and increased vacuolization. Metformin treatment effectively restored normal tissue architecture across all organs. Similarly, PPE at 400 mg/kg provided substantial protection, with regeneration of pancreatic islets, improved liver parenchyma, and normalized kidney structure, including well-preserved glomeruli and tubules. These results suggest that PPE has significant protective and restorative effects against diabetes-induced tissue damage.

In silico studies, including molecular docking, provide a rapid and cost-effective approach to predict the interaction of bioactive compounds with target proteins relevant to diabetes. These computational analyses help identify potential mechanisms of action, binding affinity, and stability of ligand-receptor interactions, thereby supporting and guiding *in vitro* and *in vivo* studies. By simulating molecular interactions, *in silico* studies allow the prioritization of promising compounds, such as phytoconstituents from plant extracts, for further experimental validation [53, 54]. The *in silico* molecular docking study revealed that compounds identified from the GC-MS analysis of the PPE exhibited strong binding affinity and hydrogen bonding interactions with the target molecules. Among them, stevioside showed particularly promising docking results, with binding characteristics comparable to the standard drug, metformin. These findings suggest that the bioactive compounds present in the PPE may contribute significantly to its observed antidiabetic activity.

CONCLUSION

In conclusion, the ethanol extract of *P. plebeium* has significant antidiabetic effects, working through several key mechanisms. The GC-MS analysis identified bioactive compounds like stevioside and oleic acid, which seem to drive much of this activity. *In vitro* analysis revealed promising inhibition of α-glucosidase and α-amylase, and while acute and long-term OGTT confirmed rapid and sustained blood glucose lowering. Treatment with PPE improved serum biochemical parameters, normalized lipid profiles, and protected pancreatic, hepatic, and renal tissues, as evidenced by histopathology. *In silico* molecular docking further supported the interaction of key phytoconstituents with diabetes-related targets. Collectively, these findings suggest that the ethanol extract of *P. plebeium* is a promising multi-targeted antidiabetic agent, and further pharmacological, molecular, and clinical studies are warranted to fully validate its therapeutic potential.

Abbreviations

ALP
Alkaline Phosphatase
ALT
Alanine Aminotransferase
AST
Aspartate Aminotransferase
GLUT-2

Glucose Transporter-2
HDL
High-Density Lipoprotein
LDL
Low-Density Lipoprotein
OGTT
Oral Glucose Tolerance Test
PPA
Aqueous extract of *Polygonum plebeium*
PPAR-γ
Peroxisome Proliferator-Activated Receptor gamma
PPC
Chloroform extract of *Polygonum plebeium*
PPE
Ethanol extract of *Polygonum plebeium*
PPEA
Ethyl acetate extract of *Polygonum plebeium*
PPP
Petroleum ether extract of *Polygonum plebeium*
TC
Total Cholesterol
TG
Triglycerides
VLDL
Very Low-Density Lipoprotein

Declarations

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Author Contribution

Miss. Sarojini Nayak: Conceptualization, Investigation, Methodology, Data curation, Writing original draft. Dr. Druga Madhab Kar: Supervision, Project administration, Validation, review, and editing. Dr. N. Saroj Kumar Choudhury: Formal analysis, Validation, and Data curation. Dr. Smrutiranjan Dash: Methodology, Software, Visualization, Formal analysis, review, and editing.

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Figures

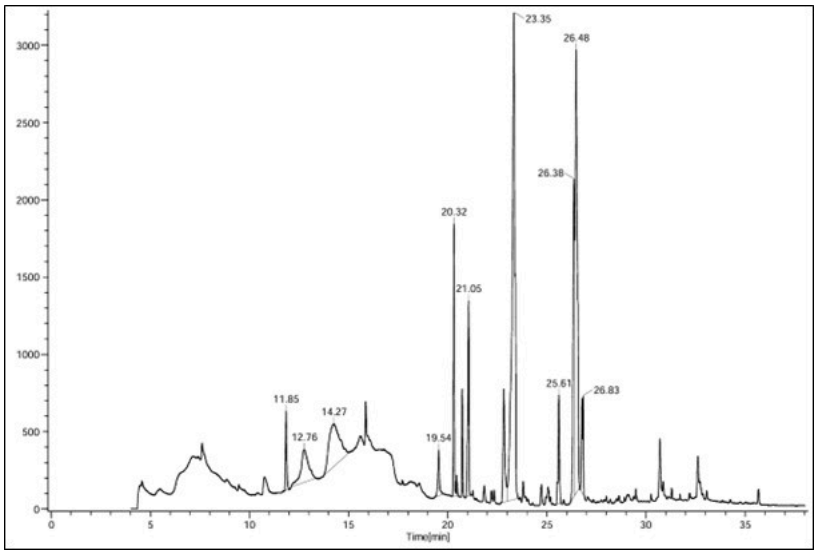


Figure 1

GC-MS chromatogram of PPE

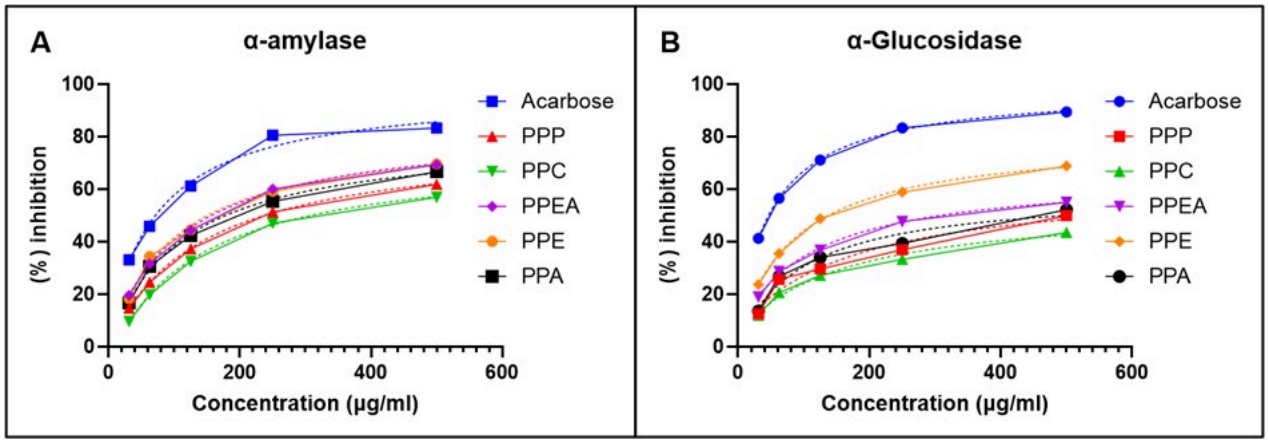


Figure 2

Percentage inhibition of (A) α-amylase, (B) α-glucosidase at different concentrations of acarbose, PPP, PPC, PPEA, PPE, and PPA

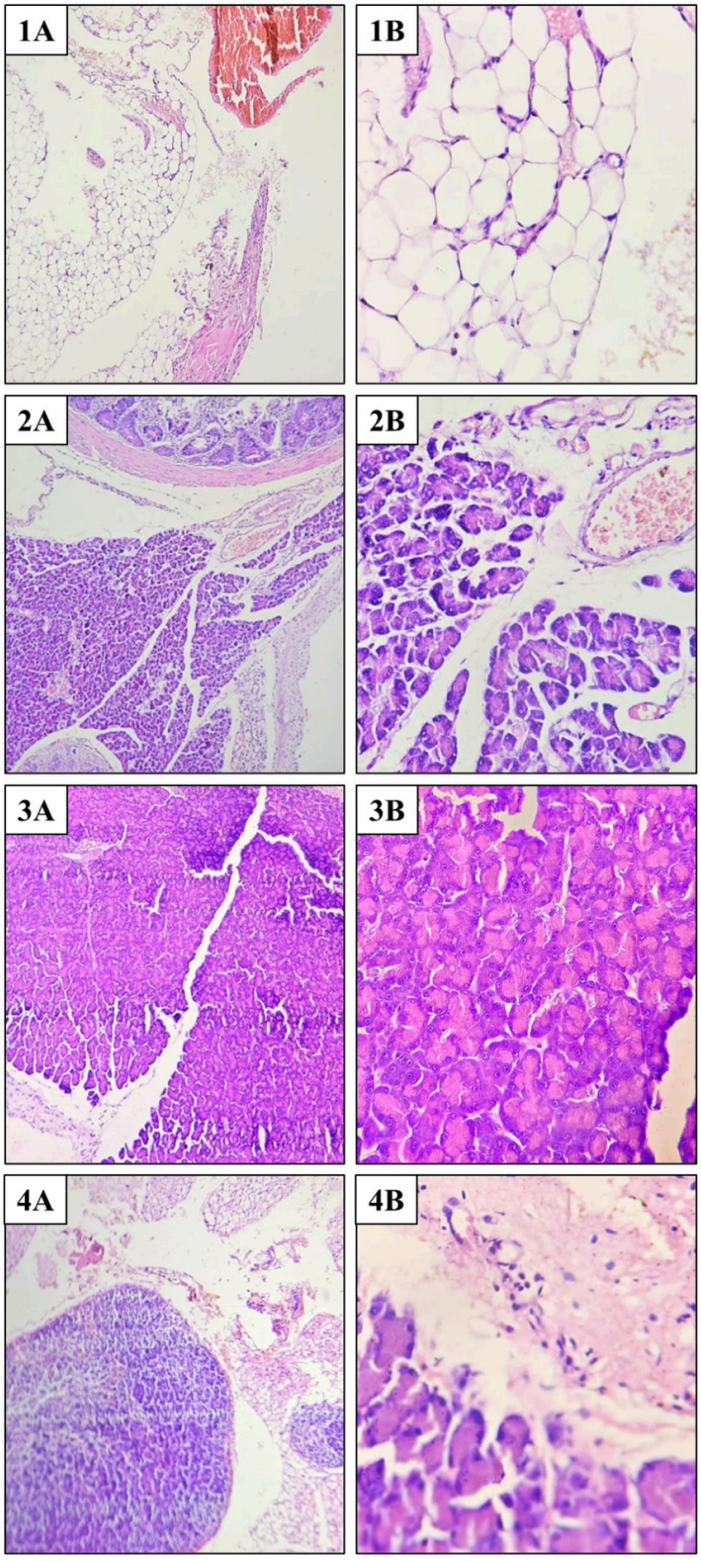


Figure 3

Histopathology of pancreatic tissue (TS) at 10x **(A)** and 40x **(B)** magnification. **1A**and **B:** Normal control showed intact acinar architecture without necrosis or inflammation. **2A** and **B:** Diabetic exhibited severe degenerative and necrotic changes in both exocrine and endocrine regions, with vacuolar degeneration and vascular congestion. **3A** and **B:** Metformin restored acinar architecture. **4A** and **B:** PPE 400 mg/kg demonstrated improvement in histoarchitecture, particularly regeneration of the islet of Langerhans.

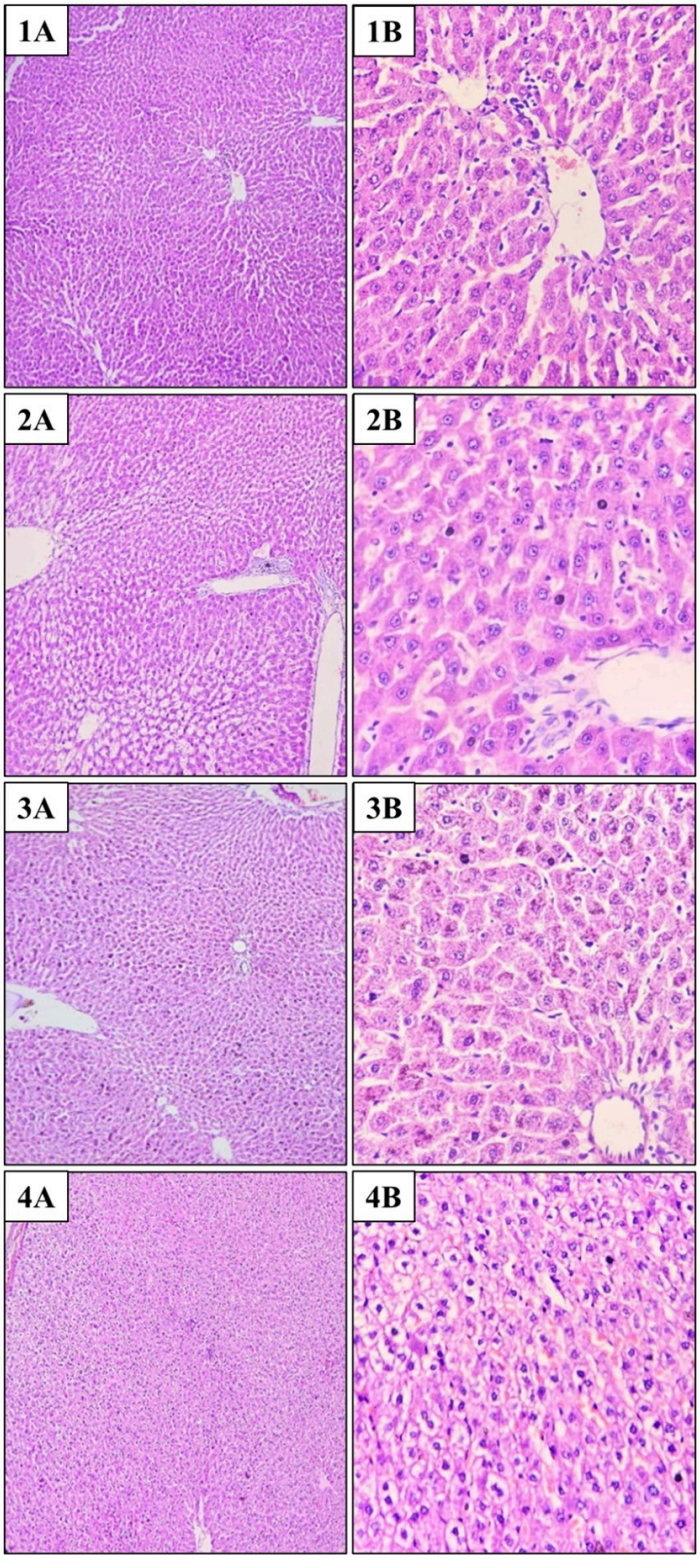


Figure 4

Histopathology of liver tissue (TS) at 10x **(A)** and 40x **(B)** magnification. **1A**and **B:** Normal control exhibited preserved hepatic architecture with normal hepatocytes and central vein. **2A** and **B:** Diabetic control group showed marked histopathological alterations characterized by distorted hepatic architecture and prominent centrilobular necrosis. **3A** and **B:** The metformin-treated group demonstrated a marked restoration of hepatic parenchyma with reduced necrosis. **4A** and **B:** PPE 400 mg/showed significant improvement in hepatic histoarchitecture.

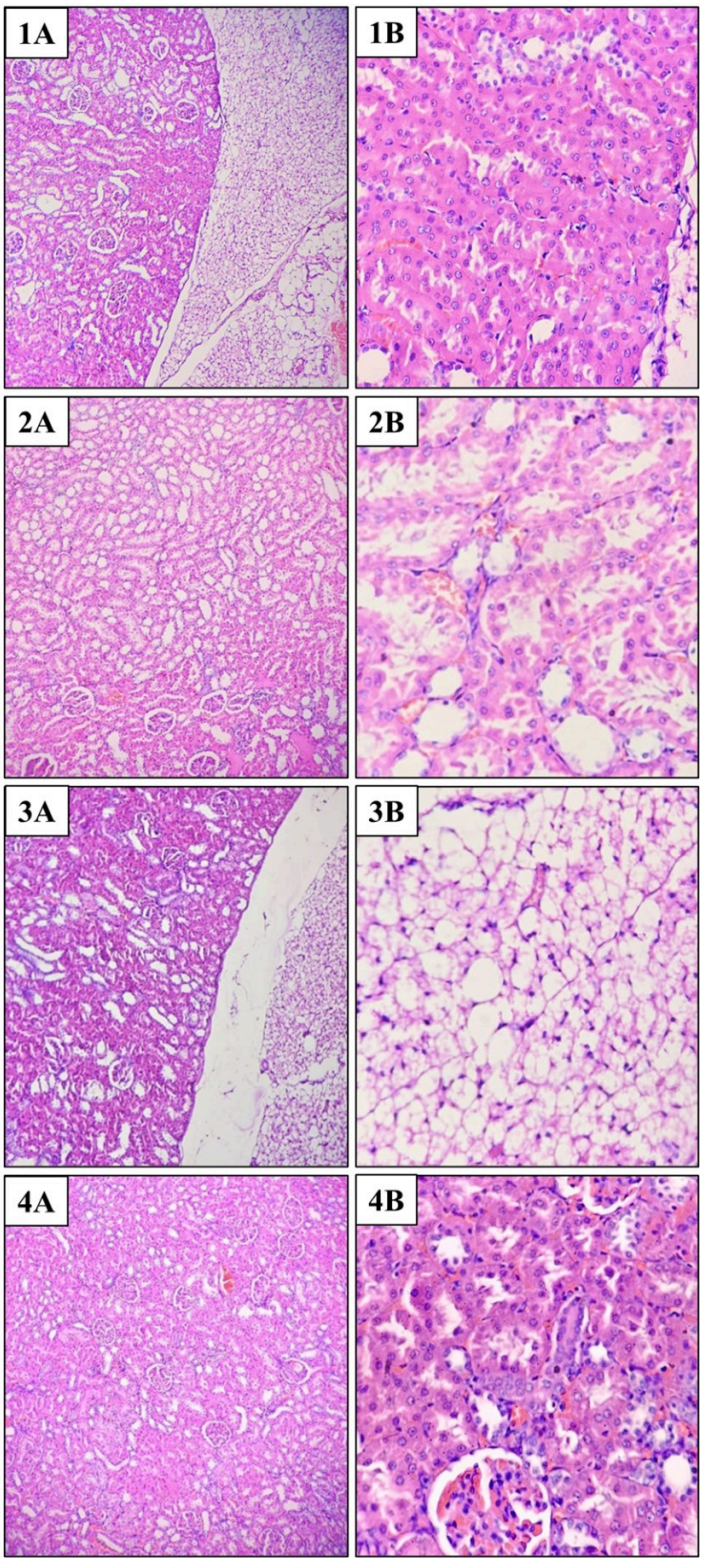


Figure 5

Histopathology of the kidney (TS) at 10x **(A)** and 40x **(B)** magnification. **1A**and **B:** Normal control showed Normal control group showed normal renal histoarchitecture with intact glomeruli and well-organized renal tubules. **2A**and **B:** The diabetic control group exhibited mild tubular dilation, disorganized nephron architecture, and increased endocytic vacuolization in tubular epithelial cells. **3A** and **B:** Metformin showed restoration of nephron architecture with improved glomerular structure and reduced endocytic vacuoles. **4A** and **B:** PPE 400 mg/kg. demonstrated normal renal histology with well-preserved glomeruli, normalized tubular arrangement, and minimal vacuolization.

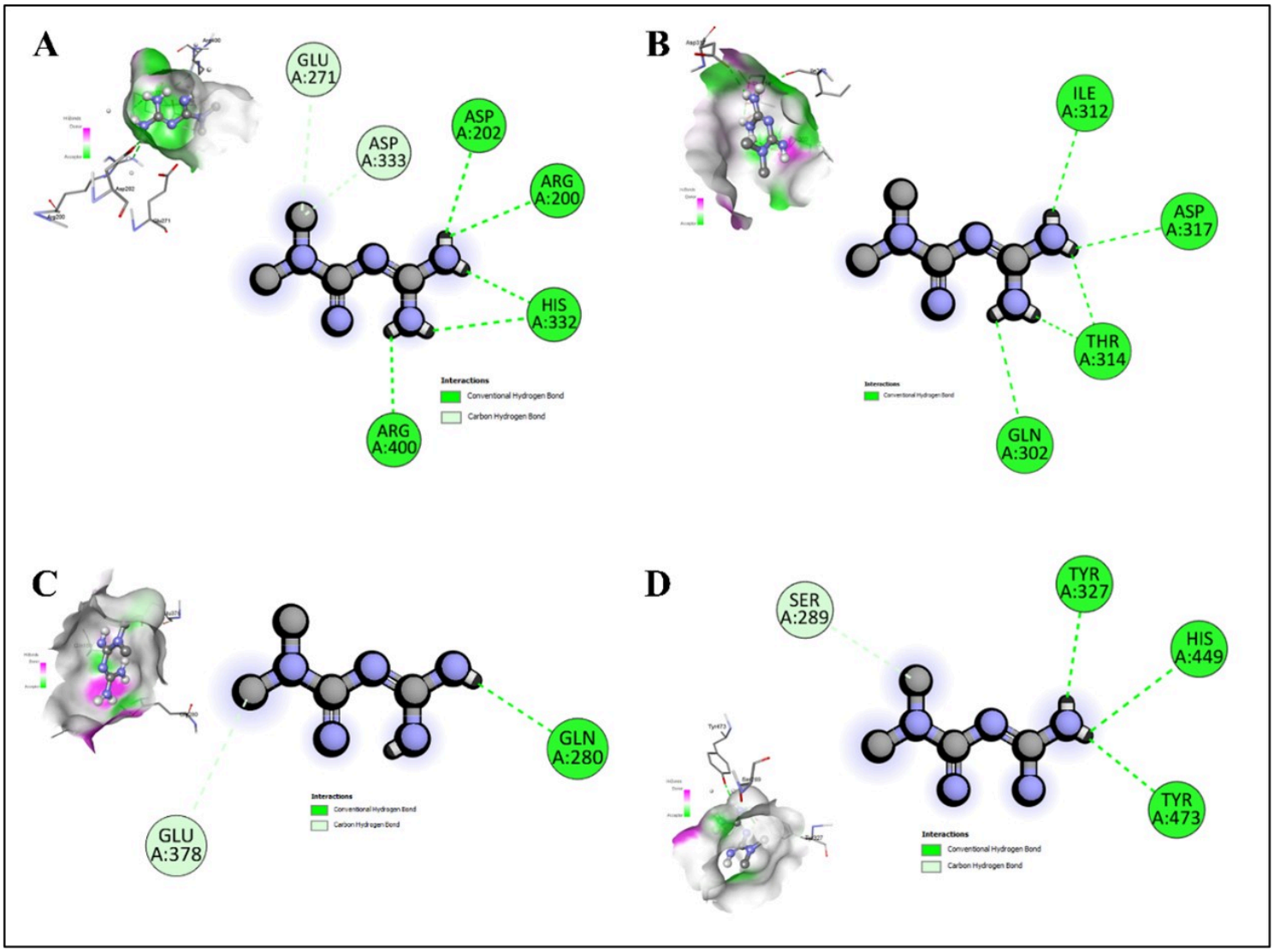


Figure 6

Binding affinity of metformin with (A) Alpha-glucosidase, (B) Alpha-Amylase, (C) GLUT-2, (D) PPAR-γ

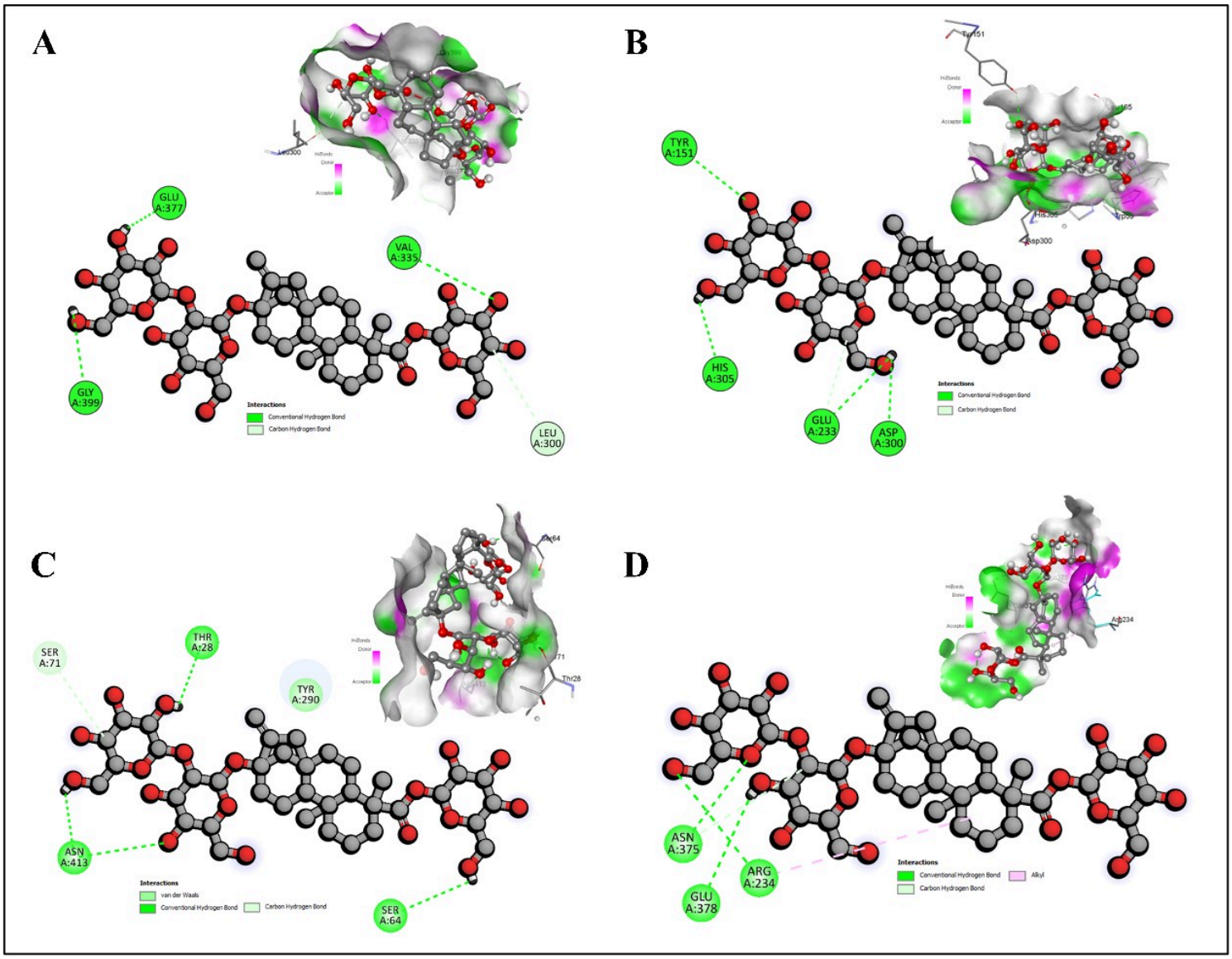


Figure 7

Binding affinity of stevioside with (A) Alpha-glucosidase, (B) Alpha-Amylase, (C) GLUT-2, (D) PPAR-γ