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Research Article

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Antioxidant Activity, *In Vitro* and *In Vivo* Antidiabetic Efficacy of the Ethanolic Extract from *Pyrus communis* Leaves.

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Abstract

One of the more complex long-term metabolic diseases, diabetes is characterised by high blood sugar levels that are either caused by insufficient or inefficient insulin production or use by the body. Antioxidant activity, *in vitro* and *in vivo* antidiabetic potency of *Pyrus communis* leaf ethanolic extract. The study involved collecting *Pyrus communis* leaves from Tamil Nadu, India, and analyzing their phytochemical properties. *In vitro*, antioxidant assays were conducted, and total antioxidant activity was determined. *In vivo*, antidiabetic activity was evaluated in streptozotocin-induced diabetic rats, and the effects of the extract with glibenclamide were evaluated over 4 weeks. Phytochemical screening identified glycosides, phenolics, flavones, flavonoids, terpenoids, and sterols in the extract, highlighting its diverse bioactive components. *In vitro*, antioxidant activity revealed notable concentration-dependent radical scavenging effects, with DPPH inhibition ranging from 18.86% to 50.97%, ABTS inhibition from 16.01% to 72.42%, and increasing reducing power from 0.2381 to 1.413. The ethanolic extract showed a strong *in vitro* antidiabetic effect by inhibiting alpha-amylase and alpha-glucosidase in a dose-dependent manner, with inhibition percentages increasing from 13.44% to 64.89% and from 7.84% to 42.79%, respectively. *In vivo*, studies on STZ-NA-induced diabetic rats revealed protective effects on body weight, with final weights of 163.0±2.08 g and 180.7±3.13 g for lower and higher doses, respectively. Blood glucose levels significantly decreased in test groups, reaching 230.5±2.30 mg/dl and 224.0±3.02 mg/dl, indicating potential antidiabetic efficacy. Histopathological examination revealed preserved pancreatic architecture akin to the normal and standard groups, suggesting protection against diabetes-induced structural alterations. This thorough analysis highlights the antioxidant and antidiabetic properties of *P. communis* leaf extract, emphasizing its potential as a medicinal agent for treating oxidative stress and diabetes. Further clinical trials and additional mechanistic research are needed to validate this drug's therapeutic efficacy and safety.

Keywords: *Pyrus communis*; ethanolic extract; DPPH; ABTS; alpha-amylase; *in vivo* diabetic activity.

1. Introduction

High blood glucose levels are a major marker of sugar metabolic diseases caused by inadequate production or inefficient utilization of insulin. Conditions like this, which affect more human beings worldwide, pose a significant health challenge owing to their potential for various complications [1, 2]. One of the most pervasive complications is cardiovascular disease, which increases

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the incidence of heart disease-related complications [3]. The interplay between elevated blood sugar levels and vascular damage contributes to a higher incidence of stroke, thereby emphasizing the intricate relationship between diabetes and cardiovascular health. In addition to the cardiovascular system, diabetes affects the kidneys, leading to various metabolic complications [4, 5]. The kidneys, tasked with filtering waste products, succumbed to prolonged exposure to high glucose levels, impairing vital functions. Nerve damage or neuropathy is another form of diabetes that manifests in various forms [6]. Peripheral neuropathy often begins in the feet and legs and causes tingling, numbness, and pain. The intricate network of nerves that control involuntary bodily functions can also be affected, resulting in autonomic neuropathy. This can lead to complications such as digestive issues, blood pressure abnormalities, and sexual dysfunction. The eyes also bear the brunt of diabetes, as the disease can precipitate diabetes-related retinopathy, which damages the blood vessels in the retina and, if ignored, can result in blindness and visual impairment. The integumentary system is not spared, as individuals with diabetes are more susceptible to skin infections and delayed wound healing. Poor circulation and nerve damage contribute to these complications, necessitating meticulous foot care to prevent ulcers and infections that may culminate in amputation in severe cases.

Additionally, hypoglycemia or low blood sugar poses a risk, particularly for those using insulin or certain diabetes medications. The symptoms of hypoglycemia, ranging from shakiness to confusion and unconsciousness in severe cases, underscore the delicate balance required for diabetes management. Comprehensive diabetes management involves a multi-faceted approach [7]. Medications, including sugar-switch hormones and oral glucose-lowering medications, are often used to regulate blood sugar concentration [8, 9]. Routine blood testing of the sugar stages is imperative to maintain treatment plans as needed. Furthermore, routine medical check-ups are crucial for the prior detection of the disease. Patient education and support are integral components that empower individuals to make informed decisions about their health [10].

Plant medicine is a compelling choice for diabetic treatment, offering a natural and holistic approach to managing the condition. Plant-based remedies exhibit promising antidiabetic properties by harnessing the therapeutic potential of diverse botanical compounds. With the ability of various plants to regulate blood glucose levels, enhance insulin sensitivity, and mitigate inflammation, individuals are increasingly considering plant medicine as a viable alternative to conventional diabetes treatment [11]. The appeal lies not only in the potential efficacy of these remedies but also in their relatively fewer side effects, aligning with a holistic philosophy that addresses overall well-being. As scientific research continues to validate the benefits of plant-based interventions, integrating plant medicine into diabetic care represents a promising avenue for those seeking a comprehensive and natural approach to managing their health. *Pyrus communis* leaves, rich in polyphenols like quercetin and kaempferol, exhibit potent antioxidant, anti-inflammatory, antimicrobial, and cardioprotective properties. These bioactive compounds help neutralize free radicals, reducing oxidative stress and inflammation, making the leaves useful in managing chronic conditions such as cardiovascular diseases, arthritis, and skin inflammation.

Additionally, they have shown antimicrobial activity, offering the potential to develop natural antibiotic agents. The leaves also hold antidiabetic potential by modulating glucose metabolism and are beneficial in skin care and wound healing due to their antioxidant and tissue-repairing properties. [12, 13]. The current investigation involved the extraction process and assessing the ethanolic extract antidiabetic and antioxidant properties of the *Pyrus communis* leaf.

2. Material and Methods

2.1. Selecting and identifying plants

Pyrus communis leaves were gathered from the hills surrounding Kodaikanal in Tamil Nadu, India, in July (**Figure 1**). The authentication process was conducted in BSI Coimbatore, India. The certificate number is no. BSI/SRC/5/23/2022/Tech/623. Pyrus communis leaves were collected in May-July before flowering because it is the best time to get more phytoconstituents from the leaves. Following the gathering, the leaves were thoroughly cleaned, dried in the shadows, and crushed to a rough powder until needed again; the resultant powder was kept in an airtight plastic container [14].

Figure 1. *Pyrus communis*.

2.2. Ethanolic extraction of leaves of Pyrus communis

The Pyrus communis leaves were originally dried and ground into coarse powder. Finely ground plant leaf powder from which ethanol was extracted (60–80°C). Ethanol was selected based on the extractive value, which had a high percentage yield and a higher amount of flavonoid phenolic contents in the extract than other solvent systems. A Soxhlet device was used to extract the ethanol for 72 h at 40°C. Whatman No. 1 filter paper (Whatman Ltd., England) filters the produced silt. The resulting plant extract was then subjected to additional vacuum concentration at 40°C using a rotating vacuum evaporator (Buchan R-V120, Switzerland). The crude extract was weighed and stored at 4°C until further examination [15].

2.3. Preliminary phytochemical screening of ethanolic extraction of leaves of Pyrus Communis.

The ethanolic extract of *Pyrus communis* leaves underwent preliminary phytochemical screening to identify key phytoconstituents, including alkaloids, flavonoids, saponins, tannins, and glycosides. The concentrations of total phenols and flavonoids in the extracts were quantified using a spectrophotometric method [16].

2.4. Ethanolic extract of Pyrus communis leaves: an in vitro antioxidant assay

2.4.1. DPPH assay

According to Leo and Ong [17], the ethanolic extract of *P. communis* leaves was examined for radical scavenging using the DPPH assay. The DPPH absorbance at 517 nm was measured using an ascorbic acid standard. Samples ranging in concentration from 12.5 µg/mL to 200 µg/mL were prepared using the stock solution and DMSO to yield a final volume of 20µl. 1.48 ml of DPPH solution (0.1 mM) was then added. The same amount of distilled water was used to make a control, but the test element was absent. After the reaction, the mixture was allowed to stand at room temperature for 20 minutes in the dark, and the absorbance at 517 nm was recorded. The baseline control was three mg/mL of DPPH [17]. The following formula was used to get the inhibition percentage: Inhibition Percentage = Control-Test/Control×100.

2.4.2. Assay ABTS

The preformed radical monocation of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+), a blue chromogen, is produced when ABTS is oxidised with potassium persulfate. Antioxidants that donate hydrogen decrease this radical cation. Ascorbic acid (10 mg/mL)

was employed as a norm. Assess antioxidant activity in various test sample concentrations (125–2000 µg/mL, prepared from 10 mg/mL); 0.2 mL of each sample was mixed with 1 mL of distilled water. Following this, 0.16 mL of the ABTS solution was added, and the volume was adjusted to the last volume of 1.36 mL. A control sample was also prepared, which included the same volume of distilled water but was free of the test ingredient. A 20-minute incubation period was followed by an absorbance measurement at 734 nm [18]. The following formula was used: Percentage of inhibition = Control Test/Control ×100 to calculate percentage inhibition.

2.4.3. Reducing power assay

Pyrus communis ethanolic extract reducing power was measured using the Paulo technique [19]. 2.5 mL of phosphate buffer was mixed with different quantities of the sample, which ranged from 125 to 2000 µg/mL and were made from a 10 mg/mL stock solution (200 mM, pH 6.6). Potassium ferric cyanide, 2.5 mL, 1% was added to this mixture, and after 20 min at 50°C, the resultant solution was heated. A control sample that included the same volume of distilled water was also prepared but did not contain the test substance. Following the incubation period, each combination received 2.5 mL of 10% trichloroacetic acid (TCA) and was centrifuged at 650 g for 10 min. Then, 5 mL of distilled water, 1 mL of 0.1% ferric chloride, and 5 mL of the top layer (5 mL) were mixed. The absorbance of the resulting solution was measured at 700 nm. Quercetin (10 mg/mL in DMSO) was used as a reference compound for this analysis [19].

2.4.4. Total antioxidant activity

Pyrus communis A reagent solution containing 0.6 mL H2SO4, 28 mM sodium phosphate, and four mM ammonium molybdate was used to make 3 mL of ethanolic extract. The ethanolic extract was made at 125–2000 µg/mL concentrations, starting at a 10 mg/mL stock concentration. For ninety minutes, the reaction solutions were incubated at 95°C. Subsequently, at a wavelength of 695 nm, the absorbance of the solutions was determined relative to a blank (0.3 mL) instead of the extract. The reference was ascorbic acid (10 mg/mL in DMSO) [20]. The antioxidant power was expressed in ascorbic acid equivalents (g). Inhibition Percentage = Control-Test/Control×100.

2.5. *In-vitro* antidiabetic activity ethanolic extraction of leaves of *Pyrus communis*

2.5.1. Alpha amylase inhibitory assay

Identification of decreasing the sugars that α-amylase produces involves their interaction with dinitrosalicylic acid, which forms nitro-amino salicylic acid, a brown-colored product. Using 25 mM phosphate buffer at pH 6.9, various concentrations of ethanolic extraction of Pyrus communis leaves were made, ranging from 62.5 µg/mL to 1000 µg/mL, resulting in a final volume of 100 µL. The stock concentration was 10 mg/mL. Each sample was incubated at 25°C for ten minutes with 25 µL of 0.5 mg/mL porcine α-amylase. After the pre-incubation period, 25 µL of a 0.5% starch solution in a 25 mM phosphate buffer at pH 6.9 was added to each reaction mixture. After that, the mixtures were incubated for ten more minutes at 25°C. 50 µL of 96 mM 3,5-dinitrosalicylic acid was added to halt the process. The microplate was placed in a bath of boiling water for five minutes after allowing it to come to room temperature. At 540 nm, the absorbance was determined with an Erba Lisascan microplate reader [21].

2.5.2. Alpha-glucosidase inhibition assay

The measurement of the reducing sugar that results from alpha-glucosidase hydrolyzing sucrose made it possible to determine the enzyme's activity. The sample was made at 62.5 µg/mL and 1000 µg/mL, starting from a 10 mg/mL stock concentration. The sample was adjusted to a final volume of 100 µL using 0.1 M phosphate buffer at pH 7.2. After adding 25 µL of α-glucosidase to each sample, the mixture was incubated at 25°C for 10 minutes. Following preincubation, 37 mM sucrose was added to the reaction mixture and 1 mL of M phosphate buffer (pH 7.2). An enzyme and phosphate buffer-containing control tube was utilised as a point of comparison. The incubation period was increased to 10 minutes after 250 µL of glucose reagent was added to each tube. Absorbance was measured using a microplate reader at 510 nm [22].

2.6. *In*-vivo antidiabetic study of ethanolic extract of *Pyrus communis*

2.6.1. Acute toxicity studies

A toxicity assessment was conducted to determine the appropriate and safest dosage range for further

investigation rather than presenting the toxicity outcomes of the entire extract. Acute toxicity tests confirmed the administration of ethanolic extract of *Pyrus communis* up to a dosage of 2000 mg/kg. No significant alterations in animal behavior were observed. Additionally, no fatalities were observed at doses up to 4000 mg/kg body weight throughout the testing period. These findings were also documented during the study period.

2.6.2. *In-vivo* antidiabetic activity study in STZ-induced diabetic rats

Animals were accommodated in polypropylene cages with sawdust litter, and 23+2°c temperature was maintained. 12 hrs light and 12 hrs dark lightening were given over 24 hrs. Based on OECD guidelines, 423 acute toxicity studies were performed, and doses selected based on the mortality and clinical signs of the animal's higher and lower dose limits were adopted. Overnight-fasted Wistar male albino rats weighing 220 g were subjected to a single intraperitoneal administration of 55 mg/kg freshly prepared streptozotocin (STZ) in 0.1M citrate buffer (pH 4.5). Simultaneously, 120 mg/kg nicotinamide (NAD) was injected intraperitoneally after 15 min. Following 96 hours, rats were fasted overnight with access to water. Those with blood glucose levels > 250 mg/dl were classified as having type 2 diabetes mellitus. Experimental animals were divided into five groups (n=6 each). Group 1 received only the normal vehicle, whereas group 2 received streptozotocin (55 mg/kg, i.p.) + nicotinamide (120 mg/kg, i.p.). Group 3 received 0.25 mg/kg of glibenclamide for 4 weeks. In Group 4, a dose of 400 mg/kg of the test I was administered, while group 5 received 600 mg/kg of test II. Subsequently, the injected rats were monitored over 7 days to assess their diabetic status [23].

2.7. Statistical analysis

Experimental data are expressed as mean + standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Tukey's test, which was used for data analysis. Multiple comparisons of the data were employed using GraphPad Prism 5.0 software. All the data were considered significance $p<0.05$.

3. Results and Discussion

3.1. Plant collection and extraction

A meticulous collection of leaves from the verified plant *Pyrus communis* was made without adulterants. We collected leaf material from healthy and good-quality plants and employed shade drying to dry it and stop the degradation of bioactive phytoconstituents. Five hundred grams of the Pyrus communis leaf sample were weighed and set aside after the leaf had dried. Using a Soxhlet device, the material was subjected to a series of continuous heated extraction processes (**Figure 2**).

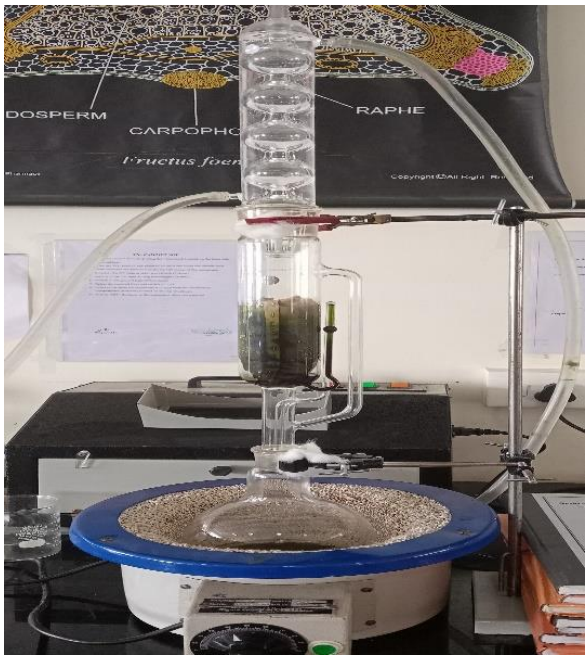

Figure 2. Ethanolic extraction of *Pyrus communis* leaf by Soxhlet apparatus

3.2. Qualitative phytochemical screening of ethanolic extraction of Pyrus communis leaf

The ethanolic extract (EE) of *Pyrus communis* leaves was subjected to qualitative phytochemical screening to identify various phytoconstituents. Notably, the extract tested positive for glycosides, indicating the potential presence of arbutin and anthraquinone glycosides. Phenolics, flavones, and flavonoids were also detected, suggesting the presence of antioxidants and anti-inflammatory agents in plant material. Additionally, terpenoids and sterols, known for their diverse pharmacological activities, were found in the extract. The presence of saponins and tannins further highlighted the extract as a basis of normal surfactants and antioxidants. However, ethanolic extracts did not detect

alkaloids, carbohydrates, and proteins/amino acids. This qualitative phytochemical profile offers insightful information on the bioactive constituents of *Pyrus communis* leaves, supporting their potential therapeutic applications and warranting further investigation into their specific health benefits.

3.3. *Pyrus communis leaf ethanolic extract in vitro antioxidant* assay *using DPPH assay*

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to assess the ethanolic extract of *Pyrus communis* leaves *in vitro* antioxidant activity percentage inhibition at various concentrations (µg/mL). Results demonstrated a concentration-dependent increase in antioxidant activity. At the lowest concentration of 12.5 µg/mL, the extract exhibited 18.86% inhibition of DPPH radicals, indicating its ability to neutralize free radicals (**Figure 3**). As the concentration increased, the percentage of inhibition also increased, reaching 50.97%, 200 µg/mL, the maximum concentration. This dose-response relationship suggests a dose-dependent scavenging effect on DPPH radicals by the ethanolic extract on DPPH radicals, showcasing its potent antioxidant potential. These findings highlight the promising antioxidant properties of *Pyrus communis* leaves and support their potential applications in combating oxidative stress-related disorders. Further studies would contribute to a comprehensive understanding of the plant's therapeutic potential, including additional antioxidant assays and identifying specific bioactive compounds responsible for the observed effects.

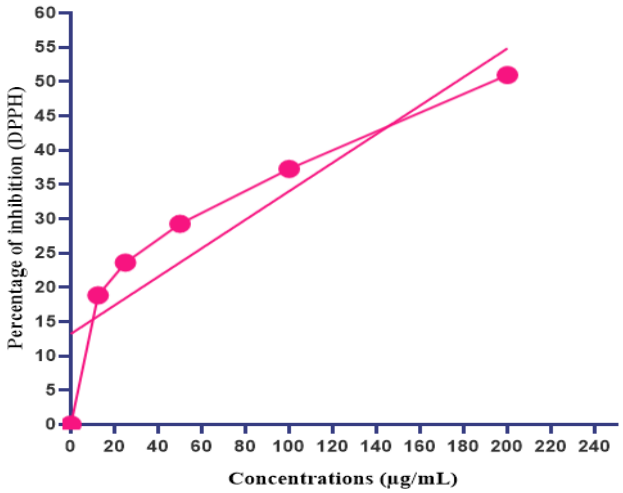

Figure 3. *Pyrus communis* leaf ethanolic extract's antioxidant capacity as determined by the DPPH test

3.4. *ABTS assay for Pyrus communis leaf ethanolic extract in vitro* antioxidant *assay*

This assay evaluates the % of inhibition at various dosages (µg/mL). Results demonstrated a concentration-dependent antioxidant effect, with the extract exhibiting increased ABTS radical scavenging activity as the concentration increased. At 125 µg/mL, the extract displayed 16.01% inhibition, indicating its ability to neutralize free radicals. As the concentration increased to 250, 500, 1000, and 2000 µg/mL, the percentage inhibition steadily increased, reaching 72.42% at the highest concentration tested (**Figure 4**). This dose-dependent response underscores the potent antioxidant potential of the Pyrus communis leaf extract, suggesting its effectiveness in quenching ABTS radicals. These findings contribute to an increasing amount of data indicating the antioxidant properties of plants, which are crucial for mitigating oxidative stress. Further investigations of specific antioxidant compounds in the extract would enhance our understanding of its therapeutic applications and potential health benefits.

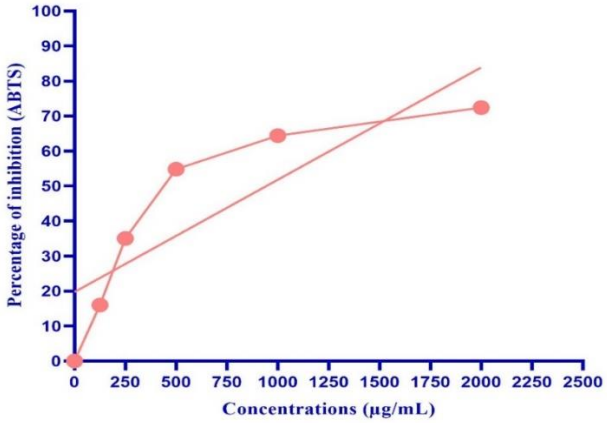

Figure 4. *Pyrus communis* leaf ethanolic extract's antioxidant capacity as determined by the ABTS test.

3.5. *Pyrus communis* leaf ethanolic extract's overall antioxidant activity

A thorough evaluation of the ethanolic extract of *Pyrus communis* leaves' % inhibition in terms of its total antioxidant capacity at different concentrations (µg/mL) was used to determine the extract's overall antioxidant activity. The findings showed a concentration-dependent

increase in total antioxidant capacity, indicating that the extract might counteract free radicals. At 125 µg/mL, the extract exhibited 14.57% inhibition; as the concentration increased to 250, 500, 1000, and 2000 µg/mL, a steady increase in percentage inhibition was observed, reaching 71.81%, the highest concentration tested (**Figure 5**). This concentration-dependent response underscores the robust total antioxidant activity of the *Pyrus communis* leaf extract, highlighting its potential as a potent source of antioxidants. These findings provide valuable insights into the overall antioxidant capabilities of the extract, emphasizing its potential in combating oxidative stress-related conditions. Further exploration of the specific antioxidant compounds responsible for these effects will deepen our understanding of this extract's therapeutic applications and health-promoting properties.

Pyrus communis leaves exhibit significant antioxidant activity due to their high content of polyphenols, particularly flavonoids like quercetin and kaempferol. These compounds effectively scavenge free radicals, reducing oxidative stress linked to chronic diseases such as cancer and cardiovascular disorders. A study by Zhang et al. [24] demonstrated the strong antioxidant potential of pear leaves, with DPPH radical scavenging assays confirming their efficacy. Comparatively, earlier research by Rodrigues et al. [25] highlighted similar antioxidant activity in *Pyrus pyrifolia* leaves, suggesting that different pear species offer comparable benefits, making Pyrus communis leaves valuable for pharmaceutical applications

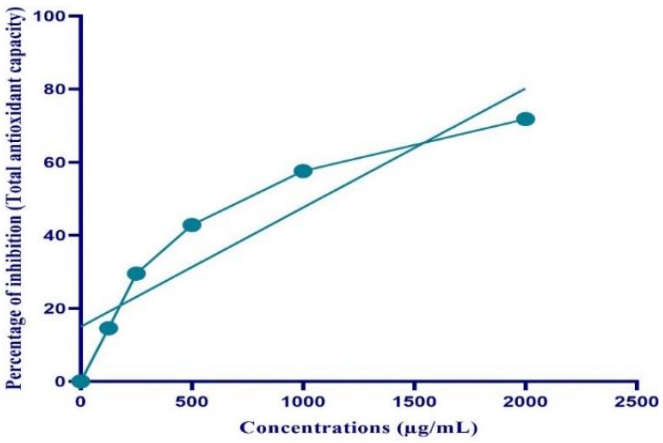

Figure 5. Total antioxidant activity of ethanolic extract of *Pyrus communis* leaves

3.6. Lowering the power assay of Pyrus communis leaf ethanolic extract

A reducing power assay was used to assess the reducing capabilities of the ethanolic extract derived from Pyrus communis leaves at various concentrations (µg/mL). The results demonstrated a concentration-dependent increase in reducing power, indicating the aptitude of the extract to donate electrons and act as a potential antioxidant. At 125 µg/mL, the reducing power was 0.2381, and as the concentration increased to 250, 500, 1000, and 2000 µg/mL, a progressive increase in reducing power was observed, reaching 1.413 at the highest concentration tested (**Figure 6**). This dose-dependent response highlights the capacity of the extract to act as a reducing agent, suggesting its potential role in mitigating oxidative stress by neutralizing free radicals. These results advance our comprehension of the antioxidant possessions of *Pyrus communis* leaves, emphasizing their possible therapeutic applications in counteracting oxidative damage. Further exploration of the specific bioactive compounds responsible for the observed reducing power will enhance our understanding of the antioxidant profile of the extract.

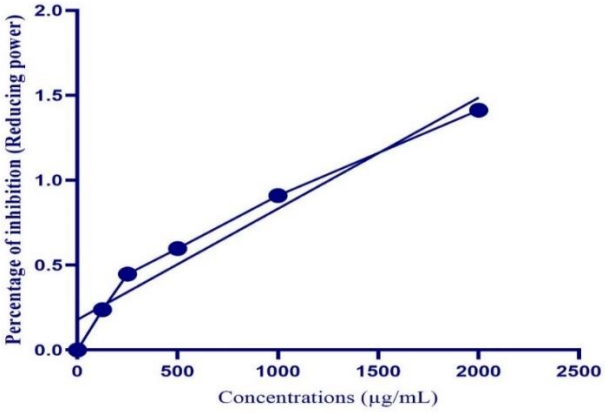

Figure 6. Reducing power assay of ethanolic extract of *Pyrus communis* leaves

3.7. In-vitro antidiabetic activity ethanolic extraction of leaves of Pyrus communis

3.7.1. Assay for inhibiting alpha-amylase

The results revealed the extract's concentration-dependent inhibition of the alpha-amylase enzyme, suggesting its potential role in diabetes management. At the lowest concentration of 62.5 µg/mL, the extract exhibited 13.44% inhibition, indicating its initial ability

to inhibit alpha-amylase activity. As the concentration increased to 125, 250, 500, and 1000 µg/mL, a progressive increase in the proportion of α-amylase reserve was experimental, reaching 64.89% of the highest concentration tested (**Figure 7**). This dose-dependent response underscores the potential of the extract as an alpha-amylase inhibitor, which is significant in diabetes management because alpha-amylase plays a crucial role in carbohydrate digestion. These findings support the exploration of *Pyrus communis* leaves as a possible origin of antidiabetic substances, and they call for additional research-specific bioactive elements accountable for observed inhibitory belongings.

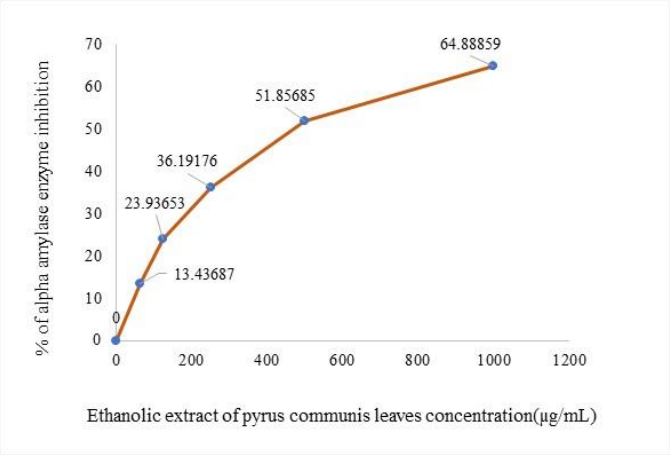

Figure 7. The leaves of *Pyrus communis* were extracted using ethanol alpha-amylase activity.

3.7.2. lpha-glucosidase inhibitory assay

Pyrus communis leaves evaluated its possible antidiabetic properties using the alpha-glucosidase inhibitory assay, which quantified the percentage of α-glucosidase enzyme inhibition at different concentrations (µg/mL). The results revealed concentration-dependent Alpha-glucosidase enzyme suppression by the extract, suggesting its role in controlling glucose absorption. At 62.5 µg/mL, the extract exhibited 7.84% inhibition, indicating its initial ability to impede alpha-glucosidase activity. As the concentration increased to 125, 250, 500, and 1000 µg/mL, a progressive increase in the percentage of α-glucosidase inhibition was observed, reaching 42.79% at the highest concentration tested (**Figure 8**). This dose-dependent response highlighted the potential of the extract as an alpha-glucosidase inhibitor, an important enzyme involved in breakdown of carbohydrates and glucose absorption. These findings support the exploration of Pyrus communis leaves as a possible supply of antidiabetic substances and call for additional research of specific bioactive constituents responsible for the observed inhibitory effects on α-glucosidase.

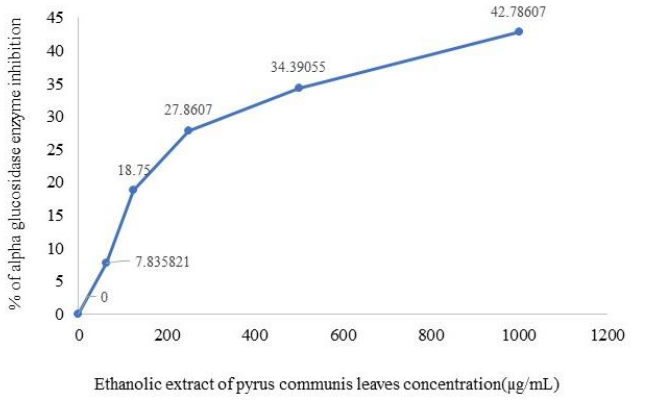

Figure 8. The ethanolic extract made from *Pyrus communis* leaves alpha-glucosidase activity.

3.8. Acute toxicity study of Pyrus communis ethanolic extract

Rather than providing complete toxicity results, a simple toxicity study of the ethanolic extract of P. communis was conducted to highlight the appropriate and safe dose range that could be utilized for subsequent investigations. Acute toxicity testing verified that *Pyrus communis* ethanolic extract of P. communis was administered at a dose of 2000 mg/kg. The ethanolic extract of P. communis did not significantly change the behavior of the animals. No deaths were reported up to a dose of 4000 mg/kg body weight.

3.9. Ethanolic extract of Pyrus communis leaves: in-vivo antidiabetic efficacy investigated in diabetic rats caused by STZ-NA.

3.9.1. Body weight analysis in normal and experimental rats

The *in-vivo* antidiabetic activity of the ethanolic-solvable compounds of *Pyrus communis* leaves was investigated in streptozotocin-nicotinamide (STZ-NA)-induced diabetic rats, focusing on body weight changes over 28 days. The study involved various experimental groups, including a normal group, a control group receiving only STZ-NA induction, a positive control group

administered glibenclamide (a known antidiabetic drug), and two test groups (G-4 and G-5) receiving different concentrations of the ethanolic extract (400 and 600 mg). On the 0th day, all groups exhibited comparable body weights. However, on the 28th day, the control group showed a significant decrease in body weight (141.8±2.75 g), indicating the detrimental effects of STZ-NA induction in the rats. In contrast, the normal group exhibited a modest increase in body weight (208.3±3.80 g to 218.7±2.51 g). Notably, the groups treated with ethanolic extract showed a partial reversal of the weight loss observed in the control group. G-1 and G-2 groups showed final body weights of 163.0±2.08 g and 180.7±3.13 g, respectively, suggesting a potential protective effect against STZ-NA-induced weight loss. The positive control group treated with glibenclamide also exhibited a protective effect, with a final body weight of 196.5±3.65 g (**Figure 9**). These results indicate the potential antidiabetic activity of the ethanolic extract of Pyrus communis leaves in ameliorating the body weight loss associated with STZ-NA induction in diabetic rats. Further investigations of glucose metabolism, insulin sensitivity, and other biochemical parameters would provide a more comprehensive understanding of the therapeutic effects of the extract on diabetes management.

3.9.2. Rats with normal and diabetic cholesterol and TG (Try glycerol) levels were measured (gm/dl).

Rats were assessed for cholesterol and triglyceride (TG) levels, with distinctions made between those in normal health and those in diabetic conditions across various experimental groups. In the normal group, the mean total cholesterol level was 169.108 gm/dL (±1.02), and the mean total TG level was 145.243 g/dL (±1.11) in the normal group. Conversely, rats in the control group exhibited higher levels, with mean total cholesterol and TG levels of 258.628 g/dL (±1.49) and 239.286 g/dL (±1.49), respectively. Treatment interventions included Glibenclamide administration, resulting in mean total cholesterol and TG levels of 164.431 gm/dL (±1.11) and 153.55 gm/dL (±1.09), respectively. Additionally, groups receiving EE Pyrus communis at doses of 400 mg and 600 mg demonstrated varying effects, with mean total cholesterol levels of 204.088 g/dL (±0.87) and 186.785 g/dL (±3.51) and mean total TG levels of 197.462 g/dL (±0.91) and 181.413 g/dL (±1.91), respectively. These results offer insightful information about regulating cholesterol and TG levels in rats under different experimental conditions, potentially informing further research on treatments for dyslipidemia and related metabolic disorders (**Table 1**).

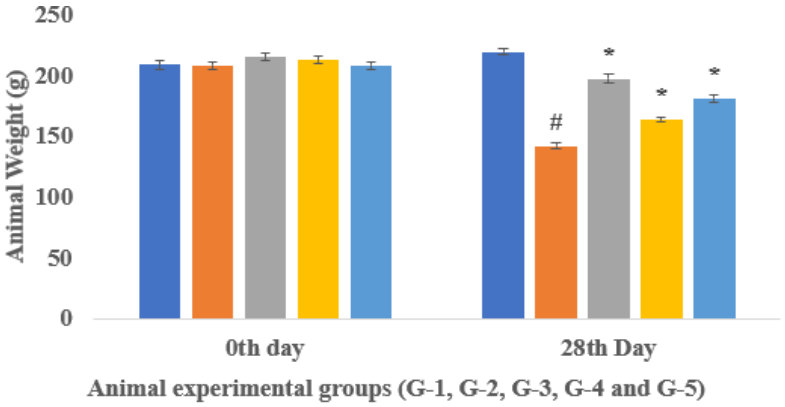

Figure 9. Analysing the body weight of experimental rats with and without diabetes. The mean ±SEM is used to represent the data. Tukey's test was conducted after a one-way analysis of variance (ANOVA) was used to analyse the data. n=6; *Treated vs. Control; #Control vs. Normal; p<0.05 was deemed significant.

Table 1. Rats were measured for their cholesterol and triglyceride (TG) levels, distinguishing between those with normal and diabetic conditions.

Group of animals	Total cholesterol level	Total TG level
Normal	169.108±1.02	145.243 +1.11
Control	258.628±1.49	239.286+1.49
Glibenclamide	164.431±1.11	153.55+1.09
EE *Pyrus communis* 400 mg	204.088±0.87	197.462+0.91
EE *Pyrus communis* 600 mg	186.785±3.51	181.413+1.91

3.9.3. Blood parameters in normal and diabetic experimental rats

A comparison of blood parameters among rats categorized by their health conditions revealed intriguing variations. In the normal group, rats exhibited relatively stable levels: total hemoglobin (Hb) averaged at 13.466 g/dL (±2.08), packed cell volume (PCV) at 37.733% (±1.15), total white blood cell (WBC) count at 13.6 x 10^3/µL (±3.50), total red blood cell (RBC) count at 4.79 x 10^6/µL (±0.427), and platelet count at 565 x 10^3/µL (±24.24). Treatment interventions exhibited varied effects: glibenclamide-treated rats showed increased Hb (14.7 g/dL ±2.47) and PCV (43.86% ±7.21), potentially indicating therapeutic benefits in managing diabetic complications. Conversely, supplementation with EE Pyrus communis at 400 mg and 600 mg yielded different outcomes, with varying Hb, PCV, WBC count, RBC count, and platelet count. These results underscore the intricate interplay between blood parameters and health conditions, providing valuable insights for further research on therapeutic interventions for diabetes-related complications (**Table 2**).

3.9.4. Rats with normal and diabetic blood glucose levels were measured (gm/dl).

The *In vivo* antidiabetic activity of the ethanolic extract of *Pyrus communis* leaves was investigated in streptozotocin-nicotinamide (STZ-NA)-induced diabetic rats, focusing on the blood glucose levels over 28 days. The study comprised different experimental groups, including a normal group, a control group subjected to STZ-NA induction, a positive control group treated with glibenclamide (an established antidiabetic drug), and two test groups (G-4 and G-5) receiving different concentrations of the ethanolic extract. On the 0th day, all groups exhibited baseline blood glucose levels, with the normal group showing consistent normoglycemia throughout the study. In contrast, the control group displayed a significant increase in blood glucose levels from the 0th day (282.2±2.08 mg/dl) to the 28th day (354.7±2.96 mg/dl), indicative of the sustained hyperglycemia induced by STZ-NA. Notably, the groups treated with the ethanolic extract demonstrated a trend toward maintaining lower blood sugar readings concerning the group under control. Test I and II groups exhibited a noticeable reduction in blood glucose levels from the 0th to the 28th day, with values of 230.5±2.30 mg/dl and 224.0±3.02 mg/dl, respectively. The positive control group, treated with glibenclamide, also showed a noteworthy drop in blood sugar levels (192.7±2.41 mg/dl) on the 28th day, indicating its efficacy in controlling hyperglycemia (**Figure 10**).

Table 2. Rats were categorized based on their blood parameters, distinguishing between those with normal and diabetic conditions.

Group of Animal	Total Hb g/dl	PCV %	Total WBC Count 10^3/µl	Total RBC Count 10^6/µl	Platelet Count 10^3/µl
Normal	13.466+2.08	37.733+1.15	13.6+3.50	4.79+0.427	565+24.24
Control	13.33+1.74	40.066+6.02	11.633+3.23	4.643+0.34	682+85.06
Glibenclamide	14.7+2.47	43.86+7.21	12.33+1.48	5.023+0.96	719+54.58
EE *Pyrus communis* 400 mg	12.9+1.15	39.13+2.857	11.8+2.74	4.77+0.13	669.66+136.87
EE *Pyrus communis* 600 mg	12.867+1.78	38.366+4.94	11.633+1.24	4.953+1.24	703.66+47.75

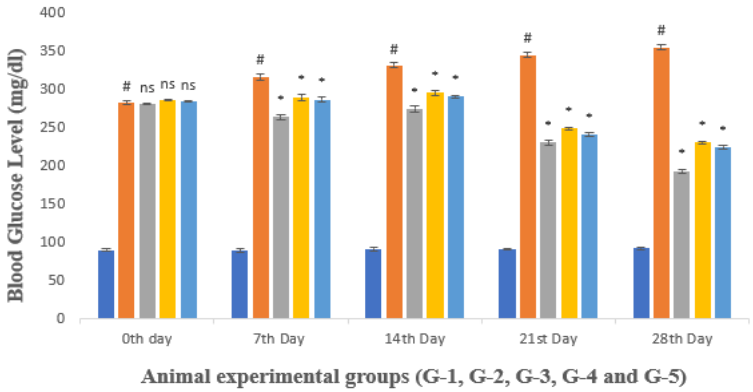

Figure 10. Normal and diabetic experimental rats blood glucose levels (gm/dl).

These findings indicated the possibility of antidiabetic activity in the ethanolic extract of Pyrus communis leaves to ameliorate STZ-NA-induced hyperglycemia in diabetic rats. Further investigation of additional biochemical parameters, insulin sensitivity, and histopathological changes would provide the extract's more complete sympathetic therapeutic effects in diabetes management.

3.9.5. histopathological examination

The effect of *Pyrus communis* ethanolic extract on animals with and without diabetes and the histological analysis of the pancreas in both groups (**Figure 11**). All animals received a singular gray-tan soft tissue measuring 4x1x0.5 cm, and each specimen was successfully embedded for histological examination. G-1) Sections from the specimen labeled as "normal" depict well-defined lobules of exocrine pancreatic acini with clusters of islet cells exhibiting a normal histological appearance. The surrounding fibrofatty tissue was also observed. G-2) Sections from the specimen labeled as control reveal lobules of exocrine pancreatic acini with clusters of islet cells appearing diminished in size and reduced in number. Adjacent fibrofatty tissues were also observed. G-3) Sections from the specimen labeled as standard display lobules of exocrine pancreatic acini with clusters of islet cells that appear normal in size and number. Adjacent fibrofatty tissue was also observed. G-4) Sections from the specimen labeled " EE *Pyrus communis* 400 mg (G-4)" depict lobules of exocrine pancreatic acini, with clusters of islet cells appearing normal in size and number, accompanied by adjacent fibrofatty tissue. G-5) Sections from the specimen labeled " EE *Pyrus communis* 600 mg (G-5)" illustrate lobules of exocrine pancreatic acini with clusters of islet cells exhibiting normal histology, and adjacent fibrofatty tissue is visible. Histopathological observations of pancreatic tissues provided crucial insights into the effects of the ethanolic extract of *Pyrus communis* in both diabetic and normal animals.

In normal and experimental groups, pancreatic architecture appeared normal, with well-defined lobules of exocrine pancreatic acini and clusters of islet cells that maintained their usual histological features. This suggests that the ethanolic extract does not adversely affect the pancreatic structure of healthy animals. In contrast, the "control" group exhibited notable alterations, including a reduction in the size and number of islet cells, indicating a negative impact of diabetes induction on pancreatic histology. The observed changes align with the expected consequences of diabetes-induced stress on the pancreatic tissues. Remarkably, both the "Lower dose" and "Higher dose" groups show histological patterns comparable to the "normal" and "standard" groups, indicating a potential protective effect of the ethanolic extract against diabetes-induced structural alterations. Maintaining normal pancreatic histology in the test groups suggests a positive influence on pancreatic health. Further analyses and exploration of specific cellular changes within the pancreatic tissues would enhance our understanding of the protective mechanisms of the extract and its potential therapeutic applications in diabetes management (**Figure 12**).

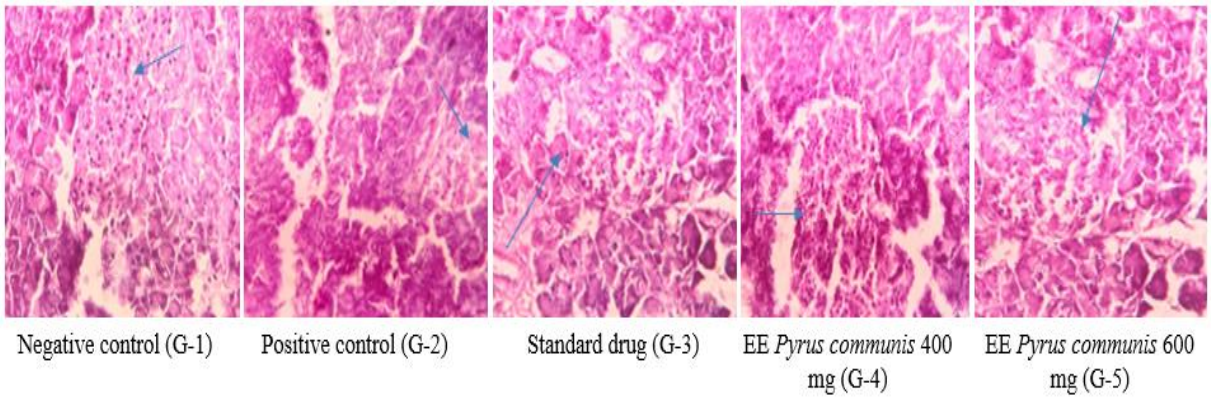

Figure 11. Properties of ethanolic extract from *Pyrus communis* on both normal and diabetes-induced animals, as well as a histological analysis of the pancreas in control and diabetes-induced animals.

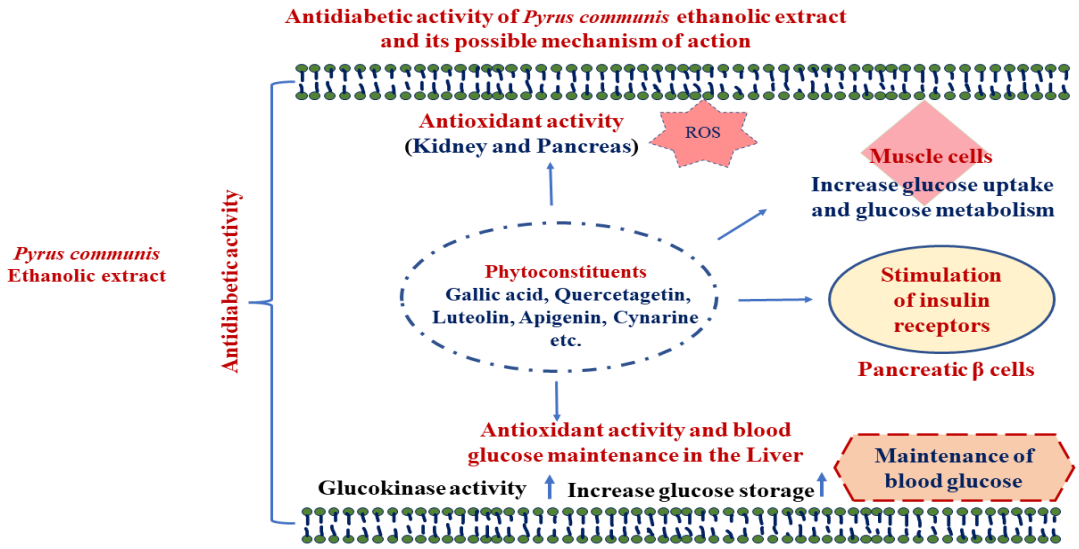

Figure 12. Ethanolic extract of *Pyrus communis* leaves possible antidiabetic activity mechanism.

4. Conclusion

In conclusion, the ethanolic extract of *Pyrus communis* leaves exhibited significant antioxidant and antidiabetic properties, both in vitro and in vivo. Its rich phytochemical profile, which includes glycosides, phenolics, flavonoids, and terpenoids, underscores its potential as a source of bioactive compounds. *In vitro* antioxidant assays demonstrated concentration-dependent scavenging effects, indicating its robust ability to neutralize free radicals. Furthermore, the extract exhibited notably in vitro antidiabetic activity by inhibiting alpha-amylase and alpha-glucosidase, crucial for carbohydrate digestion and glucose absorption. In STZ-NA-induced diabetic rats, the extract demonstrated a protective on body weight and blood glucose levels, suggesting its potential to mitigate diabetes-induced complications. Histopathological examination revealed preserved pancreatic architecture in test groups, supporting the protective role of the extract against diabetes-induced structural alterations. These findings highlight the promising therapeutic potential of *Pyrus communis* leaf extract in addressing oxidative stress- and diabetes-related complications. While these results are encouraging, further studies are warranted to elucidate specific molecular mechanisms underlying the observed effects and evaluate the extract's long-term effectiveness and safety. Clinical trials are essential to validate these therapeutic benefits in human subjects. Overall, the present study provides insightful observations about the possibilities of *Pyrus communis* leaves, the natural source of antioxidant and antidiabetic agents with potential applications in complementary and alternative medicine.

Summary of the work

This study explored the ethanolic extraction of *Pyrus communis* leaves to evaluate their bioactive potential, focusing on antioxidant and antidiabetic properties. The leaves were collected, shade-dried, and subjected to Soxhlet extraction using ethanol as the solvent, yielding an extract for further biological analysis. Phytochemical screening revealed the presence of glycosides, phenolics, flavonoids, terpenoids, sterols, saponins, and tannins, indicating the extract's potential antioxidant, anti-inflammatory, and therapeutic benefits.

The antioxidant potential of the *Pyrus communis* ethanolic extract was evaluated using various assays, all of which demonstrated significant concentration-dependent activity. In the DPPH assay, the extract achieved 50.97% inhibition at 200 µg/mL, while the ABTS assay showed an increase in inhibition from 16.01% at 125 µg/mL to 72.42% at 2000 µg/mL. The total antioxidant activity displayed similar trends, reaching 71.81% inhibition at 2000 µg/mL. The reducing power assay confirmed the extract's dose-dependent electron-donating capacity, further validating its antioxidant efficacy.

The *Pyrus communis* leaf extract exhibited promising in vitro antidiabetic activity by effectively inhibiting alpha-amylase and alpha-glucosidase, achieving 64.89% and 42.79% inhibition, respectively, at 1000 µg/mL, which suggests its potential to manage carbohydrate digestion and postprandial hyperglycemia. An acute toxicity study confirmed its safety, as no adverse effects were observed in animals treated with doses up to 4000 mg/kg. In vivo, the extract demonstrated significant benefits in diabetic rats, reducing weight loss and blood glucose levels over 28 days and improving lipid profiles. Histopathological examination revealed that pancreatic tissues in treated animals retained normal structures, while those in diabetic controls showed damage, indicating that the extract may help protect pancreatic health and support its antidiabetic effects.

Animal ethical approval: JSSCP/OT/IAEC/48/2022-23

Conflict of Interest: Authors declare that they have no conflict of interest.

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Data availability

The dataset used and/or analyzed in the current study is available to use.

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The authors declare to have no conflict of interest.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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