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RESEARCH ARTICLE

Protective and Restorative Effects of Apium graveolens Methanolic Extract on Pancreatic β-Cell Function, Oxidative Stress, and Dyslipidaemia in STZ-Induced Diabetic Rats

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Abstract: Diabetes mellitus remains a significant global health concern, necessitating the search for alternative therapeutic interventions. The present study evaluated the antidiabetic potential of Apium graveolens methanolic extract (AGME-L) in streptozotocin (STZ)-induced diabetic Wistar rats. The extract was prepared using maceration, and its phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides. Acute toxicity studies were conducted following OECD guidelines, ensuring safety at doses up to 2000 mg/kg. Diabetes was induced using a single intraperitoneal injection of STZ (50 mg/kg), and animals were divided into five groups: normal control, diabetic control, standard treatment (Glibenclamide, 10 mg/kg), AGME-L 200 mg/kg, and AGME-L 400 mg/kg. The extract significantly reduced fasting blood glucose levels in a dose-dependent manner, with the higher dose demonstrating enhanced glycaemic control. Additionally, AGME-L improved lipid profiles by reducing total cholesterol, LDL, and triglycerides while increasing HDL. Oxidative stress markers such as MDA, SOD, CAT, and GSH levels showed improvement following AGME-L treatment, indicating its antioxidant potential. Histopathological analysis of pancreatic tissue revealed partial β-cell regeneration in AGME-L-treated groups. These findings suggest that AGME-L possesses hypoglycaemic and antioxidant properties, making it a promising candidate for diabetes management.

Keywords: Diabetes, Acute toxicity study, Apium graveolens, Lipid Dysregulation, Oxidative stress, Antioxidant, Hyperglycaemia, Antidiabetic.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. It is a major public health concern affecting millions worldwide, with a rising prevalence due to sedentary lifestyles, unhealthy dietary habits, and genetic predisposition. According to the International Diabetes Federation (IDF), the global burden of diabetes is expected to rise significantly in the coming years, with type 2 diabetes mellitus (T2DM) accounting for the majority of cases (Anderson & Zochodne, 2016). The disease is associated with severe complications such as cardiovascular disorders, nephropathy, neuropathy, and retinopathy, making its management a critical focus in biomedical research (Abbate & Brunzell, 1990; O'Brien et al., 1998).

Conventional antidiabetic medications such as sulfonylureas, biguanides, and insulin therapy have been widely used to control hyperglycemia. However, these pharmacological interventions often come with adverse effects, including hypoglycemia, gastrointestinal disturbances, and drug resistance over prolonged use. The limitations of existing therapies have fueled interest in alternative treatment approaches, particularly the use of plant-based bioactive compounds with hypoglycemic and antioxidant properties. Medicinal plants have been extensively explored for their potential role in diabetes management due to their safety, efficacy, and affordability compared to synthetic drugs (Balaha et al., 2018; Cherng & Shih, 2006; CSIR, 1985; Deeds et al., 2011; Marchete et al., 2021; Pereira et al., 2007; Wei et al., 2003). Among the various plants investigated for their antidiabetic activity, *Apium graveolens* (leaf celery) has garnered attention due to its rich phytochemical profile and therapeutic properties. *Apium graveolens*, belonging to the Apiaceae family, has been traditionally used for its anti-inflammatory, antioxidant, and hepatoprotective effects (Tan et al., 2023). Recent studies suggest that the plant possesses bioactive compounds such as flavonoids, alkaloids, tannins, and terpenoids, which may contribute to its antidiabetic potential. Flavonoids, in particular, have been reported to exhibit insulin-mimetic properties, enhancing glucose

uptake and modulating carbohydrate metabolism. Additionally, the antioxidant components in *Apium graveolens* may help combat oxidative stress, a key factor in diabetes pathogenesis (Tan et al., 2023). Oxidative stress plays a crucial role in the development and progression of diabetes. Elevated glucose levels lead to increased production of reactive oxygen species (ROS), which, in turn, damage pancreatic β-cells and impair insulin signaling pathways. This oxidative imbalance contributes to the worsening of diabetes-related complications. Natural antioxidants derived from medicinal plants are known to counteract oxidative stress by scavenging free radicals and restoring endogenous antioxidant defense mechanisms. Given the antioxidant-rich composition of *Apium graveolens*, investigating its potential role in alleviating diabetes-induced oxidative stress becomes imperative (Dewanjee et al., 2018; Lipinski, 2001; Liu et al., 2019; Petchi et al., 2013; Piconi et al., 2003; Pitocco et al., 2013; Yang et al., 2011).

The current study aims to evaluate the antidiabetic and antioxidant properties of *Apium graveolens* methanolic extract (AGME-L) in streptozotocin (STZ)-induced diabetic Wistar rats. STZ, a well-known diabetogenic agent, selectively destroys pancreatic β-cells through DNA alkylation, thereby creating an experimental model that closely mimics type 1 diabetes. The use of animal models is crucial in preclinical research as it provides insights into the efficacy and safety of potential therapeutic agents before clinical trials. In this study, diabetes was induced in Wistar rats using a single intraperitoneal injection of STZ (50 mg/kg), and the hypoglycemic effect of AGME-L was assessed by monitoring fasting blood glucose levels over a specified duration. The extract's impact on lipid metabolism was also evaluated, considering that dyslipidemia is a common complication associated with diabetes. Additionally, the study investigated the oxidative stress markers, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), to determine the antioxidant potential of AGME-L. Histopathological analysis of pancreatic tissue was performed to assess any structural improvements in β-cell integrity following AGME-L treatment. The experimental design incorporated five groups: a normal control group receiving citrate buffer, a diabetic control group receiving STZ without treatment, a standard treatment group receiving Glibenclamide (10 mg/kg), and two test groups receiving AGME-L at 200 mg/kg and 400 mg/kg doses. This dose-dependent approach allowed the evaluation of AGME-L's therapeutic efficacy in a comparative manner (Chan et al., 2014; Gore et al., 2006; Menke et al., 2015; Pop-Busui et al., 2016). Preliminary phytochemical screening was conducted to identify the presence of bioactive compounds responsible for the observed pharmacological effects. Previous studies have highlighted the role of flavonoids, tannins, and saponins in regulating blood glucose levels through mechanisms such as glucose uptake enhancement, inhibition of carbohydrate-digesting enzymes, and modulation of insulin secretion. These compounds are also known to exert lipid-lowering effects, reducing the risk of cardiovascular complications in diabetic individuals. The findings of this study are expected to contribute valuable insights into the potential use of *Apium graveolens* as a natural antidiabetic agent. If proven effective, AGME-L could serve as an adjunct therapy for diabetes management, offering a safer and more sustainable alternative to conventional drugs. The study also highlights the need for further investigations into the molecular mechanisms underlying its pharmacological actions, paving the way for future clinical applications.

MATERIAL AND METHODS

Plant material collection, authentication, and extraction procedure

Fresh leaves of *Apium graveolens* (leaf celery) were collected from Bhopal region during October - December 2022. The plant material was authenticated by a botanist, and a voucher specimen (Voucher No.: MK/AG-348/23409) was deposited at Herbarium for future reference. The collected leaves were thoroughly washed with distilled water to remove surface impurities and dried under shade at room temperature (25 ± 2°C) for 15-20 days until a constant weight was achieved. The dried leaves were then pulverized into a fine powder using a mechanical grinder and stored in an airtight container under desiccated conditions until further use. For extraction, methanol extraction was carried out using the maceration extraction method. In brief, 500 g of the powdered plant material was extracted with 1000 mL of methanol at room temperature for a week. The extract was then filtered using Whatman No.1 filter paper, and the solvent was removed under reduced pressure using a rotary evaporator at 40°C. The dried extract was stored at 4°C until further use in subsequent experiments. This final extract was codenamed as AGME-L.

Preliminary Phytochemical Screening

The preliminary phytochemical screening was conducted to identify the presence of various bioactive compounds in the plant extract. A stock solution of the extract was prepared by dissolving an appropriate quantity in distilled water or respective solvents such as ethanol, methanol, or chloroform. The qualitative tests were performed using standard procedures for different classes of phytochemicals. The presence of alkaloids was determined using Mayer's test, where a few drops of Mayer's reagent (potassium mercuric iodide) were added to 2 mL of the extract solution, and the formation of a creamy white precipitate indicated the presence of alkaloids. Flavonoids were tested using the Shinoda test, in which a small amount of magnesium turnings and concentrated hydrochloric acid were added to the extract, resulting in a pink or red coloration confirming their presence. The presence of tannins and phenols was detected using the ferric chloride test, where a few drops of 1% ferric chloride solution were added to the extract,

and the appearance of a blue-green or black coloration indicated their presence. The foam test was performed to detect saponins, in which the extract was diluted with distilled water and vigorously shaken in a test tube, and the formation of a stable froth confirmed their presence. Terpenoids were identified using the Salkowski test by adding concentrated sulfuric acid to the extract, leading to the appearance of a reddish-brown color at the interface. The presence of steroids was determined using the Liebermann-Burchard test, where acetic anhydride and concentrated sulfuric acid were added, resulting in a blue-green coloration indicative of steroids. Glycosides were detected using the Keller-Killiani test, in which glacial acetic acid, ferric chloride, and concentrated sulfuric acid were added to the extract, and the formation of a brown ring at the interface confirmed their presence. The observed color changes or precipitate formations were documented, and all experiments were performed in triplicate to ensure accuracy. This qualitative screening provided an initial insight into the phytochemical composition of the extract, which was further validated using advanced chromatographic and spectroscopic techniques.

Animals

For this study, healthy adult Wistar albino rats, weighing 180–250 g, were procured from reputed animal house. The animals were housed in standard laboratory conditions, maintained at a temperature of (22 ± 2°C), with a relative humidity of (50–60%), and a 12-hour light/dark cycle. They were provided with a standard pellet diet and had ad libitum access to water throughout the study period. Prior to experimentation, the animals were acclimatized to the laboratory environment for one week to minimize stress and ensure physiological stability. All experimental protocols were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC). Efforts were made to minimize animal suffering, and all procedures, including drug administration, diabetes induction, and sample collection, were performed following ethical and humane handling practices. Proper post-experimental care was ensured, and euthanasia was carried out as per ethical guidelines when required.

Material: Reagents, chemicals, and drugs

Glibenclamide and streptozotocin (STZ), the primary drugs used in this study, were obtained from Loba Chemical Company, Mumbai, India, a trusted supplier of high-quality biochemical reagents. These compounds were essential for the experimental procedures due to their distinct pharmacological roles. Glibenclamide, a widely used sulfonylurea, was selected for its potent hypoglycaemic effect, which enhances insulin secretion from pancreatic β-cells. In contrast, STZ was utilized for its diabetogenic properties, as it selectively destroys pancreatic β-cells by inducing DNA alkylation, thereby establishing a reliable model for diabetes research. To ensure precision and reproducibility in experimental outcomes, all other chemicals and reagents used in the study were of analytical grade. These materials were sourced from reputable suppliers, including SRL Mumbai, Sigma-Aldrich, Loba Chem, and E. Merck India, known for their stringent quality control standards. The careful selection of high-purity reagents was critical in maintaining the accuracy and consistency of the study. Proper handling and storage of these chemicals were ensured to prevent degradation and contamination, thereby upholding the reliability and validity of the experimental findings.

Study of acute toxicity

The acute toxicity of the extracts was evaluated following the guidelines outlined in the Organisation for Economic Co-operation and Development (OECD) Test Guideline 423 (OECD, 2008) (OECD, 2008). The study was conducted using [specify species, e.g., female Wistar albino rats], as per regulatory recommendations, with each animal weighing 180–220 g. Prior to administration, the animals were fasted overnight but had free access to water. The extract was administered orally at an initial dose of 5 mg/kg body weight, as per the dose progression scheme recommended by OECD 423. Based on the observed effects, subsequent doses of 50, 300, and 2000 mg/kg were administered sequentially to different groups of animals. The animals were closely monitored for 14 days for any signs of toxicity, behavioural changes, autonomic dysfunctions, or mortality. Observations were made at regular intervals during the first 24 hours post-administration and then daily for the entire study duration. Key parameters such as body weight, food and water intake, and clinical signs including tremors, convulsions, lethargy, and respiratory distress were recorded. At the end of the study, the animals were humanely euthanized, and a gross necropsy was performed to examine potential abnormalities in major organs such as the liver, kidneys, heart, lungs, and spleen. The study adhered to the ethical guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and was approved by the Institutional Animal Ethics Committee (IAEC).

Diabetes induction

Diabetes was induced in Wistar rats using a single intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, prepared in 0.1 M citrate buffer (pH 4.5). Prior to administration, the rats were fasted overnight to enhance the diabetogenic effect of STZ (Ozay et al., 2020). Following injection, the animals were provided with 5% glucose solution in drinking water for the first 24 hours to prevent early hypoglycaemic shock due to acute β-cell destruction. Blood glucose levels were measured 72 hours post-injection using a glucometer by collecting blood from the tail vein. Rats with fasting blood glucose levels ≥250 mg/dL were considered diabetic and included in the

study. The diabetic rats were maintained under standard laboratory conditions, with free access to food and water, and were monitored for clinical signs such as polyphagia, polydipsia, and weight loss throughout the experimental period. The study was conducted in compliance with ethical guidelines and approved by the Institutional Animal Ethics Committee (IAEC) (Kohzaki et al., 2008).

Experimental design

The study was designed to evaluate the antidiabetic potential of the test extract in streptozotocin-induced diabetic rats. The animals were randomly divided into five groups, each consisting of an equal number of rats. Group 1 (Normal Control) consisted of healthy rats that did not undergo diabetes induction; instead, they received only citrate buffer as a vehicle. Group 2 (Diabetic Control) included diabetic rats that were administered a single intraperitoneal injection of STZ at a dose of 55 mg/kg body weight but received no further treatment, serving as a disease model. Group 3 (Standard Treatment Group) comprised diabetic rats treated with Glibenclamide, a well-established antidiabetic drug, administered orally at a dose of 10 mg/kg body weight to serve as the reference treatment. Group 4 (Low Dose Test Group) received the test extract orally at a dose of 200 mg/kg body weight to assess its hypoglycaemic efficacy at a lower concentration. Group 5 (High Dose Test Group) was administered the test extract orally at a dose of 400 mg/kg body weight to evaluate the effects of a higher concentration of the extract. The treatment was continued for a specific duration, during which the animals were monitored for changes in blood glucose levels, body weight, and other physiological parameters to determine the efficacy of the test extract in managing diabetes (Reda et al., 2016).

Table 1. the experimental design presented in a table format:

Group	**Treatment**
Group 1 (Normal Control)	Received citrate buffer (vehicle), no diabetes induction
Group 2 (Diabetic Control)	Induced diabetes with STZ (55 mg/kg, i.p.), no further treatment
Group 3 (Standard Treatment Group)	Diabetic rats treated with Glibenclamide (10 mg/kg, oral)
Group 4 (Low Dose Test Group)	Diabetic rats treated with AGME-L (200 mg/kg, oral)
Group 5 (High Dose Test Group)	Diabetic rats treated with AGME-L (400 mg/kg, oral)

General Parameters

The evaluation of general physiological parameters was conducted to monitor the health status and metabolic changes in experimental animals throughout the study period.

Body Weight: The body weight of all animals was recorded at the beginning of the study and at regular intervals during the treatment period. Changes in body weight were monitored to assess the impact of diabetes induction and the effectiveness of the administered treatments.

Fasting Blood Glucose (FBG): Blood glucose levels were measured after an overnight fast at baseline (before diabetes induction) and at specified intervals during the study using a glucometer. Tail vein blood was collected for glucose estimation, and values were expressed in mg/dL.

Food and Water Intake: Daily food and water intake were recorded for each group to evaluate changes in appetite and hydration, as diabetes is often associated with polyphagia and polydipsia.

Mortality and Clinical Signs: The animals were closely observed for any signs of distress, including lethargy, tremors, excessive urination, or significant behavioral changes. Mortality, if any, was documented along with potential causes to assess the safety and tolerability of the treatments.

Biochemical Parameters

To further understand the metabolic and oxidative stress-related changes in diabetic rats, biochemical markers were analyzed.

Markers of Oxidative Stress

Malondialdehyde (MDA): A key marker of lipid peroxidation, MDA levels were assessed to determine oxidative damage to cell membranes.

Superoxide Dismutase (SOD): SOD activity was measured to evaluate the antioxidant defense mechanism against superoxide radicals.

Catalase (CAT): Catalase activity was determined as an indicator of the enzymatic breakdown of hydrogen peroxide, a major reactive oxygen species.

Glutathione (GSH): The levels of reduced glutathione were assessed to measure the cellular antioxidant capacity and detoxification potential.

These biochemical markers were quantified using standard spectrophotometric methods to assess oxidative stress status in diabetic and treated animals (Finley & Tietz, 1996; King, 2012).

Histopathological assessments

Histopathological examinations were conducted to assess tissue alterations in response to diabetes induction and treatment interventions. At the end of the study, animals were euthanized following ethical guidelines, and vital organs, including the pancreas, liver, and kidneys, were carefully excised for microscopic analysis. The tissues were immediately fixed in 10% neutral buffered formalin for at least 24 hours to preserve cellular integrity. After fixation, the tissues were processed using standard paraffin embedding techniques. Thin sections (4–5 µm) were prepared using a microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E) for general histological evaluation. The slides were examined under a light microscope to assess structural changes such as pancreatic β-cell damage, hepatic degeneration, and renal abnormalities. Specific histopathological features analyzed included β-cell destruction, inflammatory infiltration, necrosis, vacuolization, fibrosis, and cellular hypertrophy. The severity of histopathological changes was graded based on established criteria (Culling, 2013; Tasci & Bozdayi, 2007). Images of representative sections were captured for documentation and comparative analysis across experimental groups (Culling, 2013; I. Tasci & M. Bozdayi, 2007).

Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple group comparisons. For non-parametric data, the Kruskal-Wallis test followed by Dunn's multiple comparison test was applied. Fasting blood glucose levels, body weight, oxidative stress markers, and biochemical parameters were analyzed to determine significant differences between the normal control, diabetic control, standard treatment, and test extract-treated groups. The level of statistical significance was set at $p < 0.05$, indicating a significant difference between the groups. All statistical analyses were conducted using GraphPad Prism (Version 8). The results were graphically represented using bar charts and line graphs to visualize trends and differences among the experimental groups.

RESULTS

The preliminary phytochemical screening, as presented in Table 2, revealed the presence of several bioactive compounds in the plant extract. The Mayer's test confirmed the presence of alkaloids, as indicated by the formation of a creamy white precipitate. Flavonoids were also detected through the Shinoda test, which resulted in a pink or red coloration, suggesting their abundance in the extract. The Ferric chloride test yielded a blue-green or black coloration, confirming the presence of tannins and phenols, which are known for their antioxidant and astringent properties. The foam test produced stable froth formation, indicating the presence of saponins, which are widely recognized for their surfactant and antimicrobial properties. Additionally, the Salkowski test resulted in a reddish-brown colour, confirming the presence of terpenoids, which are known to exhibit a wide range of pharmacological activities, including anti-inflammatory and antimicrobial effects. The Liebermann-Burchard test, however, did not produce the characteristic blue-green coloration, suggesting the absence of steroids in the extract. The Keller-Killiani test resulted in the formation of a brown ring, indicating the presence of glycosides, which are often associated with cardioprotective properties. These findings suggest that the extract contains a diverse range of bioactive compounds, which may contribute to its therapeutic potential. The presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides suggests possible antioxidant, antimicrobial, and anti-inflammatory properties, making the extract a promising candidate for further pharmacological investigations.

Table 2. Presenting the preliminary phytochemical screening results:

Phytochemical Test	Reagent/Test Used	Observation	Inference (Presence/Absence)
Alkaloids	Mayer's Test	Creamy white precipitate	+
Flavonoids	Shinoda Test	Pink or red coloration	+
Tannins & Phenols	Ferric Chloride Test	Blue-green/black color	+
Saponins	Foam Test	Stable froth formation	+
Terpenoids	Salkowski Test	Reddish-brown color	+
Steroids	Liebermann-Burchard Test	Blue-green coloration	-
Glycosides	Keller-Killiani Test	Brown ring formation	+

"+" indicates the presence of the phytochemical, while "-" denotes its absence.

The impact of AGME-L on blood glucose levels

The impact of AGME-L on blood glucose levels in different animal groups is presented in Table 3. The normal control group maintained stable blood glucose levels throughout the study period, with values ranging from 87.17 ± 2.06 mg/dL to 99.54 ± 2.50 mg/dL. In contrast, the diabetic control group exhibited significantly elevated glucose levels, reaching a peak of 357.47 ± 3.01 mg/dL on day 8, followed by a gradual decline but remaining substantially higher than the normal control throughout the study period. This confirms the successful induction of diabetes in this group. The standard treatment group showed a marked reduction in glucose levels compared to the diabetic control, with values decreasing from 317.80 ± 2.38 mg/dL on day 2 to 182.47 ± 3.34 mg/dL by day 30, indicating effective glycemic control. Treatment with AGME-L at both 200 mg/kg and 400 mg/kg demonstrated a dose-dependent reduction in blood glucose levels. The 200 mg/kg dose resulted in an initial drop from 208.51 ± 2.91 mg/dL on day 2 to 26.15 ± 2.58 mg/dL on day 8, followed by a slight increase to 242.65 ± 3.23 mg/dL by day 30. The 400 mg/kg dose exhibited a similar trend, with glucose levels decreasing from 202.17 ± 3.52 mg/dL on day 2 to 217.21 ± 3.06 mg/dL on day 8 and stabilizing at 231.73 ± 3.60 mg/dL by day 30. The presence of statistically significant reductions (*$p < 0.05$) in the AGME-L-treated groups compared to the diabetic control suggests that AGME-L possesses hypoglycemic potential. The dose-dependent effect observed indicates that the 400 mg/kg dose was slightly more effective in maintaining reduced glucose levels over time. However, the glucose-lowering effect of AGME-L, although notable, did not reach the efficacy of the standard treatment. These findings suggest that AGME-L may have potential as an adjunct therapy for diabetes management, warranting further investigation into its mechanism of action and long-term efficacy.

Table 3. AGME-L's impact on blood glucose levels

Animal groups	**Glucose levels in Blood (mg/dl)**					
	Day					
	1	**2 (48 hours)**	**8**	**16**	**24**	**30**
Normal control	90.49 ± 1.70	87.17 ± 2.06	89.28 ± 2.87	91.27 ± 2.06	94.47 ± 2.66	99.54 ± 2.50
Diabetic control	87.10 ± 1.64	313.18 ± 2.50***	357.47 ± 3.01***	251.42 ± 3.94***	261.64 ± 3.60***	270.76 ± 3.13***
Standard treatment	89.43 ± 1.61	317.80 ± 2.38	249.81 ± 3.10	201.39 ± 3.47	181.51 ± 2.73	182.47 ± 3.34
AGME-L 200 mg/kg	87.67 ± 1.80	208.51 ± 2.91	26.15 ± 2.58	229.12 ± 3.92*	235.43 ± 3.30*	242.65 ± 3.23*
AGME-L 400 mg/kg	89.22 ± 1.82	202.17 ± 3.52	217.21 ± 3.06	220.99 ± 4.05*	227.60 ± 4.10*	231.73 ± 3.60*

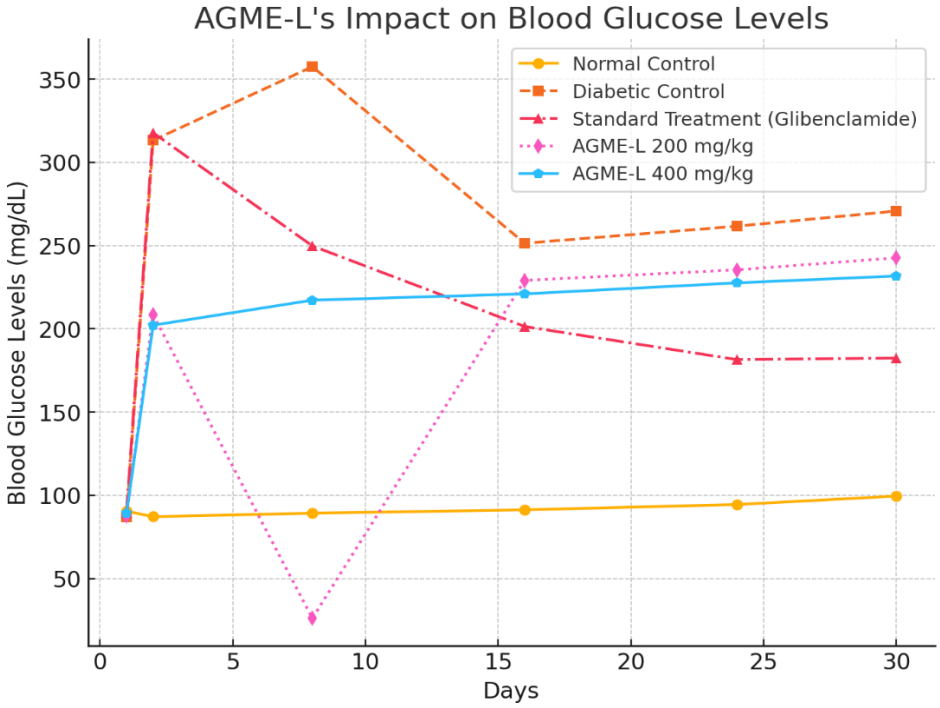

Figure 1. Impact of AGME-L on blood glucose levels

Impact of AGME-L on the lipid profile

The effect of AGME-L on the lipid profile is presented in Table 4, which highlights significant variations in total cholesterol (TC), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), and triglycerides (TG) across different treatment groups. The normal control (NC) group maintained stable lipid parameters, with TC at 68.59 ± 1.96 mg/dL, LDL at 62.40 ± 1.97 mg/dL, and HDL at 20.75 ± 2.06 mg/dL. In contrast, the diabetic control (DC) group exhibited significantly elevated TC, LDL, VLDL, and TG levels, while HDL levels were reduced compared to the normal control. Specifically, TC and LDL levels were markedly increased to 78.69 ± 1.99 mg/dL and 75.70 ± 1.98 mg/dL, respectively (**p<0.01), indicating dyslipidemia associated with diabetes. A decline in HDL levels (19.31 ± 1.75 mg/dL) was observed, further confirming the disturbance in lipid metabolism. The standard treatment (ST) group showed a reduction in lipid parameters, with TC, LDL, and TG levels close to normal, suggesting the efficacy of the treatment in mitigating diabetes-induced dyslipidemia. AGME-L treatment at both 200 mg/kg and 400 mg/kg exhibited a dose-dependent improvement in lipid profile. The 100 mg/kg dose resulted in a slight reduction in TC (71.89 ± 2.86 mg/dL) and LDL (66.70 ± 2.17 mg/dL), along with a significant increase in HDL (20.91 ± 1.97 mg/dL, *p<0.05). Similarly, the 200 mg/kg dose further improved the lipid profile, lowering LDL levels (65.94 ± 2.60 mg/dL, **p<0.01) while increasing HDL levels (21.23 ± 2.07 mg/dL, *p<0.05). These findings indicate that AGME-L exerts a positive impact on lipid metabolism, potentially reducing the risk of cardiovascular complications associated with diabetes. The dose-dependent improvement in lipid parameters suggests that AGME-L may possess hypolipidemic properties, which could be attributed to its bioactive constituents. Although its effects were not as pronounced as the standard treatment, AGME-L demonstrated significant potential in modulating lipid metabolism, making it a promising candidate for further research in diabetes-associated dyslipidemia.

Table 4: Lipid profile: the effect of AGME-L.

Treatment Groups	**TC**	**LDL**	**VLDL**	**HDL**	**TG**
	(U/l)	**mg/dl**			
NC	68.59 ± 1.96	62.40 ± 1.97	13.42 ± 0.86	20.75 ± 2.06	39.39 ± 2.07
DC	a78.69 ± 1.99**	a75.70 ± 1.98**	a15.82 ± 1.14**	a19.31 ± 1.75**	a48.29 ± 2.11**
ST	69.39 ± 2.64	65.81 ± 2.28	13.95 ± 1.16	21.20 ± 1.90	40.60 ± 2.28
AGME-L 200 mg/kg	b71.89 ± 2.86*	b66.70 ± 2.17*	13.86 ± 1.17	b20.91 ± 1.97*	b41.89 ± 2.18*
AGME-L 400 mg/kg	b72.50 ± 3.00*	b65.94 ± 2.60**	b13.55 ± 1.20*	b21.23 ± 2.07*	b41.29 ± 2.24*

The values are presented as Mean ± SD for each of the six samples. Statistical significance is indicated as p < 0.01 (), and p < 0.001 (). Abbreviations: LDL (Low-Density Lipoprotein), VLDL (Very Low-Density Lipoprotein), TC (Total Cholesterol), TG (Triglycerides), and HDL (High-Density Lipoprotein). Experimental groups include NC (Normal Control), DC (Diabetic Control), and ST (Standard Treatment).

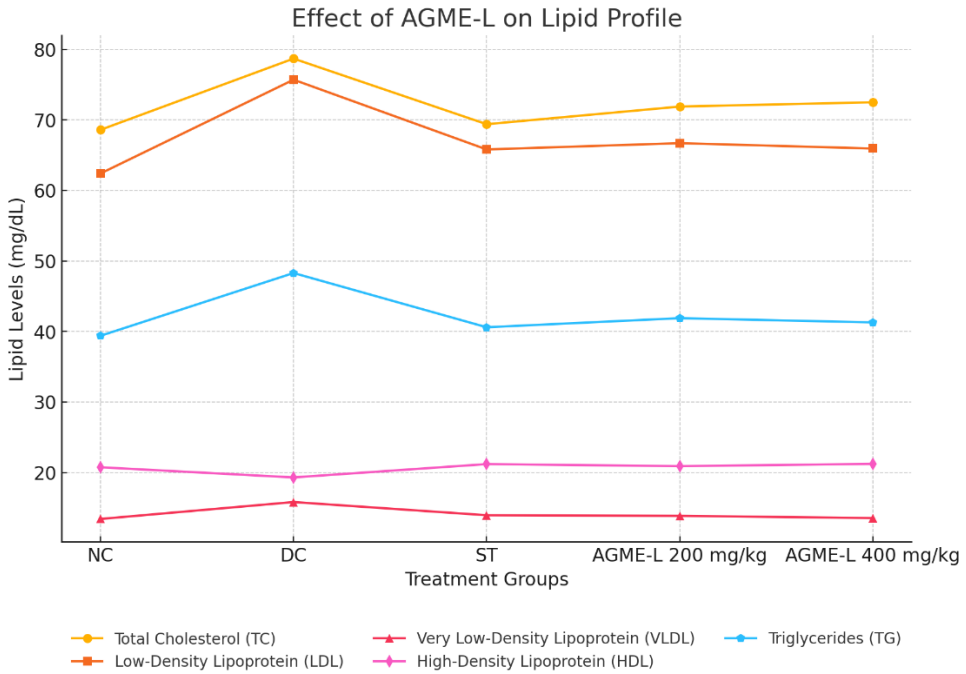

Figure 2. AGME-L's impact on the lipid profile

Assessment of oxidative stress markers

The impact of AGME-L on oxidative stress indicators is presented in Table 5, highlighting changes in catalase (CAT), thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and glutathione (GSH) levels across different treatment groups. The normal control group exhibited stable oxidative stress parameters, with CAT activity at 8.25 ± 1.16 U/min, TBARS levels at 36.27 ± 1.20 nM/min/mg protein, SOD at 27.50 ± 1.18 U/mg protein, and GSH levels at 9.70 ± 1.16 µM/g tissue. In contrast, the diabetic control group showed a significant increase in TBARS levels (60.25 ± 1.19 nM/min/mg protein, ***p<0.001), indicating elevated lipid peroxidation and oxidative damage. Additionally, a marked reduction in antioxidant enzyme activity was observed, with CAT, SOD, and GSH levels significantly lower than those of the normal control (**p<0.001), confirming increased oxidative stress in diabetes. The standard treatment group exhibited a notable reduction in oxidative stress, with TBARS levels significantly lowered (34.85 ± 1.20 nM/min/mg protein, ***p<0.001), while CAT, SOD, and GSH levels were restored close to normal. AGME-L treatment at both 200 mg/kg and 400 mg/kg demonstrated a dose-dependent antioxidant effect. The 200 mg/kg dose resulted in an increase in CAT (8.42 ± 1.15 U/min, *p<0.05) and SOD (21.49 ± 1.17 U/mg protein, **p<0.01) levels, while TBARS levels were reduced to 47.80 ± 1.20 nM/min/mg protein (**p<0.01). Similarly, the 400 mg/kg dose exhibited an even stronger antioxidant response, with a significant reduction in TBARS (39.25 ± 1.21 nM/min/mg protein, ***p<0.001) and increased antioxidant enzyme levels, including CAT (8.62 ± 1.18 U/min, **p<0.01) and GSH (9.53 ± 1.21 µM/g tissue, ***p<0.001). These results indicate that AGME-L exhibits protective antioxidant activity by reducing oxidative stress and enhancing endogenous antioxidant defenses. The observed dose-dependent improvement in oxidative stress parameters suggests that AGME-L may mitigate diabetes-induced oxidative damage, possibly through its bioactive phytoconstituents. While the antioxidant effects of AGME-L were slightly lower than those of the standard treatment, its ability to restore antioxidant enzyme levels and reduce lipid peroxidation makes it a promising candidate for further research in diabetes management.

Table 5: Impact of AGME-L on oxidative stress indicators

	CAT (U/min)	**TBARS (nM /min/mg protein)**	**SOD (U/mg protein)**	**GSH (µM /g tissue)**
Normal Control	8.25 ± 1.16	36.27 ± 1.20	27.50 ± 1.18	9.70 ± 1.16
Diabetes Control	a6.70 ± 1.13***	a60.25 ± 1.19***	a16.88 ± 1.19***	a5.08 ± 1.18***
Standard treatment	b8.78 ± 1.19**	b34.85 ± 1.20***	b25.85 ± 1.21***	b10.59 ± 1.16***
AGME-L 200 mg/kg	b8.42 ± 1.15*	b47.80 ± 1.20**	b21.49 ± 1.17**	b8.78 ± 1.18**
AGME-L 400 mg/kg	b8.62 ± 1.18**	b39.25 ± 1.21***	b24.58 ± 1.20***	b9.53 ± 1.21***

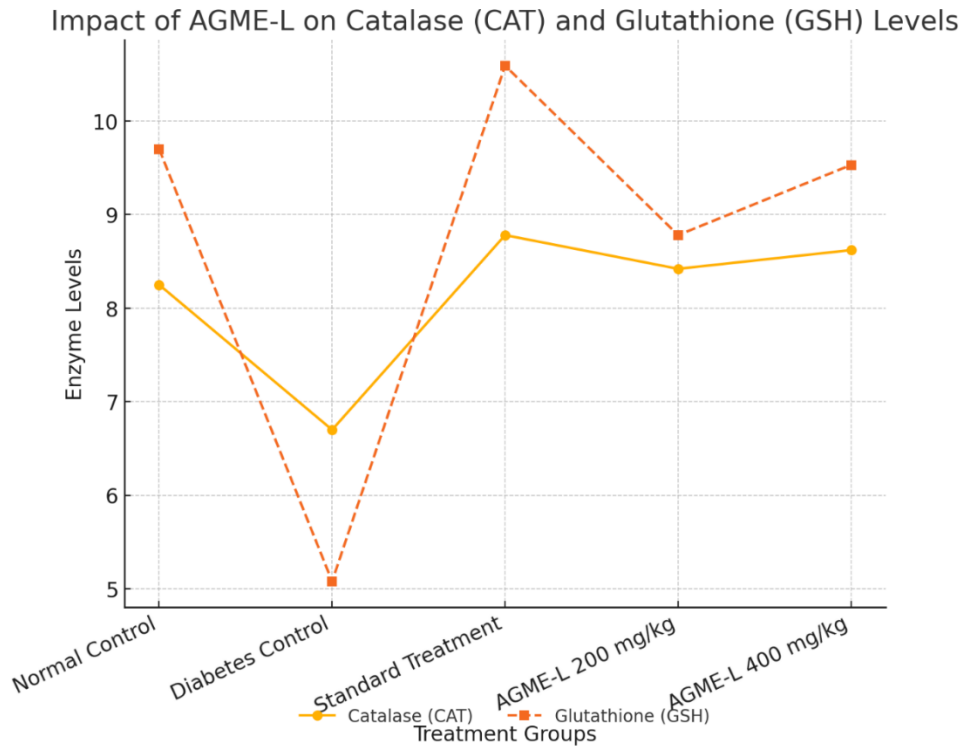

Figure 3. Impact of AGME-L on CAT and GSH levels

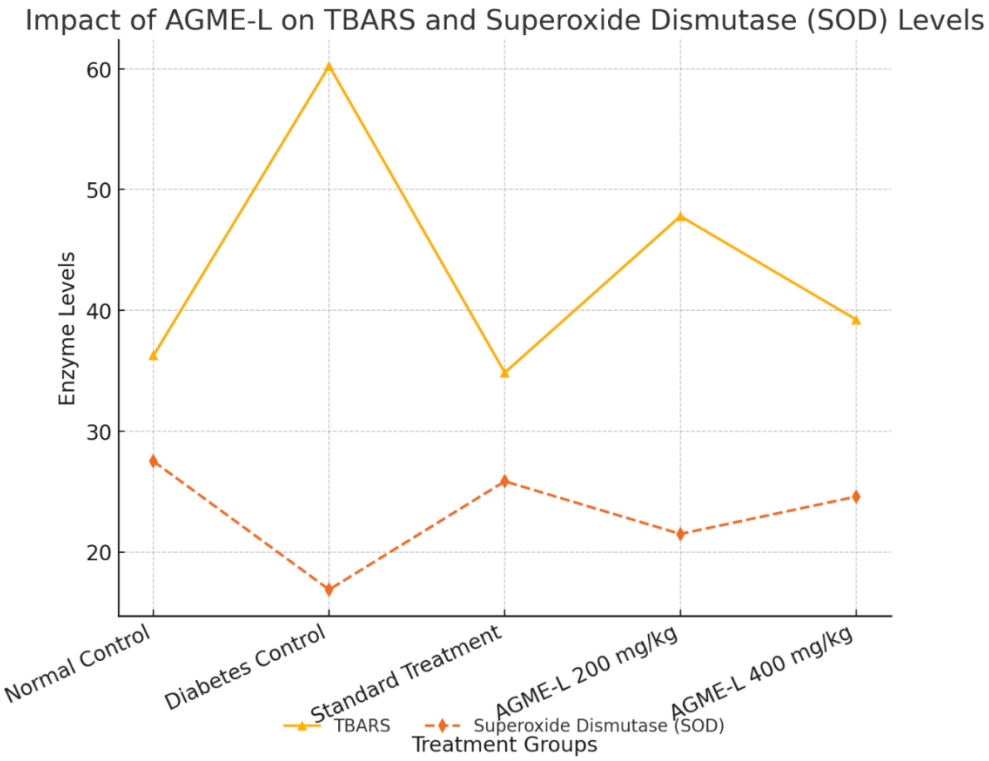

Figure 4. Impact of AGME-L on TBARS and SOD

Histopathological examination

The histopathological examination of pancreatic tissue was conducted using hematoxylin and eosin (H&E) staining at 200x magnification to assess the structural changes in different experimental groups. Photomicrographs of pancreatic sections revealed distinct histological features across the groups. In Figure A, representing the normal control group, the pancreatic tissue exhibited well-organized islets of Langerhans with intact β-cells, surrounded by exocrine acinar cells with no signs of cellular damage or inflammation. In contrast, Figure B, corresponding to the diabetic control group, showed significant

histopathological alterations, including degenerative changes, reduced islet size, cellular disorganization, and vacuolization, indicating β-cell destruction and impaired insulin secretion. The Figure C, representing the standard treatment group that received 10 mg/kg of Glibenclamide, demonstrated partial restoration of pancreatic architecture, with improved β-cell density and reduced vacuolization compared to the diabetic control, suggesting the protective effect of Glibenclamide. The Figure D, corresponding to the AGME-L 200 mg/kg-treated group, exhibited moderate structural improvement, with noticeable regeneration of β-cells, reduced inflammation, and partial restoration of pancreatic islets, indicating a protective effect against diabetes-induced pancreatic damage. The Figure E, representing the group that received a higher dose of AGME-L (400 mg/kg), displayed more prominent restoration of pancreatic morphology, with increased islet cell integrity and improved cellular arrangement, similar to the standard treatment group. Overall, the photomicrographs suggest that AGME-L treatment demonstrated a dose-dependent protective effect on pancreatic tissue, with the higher dose (400 mg/kg) showing enhanced regenerative potential. These findings indicate that AGME-L may exert a protective effect against diabetes-induced pancreatic damage, potentially contributing to its hypoglycemic activity.

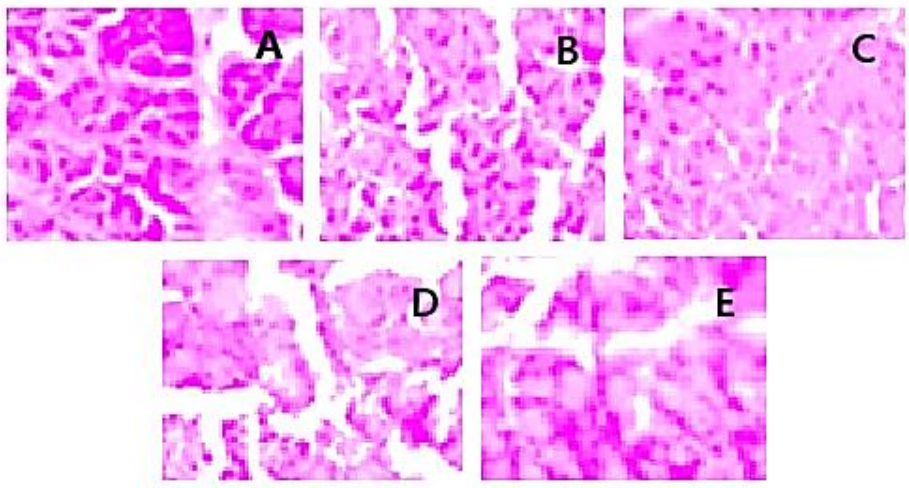

Figure 5. Photomicrographs of pancreatic tissue from each experimental group, stained with hematoxylin and eosin (H&E) and observed under 200x magnification. **A** represents the normal control group with intact pancreatic architecture. **B** corresponds to the diabetic control group, exhibiting structural alterations due to STZ-induced damage. **C** depicts the standard treatment group, which received 10 mg/kg of Glibenclamide, showing partial restoration of pancreatic integrity. **D** illustrates the AGME-L 200 mg/kg-treated group, demonstrating moderate pancreatic recovery. **E** represents the AGME-L 400 mg/kg-treated group, displaying enhanced pancreatic protection and improved β-cell morphology.

CONCLUSION

The present study demonstrated that the methanolic extract of *Apium graveolens* (AGME-L) possesses significant antidiabetic potential in STZ-induced diabetic rats. The extract, rich in bioactive phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids, exhibited a dose-dependent reduction in fasting blood glucose levels. Treatment with AGME-L at 200 mg/kg and 400 mg/kg effectively lowered blood glucose levels compared to the diabetic control, though not to the extent of the standard treatment (Glibenclamide). The observed reduction in hyperglycemia suggests that AGME-L may act through insulinotropic or insulin-mimetic mechanisms, warranting further mechanistic studies. Beyond glycemic control, AGME-L also improved lipid metabolism by significantly reducing total cholesterol, LDL, and triglyceride levels while increasing HDL, thereby potentially mitigating the cardiovascular risks associated with diabetes. The extract further exhibited strong antioxidant properties, as evidenced by enhanced SOD, CAT, and GSH levels and reduced MDA levels, indicating a reduction in oxidative stress. Given the established link between oxidative stress and diabetes progression, AGME-L's ability to restore antioxidant enzyme activity highlights its therapeutic potential. Histopathological assessments revealed that AGME-L conferred pancreatic β-cell protection and regeneration, with the higher dose (400 mg/kg) demonstrating superior restoration of islet cell integrity. This suggests that AGME-L may play a role in pancreatic preservation, an essential factor in diabetes management. In conclusion, AGME-L exhibited promising antidiabetic, hypolipidemic, and antioxidant properties, making it a potential herbal alternative for diabetes treatment. While the extract showed considerable efficacy, further studies involving molecular mechanisms, long-term toxicity, and clinical trials are necessary to establish its therapeutic applicability.

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