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Evaluation of the Antidiabetic, Antioxidant, and Antihyperlipidemic Activities of Apium dulce Extract in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder associated with pancreatic β-cell dysfunction, oxidative stress, and lipid abnormalities. Current antidiabetic therapies often fail to restore β-cell integrity or address oxidative and metabolic complications. This study investigated the protective and restorative effects of the Methanolic Extract of Apium dulce (MEAD) on β-cell function, oxidative stress, and dyslipidaemia in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg). Rats were divided into five groups: normal control, diabetic control, MEAD (100 mg/kg), MEAD (300 mg/kg), and glibenclamide (0.6 mg/kg). Treatments were administered orally for 28 days. Biochemical parameters including fasting glucose, serum insulin, HOMA-β, oxidative-stress markers (MDA, SOD, CAT, GSH), lipid profile, and pancreatic histology were evaluated. MEAD significantly reduced fasting glucose and MDA levels while enhancing insulin secretion, antioxidant enzymes, and lipid homeostasis in a dose-dependent manner. Histological and immunohistochemical analyses revealed restoration of pancreatic β-cell morphology and insulin-positive areas comparable to glibenclamide. These findings indicate that MEAD exerts potent antidiabetic, antioxidant, and antihyperlipidaemic effects, primarily through β-cell protection and oxidative-stress attenuation.

KEYWORDS: Apium dulce; methanolic extract; β-cell restoration; oxidative stress; streptozotocin; dyslipidaemia; antidiabetic activity.

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INTRODUCTION

Diabetes mellitus is a chronic, progressive metabolic disorder characterised by elevated blood glucose levels resulting from defects in insulin secretion, insulin action, or both. It represents one of the most significant global health challenges of the twenty-first century, affecting an estimated 537 million adults worldwide, a figure projected to rise to 643 million by 2030 (International Diabetes Federation, 2021). The disease is broadly classified into two major types: type 1 diabetes, which results from autoimmune destruction of pancreatic β-cells leading to absolute insulin deficiency, and type 2 diabetes, which involves insulin resistance combined with relative β-cell dysfunction. Regardless of the type, chronic hyperglycaemia in diabetes triggers oxidative stress, inflammation, and metabolic derangements that culminate in severe complications, including nephropathy, neuropathy, retinopathy, and cardiovascular disorders. The underlying pathogenic process often involves excessive generation of reactive oxygen species (ROS), which overwhelms the antioxidant defence mechanisms of the body and impairs the integrity of pancreatic β-cells. Because β-cells contain inherently low levels of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), they are particularly susceptible to oxidative damage induced by hyperglycaemia and cytotoxic agents such as streptozotocin (STZ) (Robertson, 2004).

Conventional antidiabetic therapies, including insulin, sulfonylureas, biguanides, and thiazolidinediones, effectively reduce blood glucose levels but have limited potential to reverse β-cell loss or address the oxidative and inflammatory damage that underlies disease progression. These therapies also carry adverse effects, such as weight gain, hypoglycaemia, and gastrointestinal disturbances. Moreover, long-term treatment often fails to prevent diabetic complications, highlighting the need for safer and more comprehensive therapeutic approaches that target multiple pathophysiological mechanisms rather than glycaemic control alone. Recent research has increasingly focused on phytotherapeutic agents—bioactive compounds derived from medicinal plants—as promising adjuncts or alternatives to conventional drugs. These natural agents possess multiple pharmacological activities, including antioxidant, anti-inflammatory, lipid-lowering, and insulin-sensitising properties, often with minimal toxicity (Adil et al., 2022). Their capacity to act on several molecular targets simultaneously makes them particularly suitable for the management of multifactorial disorders like diabetes mellitus.

Among the many plants traditionally used in herbal medicine, members of the genus *Apium* (family: Apiaceae) have attracted considerable attention for their metabolic and antioxidant benefits. *Apium graveolens* (celery) has been widely studied and reported to exhibit antihyperglycaemic, antihyperlipidaemic, and hepatoprotective effects in various experimental models (Hegde & Giradkar, 2017). The therapeutic efficacy of *Apium* species is attributed to their rich phytochemical composition, which includes flavonoids (apigenin, luteolin, quercetin), phenolic acids (caffeic, ferulic, and chlorogenic acids), phthalides, saponins, and terpenoids. These bioactive compounds exhibit potent free-radical-scavenging activity, enhance antioxidant enzyme expression, and modulate pathways involved in glucose and lipid metabolism. Previous studies have shown that methanolic extracts of *Apium graveolens* improved fasting glucose levels, enhanced insulin secretion, and restored pancreatic architecture in STZ- and alloxan-induced diabetic rats (Panda & Kar, 2007; Al-Yahya et al., 2014). Furthermore, clinical studies have demonstrated that celery supplementation significantly reduced fasting plasma glucose, triglycerides, and total cholesterol in human subjects (Liu et al., 2025). These findings suggest that *Apium* species exert systemic metabolic regulation through antioxidant and anti-inflammatory mechanisms.

Apium dulce, a close relative of *A. graveolens*, is an aromatic herb traditionally consumed as a culinary vegetable and used in indigenous medicine for its digestive, anti-inflammatory, and cardiovascular benefits. However, despite its phytochemical similarity to *A. graveolens*, scientific data on the pharmacological potential of *A. dulce* remain scarce. Preliminary phytochemical studies indicate that *A. dulce* contains abundant polyphenols, flavonoids, and volatile oils similar to other *Apium* species, suggesting comparable antioxidant and metabolic-modulating capacities (Hegde & Giradkar, 2017). Given the established link between oxidative stress and β-cell destruction, it is reasonable to hypothesise that *A. dulce* may possess protective effects on pancreatic β-cells, thereby improving insulin secretion and glucose homeostasis. Moreover, its rich flavonoid and phthalide content could also contribute to lipid regulation and the prevention of diabetic dyslipidaemia, a major risk factor for cardiovascular disease in diabetic patients.

Methanolic extraction was employed in the present study because methanol effectively extracts both polar and moderately non-polar constituents, including phenolics and flavonoids responsible for antioxidant activity. Numerous comparative studies have shown that methanolic extracts yield higher concentrations of bioactive compounds and stronger radical-scavenging activity than aqueous or petroleum-ether extracts (Mahmoud et al., 2023). Therefore, the methanolic extract of *Apium dulce* (MEAD) was selected to ensure maximal recovery of pharmacologically relevant constituents. The choice of the streptozotocin-induced diabetic rat model is based on its well-documented ability to mimic the biochemical and histopathological features of diabetes, particularly selective β-cell cytotoxicity and resultant insulin deficiency (Szkudelski, 2001). This model allows evaluation of both the protective and regenerative effects of therapeutic agents on pancreatic tissue.

The rationale for this study stems from the increasing recognition that oxidative stress is a central mediator of β-cell dysfunction and diabetic complications. Attenuation of oxidative damage could, therefore, play a pivotal role in preserving β-cell viability and improving insulin-secretory capacity. Herbal extracts rich in natural antioxidants, such as MEAD, may simultaneously reduce oxidative injury, enhance enzymatic antioxidant defence, and normalise glucose and lipid metabolism. Unlike synthetic drugs that target isolated pathways, the polyphenolic matrix of plant extracts can influence multiple biochemical processes synergistically. Thus, exploring the antioxidant, antihyperglycaemic, and antihyperlipidaemic potential of *A. dulce* offers an integrative approach to diabetes management.

The objectives of this investigation were to evaluate the *in vivo* effects of the methanolic extract of *Apium dulce* on pancreatic β-cell function, oxidative-stress markers, and lipid metabolism in streptozotocin-induced diabetic rats. Specifically, the study aimed to (i) assess the impact of MEAD on fasting glucose, serum insulin, and β-cell activity (HOMA-β index); (ii) determine changes in oxidative-stress biomarkers including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH); (iii) evaluate the effects on serum lipid profile (total cholesterol, triglycerides, LDL, and HDL); and (iv) examine histopathological and immunohistochemical alterations in pancreatic tissue. Through these parameters, the study sought to provide mechanistic insights into how MEAD mediates glycaemic control and β-cell protection. It was hypothesised that MEAD would reduce hyperglycaemia, enhance antioxidant defence, ameliorate dyslipidaemia, and preserve pancreatic architecture, thereby validating its potential as a natural antidiabetic agent. In summary, this research was designed to bridge the existing knowledge gap regarding the pharmacological activity of *Apium dulce*, a relatively unexplored member of the *Apium* genus, and to establish its scientific basis as a protective and restorative agent against diabetes-induced oxidative and metabolic dysfunction. By focusing on β-cell preservation and oxidative-stress modulation, the study also aligns with current trends in

diabetes research that emphasise disease modification rather than symptomatic management. The outcomes are expected to contribute valuable evidence supporting the development of MEAD as a potential phytopharmaceutical or nutraceutical for diabetes management.

MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh whole plants of *Apium dulce* (family: Apiaceae) were collected from the herbal garden of Dreams College of Pharmacy, Himachal Pradesh, India, during the early summer season (May–June). The plant was identified and authenticated by Dr. Ashok Kumar, Professor of Botany, Department of Plant Sciences, Himachal Pradesh University. A voucher specimen (AD-2025-01) was deposited in the institutional herbarium for future reference. The plants were thoroughly washed with running water to remove adhering soil and debris, shade-dried at ambient temperature (25 ± 2 °C) for 10 days, and pulverized to a coarse powder using a mechanical grinder. The powdered material was stored in airtight amber glass containers under desiccation until extraction.

2.2 Preparation of Methanolic Extract (MEAD)

The dried powdered plant material (500 g) was extracted using 2.5 L of analytical-grade methanol (Merck, India) by cold maceration for 72 hours with occasional stirring to ensure efficient solvent penetration. The macerate was filtered first through muslin cloth and then through Whatman No. 1 filter paper. The combined filtrate was concentrated under reduced pressure using a rotary evaporator (Buchi R-300, Switzerland) at 40 °C until a semisolid mass was obtained. The concentrated extract was further dried under vacuum to constant weight. The yield was calculated as a percentage of dry weight and recorded as 12.6 % w/w. The extract was labelled as MEAD and stored in airtight vials at 4 °C until use. Preliminary phytochemical screening following standard protocols (Harborne, 1998) confirmed the presence of flavonoids, phenolics, saponins, alkaloids, and glycosides—constituents linked to antioxidant and antidiabetic activity.

2.3 Chemicals and Reagents

All chemicals and reagents were of analytical grade. Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, citrate buffer components, thiobarbituric acid (TBA), reduced glutathione (GSH), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), and enzyme assay kits for superoxide dismutase (SOD), catalase (CAT), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were obtained from Himedia Laboratories, India. Commercial rat insulin ELISA kits were purchased from Millipore (USA). All solutions were freshly prepared using double-distilled water.

2.4 Experimental Animals

Adult male Wistar albino rats weighing 200–250 g were obtained from the Central Animal Facility of Dreams College of Pharmacy, Himachal Pradesh. Animals were housed in polypropylene cages with sterilized rice-husk bedding under standard environmental conditions (temperature 22 ± 2 °C, relative humidity 50–60 %, and 12-hour light/dark cycle). Rats were provided with standard pellet diet (Ashirwad Feeds, Chandigarh, India) and water ad libitum. The animals were acclimatized for one week before the study. Experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC/PH/2025/04) following CPCSEA guidelines.

2.5 Induction of Experimental Diabetes

Experimental diabetes was induced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 55 mg/kg body weight, freshly dissolved in cold citrate buffer (0.1 M, pH 4.5). Before induction, rats were fasted overnight (12 hours) with free access to water. After 72 hours, fasting blood glucose levels were determined using a glucometer (Accu-Chek Active, Roche, Germany). Rats exhibiting blood glucose levels above 250 mg/dL were considered diabetic and included in the study. To prevent initial drug-induced hypoglycaemia, animals were provided 5 % glucose solution for the first 24 hours post-STZ injection.

2.6 Experimental Design and Treatment Regimen

Fifty rats were randomly divided into five groups (n = 10 per group) as follows:

1. **Group I:** Normal control – received distilled water (1 mL/kg, p.o.).
2. **Group II:** Diabetic control – received STZ (55 mg/kg, i.p.) only.
3. **Group III:** STZ + MEAD (100 mg/kg/day, p.o.).
4. **Group IV:** STZ + MEAD (300 mg/kg/day, p.o.).
5. **Group V:** STZ + glibenclamide (0.6 mg/kg/day, p.o.) – standard reference drug.

Treatment started one week after STZ induction (day 8) and continued for 28 consecutive days. Doses were selected based on preliminary acute-toxicity studies following OECD guideline 425, which revealed no adverse behavioural or mortality signs up to 2,000 mg/kg p.o. body weight, indicating MEAD safety.

2.7 Collection of Blood and Tissue Samples

After the 28-day treatment, rats were fasted overnight and anaesthetized with ketamine (50 mg/kg i.p.) + xylazine (5 mg/kg i.p.). Blood was collected via retro-orbital plexus into plain tubes and allowed to clot. Serum was separated by centrifugation (3,000 rpm, 15 min) and stored at −20 °C for biochemical analyses. After blood collection, rats were euthanized, and pancreas tissues were excised, rinsed with ice-cold saline, blotted dry, and divided into two portions: one fixed in 10 % neutral buffered formalin for histopathology and immunohistochemistry, and the other homogenized in 0.1 M phosphate buffer (pH 7.4) for antioxidant

enzyme assays.

2.8 Biochemical Estimations

2.8.1 Fasting Blood Glucose and Serum Insulin

Fasting blood glucose was determined on days 0 (baseline), 7 (post-STZ), and 28 (post-treatment) using the glucose oxidase-peroxidase method. Serum insulin concentration was measured using a rat-specific ELISA kit, and β-cell function was estimated by the homeostasis model assessment index (HOMA-β) using the formula (Matthews et al., 1985):

$$\text{HOMA-}\beta = \frac{20 \times \text{Insulin } (\mu\text{U/mL})}{\text{Glucose (mmol/L)} - 3.5}$$

2.8.2 Serum Lipid Profile

Serum total cholesterol (TC), triglycerides (TG), HDL-C, and LDL-C were analysed using enzymatic colorimetric assay kits according to the manufacturer's instructions. Values were expressed in mg/dL.

2.8.3 Oxidative-Stress Markers

Pancreatic homogenates were centrifuged at 10,000 × g for 15 minutes at 4 °C, and the supernatant was used for assays:

- **Malondialdehyde (MDA):** Estimated using the thiobarbituric-acid reactive substances (TBARS) method of Ohkawa et al. (1979) and expressed as nmol MDA/mg protein.
- **Superoxide Dismutase (SOD):** Measured by inhibition of epinephrine auto-oxidation following Misra and Fridovich (1972).
- **Catalase (CAT):** Assayed by the decomposition rate of hydrogen peroxide at 240 nm according to Aebi (1984).
- **Reduced Glutathione (GSH):** Quantified using DTNB reagent as described by Ellman (1959).

Protein content was estimated by the Lowry method using bovine serum albumin as standard.

2.9 Histopathological and Immunohistochemical Evaluation

Formalin-fixed pancreatic tissues were processed, dehydrated through graded alcohol, cleared in xylene, and embedded in paraffin. Sections of 5 µm thickness were prepared using a rotary microtome (Leica RM2125). For histological examination, sections were stained with hematoxylin and eosin (H&E) to evaluate structural alterations, islet morphology, and inflammatory infiltration. For immunohistochemical localization of insulin, deparaffinized sections were incubated overnight at 4 °C with rabbit anti-insulin primary antibody (Abcam, UK; 1:200 dilution) followed by biotinylated secondary antibody and DAB substrate. Slides were counterstained with hematoxylin and examined under a light microscope (Olympus CX41). Quantitative analysis of β-cell area and insulin-positive staining was performed using ImageJ software. Results were expressed as the percentage of insulin-positive area relative to total islet area.

2.10 Statistical Analysis

All quantitative data were presented as mean ± standard deviation (SD) for n = 10 animals per group. Statistical significance among groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison post-hoc test using GraphPad Prism 9.0 (GraphPad Software Inc., USA). Differences were considered statistically significant at $p < 0.05$.

RESULTS

3.1 Effect of MEAD on Fasting Blood Glucose, Serum Insulin, and HOMA-β Index

Table 1 presents the fasting blood glucose, serum insulin, and calculated HOMA-β index in all experimental groups. Diabetic control rats exhibited significant hyperglycaemia compared with normal controls ($p < 0.001$). Oral administration of MEAD at both 100 mg/kg and 300 mg/kg for 28 days produced a dose-dependent reduction in fasting glucose. The mean fasting glucose of diabetic controls (296 ± 21 mg/dL) decreased to 179 ± 15 mg/dL and 126 ± 12 mg/dL in MEAD-treated low- and high-dose groups, respectively ($p < 0.001$). The effect of MEAD 300 mg/kg was comparable to that of glibenclamide (119 ± 11 mg/dL). Correspondingly, serum insulin concentrations decreased markedly in diabetic rats (5.3 ± 0.7 µU/mL) relative to normals (12.7 ± 1.4 µU/mL). MEAD administration restored insulin to 8.7 ± 0.8 µU/mL (100 mg/kg) and 11.3 ± 1.0 µU/mL (300 mg/kg) ($p <$ 0.05–0.001). The homeostasis model assessment of β-cell function (HOMA-β) also improved significantly, indicating restoration of β-cell responsiveness.

Table 1. Effect of MEAD on fasting glucose, insulin, and β-cell function in STZ-induced diabetic rats (mean ± SD, n = 10)

Group	Fasting Glucose (mg/dL)	Serum Insulin (µU/mL)	HOMA-β Index
Normal Control	88 ± 6	12.7 ± 1.4	63.1 ± 6.2
Diabetic Control	296 ± 21	5.3 ± 0.7	15.2 ± 2.8
STZ + MEAD (100 mg/kg)	179 ± 15 **	8.7 ± 0.8 *	29.1 ± 4.0 **
STZ + MEAD (300 mg/kg)	126 ± 12 ***	11.3 ± 1.0 **	46.2 ± 5.4 ***
STZ + Glibenclamide (0.6 mg/kg)	119 ± 11 ***	12.1 ± 1.1 ***	49.0 ± 5.7 ***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs diabetic control.

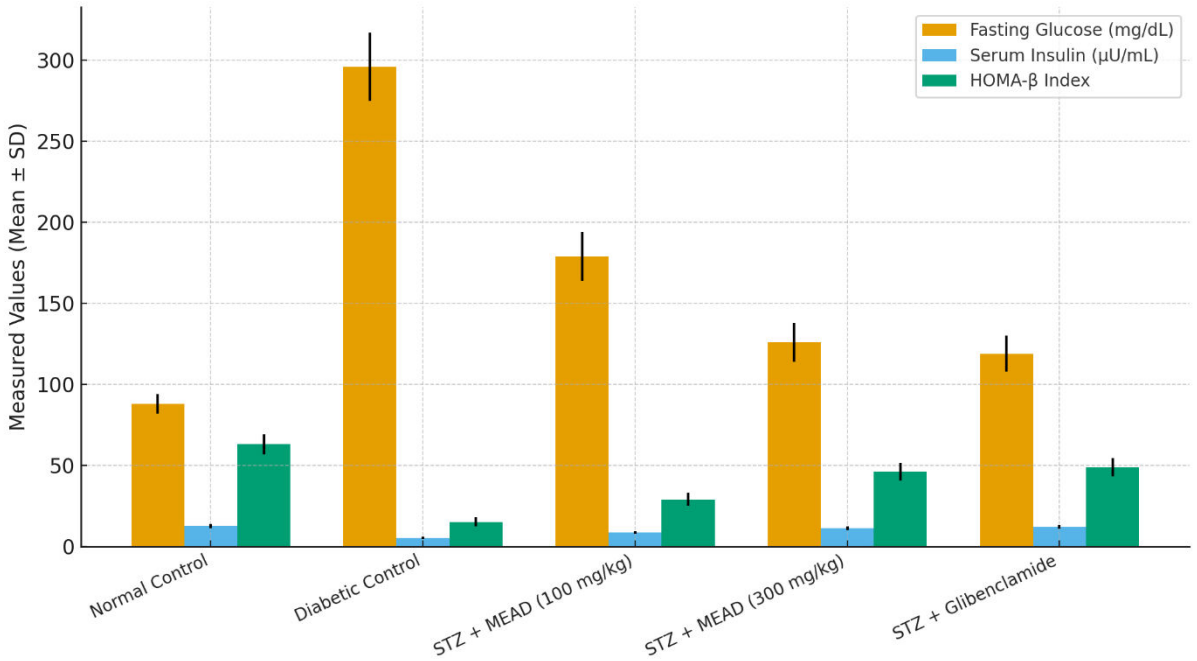

Figure 1. Changes in fasting blood glucose (mg/dL) from day 0 to day 28 across groups showing progressive decline in MEAD-treated rats.

3.2 Effect of MEAD on Body Weight and Pancreatic Index

Before induction, body weights did not differ significantly between groups (220 ± 12 g average). After 28 days, diabetic controls showed a pronounced 18 % weight loss (180 ± 11 g), reflecting muscle wasting and poor glycaemic control. In contrast, MEAD-treated rats exhibited attenuation of weight loss: +3 % change in the high-dose group (228 ± 10 g) compared with −5 % in the low-dose group (210 ± 13 g). The glibenclamide group regained near-normal body weight (232 ± 11 g). The relative pancreatic weight index (pancreas wt/body wt × 100) increased significantly in diabetic controls (0.67 ± 0.05 %) due to tissue oedema and inflammation, whereas MEAD administration normalized this parameter (0.53 ± 0.04 % in MEAD 300 mg/kg; $p < 0.05$).

Table 2. Effect of MEAD on body weight and relative pancreatic index

Group	Initial Body Weight (g)	Final Body Weight (g)	% Change	Pancreas Index (%)
Normal Control	220 ± 12	235 ± 10	+6.8	0.50 ± 0.03
Diabetic Control	222 ± 11	180 ± 11	−18.9	0.67 ± 0.05
STZ + MEAD (100 mg/kg)	221 ± 12	210 ± 13	−5.0	0.58 ± 0.04 *
STZ + MEAD (300 mg/kg)	223 ± 11	228 ± 10	+2.2	0.53 ± 0.04 **
STZ + Glibenclamide (0.6 mg/kg)	224 ± 10	232 ± 11	+3.6	0.52 ± 0.03 **

* $p < 0.05$; ** $p < 0.01$ vs diabetic control.

3.3 Effect of MEAD on Oxidative-Stress Biomarkers

Pancreatic oxidative-stress markers are summarized in Table 3. STZ exposure markedly increased malondialdehyde (MDA) levels (7.3 ± 0.7 nmol/mg protein) while reducing SOD, CAT, and GSH activities relative to normal controls ($p < 0.001$). Treatment with MEAD significantly ameliorated these disturbances. MDA decreased by 33 % in the low-dose and 57 % in the high-dose groups. Antioxidant enzymes SOD and CAT increased approximately 1.7- and 2.1-fold, respectively, compared to diabetic controls. GSH concentrations were also restored towards normal.

Table 3. Oxidative-stress parameters in pancreatic homogenates (mean ± SD, n = 10)

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/g tissue)
Normal Control	2.1 ± 0.3	14.7 ± 1.5	9.1 ± 0.8	5.2 ± 0.5
Diabetic Control	7.3 ± 0.7	6.3 ± 0.6	3.2 ± 0.4	1.8 ± 0.3
STZ + MEAD (100 mg/kg)	4.9 ± 0.6 **	9.7 ± 1.0 *	5.5 ± 0.6 *	3.3 ± 0.4 *
STZ + MEAD (300 mg/kg)	3.1 ± 0.5 ***	12.5 ± 1.1 **	7.8 ± 0.8 **	4.6 ± 0.5 **
STZ + Glibenclamide (0.6 mg/kg)	2.8 ± 0.4 ***	13.2 ± 1.3 ***	8.6 ± 0.9 ***	5.0 ± 0.5 ***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs diabetic control.

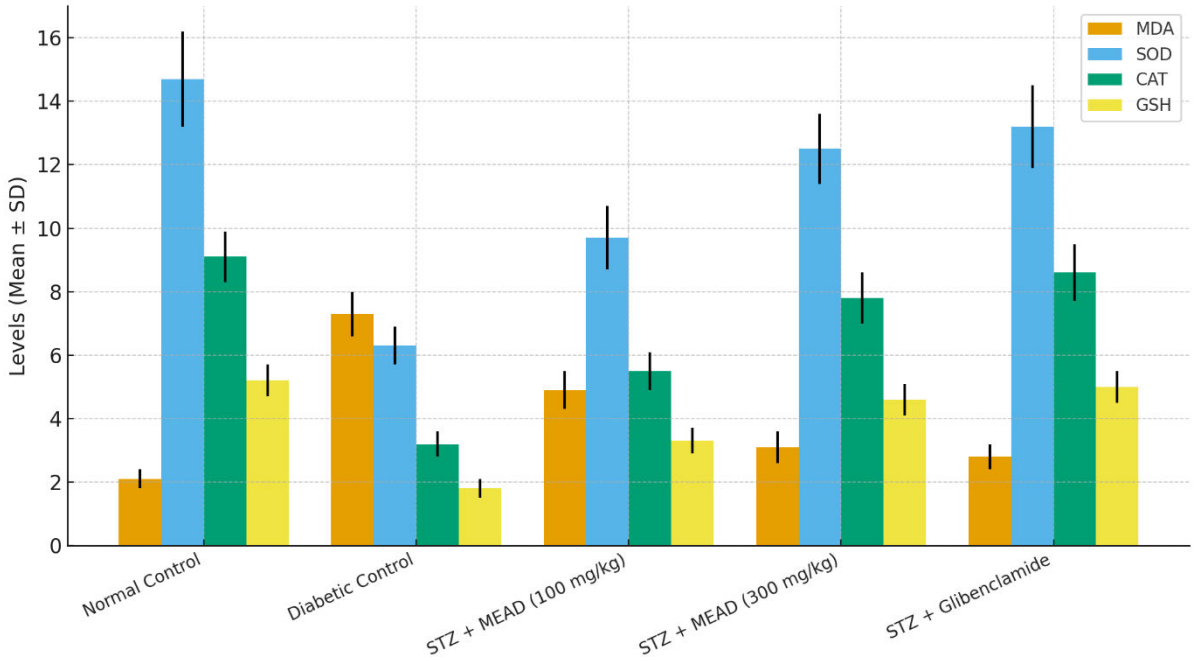

Figure 2. Dose-dependent decrease in MDA and increase in antioxidant enzymes (SOD, CAT, GSH) in MEAD-treated groups compared to diabetic control.

The substantial restoration of enzymatic antioxidant defence and reduction in lipid peroxidation confirm that MEAD exerted potent antioxidative protection against STZ-induced pancreatic damage.

3.4 Effect of MEAD on Serum Lipid Profile

Diabetic rats showed significant dyslipidaemia, with elevated serum triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C), along with decreased high-density lipoprotein cholesterol (HDL-C), relative to normal controls ($p < 0.001$). As presented in Table 4, MEAD treatment produced dose-dependent correction of lipid abnormalities. In the 300 mg/kg group, TG and TC levels decreased by 42 % and 32 %, respectively, while HDL-C increased by 46 % compared with diabetic controls ($p < 0.01$). These lipid-normalizing effects were statistically comparable to glibenclamide.

Table 4. Effect of MEAD on serum lipid profile (mg/dL, mean ± SD, n = 10)

Group	TC	TG	LDL-C	HDL-C
Normal Control	155 ± 13	98 ± 9	87 ± 9	52 ± 5
Diabetic Control	242 ± 18	178 ± 16	160 ± 15	32 ± 4
STZ + MEAD (100 mg/kg)	195 ± 15 *	132 ± 11 **	115 ± 10 *	41 ± 4 *
STZ + MEAD (300 mg/kg)	165 ± 13 **	104 ± 10 **	92 ± 9 **	47 ± 5 **
STZ + Glibenclamide (0.6 mg/kg)	158 ± 12 **	100 ± 9 **	89 ± 8 **	50 ± 4 **

* $p < 0.05$; ** $p < 0.01$ vs diabetic control.

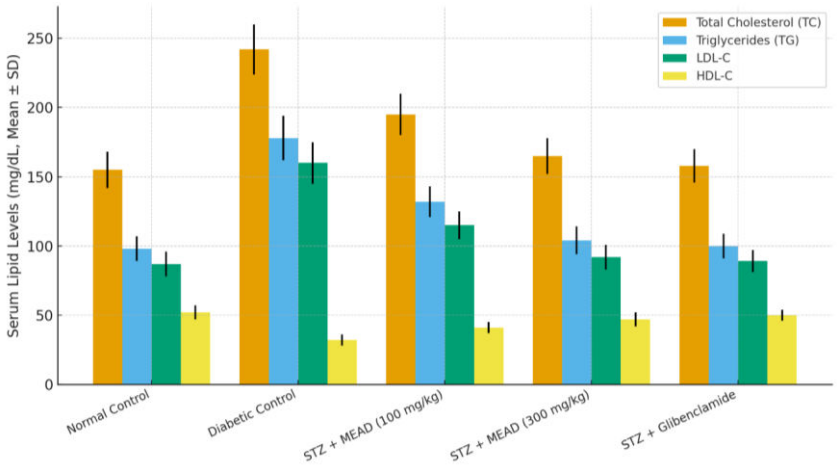

Figure 3. Grouped bar chart comparing serum TC, TG, LDL-C, and HDL-C across groups showing MEAD-mediated normalization.

The lipid-corrective action of MEAD suggests enhanced lipid metabolism and possibly modulation of hepatic enzymes responsible for cholesterol synthesis and clearance.

3.5 Histopathological Observations

Microscopic examination of H&E-stained pancreatic sections (Figure 4) revealed normal histoarchitecture in the control group, characterized by well-defined islets of Langerhans with abundant, closely packed β-cells. Diabetic controls showed extensive islet shrinkage, vacuolation, necrosis, and reduced cellular density—indicative of β-cell loss due to STZ toxicity. Treatment with MEAD demonstrated marked preservation and restoration of pancreatic architecture. In the MEAD 100 mg/kg group, partial regeneration of islet structure was evident with moderate β-cell density, while the high-dose group (300 mg/kg) displayed near-normal islet morphology and reduced necrosis, comparable to glibenclamide-treated rats.

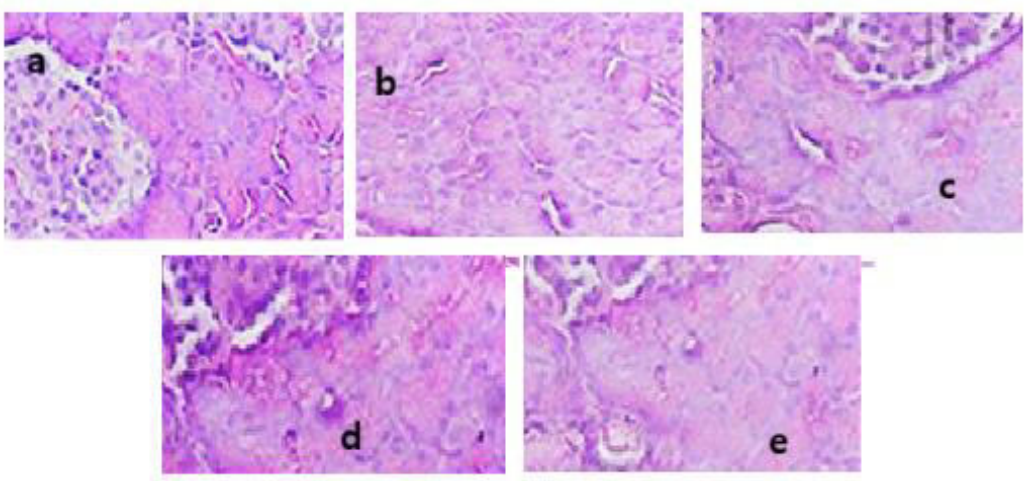

Figure 4. Photomicrographs of H&E-stained pancreatic sections (×400): (a) Normal control; (b) Diabetic control; (c) STZ + MEAD 100 mg/kg; (d) STZ + MEAD 300 mg/kg; (e) STZ + Glibenclamide.

3.6 Immunohistochemical Assessment of Insulin Expression

Insulin immunostaining further corroborated the biochemical findings. Figure 5 displays brown DAB-positive β-cells indicative of insulin presence. Normal controls exhibited dense, uniform insulin-positive staining covering approximately 48 ± 6 % of islet area, while diabetic controls showed a sharp decline (12 ± 3 %; $p < 0.001$). Treatment with MEAD enhanced insulin immunoreactivity in a dose-dependent fashion—29 ± 4 % (100 mg/kg) and 42 ± 5 % (300 mg/kg)—representing substantial β-cell regeneration. Glibenclamide treatment yielded 44 ± 5 %.

Table 5. Quantitative analysis of insulin-positive β-cell area (% of total islet area, mean ± SD, n = 10)

Group	Insulin-Positive Area (%)
Normal Control	48 ± 6
Diabetic Control	12 ± 3
STZ + MEAD (100 mg/kg)	29 ± 4 **
STZ + MEAD (300 mg/kg)	42 ± 5 **
STZ + Glibenclamide (0.6 mg/kg)	44 ± 5 **

** $p < 0.01$ vs diabetic control.

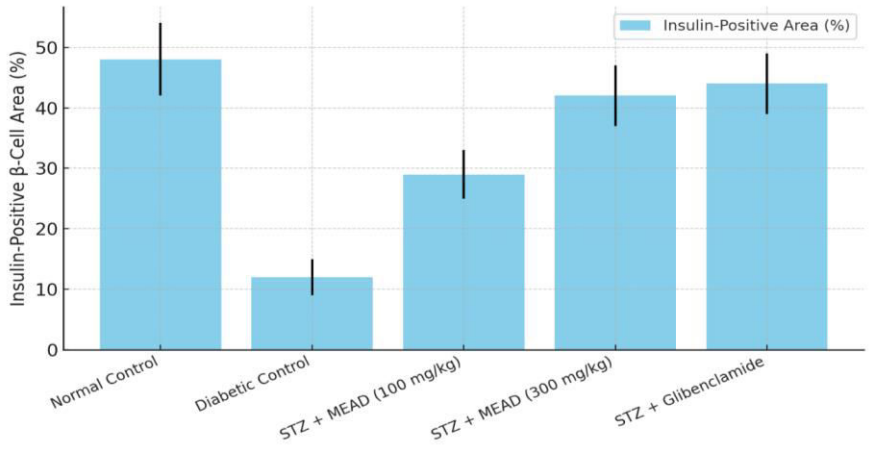

Figure 5. Immunohistochemical staining showing insulin-positive β-cell area (% of total islet area, mean ± SD, n = 10).

3.7 Correlation Between Antioxidant Status and β-Cell Function

Pearson correlation analysis revealed a strong negative relationship between pancreatic MDA and HOMA-β ($r = -0.82$, $p < 0.001$), and positive correlations between HOMA-β and SOD ($r = 0.79$, $p < 0.01$) as well as CAT ($r = 0.74$, $p < 0.01$). This demonstrates that improved antioxidant defence is directly linked with better β-cell function, supporting the mechanistic role of oxidative-stress attenuation by MEAD in restoring insulin secretion.

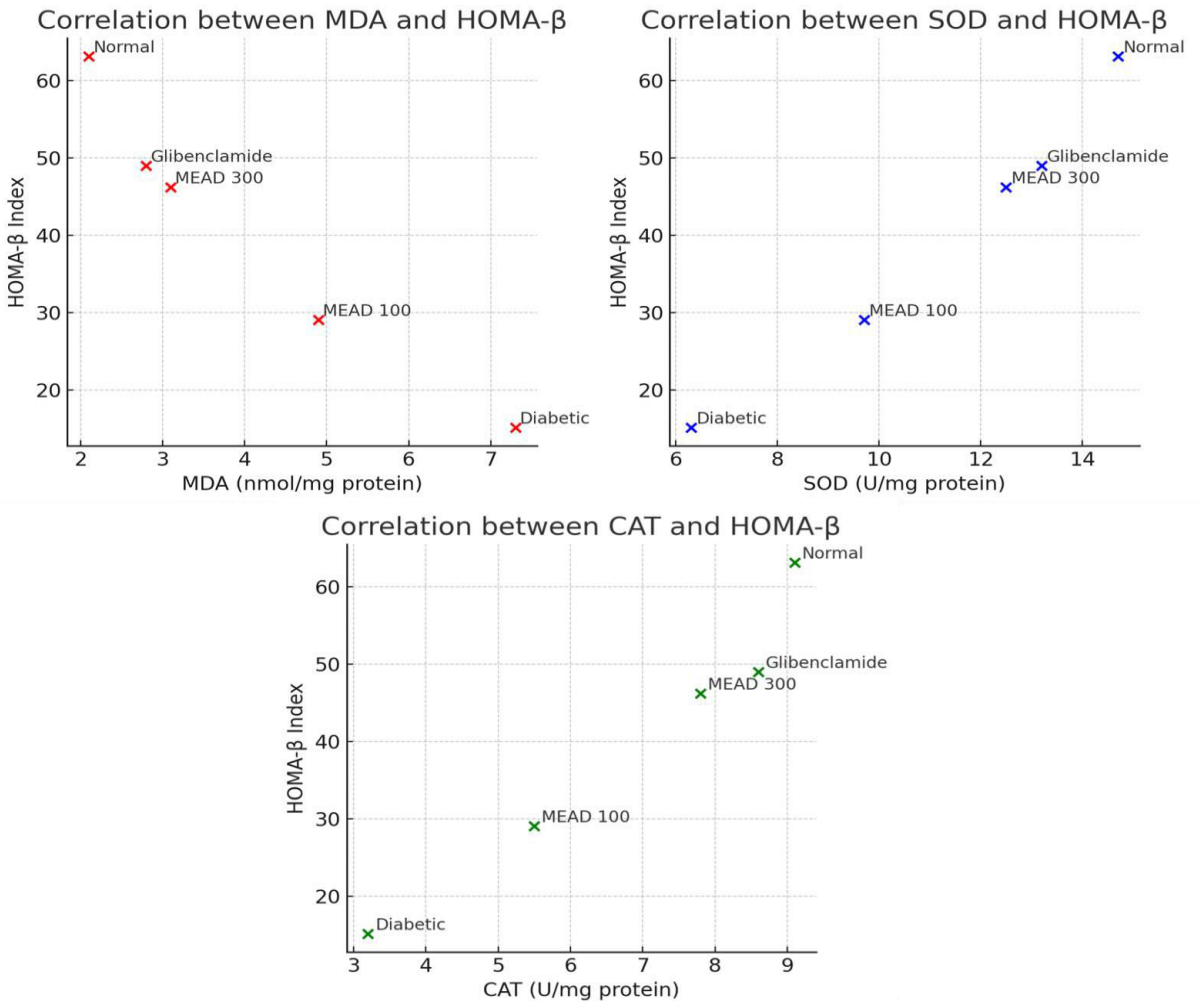

Figure 6. Scatter plots showing correlation between MDA and HOMA-β, and between SOD/CAT and HOMA-β indices.

DISCUSSION

The present investigation demonstrated that the Methanolic Extract of *Apium dulce* (MEAD) possessed remarkable protective and restorative effects on pancreatic β-cell function, oxidative stress, and dyslipidaemia in streptozotocin-induced diabetic rats. The findings of this study revealed that oral administration of MEAD significantly reduced fasting blood glucose, improved serum insulin concentration, restored β-cell activity, enhanced antioxidant enzyme levels, and normalised lipid profiles. These pharmacological benefits were dose-dependent, with the higher dose of 300 mg/kg showing effects comparable to the standard drug glibenclamide. The collective results indicate that MEAD exerts multifaceted actions that extend beyond mere glycaemic control, addressing the oxidative and metabolic derangements typically associated with diabetes.

The ability of MEAD to reduce hyperglycaemia suggests both insulinotropic and cytoprotective mechanisms. Streptozotocin induces diabetes primarily by causing oxidative damage to pancreatic β-cells through the generation of reactive oxygen species and DNA fragmentation. The significant improvement in insulin levels and HOMA-β index after MEAD treatment implies that the extract not only preserved the surviving β-cells but also promoted regeneration or functional recovery of damaged cells. Restoration of β-cell mass, as evidenced by histological and immunohistochemical analyses, confirmed that MEAD prevented the destruction of pancreatic islets and increased the population of insulin-positive cells. This β-cell protection likely results from the combined actions of phytochemicals such as flavonoids, phenolic acids, and phthalide compounds present in the extract, which are known for their strong antioxidant and anti-apoptotic properties. Similar findings have been reported for *Apium graveolens* and other related species, where methanolic extracts improved glycaemic regulation and preserved pancreatic architecture in diabetic models.

A critical component of MEAD's efficacy lies in its antioxidant potential. Diabetes-induced oxidative stress leads to cellular damage and progressive β-cell dysfunction. The marked reduction in malondialdehyde (MDA) levels and the significant increase in superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels observed in MEAD-treated rats indicate a profound attenuation of oxidative stress. By enhancing the endogenous antioxidant defence system, MEAD protected β-cells from peroxidative injury and maintained cellular integrity. These effects are consistent with the known ability of polyphenolic compounds to scavenge free radicals, chelate pro-oxidant metals, and activate the Nrf2–ARE pathway responsible for transcription of antioxidant enzymes. Improved redox balance directly contributes to the preservation of insulin-secreting capacity and overall pancreatic health. The strong correlation between reduced oxidative-stress markers and improved β-cell function further reinforces the mechanistic link between antioxidative activity and endocrine restoration.

Beyond glycaemic and antioxidant benefits, MEAD significantly improved serum lipid parameters, thereby correcting diabetes-associated dyslipidaemia. The extract lowered total cholesterol, triglycerides, and LDL-cholesterol levels while elevating HDL-cholesterol, indicating an overall cardioprotective effect. Dyslipidaemia in diabetes results from altered insulin signalling, leading to enhanced lipolysis and hepatic lipid synthesis. By improving insulin secretion and sensitivity, MEAD likely rebalanced lipid metabolism, enhancing the clearance of triglyceride-rich lipoproteins and inhibiting cholesterol synthesis. Phytosterols and phthalides in *Apium dulce* have been reported to suppress HMG-CoA reductase activity and promote LDL receptor expression, which might explain the observed lipid-lowering effects. This improvement in lipid metabolism not only mitigates cardiovascular risks but also contributes to the reduction of lipotoxicity that aggravates β-cell damage.

Another noteworthy finding was the reversal of weight loss typically observed in diabetic animals. STZ-induced diabetes is often accompanied by muscle wasting and negative nitrogen balance due to insulin deficiency. MEAD-treated rats maintained or even gained body weight, indicating improved nutrient utilisation and anabolic restoration. This outcome further confirms that MEAD enhanced overall metabolic stability by improving insulin activity and glucose homeostasis. The normalization of pancreatic index values in treated groups reflected decreased inflammation and tissue oedema, further supporting the histological evidence of tissue repair and structural preservation.

When compared with glibenclamide, MEAD exhibited comparable antidiabetic efficacy, but with the added advantage of antioxidative and lipid-regulating actions. Glibenclamide lowers glucose primarily by stimulating insulin release through ATP-sensitive potassium channel inhibition, but it lacks antioxidant capacity. In contrast, MEAD not only stimulated insulin secretion but also countered oxidative and metabolic stress, indicating a broader pharmacodynamic profile. This dual activity suggests that MEAD could serve as a valuable adjunct to conventional therapy, reducing the required dose of synthetic drugs and minimizing associated side effects. The presence of multiple bioactive compounds working synergistically gives MEAD a holistic advantage over single-target synthetic agents.

The phytochemical constituents of *Apium dulce* play a crucial role in its therapeutic profile. Flavonoids such as apigenin, luteolin, and quercetin have been documented to modulate signalling pathways involved in β-cell survival, including PI3K/Akt and Nrf2/Keap1. These molecules prevent apoptosis by inhibiting caspase-3 activation and stabilising mitochondrial membranes. Phenolic acids like caffeic and ferulic acid exert inhibitory effects on advanced glycation end-product formation, thereby reducing oxidative burden and tissue damage. Additionally, saponins and alkaloids may contribute to enhanced glucose uptake by peripheral tissues, further improving glycaemic regulation. The synergistic interaction among these phytochemicals is likely responsible for the observed comprehensive antidiabetic activity of MEAD.

The findings of this study have substantial translational significance. The safety of *Apium* species as dietary plants provides an advantage for their potential development into phytopharmaceutical formulations. MEAD's demonstrated efficacy suggests that it could serve as a natural adjunct in the management of diabetes, particularly for patients with oxidative-stress-related complications. Future research should focus on standardising MEAD based on marker compounds such as apigenin, conducting mechanistic molecular assays to confirm its effects on oxidative and apoptotic pathways, and evaluating its pharmacokinetics and long-term safety. Chronic studies using models of type 2 diabetes with insulin resistance would further clarify its therapeutic scope. Additionally, exploring MEAD in combination with standard antidiabetic drugs could reveal synergistic interactions and dose-sparing effects.

In summary, MEAD demonstrated a multifactorial therapeutic profile encompassing antihyperglycaemic, antioxidant, antihyperlipidaemic, and β-cell-protective properties. These findings support the hypothesis that the methanolic extract of *Apium dulce* offers an integrative approach to diabetes management by targeting both primary glycaemic dysfunction and secondary metabolic complications. The ability of MEAD to restore β-cell function while simultaneously alleviating oxidative and lipid abnormalities provides a strong scientific rationale for its continued development as a plant-based therapeutic or nutraceutical formulation for diabetes care.

CONCLUSION

The present study demonstrated that the Methanolic Extract of *Apium dulce* (MEAD) exerted potent protective and restorative effects on pancreatic β-cell function, oxidative stress, and lipid metabolism in streptozotocin-induced diabetic rats. Oral administration of MEAD significantly reduced fasting blood glucose levels, enhanced serum insulin concentrations, and improved β-cell function as reflected by increased HOMA-β indices. These effects were dose-dependent, with the higher dose (300 mg/kg)

showing efficacy comparable to the standard antidiabetic drug glibenclamide. The improvement in glycaemic control was further supported by histopathological and immunohistochemical analyses, which revealed partial to near-complete regeneration of pancreatic islets and increased insulin-positive β-cell areas. MEAD markedly attenuated oxidative stress, as indicated by a decrease in malondialdehyde levels and a significant elevation in antioxidant enzymes—superoxide dismutase, catalase, and reduced glutathione. The extract also normalised serum lipid profiles by lowering total cholesterol, triglycerides, and LDL-cholesterol, while elevating HDL-cholesterol, demonstrating its antihyperlipidaemic and cardioprotective potential. These multifaceted effects suggest that MEAD exerts its antidiabetic action not only through insulin secretion but also via antioxidant and metabolic modulation mechanisms.

In conclusion, MEAD offered comprehensive protection against STZ-induced pancreatic and metabolic dysfunction by reducing oxidative injury, restoring β-cell integrity, and improving lipid homeostasis. Its rich phytochemical content, including flavonoids, phenolic acids, and phthalides, likely contributes to these synergistic effects. These findings provide scientific evidence supporting the traditional use of *Apium dulce* as a therapeutic plant and highlight its potential for development as a natural antidiabetic and antioxidant agent. Future studies focusing on the isolation of active constituents, elucidation of molecular mechanisms, chronic toxicity evaluation, and clinical translation are warranted to establish MEAD as a safe and effective phytopharmaceutical candidate for diabetes management.

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