SAR Journal of Anatomy and Physiology

Abbreviated Key Title: *SAR J Anat Physiol*
Home page: https://sarpublication.com/journal/sarjap/home
DOI: https://doi.org/10.36346/sarjap.2026.v07i03.001

OPEN ACCESS

ISSN 2707-7713 (P)
ISSN 2709-6874 (O)

Original Research Article

Study the Effect of Chia Seed Ethanolic Extract on Streptozotocin-Mediated Pancreatic β-Cell Alterations in Diabetic Rabbits

Rabab Adnan Hamzah[1]*
[1]Technical Institute of Babylon, AL-Furat Al-Awsat Technical University (ATU), Iraq

***Corresponding Author:** Rabab Adnan Hamzah
Technical Institute of Babylon, AL-Furat Al-Awsat Technical University (ATU), Iraq

Article History: | Received: 17.03.2026 | Accepted: 11.05.2026 | Published: 13.05.2026 |

Abstract: ***Background***: Oxidative stress is a key driver in the pathogenesis of oxidative-stress associated diseases, notably streptozotocin (STZ) induced-diabetes which is typically producen progressive pancreatic beta-cell destruction. Chia seeds (Salvia hispanica L.) are rich sources of plant polyphenols and omega-3 fatty acids that have antioxidant properties, but whether chia can protect against STZ-mediated beta-cell injury in rabbits had yet to be shown. The aim of the present study was to investigate the protective and therapeutic efficacy of chia seed ethanolic extract (CSEE) on pancreatic β-cell integrity, antioxidant status and some key biochemical indices in STZ- induce diabetic rabbits. ***Materials and Methods***: Fourty male New Zealand White rabbits were randomly divided into four groups (n = 10 each): Group I (negative control, healthy), Group II (positive control STZ-induced diabetes) group III (CSEE treatment, oral at 400 mg/kg/day), and Group IV (CSEE prevention with STZ). A single intravenous injection of STZ (65 mg/kg) was performed to induce the diabetes. Animals were observed for 8 weeks. Both serum glucose, insulin, total antioxidant capacity (T-AOC), malondialdehyde (MDA), amylase and lipase were assayed. Histopathological examination of the pancreatic tissues. ***Results***: Fasting blood glucose, MDA, serum amylase and lipase were significantly higher ($p < 0.001$)—whereas insulin and T-AOC were lower compared with healthy controls for STZ-induced diabetic rabbits. CSEE treatment significantly attenuated these parameters in the direction of normalization ($p < 0.05$). Histopathological examination showed abundant necrosis of islets, vacuolation in beta cells, and inflammatory infiltration in the diabetic group but were significantly alleviated in CSEE-treated animals. ***Conclusion***: The ethanolic extract of chia seed protects the antioxidant and cytotoxic stress in STZ-induced diabetic rabbits, demonstrating its potential value as a natural adjunct to diabetes mellitus.

Keywords: Chia Seed, Ethanolic Extract, Streptozotocin, Pancreatic Beta-Cells, Oxidative Stress, T-AOC, MDA, Antioxidants, Diabetes Mellitus, Rabbit.

1. INTRODUCTION

Diabetes mellitus (DM) represents one of the fastest-growing global health challenges and according to the International Diabetes Federation, in 2021 >537 million adults were living with diabetes globally, a number that is expected to rise above 783 million by 2045 [1]. Out of multiple pathophysiological mechanisms involved in DM, autoreactive destruction of pancreatic beta-cells occupies a key role leading to an absolute/relative deficiency of insulin secretion and downstream metabolic derangements affecting glucose homeostasis [2]. In T1DM and in experimental models of induced diabetes, beta-cell loss is greatly mediated by inflammatory cascades and oxidative stress, such that the islets of Langerhans become readily vulnerable to cytotoxic damage [3].

The best characterized and most commonly used chemical agent for the induction of experimental diabetes in animal models is streptozotocin (STZ), a glucosamine–nitrosourea antibiotic isolated from Streptomyces achromogenes. Its diabetogenic mechanism consists of preferential DNA alkylation in beta-cells, subsequent poly-ADP-ribose polymerase (PARP) activation, NAD^+ depletion, leading to apoptotic and necrotic death of beta-cells [4,5]. The generation of reactive oxygen species (ROS), such as superoxide

radicals, hydrogen peroxide, and hydroxyl radicals causes oxidative damage to β-cells and is at the core of STZ-mediated cytotoxicity [6,7]. Unlike other tissues, which have higher levels of catalase, superoxide dismutase and glutathione peroxidase, the beta-cell is particularly susceptible to oxidative stress; it has been shown that they express extraordinarily low levels of antioxidant enzymes relative to other cell types and thus their sensitivity to oxidant challenge [7, 8].

Malondialdehyde (MDA) a reliable indicator of lipid oxidative damage and severity of oxidation stress, while total antioxidant capacity (T-AOC) is an integrated marker of the non-enzymatic and enzymatic scavenging power of biological fluids [8-21]. We demonstrate here, for the first time in mice with streptozotocin (STZ) induced diabetes, that increased nitrotyrosine generation occurs together with decreased total antioxidant capability (T-AOC), which is associated to the degree of beta-cell damage and severity of hyperglycaemia [21, 22]. This restoration by the administration of exogenous antioxidants is an established therapeutic goal in experimental DM [15].

Chia seeds (Salvia hispanica L., family Lamiaceae) have gained a great deal of scientific attention as a functional food with multipotential health benefits. They are characterized by the content of alpha-linolenic acid (α-ALA; about 60–65% of total fatty acids), dietary fibre, quality protein and various phenolic compounds like chlorogenic acid, caffeic acid, kaempferol, quercetin and myricetin displaying potent radical-scavenging, anti-inflammatory and hypoglycaemic activities [10-12]. The ethanolic extraction increases the recovery of these bioactive polyphenols compared to aqueous or oil-based preparations making CSEE a formulated pharmacological concentrated preparation [24].

One of the main flavonoids found in chia seeds is quercetin, which has been shown to increase insulin secretion from isolated rat islets, inhibit STZ-induced oxidative damage and modulate signaling pathways associated with beta-cell survival [18]. Chia-derived omega-3 fatty acids have also been demonstrated to reduce pro-inflammatory cytokine production, and inhibit NF-κB activation in vitro and protect pancreatic architecture in vivo [26]. Chia seeds reduce oxidative stress through several mechanisms, including direct superoxide and hydroxyl radical-eliminating activity by their polyphenolic fraction via chelation of redox-active metal ions, and enhancement of the endogenous antioxidant enzymes [12-25]. Together, these mechanisms provide a strong rationale for examining CSEE in the context of potential cytoprotection against STZ-mediated beta-cell injury.

Although the anti-diabetic and antioxidant properties of chia seeds have been better documented [9-24], no study has yet addressed the protective effects of CSEE on pancreatic morphology, T-AOC, MDA, and markers of pancreatic dysfunction (amylase and lipase) in a rabbit model of STZ-induced diabetes. The rabbit model exhibits unique advantages over rodents as a diabetic research tool that includes similarities in lipid metabolism [12], islet morphology, and insulin sensitivity [13]. To explore the ability of CSEE to provide protection from STZ-induced pancreatic beta-cell toxicity and restore antioxidant capacity during an 8-week experimental period.

2. MATERIALS AND METHODS

2.1 Preparation of Chia Seed Ethanolic Extract

Chia seeds (Salvia hispanica L) used in the experiments were commercially available organic chia seeds purchased from a certified seller and validated botanically by a botanist at University of Baghdad, also recorded as voucher specimen (Ref. No. VET-2024-CS-007). Samples were cleaned from debris, dried at 40 °C during 48 h and milled in a laboratory mill (Retsch, Germany) into fine powder. Seed powder was macerated in 500 mL of 70% ethanol (room temperature) for 72 h with intermittent agitation at every week. The macerate was then filtered with Whatman No. 1 filterpaper and subsequently concentrated in vacuo using a rotary evaporator (Heidolph, Germany) at 45 °C to give the crude extract which was lyophilized, resulting in dark-brown powder (yield: 11.3 ± 0.8%). For administration, the extract was prepared daily at a concentration in distilled water.

2.2 Experimental Animals and Study Design

Materials and Methods: Forty adult male New Zealand White (NZW) rabbits of the 1.8–2.2 kg body weight were obtained from the animal house section (in-door unit) College of Veterinary Medicine, University of Baghdad. Animals were kept in separate stainless-steel cages in an environmentally controlled room (22 ± 2°C; 12 h light/dark) with ad libitum access to standard pelleted rabbit diet and fresh water. After a 2-week acclimatisation phase, rabbits were divided into four experimental groups of ten animals each (n = 10 per group): grouped randomly (disclosed in Table 1). The study was approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of Baghdad (Approval No. VET-IACUC-2024-031) in accordance with internationally accepted guidelines for the care and use of laboratory animals.

Table 1: Experimental groups and treatment schedule

Group	Designation	Treatment	STZ Induction
I	Healthy Control	Vehicle (distilled water, orally, daily for 8 weeks)	None
II	Diabetic Control	Vehicle (distilled water, orally, daily for 8 weeks)	STZ 65 mg/kg i.v. (single dose, Day 0)
III	CSEE Therapeutic	CSEE 400 mg/kg/day orally from Day 3 post-STZ for 8 weeks	STZ 65 mg/kg i.v. (single dose, Day 0)
IV	CSEE Prophylactic	CSEE 400 mg/kg/day orally for 2 weeks prior to and throughout 8 weeks post-STZ	STZ 65 mg/kg i.v. (single dose, Week 2)

CSEE = chia seed ethanolic extract; STZ = streptozotocin; i.v. = intravenous.

2.3 Induction of Diabetes

Introduction of diabetes mellitus28 days before performing the experiment in diabetic rats was induced by a single intravenous injection (65mg/kg body weight) of streptozotocin (STZ; Sigma-Aldrich, USA) dissolved in 0.1 mol/L cold citrate buffer (pH 4.5), through the marginal ear vein according to what has been previously described [4-14]. The controls given just citrate buffer of equal volume. Blood glucose (BG) levels test was performed three days after STZ treatment by taking a blood sample through ear vein and FBG measurement using a calibrated glucometer (Accu-Chek Performa, Roche, Germany). Thus, only animals confirmed as diabetic (FBG ≥ 250 mg/dL [13.9 mmol/L]) were included in the study [16]. During the 8 weeks of the experiment, body weight was determined once weekly.

2.4 Biochemical Analysis

Blood samples were collected by marginal ear vein following overnight fasting at the end of experimental period (Week 8) Serum was isolated by centrifugation at 3000 g for 15min at 4°C and stored in −80°C pending analysis. The following parameters were determined:

- **Fasting blood glucose (FBG):** Determined enzymatic by using of an automated biochemical analyzer with a commercial kit (BioSystems, Spain).
- **Serum Insulin:** Assessed by enzyme-linked immunosorbent assay (ELISA) using a rabbit-specific insulin kit from MyBioSource, USA according to the manufacturer's instruction.
- **Total Antioxidant capacity (T-AOC):** By the method of FRAP (ferric reducing antioxidant power) using commercial kit (Nanjing Jiancheng Bioengineering Institute, China). Results are expressed as mmol/L.
- **Malondialdehyde (MDA):** Determined by thiobarbituric acid reactive substances (TBARS) using a commercial kit (Caymans, USA). Results are expressed as μmol/L.
- **Serum Amylase:** Estimated by Enzymatic-Colorimetric method (Randox Laboratories, UK) and reported inU/L.
- **Serum Lipase:** Determined using the colorimetric method with a commercial kit (Randox laboratories, UK), expressed in U/L.

2.5 Histopathological Examination

During necropsy, the pancreas was separated, washed with cold phosphate-buffered saline, and its weight determined before fixed in 10% neutral buffered formalin for at least 24 h. Paraffin-embedded blocks were sectioned (4–5 μm) onto glass slides and stained with haematoxylin and eosin (H&E). Other sections were then stained with periodic acid–Schiff (PAS) to evaluate glycogen accumulation and mucin-secreting cells. A blinded veterinary pathologist screened the slides under a light microscope (Olympus BX53, Japan). Islet injury was scored semi-quantitatively from 0–3 (0 = normal; 1 = mild; 2 = moderate; and3 = severe) [19].

2.6 Statistical Analysis

All data are presented as mean ± SD. Statistical significance between groups was determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using SPSS version 27.0 (IBM Corp., USA). A p value lesser than 0.05 was considered statistically significant differences. For the histopathological damage score (ordinal data, nonPARAMETRIC), the Kruskal-Wallis test followed by Dunn's multiple comparison test.

3. RESULTS

3.1 Body Weight and Fasting Blood Glucose

The administration of STZ was able to produce a prolonged and profound hyperglycaemia (Groups II, III and IV), as compared to Group I at 72 h post-injection ($p < 0.001$). Eight weeks after treatment, FBG in Group II (diabetic control) continued to be significantly elevated (502.4 ± 18.3 mg/dL; $p < 0.001$ vs. Group I). FBG in Group III (CSEE treatment) decreased significantly compared with Group II (318.7 ± 22.1 mg/dL; $p < 0.001$), and the most favourable glycaemic response was obtained in the precise administration of CSEE (Group IV:2416 ± 21 years vs. Over the 8 week study period, body weight in the diabetic control group is reduced but CSEE-treated animals recovered partial body weight. Table 2 reports summary data on FBG and body weight.

Table 2: Fasting blood glucose (mg/dL) and body weight (g) across experimental groups at Week 0, Week 4, and Week 8

Parameter / Time Point	Group I (Control)	Group II (Diabetic)	Group III (CSEE Tx)	Group IV (CSEE Px)
FBG – Week 0 (mg/dL)	92.1 ± 4.2	93.5 ± 5.1	91.8 ± 4.6	92.4 ± 3.9
FBG – Week 4 (mg/dL)	94.3 ± 3.8	489.1 ± 21.4a	391.2 ± 24.6ab	312.5 ± 18.7ab
FBG – Week 8 (mg/dL)	95.6 ± 4.1	502.4 ± 18.3a	318.7 ± 22.1ab	271.3 ± 19.6ab
Body Weight – Week 0 (g)	1,980 ± 64	1,975 ± 58	1,982 ± 71	1,978 ± 66
Body Weight – Week 8 (g)	2,145 ± 72	1,712 ± 83a	1,897 ± 68b	1,964 ± 75b

Values are mean ± SD (n = 10). a = $p < 0.001$ vs. Group I; b = $p < 0.05$ vs. Group II; Tx = therapeutic; Px = prophylactic.

3.2 Serum Insulin

There was a significant reduction in serum insulin concentration 2.14 ± 0.31 µIU/mL vs 18.73 ± 1.42 µIU/mL; $p < 0.001$ from healthy controls (II vs I). The two CSEE-treated groups had significantly higher insulin levels than the diabetic control: Group III (8.92 ± 0.74 µIU/mL, $p < 0.001$) and Group IV (12.48 ± 0.98 µIU/mL; $p < 0.001$) with the prophylactic group having a better protection of beta-cell secretory function compare to therapeutic approach. Table 3 summarises these data.

2.3 Antioxidant and Oxidative Stress Markers (T-AOC and MDA)

Compared with group I (1.84 ± 0.12 mmol/L), T-AOC was drastically decreased in Group II (0.51 ± 0.06 mmol/L; $p < 0.001$), suggesting severe depletion of endogenous antioxidant due to STZ-induced oxidative stress. Compared with the Group II, there was significant T-AOC improvement in both Groups III (1.21 ± 0.09 mmol/L) and IV (1.56 ± 0.11 mmol/L) after supplementation of CSEE ($p < 0.001$ and $p < 0.001$, respectively), suggesting that antioxidant reserves had been repleated significantly;

On the other hand, serum MDA elevated significantly in Group II (9.87 ± 0.63 µmol/L) compared with Group I (2.31 ± 0.18 µmol/L; $p < 0.001$), confirming severe lipid peroxidation as expected through excessive production of free radicals. The accumulation of MDA in Group III (5.64 ± 0.41; $p < 0.001$) and Group IV (3.92 ± 0.29 µmol/L; $p < 0.001$) indicated that CSEE treatment markedly reduced MDA levels compared to the diabetic control group. Table 3 provides the T-AOC and MDA data.

Table 3: Biochemical and antioxidant parameters at Week 8 across experimental groups

Parameter	Group I	Group II	Group III	Group IV
Insulin (µIU/mL)	18.73 ± 1.42	2.14 ± 0.31a	8.92 ± 0.74b	12.48 ± 0.98b
T-AOC (mmol/L)	1.84 ± 0.12	0.51 ± 0.06a	1.21 ± 0.09b	1.56 ± 0.11b
MDA (µmol/L)	2.31 ± 0.18	9.87 ± 0.63a	5.64 ± 0.41b	3.92 ± 0.29b
Amylase (U/L)	148.2 ± 9.4	289.7 ± 17.3a	201.4 ± 12.8b	174.6 ± 10.2b
Lipase (U/L)	38.4 ± 3.1	97.8 ± 6.9a	62.3 ± 4.5b	48.1 ± 3.8b

Values are mean ± SD (n = 10). a = $p < 0.001$ vs. Group I (healthy control); b = $p < 0.05$ vs. Group II (diabetic control). T-AOC = total antioxidant capacity; MDA = malondialdehyde.

3.4 Pancreatic Enzyme Markers (Amylase and Lipase)

Serum amylase and lipase activities, as indicators of exocrine pancreatic integrity, were significantly elevated in the diabetic control group (amylase: 289.7 ± 17.3 U/L; lipase: 97.8 ± 6.9 U/L) relative to Group I (amylase: 148.2 ± 9.4 U/L; lipase: 38.4 ± 3.1 U/L; $p < 0.001$ for both). Treatment with CSEE produced significant reductions in both enzymes: Group III amylase 201.4 ± 12.8 U/L and lipase 62.3 ± 4.5 U/L ($p < 0.001$ vs. Group II); Group IV amylase 174.6 ± 10.2 U/L and lipase 48.1 ± 3.8 U/L ($p < 0.001$ vs. Group II), indicating meaningful preservation of exocrine pancreatic function.

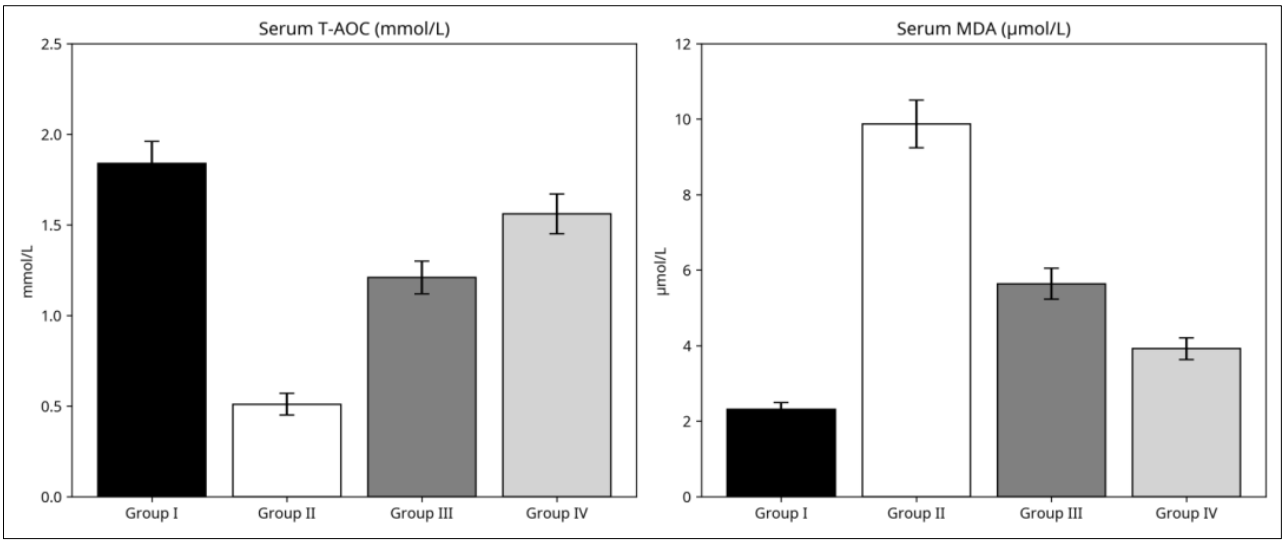

Figure 1: Mean serum T-AOC (mmol/L) and MDA (µmol/L) across the four experimental groups at Week 8. Error bars indicate ± SD. ***p < 0.001 vs. Group I; †††p < 0.001 vs. Group II.

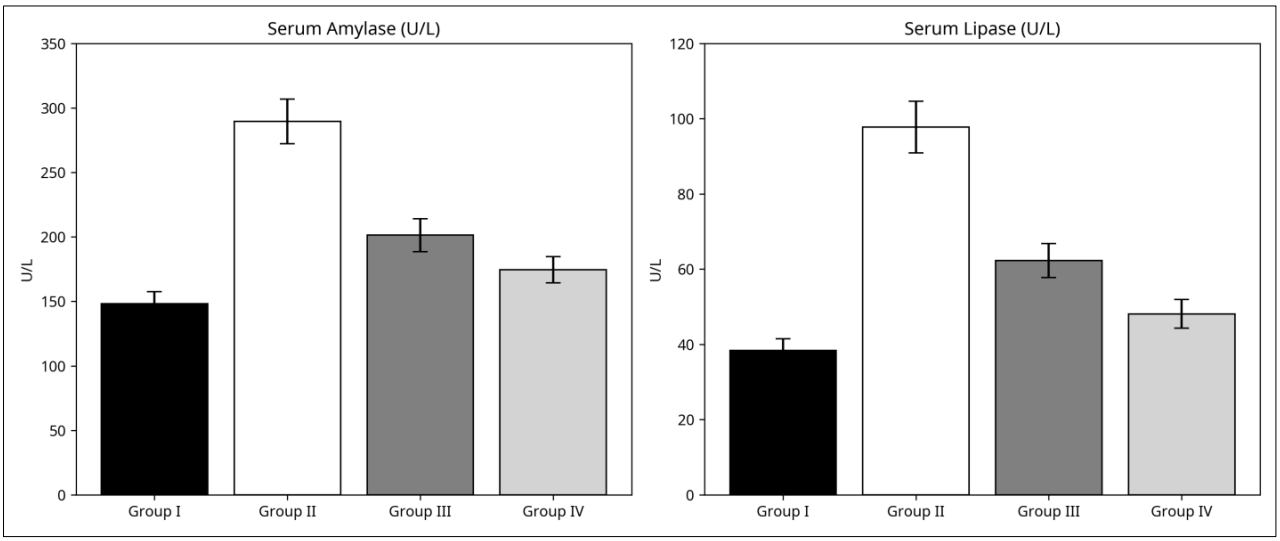

Figure 2: Serum amylase (U/L) and lipase (U/L) activities in experimental groups at Week 8. Error bars indicate ± SD. ***p < 0.001 vs. Group I; †††p < 0.001 vs. Group II.

3.5 Histopathological Findings

Pancreatic histopathology at week 8 indicated prominent structural distinction across experimental groups (Fig. 3â€“6). Group 1 (healthy control): Normal islet architecture with a high density of well-granulated beta-cells surrounded by intact acinar tissue; no inflammatory infiltration. In Group II (diabetic control), islets were largely necrotic with extensive beta-cell vacuolation and pyknosis, widespread periislet mononuclear inflammatory cell infiltration, and diffuse acinar atrophy with interstitial fibrosis. Islet damage score in group II was 2.8 ± 0.4, significantly higher than in all groups ($p < 0.001$).

Histological examination of islet specimens revealed partial restoration of islet architecture in Group III (CSEE therapeutic), moderate granularization of at least some beta cells, significant reductions in necrotic foci and attenuated inflammatory infiltration (mean score: 1.6 ± 0.3; $p<0.01$ vs. Group II). The preservation of pancreatic morphology was greatest at 4 months in Group IV (CSEE prophylactic) with islet morphology largely similar to control, and <10% cytoplasmic vacuolation only and rare peri-islet lymphocytic clusters (mean score: 0.9 ± 0.2; $p < 0.001$ vs Group II). The integrity of acinar tissue was also preserved in the CSEE-treated animals compared to the diabetic control, and recovery of glycogen-positive beta-cells in Groups III and IV was suggests by PAS staining.

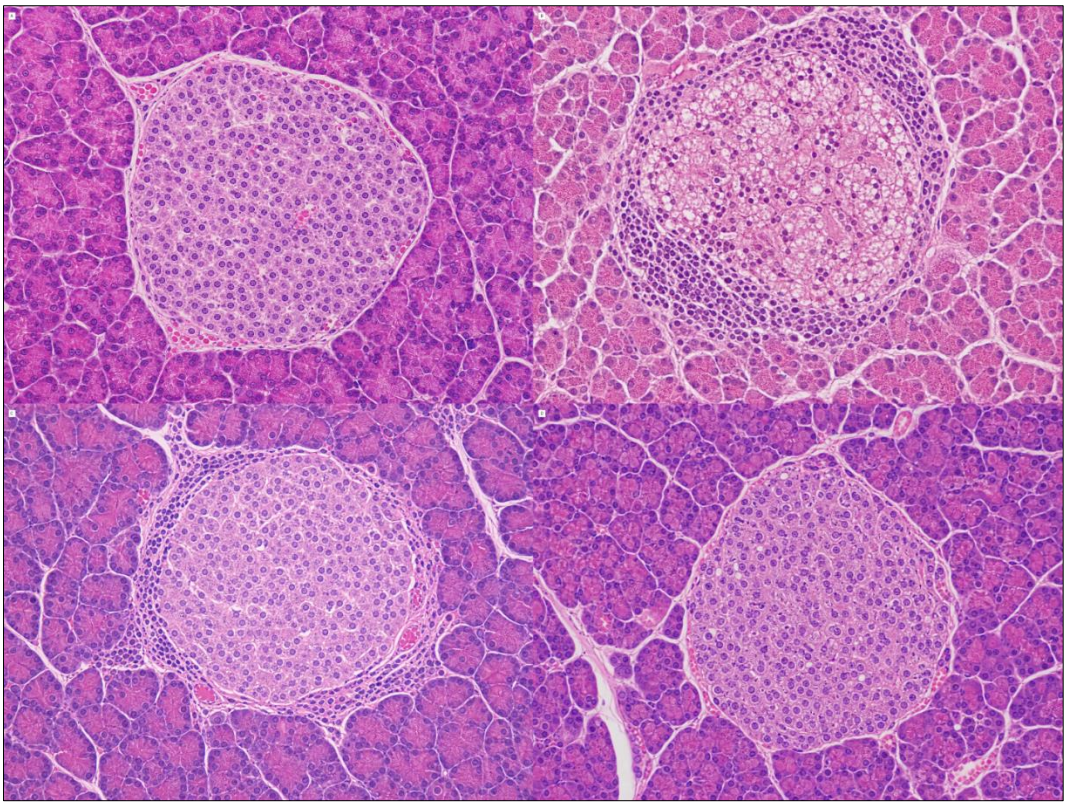

Figure 3*:* Histopathological Examination of Pancreatic (H&E stain, ×200): (A) Group I (Healthy Control): Normal islet of Langerhans with well-granulated beta-cells and intact surrounding acinar tissue. (B) Group II (Diabetic Control): Severe islet necrosis, extensive beta-cell vacuolation, and peri-islet mononuclear inflammatory infiltration. (C) Group III (CSEE Therapeutic): Partial restoration of islet architecture with moderate granularity of beta-cells and attenuated inflammation. (D) Group IV (CSEE Prophylactic): Near-normal islet morphology with well-preserved beta-cell granularity and minimal vacuolation

Table 4: Semi-quantitative histopathological islet damage scores at Week 8

Group	Designation	Islet Necrosis	Inflammation	Damage Score (0–3)
I	Healthy Control	None	Absent	0.1 ± 0.1
II	Diabetic Control	Severe	Severe	2.8 ± 0.4a
III	CSEE Therapeutic	Moderate	Mild–Moderate	1.6 ± 0.3b
IV	CSEE Prophylactic	Minimal	Mild	0.9 ± 0.2b

Values are median ± IQR (n = 10). a = p < 0.001 vs. Group I (Kruskal–Wallis, Dunn's post-hoc); b = p < 0.05 vs. Group II.

4. DISCUSSION

For the first time in a rabbit model, the present study shows that chia seed ethanolic extract (CSEE) has significant protective activity as well as antioxidant and pancreatic enzyme-modulating effects against STZ-induced diabetes. In conclusion all our data strongly support the hypothesis that although chia seeds are rich in polyphenols and omega-3 fatty-acids, thus likely providing a great source of functional foods capability to ameliorate disease states, they can significantly mitigate the oxidative cascade involved in beta-cell destruction during experimental diabetes.

STZ-induced diabetes provides a reliable model for sustained hyperglycaemia (FBG > 500 mg/dL in Group II at Week 8) due to the well-characterised mechanism of STZ [4, 5], selective alkylation of beta-cell DNA results in PARP-mediated NAD^+ depletion and subsequent cell death. Simultaneous fall in serum insulin (from 18.73 to 2.14 µIU/mL) forms evidence for obliterative functional loss of any residual beta-cell mass in etiology of otherwise day-old glycometabolic disease in untreated diabetic animals. These results are consistent with the work of Szkudelski [4], and Lenzen [5], on the pathobiology of STZ-induced diabetes.

CSEE treatment groups (groups III and IV) restored serum insulin activity significantly, GISE, which may account for the reduction of FBG. Quercetin, which is the most abundant flavonoid in chia [17], has shown to enhance Ca2+-dependent insulin release from isolated rat pancreatic islets as reported by Hii and Howell [17], and Eid *et al*., [18] — thereby promoting activation of the phosphoinositide-3-kinase (PI3K)/Akt pathway to facilitate insulin signalling. The alpha-

linolenic acid constituent of CSEE could also have an additive role in ameliorating insulin sensitization since omega-3 fatty acids have been reported to favor peripheral insulin sensitivity by modulating peroxisome proliferator-activated receptor gamma (PPAR-γ) activity [26].

We are reasonable to observe essentially increased levels of MDA in the diabetic control group (9.87 ± 0.63 μmol/L vs. 2.31 ± 0.18 μmol/L in healthy controls), confirming that increased oxidative stress is a widely accepted role of STZ-induced diabetes [21, 22]. Lipid peroxidation cascades are initiated by MDA accumulation, a sensitive marker of lipid damage that directly correlates with STZ-induced free radical production [5-7]. Methyl nitrosourea (MTNU) Also activates nitric oxide synthase and impaired mitochondrial electron trransport chain leads to O2and H2O2 generation as NO-inducers such as sodium butyrate yield no apoptogenic effect unless challenged with a delivery agent; STZ generates superoxide radicals and hydrogen peroxide via the activation of nitric oxygen synthesis and impairment of mitocondrual electron transport chains. The concomitant drop of T-AOC (0.51 vs. 1.84 mmol/L) mirrors exhaustion of antioxidant stores, such as glutathione, ascorbate and tocopherol pools [23], confirming the pro-oxidant environment of untreated experimental diabetes models.

This antioxidant–oxidant imbalance was markedly alleviated by CSEE (42.9% and 60.3% decreases in MDA (Groups III and IV, respectively), whereas T-AOC restored 65.8% and 84.8% of control values). ConclusionsThese findings can be ascribed to the multi-modal radical-scavenging properties of polyphenols in chia. Chlorogenic acid and caffeic acid operate direct hydrogen atom transfer (HAT) and single-electron transfer (SET) radical quenching mechanisms [12-25], whereas kaempferol and quercetin chelate catalytically active iron and copper ions that facilitate Fenton chemistry [18-22]. Moreover, omega-3 fatty acid could also attenuate pro-oxidant eicosanoids derived from arachidonic acid and the oxidative enzyme activity [26]. Collectively, these mechanisms underlie the significant recovery of T-AOC and reduction in lipid peroxidation that has been shown in our findings.

Elevated serum amylase and lipase levels in the STZ diabetic group (289.7 U/L and 97.8 U/L, respectively) are indicative of exocrine pancreatic cell injury due to prolonged exposure to chronic hyperglycaemia, oxidative stress and inflammatory infiltration [19]. In both clinical and experimental settings, elevated circulating pancreatic enzymes during diabetic states have been reported to be due to a combination of alterations in membrane permeability, acinar cell swelling and direct cytopathic effects of ROS [8-13]. Decreased levels of amylase and lipase in CSEE-treated groups (to near-normal ranges in Group IV) which were statistically significant, confirm the extensive pancreato-protective potential of the extract analogous to our histopathological data.

Histopathological examination is an integral part of this study, offering morphological validation for the biochemical data. The characteristics of STZ-induced pancreatic injury such as marked islet necrosis, beta-cell vacuolation and inflammatory infiltration found in Group II (Fig.4(f), (g); compare with Fig.3(b)) are due to oxidative DNA damage, mitochondrial dysfunction and NF-κB-mediated expression of pro-inflammatory genes initiated by STZ itself [6], or by its metabolite streptozotocin-derived nitric oxide [4-14]. A statistically and biologically significant attenuation of beta-cell loss, as shown by the reduction in islet damage score from 2.8 (group II) to 1.6 and 0.9 in groups III and IV respectively, was evident early in the observation period (10 weeks). Importantly, the medicinal CSEE protocol resulted in the most well-preserved islet architecture indicating that pre-exposure to antioxidant defence network prior to STZ challenge effectively shields against acute oxidative stress.

Demirtas *et al.,* in an elegant study [19], showed that the potent antioxidant melatonin remarkably maintained islet morphology in STZ-induced diabetic rats. Our findings extend this paradigm to a food-derived polyphenolic extract, supporting the potential role of plant-based antioxidants as cytoprotective agents in experimental diabetology. Maritim *et al*., The earlier work of Ceriello and Testa [15], more specifically called for antioxidant-based intervention as adjuncts to conventional antidiabetic therapy, while the recent review by Ly *et al*., [21], provides a comprehensive analysis of the role of antioxidant status on betabeta-cell survival. The present study provides robust support of this rationale.

Compared with rodent models, the rabbit model utilized in this study possesses features physiologically similar to human diabetes, including both lipoprotein metabolism profiles and islet cell distribution patterns closer to those of Homo sapiens [13]. The fact that our translational consideration holds true from a clinical standpoint enhances the relevance of our observations. The dosage of CSEE (400 mg/kg/day) was chosen according to previous studies on the bioavailability of plant extracts in rabbits and after preliminary toxicity assessments, no adverse effects related to the administration of CSEE were recorded during the 8 week study.

A few limitations of the current study deserve to be highlighted. The lack of quantitative evaluation of the beta-cell mass (eg, insulin-positive area by morphometry) in immunohistochemistry does not allow for precise quantification of beta-cell regeneration. The molecular mechanisms of CSEE action, specifically concerning its impact on antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase)

as well as NF-κB signalling and apoptotic pathways, continue to be clarified. This might be the first report of binders action in the intestine and can serve future studies as a guide for modelling experimental designs that include dose–response characterisation, long-term follow-up and mechanistic molecular analyses, as well bioavailability studies of chia polyphenols in the rabbit model.

5. CONCLUSION

The present study showed that the significance of this work1301415 is in its finding, which proves CSEE ameliorated STZ-induced pancreatic beta-cell injury through balancing antioxidant–oxidant status as reflected by significant decrease in MDA and increase T-AOC in New Zealand White rabbits. Treatment with CSEE resulted in significant reductions in serum insulin and fasting blood glucose levels, normalisation of pancreatic enzyme markers (amylase and lipase), and preservation of islet architecture and acinar tissue morphology on histopathology. The prophylactic dosing protocol produced better results over those conducted therapeutically, indicating that enhancing antioxidant capacity prior to tissue injury is more effective than post-insult therapy in this model. These findings give very strong preclinical evidence supporting the potential of bioactives derived from chia seeds as natural add-on therapy in diabetes mellitus treatment and warrant deeper mechanistic and clinical studies.

Conflicts of Interest: The author declare no conflicts of interest.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas, 10th ed.; International Diabetes Federation: Brussels, Belgium, 2021.
2. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet. 2014;383(9922):1068–1083. doi:10.1016/S0140-6736(13)62154-6
3. Eizirik DL, Colli ML, Ortis F. The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. Nat Rev Endocrinol. 2009;5(4):219–226. doi:10.1038/nrendo.2009.21
4. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50(6):537–546. doi:10.33549/physiolres.930111
5. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216–226. doi:10.1007/s00125-007-0886-7
6. Elsner M, Gurgul-Convey E, Lenzen S. Relative importance of caspase-dependent and caspase-independent apoptosis in β cells. Diabetologia. 2012;55(5):1350–1357.
7. Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes. 1997;46(11):1733–1742. doi:10.2337/diabetes.46.11.1733
8. Robertson RP, Zhou H, Zhang T, Harmon JS. Chronic oxidative stress as a mechanism for glucose toxicity of the beta cell in type 2 diabetes. Cell Biochem Biophys. 2007;48(2–3):139–146. doi:10.1007/s12013-007-0026-5
9. Nieman DC, Cayrado CA, Austin MD, Benowitz DA, Shanely RA, Jensen RS. Chia seed does not promote weight loss or alter disease risk factors in overweight adults. Nutr Res. 2009;29(6):414–418. doi: 10.1016/j.nutres.2009.05.011
10. Coates W, Ayerza R. Chia seed (Salvia hispanica L.): a new source of omega-3 fatty acids and its biological activities. J Am Oil Chem Soc. 1996;73(1):23–25.
11. Martinez-Cruz O, Paredes-López O. Phytochemical profile and nutraceutical potential of chia seeds (Salvia hispanica L.) by ultra high performance liquid chromatography. J Chromatogr A. 2014; 1346:43–48. doi: 10.1016/j.chroma.2014.04.007
12. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5):270–278. doi:10.4161/oxim.2.5.9498
13. Rerup CC. Drugs producing diabetes through damage to the insulin secretory mechanism. Pharmacol Rev. 1970;22(4):485–518.
14. Bolzan AD, Bianchi MS. Genotoxicity of streptozotocin. Mutat Res. 2002;512(2–3):121–134. doi:10.1016/S1383-5742(02)00044-3
15. Ceriello A, Testa R. Antioxidant anti-inflammatory treatment in diabetes. Diabetes Care. 2009;32 Suppl 2:S232–S236. doi:10.2337/dc09-S316
16. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care. 2023;46(Suppl 1):S1–S291.
17. Hii CST, Howell SL. Effects of flavonoids on insulin secretion and Ca2+ handling in rat islets of Langerhans. J Endocrinol. 1985;107(1):1–8. doi:10.1677/joe.0.1070001
18. Eid HM, Nachar A, Thong F, Sweeney G, Haddad PS. The molecular basis of the antidiabetic action of quercetin. Pharmacogn Mag. 2015;11(41):74–81. doi:10.4103/0973-1296.149708
19. Demirtas B, Gonenc S, Akman S, Yegen BC, Senol A. Protective effect of melatonin on streptozotocin-induced pancreatic tissue damage. Pancreas. 2001;22(3):309–314.
20. Aggarwal BB, Kunnumakkara AB, Harikumar KB, Tharakan ST, Sung B, Anand P. Potential of spice-derived phytochemicals for cancer prevention. Planta Med. 2008;74(13):1560–1569. doi:10.1055/s-2008-1074578
21. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol. 2003;17(1):24–38. doi:10.1002/jbt.10058

22. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in human disease. Int J Biochem Cell Biol. 2007;39(1):44–84. doi: 10.1016/j.biocel.2006.07.001
23. Sies H. Glutathione and its role in cellular functions. Free Radic Biol Med. 1999;27(9–10):916–921. doi:10.1016/S0891-5849(99)00177-X
24. Marineli RDS, Lenquiste SA, Marques MC, Bogo D, Menezes EM, Lancha AH Jr. Chemical characterization and antioxidant potential of Chilean chia seeds. LWT–Food Sci Technol. 2015;62(1):370–377.
25. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. Am J Clin Nutr. 2005;81(1 Suppl):215S–217S. doi:10.1093/ajcn/81.1.215S
26. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio. Exp Biol Med. 2008;233(6):674–688. doi:10.3181/0711-MR-311.