JMALS

Journal of Medical and Life Science
https://jmals.journals.ekb.eg/

SPBH

Mitigation of fibrosis and related complications in STZ-induced rats by sericin treatment

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DOI: 10.21608/jmals.2025.410469

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, which can lead to various complications, including damage to the pancreas and kidney. This study investigates the efficacy of sericin (Ser) treatment in mitigating fibrosis and related complications in streptozotocin (STZ)-induced diabetic rats. Diabetes induction led to increased serum glucose, HbA1c, oxidative stress markers (H_2O_2, AGEs), and pro-inflammatory mediators (NF-κB, TNFα, INF-γ, TGF-β), along with reduced antioxidant defenses, including total antioxidant capacity (TAC) and pancreatic glutathione reductase (GR) activity. Digestive enzyme activities (amylase and lipase) were diminished, and kidney dysfunction was evident, marked by elevated urea, creatinine, uric acid, and potassium levels, and decreased sodium concentrations. Histological analysis revealed significant collagen deposition in pancreatic tissue, indicative of fibrosis.

Ser treatment demonstrated protective effects by lowering serum glucose and HbA1c levels, restoring insulin and C-peptide concentrations, and enhancing antioxidant defenses. It reduced oxidative stress markers, pro-inflammatory mediators, and fibrosis in serum and pancreatic tissues. Additionally, digestive enzyme activities were restored, and kidney function improved, with normalization of urea, creatinine, uric acid, potassium, and sodium levels. Histological analysis confirmed reduced fibrosis and collagen deposition in the pancreatic tissue of Ser-treated rats.

These findings highlight sericin's potential to mitigate fibrosis and its associated complications in STZ-induced diabetic rats, offering promising therapeutic insights for managing diabetes-related organ damage.

Keywords: Diabetes, oxidative stress, diabetic nephropathy, inflammation, fibrosis, Sericin.

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1. INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, poses a significant global health challenge (1). Hyperglycemia-induced oxidative stress plays a pivotal role in the pathogenesis of diabetic complications, leading to cellular damage, inflammation, and fibrosis (2). The disease is associated with a complex array of complications that can affect various organs and systems, including the pancreas and kidneys (3). As both the pancreas and kidneys play vital roles in maintaining metabolic homeostasis, their dysfunction in diabetes creates a complex interplay of systemic complications.

The pancreas, a vital organ responsible for insulin production and digestive enzyme secretion, is particularly vulnerable to the damaging effects of oxidative stress (1). Hyperglycemia can impair pancreatic β-cell function, leading to insulin deficiency and further exacerbating hyperglycemia (4). Additionally, oxidative stress can contribute to

Received: November 1, 2024. Accepted: January 20, 2025. Published: February 4, 2025

pancreatic β-cell apoptosis, further compromising insulin production (5).

The kidneys are another organ that is susceptible to the detrimental effects of diabetes. Diabetic nephropathy, a major complication of diabetes, is characterized by progressive kidney damage and dysfunction (3). Oxidative stress, inflammation, and advanced glycation end products (AGEs) contribute to the development and progression of diabetic nephropathy, leading to glomerular damage, fibrosis, and renal failure (6).

Current therapeutic approaches for diabetes primarily focus on managing hyperglycemia but often lack the ability to address the underlying cellular damage. Natural products with antioxidant and cytoprotective properties have emerged as potential therapeutic candidates for diabetic complications (7).

Sericin (Ser), a protein component of silk fibroin, has also gained interest for its potential health benefits. Studies report sericin possesses antioxidant, anti-inflammatory, and hepatoprotective properties (8, 9). However, the effects of sericin on pancreatic function in diabetes remain largely unexplored.

The current research sought to examine the influence of diabetes on the function of the pancreas and kidney and explore the possible therapeutic advantages of Ser, a natural compound sourced from silk cocoons. It was proposed that Ser might counteract the harmful effects of diabetes by mitigating cellular oxidation, immune response, and fibrosis.

2. MATERIALS AND METHODS

2.1. Chemicals

Streptozotocin (STZ) and Sericin (Ser), key components of the study, were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used throughout the study were of analytical grade, ensuring the highest level of purity and suitability for experimental use.

2.2. Animals and Housing

The study involved adult male Wistar albino rats, each weighing between 150-170 g. These rats were housed in typical laboratory conditions at the Experimental Animal Center of the Medical Research Institute, Alexandria University, Egypt. They were placed in standard cages and maintained on a 12-hour light-dark cycle with a controlled temperature of 22 ± 2 °C. The rats had unrestricted access to standard rodent feed and water.

Ethical approval:

The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Alexandria University, Egypt, under approval number AU04 21 9 23 2 02.

2.3. Experimental Design

After a two-week acclimatization period, a total of 18 adult male Wistar albino rats were selected for the study and divided into two main groups based on the experimental design. The first group was a control group consisting of 6 rats, which were administered daily oral doses of distilled water for duration of 28 days. This group served as the baseline for comparison with the diabetic rats. The second group, the diabetic group, included 12 rats that underwent a two-step process to induce diabetes.

In the first phase, these rats were provided with a 10% fructose solution in their drinking water for two weeks. The purpose of this step was to induce insulin resistance, as fructose has been shown to contribute to metabolic disturbances that mimic the early stages of type 2 diabetes. After this initial two-week period, the diabetic rats were given a single intraperitoneal injection of streptozotocin (STZ) at a dose of 40 mg/kg body weight. STZ is a well-established chemical agent used to induce experimental diabetes by selectively damaging the insulin-producing β-cells in the pancreas, leading to hyperglycemia. This method of diabetes induction follows previously described protocols (10).

Three days after the STZ injection, the rats were monitored for fasting blood glucose levels. Rats that exhibited fasting blood glucose concentrations exceeding 220 mg/dL were classified as diabetic. These rats, now confirmed to have diabetes, were randomly assigned to two subgroups of 6 rats each. One subgroup continued to receive no additional treatment. It served as the Diabetic group, while the other subgroup, the Diabetic + Ser group, was treated with sericin (Ser) at a dose of 250 mg/kg per day, as described by Pachhiappan et al. (11). Ser was administered once daily. The treatment period lasted for 4 weeks. During this time, all rats were monitored for their health status, and blood glucose levels were regularly measured to assess the effectiveness of the intervention.

2.4. Collection of blood and tissue preparation

At the end of the study, blood samples were collected from fasting rats via the jugular veins under anesthesia induced by a ketamine (100 mg/kg) and xylazine (5 mg/kg) mixture. The blood was allowed to clot, centrifuged at 3000 rpm for 15 minutes, and the serum was divided into three Eppendorf tubes. The rats were then humanely euthanized by cervical dislocation, and their pancreas tissues were promptly extracted, rinsed in ice-cold saline, minced, and homogenized in PBS buffer (pH 7.4) using a Dounce glass homogenizer. The homogenates were centrifuged at 2,000 rpm for 10 minutes, and the supernatant was collected and stored at -20°C for further biochemical analysis. Some pancreas tissue portions were preserved in 10% formalin for histological examination.

3.5. Biochemical parameters

The serum glucose level was determined using a commercially available diagnostic kit that utilizes a colorimetric assay (Cat.no.GL1320; Biodiagnostic, Egypt). This method follows the protocol outlined by Trinder, (12). In this colorimetric assay, glucose in the serum reacts with specific reagents to produce a colored product. The color intensity, which is proportional to glucose concentration, is measured using a spectrophotometer. The glucose level is then calculated from the absorbance, with results expressed in mg/dL.

Insulin levels were measured using Sandwich ELISA kits (Cat.no. ER1113; FineTest, China) following the manufacturer's instructions. The assay involves coating wells with an antibody specific to insulin, adding serum containing insulin, and allowing it to bind to the antibody. An enzyme-linked antibody is then introduced, followed by a streptavidin-HRP complex that binds to the enzyme-linked antibody. The color produced by the HRP enzyme is proportional to the insulin concentration in the sample.

Glycosylated hemoglobin (HbA1C) was assessed according to the manufacturer’s instructions for the rat turbidimetric immunoassay kit (Cat.no. REF 3155005; LINEAR CHEMICALS, SPAIN). This assay was performed on the autoanalyzer (Mannheim, Germany).

C-Peptide was assessed using an ELISA Kit (Cat.no. EA0006Ra; BT LAB, China). The activity of amylase was measured using a kit (Cat.no. ECAM-100; BioAssay Systems, USA). The assay utilizes a colorimetric method based on the enzymatic hydrolysis of an amylase-specific substrate, resulting in the release of products that react with a color reagent. The activity of Lipase was measured using a colorimetric enzymatic method kit (Cat.no. LS-K298-100, LifeSpan Bioscience, USA).

The serum urea level was determined using a spectrophotometric method based on the diacetyl monoxime reaction, as described by Natelson et al. (13). The serum creatinine level was measured using the Jaffe Reaction via a spectrophotometric method (Cat.no. CR 12 50; biodiagnostic, Egypt). The serum uric acid level was determined using a spectrophotometric method (Cat.no. UA 21 20; biodiagnostic, Egypt), as described by Caraway, (14). The method utilizes the reducing property of

uric acid in the presence of alkaline phosphotungstic acid.

The serum Blood Urea Nitrogen (BUN) level was determined using an enzymatic colorimetric kit (Cat.no. E-BC-K183-S; Elabscience, USA). The serum potassium (K) and serum sodium (Na) levels were measured using commercially available kits from Biodiagnostic, Egypt (Cat.no. PT 18 20 and SO 19 10, respectively).

The levels of hydrogen peroxide (H_2O_2) were quantified using a specific assay kit from Elabscience, USA (Cat.no. E-BC-K102-M). The total antioxidant capacity (TAC) of the samples was assessed using a commercial kit from Biodiagnostic, Egypt (Cat.no. TA 25 13). Advanced Glycation End-products (AGE) were conducted using a specific ELISA kit (Cat.no. ER0268) from Fine Test, China. The activity of glutathione reductase (GR) was measured using an Assay Kit (Cat.no. ab83461) from Abcam, USA.

The levels of various key inflammatory, immune, and cellular markers were quantified using specific ELISA kits, adhering to the manufacturers' instructions for precise and reliable measurements. Nuclear Factor Kappa B (NF-κB) was estimated using the kit (Cat.no. E1817Ra) from BT LAB, China. Tumor Necrosis Factor-Alpha (TNF-α) was measured with the kit (Cat.no. E0764Ra) from BT LAB, China. Interferon Gamma (IFN-γ) was quantified using the kit (Cat.no. ER0012) from FineTest, USA. Transforming Growth Factor Beta (TGF-β) was estimated using the kit (Cat.no. ab119558) from Abcam, USA. Interleukin-6 (IL-6) was assessed with the kit (Cat.no. E0135Ra) from BT LAB, China. Caspase-3 was measured using the kit (Cat.no. E-EL-R0160) from Elabscience, USA, providing insights into cellular apoptosis mechanisms. Vascular Endothelial Growth Factor (VEGF) was estimated using the kit (Cat.no. CSB-E04757r) from CUSABIO, USA.

2.6. Masson's Trichrome Staining

Masson's Trichrome Staining was performed to evaluate the histological structure of the pancreas tissues. Initially, the tissues were fixed in 10% phosphate-buffered formalin to preserve the cellular and extracellular components. Following fixation, the tissues underwent processing using an automated tissue processor (Leica, Germany), ensuring optimal dehydration, clearing, and infiltration with paraffin. The processed tissues were then embedded in paraffin blocks and sectioned into 5-µm-thick slices using a microtome. These sections were subsequently stained with Masson's trichrome at room temperature according to the manufacturer's instructions. This staining technique highlights collagen fibers in blue, cytoplasm and muscle fibers in red, and nuclei in black, facilitating the evaluation of fibrosis and tissue architecture. Stained sections were examined under a light microscope (Olympus Corporation, Tokyo, Japan), and high-quality images were captured for analysis. The results were interpreted to assess pathological changes, including fibrosis and alterations in pancreatic structure, providing valuable insights into the experimental conditions.

2.7. Statistical Analysis

Statistical analysis was performed to evaluate the data, with all values expressed as mean ± Standard Error (SE). The significance of differences between groups was assessed using a one-way analysis of variance (ANOVA), conducted with SPSS software. To identify specific differences between groups, the post hoc test Least Significant Difference (LSD) was applied, allowing pairwise comparisons among the groups. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant, indicating meaningful differences in the measured parameters between the experimental groups. This approach ensures a rigorous and reliable interpretation of the data.

3. RESULTS

3.1. Impact of Ser on Glucose Metabolism, Insulin Secretion, and Digestive Enzyme Function

As illustrated in Table 1, a statistically marked ($P < 0.05$) increase in serum glucose and blood HbA1c levels was observed in diabetic rats when compared to the control group. These findings are consistent with the characteristic hyperglycemia and impaired glucose metabolism observed in diabetic conditions. Conversely, there were notable ($P<0.05$) reductions in serum insulin and C-peptide levels in the diabetic group, which reflect the dysfunction in insulin secretion and pancreatic β-cell activity commonly associated with diabetes. Additionally, the activities of key digestive enzymes, namely amylase, and lipase, were considerably ($P < 0.05$) decreased in the diabetic rats relative to the control group. This suggests that diabetes may impair enzyme function, potentially affecting the digestion and absorption of nutrients. Notably, treatment with Ser resulted in a marked reduction in serum glucose and HbA1c levels, which suggests enhanced glycemic control and improved long-term glucose regulation. Moreover, the supplementation with Ser effectively restored the reduced levels of insulin and C-peptide, indicating potential benefits for pancreatic function and insulin secretion. Furthermore, the activities of amylase and lipase were notably elevated following Ser treatment, suggesting an improvement in digestive enzyme function.

3.2. Impact of Ser on Renal Function Markers and Electrolyte Balance

The assessment of kidney function revealed significant ($p < 0.05$) increases in serum levels of urea, creatinine, uric acid, BUN, and K, along with a marked decrease ($p < 0.05$) in sodium (Na) levels in the diabetic group compared to the control group. These alterations suggest impaired renal function associated with diabetes. Supplementation with Ser in the diabetic group resulted in marked ($p < 0.05$) improvements in these parameters when compared to the diabetic group alone (Table 2). Specifically, treatment with Ser resulted in a reduction of the elevated urea, creatinine, uric acid, BUN, and K levels, as well as a restoration of sodium levels, compared to the diabetic group without Ser supplementation. These findings indicate that Ser supplementation may offer protective effects on kidney function in diabetic rats, potentially mitigating some of the renal disturbances observed in diabetes.

3.3. Impact of Ser on of Serum Oxidative Stress Markers and AGE

The presented data in Table 3 demonstrate that the diabetic induction triggered marked ($P<0.05$) elevation in serum H_2O_2 and AGE, indicating a notable elevation in oxidative stress and the accumulation of oxidative byproducts. In parallel, a marked ($P < 0.05$) reduction in serum TAC was observed in the diabetic group, suggesting a compromised antioxidant defense system. These alterations reflect the heightened oxidative stress and its associated metabolic disturbances in diabetic rats. Alternatively, the supplementation of Ser in the diabetic group resulted in a substantial ($P<0.05$) amelioration in serum oxidative stress markers and AGE compared to the diabetic group. This suggests that Ser supplementation effectively mitigates oxidative stress and the formation of AGEs, potentially enhancing the antioxidant defense mechanisms and reducing the harmful effects of oxidative damage in diabetic rats.

3.4. Impact of Ser on Pancreatic Oxidative Stress Markers

The data presented in Table 4 indicate that the induction of diabetes in rats led to a significant ($P < 0.05$) increase in pancreatic H_2O_2 levels, reflecting a marked rise in oxidative stress within the pancreas. In addition, there was a significant ($P < 0.05$) decrease in TAC level and GR activity in the pancreas of diabetic rats, indicating a reduced ability to combat oxidative damage. However, the administration of Ser led to a marked reduction in pancreatic H_2O_2 levels, suggesting a decrease in oxidative stress within the pancreas. Moreover, Ser supplementation caused a significant ($P < 0.05$) increase in TAC levels, reflecting an enhancement of the pancreatic antioxidant defense mechanisms. The

activity of GR was also notably restored in the Diabetic+Ser group, indicating that Ser may help to rejuvenate the enzyme's function and contribute to a more robust antioxidant response.

3.5. Impact of Ser on Serum Inflammatory Markers

As illustrated in Table 5, the induction of diabetes resulted in significant ($P < 0.05$) increases in serum levels of NF-κB, TNFα, INF-γ, and TGF β, compared to the control group. These elevated pro-inflammatory markers suggest the activation of inflammatory pathways, which are commonly associated with the pathophysiology of diabetes and its complications. In contrast, the supplementation of Ser in the diabetic group (Diabetic+Ser) resulted in a significant ($P < 0.05$) reduction in the serum levels of NF-κB, TNFα, INF-γ, and TGF β. This suggests that Ser supplementation has a potent anti-inflammatory effect, attenuating the inflammatory response typically observed in diabetes.

3.6. Impact of Ser on Pancreatic Inflammation and Fibrosis

As presented in Table 6, the induction of diabetes led to significant ($P < 0.05$) elevations in several key biomarkers in the pancreas, including NF-κB, IL-6, caspase-3, TGF β, and VEGF levels compared to the control group. These elevated markers are indicative of heightened inflammation, cellular stress, and tissue remodeling within the pancreas. In contrast, supplementation with Ser in the diabetic group (Diabetic+Ser) resulted in significant ($P < 0.05$) reductions in the pancreatic levels of NF-κB, IL-6, caspase-3, TGF-β, and VEGF when compared to the diabetic group without treatment. These reductions indicate that Ser supplementation effectively attenuates the inflammatory and apoptotic processes within the pancreas and prevents excessive fibrosis and angiogenesis preserving pancreatic health and function.

3.6. Impact of Ser on Pancreatic Collagen Fiber Deposition

Collagen fiber was examined using Masson's trichrome staining to detect the effects of Ser on pancreas fibrosis and changes in collagen fiber in the control and experimental groups. Furthermore, this staining established that collagen fiber (blue staining) was significantly high in diabetic rats. Ser treatments reduced the collagen deposition in the pancreatic tissue of diabetic rats when compared to the STZ-induced diabetes group (Figure 1).

Table (1): Impact of Ser on Glucose Metabolism, Insulin Secretion, and Digestive Enzyme Function

Parameters	Experimental groups		
	Control	Diabetic	Diabetic+ Ser
Glucose (mg/dL)	93.5±1.79	584.9±8.1[a]	196.5±6.5 [ab]
Insulin (Pg/mL)	309.0±9.4	120.0±7.8[a]	215.2±11.7[ab]
HbA1c (%)	4.4±0.24	10.1±0.23[a]	6.7±0.27[ab]
Amylase (U/L)	343.16±11.6	103.7±4.87[a]	227.3±9.33[ab]
Lipase (U/L)	254.8±9.01	104.7±2.85[a]	123.17±2.2 [ab]
C-peptide (ng/mL)	14.8±0.31	5.5±0.40[a]	8.5±0.26[ab]

The values are expressed as mean ± SE. n = 6
a The mean values are significantly different in comparison with the control group (P≤0.05).
b The mean values are significantly different in comparison with the diabetic group (P≤0.05).

Table (2): Impact of Ser on Renal Function Markers and Electrolyte Balance

Parameters	Experimental groups		
	Control	Diabetic	Diabetic + Ser
Urea (mg/dl)	22.45±0.85	53.7±1.52[a]	39.2±2.23 [ab]
Creatinine (mg/dl)	0.43±0.031	1.33±0.053[a]	0.94±0.035[ab]
Uric acid (mg/dl)	4.02±0.12	6.6±0.21[a]	5.6±0.17[ab]
BUN (mmol/L)	8.42±0.38	24.33±0.56[a]	17.6±0.56 [ab]
K (mmol/L)	4.4±0.203	7.68±0.28[a]	6.1±0.11[ab]
Na (mmol/L)	134.2±2.02	104.9±0.97[a]	116.6±2.9[ab]

The values are expressed as mean ± SE. n = 6
a The mean values are significantly different in comparison with the control group (P≤0.05).
b The mean values are significantly different in comparison with the diabetic group (P≤0.05).

Table (3): Impact of Ser on Serum Oxidative Stress Markers and AGE

Parameters	Experimental groups		
	Control	Diabetic	Diabetic + Ser
H_2O_2 (mmol/L)	7.7±0.24	31.2±1.3[a]	20.6±0.85[ab]
TAC (mM / L)	2.9±0.26	0.76±0.033[a]	1.26±0.031[ab]
AGE (ng/mL)	3.2±0.42	15.4±0.37[a]	8.8±0.53[ab]

The values are expressed as mean ± SE. n = 6
a The mean values are significantly different in comparison with the control group (P≤0.05).
b The mean values are significantly different in comparison with the diabetic group (P≤0.05).

Table (4): Impact of Ser on Pancreatic Oxidative Stress Markers

Parameters	Experimental groups		
	Control	Diabetic	Diabetic + Ser
H_2O_2 (mmol / g. tissue)	12.02±0.57	65.5±1.75[a]	41.67±1.41[ab]
GR (mU/ g. tissue)	33.72±1.03	15.35±0.55[a]	20.03±0.54[ab]
TAC (mM / g. tissue)	2.29±0.069	0.85±0.047[a]	1.29±0.040[ab]

The values are expressed as mean ± SE. n = 6
a The mean values are significantly different in comparison with the control group (P≤0.05).
b The mean values are significantly different in comparison with the diabetic group (P≤0.05).

Table (5): Impact of Ser on Inflammatory Markers

Parameters	Experimental groups		
	Control	Diabetic	Diabetic + Ser
NFKB (ng/L)	3.7±0.14	16.03±0.69[a]	11.1±0.35[ab]
TNFα (ng/mL)	34.5±0.66	78.2±1.47[a]	58.3±1.12[ab]
INF- γ (pg/ml)	29.8±0.73	85.2±1.85[a]	63.4±1.8 [ab]
TGF β (pg/ml)	34.5±0.68	68.1±1.63[a]	54.6±1.43[ab]

The values are expressed as mean ± SE. n = 6
a The mean values are significantly different in comparison with the control group (P≤0.05).
b The mean values are significantly different in comparison with the diabetic group (P≤0.05).

Table (6): Impact of Ser on Pancreatic Inflammation and Fibrosis

Parameters	Experimental groups		
	Control	Diabetic	Diabetic + Ser
NFKB (ng/g.tissue)	3.5 ± 0.21	14.1 ± 0.34[a]	9.6 ± 0.41 [ab]
IL-6 (ng/g.tissue)	18.0 ± 0.51	59.5 ± 1.75[a]	38.6 ± 1.33 [ab]
Caspase3 (ng/g.tissue)	2.8 ± 0.17	10.0 ± 0.38[a]	6.8 ± 0.28 [ab]
TGF β (pg/g.tissue)	47.8 ± 0.67	91.9 ± 1.5[a]	73.8 ± 2.1 [ab]
VEGF (pg/g.tissue)	68.5 ± 2.4	148.2 ± 2.8 [a]	122.0 ± 2.4 [ab]

The values are expressed as mean ± SE. n = 6
a The mean values are significantly different in comparison with the control group (P≤0.05).
b The mean values are significantly different in comparison with the diabetic group (P≤0.05).

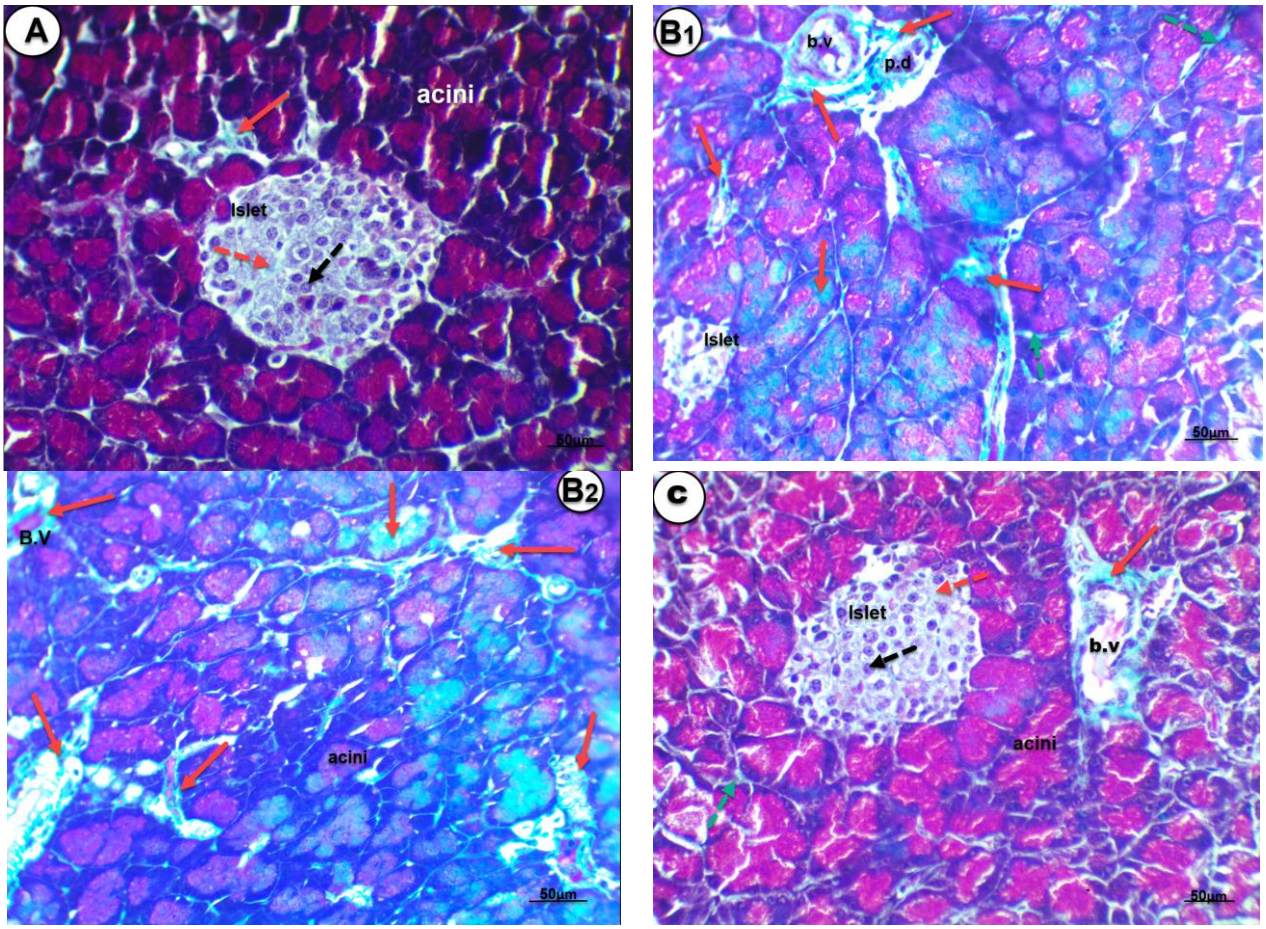

Figure 1. Photomicrographs of Masson trichrome-stained pancreatic sections. A. Section from the control group exhibiting minimal collagen fibers within the islets (red dotted arrow) and a few pancreatic ducts (red arrow). B1 & B2. Sections from the pancreas of the STZ-treated group demonstrated an increase in collagen fibers within the islets (red dotted arrow), surrounding certain pancreatic ducts (p.d.), blood vessels (b.v.), within acini (red arrow), and between pancreatic acini (green dotted arrow). C. Section from the pancreas of the STZ + Ser-treated group presenting a reduction in collagen fibers versus the STZ group. (X 400)

4. DISCUSSION

Diabetes, a metabolic disorder characterized by high blood sugar levels, is often associated with increased oxidative stress (1). This oxidative stress can damage cells, including those in the pancreas responsible for insulin production, and contribute to insulin resistance (5). Similarly, the kidneys are also highly susceptible to the damaging effects of diabetes, often resulting in diabetic nephropathy, a leading cause of end-stage renal disease (3). As both the pancreas and kidneys are crucial for maintaining metabolic homeostasis, the interplay of these complications in diabetes underscores the importance of early intervention and targeted therapies to manage and mitigate the long-term effects of the disease.

Our results indicate that diabetic rats revealed atypical serum glucose, insulin, and C-peptide levels, which are consistent with the well-established pathophysiology of diabetes mellitus. These findings underscore the key metabolic disturbances that occur in diabetic conditions, particularly concerning insulin secretion, glucose homeostasis, and pancreatic function. In the current study, the elevated serum glucose levels observed in diabetic rats are

consistent with the findings of numerous studies showing that uncontrolled diabetes leads to sustained hyperglycemia due to either a lack of insulin action or inadequate insulin secretion (15,16). This is often accompanied by other metabolic disturbances that further exacerbate the hyperglycemic state. The significant reduction in serum insulin and C-peptide levels in diabetic rats is indicative of a dysfunction in pancreatic β-cell activity, which is a hallmark of diabetes (17).

Our findings reveal that treatment with Ser noticeably amended the altered levels of serum glucose, insulin, and C-peptide levels in diabetic rats compared to the untreated diabetic group. Ser has been shown to reduce blood sugar levels in diabetic rats and may enhance insulin sensitivity (18). This was attributed to Ser's ability to enhance glucose uptake by tissues and increase insulin receptor sensitivity (19). Ser may also directly protect pancreatic β-cells from damage and improve insulin production (19). Ser has been found to increase C-peptide levels in diabetic rats. This is remarkable because C-peptide is a marker of insulin secretion and can provide insights into the functional state of beta cells (20).

Our study revealed that diabetic rats exhibited considerably higher levels of blood HbA1c compared to control rats. Consistent with the previous report, the hyperglycemic environment in diabetes leads to increased glycosylation of hemoglobin, resulting in higher HbA1c levels (21). This elevated HbA1c indicates poor glycemic control and is associated with a higher risk of diabetes-related complications. HbA1c is not only a marker of hyperglycemia but also exhibits an increased affinity for oxygen, leading to reduced oxygen delivery to tissues. This decreased oxygen supply can contribute to generalized hypoxia and metabolic dysfunction in peripheral tissues (22).

However, the treatment of diabetic rats with Ser reduced blood HbA1c levels compared to the untreated diabetic group. The study by Zhao et al. (23) provides compelling evidence that *Ser* derived from the *Bombyx mori* cocoon could be an effective therapeutic agent in diabetes management. The researchers observed a significant reduction in HbA1c in diabetic mice treated with Ser, suggesting that it may play a role in improving long-term glycemic control. While the precise mechanism of action of Ser was not fully elucidated in the study, it is hypothesized that its effects could be due to its potential to enhance insulin sensitivity, promote pancreatic β-cell function, or reduce oxidative stress, all of which contribute to better glucose metabolism.

The current study showed that diabetes is associated with defects in the activities of amylase and lipase enzymes compared to the control group. These data are similar to Aughsteen & Mohammed, (24) who observed a decline in the activities of these enzymes in diabetic rats. The observed decrease in the activities of amylase and lipase enzymes in untreated diabetic rats may be attributed to pancreatic exocrine insufficiency resulting from various mechanisms, including insulin deficiency, autoimmune processes, and direct damage to pancreatic cells (25). Research suggests that improved glycemic control can partially restore pancreatic exocrine function in diabetic patients, highlighting the importance of managing blood sugar levels to mitigate the negative effects of diabetes on pancreatic health.

The treatment of diabetic rats with Ser ameliorated the diabetic disturbance in the activities of amylase and lipase enzymes versus the untreated diabetic group. A study by Dong et al. (26) explored the potential therapeutic effects of Ser peptides on pancreatic exocrine function in the context of diabetes. The authors suggested that the beneficial effects of Ser peptides on exocrine pancreatic function could be attributed, at least in part, to their antioxidant properties.

In the current study, the diabetic rats exhibited substantial elevations in serum urea, creatinine, uric

acid, BUN, and K levels, accompanied by a noticeable decrease in Na levels relative to control rats. Studies have consistently shown that diabetic rats exhibit elevated levels of renal markers, indicating kidney dysfunction. Lai et al. (27) found that diabetic rats had markedly higher levels of serum creatinine, urea, and uric acid compared to control rats. Elevated levels of these markers can indicate kidney damage or dysfunction resulting from chronic hyperglycemia and damage to the small blood vessels in the kidneys (28). The high urea concentration observed in diabetic rats from this study indicates an excessive amount of nitrogenated amino acids derived from catabolism and also was an important test to evaluate the functional capacity of the liver (29). Moreover, it is established that diabetes can disrupt electrolyte balance in rats. Arif et al. (30) revealed that diabetes increased the serum K level along with the decrease in Na level. Diabetic nephropathy is associated with diminished renal function and can result in dysregulation of electrolyte homeostasis. This is attributed to the damage inflicted upon the nephrons by chronic hyperglycemia (31).

The findings of the present study suggest that the treatment with Ser could improve the kidney function markers in our diabetic model. These findings are in line with other studies (32-34). It was reported that Ser treatment was associated with a decrease in BUN and uric acid levels compared to the diabetic control group, suggesting its potential to improve kidney function in diabetic rats (32). Ser's potential to regulate blood pressure and improve vascular function could indirectly impact Na and K levels (35). Diabetes often leads to electrolyte imbalances, and Ser's potential to improve overall kidney function may help maintain electrolyte balance (33, 34). Moreover, the promising effects of Ser on kidney function and electrolyte balance highlight the potential of this natural compound as part of a broader therapeutic strategy for managing diabetes and its complications.

The current diabetes model exhibited increased levels of serum H_2O_2 and AGE while showing decreased levels of serum TAC compared to the control group. This finding is consistent with well-established research indicating that oxidative stress plays a crucial role in the pathophysiology of diabetes and its complications, including kidney dysfunction (36). The increase in H_2O_2 levels reflects a heightened state of oxidative stress in diabetic animals, while the accumulation of AGEs is a hallmark of chronic hyperglycemia and a contributor to the pathogenesis of diabetic complications (37). Elevated oxidative stress and AGEs are particularly detrimental to organs such as the kidneys, where they can exacerbate the progression of diabetic nephropathy, a common complication in diabetes characterized by glomerular damage, fibrosis, and eventually renal failure (38). TAC is a comprehensive measure of the body's ability to neutralize free radicals, and a reduction in TAC indicates that the antioxidant systems in the body are less effective at counteracting oxidative damage (39).

The supplementation of Ser in diabetic rats resulted in a significant reduction in serum oxidative stress markers, such as H_2O_2 and AGE compared to the diabetic control group. This finding suggests that Ser supplementation may effectively mitigate oxidative stress and the formation of AGEs, both of which are central to the pathogenesis of diabetic complications, including diabetic nephropathy. Additionally, previous investigations demonstrated that Ser derived from both mulberry and non-mulberry sources effectively mitigated H_2O_2-induced oxidative stress, leading to reduced ROS generation which in turn suppresses the MDA activity levels and decreases oxidative lipid peroxidation (40).

Our findings indicate that the induction of diabetes led to a substantial increase in oxidative stress markers and a decrease in antioxidant markers in the pancreas of diabetic rats compared to control rats. Prior investigations into the correlation between

oxidative stress and DM, along with its associated complications, have hypothesized that the toxic effects of hyperglycemia could lead to the damage of pancreatic β-cells, which are highly susceptible to oxidative stress (40, 41). Additionally, the literature suggests that alterations in energy metabolism, as well as tissue damage resulting from metabolic stress, hypoxia, and ischemia-reperfusion injury, can contribute to the increased generation of free radicals and the impairment of the antioxidant system (42). The findings of the present study align with these reports. Consequently, it is posited that the restoration of the antioxidant system's efficacy holds particular implication in the management of diabetes.

Treatment with Ser considerably reduced oxidative stress markers in the pancreas of diabetic rats. Dong et al. (26) demonstrated oral administration of Ser to diabetic mice enhances antioxidant capacity resulting in a certain degree of recovery in the pancreas tissues. Ser is rich in polar amino acids that enable Ser to chelate metal ions such as iron and copper, thereby neutralizing their oxidative potential (43).

Our study revealed that the induction of diabetes led to substantial impairment in serum levels of NF-κB, TNFα, INF-γ, and TGF-β matched to control rats. These findings indicate that diabetes promotes a systemic inflammatory response, which is a well-established characteristic of both the disease itself and its associated complications, including diabetic nephropathy (44, 45). The increase in these inflammatory mediators is indicative of the activation of various pro-inflammatory signaling pathways, which play a central role in the development and progression of diabetic complications. Elevated NF-κB activity contributes to the upregulation of inflammatory cytokines, which, in turn, promote tissue damage and the development of complications. For instance, in the kidneys, NF-κB activation leads to the expression of pro-inflammatory cytokines and the recruitment of immune cells, contributing to the fibrosis and glomerular injury characteristic of diabetic nephropathy (26). TNFα and INF-γ are pivotal cytokines in the inflammatory response. TNFα is involved in the regulation of immune cells and has been implicated in insulin resistance, a hallmark of type 2 diabetes (46-48). Similarly, INF-γ is involved in the immune response and exacerbates the inflammatory milieu in tissues affected by diabetes (49). The elevated levels of TNFα and INF-γ observed in diabetic rats suggest that these inflammatory mediators contribute to the insulin resistance and tissue dysfunction commonly observed in diabetes. Furthermore, the persistent elevation of these cytokines has been associated with the development of diabetic complications, such as nephropathy and retinopathy (50). In the context of diabetes, TGF-β plays a critical role in the pathogenesis of diabetic nephropathy, as it promotes the deposition of extracellular matrix proteins in the glomeruli and tubules, leading to fibrosis and renal dysfunction (51). The increased serum levels of TGF-β in the diabetic group suggest that fibrosis and renal injury may be progressing due to the heightened inflammatory response. Additionally, TGF-β is involved in the activation of fibrotic pathways, which can lead to scarring and further compromise renal function (52).

Interestingly, the supplementation of Ser in the diabetic rats led to a significant reduction in the levels of NF-κB, TNFα, INF-γ, and TGF-β. These findings suggest that Ser possesses potent anti-inflammatory properties that help attenuate the inflammatory response observed in diabetes. The ability of Ser to reduce the levels of these key pro-inflammatory markers may contribute to the prevention or mitigation of diabetic complications, including kidney damage. There is growing evidence supporting the anti-inflammatory effects of bioactive peptides, including those derived from silkworms such as Ser. For instance, studies have shown that silkworm peptides can inhibit the activation of NF-κB and reduce the production of inflammatory cytokines like TNFα and INF-γ (26). This suggests

that Ser may exert its anti-inflammatory effects through the modulation of these key signaling pathways, ultimately reducing the chronic inflammation that drives the progression of diabetic complications. Moreover, Ser supplementation has been shown to reduce the expression of TGF-β in various animal models, which could help to prevent or reduce fibrosis and tissue damage in the kidneys and other organs (32).

Our study revealed that the induction of diabetes led to marked elevations in pancreatic levels of NF-κB, IL-6, caspase-3, TGF-β, and VEGF versus control rats. This data is confirmed by Liu et al. (53) who reported that diabetic induction was associated with elevated expression levels of IL-6 NF-κB in pancreatic cells concurrent with a decline in pancreatic β-cell function. These results suggest a potential link between the NF-κB pathway and inflammation in the pathogenesis of pancreatic β-cell apoptosis in diabetes. In addition, another report exposed that diabetic mice exhibited a boosted mitochondrial death signaling pathway via raised executor caspase-3 activity in pancreatic islets (54). The TGF-β level is elevated in diabetes mellitus and largely involved in the initiation and progression of diabetic nephropathy (55). Hyperglycemia induces the upregulation of glucose transporter-1 (GLUT-1), resulting in the overexpression of TGF-β (56). Moreover, the activation of the renin-angiotensin system, ROS, and AGEs stimulates TGF-β production through protein kinase C (55). The activation of TGF-β signaling results in the induction of VEGF, which serves as a paracrine effector for TGF-β (56). VEGF exhibits a biphasic role in wound healing, with its sustained release potentially contributing to the development of fibrosis (57). Furthermore, VEGF plays a pivotal role in the thickening of the glomerular basement membrane, the loss of podocytes, and the development of diabetic nephropathy (55).

Treatment with Ser considerably reduced the levels of these inflammatory and apoptotic markers in the pancreas of diabetic rats. Rahimpour et al. (58) stated that Ser reduces inflammation in the liver and pancreas through the reduction of NF-κB and IL-6 as well as oxidative stress. Also, Ser demonstrates the ability to protect cells from ultraviolet irradiation-induced apoptosis by inhibiting caspase-3 activation (59).

Masson's trichrome staining is a technique employed to visualize collagen fibers. By assessing the presence or absence of these fibers, one can gain insights into the impact of ROS on extracellular matrix production. Diabetes can lead to increased collagen deposition within the pancreas, particularly around the islets of Langerhans. This fibrosis can impair the function of the islets and contribute to insulin deficiency (60). In addition, an increase in reactive oxygen species can stimulate the synthesis of extracellular matrix, as evidenced by the appearance of blue-stained collagen fibers in the pancreas tissue of the diabetic group. These findings suggest that the elevated levels of reactive oxygen species in this group may have triggered the upregulation of TGF-β, consequently leading to excessive extracellular matrix deposition (61). However, treatment with Ser resulted in a substantial reduction in fibrosis in the diabetic rats. Ser has been shown to inhibit the activation of hepatic stellate cells, which are responsible for collagen production. This can lead to reduced fibrosis in the pancreas (62, 63). Ser has anti-inflammatory properties that can help mitigate inflammation in the pancreas, which can contribute to tissue damage and fibrosis (8, 26).

5. Conclusions

In conclusion, the present study demonstrates that diabetes mellitus induces significant metabolic disturbances, oxidative stress, and inflammation, leading to impaired pancreatic function and renal dysfunction. The elevated levels of serum glucose, HbA1c, and decreased levels of insulin and C-peptide in diabetic rats highlight the dysregulated glucose metabolism and compromised pancreatic β-cell function. Additionally, the increased oxidative

stress, as evidenced by elevated H_2O_2 and AGE levels, and decreased antioxidant capacity, contribute to the development of diabetic complications. The elevated levels of inflammatory markers, such as NF-κB, TNF-α, INF-γ, and TGF-β, further exacerbate the inflammatory response and contribute to tissue damage.

The administration of Ser to diabetic rats significantly ameliorated these metabolic disturbances and oxidative stress. Ser supplementation resulted in a reduction in serum glucose, and HbA1c, and an increase in insulin and C-peptide levels, indicating improved pancreatic function. Additionally, Ser reduced oxidative stress markers, including H_2O_2 and AGE, and increased antioxidant capacity, suggesting its potent antioxidant properties. Furthermore, Ser effectively attenuated the inflammatory response by reducing the levels of inflammatory markers, such as NF-κB, TNF-α, INF-γ, and TGF-β. This reduction in inflammation likely contributed to the observed improvement in pancreatic function and renal parameters.

The findings of this study provide strong evidence for the therapeutic potential of Ser in managing diabetes and its complications. Ser's ability to mitigate metabolic disturbances, oxidative stress, and inflammation suggests that it may be a promising natural compound for the prevention and treatment of diabetes and its associated complications. Further research is needed to fully elucidate the mechanisms of action of Ser and to explore its potential clinical applications.

Conflict of interest: None

Funding: None

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