

Research Article

Histological and Immunohistochemical Study of the Protective Effect of Sodium Glucose Co-transporter 2 (SGLT2) Inhibitors on Streptozotocin Induced Pancreatic Damage in Adult Male Albino Rats



Abdel Hamid Sayed Abo Bakr¹, Mohamed Ahmed Desuky¹, Youssra Magdy Hassan¹ and Medhat Atta Salah¹

Department of Human Anatomy & Embryology, Faculty of Medicine, Minia University, El-Minia, Egypt

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Abstract

Background: Sodium Glucose Co-transporter 2 (SGLT2) inhibitor is a type of medication primarily prescribed for treatment of type 2 diabetes. By prevention the reabsorption of glucose in the kidneys, these drugs facilitate glucose removal through urine which contributes to lowering blood glucose levels. Recent studies suggest that SGLT2 inhibitors may also exhibit protective effects in various organ systems, by modulating inflammation and oxidative stress. **Aim:** The aim of this study was to investigate the possible protective effect of Sodium Glucose Co-transporter 2 (SGLT2) inhibitors on pancreatic damage induced by streptozotocin in adult male albino rats, through histological and immunohistochemical analyses. **Materials and Methods:** This study involved forty adult male albino rats, and they were divided into four groups. Group I (Control Group): consisted of 10 rats that administered three ml distilled water orally once daily. Group II: This group included 10 rats that were administered Jardiance (Empagliflozin, an SGLT2 inhibitor) at a dose of 10 mg/kg/day, dissolved in drinking water for 28 days. Group III: This group included 10 rats that were fasted for 12 hours before receiving a single high dose of streptozotocin (50 mg/kg) intraperitoneally to induce pancreatic damage. Group IV: This group included 10 rats that received Jardiance (Empagliflozin, an SGLT2 inhibitor) at a dose of 10 mg/kg/day, dissolved in drinking water for 14 days before STZ injection. After that, rats were injected with a single dose of streptozotocin (50 mg/kg) intraperitoneally, and continued to receive the daily dose of Jardiance for 14 days. At the end of the experiment (28 days), pancreatic tissue samples were collected and processed for H&E staining as well as immunohistochemical analysis. Biochemical analyses were conducted to assess serum levels of glucose. **Results:** Degenerative changes were observed in the pancreas of Group III, including destruction of the pancreatic architecture, disfigured islet of Langerhans with ill-defined demarcation between the islets and the surrounding pancreatic acini. Signs of apoptosis were also evident. Protection with SGLT2 inhibitors significantly attenuated these degenerative effects, demonstrating a protective effect on pancreatic tissue. **Conclusion:** SGLT2 inhibitors effectively attenuate the degenerative effects induced by streptozotocin in the pancreas. The protective effects were evidenced by preservation of pancreatic architecture, reduction in inflammatory infiltrates, and attenuation of apoptosis. These results suggest that SGLT2 inhibitors may offer a potential therapeutic strategy for protecting pancreatic tissue against pancreatic damage.

Key Words: Sodium glucose co-transporter 2 inhibitors, streptozotocin, Pancreas.

Introduction

Pancreas is an important organ situated behind the stomach, responsible for regulating blood

sugar levels through hormone secretion, such as insulin and glucagon, and producing digestive enzymes. Disruptions in its function due to

diseases can lead to significant complications in both glucose regulation and digestion ⁽¹⁾. Pancreatic tissue is especially vulnerable to oxidative stress due to its limited antioxidative defense mechanisms. Various factors such as medications, toxins, infections, and physical trauma can directly or indirectly induce pancreatic injury, leading to a compromise in its normal function. This damage often results in disturbances in glucose metabolism, contributing to issues such as insulin resistance or hyperglycemia ⁽²⁾.

Streptozotocin (STZ) is a naturally occurring compound primarily utilized in medical research and treatment due to its ability to selectively target and destroy insulin-producing beta cells in pancreas ⁽³⁾. This property makes it valuable for inducing experimental models of diabetes in animals. Clinically, STZ has been used as a chemotherapeutic agent, particularly in the treatment of pancreatic islet cell tumors. Its mechanism involves DNA alkylation, leading to cell death, which is why it's also being explored for its cytotoxic effects in various cancer treatments ⁽⁴⁾.

Canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin are FDA-approved medications for managing type 2 diabetes in adults, aiming to improve blood sugar control with diet and exercise. These drugs belong to the class of sodium-glucose co-transporter 2 (SGLT2) inhibitors, which target the SGLT-2 proteins in the renal proximal convoluted tubules. By inhibiting these proteins, they reduce glucose reabsorption, lower the renal threshold for glucose (RTG), and enhance glucose excretion through urine ⁽⁵⁾.

Recently, these inhibitors have attracted interest due to their potential therapeutic effects beyond controlling blood sugar levels. They have demonstrated anti-inflammatory, anti-oxidant, and organ-protective properties in experimental models ⁽⁶⁾.

Materials and Methods

1- Chemical compounds:

Streptozotocin (STZ) was purchased from Sigma Chemicals Co. (Catalog No.: S0130, Sigma Aldrich, Egypt). Citrate buffer saline (Catalog No.: ab93678, Abcam, Egypt) was used in the study. Glucose Colorimetric Assay Kit for plasma glucose levels (Catalogue No.

GLU-20 24). Empagliflozin (Jardiance 10 mg tablets), an SGLT2 inhibitor, was obtained from Bloom (Egypt).

2- Experimental animals

This study was conducted in Faculty of Medicine, Minia University, Egypt. All animal's procedures were performed according to the local guidelines of the ethical committee of Faculty of Medicine, Minia University according to the international guidelines. Approval No.336/4/2022.

3- Experimental design:

Forty adult male albino rats were used in this study; they were split into four groups:

- **Group I (control group):** included 10 rats that administered three ml distilled water orally once daily
- **Group II:** included 10 rats. Rats received Jardiance 10 mg (Empagliflozin - SGLT2 inhibitors) at dose of 10mg/kg/day dissolved in drinking water for 28 days.
- **Group III:** included 10 rats. Rats were fasted for 12 hours before being injected intraperitoneally with a single high dose of streptozotocin (50 mg/kg) to induce acute pancreatitis.
- **Group IV:** included 10 rats received Jardiance (Empagliflozin - SGLT2 inhibitor) at a dose of 10 mg/kg/day dissolved in drinking water for 14 days. After that, STZ was administered intraperitoneally at 50 mg/kg, and the rats continued receiving the daily dose of the SGLT2 inhibitor for 14 days.

. At the end of the experiment (28 days), the pancreatic tissue was removed and prepared for light microscopic analysis using H&E. Immunohistochemical staining was performed using anti-caspase 3. Biochemical analyses to measure serum glucose level.

I- Histological study:

Specimens of all rats were collected then fixed in 10% formal saline, then the tissues were dehydrated in increasing concentrations of ethyl alcohol, cleared in xylene, and then infiltrated with soft paraffin followed by hard paraffin. They were sectioned into 5-µm-thick slices. The tissue sections were stained with Hematoxylin and Eosin to evaluate the overall pancreatic structure ⁽⁷⁾.

II- Immunohistochemical study (using activated caspase 3 antibody):

Immunohistochemical study for caspase 3 was used as an indicator of apoptosis. Brown coloration was seen at color at the immune-reactive sites ⁽⁸⁾.

III- Biochemical study:

Plasma glucose levels were determined using a commercial glucose Colorimetric Assay Kits.

Results

I- Histological Results

Hematoxylin and Eosin (H&E) sections in **Group I** revealed the normal architecture of pancreas in the form of islet of Langerhans surrounded by pancreatic acini with well-defined demarcation between them; which is a delicate connective tissue capsule. Blood capillaries, central beta (β) cells with large vesicular rounded nuclei and peripheral alpha (α) cells with small dark nuclei were noticed (**Figure 1**).

Group II islets of Langerhans, centrally located beta (β) cells were identified, characterized by large, vesicular, rounded nuclei, while peripheral alpha (α) cells had smaller, darker nuclei. Blood capillaries were also observed. There was a distinct demarcation between the islets of Langerhans and the surrounding pancreatic acini. Notice the pancreatic acini exhibited basal basophilia and apical acidophilia (**Figure 2**).

Group III revealed islets cells with cytoplasmic vacuolations. Other cells had hypereosinophilic cytoplasm and pyknotic nuclei. karyolysis and mild mononuclear cells infiltrations situated peripherally were observed. Separation between the islets cells was also noticed. Some islet of Langerhans was shrunken (**Figure 3**). Disfigured islet of Langerhans with ill- defined demarcation was also noticed (**Figure 4**)

Group IV showed a clear preservation of the normal islets of Langerhans, surrounded by pancreatic acini. The acinar cells displayed basal basophilia and apical acidophilia. The central beta (β) cells exhibited large, vesicular, rounded nuclei, while the peripheral alpha (α) cells had smaller, darker nuclei. Additionally, some separation between cells and cytoplasmic vacuolation in some cells were observed. (**Figure 5**).

II- Immunohistochemical staining results against active caspase III:

Immunohistochemical results for anti-activated caspase 3 antibody in **Group I** revealed that, there was no detectable reaction for anti-activated caspase 3 antibody in the nuclei or cytoplasm of cells of pancreatic islet of Langerhans in this group (**Figure 6**).

Group II showed negative nuclear and cytoplasmic expression in cells of pancreatic islet of Langerhans (**Figure 7**).

Group III revealed many cells with positive nuclear and cytoplasmic expression in the pancreatic islet of Langerhans (**Figure 8**).

Group IV revealed scattered cells with positive nuclear and cytoplasmic expression in the pancreatic islet of Langerhans (**Figure 9**).

III- Biochemical results:

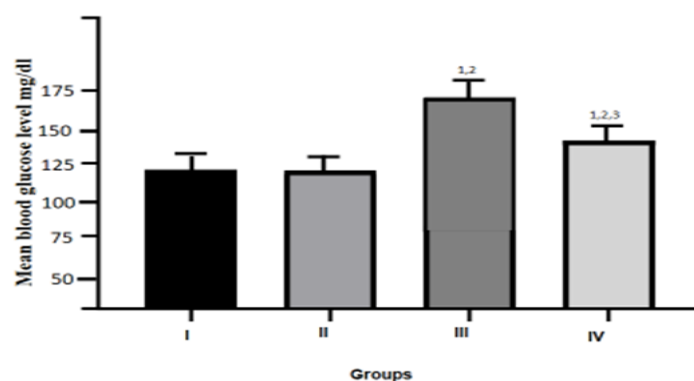
Blood glucose levels:

The statistical analysis of blood glucose levels revealed no significant difference between groups I and II ($P > 0.05$). However, group III showed a significant increase in blood glucose levels compared to groups I and II ($P < 0.0001$). Group IV demonstrated a significant decrease in blood glucose levels compared to group III ($P < 0.0001$) but a significant increase compared to groups I and II ($P < 0.05$). (**Table 1 & Bar Chart 1**).

Table 1: The mean blood levels of glucose (mg/dl) in the studied groups (n=10).

Groups	Mean $\pm$ SEM	p-value
Group I	117.60 $\pm$ 1.407	
Group II	116.60 $\pm$ 1.557	0.977 ¹
Group III	174.80 $\pm$ 3.340	< 0.0001 ^{1*} < 0.0001 ^{2*}
Group IV	130.40 $\pm$ 2.499	< 0.0001 ^{1*} 0.0002 ^{2*} < 0.0001 ^{3*}

SEM: Standard error of mean, ¹: vs group I, ²: vs group II, ³: vs group III, ⁴: vs group IV, *: Significant at $p < 0$.



Bar Chart 1: The mean levels of blood glucose level (mg/dl) in the studied groups (n=10).
(Significant: ¹: vs group I, ²: vs group II, ³: vs group III, ⁴: vs group IV).

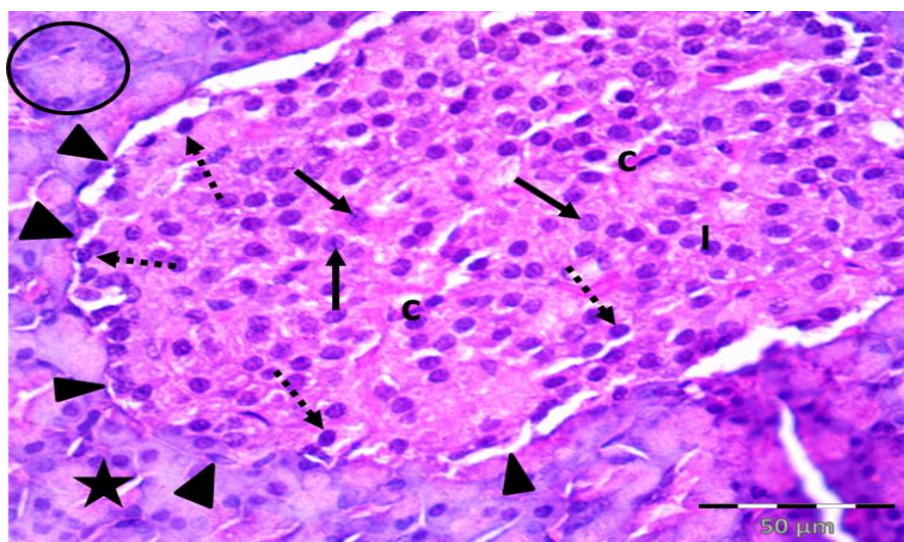


Figure (1): A representative photomicrograph of a section from rat pancreas from group I (control) showing; islet of Langerhans (I) surrounded by pancreatic acini (star) with well-defined demarcation between them (delicate connective tissue capsule) (head arrows). Notice; blood capillaries (C), central beta (β) cells with large vesicular rounded nuclei (arrows) and peripheral alpha (α) cells with small dark nuclei (dotted arrows). Pancreatic acini showing basal basophilia and apical acidophilia (circle). H&E $\times 400$; scale bar = 50 μ m.

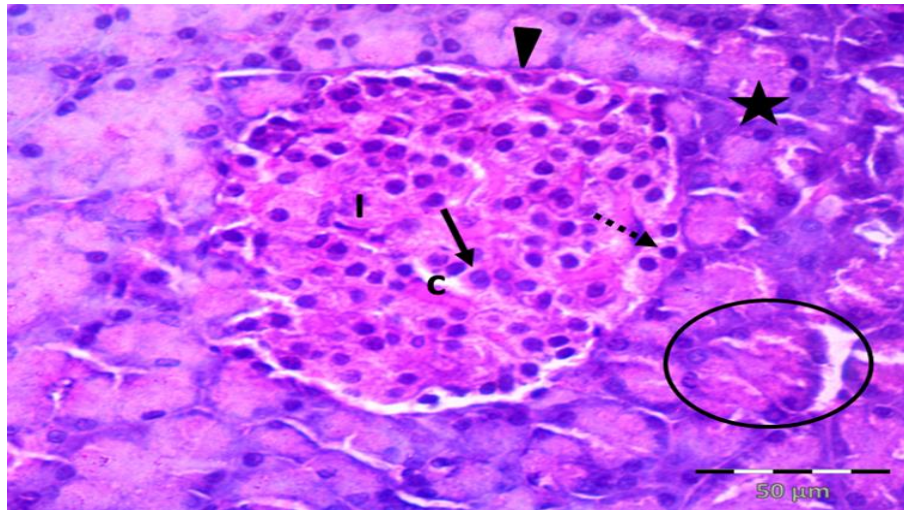


Figure (2): A representative photomicrograph of a section from rat pancreas from group II showing; islet of Langerhans (I) surrounded by pancreatic acini (star) with well-defined demarcation between them (head arrow). Notice; blood capillaries (C), central beta (β) cells with large vesicular rounded nuclei (arrow) and peripheral alpha (α) cells with small dark nuclei (dotted arrow). Pancreatic acini showing basal basophilia and apical acidophilia (circle). H&E $\times 400$; scale bar = 50 μm .

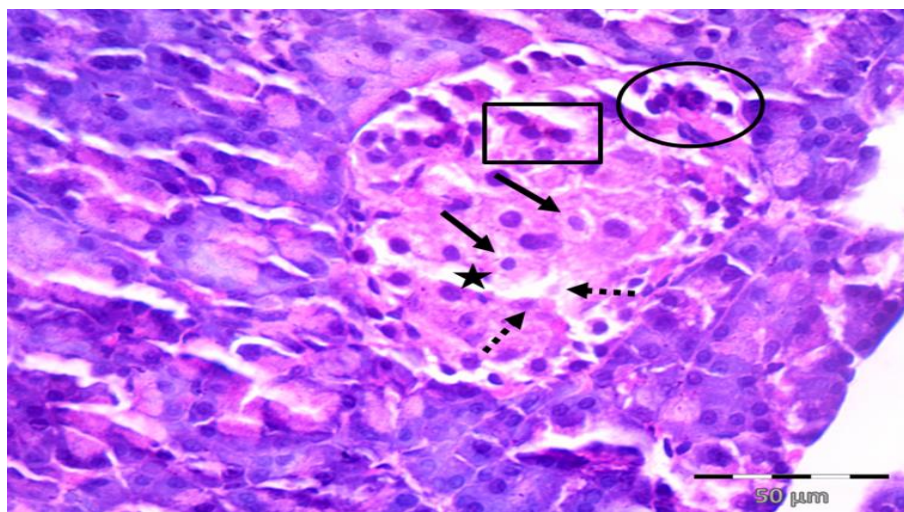


Figure (3): A representative photomicrograph of a section from rat pancreas from group III showing; cells with cytoplasmic vacuolations (arrows), other cells with hypereosinophilic cytoplasm and nuclear pyknosis (square), karyolysis (dotted arrows), and mild mononuclear cells infiltrations situated peripherally (circle). Notice the separation (star) between the islets cells. H&E $\times 400$; scale bar = 50 μm .

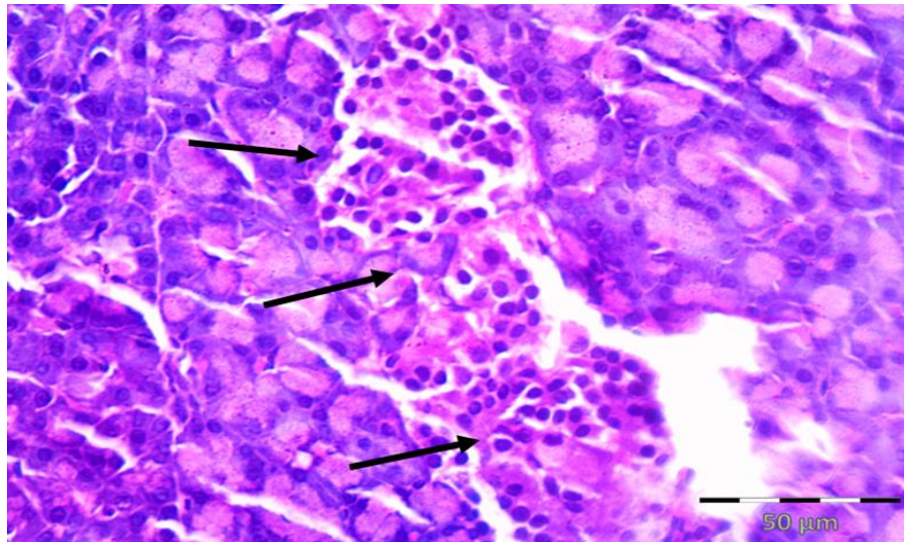


Figure (4): A representative photomicrograph of a section from rat pancreas from group III showing; disFigured islet of Langerhans (arrows) with ill-defined demarcation. H&E $\times 400$; scale bar = 50 μm .

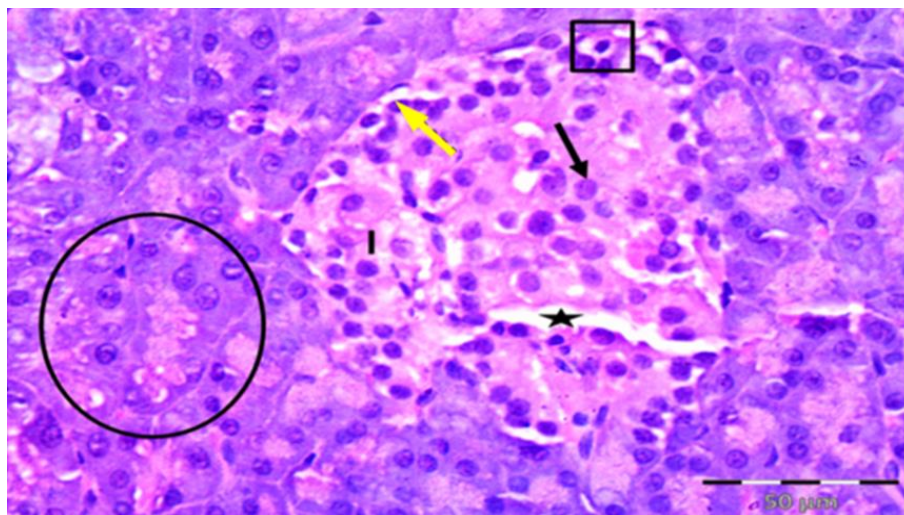


Figure (5): A representative photomicrograph of a section from rat pancreas from group IV showing; apparent normal islet of Langerhans (I) surrounded by pancreatic acini (circle) with basal basophilia and apical acidophilia. Notice; central beta (β) cells with large vesicular rounded nuclei (arrow) and peripheral alpha (α) cells with small dark nuclei (yellow arrow). Notice some separation between the cells (star) and some cells with cytoplasmic vacuolation (square). H&E $\times 400$; scale bar = 50 μm .

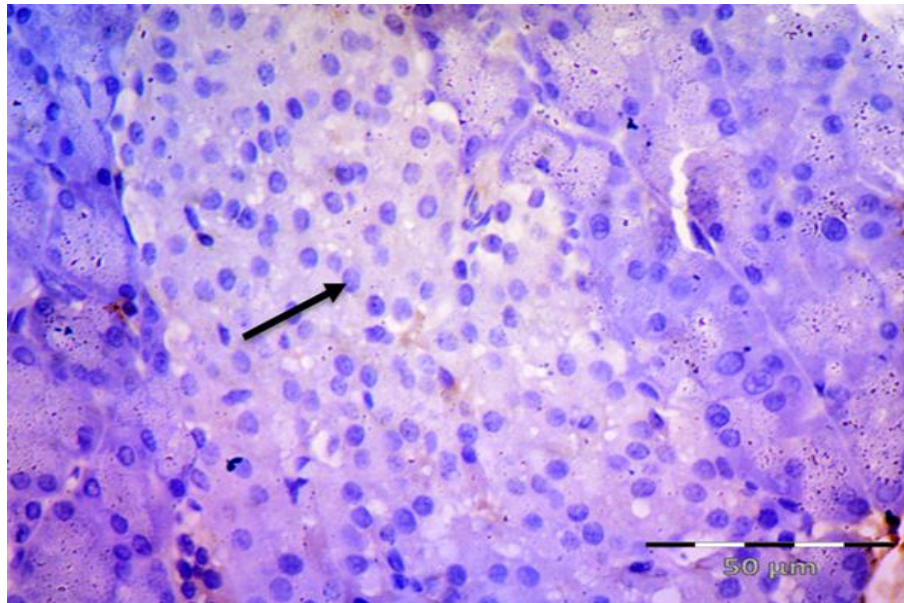


Figure 6: Representative Photomicrograph of section in rat's pancreatic tissue in group I immunolabelled for activated caspase 3 showing negative nuclear and cytoplasmic expression (arrow) in cells of pancreatic islet of Langerhans. (Immunohistochemistry for activated caspase 3 x400; scale bar = 50 μ m).

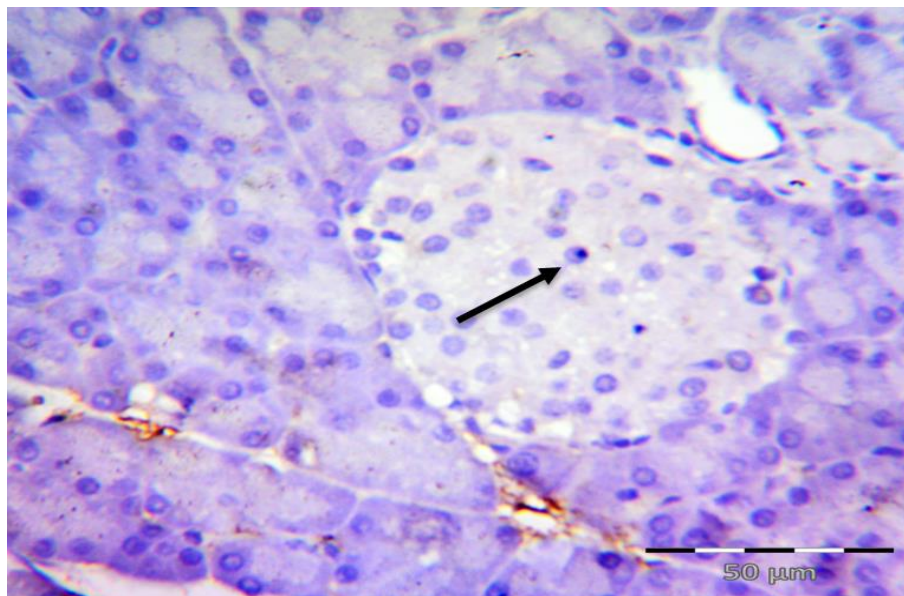


Figure 7: Representative photomicrograph of section in rat's pancreatic tissue in group II immunolabelled for activated caspase 3 showing negative nuclear and cytoplasmic expression (arrow) in cells of pancreatic islet of Langerhans. (Immunohistochemistry for activated caspase 3 x400; scale bar = 50 μ m).

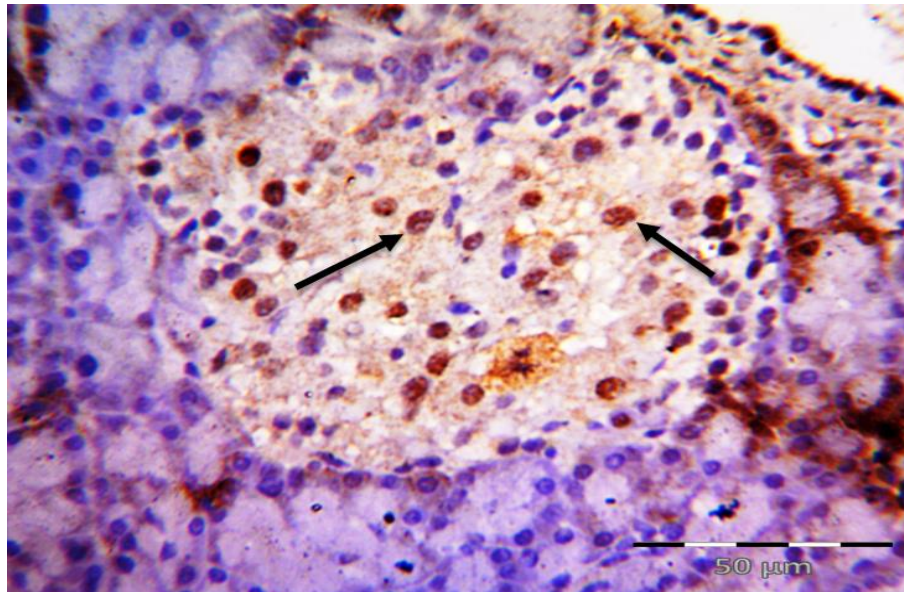


Figure 8: Representative photomicrograph of section in rat's pancreatic tissue in group III immunolabelled for activated caspase 3 showing many cells with positive nuclear and cytoplasmic expression (arrows) in the pancreatic islet of Langerhans. (Immunohistochemistry for activated caspase 3 x400; scale bar = 50 μm)

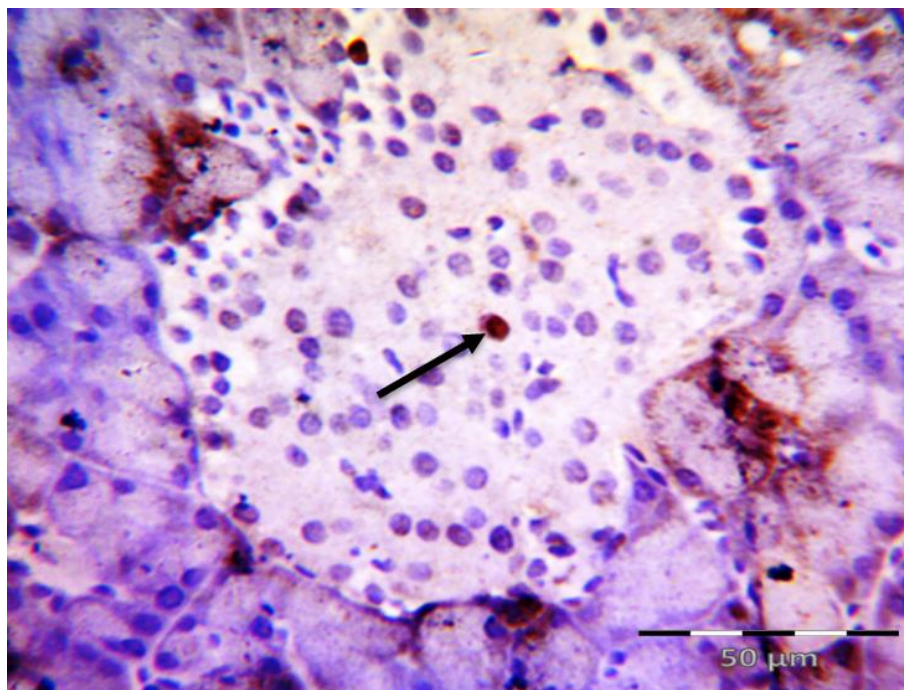


Figure 9: Representative photomicrograph of section in rat's pancreatic tissue in group IV immunolabelled for activated caspase 3 showing scattered cells with positive nuclear and cytoplasmic expression (arrow) in the pancreatic islet of Langerhans. (Immunohistochemistry for activated caspase 3 x400; scale bar = 50 μm).

Discussion

The primary objective of this research was to assess the possible protective effect of SGLT2 inhibitors on streptozotocin induced pancreatic damage.

The histological study using H&E staining in group III revealed histopathological changes, including disfigured islets of Langerhans with ill-defined demarcation. These islets contained cells with cytoplasmic vacuolations, hyper-eosinophilic cytoplasm, pyknotic nuclei, karyolysis, mild mononuclear cell infiltration, and separation between islet cells. These findings align with those reported by Abdulwahab et al., ⁽⁹⁾ and Ge et al., ⁽¹⁰⁾ who described similar histopathological changes in pancreatic tissue. In their studies, they focused on the effects of streptozotocin on pancreatic tissue reporting comparable histopathological findings, including disorganization of islet architecture and the presence of inflammatory cells within the pancreatic tissue.

Eizirik and his coworkers ⁽¹¹⁾ reported that one of the main forms of pancreatic cell death is apoptosis, which is believed to be triggered by many factors such as nutrient overload, the activation of inflammatory cytokines and oxidative stress. In severe cases, extensive damage to pancreatic tissue occurs, and the activation of the final common pathway, involving inflammatory mediators like interleukin-1 β (IL-1 β), nuclear factor- κ B (NF- κ B), and tumor necrosis factor- α (TNF- α), plays a vital role in stimulating cell death and tissue necrosis. These cytokines amplify the local inflammatory response, further damaging and impairing pancreatic function. The oxidative stress, resulting from the production of reactive oxygen species (ROS), exacerbates the inflammatory process, depleting antioxidant defenses and increasing pancreatic cell apoptosis.

The histological results were also supported by Grieco et al., ⁽¹²⁾ who reported that in streptozotocin induced pancreatic damage, the loss of functional pancreatic cells is linked to molecular events such as cellular apoptosis, dysfunction, and necrosis. Inflammatory mediators and oxidative stress contribute to pancreatic tissue damage, triggering apoptosis pathways and resulting in the loss of both exocrine and endocrine pancreatic function.

These events worsen the severity of damage and can lead to systemic complications, including hyperglycemia and insulin resistance in severe cases.

Group IV revealed near preservation of the normal histological structure of the islets of Langerhans, with only minimal histopathological changes, such as some cytoplasmic vacuolation and slight separation between the cells. These results confirm the protective role of SGLT2 inhibitors on the pancreas. This finding is supported by Okauchi et al., ⁽¹³⁾ who reported that SGLT2 inhibitors protect the pancreas by modulating glycemic control, reducing pancreatic inflammation, and alleviating oxidative stress. Furthermore, these agents have been shown to reduce pancreatic cell apoptosis and promote cellular proliferation, thereby preserving pancreatic function and preventing excessive tissue damage in animal models ⁽¹⁴⁾.

To recognize the underlying mechanism of the protective effect of SGLT2 inhibitors, we focused on the expression of activated caspase-3, a key protease in the apoptosis process. Caspase-3 plays an essential role in regulating apoptosis by initiating DNA fragmentation and protein degradation within the cell. Upon activation, caspase-3 triggers a cascade of events that lead to controlled cell death. This includes the fragmentation of DNA into small, irreparable pieces, which prevents genomic integrity. Caspase-3 also activates other proteases that degrade structural proteins in the cell, leading to the collapse of the cytoskeleton and loss of cell shape. Additionally, caspase-3 mediates the breakdown of cellular membranes, causing the cell to detach from surrounding tissues ⁽¹⁵⁾.

The expression of activated caspase-3 in group I and group II were very low, while there was a notable increase in the area percentage of activated caspase-3 expression in group III, serving as an early marker of apoptosis. This finding aligns with the work of Chen et al., ⁽¹⁶⁾ who reported that pancreatic injury increases caspase-3 expression. These results corroborate the previously mentioned histological findings, as cells with hypereosinophilic cytoplasm and deeply stained nuclei were observed in H&E stained sections.

In group IV, there was a significant decrease in the area fraction of activated caspase-3 expression compared to group III, which may explain the structural improvement observed in the histological study. This is consistent with Liu et al.,⁽¹⁷⁾ who suggested that SGLT2 inhibitors offer protection to pancreatic tissue from damage by inhibiting the NLRP3/caspase-1/GSDMD inflammasome pathway, which is involved in pyroptosis. Additionally, Yaribeygi et al.,⁽¹⁸⁾ reported that apoptosis was inhibited by SGLT2 inhibitors.

Conclusion

The present study demonstrated the potential protective effect of SGLT2 inhibitors against streptozotocin induced pancreatic damage in adult male albino rats. Histological and immunohistochemical findings revealed that SGLT2 inhibitors significantly improved pancreatic architecture, reduced inflammation, and minimized tissue damage compared to the unprotected group. These results suggest that SGLT2 inhibitors may offer a promising therapeutic strategy for mitigating pancreatic injury and could serve as a potential treatment option for conditions associated with pancreatic damage.

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