International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 5, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V27I5/077

Protective Effect of Empagliflozin (Sodium Glucose Co-transporter 2 Inhibitors) on pancreatic damage Induced by Streptozotocin in Adult Male Albino Rats

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Received: 16.10.2024 Revised: 22.11.2024 Accepted: 16.12.2024

ABSTRACT

Background: Sodium glucose co-transporter 2 (SGLT2) inhibitors are primarily utilized for controlling blood sugar levels in individuals with type 2 diabetes. These drugs work by inhibiting the reabsorption of glucose in the kidneys, leading to its elimination through urine and thereby lowering blood glucose concentrations. Recent studies have expanded their potential benefits, suggesting that SGLT2 inhibitors may also offer protective effects in various tissues by reducing inflammation and mitigating oxidative stress, both of which play a critical role in the development of organ damage damage.Streptozotocin (STZ) is a naturally occurring antibiotic derived from Streptomyces achromogenes. In medicine, it is primarily used as a chemotherapy agent to treat certain types of pancreatic neuroendocrine tumors. STZ selectively targets insulin-producing beta cells, making it useful for managing insulin-secreting tumors. Its ability to cause beta-cell destruction has also made it a key compound in diabetes research for inducing experimental models of diabetes in animals.

Aim: The aim of this study was to evaluate the potential protective effects of Sodium Glucose Co-transporter 2 (SGLT2) inhibitors on pancreatic damage caused by streptozotocin in male albino rats. This has been achieved through a combination of histological, immunohistochemical, and biochemical analyses to assess the extent of pancreatic damage and the potential therapeutic benefits of SGLT2 inhibition in mitigating inflammation, oxidative stress, and cellular injury.

Materials and Methods: Forty adult male albino rats were used in this study, divided into four distinct groups. Group I (Control Group) consisted of 10 rats thatadministered three ml distilled water orally once daily. Group II included 10 rats that were administered Jardiance (Empagliflozin, an SGLT2 inhibitor) at a dose of 10 mg/kg/day, dissolved in drinking water for a period of 28 days. Group III included 10 rats that were fasted for 12 hours and then given a single high dose of streptozotocin (50 mg/kg) intraperitoneally to induce pancreatic damage. Group IV involved 10 rats that received Jardiance (Empagliflozin) at the same dose of 10 mg/kg/day for 14 days before being injected with streptozotocin (50 mg/kg) intraperitoneally andcontinued to receive the daily dose of Jardiance for 14 days during the post-STZ period. At the end of the study (28 days), pancreatic tissues were collected from all groups and processed for H&E staining and immunohistochemical analysis.Biochemical analyses were conducted to assess levels of glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), as well as serum glucose and serum insulin levels.

Results: The structural integrity of the pancreas in Group III was compromised, with destruction of the pancreatic architecture and islet cell disruption. The demarcation between the islets and the surrounding pancreatic acini was poorly defined. Additionally, degenerative changes and apoptosis were evident. However, the use of SGLT2 inhibitors provided significant protection, leading to a marked reduction in these effects and demonstrating a notable protective role in preventing pancreatic damage.

Conclusion:.Sodium-Glucose Co-transporter 2 (SGLT2) inhibitors significantly reduce the degenerative damage caused by streptozotocin in the pancreas. The observed protective effects were reflected in the maintenance of pancreatic architecture, a decrease in inflammatory cell infiltration and a reduction in apoptosis. These findings suggest that SGLT2 inhibitors could serve as a promising therapeutic approach to protect pancreatic tissue from pancreatic damage.

Keywords:SGLT2 inhibitors, pancreas, streptozotocin, inflammation, oxidative stress.

INTRODUCTION

Empagliflozin is a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor commonly used for the management of type 2 diabetes mellitus**(1)**. It works by inhibiting glucose reabsorption in the kidneys, leading

to increased urinary glucose excretion and improved glycemic control. Beyond its antidiabetic effects, empagliflozin has shown potential benefits in cardiovascular protection and renal function preservation. Recent studies have also explored its protective role in various pathological conditions, such as inflammation and oxidative stress, making it a promising candidate for further research in non-diabetic diseases **(2).**

Streptozotocin (STZ) is a naturally occurring chemical derived from the bacterium Streptomyces achromogenes, primarily known for its cytotoxic effects on insulin-producing β-cells in the pancreas. Due to its selective uptake by GLUT2 transporters highly expressed in β-cells, STZ is widely used in research to induce experimental diabetes in animal models**(3).** Once inside the β-cells, STZ causes DNA alkylation, leading to cellular damage and the generation of reactive oxygen species (ROS**)**, which result in oxidative stress. This oxidative stress ultimately triggers apoptosisandnecrosis, leading to the destruction of pancreatic β-cells and subsequent hyperglycemia**(4).**

The pancreas, a vital organ situated behind the stomach, responsible for blood glucose regulation by secreting hormones like insulin and glucagon, in addition to producing digestive enzymes**(5).**Pancreatic tissue is particularly susceptible to oxidative stress, primarily due to its relatively low levels of endogenous antioxidant defense systems. Various external and internal factors, such as medications**,** toxins**,** infections, and physical trauma, can lead to pancreatic injury, either by direct damage or by inducing inflammatory processes. Damage to pancreatic β-cells, in particular, can lead to hyperglycemia or insulin resistance, contributing to metabolic disorders such as diabetes mellitus. Additionally, oxidative stress exacerbates inflammation, further compromising pancreatic integrity and accelerating disease progression**(6).**

MATERIALS AND METHODS

1-Chemical compounds:

Empagliflozin (Jardiance 10 mg tablets), an SGLT2 inhibitor, was purchased from Bloom (Egypt). Streptozotocin (STZ) was acquired from Sigma Chemicals Co. (Catalog No.: S0130, Sigma Aldrich, Egypt). Additionally, citrate buffer saline (Catalog No.: ab93678, Abcam, Egypt) was used in the study.Commercially available colorimetric assay kits for malondialdehyde (MDA) (Catalogue No. MD 25 29), superoxide dismutase (SOD) (Catalogue No.SD 25 21), reduced Glutathione (GSH) (Catalogue No. GSHGR 25 11),glucose Colorimetric Assay Kit for plasma glucose levels (Catalogue No. GLU-20 24) and radioimmunoassay kits for plasma insulin levels (Catalogue No. INS-20 35).

2- Experimental animals

This study was carried out at the Faculty of Medicine, Minia University, Egypt. All animal procedures were conducted in accordance with the ethical guidelines set by the Faculty of Medicine's ethical committee at Minia University, and in compliance with international standards.

3- Experimental design:

Forty adult male albino rats were used in this study, and they were divided into four groups:

- **Group I (control group):** Included 10 rats and did not receive any treatment.
- **Group II:** Included 10 rats. They received Jardiance 10 mg (Empagliflozin - SGLT2 inhibitor) at a dose of 10 mg/kg/day, dissolved in drinking water, for 28 days.
- **Group III (STZ-induced pancreatic damage):** Included 10 rats. The 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 (SGLT2-protected):** Included 10 rats. They received Jardiance (Empagliflozin - SGLT2 inhibitor) at a dose of 10 mg/kg/day, dissolved in drinking water, for 14 days before the STZ injection. Afterward, STZ was administered intraperitoneally at 50 mg/kg, and the rats continued receiving the daily dose of the SGLT2 inhibitor during the post-STZ period to assess its protective effects.

The pancreatic tissue was removed and prepared for light microscopic analysis using H&E, Mallory's trichrome and PAS stain.Immunohistochemical staining was performed using anti-cyclooxygenase 2 (COX 2). Biochemical analyses to measure GSH, MAD, SOD, serum glucose level and serum insulin level were done.

I-Biochemichal study:

A. The serum GSH, MAD, SOD were measured by using colorimetric assay kits.
B. Plasma glucose levels were determined using a commercial glucose Colorimetric Assay Kit.
C. Plasma insulin level was determined by radioimmunoassay kits.

II-Histological study:

Specimens of all rats were collected then fixed in 10% formal saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, impregnated in soft paraffin followed by hard paraffin, and sectioned in 5-µm-

thickness**.** Sections were stained with Hematoxylin and Eosin for general structureof the pancreas, Mallory's trichrome to demonstrate collagen fibers and PAS for detection of glycogen levels in pancreatic cells.

III-Immunohistochemical study (using a cyclooxygenase 2 antibody):
Immunohistochemical study for anti-COX 2 was used as an indicator of apoptosis. Positive cells for active cox 2 showed brown coloration**.**

RESULTS

Biochemichal Results:

1- The serum GSH, MAD, SOD levels:

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

The statistical analysis of malondialdehyde (MDA) levels revealed no significant difference between groups I and II (P<0.05). However, group III showed a significant increase in MDA levels compared to groups I and II (P<0.0001). Group IV demonstrated a significant decrease compared to group III (P<0.0001) but a significant increase when compared to groups I and II (P<0.0001).**(Table 2 & Bar Chart 2).**

The statistical analysis of superoxide dismutase (SOD) levels showed no significant difference between groups I and II (P<0.05). However, group III exhibited a significant decrease in SOD levels compared to groups I and II (P<0.0001). Group IV demonstrated a significant increase compared to group III (P<0.0001) but a significant decrease compared to groups I and II (P<0.0001).**(Table 3 & Bar Chart 3).**

2- 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 4 & Bar Chart 4).**

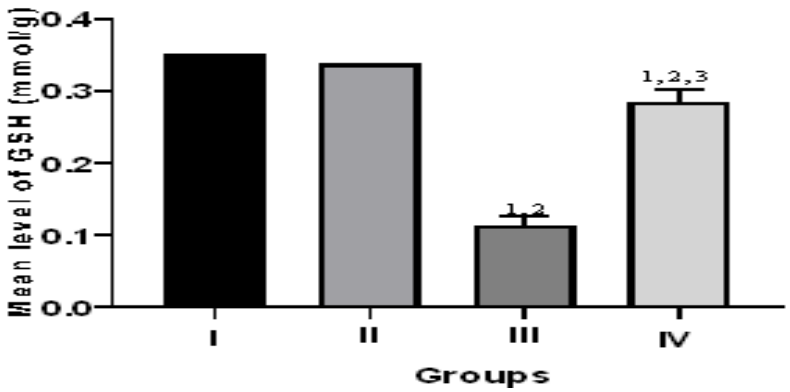
3- Serum insulin levels:

The statistical analysis of serum insulin levels showed no significant difference between groups I and II (P > 0.05). However, group III exhibited a significant decrease in serum insulin levels compared to groups I and II (P < 0.0001). Group IV demonstrated a significant increase in serum insulin levels compared to group III (P < 0.0001), but a significant decrease compared to groups I and II (P < 0.0001).**(Table 5 & Bar Chart 5).**

Table 1: The mean blood levels of glutathione (GSH) (mmol/g) in the studied groups (n=10).

Groups	Mean ± SEM	p-value
Group I	0.35± 0.02	
Group II	0.34 ± 0.02	0.688[1*]
Group III	0.12 ± 0.016	< 0.0001 [1*] < 0.0001 [2*]
Group IV	0.29 ± 0.017	< 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<0.05.

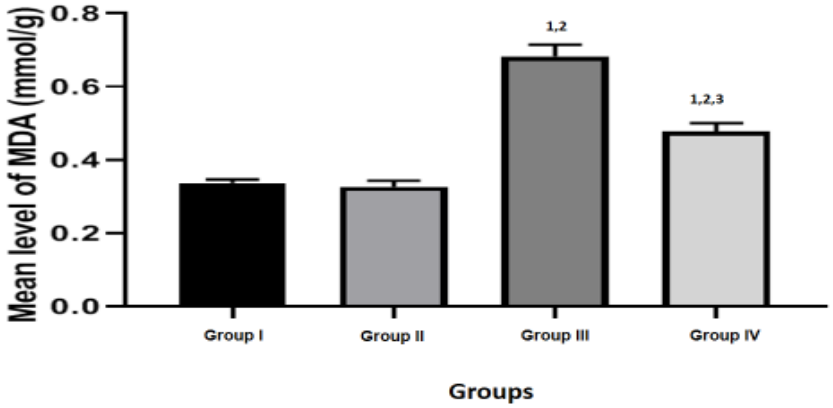


Bar Chart 1: The mean blood levels of glutathione (GSH) (mmol/g) in the studied groups (n=10). (Significant: [1]: vs group I, [2]: vs group II, [3]: vs group III, [4]: vs group IV).

Table 2: The mean blood levels of Malondialdehyde (MDA) (mmol/g) in the studied groups (n=10).

Groups	Mean ± SEM	p-value
Group I	0.34± 0.01	
Group II	0.33 ± 0.02	0.844 [1]
Group III	0.68 ± 0.03	< 0.0001 [1*] < 0.0001 [2*]
Group IV	0.48 ± 0.02	< 0.0001 [1*] < 0.0001 [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<0.05.

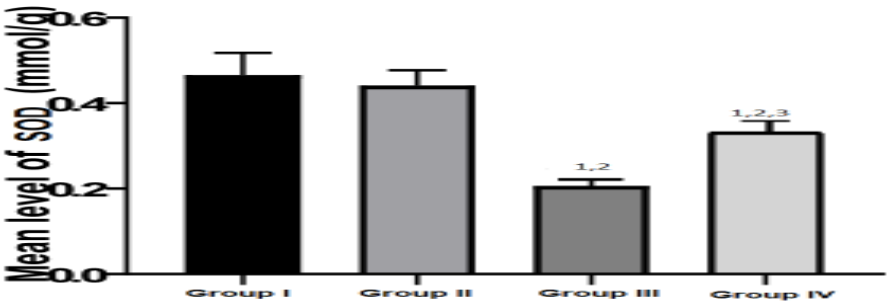


Bar Chart 2: The mean levels of Malondialdehyde (MDA)(mmol/g) in the studied groups (n=10). (Significant: [1]: vs group I, [2]: vs groupII, [3]: vs group III, [4]: vs group IV).

Table 3: The mean levels of superoxide dismutase (SOD) (mmol/g) in the studied groups (n=10).

Groups	Mean ± SEM	p-value
Group I	0.47± 0.052	
Group II	0.44 ± 0.036	0.651
Group III	0.20 ± 0.02	< 0.0001 [1*] < 0.0001 [2*]
Group IV	0.34 ± 0.027	< 0.0001 [1*] < 0.0001 [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<0.05.



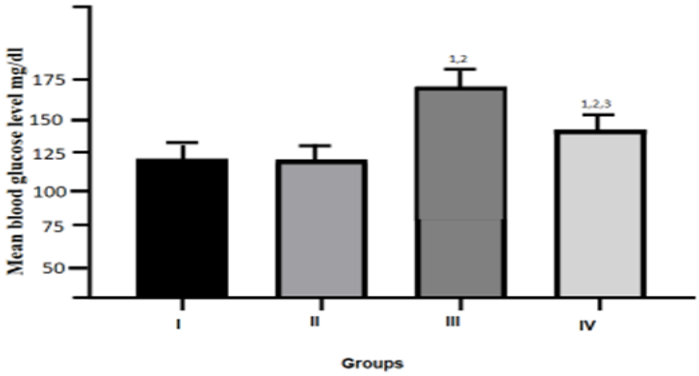
Bar Chart 3: The mean levels of superoxide dismutase (SOD) (mmol/g) in the studied groups (n=10).**(**Significant: [1]: vs group I, [2]: vs group II, [3]: vs group III, [4]: vs group IV**)**

Table 4: The mean blood levels of glucose (mg/dl) in the studied groups (n=10).

Groups	Mean ± SEM	p-value
Group I	117.60± 1.407	
Group II	116.60 ± 1.557	0.977[1]
Group III	174.80 ±3.340	< 0.0001 [1*] < 0.0001 [2*]

Group IV	130.40±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.

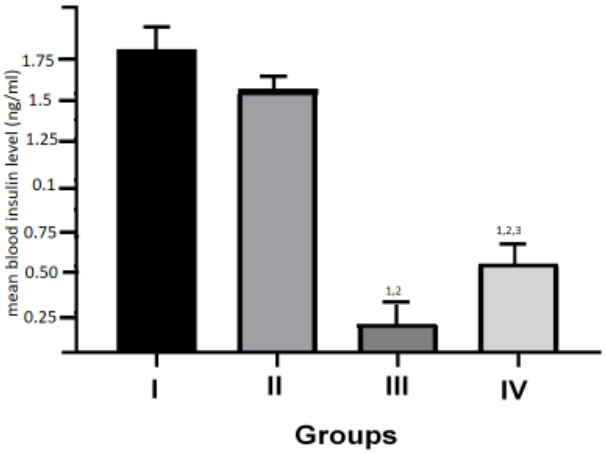


Bar Chart 4: The mean levels of blood glucose level (mg/dl) in the studied groups (n=10).**(**Significant: [1]: vs group I, [2]: vs group II, [3]: vs group III, [4]: vs group IV**).**

Table 5: The mean blood levels of insulin (ng/ml) in the studied groups (n=10).

Groups	Mean ± SEM	p-value
Group I	1.76 ± 0.015	
Group II	1.53 ± 0.036	0.73 [1]
Group III	0.23 ±0.014	< 0.0001 [1*] < 0.0001 [2*]
Group IV	0.58 ± 0.044	< 0.0001 [1*] < 0.0001 [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<0.05.



Bar Chart 5: The mean levels of blood insulin (ng/ml) in the studied groups (n=10).**(**Significant: [1]: vs group I, [2]: vs group II, [3]: vs group III, [4]: vs group IV**).**

II-Histological Results

Staining with **Hematoxylin and Eosin (H&E)**in **Group I** showed the normal structure of the pancreas, characterized by the islets of Langerhans surrounded by pancreatic acini, with a clear boundary between them formed by a delicate connective tissue capsule. Blood capillaries were visible, along with centrally located beta (β) cells that had large, vesicular, rounded nuclei, and peripherally located alpha (α) cells with smaller, darker nuclei. The pancreatic acini exhibited basal basophilia and apical acidophilia **(Figure 1).**

Group II showed islets of Langerhans surrounded by pancreatic acini, with a clear demarcation between them. Blood capillaries were observed, along with central beta (β) cells containing large, vesicular, rounded nuclei and peripheral alpha (α) cells with smaller, darker nuclei. The pancreatic acini exhibited basal basophilia and apical acidophilia**. (Figure 2).**

Group III revealed cells with cytoplasmic vacuolations. Othercells had hypereosinophilic cytoplasm and pyknotic nuclei. karyolsis and mild mononuclear cells infiltrations situated peripherally were observed. Separation between the islets cells was also noticed . Some islet of Langerhans was shrunken. Disfigured islet of Langerhans with ill- defined demarcation was also noticed **(Figures3, 4).**

Group IV revealed apparent normal islet of Langerhans surrounded by pancreatic acini. Cells of pancreatic acini had basal basophilia and apical acidophilia. Central beta (β) cells with large vesicular rounded nuclei and peripheral alpha (α) cells with small dark nuclei were noticed. Some separation between the cells and some cells with cytoplasmic vacuolation were also observed **(Figure 5).**

By Mallory's trichrome Group I revealed fine delicate collagen fiber surrounding the pancreatic islet of Langerhans **(Figure 6-a).**

Group II showed fine delicate collagen fiber surrounding the pancreatic islet of Langerhans **(Figure 6-b).**

Group III showed apparent excessive collagen fiber deposition around the pancreatic islet of Langerhans **(Figure 6-c).**

Group IV revealed minimal collagen fiber surrounding the pancreatic islet of Langerhans (Figure 6-d).

With Periodic acid Schiff (PAS) Group I revealed scattered cells with faint positive PAS reaction in the pancreatic islet of Langerhans **(Figure 7-a).**

Group II showed scattered cells with positive reaction in the pancreatic islet of Langerhans **(Figure 7-b).**

Group III showed many cells with strong positive reaction in the pancreatic islet of Langerhans **(Figure 7-c).**

Group IV showed some cells with positive reaction in the pancreatic islet of Langerhans **(Figure 7-d).**

III- Immunohistochemical staining against anti-COX-2:

Immunohistochemical results for anti-COX-2 antibody for **Group I** revealed that there was no detectable reaction for anti COX-2 antibody in cytoplasm of cells of pancreatic islet of Langerhans in this group **(Figure 8-a).**

Group II showed negative cytoplasmic expression in cells of pancreatic islet of Langerhans (Figure 8-b).

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

Group IV group revealed some cells with positive cytoplasmic expression in the pancreatic islet of Langerhans **(Figure 8-d).**

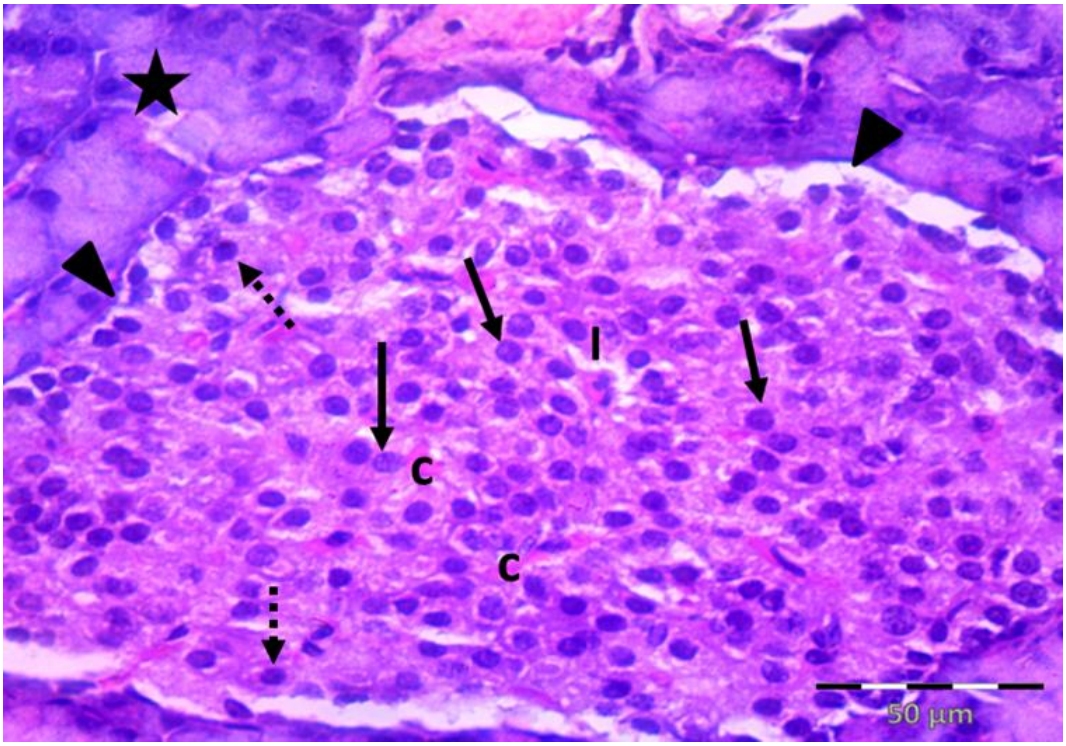


Figure 1:A representative photomicrograph of a section from rat pancreas from groupI (control) showing; islet of Langerhans (I) surrounded by pancreatic acini (star) with well-defined demarcation between them (delicate connective tissue capsule) (head arrows). Notice; blood capillaries (C), central beta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

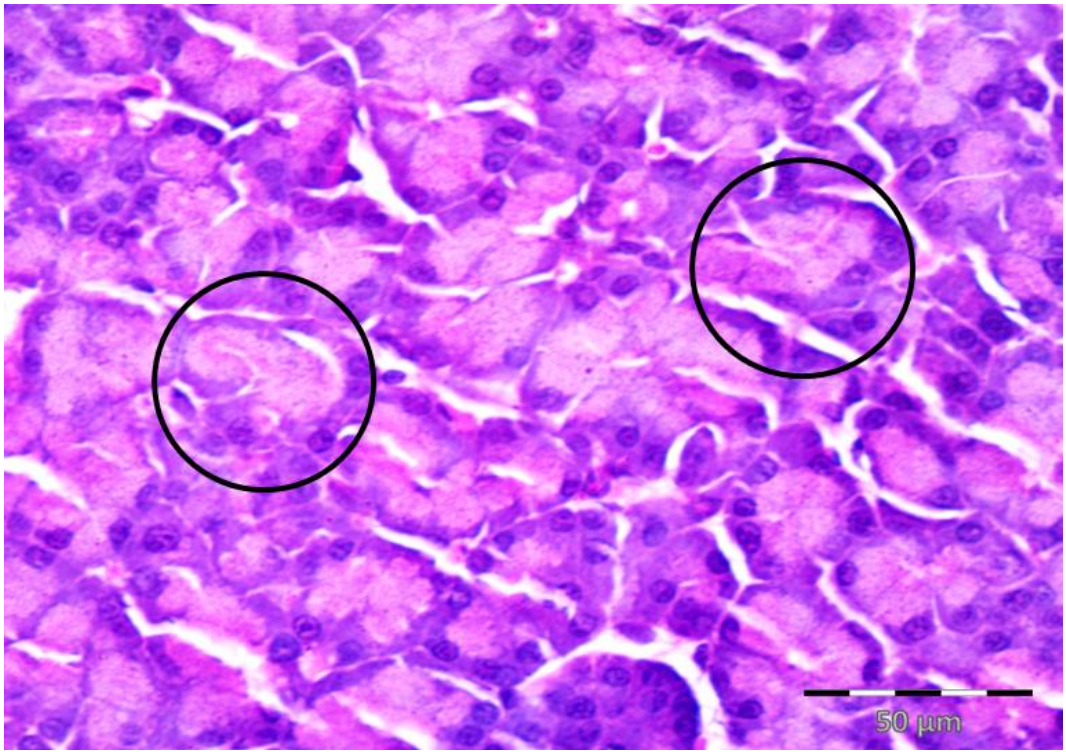


Figure 2:A representative photomicrograph of a section from rat pancreas from group IIshowing; Pancreatic acini with basal basophilia and apical acidophilia (circles). H&E ×400; scale bar=50 µm.

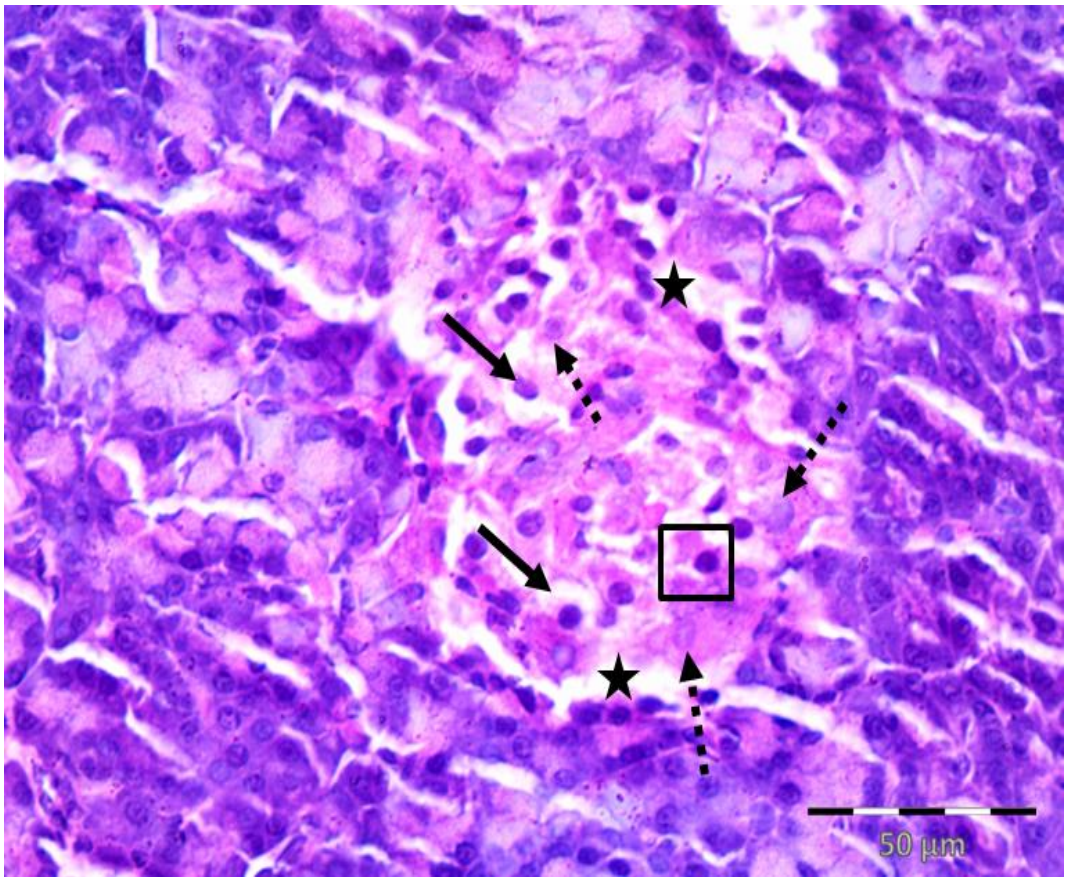


Figure 3:A representative photomicrograph of a section from rat pancreas from group III showing; cells with cytoplasmic vacuolations (arrows), othercells with hypereosinophilic cytoplasm and nuclear pyknosis (square), karyolsis (dottted arrows). Notice the separation (stars) between the islets cells. H&E ×400; scale bar=50 µm.

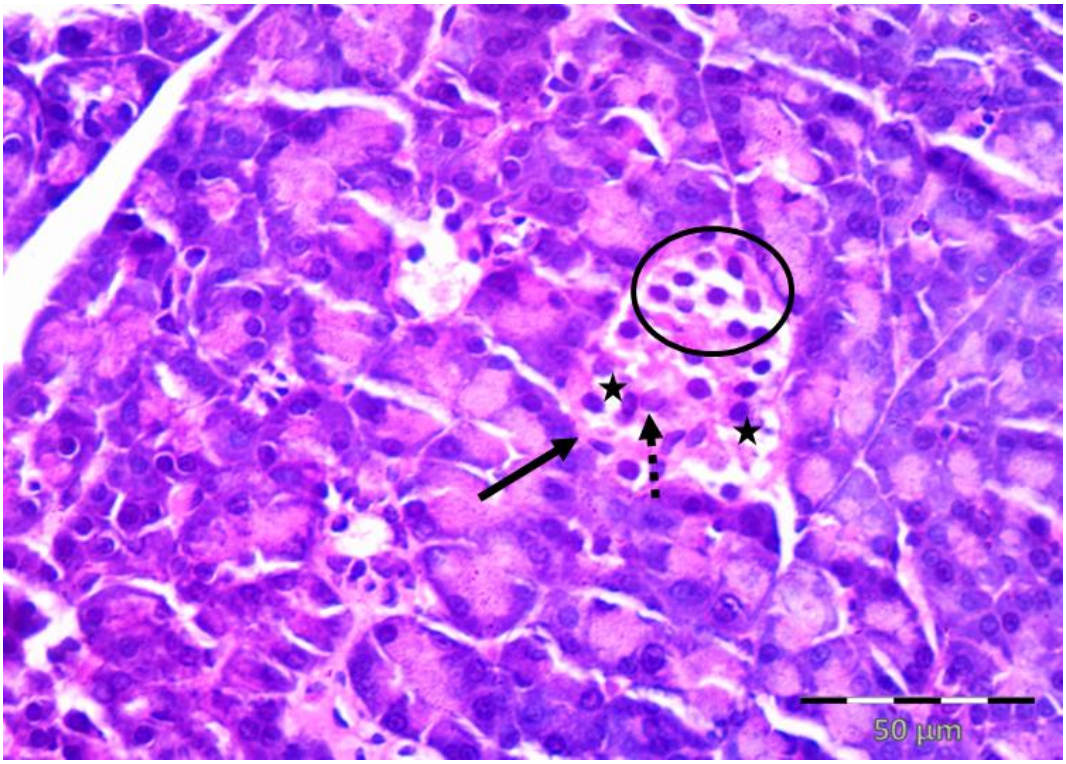


Figure 4:A representative photomicrograph of a section from rat pancreas from group III showing; shrunken islet of Langerhans (arrow), cells with cytoplasmic vacuolations (circle), karyolsis (dottted arrow), and wide spaces (stars) between the islets cells. H&E ×400; scale bar=50 µm.

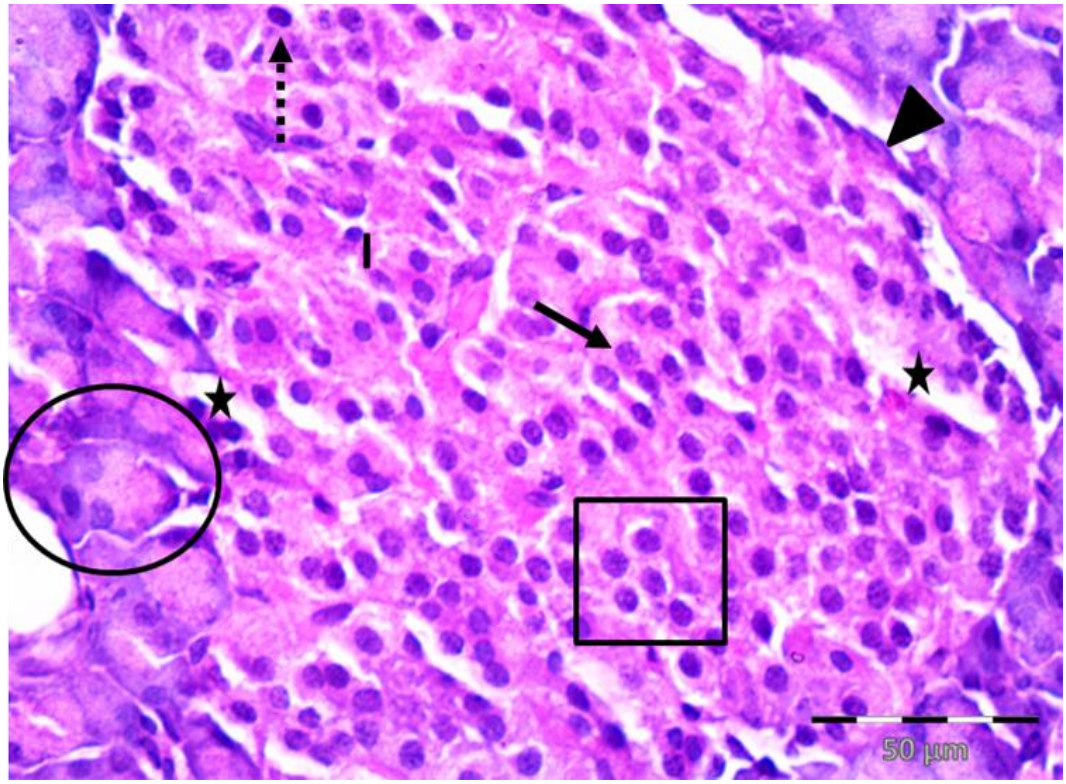


Figure 5: A representative photomicrograph of a section from rat pancreas from group IV showing; apparent normal islet of Langerhans (I) surrounded by pancreatic acini (circle) with basal basophilia and apical acidophilia. Notice; central beta (β) cells with large vesicular rounded nuclei (arrow) and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

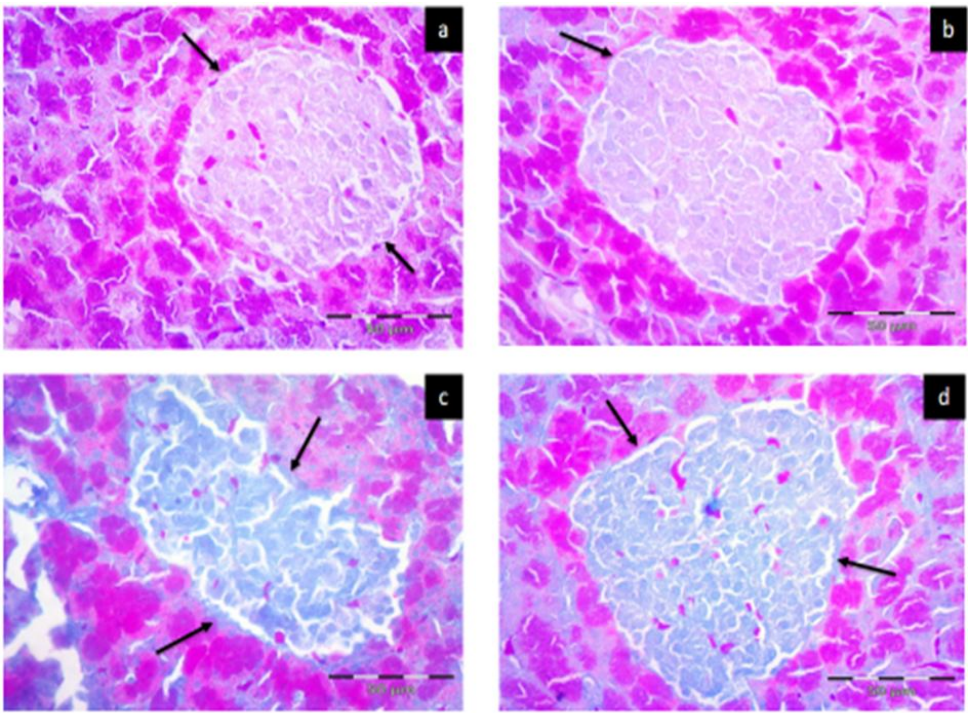


Figure 6: Representative photomicrographs of section in rat's pancreatic tissue stained by Mallory's trichrome from (a) Group I showing; fine delicate collagen fiber (arrows) surrounding the pancreatic islet of Langerhans, (b) Group II showing; fine delicate collagen fiber (arrow) surrounding the pancreatic islet of Langerhans, (c) Group III showing; apparent excessive collagen fiber (arrows) surrounding the pancreatic islet of Langerhans, (d)Group IV showing; revealing minimal collagen fiber (arrows) surrounding the pancreatic islet of Langerhans. (Mallory's trichrome x 400; scale bar=50µm).

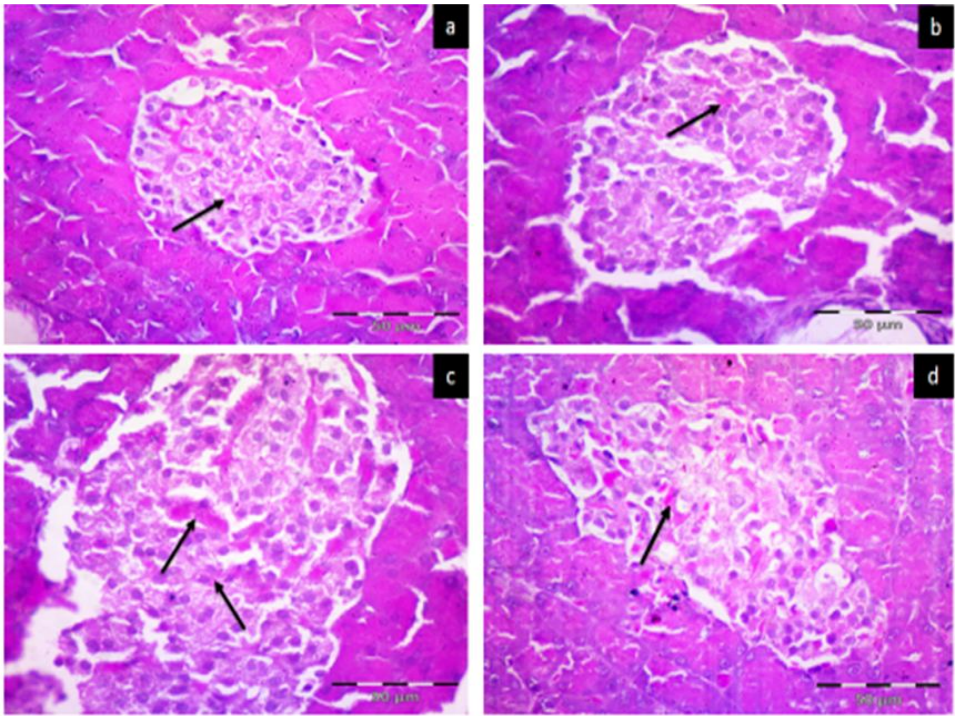


Figure 7: Representative photomicrographs of section in rat's pancreatic tissue from (a) Group I showing faint positive PAS reaction (arrows) in scattered cells in the pancreatic islet of Langerhans, (b) group II showing positive PAS reaction (arrows) in scattered cells in the pancreatic islet of Langerhans, (c) group III showing strong positive PAS reaction (arrows) in many cells in the pancreatic islet of Langerhans, (d) group IV showing positive PAS reaction (arrow) in some cells in the pancreatic islet of Langerhans. (PAS x 400; scale bar=50µm).

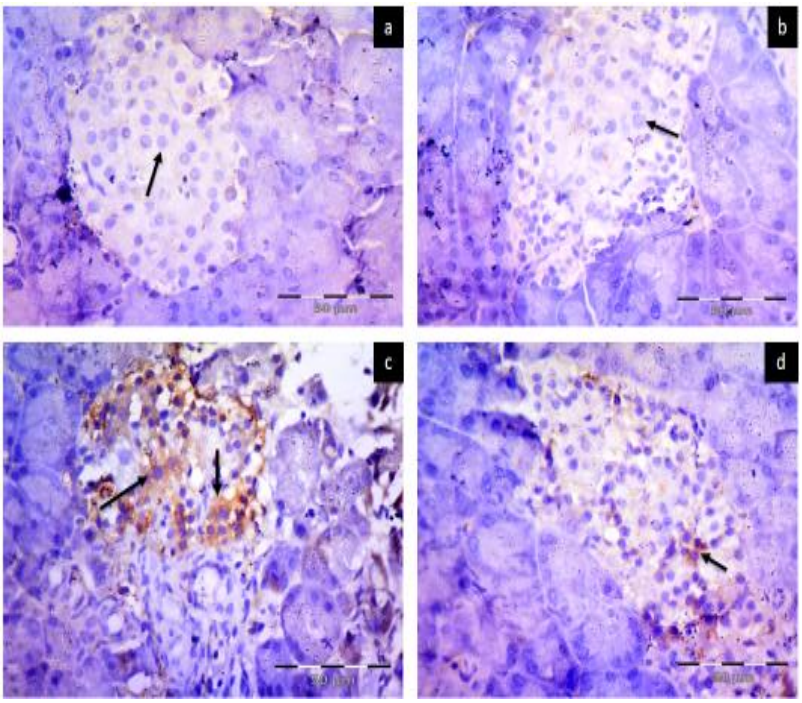


Figure 8: Representative Photomicrographs of section in rat's pancreatic tissue from (a) group I immunolabelled for COX-2 showing negative cytoplasmic expression (arrow) of cells in islet of Langerhans, (b) in group II immunolabelled for COX-2 showing negative cytoplasmic expression (arrow) of cells in islet of Langerhans, (c) in group III immunolabelled for COX-2 showing many cells with strong positive cytoplasmic expression (arrows) in the pancreatic islet of Langerhans, (d) group IV immunolabelled for COX-2 showing some cells with positive cytoplasmic expression (arrow) in the pancreatic islet of Langerhans. (Immunohistochemistry for COX-2 x 400; scale bar=50µm).

DISCUSSION

Streptozotocin (STZ) is widely recognized as a potent chemical compound used in experimental models to induce diabetes mellitus**(7)**. As an alkylating agent, STZ selectively damages the insulin-producing beta cells in the pancreas, leading to impaired glucose regulation **(8)**.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 treatment**(9)**.

The pathophysiology of pancreatic damage caused by streptozotocin (STZ)involves the selective destruction of insulin-producing β-cellsin the pancreas. STZ is taken up by β-cells via the GLUT2 transporter, where it induces DNA damage through alkylation and promotes the generation of reactive oxygen species (ROS)**.** This oxidative stress overwhelms the cells' limited antioxidant defenses, leading to mitochondrial dysfunction and triggering apoptosis. The cumulative damage results in the loss of β-cells, impairing insulin production and leading to hyperglycemia. Additionally, inflammation in the surrounding pancreatic tissue may further exacerbate the damage, contributing to pancreatic dysfunction and diabetes**(10)**

Empagliflozin is a FDA-approved for managing type 2 diabetes in adults to improve blood sugar control, alongside diet and exercise. These agents are sodium-glucose transport protein 2 (SGLT2) inhibitors, which act on the SGLT-2 proteins in the renal proximal convoluted tubules. They work by reducing the reabsorption of filtered glucose, lowering the renal threshold for glucose (RTG), and promoting urinary glucose excretion (**11).**

The primary objective of this research was to evaluate the possible protective effect of empagliflozin on Streptozotocin induced pancreatic damage.

This study investigated oxidative stress by measuring serum levels of malondialdehyde (MDA), a marker for oxidative stress **(12)**, as well as the serum levels of antioxidants glutathione (GSH) and superoxide dismutase (SOD) **(13)**. The results showed a significant increase in MDA levels and a significant decrease in GSH and SOD levels in group III compared to groups I and II. These findings align with those of **(14)**, who reported that pancreatic injury leads to an increase in reactive oxygen species, which depletes antioxidant enzymes like GSH and SOD, contributing to free radical accumulation.

On the other hand, **(15)** reported that SGLT2 inhibitors possess antioxidant properties, which help reduce reactive oxygen species and free radicals. The current study supports this finding, as treatment with SGLT2 inhibitors in group IV led to a significant decrease in the mean value of MDA and a significant increase in the mean values of GSH and SOD compared to groups I and II.

In this study, insulin levels were significantly decreased in group III, which is in line with the findings of **(16)**. They attributed this decrease to the cytotoxic effects of streptozotocin on pancreatic β-cells, which impairs insulin secretion. Streptozotocin induces oxidative stress that damages β-cells, leading to a reduced capacity for insulin production and resulting in hypoinsulinemia.

The group protected with the SGLT2 inhibitor showed a significant increase in insulin levels compared to group III. This rise is likely attributed to the protective effects of the SGLT2 inhibitor on pancreatic β-cells, which help reduce oxidative stress and inflammation, thereby preserving their insulin-secreting function. By maintaining β-cell integrity and improving glucose regulation, SGLT2 inhibitors support insulin production despite pancreatic damage. These findings align with those of **(17)**, who also observed elevated insulin levels following SGLT2 inhibitor administration, highlighting its role in protecting β-cell health under stress conditions.

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, hypereosinophilic cytoplasm, pyknotic nuclei, karyolysis, mild mononuclear cell infiltration, and separation between islet cells. These findings align with those reported by **(18)** and **(19)** who described similar histopathological changes in pancreatic tissue in pancreatitis.

(20) Reported that pancreatic damage is characterized by progressive pancreatic cell injury and inflammation. One of the primary forms of pancreatic cell death is apoptosis, which is believed to be triggered by factors such as nutrient overload, oxidative stress, and the activation of inflammatory cytokines. In severe cases, extensive damage to pancreatic tissue occurs, and the activation of the final common pathway, involving inflammatory mediators like interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and nuclear factor-κB (NF-κB), plays a crucial role in promoting cell death and tissue necrosis. These cytokines amplify the local inflammatory response, further damaging and impairing pancreatic function. In Pancreatic damage, oxidative stress, caused by reactive oxygen species (ROS) production, exacerbates the inflammatory process, depleting antioxidant defenses and increasing pancreatic cell apoptosis resulting in complications such as hyperglycemia and insulin resistance.

The histological results were also supported by **(21)**, who reported that in pancreatic damage caused by streptozotocin, 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. 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 **(22),** 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 **(23).**

Mallory's trichrome is a special stain used to highlight collagen fibers, which aids in detecting fibrosis in the islets of Langerhans in the pancreas **(24)**. Normally, a delicate layer of collagen fibers surrounds the pancreatic islets of Langerhans **(25).** In this study, group III displayed significant collagen fiber deposition around the pancreatic islets, which was confirmed by a statistically significant increase in the surface area fraction of Mallory's trichrome stain compared to groups I and II **(26).** According to**(27)**, fibrosis, characterized by the excessive deposition of extracellular matrix, is commonly observed in pancreatic damage. This fibrotic process may contribute to pancreatic dysfunction. Hyperglycemia, oxidative stress, and lipotoxic injury are known to activate the fibrotic response in pancreatic inury, either by directly stimulating matrix synthesis in pancreatic fibroblasts or by promoting a fibrogenic phenotype in immune and vascular cells. Additionally, fibrosis can arise from the conversion of endothelial and epithelial cells into fibroblast-like cells, further complicating pancreatic tissue damage and hindering recovery **(28).**

PAS (Periodic Acid-Schiff) is a special stain used to detect carbohydrate content in tissue, particularly useful for identifying glycogen deposits in pancreatic cells **(29)**. Glycogen is a normal component of pancreatic β-cells and plays a crucial role in glucose regulation. In pancreatic damage, changes in glycogen content within pancreatic cells can occur due to tissue damage and metabolic disturbances associated with inflammation **(30)**. In this study,

a significant increase in the surface area fraction of PAS staining was observed in the pancreatic islets of Langerhans in group III. **(31)** Reported that glycogen accumulation in pancreatic cells increases with hyperglycemia, possibly due to enhanced gluconeogenesis. In the context of pancreatic damage, glycogen accumulation can worsen pancreatic cell injury and contribute to β-cell apoptosis, as glycogen overload can disrupt cellular homeostasis and activate cell death mechanisms **(32).**

The immunohistological results of COX-2 in the current study, which is an inflammatory marker**,** revealed a significant increase in area fraction of COX-2 in pancreas in group III compared to group I and group II. This was in agreement with results of **(33)** who reported up-regulation of COX-2 in pancreatic damage.

Hyperglycemia-induced oxidative stress and inflammation are closely associated with the development, progression, and complications of pancreatic damage. Inflammatory cytokines, stress kinases, and reactive oxygen species (ROS) play a critical role in exacerbating pancreatic injury. These factors interfere with normal cellular signaling pathways, leading to pancreatic dysfunction and organ failure. The inflammatory response also contributes to β-cell dysfunction and apoptosis, further impairing glucose regulation and exacerbating the systemic effects of pancreatic damage **(34).**

In consistency with these results, the study of **(35)** indicated that can lead to insulin resistance, β-cell dysfunction, and impaired glucose regulation. The inflammatory response exacerbates pancreatic injury, contributing to metabolic disturbances and systemic complications.

Regarding group IV; there was a significant decrease in area fraction of COX-2 expression compared with group I and group II as reported by **(36)** who stated that SGLT2 inhibitors have antiinflammatory properties. Also **(37)** stated that SGLT2 inhibitors attenuate inflammation and oxidative stress in experimental in vitro and in vivo models. SGLT2 inhibitors have antiinflammatory effects by inhibiting the expression of CD80 in LPSinduced RAW 264.7 macrophages **(38).(39)**Observed that empagliflozin reduces the expression of pro- and anti-inflammatory mediators. These effects are mediated inhibition of ERK1/2 and NF-kB pathway.

CONCLUSION

Empagliflozin(selective SGLT2 inhibitor) demonstrate a protective effect against streptozotocin-induced acute pancreatitis in male albino rats. Their use resulted in notable improvements in pancreatic structure, reduced inflammation, and lessened tissue damage compared to the unprotected group. These results indicate that SGLT2 inhibitors help mitigate the adverse effects of acute pancreatitis, presenting a promising therapeutic option for managing pancreatitis and its associated complications.

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