

# Histological Evaluation of Pancreatic Changes Induced by Dapagliflozin in Male Rats with Alloxan-Induced Diabetes

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## ABSTRACT

Diabetes mellitus is a major global health concern, affecting individuals regardless of gender and requiring effective management strategies. Among the treatments available, sodium-glucose cotransporter 2 (SGLT2) inhibitors, such as Dapagliflozin, are emerging as effective oral anti-diabetic medications for type 2 diabetes. These drugs enhance glucose excretion via the urine by inhibiting reabsorption in the proximal renal tubules, thereby lowering blood glucose levels. This study aimed to evaluate the histological effects of Dapagliflozin on the pancreas in male rats with alloxan-induced diabetes. A total of 15 adult male rats were divided into three groups. The control group (n=5) consisted of healthy, untreated rats. The second group (n=5) included diabetic rats induced with alloxan but left untreated. The third group (n=5) consisted of diabetic rats treated with Dapagliflozin at a dose of 2 mg/kg, administered daily for 30 days. At the end of the experiment, all animals were sacrificed, and pancreatic tissue samples were collected for histological analysis. Histological examination revealed significant pathological changes in the pancreas of diabetic rats without treatment, including marked damage to both the exocrine and endocrine regions. The pancreas of Dapagliflozin-treated diabetic rats also exhibited notable histopathological changes, including vascular congestion, inter-acinar hemorrhage, and increased zymogen granules in the exocrine portion. In the endocrine portion, reductions in the size and number of Langerhans islets were observed, along with cell dissolution and internal bleeding within the islets. These findings indicate that both diabetes and Dapagliflozin treatment exert discernible effects on the pancreatic histological structure in male rats with alloxan-induced diabetes. While Dapagliflozin is effective in lowering blood glucose levels, its potential histological impact on pancreatic tissue warrants further investigation to better understand its long-term effects.

**Keywords:** Dapagliflozin, Diabetes Mellitus, Pancreas, SGLT2,

## INTRODUCTION

Worldwide, diabetes mellitus (DM) is one of the major health concerns of our time [1]. Diabetes is a chronic metabolic disorder, with the International Diabetes Federation (IDF) predicting that 10% of the global population will be diagnosed with diabetes by In 2035, 90% of diabetic patients were found to have type 2 diabetes T2DM [2]. Type 2 diabetes, which is one of the most common metabolic diseases, occurs due to the presence of two main factors: low insulin secretion by pancreatic cells and inappropriate response to insulin in tissues that contain insulin receptors, and the need for glucose to remain balanced in order to maintain insulin functions and actions [3]. Educating diabetic patients is extremely important, especially those who have a high risk of developing diabetes. Advice must be provided to patients, especially those at risk, by making adjustments to the diet, reducing excess weight, exercising regularly and continuously, checking blood sugar periodically, and staying away from... And quitting smoking [4]. Recently, a number of drugs have appeared called sodium-glucose cotransporter 2 (SGLT2) receptor inhibitors. They represent a new class of antidiabetic drugs mainly used in T2DM so far. Among these drugs, Dapagliflozin is a very powerful, specific and selective inhibitor of... SGLT2 is the only one approved for adults with T2DM in the European Union and the United States. SGLT2 inhibitors work independently of insulin by preventing reabsorption in the proximal convoluted tubules, resulting in lower glucose levels and glycosuria [5]. SGLT2 is responsible for the transport of glucose in the epithelial cells lining the proximal renal tubules, which takes place through their membranes, which are proteins (Timar et al., 2016). SGLT inhibitors have increased interest as a treatment for diabetics, which is done by inhibiting SGLTs in the kidneys [6]. Dapagliflozin is a safe and

effective non-insulin-dependent treatment for treating diabetic patients [7], and it also has many positive benefits. It can be considered an anti-inflammatory and anti-oxidant and may affect the stages that result in the occurrence of various types of diseases that affect humans [8]. It also has broad effects on the pathways involved in apoptosis and autophagy [9]. Hussain et al. [10] noted the low rates of use of SGLT2 inhibitors in patients with type 2 diabetes, heart disease, atherosclerosis, and blood vessels, as the prices of these drugs play a major role, which is likely to reduce patients' adherence to treatment [11].

## MATERIALS AND METHODS

### Induction of Diabetes Disease

Five adult male rats were isolated for the control group. The rest of the 10 male rats were injected with Alloxan under the peritoneal membrane, which is of Indian origin. At the same time as the rats were injected, Alloxan was prepared at a full dose of 150 mg/kg/day of the rat's weight, where (1 gram) was dissolved. ) of the substance in 10 ml of physiological solution [12]. After three days (72) hours have passed, a German-made diabetes device is used to check the blood sugar level and confirm whether they have diabetes.

### Division and Distribution of Animals

In this study, 15 adult white male rats were used, which were placed inside plastic cages and housed in the Educational College of Pure Sciences - University of Diyala in the place designated for them. The ages of the rats were 3-4 months, and their weights ranged between 167-200 grams of body weight. They were then divided into three

groups. The first group was the control group, which was given a physiological solution during the duration of the experiment. The second group was divided in turn into two secondary groups in which diabetes was induced by injecting Alloxan to induce diabetes. The first secondary group had induced diabetes, while the second secondary group had induced diabetes and was treated with the drug Dapagliflozin at a concentration of 2 mg/kg/day of the rat's weight, which continued for a period. The experiment lasted 30 days, and the amount of the drug administered to the rat used in this study was calculated according to the following equation: [13].  $X/D = W_{\text{Rat}}/1000$ . Where X: is the amount of drug required to be injected into rats, measured in mg/kg of body weight. D: The specific dose of the drug is (2) mg/kg/day of the rat's weight. W: Weight of male rats used in the experiment. At the end of the experiment, all the rats were dissected, the organ under study (the pancreas) was removed from them, and preparations were made to make histological sections. The samples were preserved in a 10% formalin solution for 24 hours, after which they were washed with tap water and transferred to a 70%

concentration of alcohol to preserve them. Histological sections were made according to the necessary steps.

As described in [14], the sections were examined and photographed using a microscope equipped with a digital camera after completing the staining process using hematoxylin-eosin stain.

## Results

Histological sections of the pancreas showed the presence of pathological histological changes in the group in which diabetes was induced by Alloxan during a period of 30 days of the experiment. The most important of these changes was damage to each of the internal secretory units represented by the islet of Langerhans, especially the beta cells, as well as the case with the external secretory part represented by In the secretory acini, an increase in the distance of the interlobular septa was also observed, the appearance of intralobular hemorrhage, necrosis of the islets of Langerhans, their shrinkage, and an increase in the size of the cells within them, as shown in Figure (1).

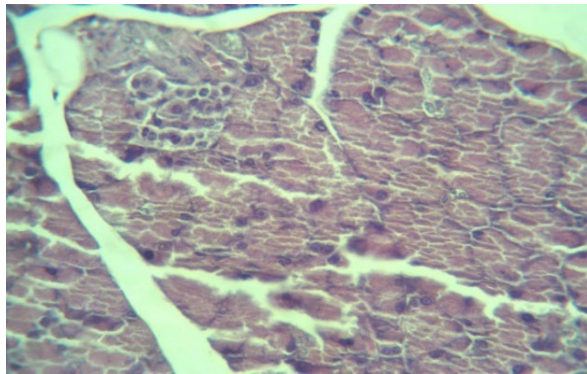


**Figure 1:** A Section of the Pancreas of Male Rats in the Group with Alloxan-Induced Diabetes For 30 Days, Showing Increased Interlobular Septal Distance T, H Hemorrhage, LBC Beta Cell Paucity, Interlobular Duct Icd, Acini A, Intralobular Necrosis N, Lobules Lo, and Atrophy of the Islets of Langerhans AIL, Colored. (H&E 40X).

The results of microscopic examination of histological sections of the pancreas in white rats induced with diabetes also showed that they suffered from pathological changes, including enlargement of the internal secretory part represented by the islets of Langerhans, an increase in the number of cells, especially beta cells in the center of the

islet, their enlargement, and then the death of some cells, the appearance of remnants of the islet, and the appearance of ruptures. There is bleeding inside it, in addition to necrosis, as well as necrosis of the external secretion part represented by the acini. We also notice an increase in the size of the cells, as shown in Figure (2).

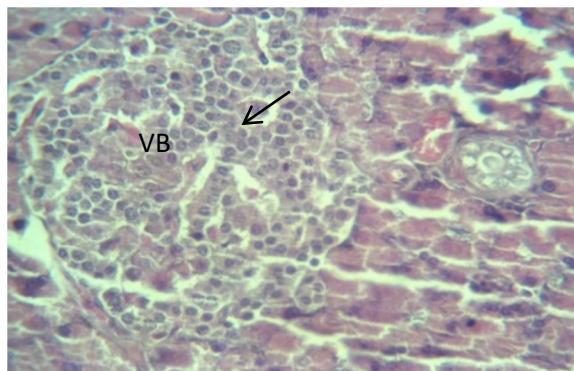


**Figure 2:** A Histological Section of the Pancreas of Male Rats with Induced Diabetes Showing an Increase in the Number of IBC Beta Cells, Necrosis between NC Cells, Enlargement of BC Beta Cells, Congestion of Blood Vessels VB, Hemorrhage within the H Islet, Death of Some BCD Beta Cells, and Necrosis of the Secretory Portion. External NEX, V-Islet Cell Rupture, LI, VB Vascular Congestion, BC Beta Cell Enlargement Stained (H&E 40X).

The results also showed, by making sections and examining them under a light microscope, the occurrence of damages represented by the occurrence of histological changes in the exocrine part in a clear manner, the most prominent of which is the appearance of yeast antigen granules in the acini spread within each of the lobules in the

pancreas, as well as the formation of the cell boundaries in a blurred manner, and some cells were observed. The acini contain two nuclei, while others were observed to be devoid of nuclei. As for the cytoplasm of pancreatic acini cells, it was characterized by containing a number of vacuoles, as shown in Figure (3).

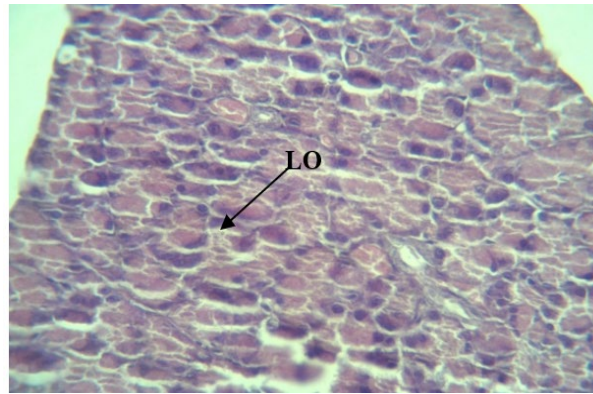


**Figure 3:** A Section of the Pancreatic Gland Tissue from a Group of Male Rats with Induced Diabetes, Showing the State of V Rupture in the Acini, ZG Granules, a Acini, CO Hyperemia, N Interacinar Necrosis, and LO Lobule. Stained (H&E 40X).

The results of the group with Alloxan-induced diabetes treated with Dapagliflozin at a concentration of 2 mg/kg/day throughout the trial period of 30 days showed the occurrence of histopathological changes, including the presence of large fatty droplets, with the occurrence of a state of congestion in both blood vessels and a blood

clot within the pancreatic duct within the lobule. In addition, a thickening of the blood vessel wall and the appearance of fibrosis around the blood vessels were observed, while in other histological sections an increase in the percentage of fatty tissue in the pancreas gland of rats, as shown in Figure (4).

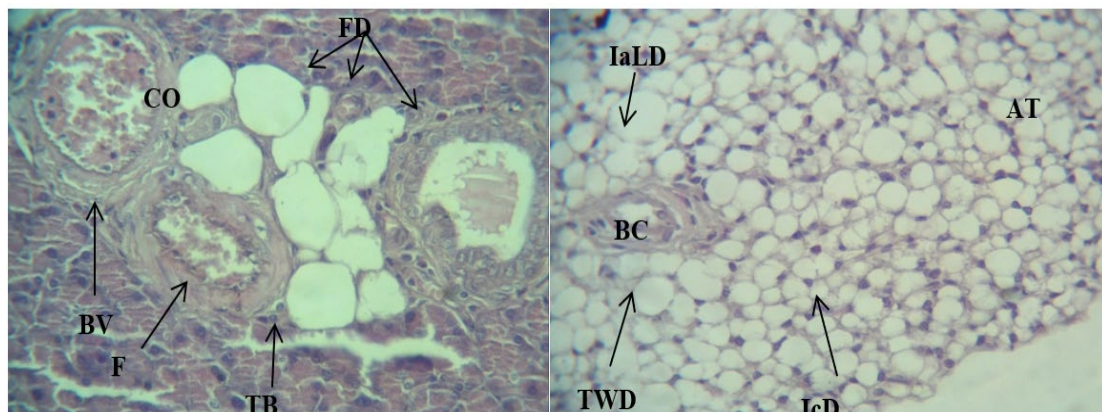


**Figure 4:** Histopathological Section of the Pancreas in a Group of Male Rats in Which Diabetes was Induced and Treated with the Drug Dapagliflozin at a Concentration Of 2 Mg/Kg/Day For 30 Days, Showing the Appearance of Large Fat Droplets (Fd), Congestion Of Large Blood Vessels (Co), Thickening of the Vessel Wall (Tbv), And Blood Clot (Bc). Intra-Pancreatic Duct (Iald), Intralobular Duct Wall Thickness (Twd), Adipose Tissue (Atr) Stained (H&E 40x).

The results of microscopic examination of histological sections of the pancreatic gland of the group treated with the drug Dapagliflozin after suffering from Alloxan-induced diabetes showed expansion of the pancreatic duct within a lobar, the appearance of bulges in its wall, thickening and fibrosis surrounding it, as well as an increase in the distance separating the lobules and congestion of the branch of the pancreatic vein, as shown in Figure (5).

The current results showed that the pancreatic vein was filled with fatty tissue and the appearance of fibrosis around it, as well as congestion of the large blood vessels and thickening of their walls, as shown in Figure (6), in addition to the appearance of nerve bundles in the gland tissue and infiltration of inflammatory cells with the occurrence of a state of rupture in the wall of the blood vessel and its congestion. This is shown in Figure (7).

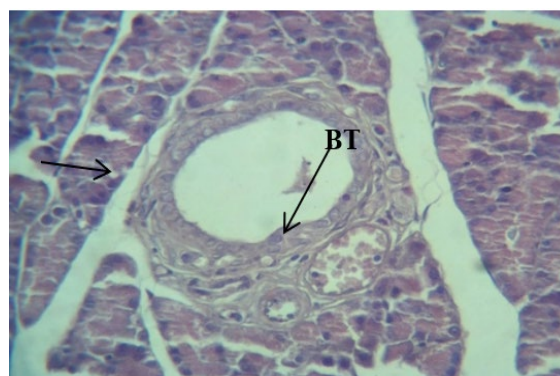


**Figure 5:** A Section Showing the Changes in the Histology of the Pancreatic Gland of Diabetic Male Rats Treated with the Drug Dapagliflozin For 30 Days. The Pancreatic Duct Expands within the Lobule (Iald) and Thickens its Wall (TWD), Congestion of the CO Vein Branch, increases in the Distance of the Septa Separating the Lobes (BT), And Rupture in the Wall of the V Duct, Branch of the Artery Ar. Stained (H&E 40X).

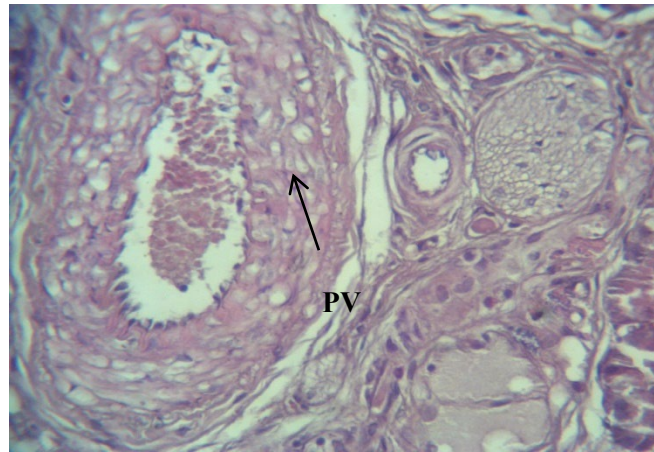


**FIGURE 6:** Part of the Pancreatic Tissue of Diabetic Male Rats Treated with Dapagliflozin at a Concentration Of 2 Mg/Kg/Day, Showing the Filling of the Branch of the Pancreatic Vein with Fatty Tissue (AT), Blood Vessel (BV), Severe Congestion of the Large Blood Vessels (CO), Thickening of the Vessel Wall (TBV), And Fibrosis Around the Blood Vessel. F, Edemae, Severe Swelling of the Vessel Wallv. Stained (H&E 40X).

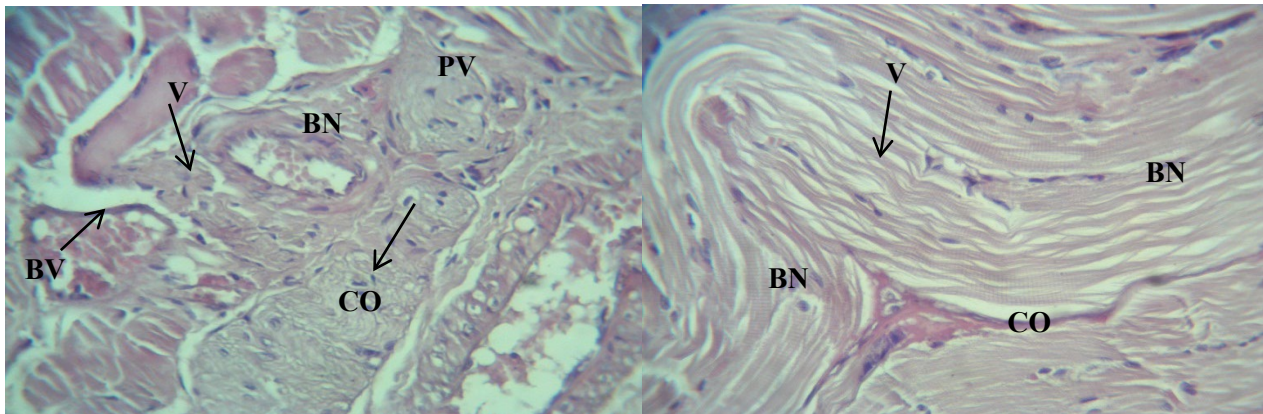


**FIGURE 7:** Shows the Changes in the Pancreatic Tissue of Rats with Induced Diabetes Treated with the Drug Dapagliflozin Throughout the 30-Day Experimental Period. Nerve Bundles BN Inside the Pancreatic Gland, V Bulges in the Blood Vessel Wall and Congests CO, And Bulges Inside the Pancreatic Vein and Congests CO, Pancreatic Vein PV, Blood Vessel BV. (H&E 40X).

## Discussion

The main factor causing diabetes is the loss of function of beta cells located in the islets of Langerhans within the pancreas. However, many studies have suggested that the possible mechanism may be insulin resistance [15], as beta cells constitute about 70% of the total islet cells. Its function is to secrete the hormone insulin, which lowers blood sugar levels [16]. Alloxan, which stimulates diabetes, causes damage to the islets of Langerhans and their enlargement, and thus they do not perform their function as a result of their deterioration [17]. Therefore, a high blood sugar level may lead to the collapse of the islets. pancreas and thus directly damage beta cells [18]. Dapagliflozin is a new synthetic drug (a subclass of gliflozin) that is an anti-diabetic agent for non-insulin-dependent type 2 diabetes. It has the ability to lower the level of glucose in the blood by inhibiting the SGLT2 protein in the renal tubules in order to prevent the reabsorption of glucose and facilitate its secretion in the kidneys. Urine This drug also helps reduce body weight and has significant antioxidant and anti-inflammatory effects, and therefore can be considered as a qualified drug candidate [13, 19] and has been found to have many other additional benefits including reduced weight, blood pressure, and recovery rate. to heart failure and death from cardiovascular causes [20, 21]. The results of the study showed,

through histological sections obtained from a group of male rats with Alloxan-induced diabetes treated with the drug Dapagliflozin, and during the experimental period of 30 days, the appearance of many damages and pathological changes, which are represented by the occurrence of enlargement of the islets of Langerhans, their shrinkage in other sections, and necrosis that occurs within the islets and their constituent cells. Atrophy in the pancreatic cells and the occurrence of edema and rupture within the pancreatic tissue. Bleeding, congestion of the blood vessels, and severe rupture of their walls were also observed. The results also showed thickening of the blood vessel wall and the density of the fibers around it. This agreed with the findings of the studies [18, 22, 23]. They confirmed a decrease in both the numbers and sizes of pancreatic islets, as well as the number of beta cells, irregularity of the pancreatic islets, necrosis of cells in some islets, and rupture of the cytoplasm of some islets.

On the other hand, some islets were completely devoid of cells, and the affected islets showed swollen cells and expansion of the pancreatic duct, with necrosis of the epithelium lining them. Vascular congestion may be due to a defect in the capillaries that feed the pancreas, or it may be due to an increase in systemic blood pressure as a result of an increase in capillary blood pressure, because there is a close connection between diabetes and blood pressure. It has also been proven that people with type 2 diabetes have High rates of high blood

pressure as well [4], in addition to that diabetes is often associated with exacerbation of complications that affect both small and large blood vessels, which lead to congestion. This result is consistent with what was reported [24]. Qadori [25] referred to providing a lot of evidence through which he stated that pancreatic tissue affected by diabetes suffered a shrinkage in the size of the islets of Langerhans, the appearance of necrosis and destruction of beta cells, and a decrease in the number of pancreatic islets. The pathological changes that were found in our study may be due to the fact that hyperglycemia may cause oxidative stress, and this exposes the cellular membranes of the tissues of the animals used (rats), in which diabetes mellitus stimulated an increase in the oxidation of animal fats and inhibition of the activity of antioxidant enzymes, which ultimately works to generate free radicals. ROS as confirmed by a study [26]. The pharmacological effects of SGLT2 inhibitors depend greatly on their

pharmacokinetics, especially their distribution and retention in the target organ, as Dapagliflozin was considered to have a strong effect, based on its pharmacokinetics, pharmacodynamics, and pharmacological results [27].

## Conclusion

The use of the drug Dapagliflozin in this study, which is considered a new class of anti-diabetic drugs of the second type to treat rats with induced diabetes. This drug may work to reduce inflammation and oxidative stress and enhance a small part of the damage to pancreatic cells and tissues caused by diabetes. It is taken orally and is considered one of the inhibitors. SGLT2 works to balance and reabsorption of glucose in the kidneys in rats with type 2 diabetes (T2DM), but it increases fatty tissue in the pancreatic tissue.

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