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## The Effect of Aqueous Extract of Fenugreek Plant on the Pancreas in Rabbits Induced with Diabetes and the Histological Changes Occurring in Them

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### Article Information

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*Fenugreek, Pancreas, White Rabbits, Diabetes*

### ABSTRACT

The study aimed to induce alloxan-induced diabetes in local white rabbits, study its effect on the pancreas, treat them with aqueous extract of fenugreek at a concentration of 200 mg/kg orally, and observe the histological changes. To complete the study 20 white rabbits were taken and divided into three groups; 1st group is the control group and this group was dosed with distilled water. 2nd group was treated with Alloxan and was injected intraperitoneally at a dose of 175 mg/kg. 3rd group is the group that was treated with the aqueous extract of fenugreek at a concentration of 200 mg/kg for 30 days. After the end of the injection and dosing period after 30 days, the animals were killed and the pancreas was removed to perform histological techniques on them. The group treated with alloxan was observed that the pancreas was composed of exocrine glandular lobules with enlarged secretory cells within the pancreatic acinus, with the presence of pale nuclei lacking pigment. Also, blood congestion of the blood vessels was found between the lobules of the gland and the pancreatic gland showed hyperplasia of its glandular epithelial cells with nuclei devoid of pigment due to the loss of the basal pigment in them. Blood congestion of the blood vessels appeared in the gland between the lobules, and dense colloidal fibers appeared in them and contained fibroblasts and phagocytic cells and the group treated with the aqueous extract of the fenugreek plant: The pancreatic parenchyma contained normal exocrine glandular secretion units, where the cytoplasm and nuclei of these cells were located in the form of regular axes, septations between the lobules of the pancreas surrounded by thick-walled arteries containing blood. and the islets of Langerhans appeared between the yeast-secreting lobules of the pancreas. They contained endocrine cells of alpha and beta cells surrounded by blood sinusoids. The interstitial tissue between the lobules is composed of loose connective tissue containing colloidal fibers the pancreatic tissue contained the serous secretory vasculature of yeasts, which were uniform in shape, and between them were found blood vessels excessive with blood, where blood was found in a decomposed form in them, with the presence of small channels inside the lobules that transport yeasts.

### INTRODUCTION

#### Diabetes Mellitus

Diabetes is one of the most common diseases in the world. Approximately three hundred and fifty million patients in the world suffer from it, and their number increases significantly every year. World Health Organization doctors believe that this disease is likely to be the seventh cause of death in the world during the next two decades, with increasing rates of diabetes. It is prevalent in both Asia and Africa for reasons related to nutrition and evidence in developing countries (Li, *et al.*, 2019). It is a chronic disease that affects the human body, making it unable to benefit from the amount of sugar present in the blood, due to the presence of two main reasons, which are: not producing insulin in sufficient quantity or a weakness in the ability of the body's cells to absorb it, and the incidence of this disease increases due to an unhealthy diet, such as: eating ready-made foods in large quantities, or drinking excessively soft drinks, and many others, so people seek to search for multiple and varied methods and methods in order to get rid of this problem, or prevent its development, and many medicinal herbs can Prepare it at home, which helps significantly lower blood sugar levels. (David.2012) Diabetes is a syndrome

characterized by metabolic disorders and high blood glucose concentrations Caused by insulin deficiency due to decreased tissue sensitivity to insulin or both. Diabetes leads to clear and serious complications or even early death, and this disease is characterized by chronic disorders in metabolic processes (Guyton and Hall, 2010). As a result of diabetes, glucose is not converted into energy, which leads to the availability of excessive amounts of it in the blood, while the cells remain thirsty for energy. Over the years, a state of hyperglycemia develops, which causes severe damage to the nerves and heart vessels and small vessel disease, which Retinopathy characterizes with possible loss of vision associated with kidney disease, which leads to kidney failure and then leads to diabetic foot injury and progresses to foot ulcers and then amputation (Poretzky, 2010).

The constant rate of glucose in the blood of a healthy person is about one gram per kilogram of blood (110-170 mg per 100 ml of blood). When the level rises in the blood, the hormone insulin is secreted to convert the excess sugar into glycogen, which is stored in the liver and muscles. (Ifthikhar, *et al.*, 2018) The disease is diagnosed clinically by an increase in the blood sugar level above the normal limit, and sugar appears in the patient's urine

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(diabetes) when its level reaches more than 180 mg/100 ml of blood. This condition is considered a standard or standardized degree standard for diabetics according to the degrees Different types of glucose tolerance (Matboli, S. et al., 2017).

## Diabetes

### Induction of Diabetes

Diabetes was induced by injecting animals after starving them for 18 hours with Alloxan at a concentration of 175 mg/kg body weight (Saikat *et al*, 2008), dissolved in a physiological solution before use, as a single dose under the peritoneal membrane according to the weight of the animal. Then, the animals were provided with food and water directly. Her blood sugar was measured three days after the injection.

### Morphological Description of Fenugreek Plant

Fenugreek is a herbaceous annual plant that resembles clover. It is a dicotyledon and is self-pollinating. Its length ranges between 30-60 cm. Its seasonal winter cultivation is that it is grown interchangeably following summer crops such as cotton and sesame. It is grown in India and Iraq from the beginning of October until January (Kumar. *et al*, 2013).

The stem is cylindrical, hollow, and dark green due to the accumulation of anthocyanins. It appears circular to oval in cross-section, with a diameter ranging between 0.5-1 cm and a length of about 50 cm or more, depending on the environment in which it is grown. It is of two types, the first of which is mono stalk. It does not contain secondary buds. The second type has multi-stalk, in which many secondary buds extend from the base to the upper nodes. As for the root, it is wedge-shaped, 8-30 cm long, and there are many root nodes on it. It is characterized by strength and branches. It has a large number of secondary branches (Prasad, 2011). As for its flowers, they are very small, yellow in color, triangular in shape, and come out in the form of a cluster. They are complete, as they contain the calyx, corolla, stamens, and pistil, and each flower carries between 10-20 seeds. Within 35-40 days, the flower sprouts after being planted. (Acharya, *et al*. 2006) (Petropoulos2002).

Its seeds are green, yellow to brown, or yellow to golden, small in size, with a rhombic, rectangular, or square shape, ranging in length from 3-5 mm and 2-3 mm in width. They are characterized by the presence of a clear groove in one of their corners located between the root and the cotyledon. It requires... Seed germination takes between 3-10 days (McComick, *et al*, 2009). The leaf is pinnate, alternately located, with the stem being cylindrical, hollow, and dark green in color due to the accumulation of anthocyanin. It appears circular to oval in cross-section, with a diameter ranging between 0.5-1 cm, and a length of about 50 cm or more, depending on the environment in which it is grown. It is of two types, the first of which is The stem has a single stalk (mono stalk) and does not contain secondary buds. As for the

second type, it has multi-stalk, in which many secondary buds extend from the base to the upper nodes. As for the root, it is wedge-shaped, 8-30 cm long, and there are many It is one of the root nodes and is characterized by strength. It branches into a large number of secondary branches (Prasad, 2011). As for its flowers, they are very small, yellow in color, triangular in shape, and emerge in the form of a cluster. They are complete as they contain the calyx, corolla, stamens, and pistil; each flower carries 10-20 seeds. Within 35-40 days, the flower sprouts after being sown (Acharya, 2002; Petropoulos, 2006). Its seeds are green, yellow to brown, or yellow to golden. They are small in size, with a rhombic, rectangular, or square shape, and their length ranges between 3-5 mm. Its width is 2-3 mm, and it is characterized by the presence of a clear groove in one of its corners located between the root and the cotyledon, and it requires between 3-10 days for seed germination (Mc Comick , *et al*, 2011).

The leaf is a feathery leaf that is alternately located on the stem, composed of three leaflets, each of which is between 0.8-1.5 cm wide and 1.5-4.5 cm long, and is green in color (Prasad, 2011). After 120-150 days of planting, the fenugreek matures.

This variation in the time it takes fenugreek plants to mature is due to many factors, including the planting date, soil type, and environmental factors such as light and temperature (Petropoulos, 2002).

20 white rabbits were taken and divided into three groups:

### The First Group

The control group. This group was dosed with distilled water.

### The Second Group

The group that was treated with Alloxan and was injected into the peritoneum at a dose of 175 mg/kg.

### The Third Group

The group that was treated with the aqueous extract of fenugreek at a concentration of 200 mg/kg for 30 days.

### Inducing Diabetes in Experimental Animals

The animals were injected with Alloxan prepared at a concentration of 175 mg/kg for a period of 3 days intraperitoneally. Immediately after the injection, the animals were provided with food and a 10% glucose solution to prevent a drop in the level of blood glucose that occurs as a result of the destruction of pancreatic beta cells. As for the control animals, they were given food and water in a regular manner. Sufficient without the use of alloxan (Dubey and Dixit (1994).

Animals with a concentration higher than (300 mg per 100 ml) were considered diabetic, as they showed signs of extreme fatigue, frequent urination, and lethargy (Witek *et al*,2001).

### Preparing the Aqueous Extract of the Fenugreek

The aqueous extract of fenugreek was prepared by

dissolving 50 gm of powdered fenugreek seeds in 1000 ml of distilled water at a temperature of 60°C and leaving the mixture in the shaking incubator for a full day at a temperature of 37°C. Then it was filtered with medical gauze and placed in test tubes in a centrifuge at a speed of 3000 r/min. For 10 minutes, it was then filtered again using filter paper. The liquid was taken and placed in a glass petri dish with a known weight. It was dried in an electric oven at a temperature of 57°C to obtain the crude aqueous extract. Then, weigh the dish containing the dry extract and subtract from it the empty dish's weight to obtain the crude extract's weight. The resulting product

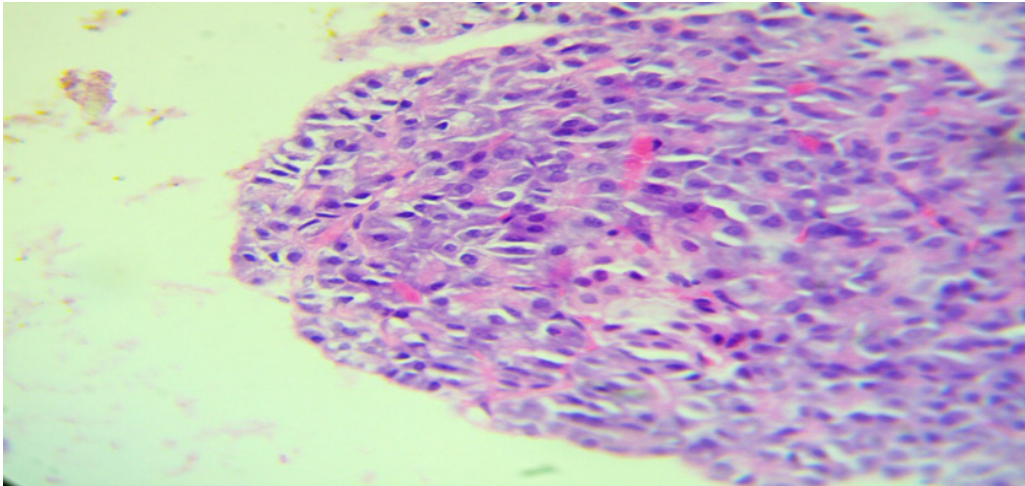
was left to dry, then the weight of the obtained extract was taken and the extract was stored in a tight container until use. (Gradelet,etal,1998).

## RESULTS AND DISCUSSIONS

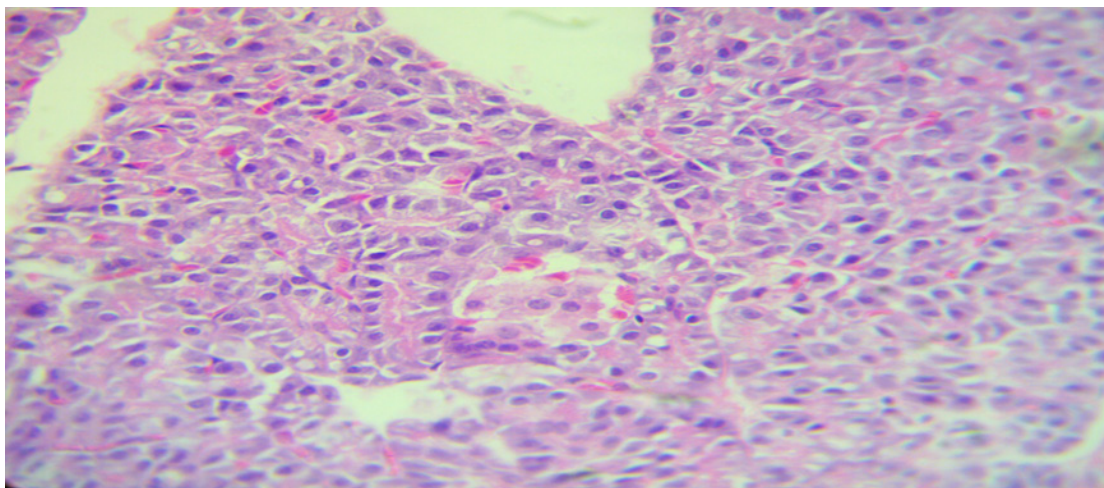
The first group (control group)

### Pancreas

The pancreatic lobules contained glandular epithelial cells in the form of small clusters called yeast-secreting acini, and among these acini were found many small blood capillaries (Figure 1).



**Figure 1:** The control group shows the lobules of the pancreas, in which the glandular secretory acini are surrounded by numbers of blood capillaries (H&EX40)

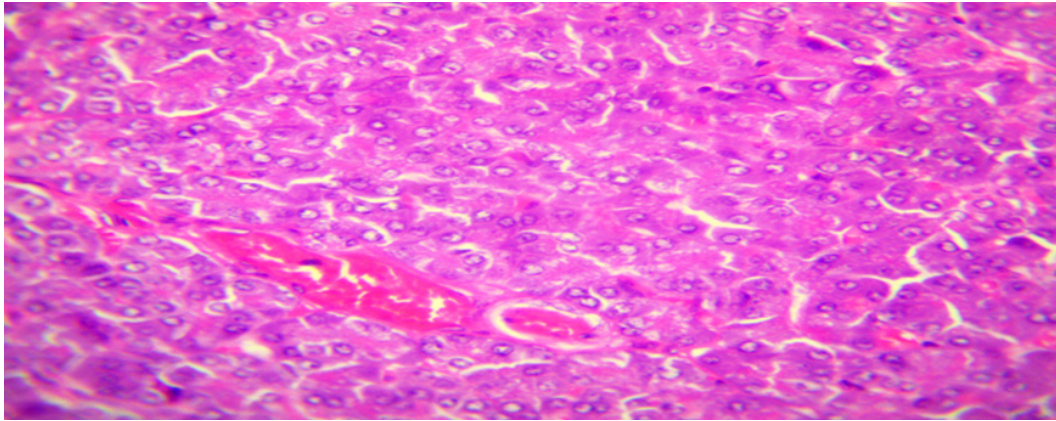


**Figure 2:** The control group shows endocrine cells (beta and alpha) in the islets of Langerhans (A), exocrine cells (B), and capillaries around them (C) (H&EX40)

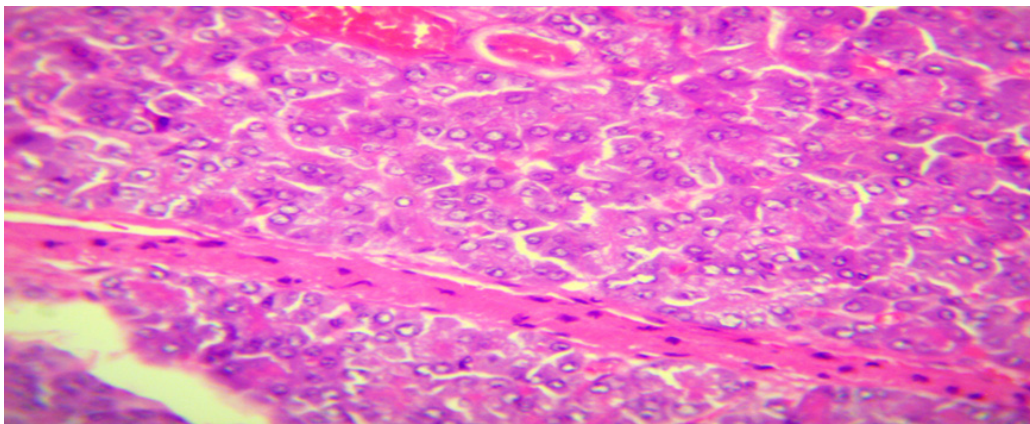
The pancreatic parenchyma in Figure (2) contained the islets of Langerhans, in which some endocrine cells secreting the hormones insulin (beta cells) and the glucocorticoid hormone produced from alpha cells were found, with capillary blood vessels around them. These islets are surrounded by the external secretion of the yeasts found with the islets. The pancreas is normal with no abnormalities (Figure 2) (H&EX40).

### The Second Group (the group treated with alloxan)

It was observed that the pancreas was composed of exocrine glandular lobules with enlarged secretory cells within the pancreatic acinus, with the presence of pale nuclei lacking pigment. Also, blood congestion of the blood vessels was found between the lobules of the gland, Figure (3).



**Figure 3:** The group treated with Alloxan shows the pancreas of the rabbits treated with Alloxan showing hyperplasia of exocrine cells in the pancreatic uvula with their pale nuclei (A), and blood congestion between the lobules (B) (H&EX40)

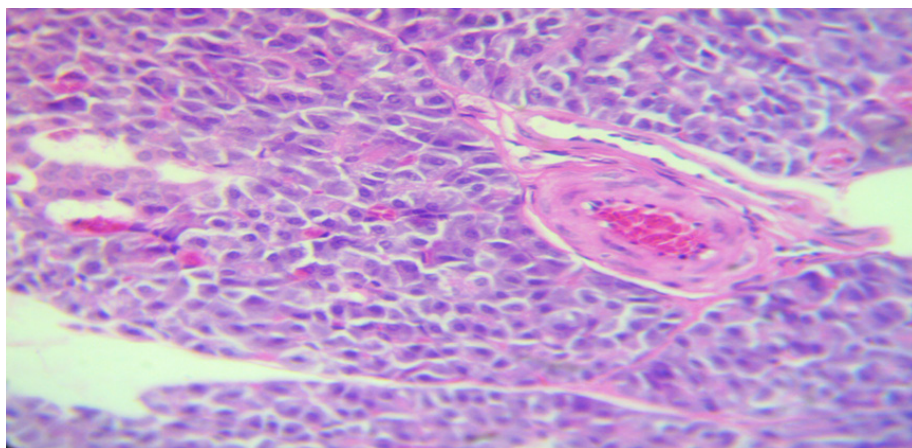


**Figure 4:** The group treated with Alloxan. The pancreatic lobules show hyperplasia of glandular epithelial cells with nuclei devoid of pigment (A), blood congestion of blood vessels (B), septations of dense colloidal fibers containing fibroblasts and phagocytic cells (C). (H&EX40)

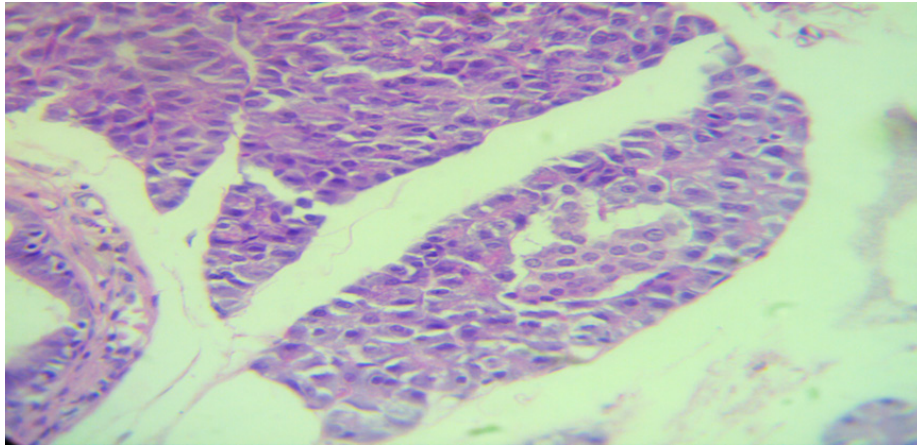
As for Figure (4), the pancreatic gland showed hyperplasia of its glandular epithelial cells with nuclei devoid of pigment due to the loss of the basal pigment in them. Blood congestion of the blood vessels appeared in the gland between the lobules, and dense colloidal fibers appeared in them and contained fibroblasts and phagocytic cells.

**The Third Group (the group treated with the aqueous extract of the fenugreek plant)**

The pancreatic parenchyma contained normal exocrine glandular secretion units, where the cytoplasm and nuclei of these cells were located in the form of regular axes, septations between the lobules of the pancreas surrounded by thick-walled arteries containing blood (Figure 5).



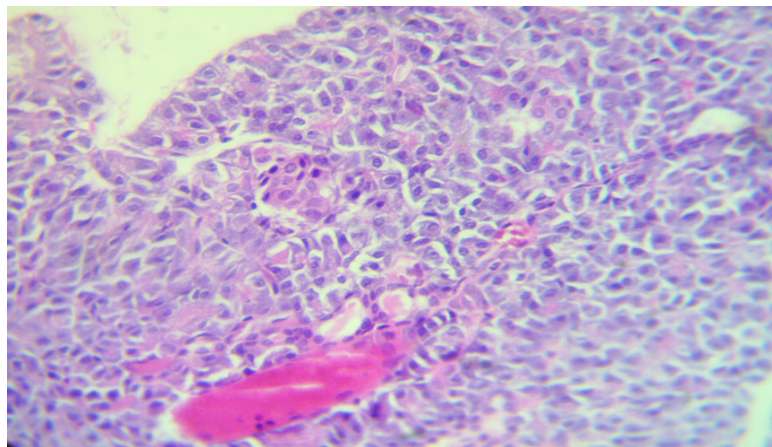
**Figure 5:** Pancreatic tissue. External secretion units (A) are uniform in shape, thick-walled septal artery filled with blood (B) (H&EX40)



**Figure 6:** Pancreatic tissue. Islets of Langerhans (A) surrounded by capillaries (B) Exocrine pancreatic lobules (C) (H&EX40)

As for Figure (6), the islets of Langerhans appeared between the yeast-secreting lobules of the pancreas. They contained endocrine cells of alpha and beta cells surrounded by blood sinusoids. The interstitial tissue between the lobules is composed of loose connective tissue containing colloidal fibers.

As for Figure (7), the pancreatic tissue contained the serous secretory vasculature of yeasts, which were uniform in shape, and between them were found blood vessels excessive with blood, where blood was found in a decomposed form in them, with the presence of small channels inside the lobules that transport yeasts.



**Figure 7:** Pancreatic tissue, exocrine glands (A), blood vessels congested with decomposed blood (B), small ducts transporting yeasts (C) (H&EX40)

## DISCUSSION

Diabetes is one of the diseases that was discovered hundreds of years ago, but its importance from a clinical and epidemiological standpoint is up to now, as it represents an important problem and a major health issue, since all the disorders that accompany it lead to the emergence of severe complications (Walter *et al.*, 1996), and this disease results from a defect in Metabolism of starchy and sugary substances, which leads to a chronic rise in the level of glucose in the blood so that the body gets rid of it by excreting it in the urine. The pancreas gland (pancreas) secretes the hormone insulin, which is responsible for regulating the metabolism of starchy and sugary substances in the body. It performs this function by attaching to specific places on the outer cell membrane to introduce the existing glucose into it so that each cell can carry out its specific function. Many studies and research

have been conducted in various parts of the world on this disease in an attempt to find ways to treat it. Given the failure of transplanting healthy pancreas into the bodies of diabetic patients to be completely successful, immune reactions occur against the transplanted cells. Therefore, science has turned to studying plants and herbs to avoid side effects of medications. And chemical compounds, because they have an effective effect in treating diabetes, and this has been confirmed by many studies, including (2007. Dixit and Chauhan) For the purpose of conducting experimental studies on the use of plant extracts, it is customary to induce diabetes in laboratory animals, and Alloxan and Streptozotocin are among the most commonly used agents in inducing diabetes in experimental animal groups, as the effect of these two drugs lies in causing changes in Pancreatic tissue (Elsner *et al.*, 2000 ; Khalil , 2004), as each of them is characterized by its selective

toxicity to pancreatic beta cells, as Streptozotocin works to impair glucose oxidation, and as a result of its toxic effect, it leads to a defect in the function of beta cells, as alloxan interferes with cellular metabolism. metabolic, as many studies have indicated its role in inducing diabetes as a result of the rapid depletion of beta B-cells in the islets of Lankarhans, and the two drugs cause damage to the DNA, which in turn stimulates the activation of poly ADP - ribosylation and thus leads to the depletion of ATP (Wrsely, 2011 ) and (Vijayanand, 1996), and this was confirmed by both NAD. In addition, Streptozotocin releases toxic molecules of nitric oxide, which contribute to the destruction of DNA, while Alloxan causes primary inflammation of the islets, which is caused by the toxic accumulation of free radicals and causes filtration of macrophage cells and active Lymphocytes in the inflammatory foci. Experimental animals The effect of these two drugs lies in causing changes in the tissue of the pancreas (Elsner *et al* ,2000 ; Khalil , 2004)Each of them is characterized by its selective toxicity to pancreatic beta cells, as Streptozotocin works to impair glucose oxidation, and as a result of its toxic effect, it leads to a defect in The function of beta cells, as Alloxan interferes with cellular metabolism, as many studies have indicated its role in the induction of diabetes as a result of the rapid depletion of beta B-cells in the islets of Lankarhans. The two drugs cause damage to DNA ATP and thus lead to the depletion of poly ADP - ribosylation, which in turn stimulates the activation of NAD + (Wrsely and Vijayanand 1996) (2011), and this was confirmed by both NAD +. In addition, Streptozotocin releases toxic molecules of nitric oxide, which contributes to the destruction of DNA, while alloxan causes inflammation. It is a primary cause of the islets and is caused by the toxic accumulation of free radicals and causes filtration of active cells in the inflammatory foci (lymphocytes and macrophage cells), which leads to a state of hyperglycemia. (skudelski. *Et al*, 2010), (Mbaka, 2001).

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This was confirmed by Wrsely (2011 and Vijayanand 1996). In addition, Streptozotocin releases toxic molecules of nitric oxide, which contribute to the destruction of DNA, while Alloxan causes primary Inflammation of the islets, which is caused by the toxic Accumulation of free radicals and causes filtration of active cells in the inflammatory foci (Lymphocytes and Macrophage cells). Which leads to Hyperglycemia.

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