Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 1 Vol 8 No 1 2023

Article

Effect of Streptozocin on the Langerhans Islands in the pancreas of birds

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Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.01.89

Abstract: In the current study, birds (*Columba livia*) were used as a new model to study the effect of streptozotocin on the pancreas gland and the blood glucose level. Three concentrations of 75,65,55 mg/kg were adopted for five consecutive days with one IP dose daily. The experimental animals showed a gradual rise in blood glucose the average glucose in the first week of the experiment was usual for the three groups compared with the control group, while there was a significant change in the blood glucose level in the three groups at the end of the experiment (4th week), where the average glucose in the streptozotocin groups was 55 mg/kg (213.80 ± 12.43) mg/dl and the group 65 mg/kg(282.60 ± 16.78) mg/dl and a group of 75 mg/kg(371.0 ± 38.39) mg/dl.

Keywords: Streptozocin, Langerhans islands, pancreas.

Introduction

Streptozotocin(STZ)2-deoxy-2-methyl (nitroso) amino carbonyl (amino β –D-glucopyranose) It is a natural chemical that was first isolated in 1960 from the bacteria Streptomyces chromogens [1,2], It has been used in the medical field as a potent alkylating agent for the treatment of pancreatic islet cell carcinoma and gastrinoma malignant[3, 4] It is one of the anti-cancer drugs approved by the Food and Drug Administration, It possesses not only anti-tumor activity but also has anti-bacterial activity through its specific interaction with cytosine, which leads to the degradation and destruction of its DNA [5]. In 1963, it was discovered that streptozocin is a cause of diabetes, as it has a selective toxicity to pancreatic beta cells and their destruction, which leads to insulin deficiency and high blood glucose, which are features of human diabetes. Since then, it has been used in the induction of diabetes in vitro to develop animal models to study diabetes and its complications and treatments. The potential for this [6, 2]. The selective toxicity of streptozocin to pancreatic beta cells is due to the glucose fraction in its chemical structure that enables entry into the cell via the glucose transporter GLUT2 at the plasma membrane (Elsher et al., 2000). The glucose transporter GLUT2 is essential in selective streptozocin toxicity, and this has been demonstrated by resistance to STZ toxicity by beta cells that do not express this transporter (Elsner et al., 2007), as well as the resistance of alpha and delta cells to STZ toxicity due to the absence of this transporter in their membranes. , since beta cells are the most expressive of GLUT2 and most active in taking up glucose, they are the most sensitive and affected by streptozocin toxicity, and liver and kidney cells are also exposed to streptozocin toxicity due to the presence of GLUT2 in their

membranes, and this explains the damage in the liver and kidneys that occurs in degrees Varying in models treated with [7]. Streptozocin affects beta cells via three different pathways, resulting in cell death due to DNA fragmentation [7]. DNA methylation represents the primary mechanism of beta-cell destruction. The chemical structure of streptozocin also contributes as an alternative or additional mechanism of action for destroying beta-cells. Streptozocin contains a nitrogen group and can release toxic nitric oxide in its further action pathway. Reactive oxygen species production is also the extra mechanism of streptozocin toxicity for beta cells[8] Ventura et al., 2011.

Material and method

Experimental animals

In the current study, 20 female and male carrier pigeons(Columba livia) of adult ages and close weight (400-500 g)obtained from the markets of Dhi Qar Governorate were used. After ensuring the birds' safety, they were housed in the animal house of the College of Education for Pure Sciences / University of Dhi Qar in wooden cages under controlled conditions of lighting, ventilation, temperatures and access to water and food.

Experience design

After acclimating the animals for a week, the body weight and blood glucose level were measured using the blood glucose monitoring system and divided into four groups; each group consisted of five animals, as follows:

1. Group A / represents the control group; it was injected(IP) with sodium citrate solution ph(4.5) 2. Group B / after the night fasting were injected(IP) with streptozocin At a dose of 55 mg/kg dissolved in a solution of sodium citrate Ph(4.5), using a 1 ml syringe, for five consecutive days.

3. Group C / after the night fasting was injected (IP) with streptozocin At a dose of 65mg/kg dissolved in a solution of sodium citrate Ph(4.5), using a 1 ml syringe for five consecutive days.

4. Group D/ after the night fasting were injected (IP) with streptozocin At a dose of 75 mg/kg dissolved in a solution of sodium citrate Ph(4.5), using a 1 ml syringe, for five consecutive days.

Preparation of streptozocin solution and injection process

The doses used were determined based on the animal's body weight. In the current study, the concentrations of 55,65, and 75 mg/kg were used for five consecutive days with one injection per day. After the experimental animals fasted overnight, STZ-streptozocin (Medchemxpress, USA) was dissolved in freshly prepared citrate solution Ph 4.5. The container was covered with aluminum foil to protect it from direct exposure to light, after which the experimental animals were injected. Five hours after the injection, a 5% glucose solution was given instead of regular drinking water to overcome the hypoglycemia caused by the high insulin released in the experimental animals. On the sixth day of the experiment, the glucose solution was replaced with normal drinking water with glycemic monitoring using an on-call plus device [6].

Preparation of sodium citrate solution

The solution was prepared by mixing (2.1 g) Citric Acid with (2.94 g) Sodium Citrate and dissolving it in 50 ml of distilled water, adjusting the pH of the solution to 4.5 using sodium hydroxide, then completing the volume to 100 ml.

Histological study

After the end of the experimental period, the animals were anesthetized and dissected according to method [9]; the pancreas was removed to prepare the glass slides for microscopy. The preparation was according to the method [10]. The tissue slide preparation included several steps represented by the fixation process: placing samples in 10% formalin solution, washing, dehydration, clearing, infiltration, embedding, trimming and sectioning, staining using hematoxylin-eosin and loading process using DPX. After completing the preparation of the tissue slides, they were examined and photographed using a light microscope prepared for imaging and by adopting the magnification power, X400, X1000 to know the histopathological changes in the pancreatic gland resulting from the use of streptozocin with different concentrations.

Statistical Analysis

The statistical analysis proceeded in all groups of study; descriptive statistics were analyzed by using an ANOVA(analysis of variations) test with LSD (least significant difference) and were performed using mean and standard deviations (SDs) for continuous variables (p_ value ≤ 0.05) was considered to be significant. All analyses were performed with a Statistical Package for the Social Sciences SPSS for Windows (version 23.0 SPSS Inc, Chicago, 111).

Results

The use of streptozocin in low doses for five consecutive days at a concentration of 55, 65, and 75 mg/kg led to apparent clinical and histological changes; these changes included blood glucose level, body weight, endocrine portion of the pancreas and beta cells in particular, where these doses caused damage Partially of the pancreas led to an inflammatory process that resulted in an increased loss of cellular activity of beta cells. Targeting and destroying beta cells responsible for the secretion of insulin led to a lack of insulin in the blood, which resulted in a defect in the metabolism of carbohydrates, proteins and fats, with a high level of sugar in the blood that appeared late, accompanied by an increase in the number of times of food intake, thirst and weight loss, which is Symptoms of human type 1 diabetes.

The results of the statistical analysis showed that there was no significant difference in the level of glucose in the blood below the probability level ($P \leq 0.05$) in the three groups treated with streptozocin compared with the control group in the first week of the experiment, i.e., on the first and second day after the last injection of the streptozocin. In contrast, there was a difference in Simple significance ($P \leq 0.05$) in the blood sugar level in the second week of the experiment in group D injected with a concentration of (75 mg/kg) compared with the control group and group B, C Table 1. During the third and fourth weeks of the experiment, the three groups treated with streptozocin showed a significant difference ($P \leq 0.05$) in the blood glucose level compared with the control group and the first and second weeks of the injection process for the three groups. Also, group D had the highest glucose level among the other groups, where the average glucose blood (371.0±38.39) mg/dl. Histological examination showed remarkable changes in the morphology of the pancreatic islets of the groups treated with streptozocin, where the islets appeared in irregular shapes with the

presence of visceral gaps and congestion in the capillaries compared to the islets of the control group, which were healthy and of regular shapes(Fig.1A, B, C). The use of streptozocin led to pathological changes in the pancreatic gland, which caused the degeneration and atrophy of the Langerhans islands, which appeared in smaller diameters compared to the control group due to their toxicity and beta-cell damage; it was also observed that its toxicity increased with the increase in the concentration used, The use of 5 doses (IP 75 mg/kg) resulted in more beta-cell damage compared to the group given lower doses. The results of the current study confirmed the selective toxicity of streptozocin to beta cells without any effect on both alpha and delta cells in the Langerhans islands, where streptozocin led to the dissolution and destruction of beta-cell membranes in addition to nucleation and necrosis. There was no effect of streptozocin on the exocrine part of the pancreas in the treated groups compared to the endocrine part that was partially damaged(Fig1D).

Weeks	**Groups**	**Mean ± S.D**	**L.S.D**
First	A control	127.80 ± 8.70[a]	**7.80**
	B (55)mg/km	124.60 ± 5.17[a]	
	C (65)mg/kg	129.60 ± 5.45[a]	
	D (75)mg/kg	131.40 ± 8.23[a]	
Second	A control	131.0 ± 12.90[b]	**9.78**
	B (55)mg/km	129.0 ± 7.77[b]	
	C (65)mg/kg	137.20 ± 7.79[b]	
	D (75)mg/kg	172.20 ± 5.11[a]	
third	A control	128.0 ± 8.74[d]	**12.31**
	B (55)mg/km	166.60 ± 5.27[c]	

	C (65)mg/kg	189.40 ± 8.87[b]	
	D (75)mg/kg	242.20 ± 17.73[a]	
fourth	A control	134.60 ± 8.61[d]	**24.17**
	B (55)Mg/kg	213.80 ± 12.43[c]	
	C (65)mg/kg	282.60 ± 16.78[b]	
	D (75)mg/kg	371.0 ± 38.39[a]	

Results represent mean ± Standard deviation (SD). * Means having different letters in the same column differed significantly (P < 0.05). * (LSD) least significant difference.

Table 1. Blood glucose concentration in birds injected with streptozocin compared with the control group.

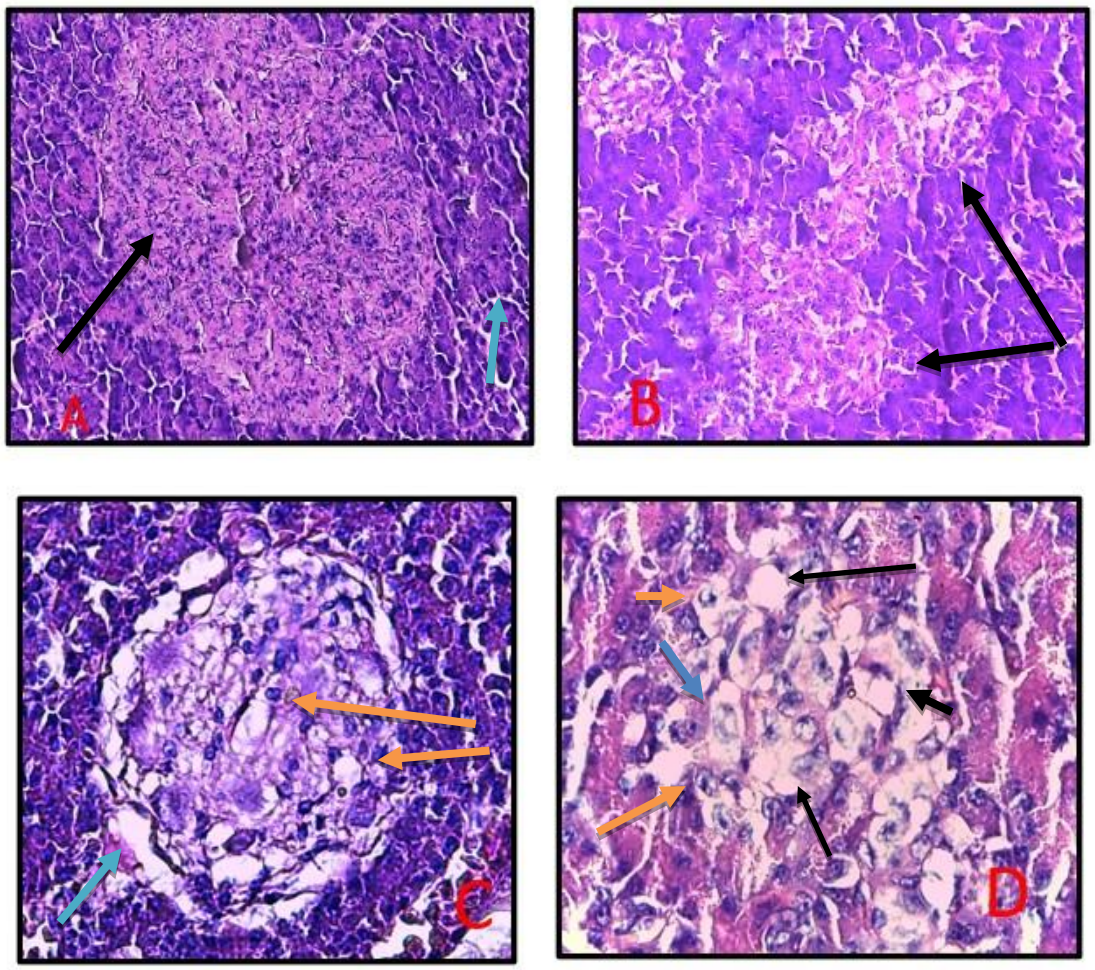

Figure 1. Cross section of pancreatic tissue: (A) control group, the black arrow indicates the Langerhans islands, and the blue arrow indicates the exocrine part. Note the details intact of the Langerhans islets and the absence of any damage;(B),(C), (D) groups treated with streptozocin, (B)note the black arrow, the irregular shape of the Langerhans islands(C); Note (blue arrow) the vacuole and contraction around the Lankerhans island and the visceral vacuoles on the island (Orange

arrow);(D) Note (black arrow) the visceral vacuoles in the Langerhans islet and the nucleus damage in the beta cells(Blue arrow)with intact alpha and delta cells(Orange arrow). (H&E) (100X,1000X).

Discussion

The use of streptozocin in multiple IP doses and at a concentration of 55,65,75 mg/kg caused significant clinical changes similar to human diabetes mellitus, defined as a metabolic disorder characterized by hyperglycemia that develops as a result of defects in insulin secretion or insulin action or both [11]. Hyperglycemia and a significant decrease in the weight of animals were observed with increased food intake and polydipsia. These results were in agreement with [12] and with [13], where they indicated that the use of streptozocin led to the induction of diabetes mellitus and the emergence of statistically significant changes. It represented a decrease in animal weight and increased water and food consumption with glucosuria; the weight loss resulted from the breakdown of structural proteins, lipolysis and oxidation of fatty acids to release byproducts of gluconeogenesis and energy production. High blood sugar results in an imbalance in blood osmosis, which leads to The transfer of water from inside the cells to the surrounding environment, causing dehydration, which is a signal to the osmotic receptors in the brain, resulting in the feeling of thirst and glucosuria in order to maintain osmotic balance [14], It also agrees with [15] which indicated the use of STZ in induction of diabetes in laboratory animals, where the use of this chemical resulted in a decrease in the level of insulin in the blood and an increase in the level of glucose due to the destruction of the beta cells responsible for secretion of insulin. STZ led to hyperglycemia and diabetes mellitus due to low insulin levels, changes that reflect the dysfunction of beta cells, where streptozocin causes beta cell destruction, lysis of secretory granules, and decreased insulin secretion. This was confirmed by examining blood sugar levels and histological examination of the pancreatic sections of groups treated with streptozocin and comparing them with the control group [16]. Streptozocin causes hyperglycemia due to low insulin levels, changes that reflect the dysfunction of beta cells. Streptozocin led to the development of diabetes due to the destruction of beta cells, lysis of secretory granules, and reduced insulin secretion. This was confirmed by the significant changes in the blood sugar level and also through Histopathological examination of pancreatic sections of treated animals and their comparison with the control group [16]. In contrast to the streptozocin-treated groups, Langerhans islands in the control group appeared normal and healthy, as showed by group D (75 mg/kg) significant morphological changes in pancreatic islets, while these changes were slight in groups with low concentration. These results are in agreement with [17] In their study of the effect of streptozocine on the structure of pancreatic islets in mice; they observed the appearance of pancreatic islets in a circular shape to the ovals regularly distributed within the parenchyma of the gland in the control group. At the same time, there were morphological changes in the pancreatic islets of the treated groups directly proportional to the concentration of the dose used. A decrease in the diameter of the pancreatic islets and the number of their cells was observed due to streptozocin destruction of beta cells. This result is consistent with the results of [18] confirmed that histological examination of the pancreatic in the rat treated with streptozocin at a concentration of 75 mg/kg showed the distribution of Langerhans islands at a lower rate as it was in small sizes and irregular shapes with a decrease in the number of beta cells and an increase in the abundance of alpha cells in the streptozocin treated group compared to the control group whose islands were regular and rich in centrally located beta cells. Histological examination showed visceral vacuoles and congestion in the capillaries, and some cells appeared

enlarged, and others lost their cellular details. These results were consistent with [19], which indicated that the use of STZ (75 mg/kg) led to the deterioration of the pancreatic islets and the emergence of Enlarged cells with clumped nuclei, loss of cellular detail and programmed death of some of them with severe congestion of blood vessels. The current study showed that STZ has a destructive effect on the endocrine part only without causing any damage to the exocrine part of the gland and has selective toxicity to beta cells without other endocrine cells. These results agree with [20,] who confirmed that streptozocin is an alkylating agent and is particularly toxic to beta cells. The reason for this is due to the presence of glucose in the chemical structure of streptozocin, which enables it to cross through the plasma membrane of beta cells via the GLUT2, where beta cells are the most expressive of this protein transporter and most sensitive to glucose [7]. Our results also agree with [21,] who confirmed the destructive effect of streptozocin on beta cells through their appearance with vacuolated cytoplasm and degenerated nuclei and indicated the emergence of inflammatory cells among pancreatic parenchyma cells.

Conclusion

The histological study revealed changes in the morphology and diameter of the pancreatic islets in addition to changes and decreases in the number of beta cells for the groups treated with streptozotocin. The streptozotocin group at a concentration of 75 mg/kg was the most affected compared to the other groups. It was also observed that the alpha and delta cells and the exocrine part of the three groups treated with streptozotocin were not damaged

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Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023
Citation: Abd, SJ; .; Faris, S.A. Effect of Streptozocin on the Langerhans Islands in the pancreas of birds. Revis Bionatura 2023;8 (1) 89. http://dx.doi.org/10.21931/RB/CSS/2023.08.01.89