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The use of alloxan to induce diabetes in rabbits and some of the changes it causes in the body

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Alloxan is frequently used to induce experimental diabetes in animals. Furthermore, by altering the dosage of administered alloxan, it has been extensively utilized to cause experimental diabetes with varying degrees of the disease severity in animals, including dogs, rats, mice, and rabbits. Alloxan significantly and dose-dependently increased blood glucose levels in rabbits. The study's objectives are to examine the effects of alloxan time effect, changes in pancreatic histopathology, and blood glucose monitoring, administer the preparation to a diabetic rabbit model. Adult local breed rabbits were provided by the College of Kerbala Veterinary Medicine Laboratory Animal Center, where the experiment was conducted. To investigate the animals, they were divided into 2 groups. The rabbits of the first (experimental) group (n=5) received an injection of alloxan monohydrate at a dose of 150 mg/kg body weight. The rabbits from the second group, the control group (n=5), were given an injection of the same volume of isotonic saline. The studies showed that before the start of the experiment, the blood glucose levels of the first and second groups were practically the same, 108.2±146 and 113.0±7.5 mg/dl, respectively. A week after the administration of alloxan monohydrate, the rabbits from the first group showed a sharp increase in glucose levels by 2.9 times ($P<0.05$) compared to the same indicator before the start of the experiment, which was also 2.8 times higher ($P<0.05$) than the indicator in the control group of rabbits. It was found that the glucose level in rabbits from the first experimental group during 2, 3, and 4 weeks of the study remained quite high, above 261 mg/dl, which was significantly higher ($P<0.05$) compared to the indicator before the start of the experiment and compared to the similar indicators in the control group. According to histological pancreas studies from the rabbits treated with alloxan monohydrate, characteristic changes were observed, indicating a negative effect of the administered drug on the cells of the studied organ. In particular, a month after the administration of alloxan monohydrate, dystrophic changes in the population of β-cells were detected in the study of pancreatic islets of Langerhans, characterized by vacuolization of the cytoplasm, pyknosis and karyolysis of the nuclei.

Keywords: alloxan, diabetes, rabbit, blood glucose, histopathology.

Використання алоксану для індукції діабету та зміни в організмі кролів за його застосування

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Алоксан часто використовується для індукції експериментального діабету у тварин. За рахунок введення різних доз алоксану науковцям вдається експериментально відтворити у тварин (включаючи собак, щурів, мишей та кролів) діабет з різним ступенем тяжкості захворювання. Мета дослідження полягала у встановленні змін вмісту глюкози у сироватці крові кролів за впливу алоксану та визначенні гістопатологічних змін підшлункової залози у кролів з алоксан-індукованим діабетом. Для проведення дослідження кролі були надані Центром лабораторних тварин Ветеринарної медицини Коледжу Кербали, де й проводився експеримент. Для дослідження тварин розділили на дві групи по 5 голів у кожній. Кролі першої групи (дослідні) отримували алоксану моногідрату в дозі 150 мг/кг маси тіла. Кролям другої групи (контрольної) вводили у тій же дозі ізотонічний розчин. Дослідженнями встановлено, що до початку експерименту вміст глюкози у сироватці крові першої та другої групи кролів був практично однаковим і становив 108,2±146 та 113,0±7,5 мг/дл відповідно. Через тиждень після введення алоксану моногідрату у сироватці крові кролів другої групи спостерігалося різке підвищення рівня глюкози в 2,9 раза ($P<0,05$) порівняно з аналогічним показником до початку експерименту та у 2,8 раза ($P<0,05$) – порівняно з показником у контрольній групі тварин. Визначено, що вміст глюкози у сироватці крові кролів другої дослідної групи протягом 2-х, 3-х та 4-х тижнів дослідження залишався досить високим, понад 261 мг/дл, що було значно вищим ($P<0,05$) порівняно з показником до початку експерименту та порівняно з показником у контрольній групі тварин. Відповідно до проведених гістологічних досліджень підшлункової залози кролів з алоксан-індукованим діабетом виявлено характерні зміни, що свідчили про негативний вплив введеного препарату на паренхіму органу. Зокрема, через місяць після введення алоксану моногідрату при дослідженні острівців Лангерганса були виявлені дистрофічні зміни в популяції β-клітин, що характеризуються вакуолізацією цитоплазми, пікнозом та каріолізисом ядер.

Ключові слова: алоксан, діабет, кролі, вміст глюкози, гістопатологія.

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Introduction

A metabolic disease with several etiologies, diabetes mellitus is characterized by problems in the metabolism of proteins, lipids, and carbohydrates as well as persistently high blood sugar levels caused by deficits in either insulin production, action, or both [1].

Diabetes is not a single disease, but rather a group of various syndromes, such as peripheral vascular disease, heart attacks, and strokes [2]. Ketoacidosis, hyperosmolar coma, macro- and microangiopathy, nephropathy, neuropathy, and recurring infections are among the acute and long-term consequences linked to diabetes. These complications are the consequences of the disease, which cause death as a result of diabetics [3].

Animals are commonly given alloxan to induce experimental diabetes [4]. Moreover, the preparation has been widely used to induce experimental diabetes with different levels of disease severity in animals such as dogs, rats, mice, and rabbits, by varying the given dose of alloxan [5]. Diabetes mellitus, or DM, is a metabolic disease that changes the metabolism of proteins, fats, and carbohydrates. It continues to be a global public health concern and can cause neurological, cardiovascular, and nephropathy problems in humans [6]. As type 1 diabetes is characterized by a total absence of insulin, it is also known as insulin-dependent diabetes mellitus. Viral invasion, chemical toxins, or autoimmune antibodies can all cause β-cells destruction. This β-cells necrosis causes insulin insufficiency and type 1 diabetes [7]. Type 2 diabetes, also known as noninsulin-dependent diabetes mellitus, is frequently linked to insulin resistance in the target organ, which limits the body's ability to respond to both endogenous and exogenous insulin [8]. Type 3 diabetes is caused by chronic pancreatitis or by long-term medication therapy with growth hormone, thiazide diuretics, diazoxide, glucocorticoids, and some protease inhibitors (such as saquinavir) [9]. About 4–5 % of pregnancies result in type 4 diabetes, which is brought on by placental hormones that encourage insulin resistance [10].

The aim of the study

The study's objectives are to examine the effects of alloxan administration time, changes in pancreatic histopathology, and blood glucose monitoring, administer the preparation to a diabetic rabbit model.

Materials and methods

The Laboratory Animal Center of the College of Kerbala Veterinary Medicine provided adult rabbits, which weighed between 1.4 and 1.6 kg and were clinically healthy. All animals were male in order to reduce the impact of hormones on the responses. The animals were from a private rabbitry and were 12 months old at the beginning of the experiment. In an animal house with a natural photoperiod and sufficient ventilation, the animals were free to roam around the Department of Physiology. As the animals acclimated to the housing and experimental protocols, they were provided with free access to fresh lucerne, high-concentration feed, and tap water for two weeks. A thorough clinical assessment was carried out in 2018 before and during the experiment.

The experimental animals were divided into two groups, five in each, and they were randomly assigned to either the negative control group or the diabetic treatment group. A (150 mg/kg body weight) injection of alloxan monohydrate was administered to the rabbits in the experimental group. The control group received an injection of isotonic saline in the same volume.

Induction of diabetes in rabbits

The rabbits were given a single intravenous injection of 150 mg/kg body weight of alloxan (Sigma, St. Louis, MO) through the marginal ear vein following a 16-hour fast. Over the course of two minutes, the physiological saline (0.9 % NaCl) was used to dissolve the injection, resulting in the animals' developing diabetes mellitus [11].

After receiving the alloxan injection, the animals were given food right after to avoid hypoglycemia shock and mortality during the hypoglycemic phase.

Additionally, a water bottle containing a 5% glucose solution in tap water was supplied for the following twenty-four hours. Three days following the alloxan injection, hyperglycemia (blood glucose >200 mg/dl) was found, confirming diabetes mellitus. Animals were classified as hyperglycemic if their blood glucose levels were higher than 170 mg/dl but lower than 400 mg/dl [12].

The control group received an injection of the same volume of isotonic saline.

Before being administered, the fasting blood glucose levels of each rabbit were measured three times, every other day, between 8:00 and 8:30. The base value was the mean value. To verify the glucose level reading, the blood extracted from the marginal ear vein was analyzed. Four-week intervals were used to collect the blood samples.

Biochemical analysis

A glucometer was used to conduct the study. The glucometer was used according to the instructions:

1. Before beginning any measurements, zero out the scales;
2. Inserting a fresh test strip into the meter;
3. A large blood drop was extracted from the peripheral vein;
4. Verifying that the meter is ready, then putting the blood onto the test strip and waiting for five to ten seconds. The result should be available in ten seconds, and frequently sooner.

Histopathological examination

On the 30th day of the experiment, the animals were killed, and their pancreases were removed, preserved in 10 % formalin, and ready for paraffin embedding. The tissues were divided into 6 mm thick slices using a

microtome. The slices were embedded in paraffin wax after being cut into 5 µm sections.

The sections were stained with hematoxylin and eosin and then mounted in Canada balsam. The standard procedure for histopathological evaluation was followed [13].

Statistical analysis

Duncan's multiple comparison tests, two-way ANOVA, and the two-tailed Student's t-test were used to study the data, which was provided as mean ± standard deviation (SD). The $P<0.05$ criterion was used to acknowledge statistical significance. Version 17 of SPSS, or the statistical software for the social sciences, was employed [14].

Ethic Statement

The research was approved by the Ethics Committee of the University of Kerbala, College of Veterinary Medicine, under the number UOK.VET.PA.2025.169.

Results and discussion

Blood glucose level

As a result of the conducted studies it was found that during the experiment (4 weeks), the glucose level in the blood of the rabbits that did not receive alloxan did not differ significantly and ranged from 105.2±17.2 to 116.8±11.5 mg/dl (***Table 1***).

The results of the study showed that when rabbits received 150 mg/kg IV of alloxan, they could develop diabetes. One week following the administration, the blood glucose level in the treated group was noticeably higher. Thus, on the 7th day of the study, the glucose level tended to increase sharply by 2.9 times compared to the same indicator before the start of the experiment ($P<0.05$). It was also found that the glucose level in the animals receiving alloxan was 2.8 times higher than the indicator in the control group on the 7th day of the study.

Table 1

The effect of alloxan on serum glucose levels in rabbits, mg/dl, n=5, (mean±SD)

Week	The control group	The treated group
0	108.2±14.6a	113.0±7.5a
1	116.8±11.5a	326.0±2.6b
2	112.0±11.9a	296.0±2.3b
3	105.2±17.2a	281.0±14.0b
4	113.2±18.4a	261.0±9.9b

Note: in a data table, values sharing the same letter are not statistically different, while different letters denote a significant difference ($P<0.05$).

In the following weeks, on the 2nd, 3rd, and 4th, a slight decrease in glucose levels was observed by 9.2, 13.8, and 19.9 %, respectively, in comparison with the indicator on the 7th day of the experiment. At the same time, in rabbits that received alloxan, the glucose level remained higher by 2.6, 2.5 and 2.3 times ($P<0.05$) in comparison with the indicator on the corresponding days before the start of the experiment (***Fig. 1***)

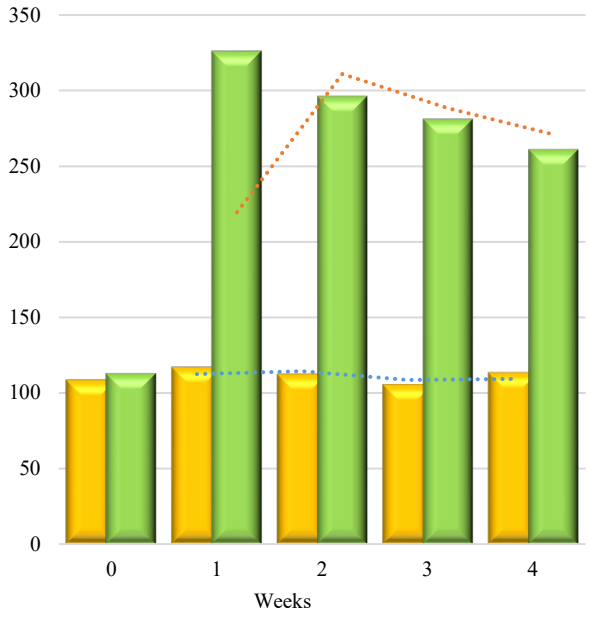

Fig. 1. The dynamics of changes in blood glucose levels in rabbits during the experiment.

It was also found that the glucose level in the rabbits treated with alloxan, compared to the control animals, was higher by 2.6, 2.7, and 2.3 times on the 2nd, 3rd, and 4th weeks, respectively ($P<0.05$).

Histopathological changes

The control group rabbit's pancreatic islet of Langerhans showed normal β-cells in histological analyses of the pancreatic endocrine region with pale rounded and ovoid β-cells in the center (yellow arrow) (***Fig. 2***).

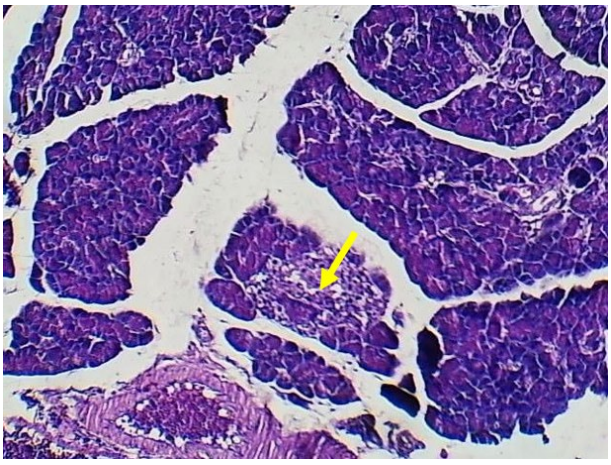

Fig. 2. Shows normal β-cells of pancreatic islet of Langerhans of the control group rabbit (H&E×100).

A damaged β-cell population was found by necrosis of β-cells with vacuolation of cytoplasm (the red arrow) and pyknotic (the orange arrow) and karyolysis (the blue arrow) of the nuclei, which is comparable to the results of the alloxan-diabetic rabbits (***Fig. 3***).

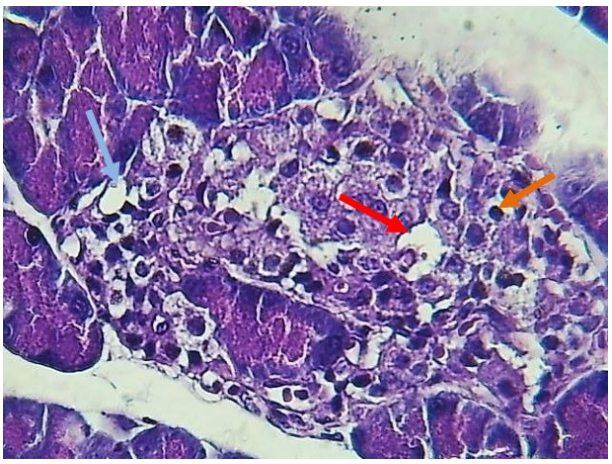

Fig. 3. Shows the destruction in pancreatic β-cells in the rabbits treated with alloxan (H&E×400).

Chemicals like alloxan are frequently used to cause diabetes in animal models. The selective absorption of lethal glucose analogs is facilitated by the beta cell's GLUT2 glucose transporter, which is one of the mechanisms by which they cause diabetes. Alloxan causes reactive oxygen species, which results in beta-cell necrosis. Therefore, when dissolving and giving alloxan to many animals at once, the induction rate can be greatly affected. The effect on the rate of DM induction is lowered when alloxan is administered freshly dissolved [15]. Mouse models of intra-islet regeneration have been demonstrated using the partial pancreatectomy paradigm and the pancreatic duct ligation and alloxan administration model [16, 17]. For instance, one research reported that 24 SD rats got diabetes but started to recover on their own two weeks after the induction, but three of the 27 SD rats that had been given 120 mg/kg of alloxan intraperitoneally died. The 24 diabetic rats in this study survived for 20 days without dying since they were not given insulin treatment. Insulin-positive islets were found in the pancreas by immunohistochemical analysis, indicating that there were a sizable number of islets that were still there and probably regenerated [18]. Groups of five SD rats were given intraperitoneal dosages of 120, 150, and 180 mg/kg of alloxan in a separate investigation. The greatest induction failure rate was 120 mg/kg, the highest mortality rate was 180 mg/kg, and the highest induction failure rate was 150 mg/kg. Within 2-4 weeks, four rats in each of the three dose groups showed signs of self-recovery from diabetes [19].

The chemical called alloxan monohydrate is used to induce diabetes mellitus. The partial loss of the β-cells in the islets of Langerhans causes diabetes. It has been discovered that alloxan targets GLUT2 [16], and glucose transporter (GLUT2) specifically absorbs it into the β-cells [15]. Hyperglycemia occurs as a result of the tissues' inability to absorb and use glucose as well as the liver's and skeletal muscles' inability to store glycogen [17].

High blood sugar levels could promote apoptosis. This leads to potential cellular damage as a result of hyperglycemia from diabetes [18]. In conclusion, this study demonstrated that in the treated groups, blood glucose levels obviously increased (diabetes) when alloxan was given intravenously to rabbits. The most common method of simulating diabetes is to use chemical preparations, such as alloxan, to specifically destroy the beta cells in the pancreas that secrete insulin [20–25].

Conclusions

The studies have shown that aloxan monohydrate injection at a dose of 150 mg/kg body weight to rabbits causes diabetes, as evidenced by a 2.9-fold increase in glucose levels ($P<0.05$) compared to baseline. Glucose levels in the rabbits that received an alloxan monohydrate injection remained consistently high, above 261 mg/dl, throughout the 4 weeks of the study, which was significantly higher ($P<0.05$) than the level before the beginning of the experiment. The adverse effect of alloxan monohydrate is confirmed by histological changes in the pancreatic islets of Langerhans of rabbits, characterized by β-cell necrosis with vacuolation of the cytoplasm, pyknosis, and karyolysis of the cell nuclei.

Conflict of interest

The author (s) state that there is no conflict of interest.

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