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Methods to Reduce the Hypoglycemic Mortality of Alloxan in Diabetic Rabbit Model

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Abstract

Objective: To explore an intervention method to reduce the mortality of alloxan diabetes model, and to preliminarily analyze the mechanism of alloxan induced animal death. **Methods:** Healthy New Zealand rabbits were randomly divided into injection group, control group, experimental group and blank group. The single injection group was injected with 100 mg/kg alloxan once. The control group was given 5% glucose solution and 100 mg/kg alloxan was injected in two times. The experimental group was given 5% glucose solution orally, 100 mg/kg alloxan, 7 mL 0.9% NaCl intravenously and 5 mL 5% glucose intraperitoneally immediately, and blood glucose was continuously monitored, 10 mL 5% glucose intravenously and 10 mL 5% glucose intraperitoneally every 4 h in the hypoglycemic stage. The blank group does nothing. Liver and kidney tissues at different time periods were stained with HE and organ index was evaluated. **Results:** 1) A single injection of 100 mg/kg alloxan without any intervention resulted in 100% mortality. Before modeling, oral administration of 5% glucose solution, divided into two injections of 100 mg/kg alloxan, mortality reached 100%; A single injection of 100 mg/kg alloxan and continuous intervention of normal saline and glucose for 20 h can significantly reduce the mortality of alloxan induced diabetic rabbit model. 2) Liver and kidney tissues were damaged in different degrees at different time periods, and liver and kidney indexes were significantly increased after alloxan injection compared with the normal group, with statistical significance ($P > 0.05$). **Conclusion:** 1) Every 4 hours of hypoglycemia, 10 ml 5% glucose was injected intravenously + 10 ml 5% glucose intraperitoneally. It can reduce the death rate of alloxan diabetic rabbit model and shorten the time of blood glucose measurement. 2) After the injection of alloxan, acute lesions of liver and kidney may occur in different degrees, or one of the causes of acute death of experimental animals.

Keywords

Alloxan, Diabetes, Model, Rabbit

1. Introduction

The incidence of diabetes mellitus (DM) is increasing worldwide. Complications such as Diabetic nephropathy, diabetic retinopathy and Diabetic foot ulcer (DFU) bring irreversible damage to patients, and the healthcare system is faced with a heavy burden [1]. DFU is a common and serious complication of diabetes without effective clinical intervention, and its pathogenesis is complex and related to multiple signaling pathways [2]. Therefore, the application of animal models in the basic experimental research of DM becomes particularly important.

Alloxouracil is often used as a chemical drug to construct DM models, and has been successfully constructed in rats, mice, rabbits and other animals [3] [4] [5] [6]. Due to its strong toxicity, the survival rate of rabbits with the same route of administration, gender, weight and age is not consistent, and the specific mechanism of death is unclear. Most of the articles did not mention the dose used and the treatment done during modeling, for example, the rabbit diabetes model was constructed with 80mg/kg alloxouracil, and the mortality rate was 75%, and the mortality rate was 100% when the dose was increased to 150 mg/kg, or vague reports such as "abnormally low mortality" and "no significant mortality" were given [7] [8] [9].

Alloxan injection has three characteristic stages (hyperglycemia-hypoglycemia-hyperglycemia), hypoglycemia is the destruction of islet beta cells by alloxan, a large amount of insulin into the blood, if no intervention, the death rate is as high as 100%. Bacevic *et al.* [10] administered 100 mg/kg intravenous injection (iv), monitored blood glucose every 1 h/time, recorded BFL <100 mg/dL, and intraperitoneally injected 5 ml 5% glucose solution (ip). Record value BGL < 50 mg/dL, intravenous (iv) 5 ml 5% glucose solution, this intervention can reduce the mortality of rabbits to about 8% [7]. Bacevic [10]'s intervention method requires 36 h continuous glucose monitoring, which consumes a lot of time and resources, and is difficult to reproduce in realistic model making. Therefore, based on the above intervention methods, we aim to propose a modeling method that can not only guarantee the success rate of diabetes model, but also greatly shorten the blood glucose monitoring time, and preliminarily explore the lethal mechanism of alloxouracil, so as to provide strategies for researchers to carry out basic research on DM.

2. Materials

2.1. Drugs and Materials

Alloxouracil, domestic Sanuo blood glucose meter, Sanuo GA-3 blood glucose

test paper (manufacturer: Zhuoyi Biochemical Co., Ltd.); 0.9%NaCl injection, specification: 100 ml/bottle (Manufacturer: Guangxi Yihe Medical Co., Ltd.); Penicillin sodium, specification: 400,000 U/bottle (manufacturer: Anhui Fengyuan Pharmaceutical Co., Ltd. Huaihai Pharmaceutical Factory); 95% alcohol (manufacturer: Guangxi Yihe Medical Co., Ltd.); Iodophor (Maoming Disinfection Supplies Factory Co., Ltd.); 5% glucose, specification: 500 ml/bottle (Henan Shuanghe Huali Co., Ltd.); Insulin injection, specification: 400 u/box (Manufacturer: Jiangsu Wanbang Biochemical Pharmaceutical Group Co., Ltd.

2.2. Laboratory Animal

Fifty-two 6-month-old healthy New Zealand rabbits were purchased from Guangxi Tiandong Longxiang Rabbit Industry Co., Ltd. (Qualification certificate No. 0009810), weighing about 2.0 - 3.0 KG. The experiments were all conducted in the Animal Experimental Operation Room of Youjiang Medical College for Nationalities (license No. SYXK GUI 2017-0004), the experiment was approved by the Animal Ethics Committee of Youjiang Medical College for Nationalities (approval number: 2021082501). All animals were routinely and adaptively fed for one week, the light was kept in circadian rhythm for 12 hours, the temperature was controlled at 24˚C - 26˚C, and the relative humidity was controlled at 50% - 70%, in accordance with the "3R principle" of experimental animals. Fifty-two experimental animals were randomly divided into four groups according to random number table method: A single injection group (n = 13, A1 - A13); B Control group (n = 13, B1 - B13); Experimental group C (n = 13, C1 - C13); D Blank group (n = 13, D1 - D113).

3. Treatment of Laboratory Animals

3.1. Preparation before Molding

The blood sugar of the four groups of experimental animals was measured before modeling to ensure that the blood sugar of all animals was normal before the experiment. B and C were given grape solution orally before surgery to antagonize excess insulin, overnight; Group A and D were given regular diet without special treatment.

3.2. Establishment of Diabetes Model

The preparation of the diabetes model was established according to Bacevic's [10] method: routine iodophor disinfection and 95% alcohol wiping filled the rabbit ear border vein. Group A received a single dose of 100 mg/kg alloxouracil without intervention. The B component was twice iv100 mg/kg alloxouracil, 1/2 of the total amount injected in 1d, and the remaining dose was injected again in 2 d. Group C single dose iv100 mg/kg alloxouracil. Group D is not processed.

3.3. Liver and Kidney Staining

The experimental animals were dissected, liver and kidney tissues were taken

out, and the tissue changes were observed by HE staining.

3.4. Statistical Analysis

Data were analyzed by GraphPad 8.02 statistical software, and the survival rate of different groups was compared by Logrank test for trend analysis. The survival rate between groups was compared by Gehan-Breslow-Wilcoxon test. By P< 0.05 was considered statistically significant.

4. Result

4.1. Animal Survival Rate

The blood glucose values of all rabbits were in the normal range before modeling. Experimental animals in group A began to die successively after 6 h injection of alloxouracil, and all died on the 2nd day. About 6 h after the first injection of alloxouracil, 3 rabbits in group B showed symptoms such as lethargy and shortness of breath. Three rabbits showed symptoms such as limbs twitching, dilated pupils and mania. Four more rabbits died; All died in 2 d. Experimental animals in group C were given 7 ml normal saline and 5 ml ip 5% glucose immediately after iv injection of alloxouracil to reverse hypoglycemia. After that, iv 10 ml 5% glucose + ip 5% glucose every 4 hours until the hypoglycemic stage was passed, only 1 animal died. No abnormality was found in the experimental animals in group D.

Team A is dead. All rabbits in Group B died within 2 days. In group C, 12 animals survived after 48 hours of induction. Blood glucose was measured for the first time after 6 hours of injection of alloxouracil, and it was found that one animal (C6) showed decreased blood glucose with a blood glucose value of 6.0 mmol/L (>100 mg/dl). 5 ml of 5% glucose solution was injected intraperitoneally according to Bacevic *et al.* [6]. 4 h later, we found that the blood glucose value dropped to 1.3 mmol/L, and symptoms such as malaise and limp limbs appeared. The patient was given 10 ml 5% glucose solution urgently in iv, and 1.1 mmol/L blood glucose was remeasured 4 h later, and 10 ml 5% glucose solution was given again, and finally died. Experimental animals in group D showed no abnormality, as shown in **Table 1**.

4.2. Hyperglycemic Phase

At 4 h after administration, the blood glucose level of all animals in group C increased, and the increase value of different animals was significantly different, with the highest being 30.3 mmol/L and the lowest being 6.0 mmol/L (rabbit numbered C6). At this time, C6 had entered the hypoglycemic stage, so we inferred: Rabbits will enter a hypoglycemic phase 6 h after injection of alloxan, and the hyperglycemic phase will not lead to death in rabbits.

4.3. Hypoglycemic Phase

The hyperglycemic phase is followed by the second phase, the hypoglycemic

phase, where glucose levels gradually decrease. We treated C6 according to previous studies: whenever the recorded value was below 100 mg/dL, 5% glucose solution (5 mL) was injected according to the individual blood glucose level (BGL) at the time of measurement; If you measure BGL < At 50 mg/dL, an iv injection of 5% glucose solution will be given; Such treatment did not make C6 recover, but remained in the hypoglycemic stage and eventually died.

For the remaining 12 rabbits, we measured the blood glucose value every 4 hours in the next 20 h, and gave iv 5% glucose solution 10 ml + ip 5% glucose solution 10 ml. For rabbits with a large decrease span of blood glucose value (>10 mmol/L) or blood glucose value below 4 mmol/L, Appropriate addition of 5 - 10 ml 5% glucose solution could be made, continuous monitoring for 20 h, and 10 ml iv 5% glucose solution + 10 ml ip 5% glucose solution was given again at 20 h. No symptoms such as malaise and limp limbs were found, and the hypoglycemic period was safely passed, as shown in Figure 1.

Table 1. Survival of three groups of experimental animals within 2 d after modeling.

Group	Death	Survive	Survival rate (%)
A	13	0	0
B	13	0	0
C	1	12	92.3
D	0	13	100%

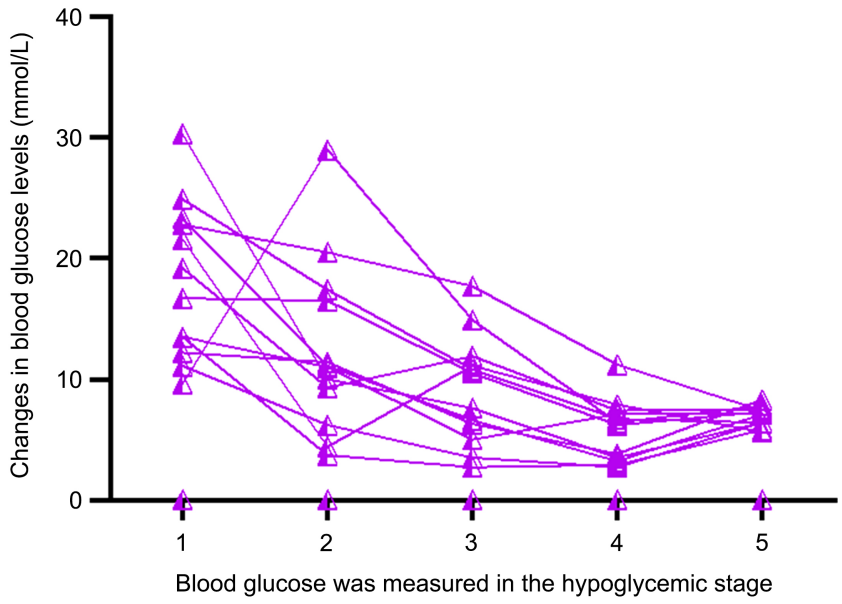


Figure 1. Changes of blood glucose in the hypoglycemic stage. 1) 1 is the hyperglycemic stage, and 2 - 5 is the blood glucose measured every 4 hours in the hypoglycemic stage. 2) Each line segment represents the variation trend of blood glucose values measured in the hypoglycemic stage of an animal.

As can be seen from the figure above, all rabbits entered the hypoglycemic stage within 6 hours. At the first time of hypoglycemia, each rabbit was given 10 ml iv 5% glucose solution + 10 ml ip 5% glucose solution. The blood glucose value showed a downward trend from the stages 2 - 4, but the decline was slow, and the blood glucose value of some rabbits increased. By the 5th blood glucose test, the blood glucose value of all rabbits tended to be stable. After iv 10 ml 5% glucose solution + ip 10 ml 5% glucose solution again, overnight observation; In 2 d, no experimental animals were found dead, and no symptoms such as lethargy and limp limbs were found.

4.4. Persistent Hyperglycemic Phase

After 20 h, iv 10 ml 5% glucose solution + ip 10 ml 5% glucose solution was given overnight. The blood sugar was measured again the next day, and it was found that all the animals passed the hypoglycemic stage safely, and in the following two days, no rabbit's blood sugar value entered the hypoglycemic stage again (Table 2), indicating that the modeling was successful. None of the animals received insulin during the two days observed.

4.5. Analysis of Changes of Blood Glucose Value in Experimental Animals during Modeling

In the whole experiment, the blood sugar value of all the experimental rabbits before the experiment was between 4 - 6 mmol/L, which was normal value. At 6 h after modeling, most of the experimental animals in group C entered the hyperglycemia stage, and no experimental animals died. After 10 h of modeling, the blood glucose of all rabbits showed a decrease (C9 was only 9.6 mmol/L in the hyperglycemic stage, and we suspected that it began to enter the hypoglycemic stage, so iv 10 ml 5% glucose solution + ip 10 ml 5% glucose solution was injected, resulting in a blood glucose increase to 29.0 mmol/L). After that, the "baseline" glucose was given once every 4 hours and continuously monitored 4 times overnight (>14 h). On the 2 d day, it was found that all experimental animals entered the hyperglycemic stage and did not enter the hypoglycemic stage again in the next 2 d, and the blood sugar of all experimental animals was >14 mmol/L, indicating successful modeling, as shown in Figure 2.

4.6. Changes of Liver and Kidney Tissue Structure in Different Days

4.6.1. Changes of Liver Tissue Structure

HE staining was performed on the liver at different time periods: 1) Without alloxan injection, rabbit liver cells were tightly arranged, with a single radial arrangement and uniform size centered on the central vein, no necrosis or infiltration of cells, and hepatic sinuses were clearly visible. 2) 3 days after injection of alloxouracil, rabbit hepatocytes still showed a single radial arrangement centered on the central vein, but some hepatocytes around the central vein showed vacuole-like degeneration. 3) 7 days after injection of alloxouracil, the hepatic

Table 2. Blood glucose levels of experimental animals in 2 days after the hypoglycemic stage.

ID	Before molding	Day 1	Day 2
C1	5.4	33.3*	31.6
C2	4.8	33.3*	33.3*
C3	6.2	33.3*	33.3*
C4	6.5	30.8	30.9
C5	6.2	21.1	21.2
C6	4.7	0	0
C7	6.4	23.0	22.0
C8	5.6	31.6	33.3*
C9	4.3	33.3*	33.3*
C10	4.9	24.6	33.3*
C11	5.6	16.6	33.3*
C12	5.6	21.1	33.0
C13	5.2	25.1	26.1
D1	4.3	5.8	5.5
D2	4.9	6.6	6.0
D3	6.1	7.1	5.8
D4	5.3	6.5	7.0
D5	6.0	6.2	6.6
D6	5.6	6.8	5.0
D7	4.2	5.5	6.1
D8	5.3	6.0	6.5
D9	5.8	5.2	6.0
D10	6.1	5.8	7.1
D11	6.3	6.0	5.6
D12	7.0	5.6	5.8
D13	4.9	6.3	7.1

Note: *in the table indicates higher than the blood glucose limit value (>33.3 mmol/L); C6 dies in the hypoglycemic stage.

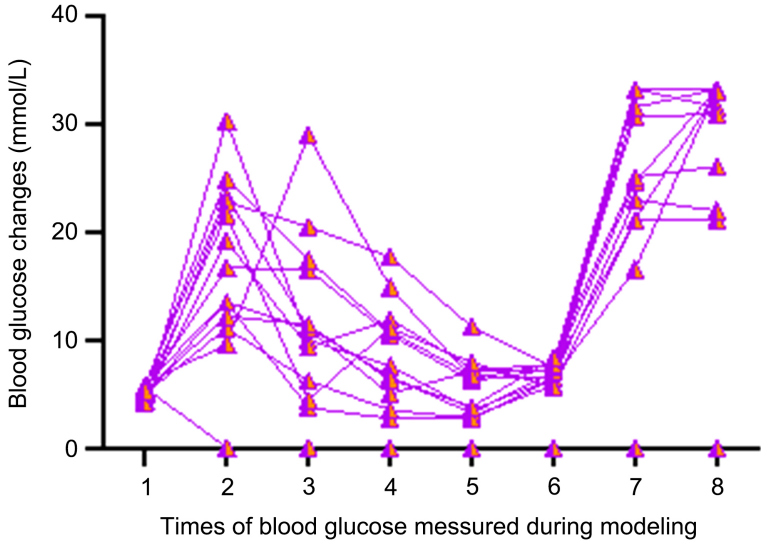


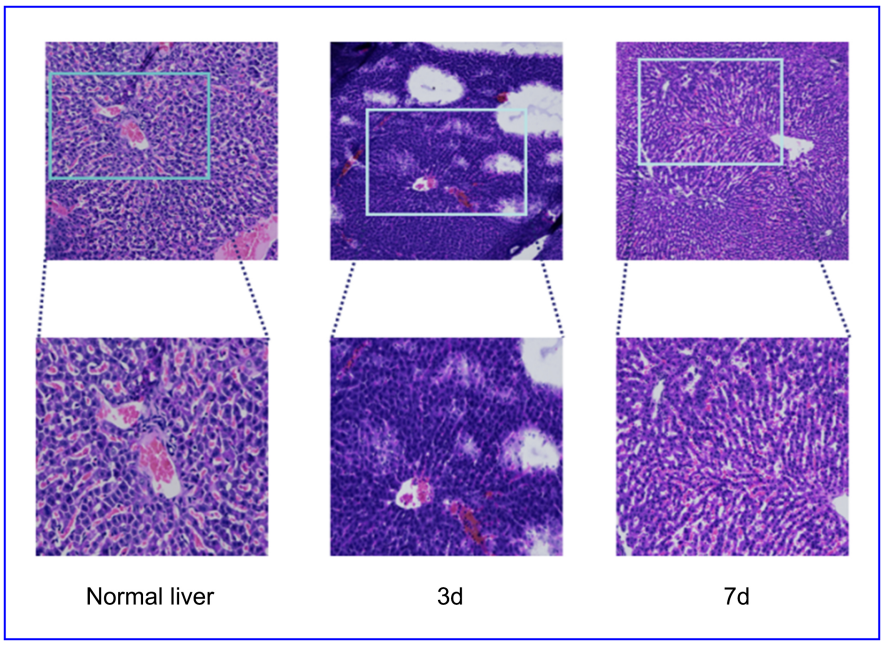
Figure 2. Changes in blood glucose of experimental animals during modeling.

lobular structure existed, the blood flow in the hepatic sinus was significantly reduced, the hepatocytes around the hepatic lobular were significantly swollen, the nuclei varied in size, and no obvious exudation or necrosis was observed. 4) 10 days after injection of alloxouracil, the outline of liver lobule and liver cord structure still existed, the arrangement of liver cells was looser than before, the shape of liver cells was irregular, the cell swelling and degeneration, the inflammatory cell infiltration, the nuclear shape was irregular and the size was different, and the structure of liver sinuses disappeared. 5) 14 days after injection of alloxouracil, the hepatic lobules showed a single radial arrangement with uniform size centered on the central vein, no necrosis or infiltration of cells, a small amount of blood flow in the hepatic sinuses, varying the size of the nuclei, loose cell arrangement, and a large number of vacuole-like changes in hepatocytes could be seen under the microscope, as shown in **Figure 3**.

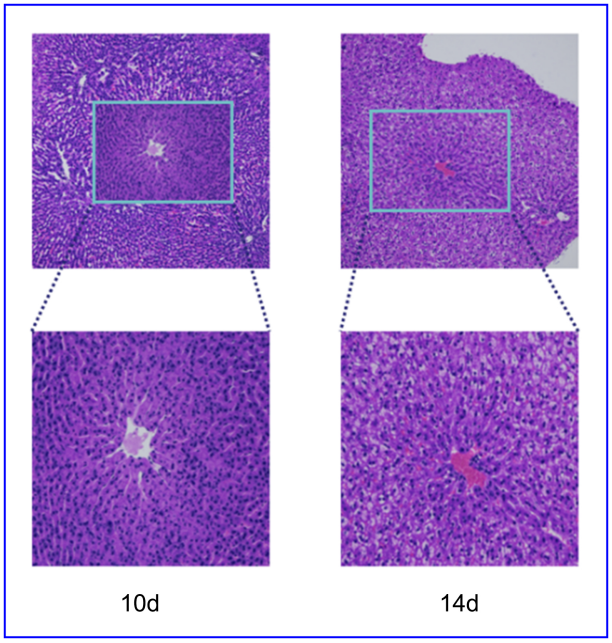
4.6.2. Renal Tissue Structure Changes

In order to more directly observe the damage of rabbit kidney tissue after injection of alloxouracil, histopathological sections of rabbit kidney tissue at different time periods were performed and stained: 1) Before injection of alloxouracil, the kidney tissue structure was clear, the cells were evenly distributed and arranged normally, the glomerular basement membrane was not abnormal, the volume of renal sacs and the space between sacs were normal. 2) 3 days after injection of alloxouracil, the number of cells in the glomerulus increased, the volume became larger and the boundary was unclear, the blood flow signal was significantly reduced than that in the normal group, and the space between the renal sacs became smaller, accompanied by the invasion of inflammatory cells. 3) 7 days after alloxouracil injection, the number of cells in the glomerulus increased, and the blood flow signal decreased significantly; The cystic space of renal sacs became smaller and inflammatory cells were still invaded. 4) 10 days after injection of

alloxouracil, the structure of some glomerulus was obviously destroyed, the number of cells was obviously increased, the boundary with the surrounding renal tissue was unclear, there was obvious exudation and necrosis, and the cystic structure of some renal sacs disappeared. 5) 14 d after injection of alloxouracil, cells in the glomerulus were significantly increased, the original contour was lost, the size of the nuclei was different, there was obvious exudation signal, and no cystic structure of the renal sacs was seen, Figure 4.

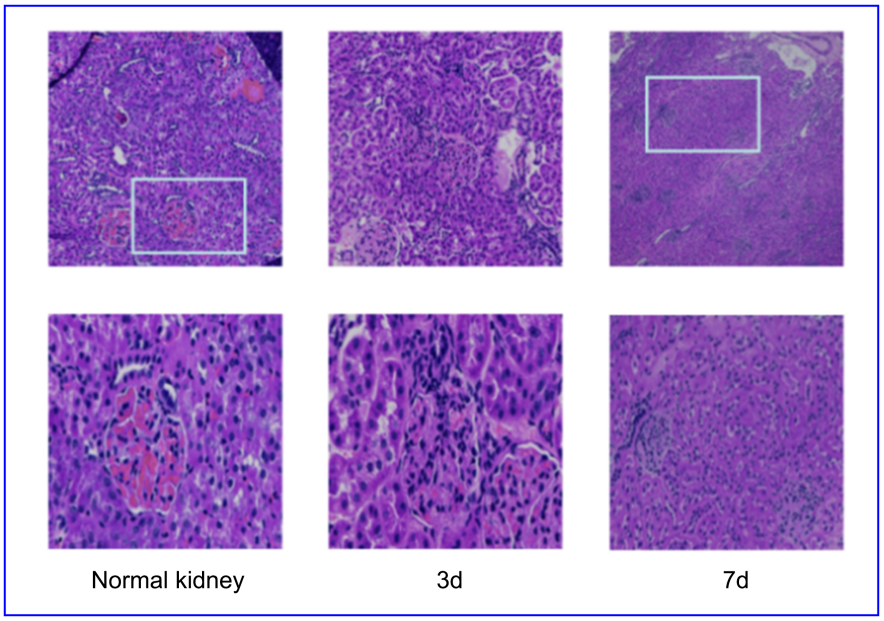


(a)

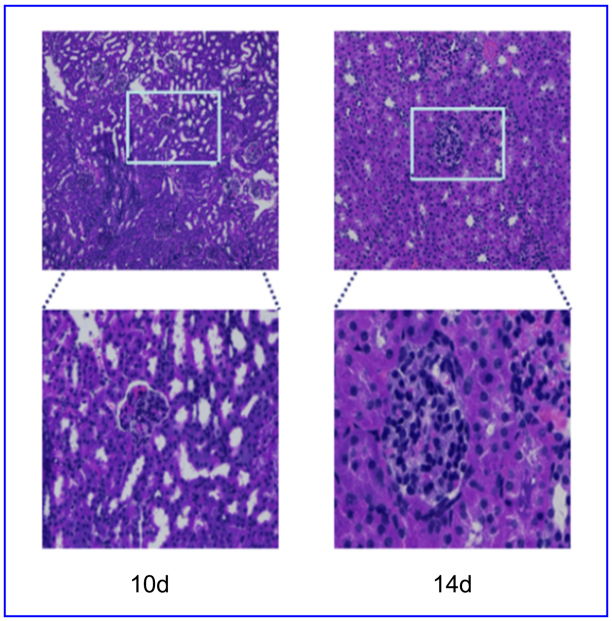


(b)

Figure 3. Liver HE staining, 40×.



(a)



(b)

Figure 4. Renal HE staining, 40×.

4.7. Statistical Analysis

Survival curve was drawn according to statistical analysis, and it was found that the survival time of group A and group B had no statistical significance ($P > 0.05$). The survival time of group A and group C was significantly different ($P < 0.001$), and the survival time of group B and group C was significantly different ($P < 0.001$). The survival time of groups A, B and D were statistically significant ($P < 0.001$). The survival time of group C and group D was not statistically significant, as shown in **Figure 5**.

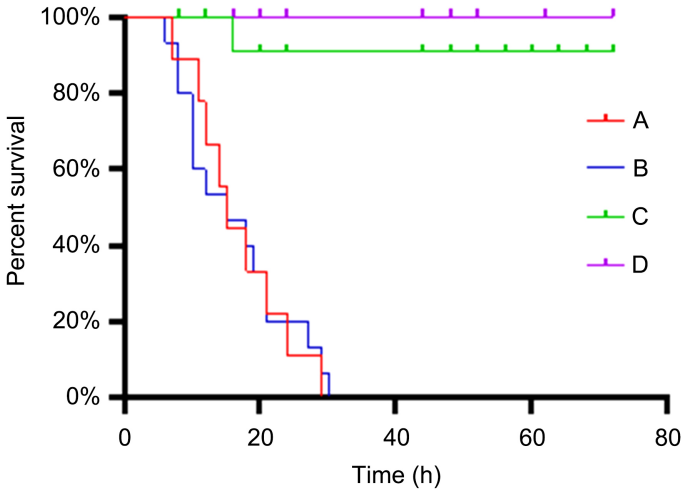


Figure 5. Survival curve analysis of rabbits in each group during the hypoglycemia stage.

5. Discussion

Bacevic *et al.* [10] found that the first stage was mainly characterized by elevated blood sugar; After 8 h, it entered the stage of malignant hypoglycemia, which lasted for 36 h. The third stage is mainly characterized by hyperglycemia and is a stable DM model stage, which is also confirmed by the research results of Lenzen [11] and Federiuk [12] *et al.*

Hypoglycemia stage is considered as the damage of alloxouracil to islet beta cells, resulting in the breakdown of secretory particles and cell membrane, resulting in a large amount of insulin into the blood, resulting in hyperinsulinemia [13] [14]. This stage is unavoidable. Relevant studies have shown [13] [15] that hypoglycemia caused by alloxouracil can lead to serious pathological changes in brain, kidney, liver and other organs. Islet beta cells are in a depolarization state, and a large number of Ca2+ ions flood into islet beta cells, damaging the genome of secreted protein of islet beta cells, resulting in DNA break and complete loss of cell structure and function. It leads to permanent cell damage, which is also one of the mechanisms by which alloxouracil induces permanent DM models and ultimately leads to the death of experimental animals [11] [16].

Sun *et al.* [17] had a mortality rate of 91.67% with a single injection of 150 mg/kg. When 150 mg/kg was injected into three times within a week, the mortality rate was 8.33%, but the rabbits were anesthetized each time. We found that the mortality rate of group A rabbits was 100% after a one-time injection of 100 mg/kg alloxouracil, which was similar to their experimental conclusion. However, the experimental animals in group B were modeled according to the concentration gradient of 50 mg/kg, 75 mg/kg, 100 mg/kg and 125 mg/kg, and half of the total amount of the first injection was found to be dead on the 2nd day, which was inconsistent with their experimental conclusions. This may be due to the rapid rate of injection of alloxouracil [18].

Previous studies have found [7] [19] that intraperitoneal injection of 20 ml

10% glucose at 4 h interval, continuous 48 h, supplemented with intravenous injection of 5 ml 10% glucose at 24 h and 34 h, the mortality rate is 10%. In this experiment, by prophylactically administering iv 10 ml 5% glucose solution + ip 10 ml 5% glucose for 4 consecutive injections, the survival rate was similar to theirs, but our method was simpler, the monitoring time was greatly shortened, and the mortality rate was also lower. Of course, our prophylactic injection of glucose does not increase blood sugar, but to competitively inhibit the absorption of alloxan by islet beta cells [20] and slow its absorption rate, thus slowing the release of insulin and the occurrence of hypoglycemia. However, the mechanism of liver and kidney injury may be related to the lipophilicity of alloxouracil [21], so the protection of liver and kidney by injection of glucose is meaningless. According to previous reports [13] [22] [23] [24] [25] [26], due to the extremely short half-life of alloxouracil, a peak will occur quickly after intravenous injection. We can antagonize the large amount of insulin in the circulatory system through iv 10 ml 5% glucose solution. Alloxouracil occurs REDOX reaction in islet beta cells, and ROS and other substances produced continue to damage islet beta cells, slowly releasing insulin, and antagonizing insulin in the circulatory system for a long time by injecting 10 ml 5% glucose solution into the abdominal cavity.

6. Conclusion

When the DM rabbit model is constructed with alloxouracil, iv 10 ml 5% glucose solution + ip 10 ml 5% glucose can be used to help the experimental animals through the stage of malignant hypoglycemia. The use of this intervention can significantly reduce the mortality of experimental animals, and greatly shorten the nursing time of experimental animals. After injection of alloxouracil, liver and kidney will appear different degrees of lesions, or one of the causes of acute death of experimental animals.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Armstrong, D.G., Swerdlow, M.A., Armstrong, A.A., *et al.* (2020) Five Year Mortality and Direct Costs of Care for People with Diabetic Foot Complications Are Comparable to Cancer. *Journal of Foot and Ankle Research*, **13**, 16.

https://doi.org/10.1186/s13047-020-00383-2

[2] Wei, J. and Zhou, H. (2019) Research Progress on the Mechanism of Wound Healing of Diabetic Foot Ulcer. *Youjiang Medical Journal*, **49**, 621-624.

[3] Min, A.Y., Yoo, J.-M., Sok, D.-E., *et al.* (2020) Mulberry Fruit Prevents Diabetes and Diabetic Dementia by Regulation of Blood Glucose through Upregulation of Antioxidative Activities and CREB/BDNF Pathway in Alloxan-Induced Diabetic Mice. *Oxidative Medicine and Cellular Longevity*, **2020**, Article ID: 1298691. https://doi.org/10.1155/2020/1298691

[4] Badin, J.K., Progar, V., Pareddy, A., *et al.* (2019) Effect of Age on Diabetogenicity of Alloxan in Ossabaw Miniature Swine. *Comparative Medicine*, **69**, 114-122. https://doi.org/10.30802/AALAS-CM-18-000037

[5] Matsuura, Y., Yamashita, A., Zhao, Y., *et al.* (2017) Altered Glucose Metabolism and Hypoxic Response in Alloxan-Induced Diabetic Atherosclerosis in Rabbits. *PLOS ONE*, **12**, e0175976. https://doi.org/10.1371/journal.pone.0175976

[6] Elseweidy, M.M., Aly, S.I., Hammad, S.K., *et al.* (2020) Early Myocardial Injury Biomarkers in Diabetic Hyperlipidemic Rats: Impact of 10-Dehydrogingerdione and Vitamin D3. *Experimental Biology and Medicine*, **245**, 1326-1334. https://doi.org/10.1177/1535370220943124

[7] Fan, X., Xiaoqin, L., Potts, B., *et al.* (2011) Topical Application of L-Arginine Blocks Advanced Glycation by Ascorbic Acid in the Lens of HSVCT2 Transgenic Mice. *Molecular Vision*, **17**, 2221-2227.

[8] Mumtaz, F.H., Lau, D.H.W., Siddiqui, E.J., *et al.* (2006) Changes in Cholinergic and Purinergic Neurotransmission in the Diabetic Rabbit Bladder. *In Vivo*, **20**, 1-4.

[9] Wang, J., Wan, R., Mo, Y., *et al.* (2010) Creating a Long-Term Diabetic Rabbit Model. *Journal of Diabetes Research*, **2010**, Article ID: 289614. https://doi.org/10.1155/2010/289614

[10] Bacevic, M., Rompen, E., Radermecker, R., *et al.* (2020) Practical Considerations for Reducing Mortality Rates in Alloxan-Induced Diabetic Rabbits. *Heliyon*, **6**, E04103. https://doi.org/10.1016/j.heliyon.2020.e04103

[11] Lenzen, S. (2008) The Mechanisms of Alloxan- and Streptozotocin-Induced Diabetes. *Diabetologia*, **51**, 216-226. https://doi.org/10.1007/s00125-007-0886-7

[12] Federiuk, I.F., Casey, H.M., Quinn, M.J., Wood, M.D. and Ward, K.W. (2004) Induction of Type-1 Diabetes Mellitus in Laboratory Rats by Use of Alloxan: Route of Administration, Pitfalls, and Insulin Treatment. *Comparative Medicine*, **54**, 252-257.

[13] Queiroz, L., *et al.* (2021) Endangered Lymphocytes: The Effects of Alloxan and Streptozotocin on Immune Cells in Type 1 Induced Diabetes. *Mediators of Inflammation*, **2021**, Article ID: 9940009. https://doi.org/10.1155/2021/9940009

[14] Radenković, M., Stojanović, M. and Prostran, M. (2016) Experimental Diabetes Induced by Alloxan and Streptozotocin: The Current State of the Art. *Journal of Pharmacological and Toxicological Methods*, **78**, 13-31. https://doi.org/10.1016/j.vascn.2015.11.004

[15] Mir, M.S., Darzi, M.M., Khan, H.M., *et al.* (2013) Pathomorphological Effects of Alloxan Induced Acute Hypoglycaemia in Rabbits. *Alexandria Journal of Medicine*, **49**, 343-353. https://doi.org/10.1016/j.ajme.2013.03.007

[16] Tasaka, Y., Inoue, Y., Matsumoto, H. and Hirata, Y. (1988) Changes in Plasma Glucagon, Pancreatic Polypeptide and Insulin during Development of Alloxan Diabetes Mellitus in Dog. *Endocrinologia Japonica*, **35**, 399-404. https://doi.org/10.1507/endocrj1954.35.399

[17] Sun, W.T., Lei, C.L., Bi, C.C., *et al.* (2010) Effect of Alloxan Time Administer Drug on Establishing Diabetic Rabbit Model. *International Journal of Ophthalmology*, **3**, 200-202.

[18] Misra, M. and Aiman, U. (2012) Alloxan: An Unpredictable Drug for Diabetes Induction? *Indian Journal of Pharmacology*, **44**, 538-539.

[19] Golay, A., Zech, L., Shi, M.Z., *et al.* (1986) Role of Insulin in Regulation of High Density Lipoprotein Metabolism. *Journal of Lipid Research*, **28**, 10-18. https://doi.org/10.1016/S0022-2275(20)38727-7

[20] Ighodaro, O.M., Adeosun, A.M. and Akinloye, O.A. (2017) Alloxan-Induced Diabetes, a Common Model for Evaluating the Glycemic-Control Potential of Therapeutic Compounds and Plants Extracts in Experimental Studies. *Medicina*, **53**, 365-374. https://doi.org/10.1016/j.medici.2018.02.001

[21] Elsner, M., Tiedge, M., Guldbakke, B., Munday, R. and Lenzen, S. (2002) Importance of the GLUT2 Glucose Transporter for Pancreatic Beta Cell Toxicity of Alloxan. *Diabetologia*, **45**, 1542-1549. https://doi.org/10.1007/s00125-002-0955-x

[22] Bingham, J.T., Etz, B.D., DuClos, J.M., *et al.* (2021) Structure and Reactivity of Alloxan Monohydrate in the Liquid Phase. *The Journal of Organic Chemistry*, **86**, 14553-14562. https://doi.org/10.1021/acs.joc.1c01389

[23] Bahar, E., Akter, K.M., Lee, G.H., *et al.* (2017) β-Cell Protection and Antidiabetic Activities of *Crassocephalum crepidioides* (Asteraceae) Benth. S. Moore Extract against Alloxan-Induced Oxidative Stress via Regulation of Apoptosis and Reactive Oxygen Species (ROS). *BMC Complementary and Alternative Medicine*, **17**, Article No. 179. https://doi.org/10.1186/s12906-017-1697-0

[24] Aloke, C., Nwachukwu, N., Obasi, N.A., *et al.* (2021) Antihyperglycemic, Antihyperlipidemic and Hepatoprotective Effects of *Ficus ottoniifolia* (Miq.) Miq. Supplementation in Alloxan-Induced Diabetic Rats. *Avicenna Journal of Phytomedicine*, **11**, 428-435.

[25] Dra, L.A., Sellami, S., Rais, H., *et al.* (2019) Antidiabetic Potential of *Caralluma europaea* against Alloxan-Induced Diabetes in Mice. *Saudi Journal of Biological Sciences*, **26**, 1171-1178. https://doi.org/10.1016/j.sjbs.2018.05.028

[26] McDaniel, M.L., Bry, C.G., Fink, C.J., *et al.* (1979) Studies on the Site of Interaction for Ninhydrin, Alloxan, and D-Glucose in Insulin Secretion by Isolated Islets *in Vitro*. *Endocrinology*, **105**, 1446-1451. https://doi.org/10.1210/endo-105-6-1446