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# **ОБРАЗОВАНИЕ И НАУКА В XXI ВЕКЕ**

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**Название публикации:** «DETERMINATION OF CHANGES IN THE LIPID PEROXIDASE INDEX IN PURULENT-NECROTIC LESIONS OF THE LOWER EXTREMITIES»

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**Annotation.** *Study of the effect of the new drug "Reomannisol" on vital organs, taking into account of diagnostics and prevention of pathophysiological aspects in the complex treatment of experimental diabetic foot syndrome.*

**Key words:** *experimental model of diabetic foot, experimental animals, diabetes mellitus, alloxan, surgical debridement, reomannisol.*

**Annotatsiya.** *Eksperimental diabetik oyoq sindromini yangi "Reomannisol" preparati bilan kompleks davolashdan keyin hayotiy organlarga ta'sirini o'rganish uchun patofiziologik diagnostika va profilaktikani olib borish.*

**Kalit so'zlar:** *diabetik oyoqning eksperimental modeli, tajriba hayvonlari, diabetes mellitus, alloksan, jarrohlik debridman, reomannisol.*

**Аннотация.** *Изучение влияния нового препарата «Реоманнисола» на жизненно-важных органов с учетом диагностики и профилактики патофизиологических аспектов в комплексном лечении экспериментального синдрома диабетической стопы.*

**Ключевые слова:** экспериментальная модель диабетической стопы, экспериментальные животные, сахарный диабет, аллоксан, хирургическая обработка, реоманнисол.

**Introduction.** Currently, one of the leading places in terms of growth rates of morbidity, disability, and mortality was occupied by diabetes mellitus (DM) among the so-called "diseases of civilization" [1,4,5,17]. Today, over 460 million people are suffering globally from diabetes; according to the predicted facts announced by the International Diabetes Federation, by 2040 the number of patients will increase up to 642 million [11,12,18].

Diabetes mellitus is accompanied by the development of complications, including diabetic foot syndrome (DFS), one of the leading clinical symptoms of which is the persistence of an ulcer on the skin of the lower extremities [4,5].

Delayed wound healing is one of the complications of the disease due to multiple factors including poor circulation [4,5,12,17], prolonged inflammation, and hyperglycemia. It is a common cause of morbidity and mortality in patients with DM [2,5,15,16]. When the wound becomes chronic, it is prone to developing foot ulcers, including neuropathy and foot deformities [6,13,17,18]. Foot ulcers in DM are the cause of more than 50% of all non-traumatic leg amputations [3,7,15]. Evidence has shown that hyperglycemia is one of the main factors contributing to slow wound healing by increasing cell apoptosis and decreasing cell survival in diabetic wounds. It has been shown to inhibit endothelial cell and fibroblast proliferation in humans [9], up to 75% slower in adult mice with DM compared to control mice [10].

At the present stage in experimental diabetology, the most widespread chemical model of diabetes mellitus uses substances that destroy  $\beta$ -cells of the islets of Langerhans [1,2, 11,12]. This study describes a model of diabetes mellitus in rats, induced by the introduction of a reduced dose of alloxan, which significantly reduces the number of animal deaths.

**Aim of the study.** Study of the effect of the new drug "Rheomannisol" on endogenous intoxication and wound healing, taking morphological aspects into the complex treatment of experimental diabetic foot syndrome.

**Materials and research methods.** The work was done on experimental material. Healthy rats were selected for the experiment. Experimental studies were carried out on 140 outbred male rats weighing 220-250 g, kept in the Tashkent Medical Academy (TMA) vivarium. The rats were kept under optimal conditions, all rats lived in a room with a 12-hour light-dark cycle and a constant temperature of 22-25°C, with free access to water. All rats were given a sufficient amount of a normal rodent diet ad libitum. (diet for rodents, State standard No. GOST R50258–92) and tap water daily. Operations and all manipulations with animals were carried out using general anesthesia, in compliance with the principles of humanity outlined in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, by the "Rules for working with experimental animals". The experimental animals were divided into 4 groups: the 1st group was intact; 2nd group –the creation of an experimental model of alloxan diabetes mellitus; 3rd control group - against the background of alloxan diabetes, the creation of an experimental model of a diabetic foot using traditional complex treatment; 4th experimental group - on an experimental model of diabetic foot - traditional treatment and reomannisol.

After a 24-hour fast, the rats were weighed. A 2% solution of alloxan diluted in 0.9% saline was administered intraperitoneally as a single dose, corresponding to a dose of 20, 15, 12 mg of alloxan per 100 g of animal weight. Food and water were given to animals only 30 minutes after drug administration. On the 3rd day, the level of glucose in the blood was assessed.

*Determination of glucose concentration in the peripheral blood of animals.* Diabetes was confirmed 3 days after the blood glucose concentration was determined. Peripheral blood glucose concentration was measured with an “AccuChek Active” glucometer (Roche Diagnostics, Germany), the linear measurement range was 0.6–33.3 mmol/L. Blood sampling to study the level of glycemia was performed from an incision in the tip of the tail. An experimental model of diabetes mellitus (type I diabetes) has been obtained. The day of verification of diabetes mellitus was considered the zero-day of its development.

**Results.** The body bodyweight before the experiment varied from 220 to 250 g. Group 1 - intact animals (10 rats each), served as controls for groups 3 and 4. 2nd group –the creation of an experimental model of a diabetic foot, against the background of alloxan diabetes; To do this, 10 rats were injected intraperitoneally with 2% alloxan in an amount of 20 mg / 100 g. In this experimental group, in the 2nd experimental group, 8 rats died in the first 3 days as a result of hyperglycemic and hypoglycemic coma, which amounted to 80%. When examining the level of glucose in the blood with a glucometer of the remaining rats, it was 33.3 mmol/l and may have been higher, since the maximum range of the glucometer is 33.3 mmol/l. The remaining 2 rats sat in the corner, there were no reactions to external stimuli, they were sedentary when picked up. The animals did not touch the food. On the next day 4, the remaining rats died.

The second series of the second group of the experimental model of diabetes mellitus was created based on alloxan at a dose of 15 mg per 100 g from 10 rats. In this series of experiments, in the first 3 days the lethality was 50% (5 rats). The glucose levels of the survivors (5 rats) ranged from 29.8 to 33.3 mmol/l. During the next 4 days, the remaining rats died.

3rd series of the experiment of the 2nd group - administration of alloxan intraperitoneally at a dose of 12 mg per 100 g per 100 rats. During the next 72 hours, no lethal outcome was observed in rats, the range of blood glucose levels in rats varied between 15.5 - 17 mmol/L. In rats on the skin of the footpad pad of the right hind paw, a full-thickness rectangular wound measuring 2 mm × 5 mm was created with a scalpel. Rats were randomly divided into 2 groups, each group of 50 rats. So it was created 3 – a control group on 50 rats and 4 experimental groups n=50 rats. In both groups, until the end of the experiment (17 days), no death was recorded.

*Visual inspection.* The first signs of diabetes were manifested in the form of a sharp increase in water consumption of 70-80 ml, polyphagia, polyuria, hyperglycemia. With alloxan-induced diabetes mellitus in animals during the experiment, lethargy, apathy, low activity, tarnishing and loss of coat, weight loss, clouding of the pupil and sclera, small-point erosion in the tails and limbs were noted. The wool of animals normally has a peculiar luster and is usually adjacent to the skin. In dynamic

observation in rats of the experimental group, by the seventh day, the condition began to improve.

**Table 1.** The product of POL is malonic dialdehyde (MDA).

Days	Control group, mkmol/l.	Maingroup, mkmol/l.	Intact group, mkmol/l.
1	1,39±0,02 <sup>***</sup>	1,35±0,02 <sup>***</sup>	0,87±0,02
3	1,43±0,02 <sup>***</sup>	1,09±0,01 <sup>***^^</sup>	-
7	1,27±0,01 <sup>***</sup>	1,02±0,01 <sup>***^^</sup>	-
10	1,22±0,01 <sup>***</sup>	0,93±0,01 <sup>^^^</sup>	-
14	1,17±0,01 <sup>***</sup>	0,90±0,02 <sup>^^</sup>	-

Note: \*- significantly compared with the intact group (\*-P<0,05; \*\*-P<0,01; \*\*\*-P<0,001)

^ - significantly compared with the control group (^-P<0,05; ^^P<0,01; ^^^-P<0,001)

Under the conditions of the model we have chosen, the state of the LPO-MDA system was also studied since this system is a key link in the pathogenesis of diabetes mellitus. On the 1st day of the experiment, the content of malondialdehyde was significantly higher in both groups compared to intact rats ( $0.87 \pm 0.02$ ), which indicates the formation of a large amount of lipid peroxidation products, indicating the processes of destruction of cell membranes (Table 2). The effect of daily administration of reomannisol at a dose of 1 ml/100 g (experimental group) on the intensity of lipid peroxidation on the 7th day was expressed by a noticeable decrease in the content of MDA ( $1.02 \pm 0.01$ ) by 1.2 times relative to the control group ( $1.27 \pm 0.01$ ). At the end of the experiment (day 14) in the group receiving traditional treatment, the animals retained a high level of MDA- $1.17 \pm 0.01$ , indicating a high content of free radicals in the animal body. While in the experimental group there is a stable decline in the level of MDA and on the 10th, 14th day fixes a normal level of MDA (respectively



0.93±0.01; 0.90±0.02). This result indicates the antioxidant, detoxifying effect of the drug reomannisol, which is characteristic of it.

MWM isolated from blood plasma and erythrocytes in rats modeled with diabetes mellitus by alloxan can activate LPO processes in the membranes of animal erythrocytes. The state of endointoxication is characterized, as a rule, by the activation of lipid peroxidation, and they also act as a factor in the aggravation of EI processes [7, 8].

**Conclusions.**1. The best option for creating an experimental model of a diabetic foot is the introduction of alloxan intraperitoneally in a single dose of 12 mg per 100 g, in which moderate diabetes develops.

2. After using the drug reomannisol intraperitoneally at a dose of 1 ml / 100 g 1 time per day for 5 days, there was a sharp decline in EI numbers. On the 10th day, the EI values in the experimental group returned to normal, similar to those in the intact group. The drug reomannisol performs "biochemical rehabilitation", due to its inherent qualities: antioxidant, improves blood rheology, detoxification, and diuretic. In rats of the control group, the EI numbers remain at high levels until the end of the experiment.

3. The results of biochemical studies demonstrate positive dynamics in experimental animals with a diabetic foot model when using the drug reomannisol. This was manifested by the fact that by the 10th day there was a decrease and normalization of the level of glucose in the peripheral blood, indicators of renal clearance (urea, creatinine), liver (ALT, AST, albumins).

4. An open, full-thickness wound of the foot, in rats with DM, had low blood circulation, prolonged inflammation, and was characterized by a violation of the inflammatory and proliferative phases of the healing process, which is associated with hyperglycemia. Thus, this model of the open foot in rats provides a good approach for studying the process of wound healing in DM, and this model can be regarded as creating an analog of the human diabetic foot syndrome in an experimental model of alloxan-induced diabetes mellitus.



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