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Improving the Complex Treatment of Experimental Diabetic Purulent-Necrotic Wounds in the Lower Extremities Based on Pathophysiological Indicators

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Annotation

Currently, there is no standardized treatment for diabetes and its complications. In treating diabetes mellitus complications, an individualized approach is applied to each patient. For this reason, our locally developed drug Reomannisol has proven effective in treating diabetic purulent-necrotic lesions. Through our biochemical, immunohistochemical, and pathomorphological studies, we have obtained the expected results confirming that the drug is effective in treating purulent-necrotic foot lesions in diabetes mellitus. Changes can be observed in MDA, the cytokine system (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, INF-γ), and vital organs of the experimentally studied rats, leading to endogenous intoxication. As a complement to our domestic drug, ozone therapy was selected.

Key words: ozone therapy, diabetic purulent-necrotic foot model, cytokine system, complex treatment, Reomannisol, Reosorbylact, inflammatory formation, diabetic foot syndrome.

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Introduction. Diabetes mellitus (DM) is a chronic autoimmune disease. When purulent-necrotic lesions of diabetic heel syndrome appear, an inflammatory reaction appears and manifests itself in the form of a local reaction, which can lead to a systemic response [1,2,3,4]. As a result of bacterial action, under the influence of humoral factors, the activation occurs in the main phagocytosis cells – neutrophils (polymorphonuclear leukocytes), monocytes (macrophages), and platelets [5,6,7,8]. Activated cells begin to produce inflammatory mediators (cytokines) in the blood, which are involved in the regulation of vascular tone, hemostasis, and cell proliferation [3,9,10,11,12]. Atherosclerosis of blood vessels in patients with diabetes occurs 8-10 years earlier than in the general population. Therefore, an

inflammatory reaction occurs against the background of changes in metabolism and circulatory disorders [13,14,15,16,17,18]. The pathogenetic mechanism of diabetes is complex, but the latest research data suggests that the participation of cytokines plays a large role in this disease. Cytokines are conditionally anti-inflammatory: natural immune regulators involved in inflammatory formation (IL-1, IL-2, IL-6, IL-12, TNF-α) and anti-inflammatory by pancreatic β-cells that depressant to insulin production: special immune regulators of inflammatory response (IL-4, IL-10, IL-13, INF-γ) these cytokines have protective and antidiabetic effects [19,20,21,22,23,24]. In addition, some cytokines regulate specific immune reactions (IL-2 and IL-4, transformative growth factor (TGF-β va et al.). It is involved in protein activation, differentiation, and growth of mature lymphocytes. Several cytokines (IL-1, IL-6, TNF-α, erythropoietin) can also have effects such as distension, which is simple hormones [25,26,27,28,29,30]. In addition, a similar single cell can secrete several different cytokines and the same cytokines at the same time, or different cells can produce several different or identical cytokines. Therefore, the importance of cytokines in the formation of diabetes mellitus, on the one hand, and in the formation of an inflammatory reaction, on the other, is interesting to obtain information about the state of the cytokine in patients with DFS [31,32,33,34,35,36,37]. The state of the cytokine system at the time of the development of purulent-necrotic processes in patients with DFS is completely unclear. Given the lack of research, DFS is solved by the dynamic state of the cytokine system in patients with purulent-necrotic processes and the recurrence of purulent-necrotic processes [38,39,40,41,42,43,44,45]. It was decided to study the dynamics of cytokine levels in the blood serum of anti-inflammatory cytokines-interleukins IL-1β, IL-2, IL-6, IL-8, tumor necrosis factor-TNF-α, as well as gamma-interferon (IFN-γ) and anti-inflammatory interleukins IL-4, IL-10 patients. In our opinion, perhaps the imbalance of cytokines will help predict the course of the purulent-necrotic process and determine the tactics of surgical treatment [46,47,48,49,50,51,52,53]. In addition, the state of the cytokine balance is of interest in patients with recurrence of purulent-necrotic complications of DFS. Cytokine management balance is currently seen as a new direction of immunotherapeutic effects in the treatment of patients with purulent-necrotic lesions of DFS [54,55,56,57,58,59,60].

The purpose of the work. Under experimental conditions, to determine the most effective ways of treating purulent-necrotic complications of the lower extremities in diabetes mellitus, taking into account pathogenetic features. Determination of the effectiveness of ozone therapy and its pathomorphological properties with new pharmacological drugs for the treatment of purulent necrotic process of the foot. By studying the dynamics of anti-inflammatory cytokine levels: analysis of the need and possibility of immunoprotection by studying cytokine status in IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, INF-γ patients on the development and recurrence of purulent-necrotic processes of DFS.

Materials and methods. Experimental stage. Experimental studies were conducted on 155 white mongrel male rats weighing 150-200 g, kept in the TMA vivarium. The rats were kept in optimal conditions, all rats were kept in a room with a 24-hour light-dark period and a constant temperature of 22-25 °C, with free access to water. Animals in sufficient numbers received a standard diet for rodents ad libitum (diet for rodents, GOST R50258-92). Before the animals were introduced into the experiment, they were quarantined for 1 month. The experimental group consisted of 155 rats, and the intact group consisted of 10 rats. The animals of the experimental group were weighed after 24 hours of fasting, and a 2% solution of alloxan diluted in 0.9% physiological water by the body weight of rats was injected once into the abdominal cavity in appropriate doses: 12, 15, 18 and 20 mg/100 g, water was given 20 minutes after administration of the drug. The development of diabetes was assessed by the level of glucose in the blood and the morphology of the beta cells of the pancreas. In series 1, 10 rats were used that had a so-called diabetes model. To do this, 2% alloxan in the amount of 20 mg/100 g was injected into the abdominal cavity of 10 rats. In this experimental group, 7 rats died within the first 4 days as a result of hyperglycemic and hypoglycemic coma, which amounted to 70%.

In the second series, 10 rats were injected with alloxan at a dose of 18 mg/100 g to create an experimental model of diabetes. In this series of experiments, mortality in the first 3 days was 50% (5 rats).

In the 3rd series of the experiment, 155 rats were used, of which 10 were an intact group, and the remaining 145 rats were caused by a diabetic foot model on the background of alloxan diabetes. Alloxan in this group was administered intraperitoneally at a dose of 12 mg/100 g. During the next 72 hours, no fatal outcome was observed in the rats, the blood glucose level in the rats ranged from 13,5 – 15,5 mmol/l. An experimental DM 1-type model has been created.

After general anesthesia, after intraperitoneal administration of sodium thiopental at a dose of 80 mg/g relative to the body weight of each rat, the experimental animal A.I.Sechenov was on the table with his stomach. The back of the rat's hind legs (only one of the left or right hind legs is selected) is treated with an antiseptic and injected under the skin of the leg using an insulin syringe of 0,25 ml of 10% calcium chloride solution ($CaCl_2$).

Before this manipulation, 20,0 ml of saline solution is poured into a Petri dish, into which 6-7 feces of the same rat are thrown. After the stool swells, it is crushed to form a suspension. The resulting solution is passed through a gauze napkin (the reason is so that during injection, fiber and tissue detritus of feces do not get into the syringe needle and become clogged). 1 ml is taken from the finished suspension and injected subcutaneously into the injection site of calcium chloride. And after 72 hours, we open the purulent-necrotic wound, and you can see its condition.

The experimental animals were divided into 4 groups: group 1 – unchanged group (intact); Group 2 – the creation of an experimental model of alloxan diabetes; control group 3 - carrying out traditional complex therapy (Ozonotherapy + Reosorbylact) against the background of the creation of an experimental model of the diabetic foot; experimental group 4 - on an experimental model of diabetic foot is the implementation of traditional complex therapy treatment and Ozonotherapy + Reomannisol.

Dynamics studies were conducted 1-3, 5-7, 10, 14, and 21 days after the start of treatment. In all groups, not a single case of rat death was recorded until the end of the experiment (18 days). At the appointed time, the general condition of the animals was assessed, the area of the wound was measured, the wound was photographed and the daily diuresis was determined. On the appointed days, the rats were removed from the experiment by decapitation, blood was taken for hematological and biochemical studies, from the lesion (diabetic purulent-necrotic foot) for morphological studies, fragments of the liver, and the endocrine part of the pancreas.

Statistical processing of numerical data was carried out using SPSS 16.0 and Statistica 6.0 for Windows applications. The average values and standard deviations, medians, and interquartile intervals, as well as nonparametric methods (Mann-Whitney, Wilcoxon, and Kruskal-Wallis tests) were revealed.

Determination of ozone concentration. To prepare an ozonated physiological solution, we ozonate 200,0 ml of physiological solution for 10 minutes at a concentration of 3-4 mg/l at a concentration of 3-4 mg/l and for 10 minutes in the apparatus "Medozons Beauty" (MEDOZONS LLC, Russia), then its dose is determined by the following formula:

$$\mathbf{D=C \times V \times t;}$$

D – ozone concentration dose;

C – ozone concentration, mg/l;

V - oxygen exit rate, l/min;

t – time, min;

The purulent area of each rat is washed 3 times a day with ozonized physiological solution for 15-20 minutes.

Results of the experimental stage

In the rats of the control group, there was a tendency to decrease the body weight of rats compared with the values of intact animals. Diuresis increased, and polydipsia persisted, on the 10th and 14th days of the experiment it significantly exceeded the values in intact rats by 4.15 (P<0.001); 3.39 (P<0.001) and 3.57 (P<0.001) times; 3.12 (P<0.001) times (Table 1).

In the comparison group (Ozonotherapy + Reosorbylact), no weight loss was observed, by the end of the experiment, the indicators of polyuria and polydipsia began to gradually decrease: on day 14, the indicators of polyuria and polydipsia decreased by 2.39 (p<0.001) and 2.38 (p<0.001) times compared with the values of the control group, but still higher than in an intact group.

No weight loss was observed in diabetic purulent-necrotic foot rats (the main group) receiving detoxification therapy with Ozonotherapy + Reomannisol. On the 14th day of the experiment, the indicators of polyuria and polydipsia were significantly lower than in the comparison group, by 1.44 (P<0.05) and 1.47 (P<0.05) times. By the end date, they did not differ significantly from the intact rats.

Table 1. Dynamics of physiological parameters of laboratory animals in the experimental diabetic foot, m±m

Animal group	Diuresis, ml/day	Weight, g	Water, ml/day
Undamaged	10,6±0,50	236,7±2,9	12,6±0,79
The control group			
Day 5-7	44,0±1,4***	229,4±2,7	45,0±1,7***
Day 10	35,9±1,0***	230,2±2,5	39,3±1,5***
Comparison of groups			
Day 5-7	24,0±0,9***^^^	231,1±1,8	24,5±0,7***^^^
Day 10	15,0±0,8***^^^	233,8±1,6	16,5±1,2***^^^
The main group			
Day 5-7	13,6±0,50***^^^&&&	236,5±1,4^&	14,1±1,3***^^^&&&
Day 10	10,4±0,45^^^&&&	239,6±1,1^^&	11,2±0,53*^^^&&

Note: *- significantly compared to the indicators of the intact group (*- P<0.05); **- P<0.01; *** - P<0.001; ^ - visibly relative to the control group (^-P<0.05); ^^- P<0.01; ^^ ^ - P<0.001); & - the comparison group, noticeable in comparison with (&- P<0,05; &&- P<0,01; &&& - P<0,001).

Modeling of diabetes has led to significant changes in basic biochemical parameters. As can be seen from Figure 1, on the 1st day of the creation of the diabetic foot model, the glucose level in the blood serum of rats increased statistically significantly by 2,86 times compared with the intact group of rats and amounted to 17,1 ± 0,29 mmol/l (P<0,001) (in the intact group – 4,3±0,19 mmol/l). In later periods, the blood glucose level in rats with diabetic purulent-necrotic foot tended to decrease, but was also high on day 14, which was 2,21 (P<0,001) times higher than in intact rats and amounted to 13,3±0,19 mmol/l.

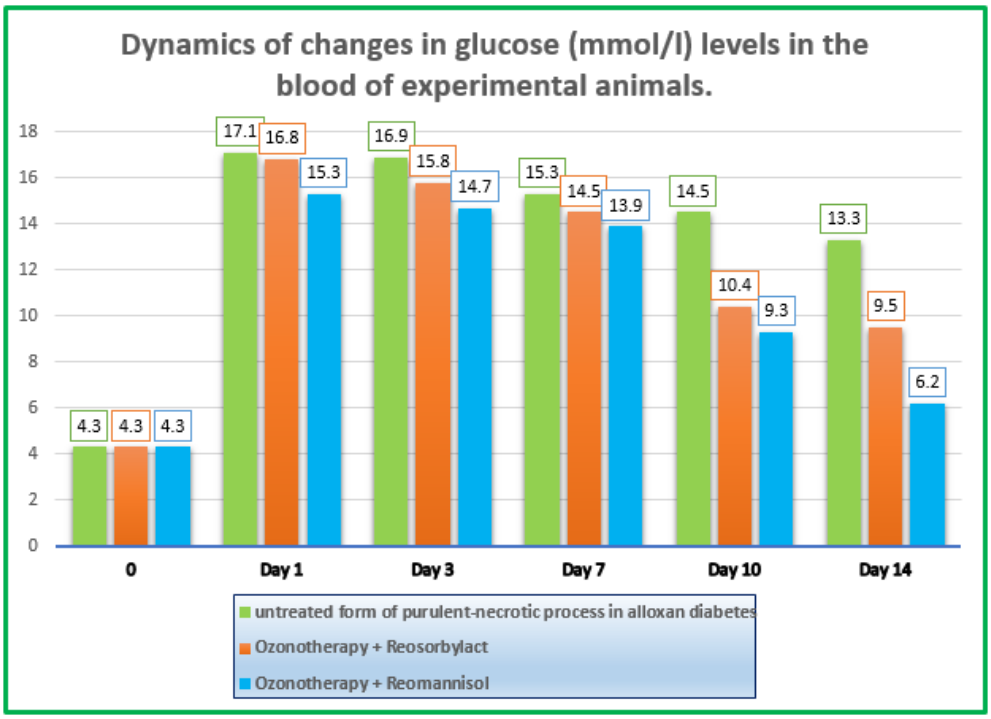


Figure 1. Results of biochemical parameters in experimental animals.

In the comparison group (Ozonotherapy + Reosorbylact), the level of glucose in the blood for 1-7 days of the experiment did not differ significantly from the values in the control group. In later periods (days 10 and 14), there was a significant decrease in blood glucose levels of experimental animals by 1,55 and 1,72 ($p<0,001$) times compared to the control group.

A gradual decrease in glycemia levels was observed in the main group (Ozonotherapy + Reomannisol). On days 7, 10, and 14, experimental animals showed 1,57; 1.89, and 1,94 ($P<0,001$) drops in blood glucose levels compared to the control group. For this reason, the components of Rheomannitol have a positive effect on pancreatic microcirculation and blood rheology, helping to increase the interactor function of the pancreas.

The main indicators of the final products of nitrogen metabolism are the amount of urea and creatinine in the blood serum. In this regard, a study showed an increase in urea and creatinine levels in diabetic foot rats (Group 3), a statistically significant increase compared to intact rats. In our opinion, such a progressive increase in the level of urea and creatinine in blood serum is associated with an increase in the catabolism of proteins and amino acids and their use for gluconeogenesis from nitrogen-free residues. In later periods, serum urea and creatinine levels gradually decreased in all groups, but in the control group, urea and creatinine significantly increased by 2.51; 2.39; 1.9 ($P<0.001$), and 2.07 ($P<0.001$) times compared to rats that are still intact. 1.84 ($P<0.001$) and 1.57 ($P<0.001$) times, respectively, on days 7, 10 and 14 of the experiment.

In the comparison group on the 10th and 14th days of the experiment, these rates are significantly higher than the values of intact rats at 1.37; 1.2 ($P<0.001$) times and 1.35; 1.17 ($P<0.001$) times. The results obtained show good detoxification properties of the drug Reosorbylact.

In the main group (Ozonotherapy + Reomannisol) on Day 10, these rates tended to exceed norm values by 1.14 and 1.12 ($P<0.001$) Times, which by the end of the experiment were not significantly different from the unbroken group indicators. The results obtained indicate that the detoxifying properties of the drug Rheomannitol are higher than those of Reosorbylact.

In diabetes mellitus, an increase in POL is observed, especially in the diabetic purulent-necrotic feet. On Day 1 of the experiment, the MDA level exceeded the standard values. By the 3rd day of the experiment,

the intensity of the POL had increased even more. Subsequently, we observed that it was gradually decreasing, despite such positive changes, on the 7th, 10th, and 14th days of the experiment, the amount of MDA in blood serum increased by 1.46; 1.4 and 1.34 (P<0.001) martagasezilar from the indicators of intact rats (Figure 2).

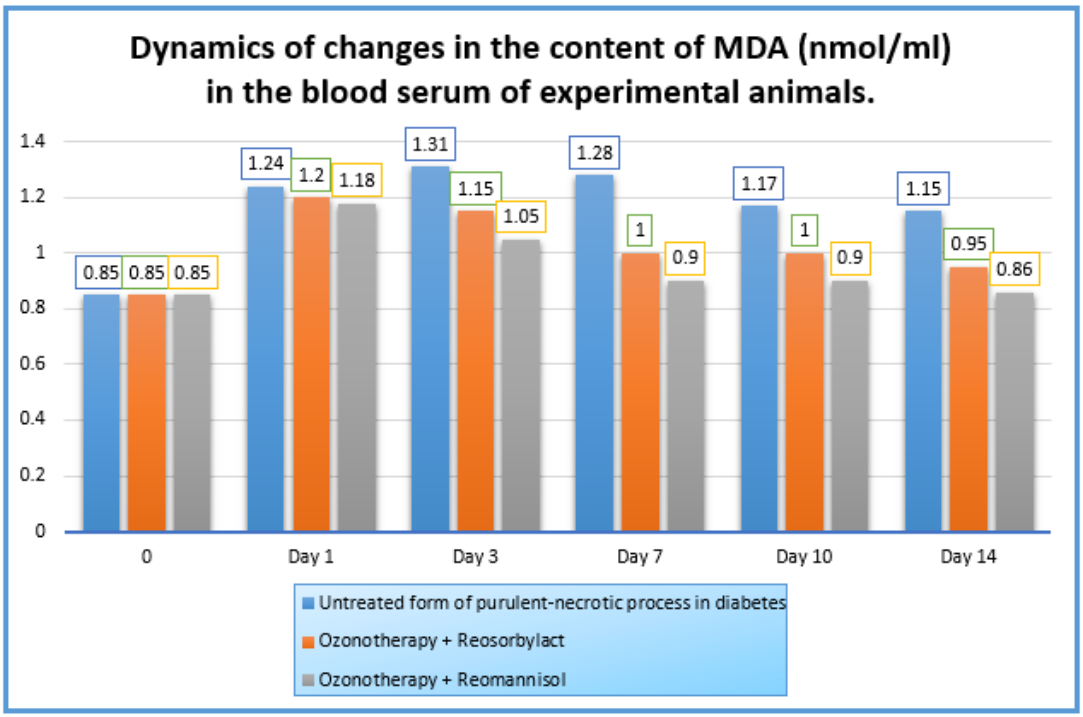


Figure 2. Results of biochemical parameters in experimental animals.

In the comparison group (Ozonotherapy + Reosorbylact), the 1st day of the study had no particular effect on LPO indicators. After longer administration of rheosorbylact, we observed a decrease in serum MDA levels on days 3, 7, 10, and 14 by 1.43; 1.3; 1.18, and 1.1 (P<0.01) times compared to the values of intact rats.

As a result of the use of the drug Reomannisol as a detoxifying treatment on the 1st and 3rd days of the experiment, the examination indicators did not differ significantly from the indicators of the comparison group. With longer use of the drug, we observed a corresponding decrease in the MDA level by 1.25; 1.31, and 1.3 (P<0.001) times compared to the parameters of the comparison group on days 7, 10, and 14 of the experiment. On the 14th day of the experiment, MDA indices did not differ from those of intact rats. As a result, the new detoxifying drug Rheomannitol not only reduces the level of the toxin but also has an antioxidant effect associated with the presence of succinate and mannitol in its composition. In his career, he also outperforms Reosorbylact.

It must be said that toxins of different molecular weights can accumulate not only in liquid biological areas of the body but also in cells, especially erythrocytes. In this regard, we study the levels of medium-weight molecular mass in diabetic purulent-necrotic foot ulcer dynamics, as well as the toxicity of oligopeptides and erythrocytes and their treatment with detoxifying drugs.

Study of the effect of the drug Rheomannitol on planimetric and morphological indicators of purulent-necrotic injury, as well as on the morphology of the pancreas and liver in diabetic-legged rats

Wound healing is a big problem for patients with diabetes. This problem is observed on a cellular and humoral basis. In this regard, we analyzed the planimetric and morphological parameters of rats in purulent-necrotic trauma of diabetes mellitus and the effect of Reomannisol on these parameters.

Studies have shown that within the control group of rats, the size of the wound was enlarged 24 hours after the injury, since the edges of the wounds were stretched apart due to the appearance of edema, the swelling spread throughout the leg. On the 2nd day after the formation of the Model, the surface of the wound defect is covered with a thin scab formed by the leakage of the wound, the shell is easily damaged and a transparent exudate leaks out of it. Clear signs of inflammation have been noted: the edges of the wound are swollen with areas of necrosis, and the bottom of the wounds are covered with fibrin areas.

A Morphological Study of wound tissue and skin surrounds in a group of diabetic-footed animals without detoxification therapy showed that on Day 1 of the experiment, the bottom and edges of the wound were covered with a mixture of blood with Necrotic mass and hematogenic pigments. Around the wound, the epidermis was in a state of change in the form of increased keratinization. In the dermis, the veins are enlarged, increased by perivascular foci of bleeding. The cellular elements of the connective tissue of the dermis near the wound were in a state of dystrophy and degeneration.

Figure 3. Image of morphological changes in the days after injury

Morphological studies on Day 7 showed the maintenance of necrotic mass and inflammatory infiltrate in the lower diabetic foot (Figure 3). In some areas, purulent inflammation in the form of neutrophil-leukocyte infiltration and abscess formation is detected around such foci, which are in a state of layered

epithelial rejection, vacuolization, and necrosis. The appearance of purulent foci of inflammation in the dermis has also been noted.

On the 10th day in the control group, a wound defect of about 1,8±0.05 sm^2 was preserved, and the regeneration coefficient was 8.4±0.21%. Massive purulent foci of inflammation in the form of both abscesses and phlegmona were found in the lower and circumference of the wound. Around the wound, the epidermis is in a state of complete rejection and necrosis, surrounded by neutrophil-leukocyte infiltration (Figure 5).

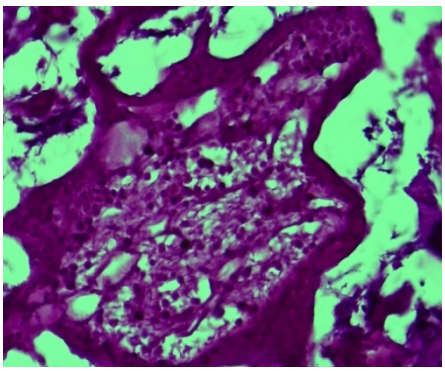
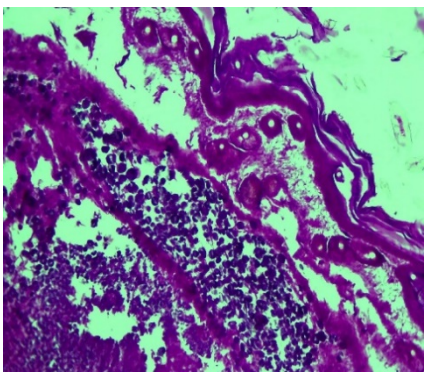
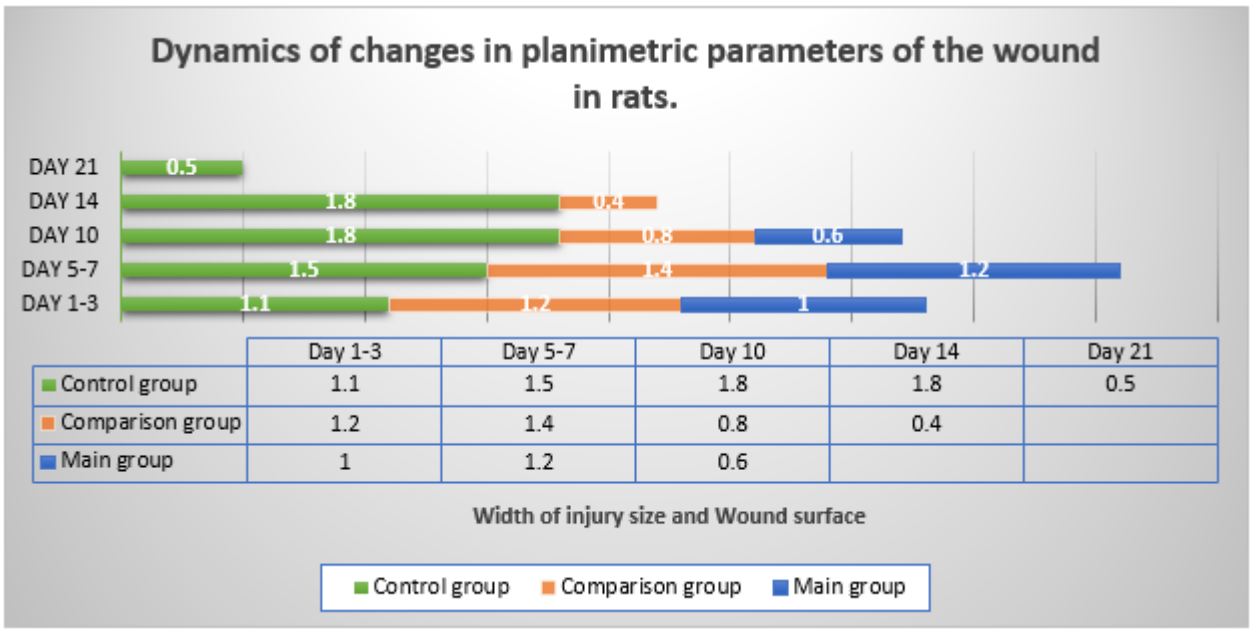
a)  b) 

Figure 5. Diabetic purulent-necrotic foot model, control group. On the 5-7th, acute foci of inflammation were found in the dermis around the wound (a). On the 10th day of the experiment, the rejection of the epidermis, and the appearance of abscess foci in the dermis (b). Color: G-E. K: 10x40.

On the 10th day of the experiment, both the epidermis and the dermis of the skin are restored, and there are signs of an inflammatory process (Figure 5b).

Thus, hyperglycemia causes microvascular complications due to impaired angiogenesis, which leads to an increase in the inflammatory effect of the wound and an extension of the wound healing period. On the 21st day of the experiment, the wound healed.



	Day 1-3	Day 5-7	Day 10	Day 14	Day 21
Control group	1.1	1.5	1.8	1.8	0.5
Comparison group	1.2	1.4	0.8	0.4	
Main group	1	1.2	0.6		

Figure 4. Dynamics of changes in planimetric parameters of the wound in rats.

In the comparison group on Day 1, we did not find significant differences in the ratio to the control group. Morphological studies have shown that swelling and relaxation of the epidermis and dermis are maintained. The epidermis was found to have dystrophic and destructive changes. On Day 7 of the

experiment, the wound defect was approximately 1,4±0.03 sm^2, with a regeneration coefficient of 8.5±0.18%, 1.42 (P<0.001) times higher than the rats' control group (figure 5.3). Morphologically, necrobiotic tissue is preserved in and around the bottom of the diabetic wound, its circumference infiltrated by leukocyte cells (see Figure 5.4 a).

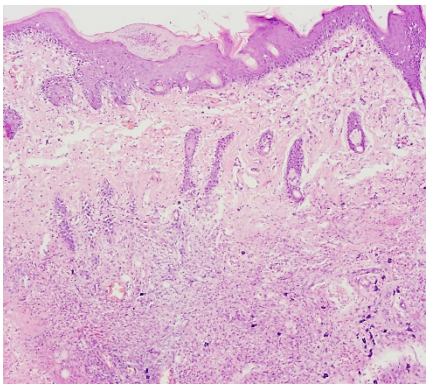
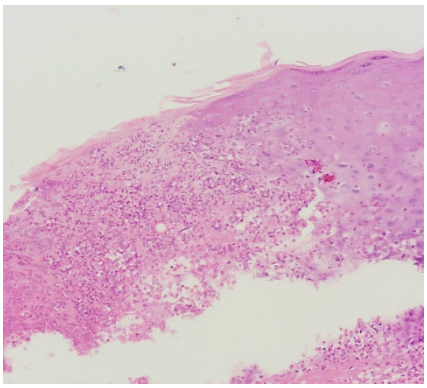
a)  b) 

Figure 6. Diabetic purulent-necrotic foot model, comparison group. On the 5-7th day of the experiment, the maintenance of necrotic mass and inflammatory infiltrate in the lower part of the wound (a). On the 10th day of the experiment, the a formation of a dense inflammatory infiltrate around the diabetic purulent-necrotic foot ulcer (b). Color: G-E. K: 10x40.

On Day 10, the formation of inflammatory granulation tissue with morphologic necrosis and foci of bleeding was found in the lower and circular areas. The inflammatory infiltrate is expressed by proliferatively active lymphoid and histiocytic cells (Figure 6b). In the center of the inflammatory infiltrate, a defect surrounded by fibrinoid necrosis and a homogeneous fibrous mass are detected. By the 14th day of the experiment in mice, the wound surface was completely closed.

In the main group (Ozonotherapy + Reomannisol) on the 1st day of the experiment, we did not find significant differences with the control group. On Day 7 of the experiment, a wound defect of about 1,2±0.05 sm^2 remains in the main group, which is 1.51 (P<0.001) times less, with a regeneration coefficient of 10.5±0.21%, which is 1.75 (P<0.001) times higher than the control group. On the 7th day after diabetic purulent-necrotic foot treatment with Ozonotherapy + Reomannisol, restriction, and rejection of destructive-necrotic tissue were recorded on the surface of the wound (Figure 7). At the same time, the epidermis tissue is compressed around the wound, and the destruction furnace of the dermis is limited.

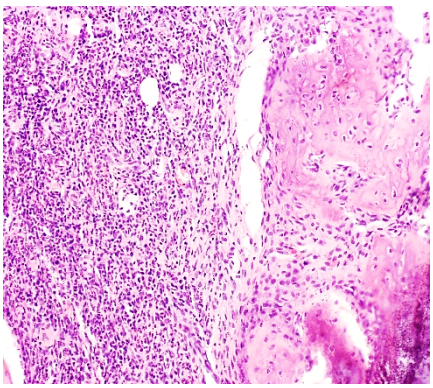
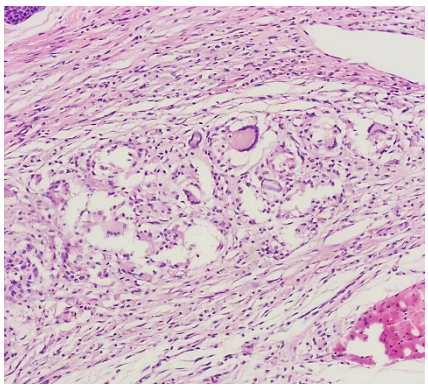
a)  b) 

Figure 7. Diabetic purulent-necrotic foot model, main group, day 5-7th, rejection of necrotic tissue on the surface of a diabetic wound (a). Day 10th, compression of the surface layers of the epidermis, fibrosis of the connective tissue of the dermis (B). Color: G-E. K: 10x40.

In rats treated with Ozonotherapy + Reomannisol, the wound surface is completely closed on the 10th day of the experiment. Morphologically, rejection of the necrotic part of epidermis tissue, increased proliferative activity of basal layer cells (Figure 7b). The connective tissue of the dermis is compressed,

without signs of an acute inflammatory process. The Epidermis is represented by several layers of active and hyperchromic epitheliocytes in the regeneration zone.

Thus, as a result of the treatment of the diabetic purulent-necrotic foot ulcer with Ozonotherapy + Rheomannitol, there is an early disappearance of signs of acute exudative inflammation in the tissues around the wound. By the 10th day, all cellular fibrous structures are fully restored without signs of dystrophy and inflammation.

Metabolic disorders, and vascular and neurological complications in DM lead to the development of changes in almost all organs and tissues. The results of morphological studies of the pancreas on Days 1 and 3 after dos modeling showed that the development of clear edema of interstitial tissues in the form of expansion, illumination, and breakdown of connective tissue structures was noted. Rotting and necrobiosis of some of them have been recorded in Langerhans cells. On the 7th day of modeling the diabetic purulent-necrotic foot ulcer, an inflammatory infiltrate appears in the pancreas. (Figure 8). On the 10th, the walls of the vessels thickened and narrowed inside, and inflammatory infiltration appeared in the circle. Endocrine islets decrease in size, there is relaxation and destruction of cellular elements. On the 14th, the development of catastrophic changes in the insular apparatus is recorded. The breakdown, and destruction of endocrine cells are recorded in the form of a violation of histography, and vacuolization of the cytoplasm of cells (figure 5.6 b). There is an inflammatory infiltrate in the circumference of the island apparatus.

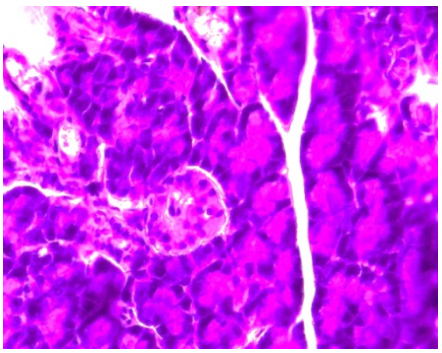
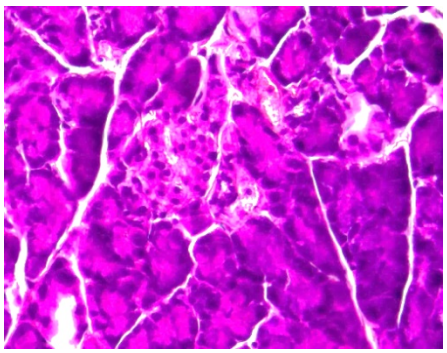
a)  b) 

Figure 8. Diabetic purulent-necrotic foot model. Control group. Pancreas. On the 5th- 7th day of the experiment, the appearance of inflammatory infiltrate in the gland intervals, and a decrease in the number of cells in the island apparatus (a). On the 14th day of the experiment, the breakdown and destruction of cellular elements of the island apparatus (b). Color: G-E. K: 10x40.

Thus, with the breakdown of cellular and fibrous structures of connective tissue in the pancreas, interstitial edema is noted. Dystrophic, destructive changes in the form of breakdown by the insular apparatus develop.

The results of a Morphological Study of the pancreas after conventional Day 1 treatment with Ozonotherapy + Reosorbylact showed that swelling and destructive changes were preserved in the glandular tissue. On the 3rd and 7th days of the experiment, a clear atrophy of the Endocrine islets and an increase in connective tissue along the glandular intervals are recorded. On the 10th day, an excess of connective tissue is observed in the form of strengthening of fibrous structures and irregular arrangement of histiocytic cells in the interglacial part of the gland. On the 14th day of the experiment, the glands of external secretion, different from the previous learning period, are gland cells of different shapes and sizes with the swelling of exocrine cells, the nuclei of which are located in the basal part of the cell.

Thus, pathomorphological changes in the pancreas remain in the form of moderate atrophy and dislocalization of endocrine cells.

Results of morphological studies of the pancreas on the dos model treated with Ozonotherapy + Reomannisol, on Day 1 of the experiment, the appearance of proliferative activity of endocrine islet cells, the preservation of endocrine and interstitial edema. exocrine parts of the gland have been recorded. In

islets of the Endocrine part of the pancreas, an increase in the number of cells is recorded on the 7th day of the experiment due to an increase in proliferative activity (Figure 9).

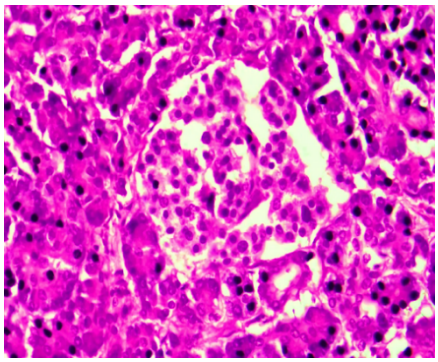
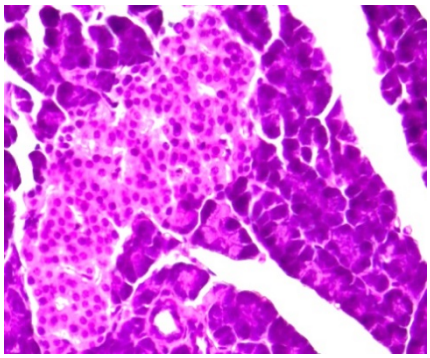
a)  b) 

Figure 9. Diabetic purulent-necrotic foot model. Experienced team. Pancreas. On the 7th day of treatment, an increase in the number of cells of the island apparatus (a). On the 10th day of the experiment, hyperplasia of endocrine Island cells (b). Color: G-E. K: 10x40.

On the 10th day of treatment of the diabetic purulent-necrotic foot model with Ozonotherapy + Reomannisol, increased hyperplasia of endocrine islet cells was noted. The area of Langerhans Island is expanded, and the entire area is filled with cells, most of which are in a state of proliferative activity (Figure 9b). On the 14th day of the experiment, the activation of the inner secretion part of the gland continues, and the outer secretion glands remain compressed due to interstitial edema.

Thus, after treatment of the diabetic purulent-necrotic foot ulcer with Ozonotherapy + Reomannisol, stabilization of dystrophic, destructive changes in the Endocrine and exocrine parts of the pancreas is noted in the pancreas.

On Day 1, the results of a Morphological Study of the liver after modeling the diabetic purulent-necrotic foot (control group), an excess of blood vessels, a diffuse tumor of the interstitial, and the development of small dropsy vacuolar dystrophy of the cytoplasm of hepatocytes were noted.

On the 7th day of the experiment, the formation of massive foci of circulatory disorders and bleeding in the liver tissue and liver parenchyma are exacerbated in the form of collapse (Figure 10). Parenchymal hepatocyte cells are most affected on days 10 and 14 after diabetic purulent-necrotic foot modeling in liver tissue. There is widespread damage to the liver parenchyma in the form of large drop vacuolar dystrophy (Figure 10).

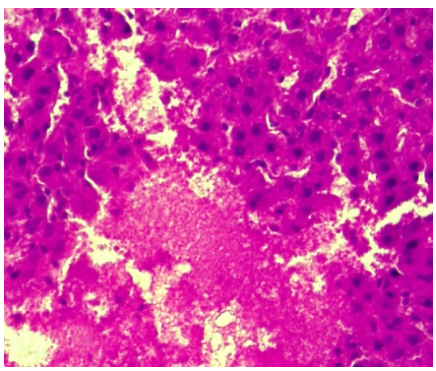
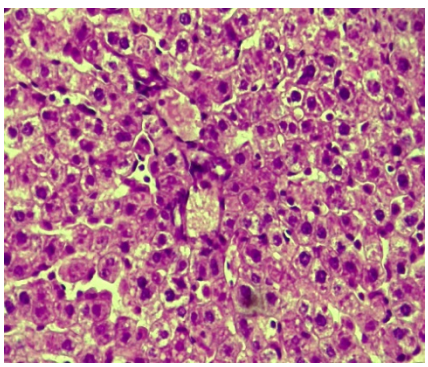
a)  b) 

Figure 10. Diabetic purulent-necrotic foot model. Control group, liver. On the 7th day of the experiment, hemodynamic disturbances with massive bleeding foci increased (a). On the 14th day of the experiment, large dropsy hydropic dystrophy of hepatocytes, the appearance of lymphoid cells in the Disse (b) cavity. Color: G-E. K: 10x40.

Thus, after modeling the diabetic purulent-necrotic foot in the liver, the development of increasing distraction, dystrophic, and dysregenerative changes in dynamics are noted.

On Day 1 of the administration of Ozonotherapy + Reosorbylact to rats with a diabetic purulent-necrotic foot, the results of a microscopic examination of liver tissue, diffuse hydropic dystrophy of the cytoplasm of hepatocytes, were preserved. On Day 7 of the administration of Ozonotherapy + Reosorbylact in rats with a diabetic purulent-necrotic foot in the liver, the level of vacuolar dystrophy of hepatocytes decreases slightly. Dystrophic changes in hepatocytes were slightly reduced on Day 10 of the administration of Ozonotherapy + Reosorbylact in rats with a diabetic purulent-necrotic foot in the liver. On Day 14 of the study, small foci of inflammatory infiltration by lymphoid cells were recorded in areas of liver tissue. Stabilization of dystrophic changes in hepatocytes has been noted by liver parenchyma compared to previous periods.

Thus, liver morphology showed the development of large dropsy vacuolar dystrophy of hepatocytes in the early stages. Next, a decrease in inflammation in animals is noted.

On the 1st day after treatment with Ozonotherapy + Reomannisol diabetic purulent-necrotic foot model, the results of microscopic examination of the liver observed the continuation of edema, dystrophic and destructive phenomena in the liver tissue. After treatment with Ozonotherapy + Reomannisol, a complete loss of vacuolar degeneration of the cytoplasm of hepatocytes is recorded in the liver in subsequent periods (days 7 and 10) (Figure 11).

By the 10th day of treatment of diabetic purulent-necrotic foot with Ozonotherapy + Reomannisol, a complete loss of dystrophic, destructive changes in hepatocytes in liver tissue is noted (Figure 11b).

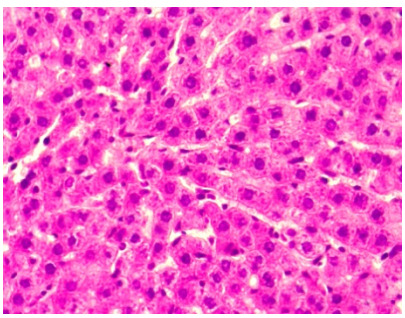
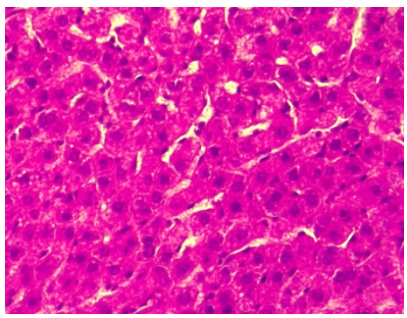
a)  b) 

Figure 11. Diabetic purulent-necrotic foot model. The main group is the liver. On the 5-7th day of the experiment, a loss of vacuolar degeneration, and an increase in the eosinophilicity of the cytoplasm of hepatocytes (a). On the 10th day of the experiment, the eosinophilicity of the cytoplasm of hepatocytes increased (b). Color: G-E. K: 10x40.

Thus, when treating a diabetic purulent-necrotic foot with Ozonotherapy + Reomannisol, stabilization of general pathological processes in the liver, loss of dystrophic and destructive changes, restoration of histotopography of liver parenchyma and radiation structure of hepatocytes are noted experience.

Summary.

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 rate of healing of wound defects in rats with diabetic foot syndrome in the control group falls on the 14th day since the terms of resorption and rejection of necrotic tissues in the wound are lengthened, damage to the vessels of the microvasculature (microangiopathy), edema is observed for a long time. The wounding process against the background of DM is characterized by the late formation of angiogenesis, slowing down and impaired maturation of granulation tissue, and marginal epithelialization. In the

experimental group, in rats, along with the local traditional method of wound treatment, the drug Reomannisol was used intraperitoneally, as a result, wound healing was recorded on the 10th day from the moment the wound was applied to the foot of the rats. The use of local treatment and Reomannisol can enhance angiogenesis in the early stages of the experiment and restore disturbed microcirculation (neoplasms of blood vessels), increase macrophage response, fibroblast proliferation, maturation and remodeling of granulation tissue and its epithelization, reduce the inflammatory reaction, which leads to more effective and early healing wound area.

4. Comprehensive treatment (application of a local traditional method of treatment on the wound and the drug Reomannisol) in an experimental model of the diabetic foot has positive effects on reparative processes and wound healing, due to the formation and enhancement of angiogenesis, as well as on the functional parameters of vital organs by reducing intoxication organism.

References

1. Ergashev, U. Y. (2022). Ernazarov Kh. I., Zohirov AR, Alzabni ID 2022. Complex Treatment of Experimental Model of Diabetic Foot Syndrome. *American Journal of Medicine and Medical Sciences*, *12*(5), 471-480.
2. Ergashev, U. Y., B. A. Abdusalomov, and A. R. Zohirov. "Eksperimental diabetik tavon sindromida hayotiy muhim a'zolarning morfologik o'zgarishlarini nazorat qilish." /Material of International scientific and practical conference" An integrated approach to the treatment of complications of diabetes", 2023.
3. Ergashev, U. Y., Zohirov, A. R., Minavarkhojayev, R. R., & Mominov, A. T. (2023). IMPROVING METHODS FOR DIAGNOSING AND MONITORING ENDOTOXICOSIS IN EXPERIMENTAL DIAETIC FOOT SYNDROME. *World Bulletin of Public Health*, *19*, 84-95.
4. Rafiqovich, Z. A. (2023). CONTROL OF INDICATORS OF ENDOTOXICOSIS IN DIABETIC FOOT SYNDROME. *Conferencea*, 83-90.
5. Rafiqovich, Z. A. (2023). MONITORING OF THE REGENERATION PROCESS IN PURULENT-NECROTIC PROCESSES OF THE LOWER EXTREMITIES. *Conferencea*, 189-194.
6. Rafiqovich, Z. A. (2023). OBSERVATION OF BIOCHEMICAL RESULTS IN EXPERIMENTAL DIABETIC FOOT SYNDROME. *Conferencea*, 181-188.
7. Rafiqovich, Z. A. (2023). STUDY OF THE EFFECT OF LIPID PEROXIDASE ANALYSIS ON THE BODY IN DIABETIC FOOT SYNDROME. *Conferencea*, 76-82.
8. Rafiqovich, Z. A. (2023, February). IMPROVING THE DETECTION OF MORPHOLOGICAL CHANGES IN PURULENT WOUNDS. In *E Conference Zone* (pp. 51-57).
9. Rafiqovich, Z. A., & Rustamovich, T. S. (2023). A Modern Approach to the Study and Analysis of Biochemical Parameters in Diabetic Foot Syndrome. *Texas Journal of Medical Science*, *19*, 39-47.
10. Rafiqovich, Z. A., Ogli, A. S. A., Ulugbekovna, K. D., Ilhomjonovna, S. U., Ogli, S. B. B., & Qizi, F. D. B. (2024). OPTIMIZATION OF COMPLEX TREATMENT OF PURULENT-NECROTIC WOUNDS ON THE FOOT IN DIABETES MELLITUS. *International Journal of Medical Sciences And Clinical Research*, *4*(09), 38-52.
11. Rafiqovich, Z. A., Sobirjonovich, S. S., Faxriddinovich, F. F., & Ubaydullaxonovich, O. S. (2023). Experimental Treatment of Purulent-Necrotic Lesions of The Lower Extremities with New Generation Drugs. *Texas Journal of Medical Science*, *18*, 30-38.
12. Ulugbek, E., Alisher, M., Nodirbek, M., Adkhamjon, Z., & Bekhzod, G. (2023). DIFFICULTIES OF LOWER LIMB AMPUTATION IN PURULENT SURGERY (LITERATURE REVIEW). *Journal of Academic Research and Trends in Educational Sciences*, *2*(2), 7-14.

13. Yusufjanovich, E. U., & Rafiqovich, Z. A. (2023). Treatment of purulent-necrotic lesions of the lower extremities with modern drugs. *Conferencea*, 88-94.
14. Yusufjanovich, E. U., Irisbaevich, M. G., Tashkarganovich, M. A., & Rafiqovich, Z. A. (2023). ACTIONS OF RHEOMANNISOLE ON THE TREATMENT OF EXPERIMENTAL DIABETIC FOOT SYNDROME. *Journal of Advanced Zoology*, *44*, 672-689.
15. Yusufjanovich, E. U., Rafiqovich, Z. A., & Irsalievich, E. K. (2023). Assessment of the Process of Epithelialization After Complex Treatment of Diabetic Foot Syndrome. *Texas Journal of Medical Science*, *16*, 19-23.
16. Yusufjanovich, E. U., Rafiqovich, Z. A., & Tohirovich, G. B. (2023). PRINCIPLES OF STUDYING LIVER MORPHOLOGY IN EXPERIMENTAL DIABETIC FOOT SYNDROME. *World Bulletin of Public Health*, *19*, 63-65.
17. Yusufjanovich, Ergashev Ulugbek, and Zokhirov Adkhamjon Rafiqovich. "DETERMINATION OF CHANGES IN THE LIPID PEROXIDASE INDEX IN PURULENT-NECROTIC LESIONS OF THE LOWER EXTREMITIES." (2022).
18. Зохиров, А. Р. (2023). ОБОСНОВАНИЕ ПРОЦЕССОВ ЭПИТЕЛИЗАЦИИ И РЕГЕНЕРАЦИИ ПРИ ГНОЙНО-НЕКРОТИЧЕСКИХ ПРОЦЕССАХ НИЖНИХ КОНЕЧНОСТЕЙ ПРИ САХАРНОМ ДИАБЕТЕ. *Conferencea*, 174-180.
19. Зохиров, А. Р., & Набиева, А. Ш. (2023). ИЗУЧЕНИЕ ПАТОМОРФОЛОГИЧЕСКИХ ОСОБЕННОСТЕЙ СОВРЕМЕННОГО ЛЕЧЕНИЯ ГНОЙНО-НЕКРОТИЧЕСКИХ ПРОЦЕССОВ ПРИ САХАРНОМ ДИАБЕТЕ. *Interpretation and researches*, *1*(2), 25-36.
20. Зохиров, А. Р., Эрназаров, Х. И., & Эргашев, У. Ю. (2022, January). ПАТОМОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ЗАЖИВЛЕНИЯ РАН ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ МОДЕЛИ ДИАБЕТИЧЕСКОЙ СТОПЫ. 64-ОЙ НАУЧНО-ПРАКТИЧЕСКОЙ КОНФЕРЕНЦИИ ОБУЧАЮЩИХСЯ «НАУКА И ЗДОРОВЬЕ» ПОСВЯЩЕННАЯ ДНЮ НАУКИ РЕСПУБЛИКИ КАЗАХСТАН С МЕЖДУНАРОДНЫМ УЧАСТИЕМ.
21. Каримов, Ш. И., et al. "Построение математических моделей оценки степени тяжести и прогноза эффективности лечения критической ишемии нижних конечностей при мультифокальном атеросклерозе." (2019).
22. Каримов, Шавкат Ибрагимович, et al. "Применение гибридных хирургических вмешательств у больных с критиче-ской ишемией нижних конечностей при мультифокальном атеросклерозе." *Тиббиет янги куни* 3 (2019): 27.
23. Эргашев, У. Ю., & Зохиров, А. Р. (2023). ИЗУЧЕНИЕ ПАТОМОРФОЛОГИИ ПЕЧЕНИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ СИНДРОМЕ ДИАБЕТИЧЕСКОЙ СТОПЫ. *European Journal of Interdisciplinary Research and Development*, *12*, 27-31.
24. Эрназаров, Х. И., Эргашев, У. Ю., Зоҳиров, А. Р., & Каримов, Х. Я. (2022). ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЕ ПРЕПАРАТА РЕОМАННИСОЛ В ЛЕЧЕНИИ ЭКСПЕРИМЕНТАЛЬНОЙ МОДЕЛИ ДИАБЕТИЧЕСКОЙ СТОПЫ.
25. Эрназаров, Х., Зохиров, А., Эргашев, У. Ю., & Исраилов, Р. (2022). ПАТОМОРФОЛОГИЧЕСКАЯ КАРТИНА ЖИЗНЕННО ВАЖНЫХ ОРГАНОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ МОДЕЛИ ДИАБЕТИЧЕСКОЙ СТОПЫ.