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**PHARMACOLOGICAL CORRECTION OF SKIN LESIONS IN  
EXPERIMENTAL DIABETES TAKING INTO ACCOUNT  
TYPOLOGICAL FEATURES**

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## TABLE OF CONTENTS

<b>INTRODUCTION.....</b>	<b>3</b>
<b>CHAPTER I. UNDERSTANDING THE PATHOPHYSIOLOGY, DIAGNOSIS AND MODERN ASPECTS OF DIABETES MELLITUS (LITERATURE REVIEW).....</b>	<b>7</b>
<b>CHAPTER II. MATERIALS AND METHODS OF RESEARCH: PHARMACOLOGIC CORRECTION OF SKIN LESIONS IN EXPERIMENTAL DIABETES TAKING INTO ACCOUNT TYPOLOGIC FEATURES.....</b>	<b>28</b>
<b>CHAPTER III. RESULTS OF THE STUDY OF TYPOLOGICAL AND TOXICOLOGICAL CHARACTERIZATION IN EXPERIMENTAL DIABETES.....</b>	<b>38</b>
<b>CHAPTER IV. MORPHOLOGIC CHANGES OF SKIN AREA TISSUES AT TRADITIONAL METHOD OF TREATMENT OF DIABETES INDUCED IN RATS IN EXPERIMENT.....</b>	<b>65</b>
<b>OVERWIEV.....</b>	<b>85</b>
<b>CONCLUSIONS .....</b>	<b>91</b>
<b>LIST OF USED LITERATURE.....</b>	<b>94</b>

## INTRODUCTION

In recent years, diabetes mellitus (DM) has evolved into a serious global public health issue, presenting challenges both to individuals and economies due to the significant cost burden it imposes. Experts from the World Health Organization (WHO) consider diabetes to be a “public health problem for all populations and age groups,” attributing its wide geographic spread, rapid disease progression, and early onset of debilitating complications that significantly impair quality of life and health status, and shorten its duration.

In a global context, the management of patients with diabetes mellitus, especially those in high-risk groups, requires an individualized typological approach, especially regarding the pathophysiology and pharmacokinetics of diabetes, as well as the development of new therapies. Scientific research has increasingly focused on understanding the pathophysiological and pharmacokinetic aspects of diabetes, which is closely linked to microcirculatory disorders and can lead to severe complications and mortality. Of primary importance is the development of a treatment system focused on preventing the severe consequences of this disease by creating a comprehensive set of preventive measures and implementing a treatment regimen.

In our country, renewed attention is being directed toward improving the quality of medical services provided to the population through the healthcare system, particularly in terms of diagnosing, treating, and enhancing the quality of care for diabetes patients. This focus is emphasized within the new phase outlined in Uzbekistan's Development Strategy for 2022-2026, which is aimed at enhancing the healthcare system, improving population health, and increasing the competence of medical professionals. A comprehensive set of measures has been devised for implementation in 2022-2023 within the framework of strategic goals such as "...strengthening the healthcare system, improving population health, and increasing the competence of medical personnel." In this regard, there is an opportunity to enhance the effectiveness of combating the pathogenic mechanisms

of diabetes and to introduce new treatment methods to achieve better outcomes through broader practical applications.

The research presented in this dissertation contributes to the realization of the tasks outlined in various regulatory documents, including Presidential Decree No. 60 of January 28, 2022, on Uzbekistan's New Development Strategy for 2022-2026, Presidential Decree No. 5590 of December 7, 2018, "On Comprehensive Measures to Improve the Healthcare System," and Resolution No. 3071 of June 20, 2017, "On the Improvement of Specialized Medical Care for the Population." The study focuses on fulfilling the responsibilities outlined in the relevant legal frameworks to achieve the stated objectives.

At present, efforts are underway to improve endocrinological legislation in foreign countries concerning the pathology and pharmacology of endocrine pathogenesis. For example, in the United States, an analysis was conducted on the diagnostic and treatment methods for individuals with clinical diabetes. Endocrinological analysis revealed that approximately 30-80% of patients with endocrine diseases experience significant damage to the sensitive tissues of the lower extremities. The quality of tissue regeneration in medicine depends on its typological features. According to research by Avdyushenko S.A. (2009), typological characteristics are determined by the relative activity of the sympathetic and parasympathetic divisions of the autonomic nervous system. Currently, a wide range of products derived from *Bombyx mori* fibroin is used in regenerative medicine, including scaffolds, films, hydrogels, microspheres, patches, electrospun dressings, ribbons, and more (Laurencin C. et al., 2006; Miron R.J. et al., 2012; Wu S. et al., 2014; Melke J. et al., 2016; Bagrov D., et al., 2017). According to the analysis of various authors' results, fibroin is a biocompatible, non-toxic natural polymer, its degradation is non-toxic, and in some cases, its breakdown can even produce beneficial by-products (Orlova A.A. et al., 2014; Moisenovich M.M. et al., 2015).

Today in Uzbekistan, a number of researchers are conducting scientific research on the negative impact on the human body, diagnosis and treatment in the

field of fibroin-based materials (Ibragimov T.K., 1992; Berdykulova D.M., 2011; Alikhanova N.M., 2016; UR. Hakimova, A.L. Alimov, 2016;). However, the exact location of fibroin-based materials has not been established. Taking into account the biocompatibility of silk fibroin, its association with long storage products, the use of these products, volumes, morphology and typological characteristics, we recommend methods to address this issue.

Studies of international literature indicate that in our country the method of processing materials based on fibroin has not been seriously studied, which indicates the relevance of the problem.

**Research objectives:**

- to evaluate the classification of animals to determine the level of skin lesions in experimental diabetes;
- to model skin lesions in groups of animals with different typological reactions in experimental diabetes and to determine the specific activity of local preparations of sulfuric acid, methylene blue and zinc complex for pharmacological correction of skin lesions;
- to study the changes of biochemical and hematological blood parameters in animals with experimental diabetes mellitus, taking into account their individual typological features;
- to analyze the comparative effect on skin lesions in the process of treatment of skin lesions with materials based on Bombyx mori fibroin in connection with typological reactions of animals.

**Scientific and practical significance of the research results.**

A new biocomposite material based on Bombyx mori fibroin from cocoons of local silkworms was developed and modeled taking into account individual typological reactions of animals with alloxan diabetes.

The processes of skin tissue regeneration as well as timely therapeutic interventions were developed.

Innovative interventions were implemented to reduce the visibility of scars at early stages, reduce the level of cosmetic deformities and minimize the expected costs of treatment, thus contributing to the development of regenerative medicine.

**Scientific and practical significance of the research results.**

The scientific significance of the research results is explained by their importance in the prevention and treatment of diabetes mellitus, therapeutic mechanisms of action of new complex drugs against inflammation, the role of their antiseptic properties in treatment, theoretical substantiation of their significance.

The practical significance of the research results is substantiated by modern screening, clinical and biochemical, pharmacological, toxicological, morphological and statistical evaluations, emphasizing the creation of new complex dosage forms based on the research results, developed through the use of domestic raw materials and ensuring the effectiveness of the drug in experimental conditions, as well as the evaluation of the mechanism of action.

## **CHAPTER I. UNDERSTANDING THE PATHOPHYSIOLOGY, DIAGNOSIS AND MODERN ASPECTS OF DIABETES MELLITUS**

### **§1.1 Current management of gestational diabetes and its epidemiologic aspects**

Diabetes mellitus (DM) is a complex metabolic disorder characterized by hyperglycemia, the different subtypes of which are closely related to the underlying causes of the disease. Currently, the prevalence of DM in our society is increasing under the influence of various factors [7, pp. 124-128; pp. 12, 27-31; pp. 18, 8-11 p] and population aging, which leads to the spread of this pathological phenomenon in the population and the development of preventive measures. Currently, there are different approaches to the study of diabetes mellitus. According to modern theories, the progression of many pathological processes is accompanied by an increase in the rate of free-radical oxidation of biosubstrates. [11, pp. 27-38; pp. 143, 133-138].

Maintaining public health through effective national health systems is an important global problem in all countries. The seriousness of the problem is evident from the increasing prevalence of chronic diseases such as diabetes, high mortality rates and early onset of diseases among patients. According to Global Health experts, "Public health is a concern for all ages and all countries." Diabetes mellitus is currently the third leading direct cause of death after cardiovascular disease and cancer; therefore, addressing this disease is a priority for many countries at the governmental level. Despite advances in modern treatments for diabetes, the disease remains a serious problem and is the eighth leading cause of death for people worldwide. Although efforts are being made to increase the life expectancy of diabetic patients with modern treatments, the number of patients continues to increase year after year. When a person becomes ill, considering the fact that his condition remains unresolved until his last day, this disease, known since ancient times, not only causes medical problems but also presents social problems in the modern world.

According to meta-analyses, there is a variety of data on the impairment of energy metabolism in chronic kidney disease [24, pp. 50-56; 31, pp. 67-77; 69, pp. 22-26; 80]. These studies demonstrate the relationship between the depth of disturbances of the energy apparatus and the severity of metabolic disorders, revealing the disruption of the close relationship between energy and metabolism. To understand the mechanism of action of various anti-diabetes drugs, products of preclinical testing stage are widely used in experimental settings to obtain detailed information about the functions of biological molecules. beradi [41, pp. 52-57; 42, pp. 005-010].

In the field of global health, the prevalence of two types of diabetes represents a major challenge in medical practice. The continuous increase in the number of patients, the progression of diabetes symptoms and the overall mortality rate represent a major barrier. Economically, countries affected by diabetes, including various countries around the world, allocate 10-15% of their health budget to the treatment of diabetes and its complications [20, p. 5-22].

Diabetes is spreading globally at a steady pace. Primarily affecting developing countries, the prevalence of diabetes is steadily increasing worldwide. However, in middle-income countries, the increase is occurring at an alarming rate. Unfortunately, the lack of effective policies to ensure access to and utilization of quality health services is a major challenge, especially for people with diabetes-related complications.

It is crucial to emphasize that the provision of health care, especially for those suffering from diabetes-related complications, is the most important task, rather than the management of the overall health of the population.

Ensuring the health and well-being of patients with diabetes is essential to prevent negative consequences. Moreover, the financial impact of the disease and its complications on individuals, families and entire national economies is significant. As part of the Sustainable Development Goals program, Member States have set ambitious targets to be achieved by 2030: reducing premature

mortality from diabetes by one third; ensuring access to essential medicines and basic health services for all.

The significance of diabetes lies in its manifestation in different countries and ethnic groups, as well as its epidemiology. Part of diabetology aims to understand the underlying principles of the epidemiologic situation and conditions associated with the disease, as well as the study of the environmental, social, and biological factors that shape its dynamics. The sources of scientific literature have provided information on the progression of the disease in different populations of the world and the microvascular complications associated with its progression, based on the analysis of studies conducted. This helped in identifying the risk factors for the development of diabetic complications.

Rapid economic development and urbanization in many parts of the world have increased the prevalence of diabetes [151, p. 1]. Diabetes affects a person's functional capacity and quality of life, leading to a significant burden of disease and early mortality [155, pp. 35-43]. Recent studies have shown that most diabetes-related deaths occur in people under 60 years of age, raising concerns about premature mortality [85, pp. 211-218]. These trends are associated with changes in diet and decreased physical activity levels, leading to an increase in body mass index (BMI) and elevated fasting blood glucose levels [135, pp. 235-239]. In particular, people with high BMI are more likely to develop the two types of diabetes [137, pp. 147-156]. Public awareness is another important factor, as diabetes affects both young and old people [149, pp. 164-174]. The cost of diabetes treatment is about 3.2 times the average cost of preventive medical care per person, and this figure increases to 9.4 times if complications are present [83, pp. 1-10]. Monitoring of factors such as glucose levels, blood pressure and other indicators remains suboptimal for many patients [80, 29-36]. Providing necessary education and health awareness is critical for effective diabetes management [120, 742-748]. According to the literature, approximately 462 million people worldwide suffer from any type of diabetes, representing 6.28% of the global population. In 2017 alone, more than 1 million deaths were directly attributed to this disease, placing it

as the ninth leading cause of death. The epidemic rise in diabetes-related deaths can be attributed to the alarming trends observed since 1990. Diabetes ranks fourth in disability-adjusted life years (DALYs) among all disease burdens.

The increasing incidence of type 2 diabetes demonstrates a distribution consistent with socioeconomic development. In developed regions such as Western Europe, despite the availability of health centers and preventive measures, the prevalence of the disease continues to increase at an alarming rate, resulting in serious health complications and persistent morbidity.

Notably, some regions such as Pacific Island countries like Fiji (20,277 cases per 100,000 people), Mauritius (18,545), American Samoa (18,312) and Kiribati (17,432) have the highest increase in diabetes cases. Southeast Asian countries such as Indonesia, Malaysia, Thailand and Vietnam have also risen in the rankings over the past twenty years.

In terms of affected population, China leads with 88.5 million cases of type 2 diabetes, followed by India with 65.9 million and the US with 28.9 million. These countries top the list for the highest number of people affected by the disease.

Although type 2 diabetes affects older people, it contributes significantly to the number of disability adjusted life years (DALYs). In 1990, it rose from 19th to 17th place, demonstrating the global importance of combating non-communicable diseases [60, pp. 379-378].

Men are more susceptible to the disease than women (6,219 cases per 100,000 compared to 5,898 cases). However, this difference is minimal. Men tend to be diagnosed slightly earlier, with the highest prevalence occurring between the ages of 55 and 59. Scientific studies have shown that from 1990 to 2017, there was no significant change in the distribution by age group [71, pp. 661-674].

In recent decades, the prevalence of patients with type 2 diabetes mellitus with complications affecting the kidneys has been increasing, indicating a significant increase in the severity of this condition. According to the literature [13, pp. 103-106; 59, pp. 54-58], the development of diabetic nephropathy accounts for

29-32% of the total progression of diabetic kidney disease. Traditionally, diabetic nephropathy progresses at a slow rate in individuals aged 35-40 years. However, recent studies have revealed an increasing trend in the incidence of type 2 diabetes in persons under 30 years of age. the percentage increased to 3.7% in persons aged 40-49 years and 6.7% in persons aged 60 years and older [159, pp. 1034-1039]. Thus, type 2 diabetes screening programs should not only target people aged 45 years and older, but also consider younger individuals who may have one or more risk factors for diabetes [72, pp. 502-512].

The DECODE (Diabetic Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) study, initiated by a group of European epidemiologists, examined the prevalence of type 2 diabetes taking into account age and gender differences [101, pp. 61-69]. The study was conducted in 8 European countries: Sweden, Finland, Denmark, Great Britain, Poland, Italy, Spain and Malta with the participation of 13 325 people aged 30 to 89 years. Among them, 1325 were diagnosed with type 2 diabetes for the first time. The prevalence of type 2 diabetes in most countries ranged from less than 10% among those under 60 years of age to 10-20% in the 60-79 age group. The highest prevalence of type 2 diabetes was found in Malta: 5.6% among men and women aged 30-39 years, 41.2% among men and 54.2% among women aged 70-79 years. The lowest prevalence was reported in Italy (2.1%) and Poland (2.6%).

According to the World Health Organization (WHO), a global health crisis caused by the novel coronavirus COVID-19 in 2020 [30, pp. 58-66] has created unprecedented public health challenges worldwide [75, pp. 132-139]. It has become a critical problem for public health systems in all countries. Individuals with poorly controlled blood glucose levels, such as diabetic patients, were particularly vulnerable because their immune response to viral attacks was compromised [75, pp. 132-139; 67, pp. 417-422]. In the presence of hyperglycemia, the virus exhibits a higher level of activity, leading to an increased risk of developing diabetes-related complications and worsening existing health conditions. COVID-19 disease progression and severity in diabetic patients have

shown an alarming trend of worsening outcomes. The most vulnerable categories of people prone to this disease are patients with severe chronic diseases such as heart and vascular diseases (coronary heart disease, heart failure, arterial hypertension, cerebrovascular disease), chronic obstructive pulmonary disease, chronic kidney disease, and diabetes mellitus (DM) [43, pp. 7-13]. According to data from medical institutions and monitoring centers, the incidence of COVID-19 in patients with comorbidities in China is recorded at 5.3% [9, pp. 227-231; 10, pp. 88-93], 10.9% in the USA with 7162 cases, and 35.5% in Italy with 355 cases [150, pp. 1775-1776]. In the Russian Federation, data on patients with COVID-19 and diabetes mellitus have not yet been analyzed. The collection of information on patients with diabetes mellitus during the epidemic, as well as the registration of infections and their outcomes in the database of patients with diabetes mellitus in Russia continues.

The number of people with DM in Uzbekistan has exceeded 245 thousand, of whom more than 2300 are children and 879 require intensive care. The results of surveys conducted by specialists in certain regions have shown a significantly higher prevalence of diabetes complications compared to official statistics. This emphasizes the need for endocrinologists to urgently address this problem using modern innovative methods of early diagnosis and treatment. The recent international scientific conference “Diabetes of the 21st Century, Global Challenges, Local Solutions” held in the capital aimed to draw attention to the problems faced by endocrinologists, neurologists, ophthalmologists, vascular surgeons and medical professionals, and to promote cooperation in developing innovative treatment approaches.

Treatment and exchange of experience. In order to further optimize the tasks to be solved, to fight endocrine diseases at an early stage, to ensure accurate diagnosis and treatment, the National Program for 2019-2023 has been developed by the Republican Specialized Scientific and Clinical Center of Endocrinology. This program includes a comprehensive plan of actions and measures, as well as the preparation of a draft “Road Map”.

Providing therapeutic and preventive care to children and adolescents with diabetes mellitus (DM) is considered a priority in the health care system of all countries of the world. DM is the most frequent endocrine and metabolic disease in children. According to the International Diabetes Federation (IDF), 395,000 children worldwide were diagnosed with type 1 DM in 2000. In 2017, according to the IDF, the total number of patients under 20 years of age with type 1 DM reached 1,106,000, of which 586,000 were children (under 15 years of age). An estimated 96,100 children develop type 1 diabetes each year, with the highest rates in the US, Russia, India and Brazil. According to the IDF, new cases of type 1 diabetes in children occur at a rate of 6 per 1,000 (3,100 per year).

In the 1990s, epidemiological studies of diabetes mellitus among children and adolescents in Russia were initiated by the Endocrinology Research Center in Moscow. The first data on the prevalence of diabetes mellitus in children were published in 1999. Currently, the main source of epidemiologic description of diabetes mellitus in different age groups is the federal register of patients with diabetes mellitus. Since 1996, the National Endocrinology Research Center of the Ministry of Health of the Russian Federation has been established. The federal register of children with diabetes has become a central repository of important clinical and organizational information. Since 2014, access to the diabetic register required authorization in the unified electronic database of the Russian Federation, which allowed online access, which by 2017 covered most regions of Russia, thus improving quality indicators. This system allows estimating the prevalence and outcomes of diabetes and related diseases in the Russian Federation.

During the period of illness and recovery, the main medical and social problems associated with chronic diseases are reduced life expectancy of patients, increased severity and unpredictability of complications.

From the point of view of modern medicine, the Register of Chronic Diseases is an automated system for analyzing information covering the entire chronic disease clinic and epidemiological monitoring, providing registration of patients and tracking the course of the disease. It also provides information on the

presence and type of complications after the disease, carbohydrate metabolism and other laboratory values, as well as the possibility of analyzing therapeutic outcomes and mortality rates.

It is important to realize that addressing diabetes prevention is not only a medical problem but also a social problem involving healthy lifestyle choices, nutrition, regular physical activity and timely detection of disease. Making such global psychological and personal changes in a short period of time may not be achievable without raising awareness. Precautionary measures, hygiene and health care delivery are areas that can facilitate the realization of the many objectives of diabetes prevention today. For every person diagnosed with diabetes, psychological support is crucial not only after diagnosis but also very important in learning to manage the disease. However, however comprehensive this knowledge may be, the main goal will not be achieved until various prevention strategies are implemented in a way that inspires confidence through simple recommendations that can be obtained from reliable sources and in a publicly accessible manner.

### **§ 1.2. Physiological and biochemical aspects of metabolic adaptation in diabetes mellitus in animals.**

The most convenient state of the organism is always called adaptation to changing environmental conditions. After a detailed analysis of the literary data, it became obvious that the development of society has caused the need to classify biochemical and physiological processes in the functioning of the human body in connection with the disorder. Disease progression involves the interaction and amplification of parallel processes. One of these processes has a pathophysiological peculiarity related to the disturbance of microcirculation and metabolism in the liver. Two types of autocatalytic processes have been identified: autocatalysis in the cascade of biochemical reactions and “self-activity” catalysis, when biochemical and pathophysiological processes enhance each other [45, p. 283-292].

A widely used model in experimental diabetology for modeling type I diabetes is the chemical model. According to scientific publications in the field of

entopharmacology, over a ten-year period from 1996 to 2006, 69% of the cases studied were associated with the use of streptozotocin and 31% with the use of the chemical compound alloxan [112, pp. 139-142]. Alloxan has been of interest since 1943, when its acute toxic effect was found to lead to selective necrosis of pancreatic beta cells, which later became recognized as a classic sign of type I diabetes, which was first demonstrated in the year of its initial discovery [105, pp. 384-387]. The presence of endogenous alloxan in biological substrates has been reported. Its concentration in humans, dogs, and rodents is reported to be 0.15025 mg% [159, pp. 1034-1039].

Alloxan is a derivative of pyruvic acid, which forms a white crystalline substance that oxidizes adipose tissue in the body. This drug shows diabetogenic effect only when administered parenterally - intraperitoneally, subcutaneously, intramuscularly or intravenously. In experimental studies, it is usually used to induce type 1 diabetes. The effective dosage varies depending on the animal species, diet, method of administration, and condition. When administered intravenously, diabetogenic doses of alloxan (mg/kg) vary between animal species. Despite numerous studies confirming the toxic effects of alloxan on  $\beta$ -cells, a generally accepted theory universally explaining this phenomenon has not yet been established. There are several hypotheses regarding the mechanism of action. According to A. Lazarov, alloxan penetrates the plasma membrane of  $\beta$ -cells and reacts with thiol groups, causing damage and disruption of membrane integrity to preserve  $\beta$ -cells. Ultimately,  $\beta$ -cells.

Based on the above information, the variability of multiple factors can be utilized to demonstrate the interaction of various factors in the pathogenesis of experimental alloxan-induced diabetes. These factors may have a synergistic effect leading to the severity of the condition as well as contributing to the reduction in their number. Further studies are needed in this area to unravel the subtle mechanisms of alloxan's effects.

The presence of liver disease in patients diagnosed with type 2 diabetes has drawn increased attention to the long-term health consequences and national health

services for the management of such conditions [127, pp. 140-148]. Individuals with type 2 diabetes are more likely to have abnormal liver function parameters compared to those without diabetes, emphasizing the importance of early detection and monitoring [81, pp. 1339-1349]. Epidemiologic studies have demonstrated a correlation between various liver diseases in type 2 diabetes, including nonalcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma [88; 129, pp. 3669-3679]. These liver diseases are considered important mortality factors in people with type 2 diabetes [117].

The important role of important metabolic organs in the regulation of glucose homeostasis in the liver has been emphasized [81, pp. 1339-1349; 148, pp. 1-12]. Indicators of liver dysfunction such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) have been proposed as good indicators to assess liver health as well as liver resistance to insulin [113, p. 14420] and risk of nonalcoholic fatty liver disease [129, pp. 3669-3679]. ALT is considered a specific marker of liver damage and is found predominantly in this organ [81, pp. 1339-1349; 142, p. 14040], whereas GGT is abundant predominantly in the bile ducts, liver, small intestine, and kidneys [81, pp. 1339-1349].

The main biological markers associated with liver diseases and oxidative stress can be identified through rigorous morphological studies, despite the paucity and inconsistency of research data [47, pp. 171-175; 90, pp. 657-666]. The assessment of various metabolic pathways in animals with alloxan diabetes is aimed at assessing the characteristics of these pathways and studying the potential participation of liver enzymes in their regulation.

### **§1.3. Pathophysiology of wounds and their relationship with diabetic complications.**

In recent years, more and more attention has been paid to political injuries by both domestic and foreign specialists. According to WTO statistics, the increase in the number of injuries worldwide is undeniable. Trauma plays a decisive role in the case of injury. Today, a severe combination of injuries and political trauma is

the most difficult problem. The unpredictability of the consequences of treating victims indicates that the problem has not been solved: mortality in severe injuries reaches 50% [14, p. 22] and a high degree of disability from 25% to 74% [61, p. 1-6].

Skin is the largest, most complex and multifunctional organ from an anatomical point of view. It plays a vital role in maintaining thermoregulation, water-electrolyte balance in the human body, serving as a barrier to external influences, including microorganisms, and a habitat for a wide variety of sensory receptors. Therefore, if the integrity of the skin is damaged, the healing process begins, ensuring complete tissue restoration. Skin wound healing is a complex, dynamic and fascinating process characterized by four main sequential and interrelated phases, each of which is regulated by several factors and overlapping in time: hemostasis, inflammation, proliferation and tissue remodeling [12]. When teriplication occurs, damage to microvascular channels can occur, leading to bleeding. To prevent bleeding, rapid vasoconstriction and coagulation processes are activated, leading to platelet aggregation and thrombus formation. Components such as fibrin, fibronectin, vitronectin, von Willebrand factor, and thrombospondin form a scaffold for the migration of blood vessel cells. Platelets play a critical role in hemostasis and provide the necessary response to prevent excessive bleeding. The process of platelet degranulation releases vasoactive substances and growth factors, including platelet-derived growth factors (PDGF), which promote the growth of new blood vessels. Modulation of key growth factors plays a critical role in various cellular processes. These include transforming growth factor- $\beta$  (TGF- $\beta$ ), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), keratinocyte growth factor (KGF), connective tissue growth factor (CTGF), etc. [109, pp. 1-17; 110, pp. 213-229]. The healing process begins with the attraction and activation of fibroblasts, endothelial cells, and macrophages. The inflammatory phase initiates the recruitment of these cells to the site of injury, the next stage is the activation of the complement system and wound infiltration with granulocytes and

polymorphonuclear leukocytes (PMN). This initiates a classic molecular cascade whereby these cells migrate to the wound site within 24-48 hours after injury in response to a variety of agents such as complement component C5a, platelets, bacterial formyl-methionyl peptides and TGF- $\beta$ . In addition, blood vessels undergo diapedesis allowing immune cells to gain access to the tissues surrounding the wound where bacteria and tissue debris are actively phagocytized and destroyed by lysosomal enzymes, peroxide and its radicals. PNL therapy is aimed at rapid resolution of wound infections, however these interventions do not directly promote the healing process. In later stages of inflammation (48-72 hours), PNL numbers decrease and monocytes migrate to the wound site, acquiring a macrophage phenotype. Macrophages play a crucial role in the resolution phase of inflammation, as they are able to produce cytokines and growth factors necessary for the proliferative phase of healing, in addition to their bactericidal function [161, pp. 866-881].

In case of development of wound infection in a patient on hemodialysis, during the subsequent healing process there may be direct mechanical violations of the boundaries of the affected area, leading to violation of tissue integrity, infection and disruption of blood supply, which leads to delay in wound healing. It should be noted that there are other factors in the literature indicating the likelihood of prolonged wound healing in hemodialysis patients, such as younger age, male gender, presence of cardiovascular diseases, late stage of chronic kidney disease, impossibility of providing external assistance, and inadequate self-care. Before starting treatment, attention should be paid to the size of the wound and the dynamics of its development during the first 4 weeks of treatment. These factors can significantly affect the progression of wound healing in patients with diabetic nephropathy, but some mechanisms of their interaction remain poorly understood. Pathogenic effects lead to more than 100 molecular and cellular disorders that cannot be cured by conventional methods of wound care, which often leads to ulceration. It is known that timely and adequate therapy of diabetic foot syndrome allows in most cases to ensure wound healing, reducing the fear of amputation.

However, the treatment of diabetic foot ulcers remains a difficult task to this day. The reasons for this are the incomplete implementation of all recommended measures by patients and the inadequacy of therapy carried out by health workers. A disease that affects all tissues and organs of the human body is called a complex disease. People with diabetes often experience changes in various organs, including the skin, eyes, kidneys, cardiovascular system and various skin diseases. Patients with a complex disease such as diabetes have unique general skin changes. In the acute phase of the disease, the skin can become dry, flaky and overly sensitive, affecting mainly the scalp and reducing the elasticity of the skin. Blisters and ulcers can form in certain areas. The skin color may appear pale. Nails can become deformed and brittle. Some dermatological manifestations can serve as “warning signs” of hidden diabetes that has not yet been diagnosed. According to the recommendations for these conditions, the occurrence of skin itching, rashes and skin lesions, as well as recurrent skin infections (such as candidiasis, pyoderma) are evidence. The etiology of skin irritation in dermatitis is certainly associated with carbohydrate metabolism disorders. To prevent the progression of symptoms, health care professionals need to regularly monitor patients' blood glucose levels. This helps reduce the occurrence and development of symptoms. When blood glucose levels drop, people with diabetes may experience excessive urination and dehydration. This condition also indicates dehydration of the skin: it becomes dry and cracked. The function of sweat and sebaceous glands is impaired. This can lead to itching, rashes and an increased risk of skin infections. Following proper hygiene rules will help prevent deterioration of skin health. However, traditional cosmetic products may not be suitable for sensitive skin, as they can disrupt the acidity of the skin and the microbial balance. Therefore, it is important to use pH-neutral soaps and cleansers when washing your hands and face. It is recommended to wash your face regularly with water or gentle cosmetic milk.

Hyperkeratosis (excessive callus formation) is one of the main causes of diabetic ulcers. In severe foot deformities, constant pressure can lead to the formation of persistent calluses in certain areas. Long-term pressure on acquired

calluses can lead to the development of trophic ulcers. The formation of wounds in the diabetic field is facilitated by its keratinization, causing cracking of the skin and making it susceptible to infection. During this process, there is a high risk of complications, including infection. Diabetic wounds can occur as a result of improper treatment of infected wounds. Common treatment methods include the use of modern wound dressings (such as alginates, polyurethane foams, hydrogels, etc.), regular treatment with alcohol-free antibacterial agents, and judicious use of antibiotics. Patients with diabetes who frequently receive insulin injections and visit places where blood samples are collected are at increased risk of skin infections. Even minor cuts from cutting toenails can also lead to infection. The occurrence of neuropathic pain (diabetic neuropathy) in patients with diabetes can lead to increased sensitivity to pain and even severe damage to the skin, which can potentially lead to skin infections. Therefore, it is extremely important to pay close attention to the skin condition of patients with diabetes. Avoid using alcohol-based antiseptics (e.g. iodine, brilliant green) or potassium permanganate to treat small wounds. It is recommended to use hydrogen peroxide, furacilin, chlorhexidine or other antibacterial agents in specialized wound care cosmetics. If there are signs of inflammation (e.g. redness, swelling, pain), the patient should immediately seek medical attention. Medical specialists in the field of endocrinology deal with pathological changes in the patient's skin that require attention. Skin changes can be a sign of a serious underlying disease and should not be overlooked. Even seemingly harmless skin changes can indicate a serious disease. The skin is considered a convenient organ for examination and at the same time provides important information. Skin changes can help to identify various internal diseases, including diabetic manifestations. Skin changes are associated with many internal diseases, especially those closely related to metabolic disorders, the pathogenesis of which is based on endocrine disorders affecting various organs and tissues, including the skin. The presence of some skin markers with metabolic changes, such as hyperglycemia and hyperlipidemia, is a direct result of diabetic complications. The progression of skin manifestations is significantly affected by

vascular, nervous or immune system dysfunction. The mechanisms of other dermatological diseases associated with diabetes remain unclear. Hyperinsulinemia observed in the early stages of insulin-resistant diabetes type 2 can also contribute to skin changes. Both macro- and microangiopathy significantly aggravate the skin manifestations of diabetes mellitus. Patients with diabetic dermopathy have increased vascular permeability, increased sympathetic innervation of vessels, and are susceptible to hypoxemic stress. The association of large vessel atherosclerosis with microvascular disease plays a role in the development of diabetic ulcers. In addition, diabetic neuropathy reduces skin sensitivity, making people more susceptible to infections and injuries. According to Khlebnikova A.N. and Marycheva N.V. (2011), there are several classifications of skin lesions in diabetic dermatopathy based on the characteristics and changes in pathogenesis. Within the framework of the pathogenesis of diabetic skin lesions, skin pathology in diabetic dermatopathy can be divided into five main groups: 1) dermatoses associated with diabetes mellitus; 2) skin pathologies associated with diabetes mellitus and insulin resistance; 3) skin pathology associated with angiopathy; 4) idiopathic rashes; 5) bacterial and fungal infections. According to the classification provided by Andrea A. Kalus, Andy J. Chien, John E. Olerud (2012), the following groups can be distinguished:

Skin manifestations of diabetes mellitus associated with metabolic, vascular, neurological or immunological diseases (diabetic scleredema, skin darkening, thickening of diabetic skin, joint movement disorders and scleroderma-like syndrome, xanthomas, skin infections (bacterial, fungal), diabetic ulcers);

Diseases associated with diabetes mellitus with unclear pathogenesis (lipoid necrobiosis, annular granuloma, diabetic dermopathy). These classifications do not act in isolation, but complement each other.

#### **§ 1.4. Modern methods of wound treatment in patients with diabetic foot ulcers involve the use of pharmacological agents that promote healing.**

After the process of wound formation, wound healing is influenced by a number of factors in the body, including the severity of the pathology, the state of

the homeostasis system, the activity of phospholipases, the presence of hypoxic phenomena, and impaired microcirculation. Healing of body wounds is characterized by gradual epithelialization and a tendency to develop granulation tissue. It is known that wound healing in the body has its own unique features, since it is associated with impaired microcirculation, the formation of microthrombi, necrotic and dystrophic wounds, and the reparative dominance of the granulation component. To enhance regenerative and reparative processes after operations to restore tissue and biochemical damage, pharmacological agents and various physiotherapeutic methods are used. An integrated approach to treatment is aimed at restoring the impaired functions of vital organs, maintaining acid-base balance, detoxifying the body, regulating metabolic, glycemic and lipid profiles, improving the hemorheological properties of blood, enhancing the body's natural defenses, and strengthening tissues. prevention of necrosis. At all stages of treatment and rehabilitation, it is necessary to involve specialists such as endocrinologists, diabetologists, orthopedists, surgeons and psychologists in caring for patients with complications of diabetic foot. Currently, one of the pressing problems of podiatry is the treatment of postoperative wounds after diabetic foot surgery against the background of diabetic vasculopathy. This includes the development of severe ischemic-necrotic conditions, a high level of lower limb amputations and the continuing problem of providing daily care to patients with diabetic foot ulcers. The pharmaceutical industry is one of the most complex branches of the chemical industry, characterized by numerous small enterprises, a high level of research and development activities and significant capital expenditures. Products of the modern pharmaceutical industry play a crucial role in protecting the health of our growing population. The production and benefit from these products are facilitated by advanced manufacturing processes and high growth rates, satisfying the growing demand for pharmaceutical products in the global economy and in individual countries through exports and imports. The pharmaceutical industry is currently one of the key sectors of the global economy, significantly influencing the state of affairs in related areas such as healthcare,

insurance, finance and others. At the same time, the knowledge-intensive nature of pharmaceutical production stimulates close development of relations with many sectors, ensuring their continuous development. In the context of global economic globalization, the pharmaceutical industry is undergoing significant changes due to broad geopolitical events. These changes affect the dynamics of the pharmaceutical market, eliminate enclaves of countries and regions associated with the global patent system, and integrate clinical and preclinical research methods into the development of drug delivery systems. Understanding the economics of the pharmaceutical industry requires consideration of the complex nature of public health measures that shape community responses to disease and the importance of aligning them with the various layers of drug delivery systems. Thus, in addition to the physical and psychological effects of a drug, its social impact is also critical. It is essential to improve patient interactions with others and to return the patient to an active lifestyle and work. The unique impact of public health programs in different countries must be taken into account.

When analyzing the problems of creating pharmaceutical products, it is important to study the phenomenon known as “disease evolution”. This phenomenon arises from various natural factors and affects human behavior and the evolution of pharmaceutical products. Therefore, it is necessary to take into account relevant fields of knowledge such as chemistry, biology, medicine, sociology, politics, statistics, psychology and management. This thesis is confirmed by the activities of well-known pharmaceutical companies engaged in the search, selection, creation, production, distribution and sale of pharmaceutical products, as well as their solution of marketing issues. The importance of the development, production, storage and distribution of pharmaceuticals and the protection of public health from an economic perspective is highlighted by the national security implications associated with the development of the pharmaceutical industry [48].

There are various creams available for the treatment of diabetic wounds and the prevention of their recurrence. However, the most effective products for wound

care are therapeutic gels and dressings that do not adhere to the wound and have high absorbency. These gels and creams prevent bacterial growth and the spread of infection. The latest development from the Russian company NPV "LitA-Tsvet" in the treatment of diabetic wounds, "LitA-Tsvet-2," is a therapeutic gel that provides high efficacy in combating microbes and stimulating biostimulation due to its unique composition. The main components of the product include "Echolin," a peptide bioregulator containing minerals, vitamins, and amino acids that accelerate tissue healing processes and provide reliable protection against bacteria.

BANEOCIN® (ointment) is used in the comprehensive treatment of lower limb ulcers in patients with diabetic foot syndrome. It is effective in treating necrotic lesions in these patients. Baneocin is an antibacterial agent used for the treatment and prevention of bacterial infections in cleansed and treated skin. It is effective in cases such as impetigo, infected trophic ulcers, infected eczema, bacterial dermatitis, and others. Additionally, in infants, Baneocin is applied to prevent the spread of bacterial infection in umbilical wounds. The ointment form of the antimicrobial agent Baneocin facilitates the treatment of burns in children and can be used on open wounds.

Povidone-iodine is an antiseptic that surpasses even alcohol-based antiseptics. Upon contact with the skin, its active ingredient, povidone, interacts with bacterial cell walls, leading to their destruction, coagulation, and eventual death of microorganisms. Povidone-iodine (Betadine®) is effective against various bacteria, and microbial resistance has not been documented. It effectively combats bacteria, unicellular microorganisms, herpes viruses, and the pathogens of fungal infections. This antiseptic is not intended for internal use. In the treatment of diabetic foot ulcers, it can only be applied topically. For optimal functioning of drainage systems, the primary components of the wound, including the ulcer and surrounding tissues, must be cleaned, disinfected, and mechanically debrided, with irrigation repeated 10 to 100 times. This irrigation helps treat any infected tissue in the wound area, promoting proper healing. Betadine solution is preferred due to its high safety profile, as it prevents iodine reabsorption when applied topically.

Therefore, povidone-iodine-based products are recommended for inclusion in wound care regimens for both adults and children. Additionally, healthcare professionals use povidone-iodine (Betadine) as an antiseptic ointment for treating skin lesions in patients with diabetic foot syndrome.

It is well known that more than 70 different enzymes are involved in the wound healing processes of the human body. Experimental data indicate that the demand for zinc significantly increases during the healing process. Thus, zinc application has shown positive results in the treatment of trophic ulcers of any etiology and diabetic foot syndrome. Some studies have demonstrated that zinc improves glucose levels (glycemic control) in diabetic patients. The accumulation of zinc-insulin complexes on the surface of pancreatic islet beta cells, enhanced insulin binding to hepatocyte membranes, inhibition of lipolysis, and stimulation of lipogenesis have been identified as key mechanisms by which zinc benefits the body.

Ternin is used as first aid in the treatment of skin injuries (deep wounds, minor cuts, pressure sores, skin blisters). It contains zinc oxide, which promotes healing, absorption, and disinfection. It reduces exudation, local inflammation, and irritation.

Chlorhexidine, in the form of chlorhexidine digluconate solution, is effective against both gram-positive and gram-negative bacteria. It establishes contact with the skin surface for no less than 10 minutes, influencing biofilm formation. Its effect on wounds and skin lesions is generally positive, although some microorganisms exhibit resistance to chlorhexidine. The product is primarily used by healthcare professionals for hygienic hand cleaning, disinfection of injection sites, and infection prevention in wound care.

*Bombyx mori*, also known as the silkworm, is a protein polymer widely utilized as a biomaterial. When silk fibroin is processed into various forms, it demonstrates remarkable mechanical properties, biological compatibility, a controlled degradation rate over years, and can be chemically modified to alter its physical properties or immobilize biologically active factors. Various methods,

including treatment with aqueous or organic solvents, can be used to fabricate silk-based biomaterials for a range of applications.

Currently, «*Bombyx mori*» silkworms are widely used in the production of hydrogels, scaffolds, sponges, composites, frameworks, microspheres, and thin films. These materials are frequently employed as biomaterials for direct implants, tissue engineering, in vivo disease models, and for the efficient delivery of therapeutic agents.

Throughout history, «*Bombyx mori*» silk has been highly valued for its durability and strength. Medical practitioners have used silk as a healing material for centuries, recognizing its biomaterial properties and unique characteristics. Its biological compatibility, ease of chemical modification, rapid degradation in vivo, and the ability to be processed into various material forms from aqueous or organic solutions make it an ideal candidate for biomedical applications. Due to its widespread availability for the textile industry, silk offers a cost-effective and sustainable solution. However, in medical applications, the purification of silk fibroin and proper processing are crucial requirements. The sericin component must be removed from the raw cocoons at the core of the fibroin fibers. Sericin is a group of water-soluble glycoproteins expressed in the silk gland of «*Bombyx mori*». These proteins coat the surface of the fibroin core of the silk thread in the cocoon filament. After removing this sticky protein, the fibroin fibers dissolve in an aqueous solution, which can then be processed into various materials.

### **Summary of Chapter One**

In modern medicine, one of the most critical issues is the increasing mortality due to the progression of diabetes and related causes. According to the International Diabetes Federation, over the past 20 years, the number of people worldwide suffering from diabetes has more than tripled.

A review of the scientific literature shows that type 2 diabetes has a high prevalence, accounting for 90% of all diabetes cases. The majority of this burden is associated with high BMI, dietary risks, environmental factors, occupational

hazards, tobacco use, alcohol consumption, and lack of physical activity. These factors together pose a significant threat to both our environment and public health.

Experimental induction of diabetes in animal models using alloxan is a complex process involving several stages, the primary element being the alteration of cellular metabolism. The induction of enzymes in animal tissues plays a key role in modifying metabolic pathways that lead to glycogen resynthesis in the liver in response to pathological conditions associated with experimental diabetes and feeding efficiency. By altering the main metabolic pathways triggered by glycogen resynthesis in the liver, enzyme induction in animal tissues helps regulate various factors that determine the rate of energy and synthetic processes. The intensity of enzymatic activity, which highlights the efficiency of energy and synthetic processes, underscores, based on numerous studies, the multiple factors contributing to the complexity of this pathological phenomenon.

Therefore, it is crucial to develop a mechanism that meets the restorative and compensatory needs of the population suffering from diabetes. The economic benefits of therapeutic and preventive measures lie in the increased life expectancy due to the effective use of treatment methods, the reduction of complications associated with the studied pathology, and, most importantly, the alleviation of human suffering.

## **CHAPTER II. MATERIALS AND METHODS OF RESEARCH: PHARMACOLOGIC CORRECTION OF SKIN LESIONS IN EXPERIMENTAL DIABETES TAKING INTO ACCOUNT TYPOLOGIC FEATURES**

Diabetes mellitus (the most common endocrine disorder) and its complications represent one of the most serious medical, social, and economic challenges in maintaining modern health. The late complications of diabetes, combined with other chronic diseases, are often a leading cause of disability. We studied the possibilities of early diagnosis and treatment of skin lesions in individuals with diabetes mellitus, taking into account their individual typological characteristics, ensuring the ability to anticipate the development of these complications.

### **§ 2.1. Description of Drugs Used in the Study**

The local silk of the silkworm «*Bombyx mori*» consists of fibroin proteins bound to sericin proteins. Sericin proteins are adhesive proteins, comprising 25-30% of the total mass of the silk cocoon. Fibroin consists of a heavy chain connected by disulfide bonds to a light chain (MM ~26 kDa) and a heavy chain (MM ~390 kDa). Fibroin is a copolymer composed of hydrophilic segments or blocks linked by hydrophobic blocks that form  $\beta$ -sheets. The crystalline regions mainly consist of glycine-X repeats, where X can be alanine, serine, threonine, or valine. Subdomains rich in glycine, alanine, serine, and tyrosine are distributed within these domains. As a result, a hydrophobic core is formed within the fibroin structure, leading to the creation of strong and elastic materials.

The dominance of  $\beta$ -sheet formation mechanisms in fibroin structures leads to materials with high mechanical strength and toughness. The strength of silk fibers is comparable to leading synthetic materials, including Kevlar®. In terms of biocompatible biomaterials used in medicine, silk fibroin stands out as a frequently used polymer due to its purity and ability to be easily processed into various biomedical materials. The ultimate tensile strength (UTS) of «*Bombyx mori*» silk fibers is 740 MPa, significantly higher compared to collagen (0.9-7.4 MPa) and

PLA (28-50 MPa). Thus, silk fibroin is a highly suitable polymer for biomedical applications.

## **§2.2. Study Materials and Design**

The scientific research was conducted in collaboration with the Institute of Pharmaceutical Education and Research (Uzbekistan) at the Laboratory of Pharmacotoxicology of the Tashkent Pharmaceutical Institute from 2018 to 2021. A total of 100 experiments were carried out on rabbits and 213 on guinea pigs. The research activities were conducted in accordance with the recommendations of the Institute for Laboratory Animal Resources (ILAR), which ensures the protection of animals used for experiments and other scientific purposes, following the European Convention (Strasbourg, March 18, 1986). Throughout the research process, animal care and handling were performed in accordance with the fundamental principles of the Helsinki Declaration and relevant guidelines. The experiments adhered to the Helsinki Declaration, emphasizing the importance of considering animal welfare alongside human interests. All procedures were closely monitored in the laboratory .

In the scientific studies, laboratory rats weighing between 140 and 320 g were used. The animals were kept under conditions providing free access to food and water, with a natural light-dark cycle. The rats were fed a standard diet in the vivarium. The experiment consisted of four series.

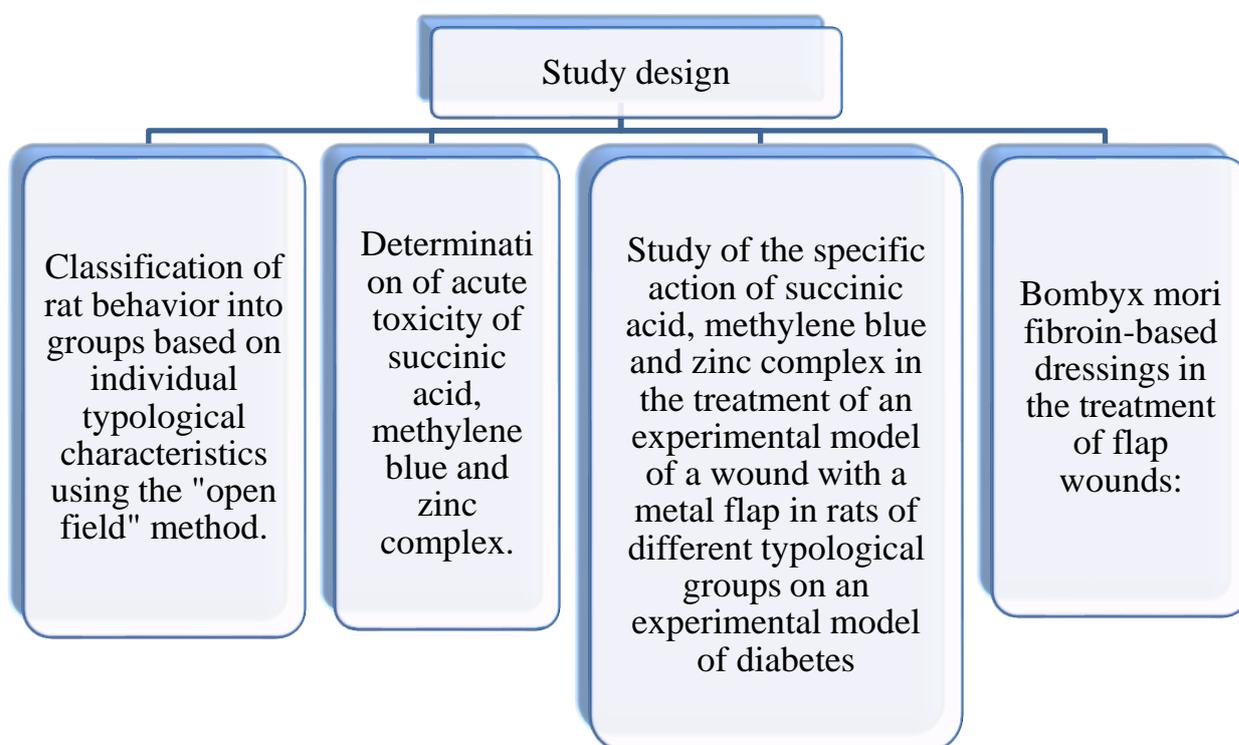
Using the "open field" method, experiments were conducted on 56 large adult laboratory rats weighing 250-300 g to determine individual typological characteristics. In a separate experimental study on 24 laboratory rats weighing 140-160 g, the toxicity of gastric and intragastric complexes of methylene blue and zinc was investigated.

For the treatment of wounds in various typological groups affected by experimental diabetic ulcers, the effects of acetic acid, methylene blue, and zinc complexes were studied on 36 rats. In another experiment, the effects of silk dressings were observed on 24 male and female rats weighing 200-220 g with skin wounds.

A study was conducted to examine skin diseases in combination with biochemical (ALT, AST, total bilirubin, creatinine and urea) and hematological parameters. Morphological parameters were also examined.

### §2.3. Research Methods

**Experiment 1. Determination of individual typological features of rat behavior using the "open field" test.**



Reactivity to stress, adaptability to stressful situations, changes in living conditions, extreme circumstances, and other stressors are considered key components of an organism's vitality. Moreover, the typological characteristics of both humans and animals are closely related to various physiological indicators. The literature describing the nervous system, based on hereditary factors, reveals different reactions and adaptation patterns to various stressors. Typological classification is a critical aspect of adequately assessing the state of physiological

systems in both normal and extreme conditions, as supported by numerous studies in physiology and experimental medicine.

Behavioral components of an animal placed in a novel open field were quantitatively assessed through observational measurements. The walls surrounding the field prevent the animal from escaping, inducing anxiety. Behaviors such as grooming, searching for food, retreating, and other active manipulations were recorded at least 1 hour prior to the experiment. Based on the "open field" test results, all animals were divided into three clusters: low physical activity, moderate physical activity, and high physical activity. The experimental trials were conducted without the presence of unfamiliar stimuli. The "open field" arena was a well-designed square platform measuring 1 x 1 m with 45 cm-high walls, with corners marked by square lines. The central sector of the arena was used to observe horizontal and vertical movements, head turns, fecal counts, and other behaviors. Animal temperament was evaluated in groups of three over a 3-minute observation period, and the results were subsequently analyzed.

**Experiment 2. Experimental study of the acute toxicity of succinic acid, methylene blue, and zinc complex in intragastric and dermal administration.**

An experiment was conducted on male and female mice and rats to study the toxicity of a complex composed of succinic acid, methylene blue, and zinc (SAMBZ), administered orally. The condition of the animals was monitored for 14 days after substance administration under controlled laboratory conditions, and the results were analyzed.

The complex was initially proposed for use in wound healing and dermatological procedures, and its acute toxicity and effects on depilated rabbit skin were investigated. The experimental animals were divided into 4 groups (6 animals per group).

The first series involved applying the tested substance to the clean skin of rabbits using a metal rod in volumes of 0.15 ml, 0.3 ml, 0.5 ml, and 0.75 ml. The substance was applied to the skin of rabbits (with an average group weight of 150

g) at doses of 10 mg/kg, 20 mg/kg, 30 mg/kg, and 50 mg/kg. The animals were observed for 14 days.

**Experiment 3. The effects of succinic acid, methylene blue, and zinc complex on wound healing in rats of different typological groups with experimental diabetes.**

In a series of experiments, we examined the effects of methylene blue and citric acid on tissue wound healing in different typological groups of experimental animals at the Center for Comprehensive Experimental Medicine (CCEM). A model of alloxan-induced diabetes was developed by administering alloxan hydrochloride at a dose of 150 mg/kg of body weight, leading to single-onset diabetes in experimental animals. In animals with experimental alloxan-induced diabetes (EAD), severe hyperglycemia was observed 10-17 days after induction, as reported in the literature, and on day 15 of the experiment, morphological and histological studies were conducted.

After grouping animals by individual typological characteristics, some exhibited distinct features of high or low motor activity. This method was used to evaluate the therapeutic efficacy of the wound healing model in diabetes mellitus (DM).

Skin wounds on the dorsal area were created to establish a wound healing model under general anesthesia using urethane at a dose of 1 g/kg. Skin flaps measuring 20x20 mm (including the underlying subcutaneous tissue) were excised. The skin defects were left open throughout the observation period. The wound surface was treated with appropriate dressings until full closure (see Figure 2.1).



**Figure 2.1. Comparative tissues during the experimental period.**

During the study, the animals were divided into six groups, each consisting of six subjects:

1. Group 1: Control group of animals with low physical activity (LPA);
2. Group 2: Control group of animals with high physical activity (HPA);
3. Group 3: Comparison group of animals with low physical activity receiving methylene blue;
4. Group 4: Comparison group of animals with high physical activity receiving methylene blue;
5. Group 5: Comparison group of animals with low physical activity receiving the succinic acid, methylene blue, and zinc complex (SAMBC);
6. Group 6: Comparison group of animals with high physical activity receiving the SAMBC.

After the wound healing process, animals were separated into individual compartments. The final wound healing dynamics were assessed using the following formula:

$$S = \frac{A \times B \times \pi}{4} \text{ (mm}^2\text{)}$$

Where:

- A - is the width of the wound in millimeters,
- B - is the length of the wound in millimeters.

Measurements were taken using an electronic caliper every two days, starting from the initial experiment. The treatment efficacy was calculated using the formula:

$$C = \left( \frac{S - S_1}{S} \right) \times 100\%$$

Where:

- S - is the initial wound area,

-  $S_1$  - is the wound area measured on the current day.

After determining the wound reduction area in each experimental group, the average wound area ( $M \pm m$ ) was calculated by subtracting the initial measurement from the healing wound area.

#### **Experiment 4: Study on the Impact of Flap-like Dressing Materials in Wound Treatment**

This experiment examined the corrective effects of a phytotherapeutic ointment based on mulberry silk extract. The experimental groups were distributed according to the dressing material used as follows:

1. Group 1 (Low emotionality): Therapeutic effect on animals with low emotional activity (control group, intact).
2. Group 2 (High emotionality): Therapeutic effect on animals with high emotional activity (control group, intact).
3. Group 3 (Low emotionality): Therapeutic effect on animals with low emotional activity treated with 50% fibroin + 50% cotton ointment (experimental group).
4. Group 4 (High emotionality): Therapeutic effect on animals with high emotional activity treated with 50% fibroin + 50% cotton ointment (experimental group).

To create the wound models, rats were placed under general anesthesia using urethane at a dose of 1.0 g/kg. In the control group, a cotton dressing was applied to the wounds. In the first experimental group, a 100% silk dressing was applied, in the second group, a 50% silk and 50% cotton dressing, and in the third group, a 30% silk and 70% cotton dressing was used. The wounds were created in the dorsal area of the rats, and skin flaps measuring 15x15 mm (including subcutaneous fat) were excised. The skin defects were left open throughout the healing period, and after complete closure of the wounds, the wound area was treated and the results were analyzed.

##### **2.2.1. Methods of Biochemical and Hematological Analysis**

The concentration of glycated hemoglobin (HbA1), derived from glucose-bound hemoglobin in the blood, was determined using human reagents. Hemolysis was induced, during which red blood cells release high concentrations of hemoglobin and borate ions into the surrounding medium. The hemolyzed mixture was then incubated with a Schiff reagent to remove interference. The hemolysate was mixed with cation exchange resin for 5 minutes, during which HbA0 was bound to the resin. A special resin was used to separate the supernatant containing HbA1 from the resin. The ratio of glycated hemoglobin to total hemoglobin in the blood was calculated by measuring the optical density of glycated hemoglobin at 415 nm or Hg 405 nm relative to total hemoglobin. All procedures were performed alongside standard samples.

Glucose levels in the blood serum were enzymatically oxidized by glucose oxidase (human reagents) in a glucose meter. In the reaction, hydrogen peroxide participates with phenol and 4-aminoantipyrine in the presence of peroxidase, resulting in the formation of a red-violet quinoneamine compound, which is then measured with a photometer.

According to IFCC (International Federation of Clinical Chemistry) recommendations, the activity of ALT and AST in blood serum was determined using a kinetic method with human reagents, utilizing pyridoxal phosphate.

All biochemical blood analyses were conducted using the semi-automatic biochemical analyzer Mindray BA-88A (China). Hematological parameters were assessed using a 5-Diff hematology analyzer from Mindray Co. Ltd (China).

### **2.2.2. Methods for Studying the Effects of Tissue Dressing Materials on Wound Healing**



The corrective effect of the dressing material made from mulberry silk fiber was studied on 24 rats of both sexes weighing 200-220 g. To create a model of skin

wounds, rats were placed under general anesthesia with urethane at a dose of 1.0 g/kg. In the experimental groups, the animals' skin was covered with a cotton dressing, while in the control group, a 100% silk dressing was applied. In the next experimental group, a dressing consisting of 50% silk and 50% cotton was applied. For the third experimental group, the dressing was composed of 30% silk and 70% cotton. After covering the animals' skin, skin flaps measuring 15x15 mm (including subcutaneous fat) were created by cutting the skin. The skin defects were left open throughout the observation period, and by the end of the treatment, the wounds had completely healed. The wound surface was then analyzed.

Following the treatment, the animals were placed in individual cages. Wound healing dynamics were evaluated by measuring changes in the wound area. After determining the wound area in each experimental animal, the mean wound area ( $M \pm m$ ) and the percentage reduction in the original wound size (i.e., the percentage of healing) were calculated for each group.

### **2.2.3. Morphological Methods**

Experimental animals were sacrificed using the decapitation method to study the morphological changes that occurred in the skin after treatment with various substances. The skin was then fixed in 10% neutral buffered formalin for 48 hours and dehydrated in increasing concentrations of alcohol (70%, 80%, 96%, 96%, 100%) and chloroform. After dehydration, the tissue was embedded in paraffin before sectioning into slices 5-6  $\mu\text{m}$  thick. The sections were stained with hematoxylin-eosin and histochemie dyes using alcian blue for visualization.

For histological analysis of the skin, the fixed tissues were dehydrated in alcohols of increasing concentration (70%, 80%, 90%, 96%, 100%) and chloroform before embedding in paraffin. The paraffin blocks were then sectioned, and the slices were dehydrated in xylene before further processing at 57°C.

### **§ 2.4. Statistical Method**

The results were analyzed using the statistical software package STATISTICA 6.0 on a personal computer. For the analysis of experimental research results, the mean values and standard errors ( $M \pm m$ ) were calculated. The

significance of differences between the results from the research groups for normally distributed samples was determined using Student's t-test. Quantitative results were summarized into variation series and reanalyzed using Microsoft Excel on an IBM-PC-AT-Pentium IV personal computer, following the confidence interval calculation method proposed by Student-Fisher (1962). Differences were considered significant when  $p < 0.05$ . Additionally, recommendations for the statistical reanalysis of laboratory research results were taken into account.

## **CHAPTER III. RESULTS OF THE STUDY OF TYPOLOGICAL AND TOXICOLOGICAL CHARACTERIZATION IN EXPERIMENTAL DIABETES**

### **§ 3.1. Determination of Individual-Typological Behavioral Characteristics in Experimental Animals Using the "Open Field" Method**

Adaptability to stress, stress-coping mechanisms, and responses to changes in living conditions or extreme situations are considered key components of an organism's resilience and viability. The nervous system, which has a hereditary basis as described in the literature, determines the individual typological differences in reactions and adaptation mechanisms to various stress factors. Numerous studies in physiology and experimental medicine confirm that typological differentiation, under normal and extreme conditions, allows for an adequate assessment of the state of physiological systems. This typological analysis is essential for evaluating test results and laboratory analyses, including "open field" experiments, involving various motor activities.

The design of the "open field" model has been described in the second section. The central area of the field was equipped with sensors to record horizontal and vertical movements, as well as to track traces and defecation counts. The behavioral patterns of the animals were observed during a 3-minute spontaneous group observation period. Based on the results of the "open field" study, all animals were divided into three clusters for analysis: high physical activity, low physical activity, and moderate physical activity.

To determine the individual-typological movement characteristics of the animals, their movement parameters in the "open field" were analyzed (see Table 3.1). The analysis showed no significant differences in the average body mass among all animals. The primary characteristics of individual typological behavior were assessed based on the number of squares crossed in the "open field."

Horizontal activity was evaluated by observing the animals' movements across peripheral and central squares (see Figure 3.1).

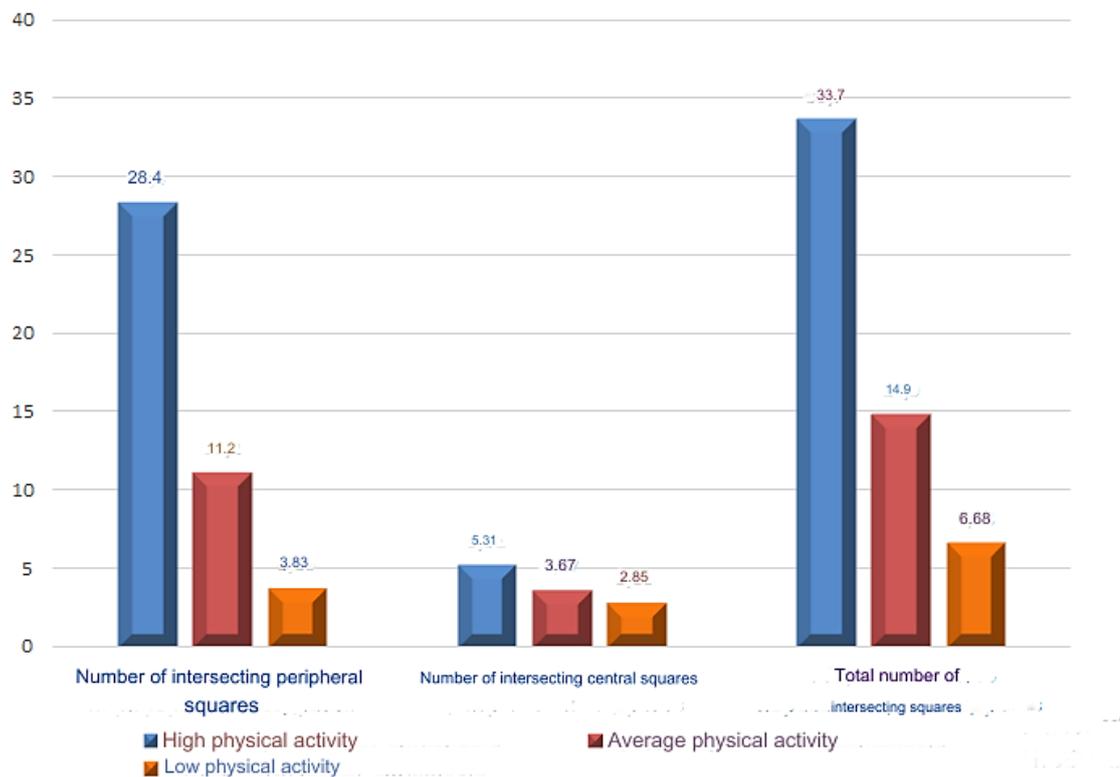


**Figure 3.1. Open Field Model**

The number of peripheral square crossings, indicative of a higher level of physical activity, was significantly higher in animals belonging to the high upper body activity type compared to other groups of animals. On average, the high-activity animals crossed  $28.4 \pm 0.9$  peripheral squares, while the low-activity animals showed a statistically significant decrease, with 7.4 times fewer crossings. The animals with moderate body activity showed an intermediate performance compared to the other groups.

The number of central square crossings also demonstrated a noticeable change, though this difference was less pronounced than the peripheral square crossings. Animals with higher physical activity crossed the central squares 1.86 times more frequently compared to those with lower physical activity (see Figure 3.2).

In analyzing the total number of crossings in the group with moderate physical activity, the number of crossings increased by 1.4 times. When examining the total number of square crossings, animals with higher physical activity crossed five times more squares compared to animals with lower physical activity. Those with moderate physical activity crossed squares 2.3 times more than animals in the low-activity group. It was also established that animals with lower physical activity showed 2.2 times fewer crossings compared to those with moderate physical activity.



**Figure 3.2. Activity indicators of laboratory rats with different types of behavior in the open field test.**

Analyzing the data obtained, animals with a high level of physical activity move mainly along the periphery of the "open field", less often heading towards its central part. In the category of animals with high physical activity, 84.3% were on the periphery of the "open field", and 15.7% were in the center. In animals with low physical activity, 57.3% were on the periphery and 42.7% were in the center, which contributed to overall physical activity. In the group of animals with moderate physical activity, 75.2% and 24.6% were evenly distributed. When analyzing the groups, no significant difference in the levels of vertical activity was found.

**Table 3.1**

**Behavioral activity indicators of laboratory rats with different types of behavior in the "open field" test**

Groups	Indicators						
	Body weight, g	Number of intersecting peripheral squares	Number of intersecting central squares	Total number of squares cut out	Vertical activity	Number of groomings, pcs.	Number of bowel movements
Average physical activity	288.7±1.5	11.2±0.3	3.67±0.15	14.9±0.3	3.18±0.20	4.80±0.22	1.85±0.16
Low physical activity	298.3±7.3 P <sub>2</sub> >0.05	3.83±0.37 P <sub>2</sub> <0.001	2.85±0.13 P <sub>2</sub> <0.01	6.68±0.32 P <sub>2</sub> <0.001	2.73±0.31 P <sub>2</sub> >0.05	4.24±0.21 P <sub>2</sub> >0.05	1.41±0.21 P <sub>2</sub> >0.05
High physical activity	271.8±1.4 P <sub>1</sub> >0.05 P <sub>2</sub> >0.05	28.4±0.9 P <sub>1</sub> <0.001 P <sub>2</sub> <0.001	5.31±0.24 P <sub>1</sub> <0.001 P <sub>2</sub> <0.01	33.7±1.0 P <sub>1</sub> <0.001 P <sub>2</sub> <0.001	3.76±0.24 P <sub>1</sub> >0.05 P <sub>2</sub> >0.05	5.26±0.20 P <sub>1</sub> >0.05 P <sub>2</sub> >0.05	2.32±0.17 P <sub>1</sub> >0.05 P <sub>2</sub> >0.05

Note: P<sub>1</sub> - applies to the group of passive type animals;

P<sub>2</sub> – indicator for a group of moderately active animals

In all groups of animals, the grooming duration was significantly brief and short, without statistically significant differences. Most animals in the sample exhibited a moderate level of defecation in the arena, with no significant differences observed between the groups. This indicates a high level of distress among the animals, as evidenced by the low baseline anxiety level before the experiment. It is likely that a close correlation had already been established between the general well-being of the animals and their healthy feeding habits. Both animals with low and moderate physical activity demonstrated a reduction in horizontal activity in the central and peripheral areas of the "open field." An increase in this indicator reflects the emergence of positive sensory tension, such as anxiety, fear, or restlessness.

The results of these experiments showed that, based on the characteristics of horizontal motor activity assessed using the "open field" test, the animals could be divided into three groups: high motor activity, moderate motor activity, and low motor activity types. According to scientific literature, physiological and biochemical differences can be assessed by dividing animals into groups based on stress-induced behavior in the unfamiliar environment of the "open field" test. Therefore, the presence of various individual typological groups among the experimental animals may lead to pathological conditions, causing differences in the organism's response to pathologies.

### **§ 3.2. Results of Acute Toxicity Assessment of Succinic Acid, Methylene Blue, and Zinc Complex**

The search for new effective treatments for diabetes mellitus (DM) is one of the most critical issues in global healthcare and disease prevention [68, pp. 71–78]. The rapid development of modern medicine has provided a range of possibilities for the diagnosis and treatment of this complex disease. Our country places significant emphasis on ensuring the supply of effective and affordable medical products produced domestically, not only from an economic standpoint but also in terms of public health. Various pharmaceutical companies and research laboratories are actively involved in developing and producing new medical products through ongoing research and development. The Central Research Laboratory at the Institute of Pharmaceutical Education and Research leads this effort, producing not only the drug *Bombyx mori*.

Currently, the significant role of chemical etiology in clinical intoxications is recognized in most countries worldwide [78]. Given this fact, we considered it necessary to study the acute toxicological properties of the *Bombyx mori* preparation, and based on the results of the research, we found the following:

After the administration of 0.5 ml/kg of the preparation, no significant changes were observed in the activity, appearance, or functional state of the animals. Their skin and fur remained unchanged, and they did not refuse food or water. No deviations in behavior or physiological parameters of the animals were

noted on the second and subsequent days. Water and food consumption remained within normal limits, with no signs of dehydration or poor growth. No mortality occurred in the animals during the 14-day observation period.

The dosage for the second group was 0.75 ml/kg: the drug was effective without any noticeable changes in the behavior and functional condition of the pigs following administration. Water and food intake remained normal, and no delays in growth or development were observed. No mortality was recorded in piglets during the 14-day observation period. The condition and appearance of the skin and fur remained largely unchanged; the animals continued to consume food and water normally, and no signs of impaired muscular function were observed. Throughout the observation period, on the second day and afterward, no pathological changes in the behavior or physiological indicators of the piglets were detected. Their skin and fur maintained a normal appearance without alterations, and food and water intake were regular, with no mortality observed within the 14-day period.

In the third group, the drug was administered at a dose of 1.0 ml/kg, and soon after administration, temporary muscle weakness and immobility lasting 30-40 minutes were observed. Within an hour, the mice returned to their previous state without any increase in normal physical activity or body weight. The mice readily consumed food and water, and their reactions to handling and care remained normal, with clean fur, skin, and regular breathing. The mice showed no signs of distress or abnormal growth. No mortality was observed in the mice.

Some changes were observed in mice administered higher doses of QCMKC (0.75 ml/kg and 1.0 ml/kg), such as reduced activity levels and seizures in the animals. These changes were noted to begin approximately 45-50 minutes after the average time of administration. The experimental results suggest that the administered drug proved more toxic when taken orally.

Due to the absence of any recorded deaths in the experimental animals, the LD50 (median lethal dose) was not determined (see Table 3.2).

**Table 3.2**

### Average toxicity indices

Dose, mg/kg	Total/deaths
0,15	6/0
0,3	6/0
0,5	6/0
0,75	6/0

During the 4-hour study, the potential for local skin irritation from plant extract samples applied to the animals was assessed. The extracts were applied to the skin in doses specified in Table 3.2. The skin was covered with gauze soaked in the extract suspension and left in place for 4 hours. Afterward, the test area was examined for signs of irritation immediately upon removing the bandage, then again after 1 hour, and finally after 16 hours. At no point during the single-application test was any irritation observed, nor were there any signs of intoxication or mortality among the animals.

In repeated toxicity tests of the drug, laboratory mice underwent a total of 20 skin applications over the course of one week, applied daily for 4 hours per day. Several skin tests were conducted throughout the experiment to assess the reaction, as well as monitoring for signs of mortality and clinical symptoms of intoxication. Special chambers were used on the mice to test the absorption effects of the drug. Skin samples from the experimental animals were excised two-thirds of their length and placed in tubes containing an erythema solution at a temperature of 36-37°C for 4 hours.

After the experiment, the animals were calm and hydrated. Throughout the 3-week observation period, no signs of intoxication or mortality were observed.

Thus, when the test substance—pesticides derived from QCMKC—was applied to the animals' skin, no abnormal reactions or fatalities were detected.

### **§ 3.3. The Effect of Succinic Acid, Methylene Blue, and Zinc Complex on Wound Healing in Rats of Different Typological Groups with Experimental Diabetes**

Localization of common musculoskeletal injuries often occurs in areas of maximal vertical load on the lower limbs. These injuries typically affect the metatarsal heads and claw prominences. Pain and proprioceptive deficits contribute to a lack of awareness of the injury and may prolong patient recovery. Failure to address these injuries in a timely manner can lead to further trauma and complicate treatment. Thus, treating trophic lesions is crucial to minimizing the impact of injury on healing. Even a few simple steps taken during the day can significantly enhance the healing process. Therefore, mitigating damage caused by various etiopathogenetic factors presents both scientific and practical challenges. Effective trauma treatment requires addressing the damage induced by specific etiopathogenetic factors.

Currently, various local plant-based remedies have shown potential to significantly enhance wound healing, including wounds that arise during recovery from pressure sores. More than 300 types of wound dressings are in clinical use, yet scientific studies have identified a few types with unique beneficial properties that can affect the various pathogenetic processes associated with different types of injuries. In the development of plant-based medicinal products that can act on wounds related to different pathogenetic processes, we set out diverse goals aimed at producing innovative wound dressings. Based on scientific literature, we conducted an experiment using *Bombyx mori* extract in the treatment of wounds from different etiological groups, particularly those affected by pressure ulcers. The study concluded that investigating the effects of hydrocolloids, methylene blue, and zinc complexes on the treatment of wounds in different typological groups led to the following results.

The results of the study indicated that the initial values of the lowest and highest levels of physical activity in animals treated with the zinc complex (ZC) in the observation groups, from 3 to 6 days post-injury, significantly decreased compared to baseline. The healing rate was  $9.90 \pm 0.05\%$  and  $6.7 \pm 0.2\%$ , respectively, which did not show significant differences between animals with moderate physical activity. The intensity of the inflammatory response was

objectively recorded, including swelling, hyperemia, and infiltration of atrophic areas. By the 9th day, the wound area had reduced, with healing rates of  $23.2\pm 0.75\%$  and  $19.4\pm 1.2\%$ , respectively. By days 12-15 of the experiment, the wound area continued to shrink, becoming more noticeable.

The clinical picture of the infiltrated tissues—characterized by edema, hyperemia, and atrophy, combined with hemorrhagic fibrin clots—was observed only on day 18. Minor bleeding and persistent hyperemia were noted as well. By day 24, the initial wound healing rate had reached  $91.3\pm 2.1\%$  and  $92.5\pm 0.9\%$ , respectively (see Table 3.3).

**Table 3.3**

**Low activity and high activity in experimental diabetes: dynamics of changes in wound area in rats of control groups.**

Duration of experience	NFA Control		VFA Control	
	Wound area , mm <sup>2</sup>	Wound healing , %	Wound area , mm <sup>2</sup>	Wound healing , %
3rd	303.5±3.3	-	300.9±2.5	-
6th	273.4±3.1	9.90±0.05	280.8±4.6	6.7±0.2
9th	233.1±3.2	23.2±0.75	242.7±4.2	19.4±1.2
12th	164.1±5.2	46.0±1.59	190.6±3.8	36.7±1.1
15th	118.3±3.3	61.0±0.99	137.8±4.5*	54.2±1.5*
18th	81.1±1.9	73.3±0.65	79.5±2.4	73.6±0.7
21st	50.9±2.3	83.2±0.7	44.2±2.2	85.3±0.7
24th	26.4±2.1	91.3±2.1	22.5±2.6	92.5±0.9
27th	11.3±1.7	96.3±0.6	4.3±0.8	98.6±0.3
30th	4.6±1.1	98.5±0.4	1.2±0.2*	99.6±0.2*
33rd	0.8±0.3	99.7±0.1	-	100,0

Note: \* - relative to animals with low activity at  $p<0.001$ .

\*\* - Statistical data for animals with low activity at  $p<0.001$ ;  $p<0.01$ ;  $p<0.05$ .

\*\*\* - Statistical data regarding animals with high activity at  $p<0.001$ .

In the studies conducted on days 27 and 30 of observation in both groups, wound healing area and healing rate were assessed using the method of I.V. Berezovskaya. The results indicate a significant difference in wound healing between animals with lower levels of physical activity, where wound closure

reached  $96.3\pm 0.4\%$ , compared to animals with higher levels of physical activity, where wound closure reached  $98.6\pm 0.3\%$ . It is noteworthy that complete wound healing in animals with high physical activity occurred 3 days earlier than in groups of animals with lower physical activity.

During experimental treatment with the antiseptic methylene blue in animals with a lower level of physical activity, there was a reduction in inflammatory processes from the start of experimental therapy, leading to a decrease in cases of swelling. The wound healing rate from days 6 to 18 did not significantly differ from the control group.

Clinically, by day 12 of the experiment, a reduction in hyperemia and the absence of edema and inflammatory reactions were observed compared to the control group. By days 21, 24, and 27 of the study, the wound healing process in the experimental group, which had lower physical activity, was significantly higher—by 9.3% and 7.3%, respectively.

On day 27 of the experiment, complete wound healing in the experimental group reached  $99.6\pm 0.2\%$ , which was an increase of 10.3% compared to the control group. Based on the data obtained, it can be concluded that by day 27, wound healing in this group had significantly accelerated.

In animals with type 2 diabetes and high physical activity treated with methylphenidate, the wound healing process was significantly accelerated compared to animals with type 2 diabetes and low physical activity. On days 6, 9, and 12 of the study, the wound healing duration in the control group and in animals with type 2 diabetes and low physical activity showed an increase of 182.1%, 84.5%, and 59.1%, respectively, compared to 87.1%, 43.2%, and 39.7% in the group with high physical activity. Experiments from days 9 to 12 showed a reduction in erythema, absence of swelling, and a decrease in inflammatory reaction in the control group compared to the low-activity group. With the continuation of treatment, the effect became more pronounced. On days 18, 21, and 24, the wound healing rates were 18.3%, 12.8%, and 7.4% in the low-activity group compared to 15.8%, 5.8%, and 1.0% in the high-activity group. Notably,

complete wound healing in animals with high physical activity occurred 6 days earlier than in the control group, and 3 days earlier than in the low-activity group (see Table 3.4).

Table 3.4

**Dynamics of changes in wound area during treatment with methylene blue in rats of low-physical and high-physical groups with experimental diabetes mellitus.**

Duration of experience	Animals with low physical activity treated with methylene blue (comparison group)		Animals with high physical activity treated with methylene blue (comparison group)	
	Wound area , mm <sup>2</sup>	Wound healing , %	Wound area , mm <sup>2</sup>	Wound healing , %
3rd	293.3±3.1	±	290.6±3.5	±
6th	263.8±2.9	10.1±0.1	235.6±4.7*,**	18.9±0.8*,**
9th	220.1±2.9*	25.0±0.7	186.6±4.8*,**	35.8±1.4*,**
12th	170.6±2.6	41.8±1.1	121.0±4.6*,**	58.4±1.4*,**
15th	122.3±3.7	58.3±1.3	65.7±3.0*,**	77.4±1.1*,**
18th	72.7±2.6*	75.2±0.8	37.4±2.2*,**	87.1±0.8*,**
21st	26.7±0.7*	90.9±0.7	11.0±0.4*,**	96.2±0.4*,**
24th	5.8±1.4*	98.0±0.5*	2.0±0.3*,**	99.3±0.1*,**
27th	1.3±0.5*	99.6±0.2*	-	100,0
30th	-	100,0	-	-

Note: \* - relative to animals with low activity at  $p<0.001$ .

\*\* - Statistical data regarding animals with low activity at  $p<0.001$ ;  $p<0.05$ .

\*\*\* - Statistical data regarding animals with high activity at  $p<0.001$ .

In the local treatment of animals with experimentally induced type 2 diabetes and low physical activity using the antiseptic complex QCMKC, inflammatory processes were observed less frequently from the start of experimental therapy compared to the control group and animals treated with methylene blue.

Compared to the control group and the methylene blue-treated group, the percentage of wound healing on days 6, 9, and 12 was 32.3%, 86.6%, and 50.4%, respectively, versus 27.7%, 73.2%, and 65.6%, showing a significantly higher rate of healing. Clinically, on day 9 of the experiment, there was less hyperemia, swelling, and inflammatory reaction in comparison to both the control group and the methylene blue-treated animals.

On days 15, 18, 21, and 24 of the experiment, the wound healing process in the control group with low physical activity was significantly higher by 34.5%, 24.7%, 16.5%, and 8.8%, respectively. In animals treated with methylene blue, these percentages were notably higher at 40.8%, 21.5%, 6.6%, and 1.3%, respectively. According to the data obtained, complete wound healing in this group occurred on day 27 of treatment.

Animals with high physical activity that received QCMKC, as well as animals in the control group treated with methylene blue, demonstrated a significantly accelerated wound healing process compared to animals with higher physical activity. This indicates a promising wound healing rate in the high-activity group.

On days 6, 9, and 12 of the experiment, the control group treated with methylene blue and animals with high physical activity had a significantly higher wound healing rate of 274.6%, 165.9%, and 116.3%, compared to 32.8%, 44.1%, and 36.0%, respectively. Moreover, by day 9 of the experiment, the control group treated with methylene blue showed reduced swelling, absence of erythema, and faster healing responses. As the treatment continued, the effect became more pronounced. On days 15 and 18 of observation, the percentage of wound healing was 71.2% and 34.5%, respectively, which was significantly higher by 13.7%. Notably, animals with a high level of physical activity achieved complete wound healing 12 days earlier than the control group and 6 days earlier than the methylene blue-treated animals with high physical activity (see Table 3.5).

Table 3.5

**Dynamics of changes in wound area during treatment of rats with low- and high-active type of experimental diabetes mellitus QCMKC.**

Duration of experience	Treatment of wounds in low physical activity type animals.		Treatment of wounds in animals of high physical activity type.	
	Wound area , mm <sup>2</sup>	Wound healing , %	Wound area , mm <sup>2</sup>	Wound healing , %
3rd	288.1± 2.4	-	283.3±3.9	-
6th	222.0±7.5	51.1 ±2.0 *	212.2±8.1 **	53.2 ±2.6 *,***

9th	163.5±12.9	70 , 8 ±4.2 *	137.2±4.4 * , **	70 , 8 ±1,3 * , ** *
12th	89.2±17.8	61 , 7 ±5.8	58.5±3.1	46,4 ±1,0 * , ** , ***
15th	52.1±18.2	42 , 8 ±6.1	20.4±1.5 **	18 , 9 ±0,5 **
18th	25.1±14.0	22 , 9 ±4.7	2.9±0.4 **	2 , 9 ±0.2 **
21st	9.0±7.0	8 , 7 ±0.8	1 , 7 ±0, 1 **	1 , 7 ±0, 5 **
24th	2.1±1.9	2 , 1 ±0.7	-	-
27th	0.3±0.2	0 , 3 ±0.1	-	-
30th	-	-	-	-
33rd	-	-	-	-

Note: \* - relative to animals with low activity at  $p<0.001$ .

\*\* - Statistical data regarding animals with low activity at  $p<0.001$ ;  $p<0.05$ .

\*\*\* - Statistical data regarding animals with high activity at  $p<0.001$ .

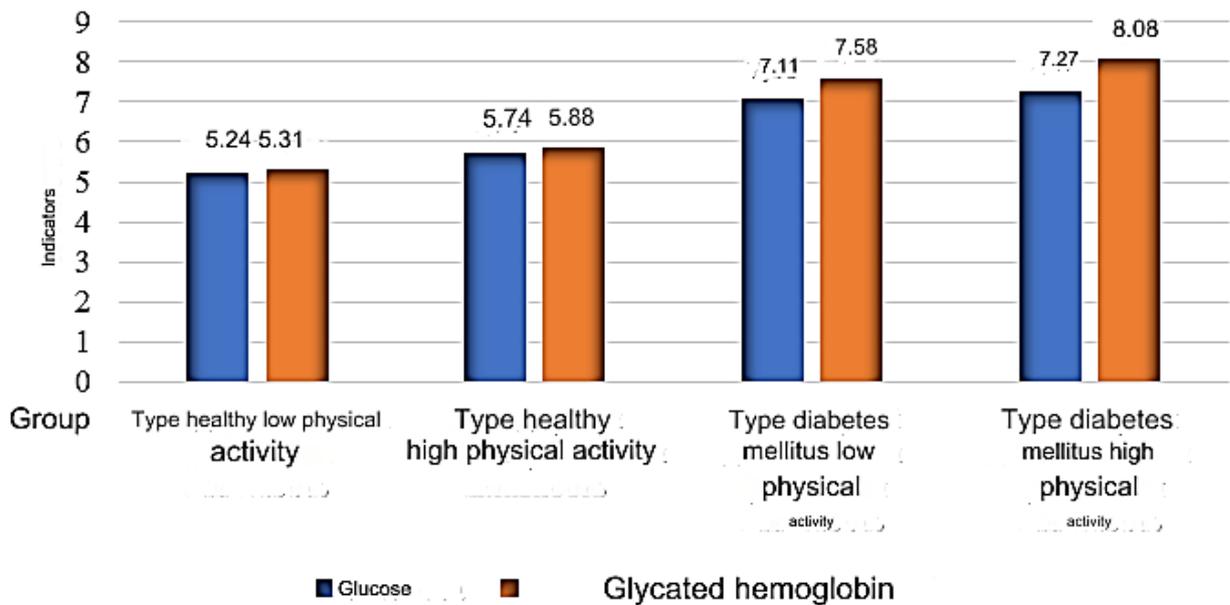
When comparing the wound healing rate in the low-physical activity group with the results of previous experiments, it was noted that during the observation periods of 6, 9 and 12 days, the wound healing rates were significantly higher - 9.1%, 19.2% and 14.7%, respectively. The strength of the effect obtained with continued treatment was recorded; that is, over 15 and 18 days of observation, wound healing significantly increased to 13.0% and 8.8%, respectively. It should be noted that complete wound healing in highly physically active animals was observed 6 days earlier than in the low-physical type groups of animals receiving YAKMSC. According to the results of the experiment, it was shown that in rats with diabetes, the group of animals with high physical activity and low physical activity had different results when discussing the wound healing process. The wound healing period in the high physical activity group was shorter than in the low physical activity group. Treatment of experimental animals with methylene blue and YKMSC resulted in a statistically significant reduction in the overall wound healing time compared to the control group.

When comparing the results of treatment of animals with high and low physical activity in this experimental group, it was found that wound healing occurred somewhat faster in animals with high physical activity compared to rats with low physical activity in the experimental group. control group. It should be noted that YKMSC treatment of animals suffering from both types of experimental

diabetes had a positive effect on the duration of complete wound healing in rats, i.e. the duration of complete wound healing was statistically significantly reduced compared to the control. group and animals treated with methylene blue.

### **§ 3.4. Study of changes in biochemical and hematological blood parameters according to individual typological characteristics of animals with experimental diabetes mellitus.**

In a series of recent studies devoted to the comparative aspect, biochemical blood parameters of animals with experimental diabetes mellitus, exhibiting low and high types of physical activity, were studied. One of the methods for determining carbohydrate metabolism disorders is the assessment of glycemia levels. Glucose concentration reflects the result at the time of blood sampling, while glycemia values assume the presence of stable fluctuations during the day. Therefore, due to the weak correlation between blood glucose concentration and the actual glycemia level, it is not possible to definitively determine the presence or absence of carbohydrate metabolism disorders in patients. According to WHO recommendations (2006), in 30% of cases it is impossible to diagnose diabetes only by assessing glycemia without determining the amount of glucose in the blood. Glycated hemoglobin (HbA1c) is considered an integrated indicator of long-term glycemia levels. Numerous studies have confirmed the correlation between HbA1c and glycemia levels in patients [38, pp. 7-11]. Therefore, in our study, we emphasized the importance of identifying glucose and glycated hemoglobin to test the KD model in experimental animals, which led to the following results. The results of the study showed that there was no statistically significant difference in the levels of glucose and glycated hemoglobin in healthy animals with low physical activity and healthy animals with high physical activity (see Figure 3.3).



**Figure 3.3. Hemoglobin, glucose and glucose-saturated hemoglobin indices in animals of low- and high-physical types against the background of experimental diabetes mellitus**

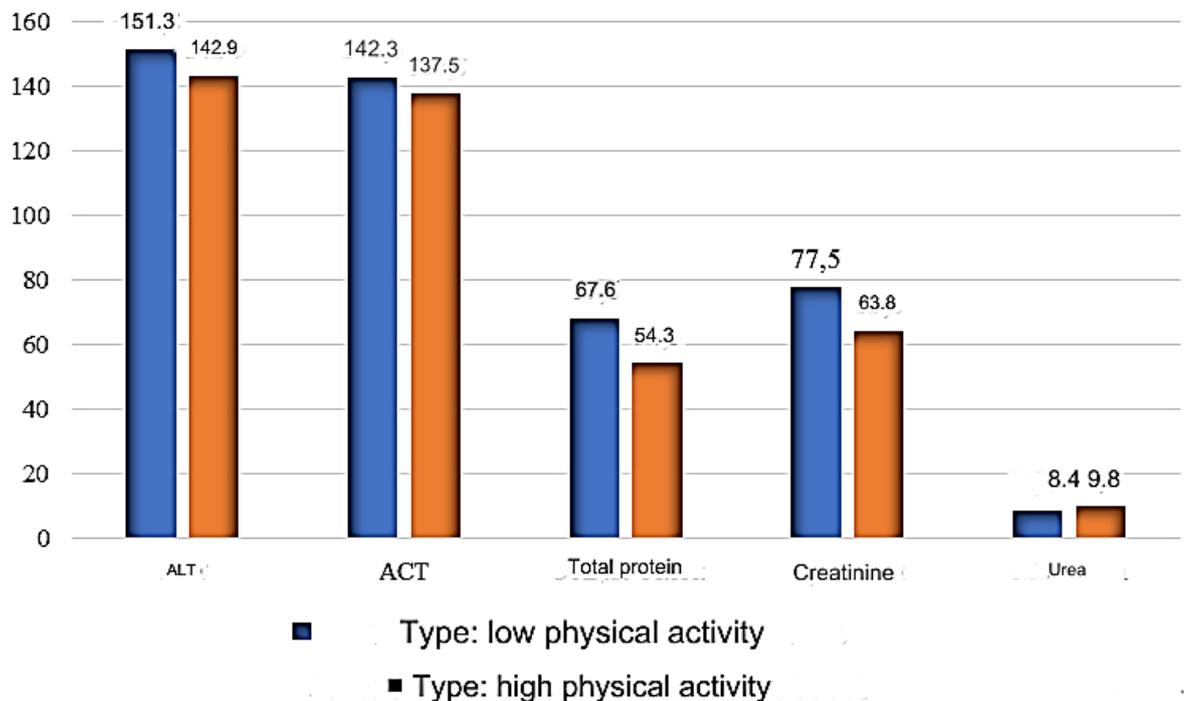
Animals with induced alloxan-induced diabetes in the present study showed statistically significant increases in blood glucose and glycated hemoglobin levels, reaching 35.7% and 42.7%, respectively.

Similar results were observed in animals with high physical activity, with a 26.7% increase in blood glucose levels and 37.4% increase in glycated hemoglobin levels compared to healthy animals with high physical activity.

We examined experimental animal models in both groups, observing comparable levels of glucose and glycated hemoglobin within the animal group. The development of steatotic-dystrophic or steatotic-necrotic liver processes may progress as a result of metabolic disturbances in the liver. Consequently, for diagnostic studies of the liver, we consider it necessary to obtain indices such as ALT, AST, total protein, urea and creatinine and obtained the following results.

In a series of recent experiments involving animals with low physical activity levels, ALT and AST activity levels were found to be 5.8% and 3.5% higher, respectively, compared to animals with high levels of physical activity. In addition, total oxalate and creatinine levels were 24.5% and 21.5% higher in the

low-activity animals, respectively. Meanwhile, the amount of urea in the blood was 16.7% lower in animals with low physical activity compared to animals with high physical activity levels (see Figure 3.4).



**Figure 3.4. Biochemical blood parameters in animals with low physical activity and high physical activity against the background of experimental diabetes mellitus**

Proper and timely use of modern treatment methods can accelerate the recovery process, reduce the risk of amputation, and decrease healthcare costs for patients with diabetic foot syndrome (DFS). However, alongside the emergence of new approaches to local wound treatment, they may ultimately lead to positive outcomes when combined with effective overall healthcare management. It is crucial not to underestimate the importance of appropriate treatment of affected limbs and improving the patient’s well-being through rehabilitation measures.

Physical activity has long been recognized as a fundamental component of human health. Most forms of physical activity and exercise are intensive, creating specific goals and expectations for patients suffering from musculoskeletal disorders. Understanding physical activity and exercise in the context of injury prevention, treatment, and rehabilitation is vital. In the case of injury, overloading

a weakened limb can negatively affect its recovery, potentially causing additional harm due to the presence of devices or equipment that increase pressure on damaged areas. Therefore, exercises should be performed cautiously to avoid exacerbating the injury or straining weakened muscles.

Due to the inability of most individuals with diabetic neuropathy to regenerate tissues, improving the effectiveness of regenerative medicine in restoring damaged tissues is of paramount importance in today's world. Research shows that physical exercise can promote tissue regeneration, accelerate healing, and enhance the use of "exercise mimetics" to facilitate recovery. These findings emphasize the importance of integrating physical activity into treatment protocols for individuals who are unable to benefit from the advantages of physical exercise.

In the context of experimental diabetic neuropathy, certain biochemical blood markers may exhibit significant variations depending on individual typological characteristics. Diabetic foot syndrome is one of the most serious complications of diabetes. Damage to the structure of small blood vessels is the primary cause of diabetic foot ulcers, indicating that factors such as neuropathy and increased pressure are key contributors. The role of blood supply in the state of macrocirculation, damage to the structure of small blood vessels, and skin microcirculation has not been fully studied.

Microcirculation is essential for the effective exchange of gases and nutrients, as well as for the delivery of metabolites. Additionally, skin microcirculation plays a crucial role in thermoregulation. Some common manifestations of diabetic microvascular complications include retinopathy, neuropathy, nephropathy, peripheral vascular disease, and diabetic foot syndrome. Microcirculation is a key aspect interrelated with all these manifestations. Structural and functional microvascular diseases (referred to as microangiopathy or small vessel disease) are often observed in individuals with diabetes during prolonged periods of hyperglycemia, leading to glycemic changes. Moreover, hyperglycemia can directly lead to microvascular changes associated with both sensory and autonomic neuropathies, contributing to microvascular functional

alterations. Parving et al. introduced the "hemodynamic theory" to explain diabetic microangiopathy. Increased resistance to microvascular blood flow results in endothelial damage and subsequent microvascular sclerosis. This, in turn, can lead to functional impairments, such as diminished maximal hyperemic response, impaired response to injury or repair stimuli, dysregulated blood flow, and altered vascular tone.

The development of ischemic lesions in diabetic neuropathy is largely attributed to the thickening of vessel walls, leading to insufficient blood supply. This may result in the formation of double ischemia, where tissues experience both impaired blood flow and capillary occlusion. Damage to the autonomic division of the peripheral nervous system can disrupt the trophic function of smooth muscle cells in blood vessels, leading to calcification and thickening of the arterial media. Consequently, arteries undergo stenosis, significantly reducing their diameter. These conditions create a favorable environment for the progression of "functional" ischemia, for instance, the increased blood flow demand during physical exertion, which may exacerbate tissue hypoxia. The combined effects of inflammatory arterioles and venules, along with neutrophil migration, can further compromise blood supply. This phenomenon worsens the progression of complications in patients with chronic kidney disease (CKD), shedding light on the pathogenesis of its formation and emphasizing the importance of anemia development and progression. Understanding the microvascular and macrovascular effects of CKD, accurate and timely differential diagnosis of anemia, and individualized treatment approaches are crucial aspects that were further explored in experimental studies involving animal models treated with CKD-MCS complex, leading to a deeper understanding of its impact on hematological parameters.

According to experiments conducted on animals with induced diabetes, grouped based on individual typological characteristics, specific changes were observed when assessing the hematological parameters of the control group (affected by experimental diabetic conditions). It was noted that hypothermia, stress, and various external factors, such as physical activity or pain, can enhance

sympathetic responses and impair tissue perfusion. Reduced oxygen concentration worsens the phagocytic capacity of leukocytes, impairs collagen production, and delays epithelialization. Hematological parameters, specifically leukocyte counts, in the low-physical-activity group increased 2.35 times compared to the control group animals, and basophil counts increased 2.83 times. Other parameters showed no statistically significant changes in comparison, highlighting the importance of these observed changes. The results of this study indicated that in animals with high levels of physical activity, leukocyte counts in the stimulated group were 1.9 times higher compared to the control group animals with higher physical activity levels, and basophil counts increased by 2.3 times. When comparing the results of the two groups (low and high levels of physical activity) of animals with experimentally induced diabetes, no significant changes were observed. It is noteworthy that the success of using even high-tech modern dressings is only achieved when they are applied to a well-prepared and cleansed wound bed. Optimal wound preparation requires the complete removal of all nonviable tissues and the establishment of moisture balance.

Several years ago, a new wound healing concept was developed, focusing on the essence of wound healing and the tissue regeneration process. The fundamental principle involves transitioning the wound from a chronic state to a healing state by removing necrotic tissue, altering phenotypic changes, and stimulating exudate production. Understanding the molecular processes involved in wound closure is crucial to appreciating the necessity of this type of treatment.

The healing processes of burn wounds and ulcerative wounds differ significantly. Surfaces beyond the hypodermis are improperly reconstructed, with a lack of reepithelialization and a prolonged healing phase described.

Hyperproliferation of the epidermal marginal zone, occurring at the site of injury, prematurely affects normal cell migration, likely inhibiting apoptosis in keratinocytes and fibroblasts. Fibroblasts isolated from the edges of burn wounds exhibit impaired proliferative capacity, demonstrating a lower response compared to the use of external growth factors. One important distinction, as highlighted by

Yana, is the level of cytokine secretion. In chronic wounds, it peaks after several days to several weeks, then begins to decline, returning to baseline if there is no infection. In superficial wounds, it remains active continuously. Exudate from untreated wounds contains factors that enhance the proliferation of healing cells at lower levels, such as peptide growth factors released by platelets, interleukin-6, and growth factors. Additionally, it reduces glucose levels and increases proteolytic activity, promoting epithelialization while slowing the healing process.

The high concentration of matrix metalloproteinases (MMPs) and serine proteases in chronic wound exudate contributes to the continuous degradation of tissues, facilitating the re-epithelialization process necessary for wound healing. Furthermore, these proteases may inhibit growth factors and cytokines critical for healing. The identification of elevated levels of inflammatory cytokines and proteases in chronic wounds, along with decreased mitogenic activity and impaired immune responses, underscores the importance of restoring the molecular environment of chronic wounds to accelerate healing. This wound healing approach aims to shift the chronic wound from an inflammatory state to a healing state by enhancing the activation of endogenous healing mechanisms.

To effectively cleanse and manage wound healing, local wound care must focus on consistent wound debridement and sanitation, monitoring exudate levels, and preventing bacterial contamination. The use of modern wound care products facilitates this process. The choice of wound dressing depends on factors such as the type of wound, the presence or absence of local infection, the amount of exudate, and the stage of wound healing. Dressing changes should be painless and gentle to the fragile epithelium and granulation tissue to avoid traumatizing the healing skin. Additionally, the dressing material should be capable of absorbing excess moisture, preventing maceration of the wound bed, maintaining a moist wound environment, providing adequate gas exchange, and regulating temperature to prevent secondary wound infections. It should be noted that no single dressing material currently meets all these requirements simultaneously. Therefore,

different tools with various properties are used at different stages of the wound healing process.

Recent advances in biotechnology and morphology have led to the development of new high-tech treatment methods that can accelerate the healing of chronic wounds. For example, the use of platelet-derived growth factor gel has proven effective and safe in promoting the closure of non-healing wounds. Research on the use of collagen-based dressings and MMP inhibitors continues to assess their effectiveness in wound healing. Current evidence suggests that interventions that modify pH levels and bind with non-crystalline silver particles may help inhibit MMP activity and expedite wound closure.

In the subsequent stage of the study on animals with experimentally induced diabetes of the hypoactive type treated with the QCMKC complex, the following changes were observed: in the experimental group, there was a statistically significant increase in the number of leukocytes and lymphocytes compared to the control group, with a 1.81-fold and 1.98-fold increase, respectively. Positive changes were identified when comparing the results to the control group, with a reduction in leukocyte and lymphocyte counts by 1.31-fold and 1.26-fold, respectively. In animals with experimentally induced diabetes of the hyperactive type treated with the QCMKC complex, there was a 1.3-fold increase in leukocyte count compared to the control group; however, a statistically significant reduction in leukocytes was observed in the control group, with a 1.42-fold decrease. The study showed a 1.43-fold decrease in lymphocyte count compared to the control group. When comparing the results of animals with diabetic neuropathy, no significant changes were observed (see Table 3.6).

Table 3.6

**Hematological indices of blood of animals of low-physical and high-physical types with experimental diabetes mellitus (n=6, M±m)**

Indicators	Groups					
	Healthy NFA type	Healthy VFA type	Type control NFA	Control type VFA	Type experimental NFA	Type: experimental VFA

Leukocytes, 10 <sup>9</sup> /l (WBC)	7.28±0.83	9.22±0.85	17.1±1.2 *	17.50±1.25 *	13.04± 0.81 *,**	12.30±0.20 *,**
Neutrophils, # ( Neu )	2.24±0.45	2.34±0.19	4.5±1.0	4.23±1.41 *	3.01± 0.44	3.10±0.17
Lymphocytes, # ( Lym )	5.01±0.45 *	17.47±10.79	12.5±1.3	13.10± 1.04	9.96± 0.76 *	9.15±0.24 **
Monocytes, # ( Mon )	0,000	0.02±0.02	0.003± 0.002	0.03±0.02	0.01± 0.01	0
Eosinophils, # ( Eos )	0.01±0.002	0.01±0.00	0.012± 0,005	0.010± 0,001	0.02± 0.01	0.01±0.003
Basophils , # ( Bas )	0.03±0.008	0.03±0.01	0.085±0.011 *	0.07±0.01 *	0.06± 0.01	0.04±0.01
Neutrophils, % ( Neu )	29.6±3.01	25.50±1.03	26.4±5.7	23.35± 6.03	23.17± 3.13	25.25±1.37
Lymphocytes, % ( Lym )	69.9±3.0	73.93±0.99	73.1±5.7	76.03± 6.04	76.27± 3.12	74.40±1.41
Monocytes, % ( Mon )	0	0.25±0.21	0	0.17±0.11	0.03± 0.03	0,00
Eosinophils, % ( Eos )	0.08±0.03	0.07±0.03	0.05±0.02	0.07±0.03	0.12± 0.04	0.03±0.02
Basophils, % ( Bas )	0.35±0.08	0.27±0.07	0.5±0.1	0.38±0.07	0.42± 0.05	0.33±0.11
Erythrocytes, 10 <sup>12</sup> /l (RBC)	7.38±0.11	7.60±0.25	7.7±0.2	7.89±0.32	7.62± 0.23	7.49±0.13
Hemoglobins, g/l (HGB)	135.5±3.7	143.00±4.04	141.2±2.6	144.50± 2.56	141.83± 4.27	142.8±2.01
Hematocrits, % (HCT)	39.5±0.9	41.92±1.21	41.2±0.6	42.38±0.75	41.37± 0.88	41.48±0.61
Mean corpuscular volume, fl (MCV)	53.6±1.2	55.20±0.8 5	53.5±1.0	53.95±1.46	54.38± 1.08	55.40±0.49
Mean corpuscular hemoglobin , p/g (MCH)	18.4±0.5	18.82±0.32	18.4±0.5	18.37±0.49	18.63± 0.34	19.03±0.17
Mean corpuscular hemoglobin concentration , g/l (MSN C )	343.5±2.8	341.00±1.82	343.2±3.1	340.17±1.2	342.83± 3.75	343.50±1.94
Coefficient of variation of the red blood cell distribution width, % (RDW-CV)	16.5±1.5	15.17±0.57	13.4±0.6	15.78±0.66 *	15.08± 0.96	16.02±0.83
erythrocytes standard spread of deviation width , fl ± (RDV-SD)	35.0±3.2	33.43±1.16	28.4±0.9	34.13±1.11	32.77± 2.10	35.58±1.74
Platelets, 10 <sup>9</sup> /l (PLT)	842.3±57.4	486.00± 115.76	686.8±58.6	564.83± 131.79	631.00± 116.22	813.17± 67,63
Mean platelet volume, fl (mpv)	5.6±0.2	6.22±0.33	5.8±0.1	6.17±0.34	6.03± 0.19	5.53±0.06 *
Platelet distribution width (PDW)	14.9±0.1 **	15.67±0.27	15.1±0.1	15.47±0.24	15.43± 0.14	15.08±0.07

Thrombocrit , % (PCT)	0.46±0.03 **	0.28±0.06	0.39±0.04	0.33±0.08	0.38± 0.07	0.45±0.04
ESR	1.67±0.43	1.67±0.43	0.83 ± 0.16	1.0	1.67± 0.43	1.00

Note: \*- statistically significant compared to healthy animals;

\*\* - statistical significance compared to control animals.

In cases of experimental diabetes in animals, an increase in certain hematological parameters, particularly the number of leukocytes, was observed. Treatment of the animals with the phytopreparation complex (QCMKC) demonstrated a positive impact on disease progression and a reduction in these changes.

### **§ 3.5. Results of the Study on the Effects of Silk Dressings in Treating Non-healing Wounds**

Recent studies have shown significant advancements in local treatment strategies for chronic wounds. Many authors advocate for classifying chronic wounds as either "healing" or "non-healing," suggesting that current standard treatment methods may be insufficient. As a result, the use of new, cutting-edge technologies is recommended, including growth factor-based preparations, metalloproteinase inhibitors, autotransplantation, and additional therapies such as electrical stimulation, ultrasonic cavitation, vacuum-assisted closure, and others. For accurate diagnosis and assessment of treatment efficacy, it is essential to employ objective and standardized methods of monitoring the wound healing process.

Based on a review of the literature, it is evident that silk textiles—products crafted using specialized techniques by skilled artisans—play an important role. The silkworm, in particular, is crucial to the textile industry due to its significant contributions. Silk fabric is primarily composed of fibroin and sericin (silk gum). The main components of silk include glycine, alanine, tyrosine, and serine. Sericin, a component of silk, is widely used in the food industry, cosmetics, and medicine. Its beneficial properties, such as promoting cell proliferation, make it a potential therapeutic agent in wound healing. Based on this information, the study of the

effects of silk dressings made from silk in previous experiments has sparked interest in understanding their effectiveness in the healing process.

The presented results showed that, four days post-operation, the wound bed area in the animal care group averaged  $128.2 \pm 9.7 \text{ mm}^2$ , with a wound closure rate of 9.8%. Objective evaluation of the wound flap revealed increased signs of inflammation, including edema, hyperemia, and infiltration of atrophied tissues. By the seventh day, the healing area constituted 32.4% of the initial wound size. A continuous reduction in wound size was noted on the 13th day of the experiment. Clinical manifestations of edema, hyperemia, and infiltration of atrophied tissues were observed only until the 15th day. By the 17th day, the healing wound site showed a slight elevation of atrophied tissues and intense granulation tissue formation due to the significant ingrowth of blood vessels. Pronounced hyperemia of the atrophied tissues was also observed. The monitoring group reached a healing rate of 99.9% within just 19 days. Cases of reduced tolerance were noted at the onset of intravenous administration of the material in the experimental group of animals (see Figure 3.5).



**Figure 3.5. Phenomenon observed in experimental animals when applying a dressing to a wound**



**Figure 3.6. Expression of the wound healing effect of silk-fibrin dressings.**

The analysis of the obtained results shows that the fabric dressings based on fibrin glue have a positive effect on the healing process of lacerations. In particular, when comparing the results of animal observations during the first 10 days, we can observe noticeable favorable changes in the recovery index. The use of 100% silk thread material in these dressings significantly accelerated wound healing and led to complete wound closure three days earlier compared to other types of dressings. Therefore, the use of fabric materials based on silk fibers in a patch wound model has a beneficial effect on wound healing and accelerates the healing process. The most effective result is shown by the effect of a fabric dressing made of 100% silk thread (see Fig. 3.6). At the initial stage of wound healing, the preliminary values of the wound closure percentage for fibroin-based dressings were 34.4%, 61.6% and 70.4% for 100% silk fibroin, while the results for 50% silk fibroin showed a percentage of 23.7%. For 41.4%, 74.6% and 70% silk dressings, the closure rates were 37%, 46.4% and 75.5%, respectively (see Table 3.7). At subsequent stages of the experiment, it was noted that the healing process continued steadily. In particular, in animals treated with 100% silk fibroin dressings, the closure percentage after 13 and 16 days was 92.3% and 95.7%, respectively. Similarly, in animals receiving 50% silk fibroin dressings, the healing rate was 94.6% and 96.1%. In addition, in the group receiving 70% silk dressings, the closure rate was 89.1% and 98.6%.

Table 3.7.

**Experimental study of the effect of silk dressing material on the healing time of flap wounds.**

Duration of experience	Control dressing material		Silk 100%		Silk 50% Cotton 50%		Silk 70 % Cotton 30%	
	Wound area, mm <sup>2</sup>	Wound healing, %	Wound area, mm <sup>2</sup>	Wound healing, %	Wound area, mm <sup>2</sup>	Wound healing, %	Wound area, mm <sup>2</sup>	Wound healing, %
1st	180.6±2.4	-	180.7±3.9	-	176.5±0.1	-	180.6±2.4	-
4th	128.2±9.7	9.8±1.8	118.3±0.5	34.4±1.4	134.8±12.9	23.7±7.3	113.2±14.2	37.0±8.3
7 - oh	122.2±7.2	32.4±3.5	68.7 ±6.5*	61.6 ±4.1*	103.4±6.9	41.4±3.9	96.2±12.9	46.4±7.6
10th	42.9±4.0	76.3±2.0	52.9 ±13.0	70.4 ±7.5	27.2±3.7	94.6±2.1	43.8±9.9	75.5±5.7
13th	26.2±4.9	85.6±2.5	13.9 ±1.7	92.3 ±0.8*	9.6±3.7*	94.6±2.1*	19.6±2.8	89.1±1.5
16th	10.2±3.0	94.3±1.7	7.7 ±2.4	95.7±1.3	6.8±1.7	96.1±1.0	2.5±0.8	98.6±0.5
19th	0.3±0.2	99.9±0.1	-	-	2.7±1.2	98.4±0.7	0.8±0.1	99.6±0.4
22 - oh	-	-			-	-	-	-

Note: \*-  $P < 0.05$  statistical significance compared to the control group.

Thus, according to the results of the studies, the new method showed a positive effect and demonstrated a positive effect on the physiological state of the experimental animals.

### **Chapter III Summary**

Studies have shown that medical drugs used in the treatment of alloxan-induced diabetes have approximately equivalent effects. The most favorable results were obtained in the *Bombix mori* study. Wound treatment was found to be effective on a flap wound model based on silkworm chitin material to accelerate wound healing. No adverse reactions and mortality were observed when the study material was applied to the skin of laboratory animals.

The preparations improved skin and hematologic parameters by slowing down erythrocytosis, erythrodiaphoresis and inflammatory proliferation. Further positive effects were observed with *Bombix mori*.

The mechanism of positive effects of the tested drugs on hyperglycemia in alloxan diabetes model leads to promising results. Analysis of the results suggests that among the tested drugs, *Bombix mori* exhibits the most favorable effects. Their beneficial effects are not only useful in clinical practice, but also stand out among other commonly used research materials.

Thus, the newly developed complex preparations developed by the Institute of Pharmaceutical Education and Research (Uzbekistan) are not prone to toxic effects, do not pose a risk of pharmacological toxicity and do not have significant side effects, which makes them a safer option for clinical use.

## **CHAPTER IV. MORPHOLOGIC CHANGES OF SKIN AREA TISSUES AT TRADITIONAL METHOD OF TREATMENT OF DIABETES INDUCED IN RATS IN EXPERIMENT**

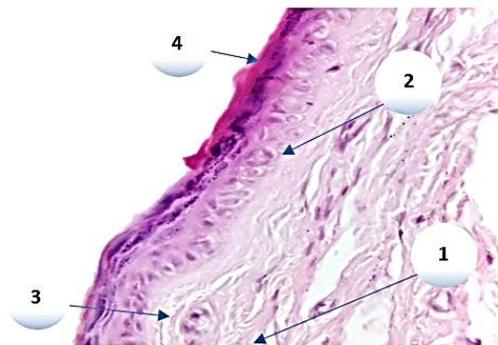
A rat model of alloxan-induced diabetes was used on experimental animals and morphometric changes in the tissues were revealed. The following changes were observed in the collected micro specimens.

When examining skin samples in DM, the thickness of epidermis and dermal layer, as well as the presence of edema in subcutaneous tissues were observed microscopically. Inflammatory reactions in the vasculature were detected, including vascular dilation, edema in the dermal layer, changes in the form of atrophy, necrosis, and dystrophy in the surrounding tissues, and marked infiltration by resident macrophages in this area. The predominant changes in the dermis and subcutaneous tissue included extensive steatonecrosis, significant vasodilation of the serous layers, and foci of interstitial edema. Inflammation is a response of blood vessels and mesenchymal tissues in the affected tissue. The progression of inflammation is continued by factors leading to destruction of cellular components such as paranecrosis, necrobiosis and development of necrotic processes. According to studies, the rapid vascular response in the tissues surrounding the affected area within 24 hours is primarily due to the degranulation of mast cells that secrete leukotrienes (inflammatory mediators). This process leads to a sharp increase in alterative processes in the affected tissues with the subsequent development of secondary alteration. The changes observed within 24 h are mainly due to vascular changes, tissue hypoxia and progression of dystrophic-necrotic changes. Over time, hemorrhage, leukocyte adhesion, and migration of numerous neutrophils may be observed in the area of tissue damage. These changes usually manifest clinically as edema, redness, localized fever, pain, and functional impairment. Laboratory values usually show leukocytosis and a left shift of neutrophil appearance. Eventually, manifestations of extensive interstitial

infiltration in the tissues lead to a marked disturbance of blood circulation and lymphatic outflow, which leads to a sharp increase in the rheological characteristics of blood in the vessels and the occurrence of sludge phenomenon in the capillaries of the stomach. . In addition, there is a dramatic release of substances in the tissues, accumulation of intermediate metabolites, and a significant decrease in resistance to secondary infectious agents, which emphasizes the efficacy of antibiotic therapy in the treatment of this condition. These changes further contribute to the rapid progression of atrophic changes in tissues and significantly alter the course of inflammatory processes.

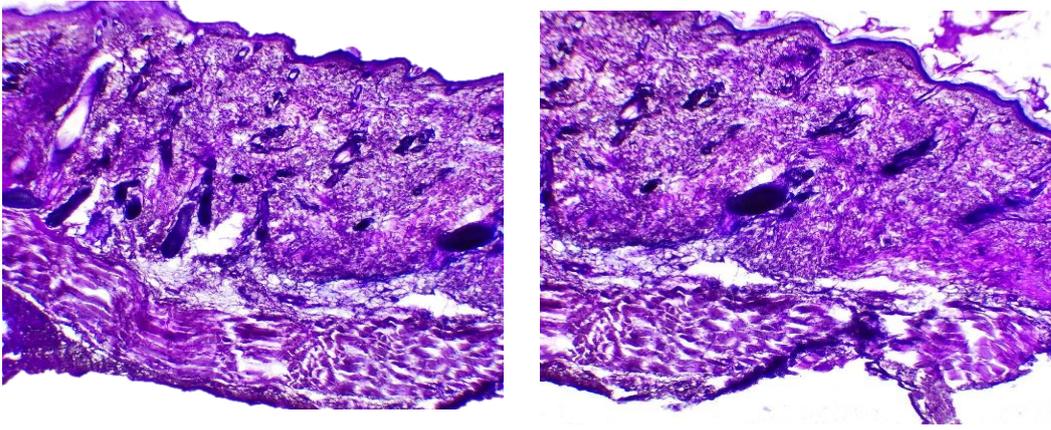
Microphotographs of skin and subcutaneous adipose tissue perimeter sections and tissue samples of rats of group 1.

1 - bandage (gauze) - control (intact) in animals with low (pastroemotional) sensitivity.



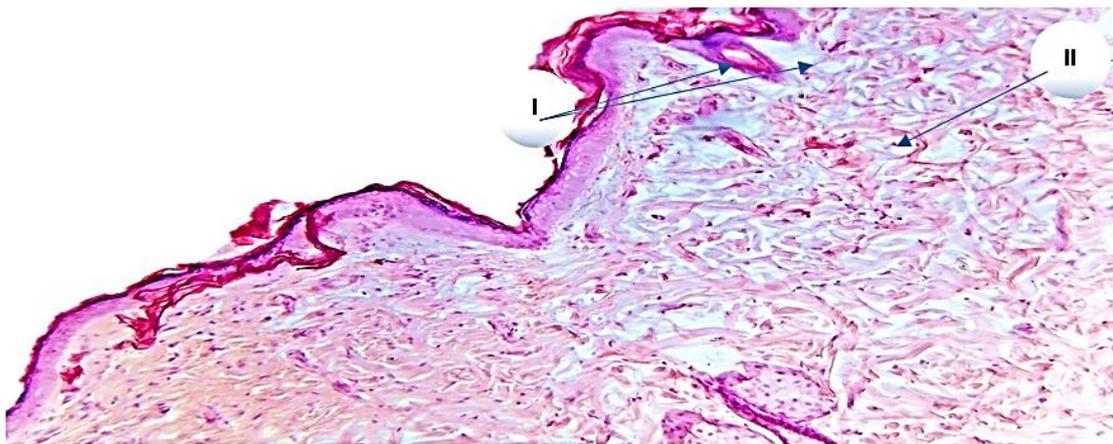
**Figure 4.1. Group 1. Preparation of intact skin of an experimental rat.**

The epidermis does not have a shiny layer, so it consists of 4 layers: basal (1), spinous (2), granular (3) and horny (4). Undoubtedly, the horny layer is thin: it consists of only 3-4 rows of freezing cells. As a result, the overall thickness of the epidermis becomes very thin. Paint G-E.10x4.



**Figure 4.2. Group 1. White rat skin tissue preparation.**

The histioarchitectonics of the dermis is unchanged; hematomas and foci of sclerosis are practically not detected between the hypodermis and muscular fascia. Hair shafts and follicles are unchanged. In the dermis without histioarchitectonic changes, interstitial swellings of varying degrees are detected between the hypodermis and muscular fascia. Size 10x10. Stained with Alcian blue.



**Figure 4.3. Group 1. Skin of the experimental animal from the intact group.**

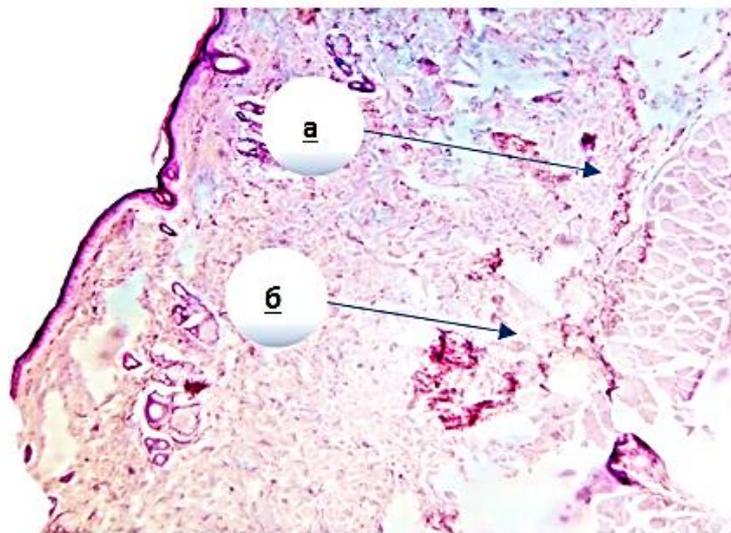
The dermis consists of two layers:

I. The papillary layer, which is composed of connective tissue with deep papillae extending into the epidermis (increasing the surface area of the proliferating layer of the epidermis) and non-porous connective tissue.

II. Beneath it lies the reticular layer, which is made up of loose connective tissue. In this layer, robust oxyphilic bundles of collagen fibers predominate, running in various directions to form a three-dimensional network. Additionally, a network of elastic fibers is present. Among the skin appendages, only sweat glands

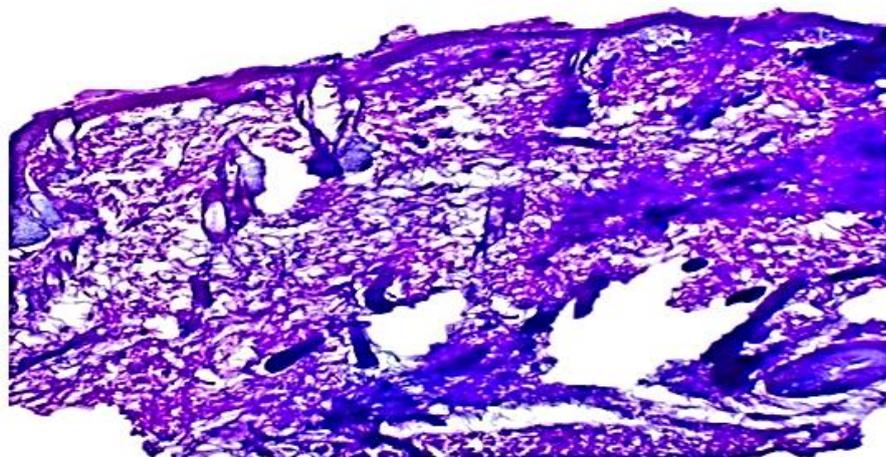
are observed. Hair follicles and sebaceous glands are not detected. Stain: H&E, magnification 10x4.

The specimen reveals intact ciliated epithelium, cilia, and a mucosal epithelial layer. There is notable thickening of the epithelial lining, interstitial edema, well-defined structures, and other tissue components (see Figures 4.1 and 4.2).



**Figure 4.4. Group 1. Skin of the intact experimental animal.**

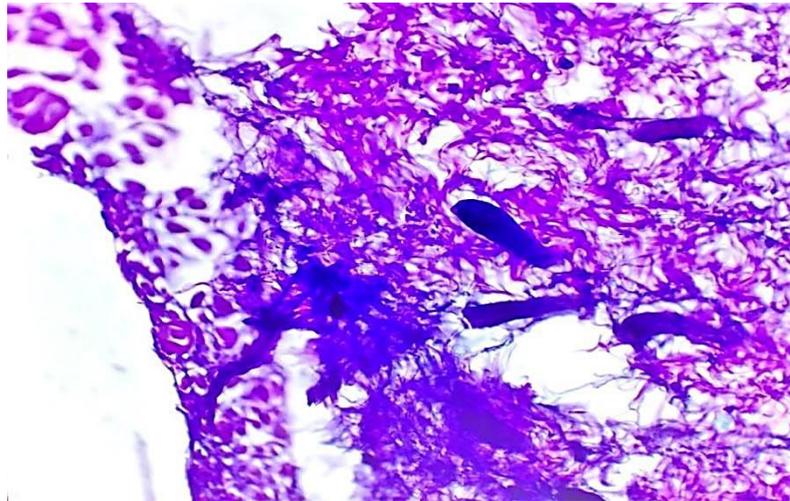
The preparation contains the skin of a white rat (a-spiny layer, b-granular layer). Hair follicles are visible on the skin. Dye G-E.10x10.



**Figure 4.5. Group 1. Skin tissue specimen from a white rat.**

In the dermis, there are no observed changes in the histoarchitectonics, and no foci of hemorrhages are detected. Size: 4x10. Staining with Alcian blue.

The overall morphology of the skin and the wound healing process: In the intact skin of the rats, stained with hematoxylin and eosin, the basal, spinous, and granular layers in the homotopic area of the wound in the experimental animals are clearly visible (see Figures 4.1 and 4.2 a and b).



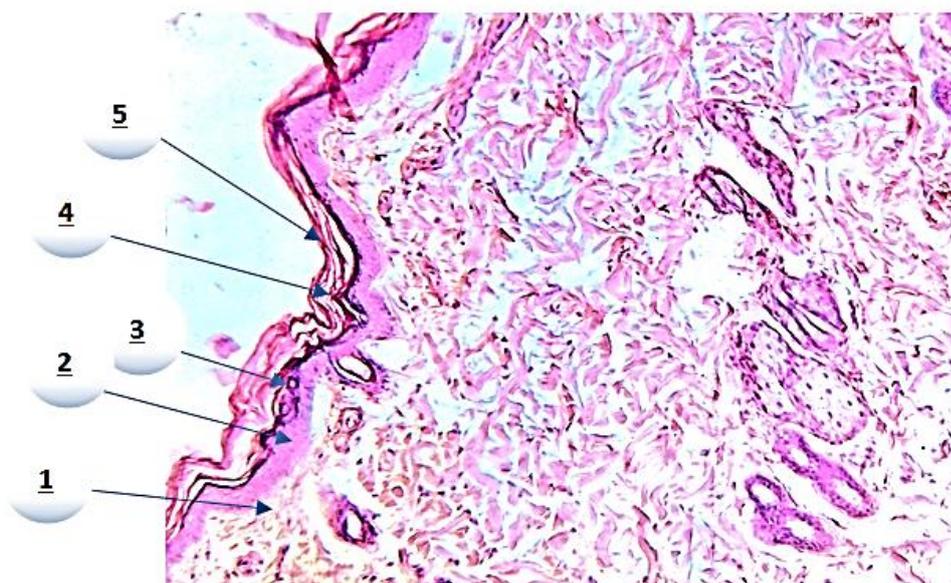
**Figure 4.6. Group 1. Skin tissue specimen from a white rat.**

In the dermis, Schiff-positive structures (acid mucopolysaccharides) are detected. No hematoma foci are identified. Size: 10x10. Stained with Alcian blue.

The results of the study indicate that the processes of reparative skin regeneration in diabetic conditions, induced in the skin tissue of this group of rats, progress relatively slowly. There is the growth of coarse fibrous granulation tissue structures around the wound, with improvement in defect areas at the wound edges. It was found that hyaline droplet dystrophy develops in the cells around blood vessels with impaired trophic function, reducing the permeability of the vascular wall, and early signs of coagulation necrosis appear. Particularly, fibrous structures of varying thickness and structures with irregular, chaotic arrangement of muscle components, as well as interstitial swellings, were detected in the interstitial tissue. Foci of coagulation necrosis were identified in most cellular structures. In the surrounding walls of small-caliber vessels, mucous secretion, fibrinoid structures, and foci of wall thickening were found, which serve as the

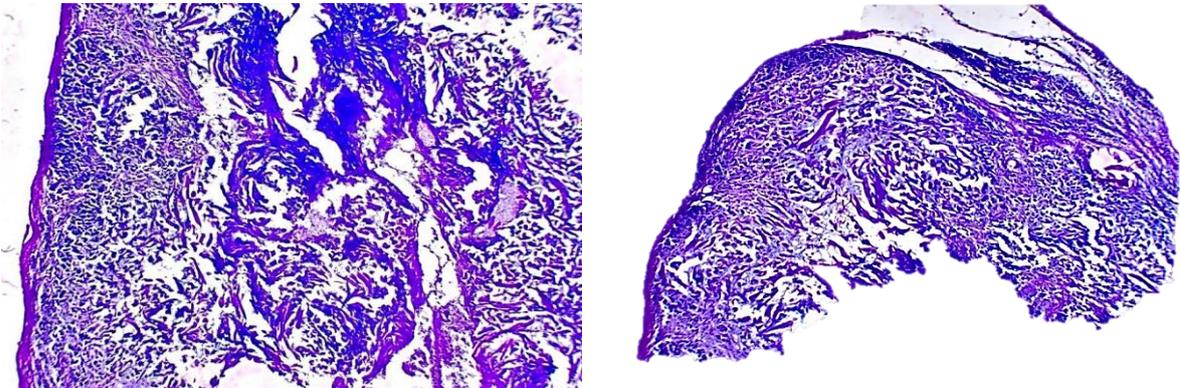
morphological substrate for diabetic angiopathy. Homogeneous dark pink protein-rich structures were identified in the perivascular branches. Due to destructive and degenerative changes in the fibers, foci of fibrinoid and fibrinoid necrosis were observed. In the same experiment, after traditional treatment, proliferatively active surfaces of various crater-like sizes were identified at the edges of flat epithelium around the wound, along with localized foci of hemosiderosis, which developed after pinpoint hemorrhagic foci in the pit area. The detection of coagulation necrosis foci in the reticular and donor areas of the dermis emphasizes the influence of histological preparation on dermatological assessment. The reduction in intraepithelial lymphocytes and migrated macrophages in atrophic areas indicates a decrease in dermal thickness and skin moisture content. Therefore, the development of coagulation necrosis due to vascular complications in diabetic angiopathies, neuropathies, and circulatory disorders creates the problem of eliminating necrotic tissue and accelerating wound healing. The progression of hyperpigmentation foci in atrophic scars may lead to the formation of defective hyperplastic keloid nodules, indicating the ongoing process of scar tissue remodeling in atrophic wounds.

**Group 2: bandage (gauze) - control (intact) in animals with high (high emotional) sensitivity.**



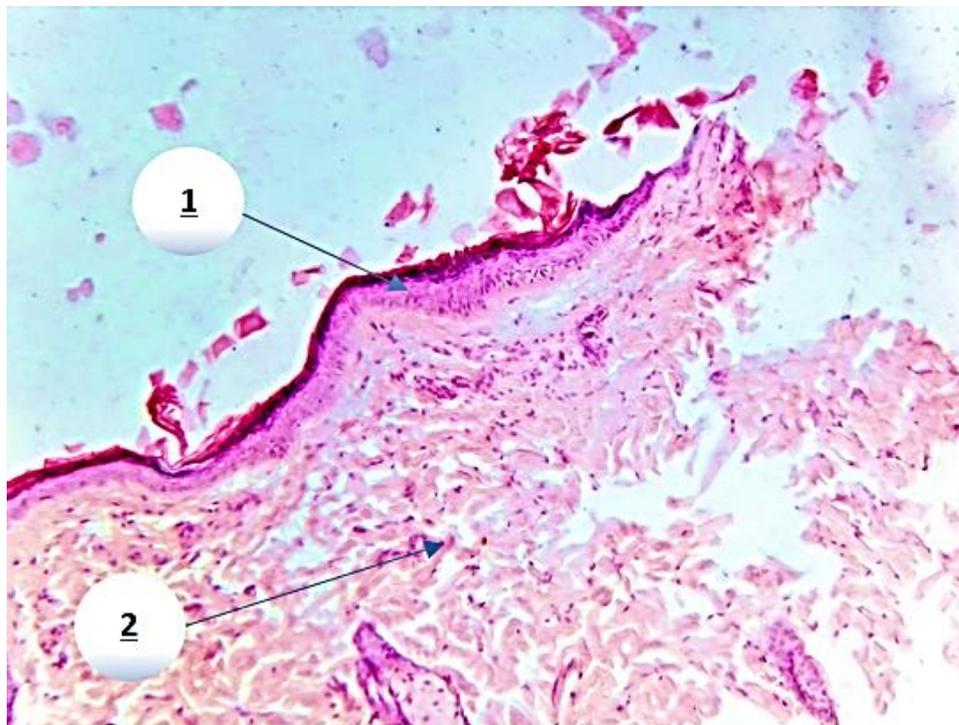
**Figure 4.7. Group 2. Skin preparation of intact white rats.**

The epidermis consists of five layers: basal (1), spinous (2), granular (3), transparent (4) and horny (5). The horny layer is somewhat thicker compared to the layers located above it. Stained with G-E.10x10.



**Figure 4.8. Group 2. Morphological appearance of hemostatic fluff based on Bombyx mori fibroin on the surface of white rat skin in the preparation.**

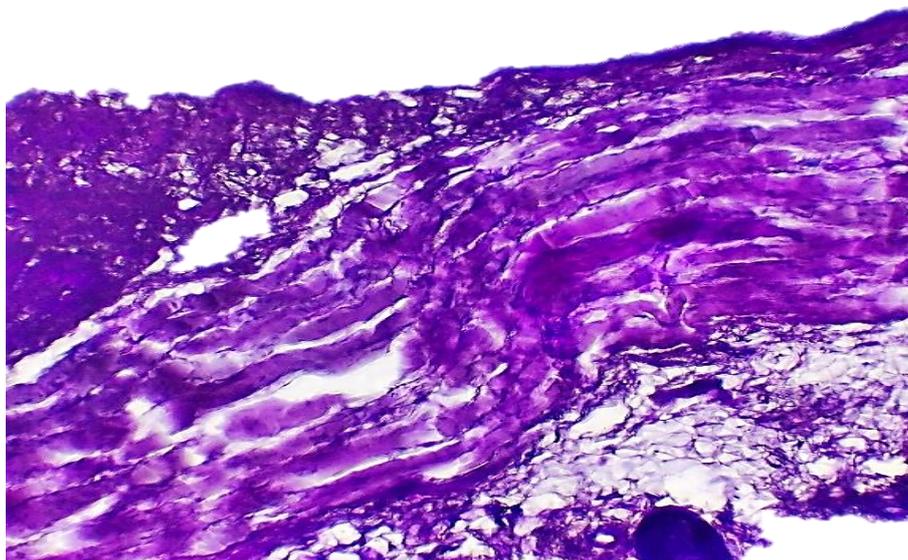
Contains collagen fiber structures and positive Schiff structures. Size 4x10 and 10x10.



**Figure 4.9. Group 2. Skin of a white rat in the specimen. Intact skin.**

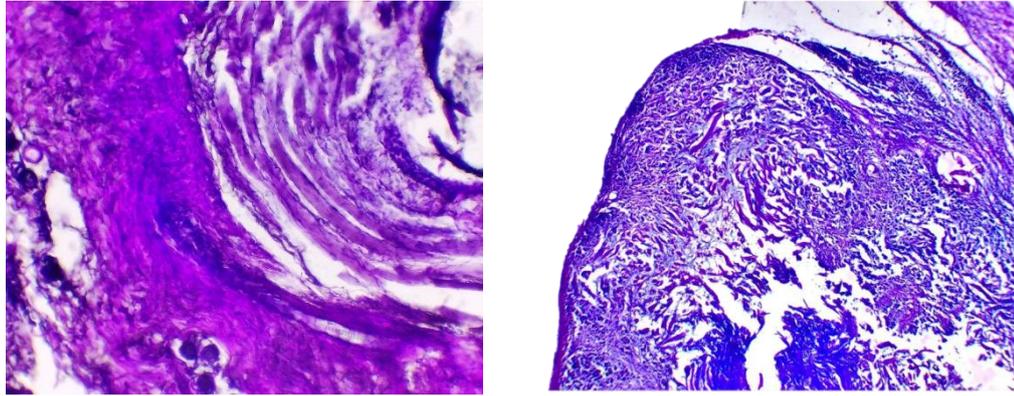
In the dermis, the papillary layer (1) is thinned, and the papillae are relatively underdeveloped. The reticular layer (2) remains unchanged. Stain: H&E, 10x10.

Based on the measured area of the lesion, there was no significant difference between Group 1 and Group 2 during this period. An alternative segment of extravasation at rupture sites maintained the integrity of the lamina propria and the destructive-degenerative changes in the muscle layer. In most extravasation margins, neutrophil infiltration in fibrous form and a decrease in the number of infiltrating lymphocytes and plasma cells were observed. Foci of coagulation necrosis were detected in the subcutaneous muscle layers. Healthy tissue components and irregular interstitial swellings were preserved in the stroma. Macroscopically, the cross-sectional area of the wound was significantly reduced, with focal reepithelialization of the wound surface observed in some rats of this group. Specific indicators of unique cellular changes were identified in the blood vessels. Leukocytes with low migration activity were found in the perivascular spaces. Regenerative foci were observed in the damaged skin structures, with proliferative foci of mesenchymal cells. However, the effectiveness of markers for reparative regeneration in wounds associated with scar formation remains inconclusive. (See Figures 4.10 and 4.11).



**Figure 4.10. Group 2. White rat skin tissue preparation.**

Adipose tissue is preserved between the hypodermis and muscle tissue. Weakly formed intermediate tumor foci are detected in the muscle tissue. Stain Alcian blue. Size 40x10.



**Figure 4.11. Group 2. Skin tissue specimen of a white rat.**

"Subcutaneous hypodermis tissue reveals punctate wounds. The wound appears as transparent Alcian blue, measuring 40x10."

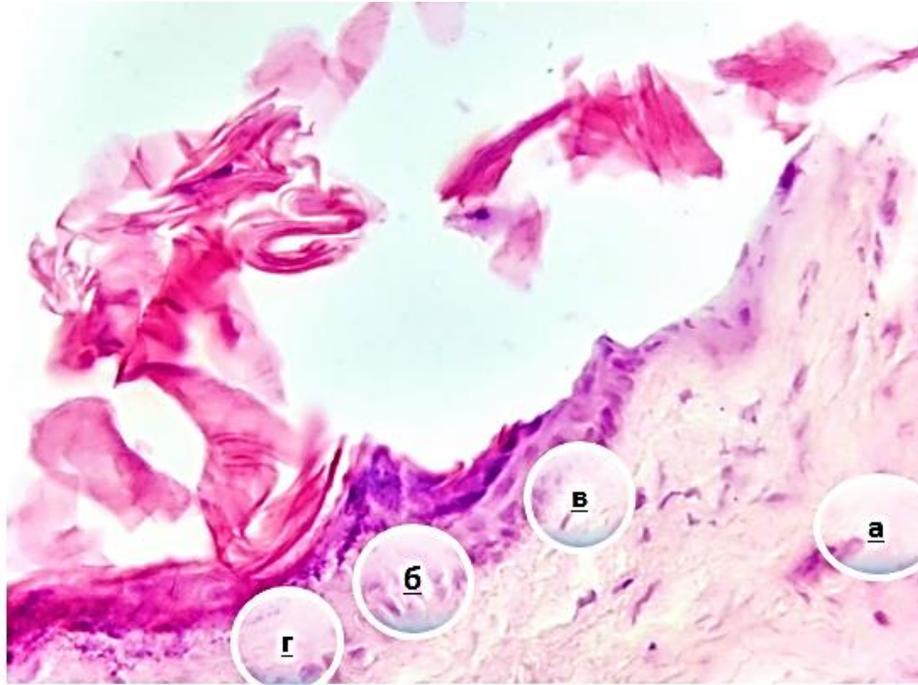
Upon comprehensive examination of the aforementioned microphotographs, it was determined that in the context of experimental wounds inflicted on rats with known inflammatory disease, the reparative regeneration process of wounds treated with dressings showed no moderate levels of fibroblast proliferation or accumulation of extracellular matrix components. Neutrophilic infiltration, macrophage necrosis, and atrophy of the epithelial and stromal components due to necrobiosis were observed, alongside a reduction in plasma cells, indicating the ongoing nature of wound healing. This is further supported by a marked increase in fibroblast proliferative activity at the wound edges, the synthesis of abundant collagen structures, and the formation of keloid nodules at sites where the basal layer of the dermis was disrupted. Epitheliation defects persisted in areas with major disruptions.

It is important to note that the use of dressings, while applied with a rational wound care approach, does not substantially accelerate healing efficiency from a morphological standpoint in clinical settings.

### **Group 3: 50% Fibroin + 50% Cotton Fiber in Animals with Low Sensitivity (Pasto-Emotional) (Experiment)**

During the wound healing process in experimental animals, the application of a specialized composition made from 50% fibroin and 50% Bombyx mori silk fabric resulted in a faster healing time for traumatic injuries, showing a 40%

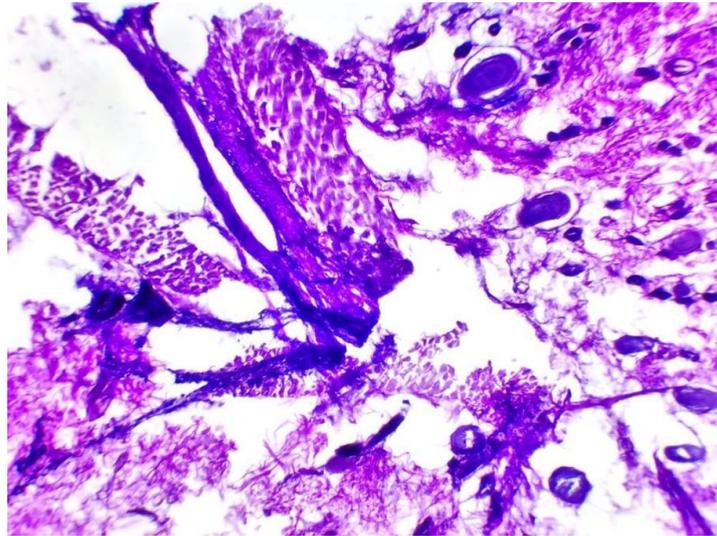
improvement compared to Groups 1 and 2. This accelerated healing is attributed to the reduction in excitability of the sympathoadrenal system under conditions of reduced emotional stress, which enhances tolerance to stress factors, promotes early apoptosis of immune cells, and decreases susceptibility to stress-induced memory material impairment.



**Figure 4.12. Group 3. Rat skin with experimental diabetes, 8th day of skin healing; a – zone of inflammation and granulation tissue formation; b – regenerating edge of the epidermis; v – basal layer; g – spinous layer. Paint G-E.10x4.**

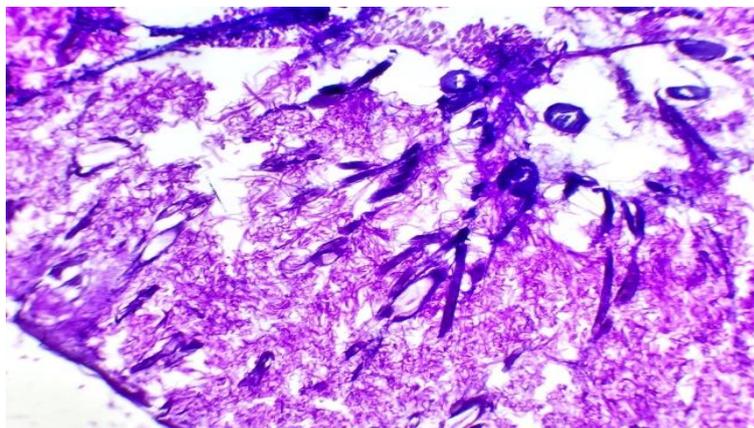
The morphological basis of these changes was characterized by the following: the superficial appearance of the epithelium, deteriorating within the epidermis and extending into the basal layer of the dermis, leading to the formation of specialized membranous structures; damage from environmental factors, such as air and other irritants on the surface of the ulcer; atrophy of scar tissue and lymphatic vessels due to environmental influences; subtle signs of fibrosis in dilated and capillary vessels; and the identification of stromal interstitial nodules in a fibrotic state. Furthermore, individuals experiencing high levels of stress within the lower emotional group exhibit an amplified stress response, resulting in

elevated concentrations of glucocorticoids in the bloodstream and a delay in the apoptotic process in immunocompetent cells.



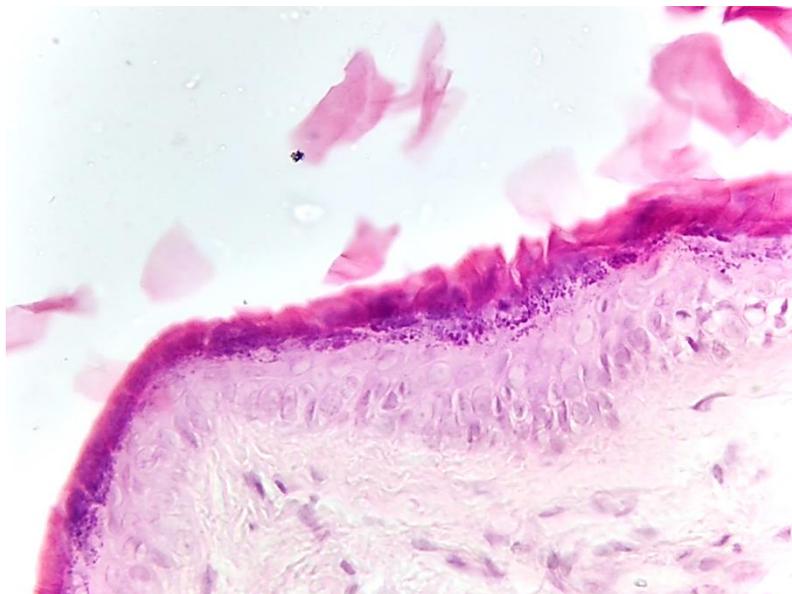
**Figure 4.13. Group 3. In the preparation, Schiff-positive structures of dark blue color are identified in the epidermis and dermis of the skin of the white rat, surrounding the hair shaft and hair follicle. Stained with Alcian Blue. Size: 20x10.**

During the acute phase of wound healing, monocytes, lymphocytes, and plasma cells are extensively infiltrated around wounds caused by traumatic emotional stimuli. This influx of cells, along with the deposition of membranous layers in response to the tissue condition, initiates the processes of repair and regeneration. The reduction of necrotic phenomena in the dermal and muscular layers, coupled with the stimulating effect of the deposited membranous layers and reparative processes at the wound surfaces, leads to the formation of multifocal areas of re-epithelialization in the atrophic regions of the wounds.



**Figure 4.14. Group 3. In the preparation, Schiff-positive structures of dark blue color are identified in the epidermis and dermis of the skin of white rats, surrounding the hair axis and hair follicle. Stained with Alcian Blue. Size: 20x10.**

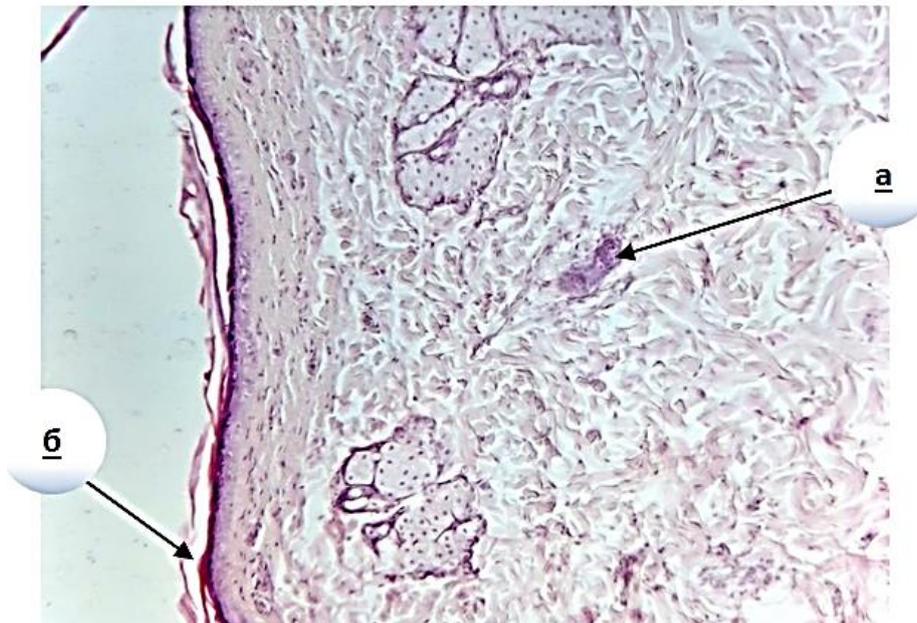
In the microphotographs of healing wounds, the reduction of infiltrate formation in isolated foci of wounds and areas of rupture leads to local reactions of mesenchymal cells: active proliferation of fibroblast foci, increased synthesis of extracellular matrix components, appearance of demarcation lines, their reduction, and uneven arrangement of fibers, decreased hematogenous migration of cells in damaged areas, and reduced vascularization of granulation tissue. These changes contribute to the predominance of humoral local responses at the sites of rupture and, under experimental conditions, the formation of a membranous layer on the surface of the wound in the area where 50% fibrous and 50% granulation tissue has developed, indicating the effectiveness of the reparative regeneration process from a clinical and morphological perspective.



**Figure 4.15. Group 3. Subcutaneous adipocytes of the experimental animal on the 8th day of the experiment.**

The texture of the subcutaneous adipose tissue varies, with emerging connective tissue foci currently being identified. In this layer, edema is also

observed, developing around the connective tissue fibers. Stained with G-E.  
Magnification: 10x4.



**Figure 4.16. Group 3. The skin of white rats is designated QD (diabetes mellitus) due to the administration of Alloxan in the preparation.**

The post-injury appearance of the epidermal portion of the skin is characterized by the presence of granulation tissue infiltrated by neutrophils, macrophages, and lymphocytes (a). Due to the abundance of cells, the regenerating edge of the epithelium is thickened (b). Stained with G-E. Magnification: 10x10.

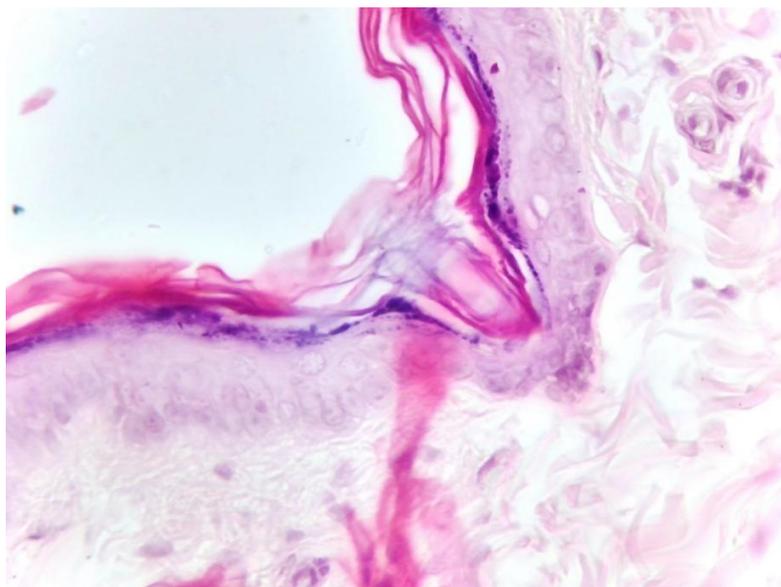
In the study of the regenerative process of wounds under conditions of reduced emotional stress in Group 3, the application of a membranous layer formed from a combination of 50% *Bombyx mori* fibroin and 50% silk stimulated reparative regeneration. This led to the formation of a membranous layer on the wound surface, acceleration of the local inflammatory response in the damaged tissue, marked proliferation of fibroblasts, an increase in well-organized structures in atrophic areas, and a reduction in granulation tissue. There was also a decrease in neutrophilic leukocyte infiltration, plasma cell infiltration, and a significant increase in the infiltration of intraepithelial cells in MALT structures, ultimately accelerating the process of reparative regeneration. Clear evidence of epidermal tissue reforming without scar formation, revascularization in atrophic tissues within the hair follicle bulb, and enhanced angiogenesis processes were observed.

Changes in the structure of silk produced by *Bombyx mori*, consisting of 50% fibroin and 50% sericin, resulted in the formation of a membranous-like layer with minimal shape disruptions in groups 1 and 2, which were used in wound treatment. The high effectiveness of this process in stimulating reparative regeneration is supported by data obtained from microphotographs.

**Group 4: (highly sensitive) animals with 50% fibroin + 50% cotton fiber (experiment).**

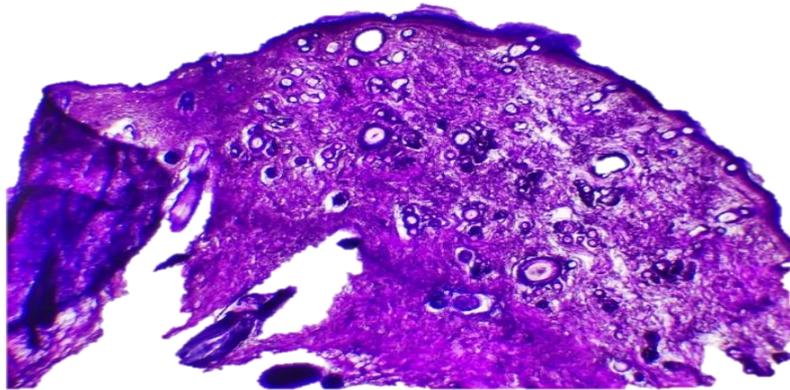
In the high-stress group, the presence of factors inducing tension in the neuroendocrine system, coupled with blood diabetes, leads to manifestations of cortico-hypothalamic-pituitary disintegration. Consequently, excessive production of catecholamines and glucocorticoids is observed in the plasma, resulting in apoptosis of all types of immunocompetent cells, including T- and B-lymphocytes in MALT and SALT systems. This results in hypermetabolism and disruption of the reparative regeneration process in the cells.

Additionally, a sharp decline in the necessary humoral environment for an active immune system response in the skin, disruption of skin tissues, dehydration of sweat glands, and necrosis in the affected areas lead to significant progression of the alteration process, profound dystrophy, and necrosis of damaged tissues, along with an increased demarcation line.



**Figure 4.17. Group 4. Preparations from rat skin, skin of rats with diabetes; After dressing with Bombyx mori fibroin-based gauze, day 2; a - basal layer; b - spinous layer; v - connective tissue scar. G-E dye.10x10.**

Ultimately, the intense degranulation of seven mast cells in this series and the exacerbation of the exudative inflammatory process will continue sharply, causing a strong reaction to opportunistic infectious agents from the outside, since the disturbed inflammatory landscape worsens, forming a vicious circle. In the same experiment, under conditions of stress caused by alloxan diabetes in the blood and high emotional arousal, secondary changes are expected to occur in four groups of rats, leading to a severe exacerbation of the inflammatory process.

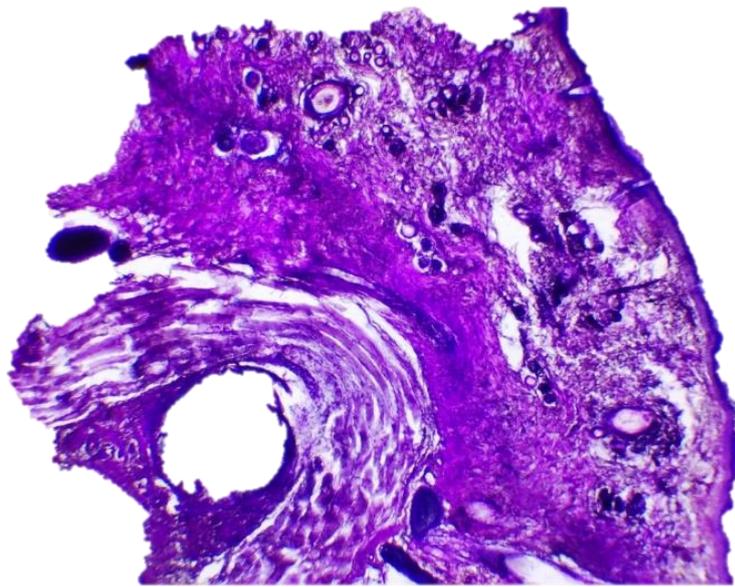


**Figure 4.18. Group 4. Experimental tissue from the skin of a rat.**

In conclusion, it should be noted that the ongoing degranulation of mast cells in the affected tissue and the external exacerbation of the inflammatory process lead to a strong response to secondary opportunistic infectious agents, resulting in the progression of the altered inflammatory landscape. The histoarchitecture of the dermis remains unchanged, with no identifiable hematoma foci in the hypodermis and fascial planes. A small number of Schwann cells are found in the dermal structures. Schwann bodies are observed around the periphery of the hair follicles at a magnification of 10x10. A pale Alcian blue stain is present.

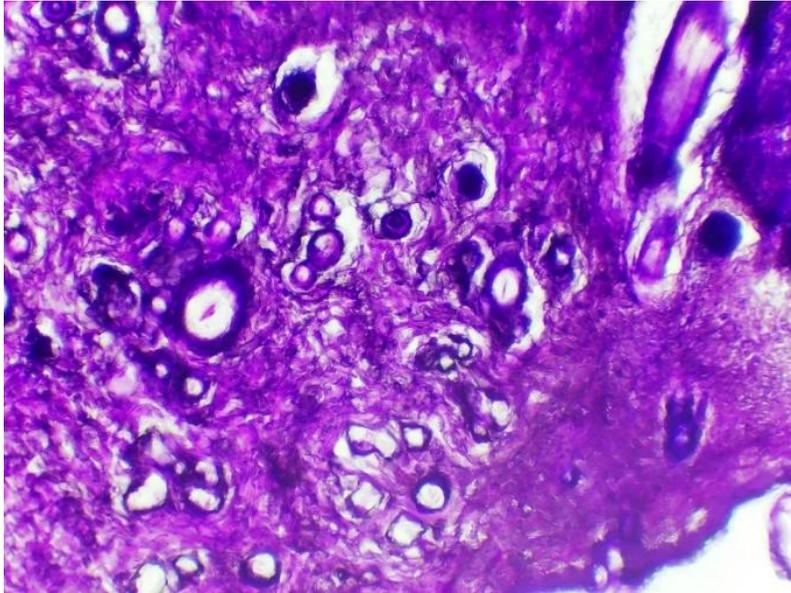
Experimental studies on skin healing in the affected area demonstrated that the membranous-like layer formed from a mixture of 50% fibroin and 50% Bombyx mori silk wool was successful. However, abnormalities in the blood

vessels of the dermis, accumulation of nodules in the perivascular areas, and the presence of multiple fibrinoid necroses in the stroma, along with a demarcation line indicating tissue rupture, were noted. Based on observations of the ongoing changes in the response of the neuroendocrine system, in most cases of sustained distress, a decrease in T- and B-lymphocytes was observed, accompanied by the appearance of 3-5% plasma cells within the field of view at 200x, and interstitial edema in the stroma of the affected tissues, along with fragmentation and an increase in the presence of myomatous structures.



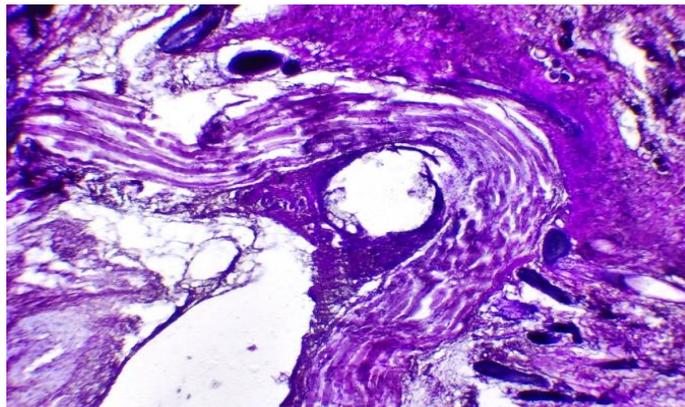
**Figure 4.19. Group 4. Experimental rat skin tissue.**

In the dermis, Schiff-positive structures with homogeneous foci without histioarchitectonic changes in the hypodermis are revealed. Cellulose fragments and poorly formed Schiff-positive structures are revealed under the hypodermis. Size 20x10. Stain Alcian blue.



**Figure 4.20. Group 4. Experimental rat skin tissue.**

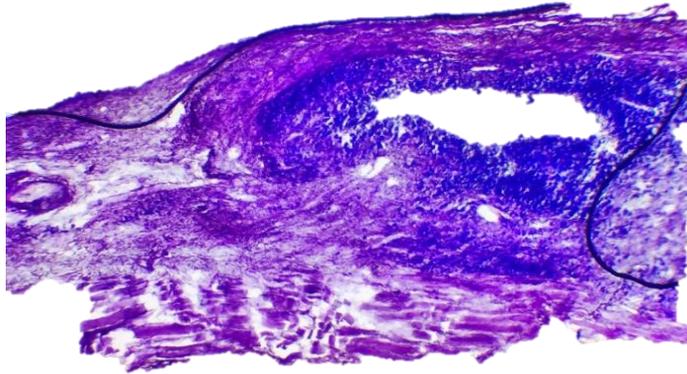
The histioarchitecture of the dermis is unchanged, no hematoma foci were detected. Size 10x10. Paint Alcian blue.



**Figure 4.21. Group 4. Experimental tissue from the skin of a rat.**

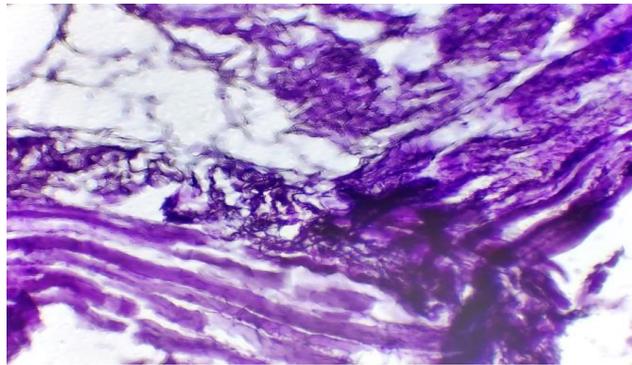
On the surface of the air silk, fragments of cellulose fibers have been identified, resembling wool under the microscope. These fragments measure 10x10 micrometers. The blue staining indicates the presence of numerous disordered mucopolysaccharides in the damaged and fragmented areas, as well as significant disorganization of cytolitic processes and structural disruptions. In particular, it is evident that the membranous lamellae derived from silk composed of 50% fibroin and 50% sericin from *Bombyx mori* have significantly evolved in distant regions. This morphological substrate, primarily consisting of silk obtained from *Bombyx mori*, illustrates the direct influence of the silk membrane on positive outcomes in

contact areas, while pronounced processes of destruction and aggregation are observed in non-contact areas.



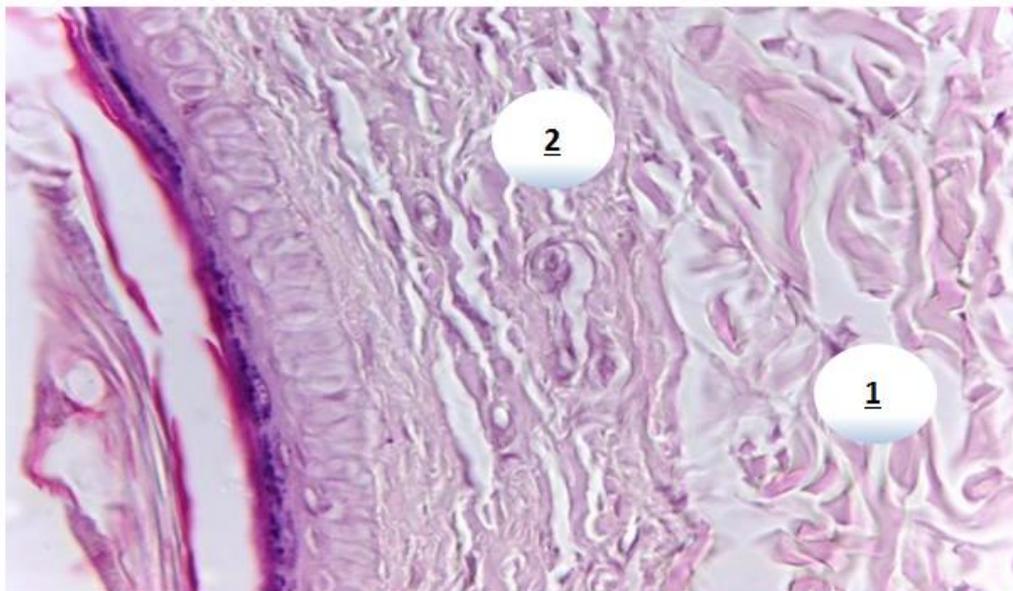
**Figure 4.22. Group 4. Experimental rat skin tissue.**

A positive Schiff result of dark blue color is revealed under the hypodermis. Remains of hemostatic fluff are found. Size 20x10. Paint Alcian blue.



**Figure 4.23. Group 4. Experimental rat skin tissue.**

Fragments of hemostatic fluff and Schiff-positive structures are found under the rat skin. Hematoma foci are not detected. Size 40x10. Paint Alcian blue.



**Figure 4.24. 4-group. The skin of white rats was named DM (diabetes mellitus) due to Alloxan in the preparation.**

Under the dermis, varying degrees of irregular interstitial edema (1), foci of coagulation necrosis around the subcutaneous muscle layer, and defragmented muscle bundles (2) are detected. G-E dye.10x10.

**Group 4.** It was found that the therapeutic efficacy of Bombyx mori 50% fibroin and 50% cotton fiber in the treatment of trophic wounds on the skin of severely emotionally affected experimental rats of group 4 was positive compared to the changes in group 4. Group 1 and Group 2 and low compared to the changes in Group 3. In experimentally induced diabetes in (highly emotional) rats, the rate of wound regeneration was determined after closure of wounds caused by skin tissue damage with special trophic-enhancing pads. The following morphologic changes were found in these studies. In particular, macroscopic examination determines that a demarcation boundary is clearly formed around the wound, the wound edges are smooth, and a thin reticulated sparse fibrous sheath is formed on the surface of the crater-shaped foci around. At the same time, foci of focal healing are identified along the edges of the wound. Microscopically in the wound area it is determined that in the damaged border areas of the skin most intermediate and muscle bundles, sparse fibrous structures are formed. Chaotic arrangement of fibrous structures is determined, between small foci of angiogenesis and foci of

active proliferation of fibroblasts, infiltration of plasmocytes into fibrous structures and destructively changed area. Foci of monocytes and lymphocytes migrating around the developing capillary networks, the presence of poorly formed tumors in the interstitial tissue are determined. At the same time in this area a lot of macrophage cells rich in basophilic cytoplasmic inclusions are determined, actively phagocytizing the structures subjected to destructive necrosis in the focus, and neutrophils are found numerically only in the focus. A sharp decrease of fat cells along the wound perimeter, strongly active proliferative foci of plasma cells, lymphocytes, a small number of macrophages and fibroblasts in the extracellular matrix are also revealed. A decrease in the number and size of cells subjected to inflammation and destruction along the demarcation line, as well as a sharp decrease in interstitial edema around it indicate the development of the reparative regeneration process. It should be noted that sharp detection of fibrous structures rich in various levels of intermediates around active proliferative foci of fibroblasts, increase in the content of acidic glycosaminoglycans in the process of reparative regeneration and sharp synthesis of fibrous structures are considered to be among the criteria proving from the morphodynamic point of view. Very few fat cells are found around this focus.

Thus, in a study on the use of silk-based dressings for the treatment and rapid healing of wounds in experiments on alloxan diabetic rats, the combination of active substances derived from “*Bombyx mori*” fibroin and hydrophilic cellulose scaffolds demonstrated the ability to effectively promote coagulation with obliteration of wound cavities, as well as a dramatic reduction in scarring, which is crucial for the process of reparative regeneration. These materials promoted the manifestation of the necessary factors to accelerate clinical morphologic replacement (tissue healing). Compared to other groups, the clinical efficacy of silk-based hemostatic dressing in four groups was demonstrated by studies based on morphological evaluation, showing promising results in healing trophic wounds (of various origins: skin abrasions, pressure sores, infections). etc.), which opens up potential applications in medical practice.

## OVERWIEV

The prevalence of diabetes mellitus is rapidly increasing as it is an endocrine disease that presents with alarming symptoms and significantly reduces the life expectancy of people. Among the most effective strategies to mitigate the health and social consequences of this disease is the global reduction in the incidence of DM and its symptoms [73, pp. 4-13; 74]. According to the literature, DM is one of the four major types of diseases that have not decreased in number. According to the International Diabetes Federation, this disease currently affects more than 425 million people worldwide, with the majority of them diagnosed with one of two types of diabetes. In Uzbekistan, the number of people with diabetes exceeds 245,000, including 2,300 children and 879 adolescents. Based on the above information, DM is one of the fastest growing problems facing the health care system in the 21st century, and research, studies and interventions are being conducted to prevent and treat these pathological phenomena. [21, c. 6-15].

It is known that the behavioral strategy of individuals adapting to specific environmental conditions is characterized by its neurophysiological features. Thus, animals that exhibit active coping mechanisms during prolonged periods of stress, affecting even the cardiovascular circulation and digestive system, may be susceptible to disease development; however, short-term stress can significantly increase the efficiency of the nervous and immune systems in animals of passive coping type. It is recognized that chronic stress (CS) and its consequences are considered potent stressors [33, pp. 55-62; 32, 29]. However, current research data do not reflect the different animal behaviors in the long-term outcome of a particular disease that we observe in laboratory studies using the open-field test, and there is a lack of scientific studies dedicated to understanding these phenomena. Based on the results of our study using the open-field test, all animals were categorized into three groups using cluster analysis: highly active, low active, and moderate. The animals were further modeled by their behavioral and physical characteristics [15, pp. 47-55]. According to sources, various studies have shown that animals within a population can differ in “open field” temperament traits [63],

which was confirmed in our research. The results of the analysis show that in the group characterized by mild to moderate obesity, 84.3% were on the periphery of the “open field” and 15.7% were in the center. In the group characterized by low physical activity, 57.3% and 42.7% were found, and in the moderate physical activity group, the figures were 75.2% and 24.6%, respectively, which is in agreement with other researchers [63].

Hence, in the studies, the baseline behavior of the animals revealed their susceptibility to subsequent stressors. Experimental studies have shown that proactive coping strategies are more effective in dealing with stress. Moreover, the focus on typological characteristics of coping styles and the relationship between the nature of reactions may help in the development of treatments for illnesses and emphasize their importance in recovery processes.

Pharmacological optimization of treatment is one of the current problems in our country. Achieving the goal of optimizing pharmacotherapeutic regimens for DM and its derivatives is one of the important tasks requiring compliance with recommended standards and timely provision of adequate glycemic control for effective choice of treatment in terms of basic clinical and economic aspects [62, p. 75-82]. Discoveries of global importance in the field of natural sciences have opened up many opportunities, one of which is the scientific research conducted by the Institute of Pharmaceutical Education and Research on the drug *Bombyx mori*. Conducting scientific research on *Bombyx mori* drug with high therapeutic efficacy and minimal side effects involves identifying the need to confirm the increased toxicity of oxalic acid, methylene blue and zinc complex for inclusion in the list of drugs.

Our results indicate that *Bombyx mori* preparation does not have toxic effects. The preparation *Bombyx mori* is considered safe from pharmacological point of view as it has no toxic properties.

One of the key objectives is to identify novel and alternative therapeutic agents that enhance reparative regeneration. In the subsequent phase of our study, we investigated the effects of hydroxy acids, methylene blue, and zinc complexes

in the treatment of wounds on experimental animal models with different types of injuries. The results showed that the preparation \**Bombyx mori*\* achieved a wound healing success rate of  $98.6 \pm 0.3\%$  in animals with high levels of physical activity during the healing process, compared to a baseline of 2.3% in animals with low physical activity. This rate was three times higher on the third day post-treatment in the high physical activity group.

Our experiments demonstrated [36, pp. 71-81] that the topical application of the antiseptic methylene blue to wounds, which had developed rapidly due to infection in the context of community-acquired pneumonia, can have a beneficial effect. Notably, a significant improvement in wound healing was observed on the 30th day of the study in the low-activity group, whereas complete wound closure was achieved by the 27th day in the high-activity group. This suggests that incorporating the antiseptic methylene blue into the treatment regimen may positively influence wound healing in patients with pneumonia-related complications.

The complex and dynamic process of skin healing involves the proliferation and integration of various tissues. Zinc plays a critical role in the remodeling phase of each stage of healing, functioning as a physiologically important element. Retrospective studies in the scientific literature indicate that zinc enhances the migration and proliferation of keratinocytes, promotes epidermal re-epithelialization, and exhibits anti-inflammatory, antioxidant, immunomodulatory, and antimicrobial properties [36, pp. 71-81]. Our findings demonstrate that zinc positively affects wound healing in animals with high levels of physical activity, showing significant benefits compared to groups with lower activity, as observed six days earlier.

It is worth noting that discussions with monitoring groups treating wounds at YKMSR, along with experiments in treatment groups using methylene blue in animals with induced diabetes, showed a significant positive effect on complete healing within the treatment period. Today, the medical community considers the confirmation of the effectiveness of early diagnosis and optimal diabetes

management as a crucial task. The final confirmation of the diabetes diagnosis relies on diagnostic methods focused on blood glucose and glycated hemoglobin levels, involving a range of laboratory tests [8, pp. 48-53].

The study of carbohydrate profiles (glucose, glycated hemoglobin) revealed the ability to distinguish their unique characteristics based on changes in their blood concentrations. Experimental animals demonstrated a significant increase in glucose levels across all study groups ( $p < 0.05$ ). By comparing the physical activity of animals with high blood glucose levels to those with lower levels, we were able to identify differences in their physical activity levels. These findings indicate the presence of metabolic syndrome in all experimental animals.

The concentration of glycated hemoglobin in the experimental animals was significantly higher than the normal range ( $p < 0.001$ ). It is important to note that the glycation of the N-terminal valine in the  $\beta$ -chain of hemoglobin A molecules within erythrocytes results in the formation of glycated hemoglobin, reflecting the percentage of hemoglobin that undergoes non-enzymatic glycation with glucose [8, pp. 48-53]. Therefore, the assessment of glycated hemoglobin is essential at all stages of diabetes diagnosis. Our research indicates that this parameter is a key factor in the development of diabetes in experimental animals and may act as a trigger for the pathological processes under investigation.

The results demonstrate a consistent elevation of both glucose and glycated hemoglobin levels across all study groups. Our findings align with previous studies [52, pp. 49-54] and confirm significant differences in all biochemical blood parameters, which were typologically identified. Glycemia was monitored in all experimental animals with diabetes, confirming the diagnosis through laboratory analysis. Additionally, disturbances in other blood biochemical markers were recorded. Animals with diabetes exhibited a moderate compensation of carbohydrate metabolism, with differences in ALT and AST levels between typological groups with low and high physiological function amounting to 5.56% and 3.4%, respectively. The overall blood glucose level also showed a slight increase, with a difference of 19.67% between the two groups.

Comparative analysis revealed differences in ALT, AST, total protein, creatinine, and urea levels, which were 5.56%, 3.4%, 19.67%, 17.67%, and 16.67%, respectively. When interpreting hematological parameters in diabetes, distinctions between different studies are observed, although such differences are not always consistent across all research. Some reports indicate no changes in hematological markers with BK, while others suggest a significant increase compared to those seen in diabetes [170, pp. 327-345]. Other studies report no substantial differences in hematological parameters in diabetes [64, pp. 26-34]. There is also information on pathological aggregation in the vessels associated with diabetes. Some data suggest that these differences may not be detectable in patients without microvascular changes or in those with microvascular changes at the subcompensation stage [65]. In various studies of patients with diabetes, including those with both macro- and microvascular complications, blood parameter differences have been observed [3, p. 672; 107, pp. 825-834; 108, pp. 216-226; 131, pp. 64-76; 133, pp. 212-226]. In the experimental pharmacotherapy of chronic kidney disease (CKD) in diabetic neuropathy, no significant differences in hematological parameters were noted. Although the administered medications could lead to an increase in leukocytes, their levels did not differ substantially from those in the control group. Thus, the experimental use of drugs did not significantly affect the hematological parameters of animals with diabetes in the models and pharmacotherapy.

Studying wound healing mechanisms can be challenging due to the complex nature of the catecholaminergic system, which involves the absence of neurons in the skin [138], as well as the response of keratinocytes to epidermal cells. In the research group monitoring healing processes, it was observed that, despite an expected 19-day completion time for wound closure, full healing occurred 3 days earlier in the group receiving 100% silk bandages. Furthermore, in animal models treated with bandages containing 100% silk fibroin and 50% silk fibroin, complete wound healing was observed by day 16. The rate of wound

healing in animals treated with silk bandages varied and potentially depended on the sensitivity of skin components to the materials used in the bandages.

The study results demonstrated that the use of localized binding materials with a positive therapeutic effect in an experimental model of alloxan-induced diabetes (DM) provided new insights. Wound healing involves receptor interactions, modulation of receptor activity by signaling molecules, and dynamic processes involving various interconnected factors such as diabetes and others. The morphological findings confirm the positive effects of the binding materials derived from *Bombyx mori*. Our research identified a significant reduction in proteolytic activity within the wound tissues.

Therefore, the findings align with the global literature on the topic. Previous studies have shown that the use of connective tissue materials in wound healing can be effective in treating wounds and injuries [130, pp. 72-79]. This work highlights the importance of the type of connective tissue material used for positive outcomes, as evidenced by our research on *Bombyx mori* - based materials, which demonstrate favorable healing indicators.

Economic efficiency refers to achieving the maximum possible benefit from existing resources, maintaining a consistent balance between the benefits obtained and the associated costs. It necessitates rational behavior. The primary criterion for socio-economic well-being is the degree to which society's ultimate consumption needs are met, particularly those related to human personal development. We calculated the socio-economic effectiveness of the dressing material we developed. The advancement of innovative healthcare technologies allows for a reduction in the number of days patients spend in the hospital, thereby increasing the efficiency and cost-effectiveness of outpatient treatment. The economic impact of measures that reduce the length of hospital stay could amount to 19,616,940.0 UZS. Utilizing modern treatment methods and selecting appropriate treatment options without hospitalization enables the final diagnosis to be made, reduces hospitalization days by 50%, and optimizes costs related to healthcare provision and the workload on medical staff.

Thus, the scientific significance of the results in the context of the individual typological characteristics of the body is crucial for studying the pharmacological correction of skin lesions in diabetic patients. This methodology is based on experimental data regarding the production of pharmaceutical agents and materials derived from them, as well as the selection system for these materials and the enhancement of their pharmacological activity.

## **CONCLUSION**

On the basis of the conducted studies of the monograph: “Pharmacological correction of skin lesions in experimental diabetes taking into account typological features” the following conclusions can be formulated:

1. It was found that the effectiveness of experimentally induced diabetic animal models in studying the healing of skin lesions is optimal. Animal typology plays a crucial role in assessing this efficacy: some animal species may exhibit a higher ability to regenerate diabetic wounds than others. These results may be useful in developing methods for wound healing in diabetic patients and further improving the treatment outcomes of this disease.

2. The experimental pharmacological treatment of skin lesions in animals with different reactions in diabetic conditions is based on studies conducted using a combination of chelating agents, methylene blue and zinc complexes as therapeutic agents. By reviewing the typology of selection of appropriate therapies based on the results of these studies, their essential importance can be appreciated. Various preparations have shown their unique efficacy in the treatment of skin lesions as well as in modeling diabetic skin lesions. The conducted studies allow us to determine the optimal drugs for pharmacological treatment of skin lesions in diabetic conditions, taking into account the individual characteristics of each group of animals. This approach makes it possible to choose the most effective treatment strategy and improve the effectiveness of treatment. This approach in the context of pharmacological treatment of skin lesions in diabetes explains the mechanism of action of chelating agents, methylene blue and zinc complex as therapeutic agents.

3. The study of changes in biochemical and hematological blood parameters in animals with experimental diabetes mellitus allows us to determine the individual-typological features of the organism's reactions to this disease. The observed changes may be different depending on the peculiarities of the organism of each animal and have different stress.

4. From the rejected studies, the following conclusions can be drawn: animals with experimental diabetes showed changes in blood biochemical parameters such as glucose, hemoglobin glycosylated to glucose, ALT, AST, creatinine, electrolytes, and total blood parameters. induced pain; Hematologic blood parameters in diabetic animals, including erythrocyte count, platelet count, leukocyte count, and hemoglobin level, were also found to be affected; the nature of these changes was related to the individual-typological characteristics of the animals. Some animals may show severe biochemical or hematologic abnormalities compared to others. The study of changes in biochemical and hematological blood parameters according to the typological characteristics of animals with diabetes mellitus may contribute to the understanding of metabolism and correction of metabolic and blood composition abnormalities, facilitating individualized treatment and recovery. Thus, these parameters may provide insight into the typological characteristics of animals with diabetes mellitus.

A comparative analysis of the use of *Bombyx mori* fibroin-based dressings in the treatment of skin wounds in animals with Turley typological reactions allows us to draw the following conclusions: *Bombyx mori* fibroin-based dressings have the potential to improve skin wound healing in animals with diabetes mellitus. The use of these dressings may help improve wound healing and accelerate the healing process; the observed results emphasize the difference in the potential of using these dressings depending on the diabetic state of the animal. Some animals showed higher efficacy of *Bombyx mori* fibroin-based skin wound treatment compared to others; the use of *Bombyx mori* fibroin-based dressings in the treatment of skin wounds allows optimization of the treatment strategy by understanding the influence of typological characteristics on efficacy, ultimately

allowing the selection of the most appropriate treatment method for each animal; further research in this area will focus on *Bombyx mori* fibroin-based dressings.

Thus, a comparative-analytical experimental study of the use of *Bombyx mori* fibroin-based dressings for the treatment of skin wounds in diabetic rats revealed the importance of the typological characteristics of the animals.

## **PRACTICAL RECOMMENDATIONS**

On the basis of systematized information and conducted studies, practical recommendations for pharmacological intervention in skin tissue lesions in experimental animals with diabetes taking into account the typological characteristics of animals are proposed.

1. Definition of personalized treatment. When choosing a method of pharmacological correction for a patient, it is important to consider the individual characteristics of the patient, taking into account both typological features of each patient.

2. Choosing the optimal dosage: understanding the effects of different dosage forms based on the typological characteristics of patients. Evaluating the efficacy of the combination of folic acid, methylene blue and zinc complexes based on the body's response.

3. Monitoring of biochemical and hematologic parameters: Investigation of changes in biochemical and hematologic blood parameters in diabetic dermatitis patients based on typological features for effective treatment of skin lesions.

4. Regular monitoring and outcome assessment: Systematic monitoring and outcome assessment of skin lesions based on patient typology for structured evaluation of the efficacy of pharmacologic interventions.

5. Conducting research on diabetes treatment with pharmacological intervention methods and treatment optimization based on typological characteristics in order to further study the progression of the disease.

By taking into account these practical recommendations and considering the typological characteristics of diabetic patients, it is possible to significantly improve the effectiveness of treatment and organization of experimental control of blood glucose levels to prevent deterioration of the condition.

#### **LIST OF USED LITERATURE**

1. Айрапетянц М. Г. и др. Реакции на умеренные функциональные нагрузки у крыс с индивидуальными особенностями поведения //Журн. выс. нерв. деят. – 1980. – Т. 30. – №. 5. – С. 994-1002.
2. Бабушкина И. В. и др. Регенерация экспериментальной раны под влиянием наночастиц цинка //Вестник новых медицинских технологий. – 2012. – Т. 19. – №. 4. – С. 16-18.
3. Балаболкин М.И. Диабетология: монография. – М.: Медицина, 2000. – 672 с.
4. Балтаева М. М., Бабаджанова Д. Д., Эшчанов Х. О. Серицин и его значение //Universum: технические науки. – 2022. – №. 1-2 (94). – С. 89-92.
5. Баранов В. Г. и др. Экспериментальный сахарный диабет. – 1983. – 240 с.
6. Баранов В.Г., Соколоверова И.М., Ситникова А.М., Онегова Р.Ф. Развитие сахарного диабета у потомства крыс и самок с аллоксановым диабетом в 6 изученных поколениях // Бюлл. exper. биол. и мед. 1988. - Т. 105. - №1. - С. 13-15.
7. Банзаракшеев В. Г. Экспериментальная оценка патофизиологии и фармакотерапии аллоксанового диабета у крыс //Забайкальский медицинский вестник. – 2016. – №. 4. – С. 124-128.
8. Бирюкова Е. В. Роль гликированного гемоглобина в диагностике и улучшении прогноза сахарного диабета //Медицинский совет. – 2017. – №. 3. – С. 48-53.
9. Бобоева Р. Р. Определение Влияния Рутана На Биотрансформацию И Глюкуронирование Лекарственных Средств На Модели Острого

Токсического Гепатита //Central Asian Journal of Medical and Natural Science. – 2023. – Т. 4. – №. 1. – С. 227-231.

10. Бобоева Р.Р. Оценка влияния силибора и рутана на желчевыделительную активность печени при экспериментальном остром гепатите //Scientific progress. – 2023. – Т. 4. – №. 2. – С. 88-93.

11. Быков И. М. и др. Особенности свободнорадикального окисления и антиоксидантной защиты у детей с сахарным диабетом первого типа //Кубанский научный медицинский вестник. – 2017. – №. 4. – С. 27-38.

12. Вечканова Н. А. и др. Аналитический подход к оценке роли системы IL-1 и IL-33 в аспекте сбоя рецепторного аппарата клетки при сахарном диабете как предпосылка применения иммунофармпрепаратов //Вестник современных исследований. – 2019. – №. 2.12. – С. 27-31.

13. Галстян Г. Р. и др. Возможности липидснижающей терапии в улучшении прогноза у пациентов с атеросклеротическими сердечно-сосудистыми заболеваниями и сахарным диабетом: роль ингибиторов PCSK9 //Российский кардиологический журнал. – 2018. – №. 12. – С. 103-106.

14. Гальцева И. В. Анализ летальных исходов острого периода травматической болезни при тяжелых сочетанных травмах: Автореф. дисс.... канд. мед. наук //С-Пб., 2002.-22 с. – 2002.

15. Гостюхина А. А. и др. Индивидуально-типологические особенности реагирования лабораторных крыс на многокомпонентный стресс //Современные вопросы биомедицины. – 2022. – Т. 6. – №. 2 (19). – С. 47-55.

16. Гостюхина А. А. и др. Поведенческая активность крыс в «открытом поле» после световой или темновой деприваций и физического переутомления //Бюллетень сибирской медицины. – 2016. – Т. 15. – №. 3. – С. 16-23.

17. Гринева Е. Н. и др. Рекомендации по ведению больных COVID-19 и эндокринными заболеваниями в период пандемии //М.: ФГБУ «НМИЦ им. В.А Алмазова. – 2020.

18. Дедов И. И. и др. Алгоритмы специализированной медицинской помощи больным сахарным диабетом //Сахарный диабет. – 2017. – №. 1S. – С. 8-110.

19. Дедов И. И., Петеркова В. А., Кураева Т. Л. Российский консенсус по терапии сахарного диабета у детей и подростков //Сахарный диабет. – 2010. – №. 5. – С. 1-8.

20. Дедов И. И., Шестакова М. В., Викулова О. К. Государственный регистр сахарного диабета в Российской Федерации: статус 2014 г. и перспективы развития //Сахарный диабет. – 2015. – Т. 18. – №. 3. – С. 5-22.

21. Демидова Т. Ю., Зенина С. Г. Коррекция инсулинорезистентности-эффективный путь управления сахарным диабетом 2-го типа и другими компонентами метаболического синдрома //Лечебное дело. – 2020. – №. 2. – С. 6-15.

22. Древаль А. В., Садыкова Р. Е., Мазо В. К. Влияние несбалансированности пищевого рациона на индуцирование и течение аллоксанового сахарного диабета у крыс //Пробл. эндокринологии. – 1991. – Т. 37. – №. 6. – С. 56-58.

23. Европейская конвенция о защите позвоночных животных, используемых для экспериментов или в иных научных целях/ Серии европейских договоров - № 123. Страсбург, 18 марта 1986 года

24. Ершова Е. В., Трошина Е. А. Применение бариатрических операций при сахарном диабете 2 типа: в помощь практическому врачу //Ожирение и метаболизм. – 2016. – Т. 13. – №. 1. – С. 50-56.

25. Закирьянов А. Р. и др. Диабетические осложнения у крыс при длительных сроках моделирования сахарного диабета 1-го типа //Патологическая физиология и экспериментальная терапия. – 2007. – №. 4. – С. 21-25.

26. Иваницкий Г. Р., Хижняк Е. П., Деев А. А. Биофизические основы медицинского тепловидения //Биофизика. – 2012. – Т. 57. – №. 1. – С. 130-139.

27. Исаев Э. И. и др. Содержание сиаловых кислот индивидуальных гликолипидов тканей в норме и при аллоксановом диабете у крыс //Пробл. эндокринолог. – 1983. – Т. 29. – №. 1. – С. 67-71.

28. Исмаилова Х. Ю., Агаев Т. М., Семенова Т. П. Индивидуальные особенности поведения (моноаминергические механизмы). – 2007.

29. Кендыш И. Н. Регуляция углеводного обмена. – 1985. – 272 с.

30. Коваленко Ю. С. и др. Сахарный диабет и COVID-19. Особенности взаимного влияния двух пандемий //Вестник современной клинической медицины. – 2021. – Т. 14. – №. 4. – С. 58-66.

31. Калапко Е. Н. и др. Сравнительный анализ эффективности n, n'-(этан-1, 2-диил) бис (хинолин-2-карбоксамид), диакамфа гидрохлорида и метформина в остром периоде аллоксанового сахарного диабета у крыс //Вестник фармации. – 2016. – №. 3 (73). – С. 67-77.

32. Корягина Ю. В., Тер-Акопов Г. Н., Корягина Ю. В. Хронобиологические особенности спортсменов при различных физических нагрузках, тренировочных циклах и условиях среды //Лечебная физкультура и спортивная медицина. – 2017. – №. 5 (143). – С. 29.

33. Котельникова С. В., Котельников А. В., Теплый Д. Л. Влияние режима освещенности на интенсивность перекисного окисления липидов в норме и при кадмиевой интоксикации //Естественные науки. – 2014. – №. 3. – С. 55-62.

34. Крупаткин А. И. и др. Лазерная доплеровская флоуметрия микроциркуляции крови //Медицина. – 2005. – Т. 256. -125 с.

35. Курьянова Е. В., Укад А. С., Жукова Ю. Д. Половые и типологические различия поведенческой активности нелинейных крыс в тесте " Открытое поле" //Современные проблемы науки и образования. – 2013. – №. 5. – С. 460-460.

36. Лебедева С. А. и др. Ранозаживляющее действие металлоорганического комплекса цинка на модели плоскостной кожной раны

у крыс //Научные результаты биомедицинских исследований. – 2022. – Т. 8. – №. 1. – С. 71-81.

37. Лебкова Н. П. и др. Ультраструктурное проявление ранних метаболических нарушений в миокарде собак при аллоксановом диабете //Бюл. exper. биол. мед. – 1980. – Т. 89. – №. 5. – С. 614.

38. Луценко Л. А. Роль гликированного гемоглобина в диагностике и мониторинге сахарного диабета //Почки. – 2014. – №. 4 (10). – С. 7-11.

39. Лушников К. В. и др. Влияние крайневысокочастотного электромагнитного излучения низкой интенсивности на показатели гуморального иммунитета здоровых мышей //Биофизика. – 2001. – Т. 46. – №. 4. – С. 753-760.

40. Миронов А. Н. и др. Руководство по проведению доклинических исследований лекарственных средств //М.: Гриф и К. – 2012. – Т. 944.

41. Михайличенко В. Ю., Пилипчук А. А., Самарин С. А. Сравнительная оценка различных методов хирургической коррекции аллоксанового сахарного диабета //Крымский журнал экспериментальной и клинической медицины. – 2018. – Т. 8. – №. 3. – С. 52-57.

42. Можейко Л. А. Экспериментальные модели для изучения сахарного диабета часть II. Хирургический, стрептозотоциновый и дитизоновый диабет //Журнал Гродненского государственного медицинского университета. – 2013. – №. 4 (44). – С. 005-010.

43. Мокрышева Н. Г. и др. Пандемия COVID-19 и эндокринопатии //Проблемы эндокринологии. – 2020. – Т. 66. – №. 1. – С. 7-13.

44. Немец В. В., Виноградова Е. П. Стресс и стратегии поведения //Национальный психологический журнал. – 2017. – №. 2 (26). – С. 59-72.

45. Нестеров С. В. и др. Автокаталитический цикл в патогенезе сахарного диабета: биохимические и патофизиологические аспекты метаболической терапии с помощью натуральных аминокислот на примере глицина //Сахарный диабет. – 2018. – Т. 21. – №. 4. – С. 283-292.

46. Нечаева О. В. и др. Оценка ранозаживляющей способности гелевой формы препарата «Меллисол» на модели экспериментальных плоскостных ран //Саратовский научно-медицинский журнал. – 2017. – Т. 13. – №. 3. – С. 635-640.

47. Обухова Л. А. и др. Влияние длительного приема пробиотика на морфофункциональное состояние эндокринной части поджелудочной железы у экспериментальных животных с аллоксановым диабетом //Сибирский научный медицинский журнал. – 2006. – №. 2. – С. 171-175.

48. Овчаров Е. Г. Мировая фармацевтическая промышленность: современное состояние и тенденции развития в условиях глобализации: дис. – 2005.

49. Пальчикова Н. А. и др. Особенности течения экспериментального сахарного диабета при введении в рацион животных природного инулинового комплекса //Сибирский научный медицинский журнал. – 2007. – №. 2. – С. 114-118.

50. Пальчикова Н. А., Кузьминова О. И., Селятицкая В. Г. Влияние перфторана на чувствительность животных к диабетогенному действию аллоксана и течение экспериментального диабета //Сибирский научный медицинский журнал. – 2006. – №. 3. – С. 113-116.

51. Пальчикова Н. А., Селятицкая В. Г., Шорин Ю. П. Количественная оценка чувствительности экспериментальных животных к диабетогенному действию аллоксана //Проблемы эндокринологии. – 1987. – Т. 33. – №. 4. – С. 65-68.

52. Прозорова И. В. и др. Особенности клинико-биохимических различий у больных с сахарным диабетом различной степени компенсации //Вестник Новгородского государственного университета им. Ярослава Мудрого. – 2016. – №. 6 (97). – С. 49-54.

53. Рогаткин Д. А. Физические основы оптической оксиметрии //Медицинская физика. – 2012. – №. 2. – С. 97-114.

54. Самотруева М. А., Сергалиева М. У. Сахарный диабет: особенности экспериментального моделирования //Астраханский медицинский журнал. – 2019. – Т. 14. – №. 3. – С. 45-57.

55. Селятицкая В. Г. и др. Активность адренокортикальной системы у крыс с высокой и низкой устойчивостью к диабетогенному действию аллоксана //Фундаментальные исследования. – 2011. – Т. 3. – С. 142-148.

56. Сидоренко Д. С. Включение 14С-глицина и 3Н-метионина в общие и цитоплазматические белки скелетных мышц крыс при дефиците инсулина и избытке глюкокортикоидных гормонов //Пробл. эндокринологии. – 1981. – Т. 27. – №. 5. – С. 59-61.

57. Симонов П. В. Мотивированный мозг/Павел Васильевич Симонов. – 1987. – 105 с.

58. Смирнов Л. Д. и др. Возможности фармакологической коррекции метаболических нарушений при экспериментальном диабете препаратами антиоксидантного типа действия //Биомедицинская химия. – 2004. – Т. 50. – №. 5. – С. 502-508.

59. Соболева Г. Н. и др. Прогностическое значение дисфункции эндотелия коронарных и плечевой артерии, традиционных факторов риска в развитии сердечно-сосудистых осложнений у пациентов с микрососудистой стенокардией //Российский кардиологический журнал. – 2017. – №. 3 (143). – С. 54-58.

60. Танирбергенова А. А., Тулебаев К. А., Аканов Ж. А. Распространение сахарного диабета в современном мире //Вестник Казахского национального медицинского университета. – 2017. – №. 2. – С. 376-378.

61. Терновой К. С., Шумада И. В. Некоторые вопросы организации восстановительного лечения больных и инвалидов с последствиями травм и заболеваниями опорно-двигательного аппарата в Украинской ССР //Ортопедия, травматология и протезирование. – 1982. – №. 5. – С. 1-6.

62. Толкачева В. В., Кичигина Т. М., Кобалава Ж. Д. Современные антигипергликемические препараты: механизмы действия и клинические эффекты //Клиническая фармакология и терапия. – 2009. – Т. 18. – №. 2. – С. 75-82.

63. Томова Т. А. и др. Влияние карбахолина и глицилпролина (GLY-PRO) на секреторную функцию желудка в зависимости от реактивности ЦНС у крыс. – 2015.

64. Фабричнова А. А. и др. Изменения реологических свойств крови при сахарном диабете //Эндокринология: Новости. Мнения. Обучение. – 2018. – №. 2 (23). – С. 26-34.

65. Федюшина О.Г. Особенности гемостаза и реологии крови человека при сахарном диабете в сочетании с артериальной гипертензией : дис. ... канд. биол. наук. Н. Новгород, 2005.

66. Храмин В. Н. Современные аспекты местного лечения хронических ран нижних конечностей у больных сахарным диабетом //Сахарный диабет. – 2005. – №. 4. – С. 26-30.

67. Худоерова Д. Р. Сахарный диабет в условиях вирусной пандемии COVID-19 //Ta'lim va rivojlanish tahlili onlayn ilmiy jurnali. – 2022. – Т. 2. – №. 10. – С. 417-422.

68. Чекина Н. А., Чукаев С. А., Николаев С. М. Сахарный диабет: возможности фармакотерапии с использованием средств растительного происхождения //Вестник Бурятского государственного университета. Медицина и фармация. – 2010. – №. 12. – С. 71-78.

69. Черепанова К. А., Корчина Т. Я. Показатели эхокардиографии у больных сахарным диабетом 2 типа, проживающих в северном регионе //Научный медицинский вестник Югры. – 2018. – №. 1. – С. 22-26.

70. Чуян Е. Н. и др. Типологические особенности микроциркуляции животных //Ученые записки Крымского федерального университета имени В.И. Вернадского. Биология. Химия. – 2012. – Т. 25. – №. 3 (64). – С. 222-239.

71. Шарофова М. У., Сагдиева Ш. С., Джаббор Ю. С. Сахарный диабет: современное состояние вопроса (часть 2) //Вестник Авиценны. – 2019. – Т. 21. – №. 4. – С. 661-674.

72. Шарофова М. У., Сагдиева Ш. С., Юсуфи С. Д. Сахарный диабет: современное состояние вопроса (часть 1) //Вестник Авиценны. – 2019. – Т. 21. – №. 3. – С. 502-512.

73. Шестакова М. В. и др. Эпидемиология сахарного диабета в Российской Федерации: что изменилось за последнее десятилетие? //Терапевтический архив. – 2019. – Т. 91. – №. 10. – С. 4-13.

74. Шестакова М. В., Дедов И. И. Диабетическая нефропатия //М.: Универсум Пабблишинг. – 2000.

75. Шестакова М. В., Мокрышева Н. Г., Дедов И. И. Сахарный диабет в условиях вирусной пандемии COVID-19: особенности течения и лечения //Сахарный диабет. – 2020. – Т. 23. – №. 2. – С. 132-139.

76. Штемберг А. С., Узбеков М. Г., Шихов С. Н. Некоторые нейротропные эффекты электромагнитных волн малой интенсивности у крыс с разными типологическими особенностями высшей нервной деятельности //Журн. Высшей нервной деятельности. – 2000. – Т. 50. – №. 5. – С. 867-877.

77. Эльбекьян К. С., Ходжаян А. Б., Муравьева А. Б. Особенности нарушения макро- и микроэлементного спектра сыворотки крови при экспериментальном сахарном диабете //Фундаментальные исследования. – 2011. – №. 10-2. – С. 411-413.

78. Якушева Э. Е., Жебентяев А. И. Практико-ориентированные задания как средство формирования практических навыков при изучении аналитической и токсикологической химии. – 2023.

79. Afanasiev S. A. et al. Comparative analysis of changes of myocardial angiogenesis and energy metabolism in postinfarction and diabetic damage of rat heart //Journal of diabetes research. – 2014. – Т. 2014.

80. Al Slail F. Y. et al. Cardiovascular risk profiles of adults with type-2 diabetes treated at urban hospitals in Riyadh, Saudi Arabia //Journal of epidemiology and global health. – 2016. – T. 6. – №. 1. – C. 29-36.

81. Ali N. et al. The prevalence of general obesity, abdominal obesity, and hypertension and its related risk factors among young adult students in Bangladesh //The Journal of Clinical Hypertension. – 2022. – T. 24. – №. 10. – C. 1339-1349.

82. Allen J. Photoplethysmography and its application in clinical physiological measurement //Physiological measurement. – 2007. – T. 28. – №. 3. – C. R1.

83. Al-Maskari F., El-Sadig M., Nagelkerke N. Assessment of the direct medical costs of diabetes mellitus and its complications in the United Arab Emirates //BMC public health. – 2010. – T. 10. – №. 1. – C. 1-10.

84. Al-Maskari F., El-Sadig M., Nagelkerke N. Assessment of the direct medical costs of diabetes mellitus and its complications in the United Arab Emirates //BMC public health. – 2010. – T. 10. – №. 1. – C. 1-10.

85. Alotaibi A. et al. Incidence and prevalence rates of diabetes mellitus in Saudi Arabia: An overview //Journal of epidemiology and global health. – 2017. – T. 7. – №. 4. – C. 211-218.

86. Antia B. S. et al. Hypoglycemic activity of aqueous leaf extract of *Persea americana* Mill //Indian journal of pharmacology. – 2005. – T. 37. – №. 5. – C. 325.

87. Aravamudhan A. et al. Insulin oedema in a child with newly diagnosed diabetes mellitus //European journal of pediatrics. – 2014. – T. 173. – C. 685-687.

88. Baek J. W., Jee S. H. Incidence of diabetes mellitus according to body mass index and gamma-glutamyltransferase level in Korea //Journal of Global Health Science. – 2023. – T. 4. – №. 2.

89. Baydas B., Karagoz S., Meral I. Effects of oral zinc and magnesium supplementation on serum thyroid hormone and lipid levels in experimentally induced diabetic rats //Biological trace element research. – 2002. – T. 88. – C. 247-253.

90. Behr G. A. et al. Pancreas  $\beta$ -cells morphology, liver antioxidant enzymes and liver oxidative parameters in alloxan-resistant and alloxan-susceptible Wistar rats: a viable model system for the study of concepts into reactive oxygen species //Fundamental & clinical pharmacology. – 2008. – T. 22. – №. 6. – C. 657-666.

91. Benus R. F. et al. Behavioural differences between artificially selected aggressive and non-aggressive mice: response to apomorphine //Behavioural brain research. – 1991. – T. 43. – №. 2. – C. 203-208.

92. Beretta A. Campanha de prevencao e diagnostico do diabetes realizada pela UNIARARAS e prefeitura municipal na cidade de Araras //Laes and Haes. – 2001. – T. 22. – №. 131. – C. 188-200.

93. Birlea S. A., Fain P. R., Spritz R. A. A Romanian population isolate with high frequency of vitiligo and associated autoimmune diseases //Archives of dermatology. – 2008. – T. 144. – №. 3. – C. 310-316.

94. Bonura C. et al. Necrobiosis Lipoidica Diabeticorum: A pediatric case report //Dermato-endocrinology. – 2014. – T. 6. – №. 1. – C. e983683.

95. Boquist L. Alloxan diabetes in mice-study of potentiating and antagonizing factors //Diabetologia. – 175 Fifth ave, New York, NY 10010: Springer verlag, 1977. – T. 13. – №. 4. – C. 383-383.

96. Briede J. et al. Effect of cerebrocrast, a new long-acting compound on blood glucose and insulin levels in rats when administered before and after STZ-induced diabetes mellitus //Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease. – 2007. – T. 25. – №. 6. – C. 673-680.

97. Calatayud F., Belzung C., Aubert A. Ethological validation and the assessment of anxiety-like behaviours: methodological comparison of classical analyses and structural approaches //Behavioural processes. – 2004. – T. 67. – №. 2. – C. 195-206.

98. Chatzigeorgiou A. et al. The use of animal models in the study of diabetes mellitus //In vivo. – 2009. – T. 23. – №. 2. – C. 245-258.

99. Craig C. L., Riekel C. Comparative architecture of silks, fibrous proteins and their encoding genes in insects and spiders //Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. – 2002. – T. 133. – №. 4. – C. 493-507.

100. Das J., Vasan V., Sil P. C. Taurine exerts hypoglycemic effect in alloxan-induced diabetic rats, improves insulin-mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress and apoptosis //Toxicology and applied pharmacology. – 2012. – T. 258. – №. 2. – C. 296-308.

101. DECODE Study Group. Age-and sex-specific prevalences of diabetes and impaired glucose regulation in 13 European cohorts //Diabetes care. – 2003. – T. 26. – №. 1. – C. 61-69.

102. Dedov I. I. et al. Atlas of diabetes register in Russian Federation, status 2018 //Diabetes mellitus. – 2019. – T. 22. – №. 2S. – C. 4-61.

103. Dedov I. I. et al. The quality of medical aid provided to the children with type 1 diabetes mellitus //Problems of Endocrinology. – 2015. – T. 61. – №. 4. – C. 29-42.

104. Dedov I. I., Peterkova V. A. Federal clinical guidelines (protocols) for the management of children with endocrine diseases. M //Practice. – 2014.

105. Dunn J. S., McLetchie N. G. B. Experimental alloxan diabetes in the rat //The Lancet. – 1943. – T. 242. – №. 6265. – C. 384-387.].

106. Ebner J., Maytin E. V. Cutaneous wound healing //Dermatologic Surgery. – 2008. – C. 81-100.

107. Elsner M. et al. Importance of the GLUT2 glucose transporter for pancreatic beta cell toxicity of alloxan //Diabetologia. – 2002. – T. 45. – C. 1542-1549.

108. Elsner M., Gurgul-Convey E., Lenzen S. Relative importance of cellular uptake and reactive oxygen species for the toxicity of alloxan and dialuric acid to insulin-producing cells //Free radical biology and medicine. – 2006. – T. 41. – №. 5. – C. 825-834.

109. Enoch S., Harding K. Review Wound Bed Preparation: The Science behind the Removal of Barriers to Healing //Wounds. – 2003. – T. 15. – №. 7. – C. 213-229.
110. Enoch S., Price P. Cellular, molecular and biochemical differences in the pathophysiology of healing between acute wounds, chronic wounds and wounds in the aged //World Wide Wounds. – 2004. – T. 13. – C. 1-17.
111. Etuk E. U. et al. Animals models for studying diabetes mellitus //Agric Biol JN Am. – 2010. – T. 1. – №. 2. – C. 130-134.
112. Etuk E. U., Muhammed B. J. Evidence based analysis of chemical method of induction of diabetes mellitus in experimental rats //Int J Res Pharm Sci. – 2010. – T. 1. – №. 2. – C. 139-142.
113. Ezeonwumelu I. J. et al. Coadministration of L-alanine and L-glutamine ameliorate blood glucose levels, biochemical indices and histological features in alloxan-induced diabetic rats //Journal of Food Biochemistry. – 2022. – T. 46. – №. 12. – C. e14420.
114. Fajre X. et al. Manifestaciones cutáneas de diabetes mellitus //Revista médica de Chile. – 2009. – T. 137. – №. 7. – C. 894-899.
115. Farhan J. et al. Interleukin-6: a possible inflammatory link between vitiligo and type 1 diabetes //British journal of biomedical science. – 2014. – T. 71. – №. 4. – C. 151-157.
116. Federiuk I. F. et al. Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: route of administration, pitfalls, and insulin treatment //Comparative medicine. – 2004. – T. 54. – №. 3. – C. 252-257.
117. Ghaffari M. et al. The effect of aerobic exercise on metabolic parameters of patients with non-alcoholic fatty liver disease: systematic review and meta-analysis //The Journal of sports medicine and physical fitness. – 2022.
118. Goturu A., Jain N., Lewis I. Bilateral cataracts and insulin oedema in a child with type 1 diabetes mellitus //Case Reports. – 2013. – T. 2013. – C. bcr2012008235.

119. Gurfinkel Y. I. Computer capillaroscopy as a channel of local visualization, noninvasive diagnostics, and screening of substances in circulating blood //Saratov Fall Meeting 2000: Optical Technologies in Biophysics and Medicine II. – SPIE, 2001. – T. 4241. – C. 467-472.

120. Hashim M. J., Mustafa H., Ali H. Knowledge of diabetes among patients in the United Arab Emirates and trends since 2001: a study using the Michigan Diabetes Knowledge Test //EMHJ-Eastern Mediterranean Health Journal. – 2016. – T. 22. – №. 10. – C. 742-748.

121. Hasselmann C. et al. Continuous subcutaneous insulin infusion allows tolerance induction and diabetes treatment in a type 1 diabetic child with insulin allergy //Diabetes & metabolism. – 2013. – T. 39. – №. 2. – C. 174-177.

122. <https://www.gazeta.uz/ru/2021/11/15/diabetes/>

123. Inoue R. et al. Localization of serine racemase and its role in the skin //Journal of Investigative Dermatology. – 2014. – T. 134. – №. 6. – C. 1618-1626.

124. International Working Group on the Diabetic Foot et al. International consensus on the diabetic foot and practical guidelines on the management and prevention of the diabetic foot: Amsterdam //www. idf. org/bookshop. – 2007.

125. Iranloye B. O. et al. Anti-diabetic and anti-oxidant effects of Zingiber officinale on alloxan-induced and insulin-resistant diabetic male rats //Nigerian journal of physiological sciences. – 2011. – T. 26. – №. 1.

126. Jorns A. et al. Comparative toxicity of alloxan, N-alkylalloxans and ninhydrin to isolated pancreatic islets in vitro //Journal of endocrinology. – 1997. – T. 155. – №. 2. – C. 283-294.

127. Khalili P. et al. Serum liver enzymes and metabolic syndrome from the Rafsanjan Cohort Study //Journal of Investigative Medicine. – 2023. – T. 71. – №. 2. – C. 140-148.

128. King H., Aubert R. E., Herman W. H. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections //Diabetes care. – 1998. – T. 21. – №. 9. – C. 1414-1431.

129. Kokabiyani Z. et al. Effect of eugenol on lipid profile, oxidative stress, sex hormone, liver injury, ovarian failure, and expression of COX-2 and PPAR- $\alpha$  genes in a rat model of diabetes //Molecular Biology Reports. – 2023. – T. 50. – №. 4. – C. 3669-3679.
130. Korejba K. A. et al. Diabetic foot syndrome. Replacement for inpatient treatment technique based on the FAST TRACK surgery //Ambulatomnaya khirurgiya= Ambulatory Surgery (Russia). – 2019. – №. 1-2. – C. 72-79.
131. Kruger D. F. et al. Managing diabetes with integrated teams: maximizing your efforts with limited time //Postgraduate Medicine. – 2012. – T. 124. – №. 2. – C. 64-76.
132. Lenzen S. et al. Alloxan derivatives as a tool for the elucidation of the mechanism of the diabetogenic action of alloxan //Lessons from Animal Diabetes VI: 75th Anniversary of the Insulin Discovery. – 1996. – C. 113-122.
133. Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes //Diabetologia. – 2008. – T. 51. – №. 2. – C. 216-226.
134. Liu Z., Bing X., Zhi X. Z. Epidemiology working group for NCIP epidemic response //Chinese Center for Disease Control and Prevention. The epidemiological characteristics of an outbreak of. – 2019. – C. 145-151.
135. Lone S. et al. Assessment of metabolic syndrome in Kashmiri population with type 2 diabetes employing the standard criteria's given by WHO, NCEPATP III and IDF //Journal of epidemiology and global health. – 2017. – T. 7. – №. 4. – C. 235-239.
136. Lymarenko M. P. Cutaneous manifestations of diabetes mellitus in children //Rossiyskiy Vestnik Perinatologii i Pediatrii (Russian Bulletin of Perinatology and Pediatrics). – 2017. – T. 62. – №. 2. – C. 17-21.
137. Mahanta T. G. et al. Prevalence of modifiable cardiovascular risk factors among tea garden and general population in Dibrugarh, Assam, India //Journal of epidemiology and global health. – 2013. – T. 3. – №. 3. – C. 147-156.

138. Maksymova O. S., Tkach G. F. Histological and planimetric characteristic of skin regeneration of young rats under the chronic hyperglycemia //Вісник проблем біології і медицини. – 2020.
139. Malaisse W. J. et al. Pancreatic uptake of [2-14C] alloxan //International journal of molecular medicine. – 2001. – Т. 7. – №. 3. – С. 311-315.
140. Maschio M. et al. A rare case of granuloma annulare in a 5-year-old child with type 1 diabetes and autoimmune thyroiditis //The American Journal of Dermatopathology. – 2013. – Т. 35. – №. 3. – С. 385-387.
141. Masiello P. Animal models of type 2 diabetes with reduced pancreatic  $\beta$ -cell mass //The international journal of biochemistry & cell biology. – 2006. – Т. 38. – №. 5-6. – С. 873-893.
142. Msomi N. Z. et al. Xylitol improves antioxidant, purinergic and cholinergic dysfunction, and lipid metabolic homeostasis in hepatic injury in type 2 diabetic rats //Journal of Food Biochemistry. – 2022. – Т. 46. – №. 4. – С. e14040.
143. Murotomi K. et al. Switching from singlet-oxygen-mediated oxidation to free-radical-mediated oxidation in the pathogenesis of type 2 diabetes in model mouse //Free Radical Research. – 2015. – Т. 49. – №. 2. – С. 133-138.
144. Murphy A. R., Kaplan D. L. Biomedical applications of chemically-modified silk fibroin //Journal of materials chemistry. – 2009. – Т. 19. – №. 36. – С. 6443-6450.
145. Mythili M. D. et al. Effect of streptozotocin on the ultrastructure of rat pancreatic islets //Microscopy research and technique. – 2004. – Т. 63. – №. 5. – С. 274-281.
146. Narita T. et al. Generalized vitiligo and associated autoimmune diseases in Japanese patients and their families //Allergology International. – 2011. – Т. 60. – №. 4. – С. 505-508.
147. Neville K. A. et al. Insulin allergy desensitization with simultaneous intravenous insulin and continuous subcutaneous insulin infusion //Pediatric Diabetes. – 2008. – Т. 9. – №. 4pt2. – С. 420-422.

148. Noroozi Karimabad M. et al. Serum liver enzymes and diabetes from the Rafsanjan cohort study //BMC Endocrine Disorders. – 2022. – T. 22. – №. 1. – C. 1-12
149. Oluyombo R. et al. Cardiovascular risk factors in semi-urban communities in southwest Nigeria: Patterns and prevalence //Journal of epidemiology and global health. – 2015. – T. 5. – №. 2. – C. 167-174.
150. Onder G., Rezza G., Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy //Jama. – 2020. – T. 323. – №. 18. – C. 1775-1776.
151. Onyango E. M., Onyango B. M. The rise of noncommunicable diseases in Kenya: an examination of the time trends and contribution of the changes in diet and physical inactivity //Journal of epidemiology and global health. – 2018. – T. 8. – №. 1-2. – C. 1.
152. Onyiriuka A. N., Ehirim F. A. Insulin-induced oedema in a patient with diabetes mellitus complicated by ketoacidosis //Acta Medica Indonesiana. – 2014. – T. 46. – №. 4.
153. Pătrașcu V. et al. Ulcerated necrobiosis lipoidica to a teenager with diabetes mellitus and obesity //Rom J Morphol Embryol. – 2014. – T. 55. – №. 1. – C. 171-176.
154. Pavlovic M. D. et al. The prevalence of cutaneous manifestations in young patients with type 1 diabetes //Diabetes care. – 2007. – T. 30. – №. 8. – C. 1964-1967.
155. Ramtahal R. et al. Prevalence of self-reported sleep duration and sleep habits in type 2 diabetes patients in South Trinidad //Journal of epidemiology and global health. – 2015. – T. 5. – №. 4. – C. S35-S43.
156. Rees D. A., Alcolado J. C. Animal models of diabetes mellitus //Diabetic medicine. – 2005. – T. 22. – №. 4. – C. 359-370.
157. Rostami P. et al. Insulin edema in a child with diabetes mellitus type 1 //The Turkish Journal of Pediatrics. – 2012. – T. 54. – №. 3. – C. 309.

158. Roustit M., CRACOWSKI J. L. U. C. Non-invasive assessment of skin microvascular function in humans: an insight into methods //Microcirculation. – 2012. – T. 19. – №. 1. – C. 47-64.
159. Sayeed M. A. et al. Diabetes and impaired fasting glycemia in a rural population of Bangladesh //Diabetes care. – 2003. – T. 26. – №. 4. – C. 1034-1039.
160. Schreml S. et al. Wound healing in the 21st century //Journal of the American Academy of Dermatology. – 2010. – T. 63. – №. 5. – C. 866-881.
161. Shahzad M. et al. Skin manifestations in diabetic patients attending a diabetic clinic in the Qassim region, Saudi Arabia //Medical Principles and Practice. – 2011. – T. 20. – №. 2. – C. 137-141..
162. Shao S. et al. Interaction of severe acute respiratory syndrome coronavirus 2 and diabetes //Frontiers in Endocrinology. – 2021. – T. 12. – C. 731974.
163. Shaw J. E., Sicree R. A., Zimmet P. Z. Global estimates of the prevalence of diabetes for 2010 and 2030 //Diabetes research and clinical practice. – 2010. – T. 87. – №. 1. – C. 4-14.
164. Sofia S. et al. Functionalized silk-based biomaterials for bone formation //Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials and The Japanese Society for Biomaterials. – 2001. – T. 54. – №. 1. – C. 139-148.
165. Stolar M. W. et al. Managing type 2 diabetes: going beyond glycemic control //Journal of Managed Care Pharmacy. – 2008. – T. 14. – №. 5 Supp B. – C. 1-22.
166. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas //Physiological research. – 2001. – T. 50. – №. 6. – C. 537-546.
167. Tasaka Y. et al. Changes in plasma glucagon, pancreatic polypeptide and insulin during development of alloxan diabetes mellitus in dog //Endocrinologia japonica. – 1988. – T. 35. – №. 3. – C. 399-404.

168. Vepari C., Kaplan D. L. Silk as a biomaterial //Progress in polymer science. – 2007. – T. 32. – №. 8-9. – C. 991-1007.
169. Viswanathaswamy A. H. M. et al. Antihyperglycemic and antihyperlipidemic activity of *Plectranthus amboinicus* on normal and alloxan-induced diabetic rats //Indian journal of pharmaceutical sciences. – 2011. – T. 73. – №. 2. – C. 139.
170. Williams A. et al. Pathophysiology of Red Blood Cell Dysfunction in Diabetes and Its Complications //Pathophysiology. – 2023. – T. 30. – №. 3. – C. 327-345.
171. Wright C. I., Kroner C. I., Draijer R. Non-invasive methods and stimuli for evaluating the skin's microcirculation //Journal of pharmacological and toxicological methods. – 2006. – T. 54. – №. 1. – C. 1-25.
172. Yu K. et al. Accelerated wound-healing capabilities of a dressing fabricated from silkworm cocoon //International journal of biological macromolecules. – 2017. – T. 102. – C. 901-913.
173. Zabeen B. et al. Hypertriglyceridemia associated with eruptive xanthomas and lipemia retinalis in newly diagnosed diabetes mellitus //Mymensingh Medical Journal: MMJ. – 2013. – T. 22. – №. 3. – C. 591-595.
174. Zaccone C. et al. Necrobiosis lipidica diabetorum in children. Description of a case //Giornale Italiano di Dermatologia e Venereologia: Organo Ufficiale, Societa Italiana di Dermatologia e Sifilografia. – 1990. – T. 125. – №. 5. – C. 225-228.
175. Zhang Z. et al. The analysis of genetics and associated autoimmune diseases in Chinese vitiligo patients //Archives of dermatological research. – 2009. – T. 301. – C. 167-173.