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EXPERIMENTAL JUSTIFICATION FOR THE CLOSURE OF WOUND DEFECTS USING COMPOSITE COLLAGEN IN DIABETIC FOOT SYNDROME

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Abstract:
Objective: To treat wounds caused by alloxan-induced diabetes under experimental conditions using a modern topical agent.
Materials and methods: Healthy rats were selected for the experiment. Experimental studies were conducted on 155 male white rats weighing 150-200 g, housed in the vivarium of the Tashkent Medical Academy (TMA).
Results: The use of this type of collagen prevented endogenous intoxication, and the high efficacy of the new topical agent was demonstrated. It proved effective in treating systemic intoxication and mechanical injuries in diabetic rats, leading to its recommendation for use in the treatment of patients with diabetic foot defects.
Conclusions: Collagen with quercetin helped to understand the molecular factors involved in the development of diabetic foot syndrome, the pathogenetic mechanisms of endothelial dysfunction, and the processes of neoangiogenesis in the progression of the pathology.

Keywords: composite collagen, diabetic foot, quercetin, alloxan, experiment.

Introduction

Diabetes mellitus is one of the most prevalent chronic diseases, gradually acquiring the character of a non-infectious epidemic. According to projections by the International Diabetes Federation, by 2030, the number of people with diabetes is expected to reach 578 million; by 2035, the prevalence will rise to 600 million; and by 2045, it is anticipated to reach 700 million. The diabetic foot syndrome is characterized by chronic purulent-necrotic processes in the leg (trophic ulcers), leading to damage of the skin,

soft tissues, and osteoarticular apparatus (Belozertseva Y.P. et al., 2016; Madyanov I.V., 2016). This often results in severe consequences, including disability and mortality (Koreyba K.A., 2016).

The spontaneous healing of diabetic ulcers in diabetic foot syndrome is typically impossible (Wella L., Formoza C., 2017). Diabetic ulcers on the feet are treated successfully in only two-thirds of all cases, and the recurrence rate reaches 60-70% (Spichkina O.G. et al., 2012). In 28% of patients, diabetic ulcers can lead to limb amputation (Guryeva I.V., 2016; Chakanov T.I., Kultayev U.T., 2016). Each year, more than 1 million people die as a result of diabetes and its complications. Every 20 seconds, a limb is amputated due to diabetes (Guryeva I.V., 2016). However, an unresolved issue remains the absence of a pathophysiologically oriented program for treating diabetic ulcerative defects. Practice has shown that even if a physician selects an appropriate treatment strategy for a patient with diabetic foot ulcers, the desired outcome may not be achieved within the expected timeframe.

Products derived from medical research—collagen—already possess the desired clinical value and are, therefore, promising for effectively stimulating the regenerative and proliferative phases of wound healing. Type I collagen is an extracellular matrix protein and the most abundant component of connective tissue in most tissues, possessing properties of cellular chemotaxis and capillary permeability.

Undoubtedly, the importance and effectiveness of bioplastic materials in the treatment of acute and chronic wounds have been confirmed by many researchers. However, there is currently no reliable evidence for experimental treatment methods for diabetic wounds using various forms of collagen in animals with diabetic foot syndrome.

MATERIALS AND METHODS

«Characteristics of Experimental Material and Description of Research Methods».

This study provides a general description of the experimental material and methods used. The research is prospective and randomized, based on modern hematological, biochemical, and morphological research techniques.

Healthy rats were selected for the experiment, with studies conducted on 155 male white sterile rats weighing 150-200 g, housed in the Tashkent Medical Academy (TMA) vivarium. The rats were kept in optimal conditions with continuous lighting and a constant temperature of 22-25°C, having free access to water. They were fed ad libitum with a standard rodent diet (GOST R50258-92) and provided with drinking water daily. All procedures involving animals were performed under general anesthesia, adhering to the humane principles outlined in the European Community Guidelines (86/609/EES) and the Helsinki Declaration, as well as in accordance with the "Rules for Conducting Research Using Experimental Animals."

The experimental animals were divided into four groups:

1. Control group (intact).
2. Group for creating an experimental model of alloxan diabetes.

3. Control group for traditional comprehensive treatment (Levomekol - "НИЖФАРМ," Russia) against the backdrop of the diabetic foot model.
4. Experimental group for traditional treatment combined with collagen and quercetin.

After a 24-hour fasting period, the rats were weighed, and a single intraperitoneal dose of 2% alloxan solution diluted in 0.9% saline was administered, corresponding to doses of 20, 15, or 12 mg of alloxan per 100 g of body weight. Food and water were provided 30 minutes after drug administration. Blood glucose levels were monitored over three days. Following prior scientific work, the optimal alloxan dose for rats was determined to be 12 mg/g.

Diabetes was confirmed three days later through blood glucose concentration measurements. Glucose levels were assessed using the Satellit Express glucometer ("ELTA," Russia), with a measurement range of 0.6 - 35.0 mmol/L, based on electrochemical measurement principles and adhering to international standard ISO 15197:2013.

On the third day of observation, a primary surgical treatment of the skin on the right hind limb of the rat was performed using an antiseptic device. A small puncture wound was created with a scalpel, and a single-use “Aortal Punch” instrument was employed to establish a skin defect on the dorsal side of the right hind limb, minimizing the risk of lateral tearing in surrounding tissues. This method enables the creation of an accurate, consistent, and reproducible round hole. The Perfect Cut and Clean Cut instruments provide uniform openings with a rotating, self-centering output mechanism, requiring less effort and time (Rationalization Proposal "Modeling the Injury Defect in Experimental Diabetic Foot Syndrome," No. 1399, TTA, 2023).

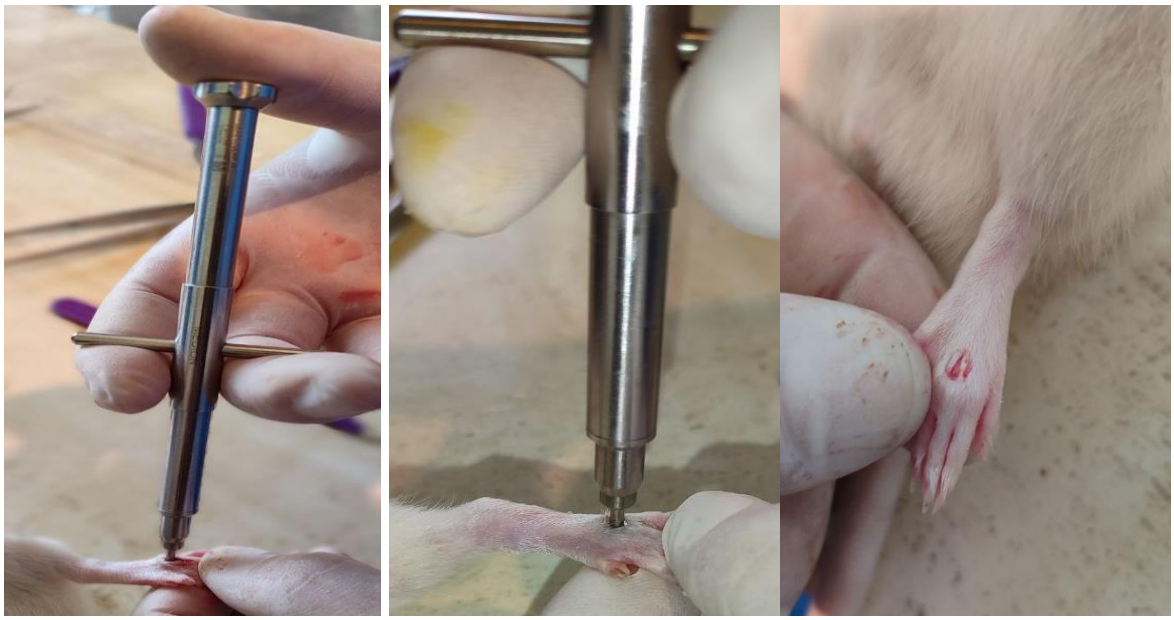

Figure 1. Appearance of the “Perfect Cut” instrument and the creation of a wound defect on the skin of rats.

The study of peripheral blood was conducted using a hematological analyzer to calculate the leukocyte formula. Based on the obtained results, the leukocyte intoxication index was calculated according to the recommendations of Kalff-Kalifa. Hematological parameters were used to determine the Shift Index (SI), Leukocyte Intoxication Index (LII), and Neutrophilic Reactive Response (RON). The activity of enzymes such as total protein, glucose, creatinine, urea, ALT, and AST was determined using a set of chemical reagents produced by the Mindray BS-380 biochemical analyzer (Germany).

In accordance with modern concepts, to assess wound healing indicators, the Vascular Endothelial Growth Factor (VEGF) and Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) were analyzed using the Wright analyzer from Elabscience (USA). Statistical processing of the data was performed using SPSS 16.0 and Windows Statistica 6.0 for applied programs. The means and standard deviations, medians, and quartiles were calculated, along with non-parametric methods (t-Student tests, Mann-Whitney tests).

RESULTS OF THE STUDY

There was a trend towards increased body weight in the rats compared to the values of animals in the unchanged control group. Diuresis, weight, and polyuria on the 10th and 14th days of the experiment significantly exceeded the values of the unchanged rats by 6.38 ($P < 0.001$), 1.14 ($P < 0.001$), and 4.69 ($P < 0.001$) times, respectively (Table 1).

In the comparison group (Levomekol), no weight loss was observed; instead, there was a weight gain. By the end of the experiment, polyuria, polydipsia, and polyphagia gradually decreased: by the 14th day, polyuria, weight, and polydipsia were reduced by 3.85 ($P < 0.001$), 1.06 ($P < 0.001$), and 3.04 ($P < 0.001$) times, but they remained higher than in the unchanged group.

In the experimental group (collagen with quercetin), there was no observed reduction in body weight on the dorsal part of the hind limbs of the rats. On the 10th day of the experiment, the indicators of polyuria, weight, and polydipsia were significantly lower than those of the control group, by 1.94 ($P < 0.05$), 1.016 ($P < 0.05$), and 2.13 ($P < 0.05$) times, respectively.

By the 14th day of the experiment, these indicators were almost indistinguishable from those in the untreated group of rats.

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Table 1. Results of Physiological Parameters in Alloxan-Induced Diabetic Rats.

GROUPS	Diuresis, Ml/day	Weight, gr	Volume of water, Ml/day
Unchanged Control Group	**9,6±0,50**	**215,7±2,9**	**15,6±0,79**
Control Group			
Day 1	**44,0±1,4*****	**229,4±2,7**	**45,0±1,7*****
Day 3	**50,6±1,2**	**231,4±1,3**	**55,3±1,4*****
Day 7	**56,1±1,0**	**235,8±1,7**	**62,5±1,7*****
Day 10	**61,3±2,4**	**245,2±1,5**	**69,2±1,8*****
Day 14	**73,3±1,6**	**235,2±1,8**	**73,2±1,4*****
Comparison Group			
Day 1	**24,1±0,9**$^{***\wedge\wedge\wedge}$	**231,1±1,8**	**24,5±0,7**$^{***\wedge\wedge\wedge}$
Day 3	**24,5±0,8**$^{***\wedge\wedge\wedge}$	**235,3±1,3**	**29,2±0,8**$^{***\wedge\wedge\wedge}$
Day 7	**23,1±1,9**$^{***\wedge\wedge\wedge}$	**230,6±1,5**	**27,3±1,4**$^{***\wedge\wedge\wedge}$
Day 10	**22,5±0,5**$^{***\wedge\wedge\wedge}$	**226,4±0,5**	**25,1±0,6**$^{***\wedge\wedge\wedge}$
Day 14	**19,0±0,4**$^{***\wedge\wedge\wedge}$	**221,8±1,6**	**21,5±1,2**$^{***\wedge\wedge\wedge}$
Experimental Group			
Day 1	**13,6±0,35**$^{\wedge\wedge\wedge\&\&\&}$	**231,4±1,5**$^{\wedge\wedge\&}$	**13,8±0,75**$^{*\wedge\wedge\wedge\&\&}$
Day 3	**14,4±0,65**$^{\wedge\wedge\wedge\&\&\&}$	**237,6±1,6**$^{\wedge\wedge\&}$	**15,2±0,85**$^{*\wedge\wedge\wedge\&\&}$
Day 7	**12,6±1,03**$^{\wedge\wedge\wedge\&\&\&}$	**234,1±1,6**$^{\wedge\wedge\&}$	**12,8±1,53**$^{*\wedge\wedge\wedge\&\&}$
Day 10	**10,8±0,45**$^{\wedge\wedge\wedge\&\&\&}$	**225,6±1,1**$^{\wedge\wedge\&}$	**11,2±0,33**$^{*\wedge\wedge\wedge\&\&}$
Day 14	**9,8±0,45**$^{\wedge\wedge\wedge\&\&\&}$	**218,2±1,3**$^{\wedge\wedge\&}$	**10,1±0,63**$^{*\wedge\wedge\wedge\&\&}$

Indicators of VEGF Factor in Alloxan-Induced Diabetes

Observations conducted in the control group showed that the amount of VEGF in the blood was lower compared to the experimental groups. This indicates that primary surgical treatment of the wound is essential; regardless of the medication used, it is absorbed from the wound and stimulates VEGF factors that originate in the bone marrow. Consequently, the higher the amount of VEGF in the blood, the greater the degree of damage. However, it should not be forgotten that hyperglycemia in the blood causes endothelial dysfunction, which reduces VEGF production. To monitor this, we

checked the blood glucose levels of the rats twice a day—morning and evening after feeding.

On the first day in the control group, the amount of VEGF was 1.34 times higher than the norm ($P < 0.005$). On the third day, the average glucose level was 15.6 mmol/L, and the amount of VEGF did not change significantly compared to the first day. By the seventh day, as the glucose level rose to 18.7 mmol/L, the VEGF level decreased by 1.1 times compared to the first day ($P < 0.005$). By the tenth day, gradual wound healing was established according to contour indicators. By the fourteenth day, the injury had completely healed, and due to the decrease in glucose levels, the amount of VEGF in the blood decreased by 1.26 times ($P < 0.005$).

Table 2. VEGF (Vascular Endothelial Growth Factor) Indicators in Experimental Animals VEGF-A, pg/ml

VEGF-A, pg/ml				
Days	**Groups**			
	Control Group	**Comparison Group**	**Experimental Group**	**Norm, pg/ml**
Day 1	**206,4±0,8*****	**215,5±1,5$^{\wedge\wedge\wedge}$**	**212,4±1,2$^{\&\&\&}$**	**153,6±0,6**
Day 3	**208,3±1,1*****	**223,6±1,7$^{\wedge\wedge\wedge}$**	**238,6±1,7$^{\&\&\&}$**	
Day 7	**186,1±1,6*****	**231,1±0,8$^{\wedge\wedge\wedge}$**	**254,5±0,6$^{\&\&\&}$**	
Day 10	**175,4±1,2*****	**212,6±0,7$^{\wedge\wedge\wedge}$**	**208,1±1,3$^{\&\&\&}$**	
Day 14	**163,7±0,3*****	**203,8±1,2$^{\wedge\wedge\wedge}$**	**178,6±1,8$^{\&\&\&}$**	
Примечание: p<0,005				

Effects of Levomekol and Quercetin Collagen on Contour and Morphological Changes of Wounds

We analyzed the effects of Levomekol and quercetin collagen on the contour properties of diabetic rats. The study showed that in the control group of rats, 24 hours after the injury, the wound size increased as the edges of the wound spread due to edema, which also extended throughout the leg. By the third day after the model was formed, the surface of the wound defect was covered with a thin black scab formed by tissue fluid. This crust was easily damaged, releasing a clear exudate. Clear signs of inflammation were observed: the edges of the wound were swollen with areas of necrosis, and the bottom of the wound was covered with fibrin deposits.

Thus, hyperglycemia causes microvascular complications due to impaired angiogenesis, leading to wound inflammation and prolonged healing times.

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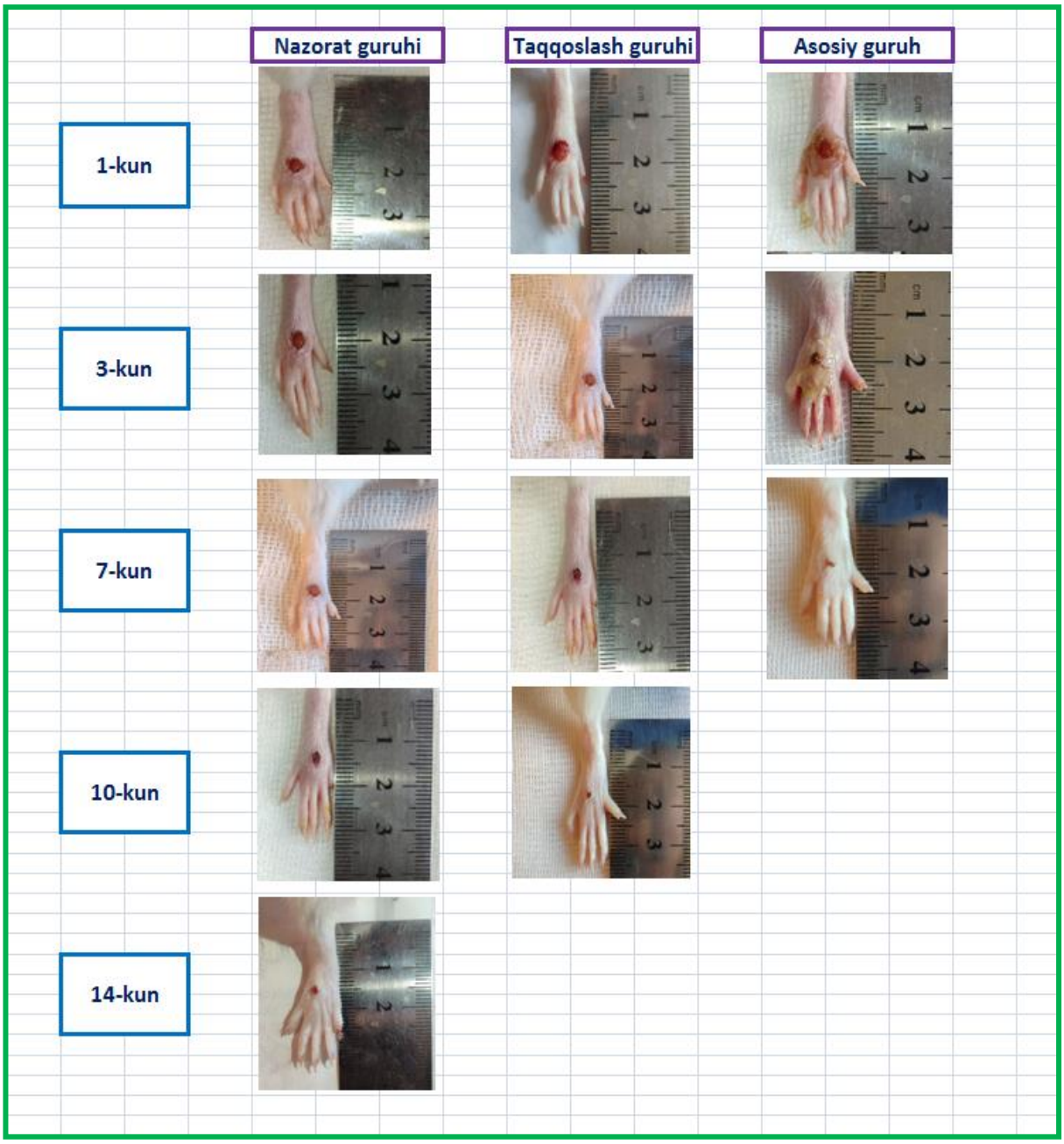

Figure 2. Planimetric Images of Experimental Laboratory Animals and Their Results

CONCLUSIONS

1. Collagen with quercetin contributed to the understanding of the molecular factors involved in the development of diabetic foot syndrome, pathogenic mechanisms associated with endothelial dysfunction, and neoangiogenesis as the severity of the pathology increased.
2. The aforementioned findings revealed the mechanisms by which VEGF influences the angiogenesis process, developed a model of diabetic foot in small laboratory

animals for studying the pathogenesis of VEGF, and established effective methods for conservative treatment.

3. The use of quercetin collagen improves the clinical signs of chronic diabetic wounds compared to Levomekol, accelerates the healing of these wounds, and restores the physiological functions of cells in rats with diabetic foot syndrome, confirming its appropriateness for use in diabetic foot syndrome.
4. The application of this type of collagen prevented endogenous intoxication, demonstrating the high effectiveness of the new topical agent in treating systemic intoxication and mechanical injuries in rats with diabetes, allowing for its recommendation in the treatment of patients with diabetic foot syndrome.

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