Evaluation of the role of the body's response to skin damage in the background of experimental alloxan diabetes.

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ABSTRACT

Chemical models of diabetes occupy the first place in experimental diabetology. Modeling of artificial skin damage in alloxan-induced diabetes, assessment of skin defect recovery, and the use of products developed on the basis of Bombyx mori fibroin for pharmacological repair of wounds, a comparative study of the regeneration process of skin wounds based on the typological response of animals

Keywords: experimental diabetology, alloxan diabetes, Bombyx mori fibroin

The most favorable state of the organism, its adaptation to the constantly changing conditions of the external environment, is called norm (norm). Physiological activity of the body's organs and systems always varies widely depending on the state of the internal environment and the conditions of the external environment: sometimes it increases, sometimes it weakens. But these changes usually do not cause disease. Adaptation of the animal body to experimental alloxan diabetes is a complex multi-step process, the main link of which is the change in cell metabolism. The induction of glyoxylate and tricarboxylic acid cycle enzymes in animal tissues provides changes in the main metabolic pathways due to glycogen resynthesis in the liver of rats in pathologies associated with food deprivation and experimental diabetes [Eprintsev A.T. i dr. 2007, Popov V.N. et al., 1998]. Earlier, the induction of marker enzymes of the glyoxylate cycle in hepatocytes of rats with alloxan diabetes was determined [Popov V.N. et al., 1996]. In addition to being the most important process in the body's response to extreme conditions, gluconeogenesis also plays an important role in energy metabolism, which is mainly related to the Krebs cycle. Therefore, the adaptation of cellular metabolism is provided by the ratio of the intensity of glucose catabolism and anabolism in liver cells and other organs of the animal body. Despite many studies on the intensity of enzymatic activity, which determines the speed of energy and synthetic processes, many regulatory factors, such as the concentration of metabolites, the effect of hormones, etc., remain unclear [Popov V.N. et al., 2005]. It is known that the healing of wounds in diabetes has its own characteristics, because it is associated with severe microcirculation disorders, microthrombi formation, necrotic and dystrophic processes, and the predominance of the inflammatory component over the reparative one. Pharmacological drugs and various physiotherapeutic methods are used to correct cellular and biochemical disorders and, as a result, stimulate regenerative and reparative processes in the postoperative period. Comprehensive treatment of injuries includes restoration of impaired functions of vital organs, correction of acid-alkaline balance, detoxification, control of

metabolic, glycemic and lipidemia, improvement of hemorgeological properties of blood, stimulation of natural resistance of the body, limitation of necrosis.

Silk fibroin from the cocoon of Bombyx mori is a widely used and studied protein polymer for biomaterial applications. Silk fibroin has excellent mechanical properties when processed into a variety of materials, exhibits biocompatibility, has a controlled degradation rate over hours to years, and can be chemically modified to alter surface properties or immobilize growth factors. A variety of aqueous or organic solvent processing methods can be used to produce silk biomaterials for a number of applications. Currently, Bombyx mori cocoons are widely used to produce hydrogels, tubes, sponges, composites, fibers, microspheres, and thin films. These materials can be used directly as biomaterials for implants, for use in tissue engineering and in vivo disease models, and for drug delivery. Bombyx mori (silkworm) silk is a unique material that has been prized throughout history for its durability and luster. Doctors have used silk as a suture material for centuries, and recently it has gained attention as a biomaterial due to several desirable properties. Specifically, these properties include its biocompatibility, ease of chemical modification, slow in vivo degradation rate, and ability to process from aqueous solution or organic solvent into multiple material form.

The purpose of the study: to study the role of the body's reaction to skin damage with pharmacological correction against the background of experimental alloxan diabetes.

Material and research method.

Rats in the experiment were divided into 2 groups.

Group 1: 50% fibroin + 50% cotton fiber in animals with low sensitivity.

Group 2: 50% fibroin + 50% cotton fiber in highly sensitive animals.

Diabetes was induced by alloxan in rats under experimental conditions. A wound was made on the skin, and it was decapitated in order to check the morphological changes that occurred after the traditional treatment. The isolated skin tissue was studied histologically using the hematoxylin-eosin method. Tissue pieces fixed in 10% neutralized formalin for 72 hours were washed in running water for 3-4 hours, then dehydrated in 70, 80, 90, 96, 100% alcohols and chloroform, and blocks were prepared by embedding in paraffin wax. The paraffin in the sections was removed by melting with xylene in a 57oC thermostat, and then stained in hematoxylin-eosin solutions to study the general histological condition of the tissues. The following changes were detected in the obtained micropreparations. When diabetes was initially induced in rats, vascular reactions were observed microscopically in the skin tissue and subcutaneous fat layer, as well as surrounding soft tissues: fullness, interstitial tissue swelling, dystrophic and necrotic changes, and sharp infiltration of resident macrophages in this area. Steatonecrosis with multiple foci, focal fullness and interstitial tumor foci are detected mainly in the surrounding tissue of the skin and adipose tissue. Inflammation is essentially a response of the vascular and mesenchymal tissues in the affected tissue area. In our work, the damage of cell components caused by inflammatory factors continues with the development of paranecrosis, necrobiosis, and necrosis. In particular, the vascular response that occurred in the surroundings of the affected tissue in 1 day, the degranulation of fat cells under the influence of leukotrienes (pro-inflammatory mediators) released by necrotic cells, leads to the rapid development of the alteration process in the surrounding tissues, and this, in turn, occurs with the emergence of secondary alteration. The changes that occurred in 1 day are mainly manifested by the involvement of vascular components in the process, tissue hypoxia, and the development of dystrophic-necrotic changes. Over time, plasmorrhagia, leukodiapedesis and migration of many neutrophils are observed in the damaged area. These changes are clinically manifested by the development of swelling, redness, increased local temperature, pain, and a dysfunctional state in the affected area. In laboratory indicators, it is mainly manifested in the form of leukocytosis, neutrophilia. As a result, the formation of many inflammatory infiltrates in the skin leads to a sharp violation of blood

circulation and lymph drainage function in this area, a sharp increase in the rheological properties of blood in the vein and the occurrence of a sludge phenomenon in small capillaries, a sharp derailment of metabolism in tissues, a large accumulation of intermediate metabolites, and a decrease in resistance to secondary infectious factors. it is explained by the low effectiveness of antibacterial therapy in the treatment of this disease. These changes develop more sharply and begin to spread to the surrounding tissues and lead to further development of the secondary alteration process.

Research results and their discussion

Group 1: 50% fibroin + 50% cotton fiber in animals with low sensitivity (low emotional) (experiment)

In the experimental conditions of the skin of rats in the 1st group, it was determined that the healing period of the wound process in the experiment was accelerated by 40% due to the use of gauze made of Bombyx mori 50% fibroin and 50% cotton fiber with a special composition. It is explained by the low excitability of the sympatho-adrenal system in low-emotional rats, the low release of catecholamines and glucocorticoids in the blood, resulting in tolerance to stressor factors, premature apoptosis of immunocompetent cells, and non-reduction of resistance to iodine substances in the damaged area.

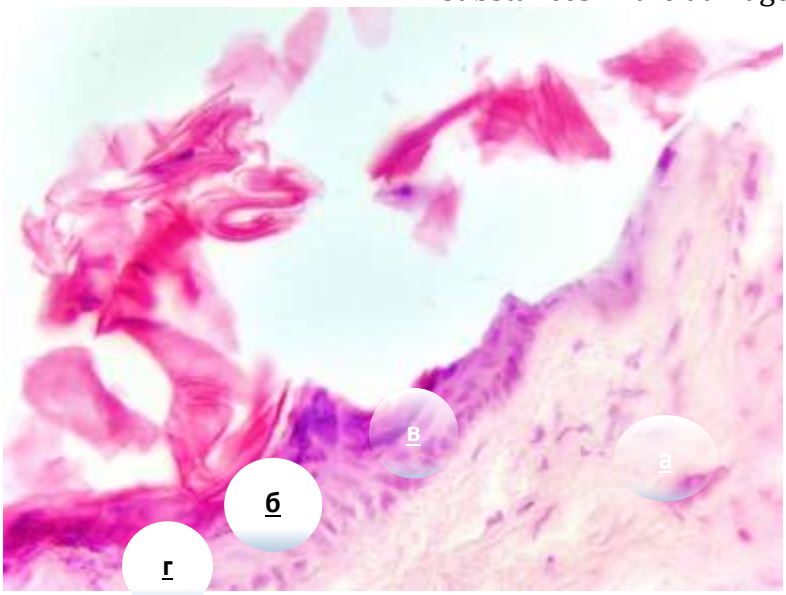

Figure 1. Group 1. - in the drug, rat skin in diabetes mellitus, on the 8th day of skin healing; a – area where inflammation and granulation tissue are formed; b - the regenerating boundary of the epidermis; v – basal layer; g - spinous layer. Paint G-E.10x4.

The morphological basis of these changes continued as follows: the appearance of a special membranoid structure in the branches of the epidermis and dermis that are damaged up to the basal layer, the wound surface is protected from air and other influencing factors, and the damaged vessels and lymph nodes are cut off from the environment, medium-level unevenness in small-caliber and capillary vessels signs of fullness, slow formation of interstitial tumors in the stroma are detected. At the same time, the low response to the stress process in the rats of the low emotional group is also explained by the fact that the concentration of glucocorticoids in the blood of the rats did not increase sharply and the apoptosis process in immunocompetent cells was slow.

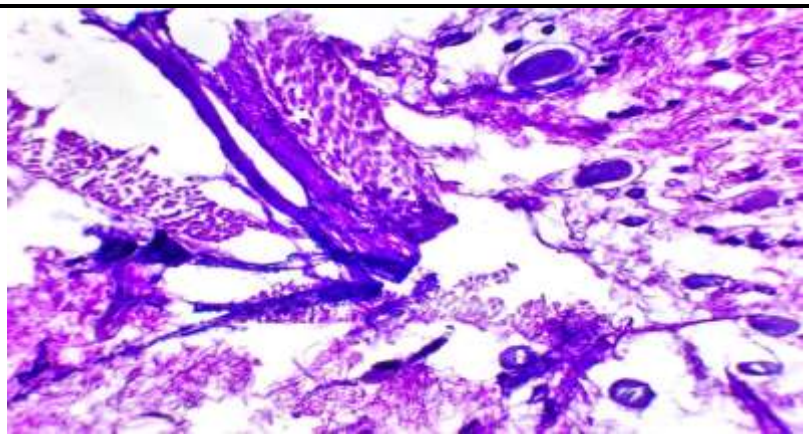

Figure 2. Group 1. In the drug, Schiff-positive structures are detected around the hair shaft and hair follicle in dark blue in the epidermis and dermis of white rat skin tissue. Paint Altsian blue. The size is 20x10.

This, in turn, is explained by the increase in reparative regeneration due to the large number of infiltration of monocytes, lymphocytes and plasma cells around the wounds caused in low emotional rats and the membranoid layer acting as a barrier under experimental conditions. The sharp reduction of necrotic processes in the subcutaneous and superficial muscle layers is based on the increase of multifocal reepithelialization foci around wound defects as a result of the barrier function of the membranoid layer formed on the wound surface and the stimulating effect of the reparation process.

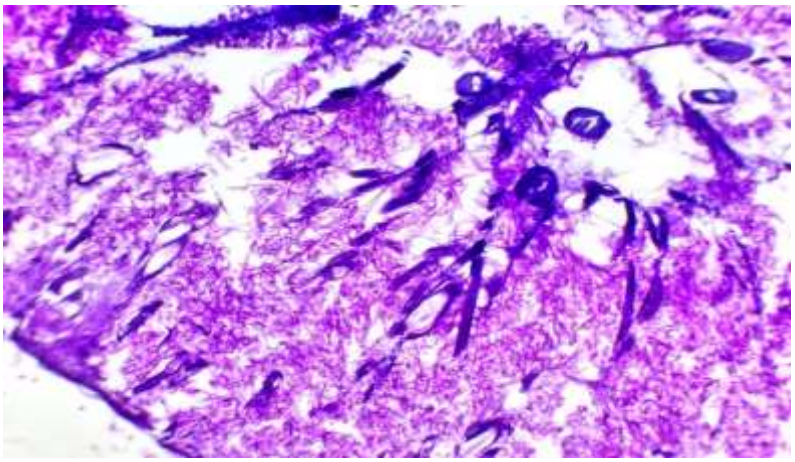

Figure 3. Group 1. In the drug, Schiff-positive structures are detected in the epidermis and dermis of white rat skin tissue around the hair axis and around the hair follicle in dark blue color. Paint Altsian blue. The size is 20x10.

In the given micrographs, as a result of the formation of a small number of inflammatory infiltrates in the isolated foci of wounds and damaged areas, local response reactions of mesenchymal cells: the formation of foci of active proliferation of fibroblasts, the synthesis of a large amount of sparse fibrous structures, the appearance of a narrow boundary of the demarcation line, the few and uneven filling of vessels, hematogenous It is explained by the low occurrence of cell migration in the damaged areas and the small number of interstitial tumors. These changes indicate the dominance of humoral local response reactions in the damaged area and formation of a membranoid layer on the surface of the wound of the gauze composed of 50% fibroin and 50% cotton fiber used in the experimental conditions, and the acceleration of the reparative regeneration process means that the treatment efficiency is high from a clinical and morphological point of view.

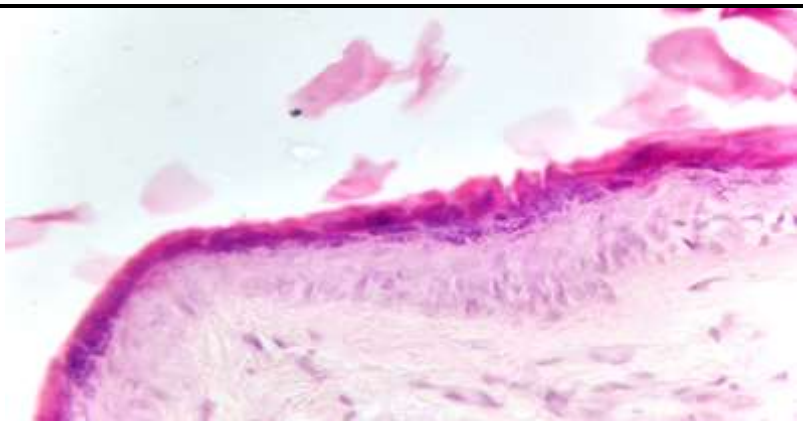

Figure 4. Group 1. 8th day. The texture of the subcutaneous adipose tissue is different, and now forming connective tissue foci are identified. In this layer, the swelling developed around the connective tissue fibers is detected in parallel. Paint G-E.10x4.

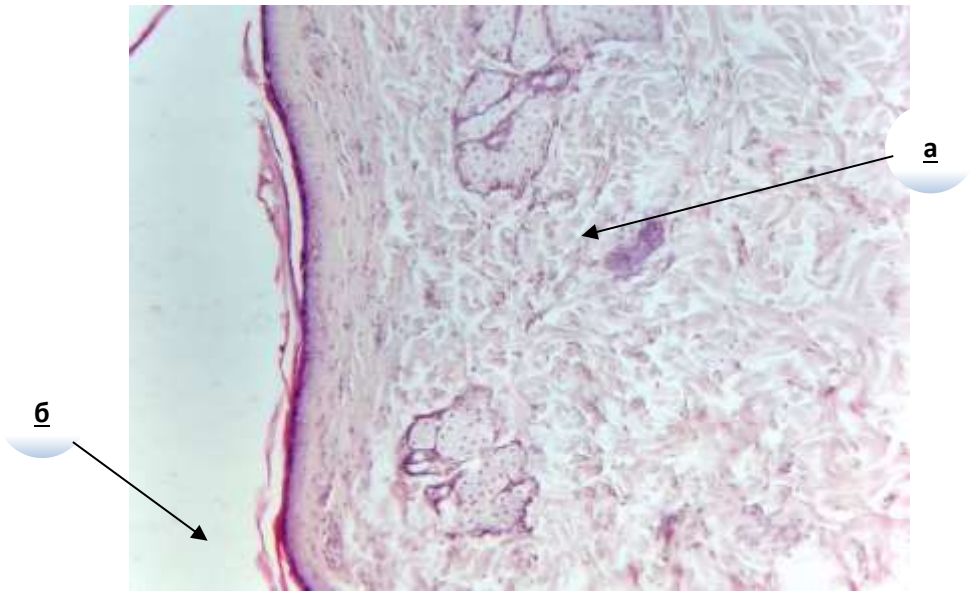

Figure 5. Group 1. The skin of white rats called QD (diabetes mellitus) through Alloxan in the preparation. Post-wound appearance, the epidermal part of the skin is characterized by granulation tissue infiltrated with neutrophils, macrophages and lymphocytes (a). Due to the large number of cells, the regenerating edge of the epithelium is thickened (b). Paint G-E.10x10

2nd group: 50% fibroin + 50% cotton fiber in highly sensitive animals (experiment)

In the highly susceptible group of rats, stress factors in the neuroendocrine system and diabetes mellitus, the occurrence of cortico-hypothalamo-pituitary disintegration, the hyperproduction of catecholamines and glucocorticoids in the blood plasma, lead to massive apoptosis of all types of immunocompetent cells, T and V lymphocytes in the MALT and SALT system. Causes hypermetabolism in cells and disruption of the reparative regeneration process. At the same time, as a result of dehydration and drying of mucous membranes, a sharp decrease in the moist environment necessary for an active cellular and humoral immune system on the skin and damaged skin surfaces, a strong development of the alteration process, deep dystrophy in damaged areas and massive death of cells in necrobiosis, expansion of the demarcation border are determined.

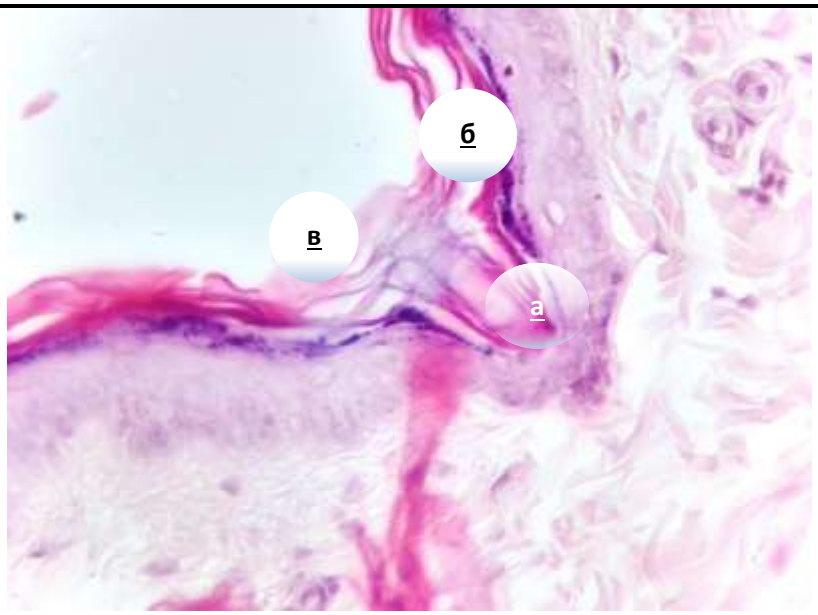

Figure 1. Group 2. Preparations made from rat skin, skin of diabetic rats; After dressing with gauze based on Bombyx mori fibroin, day 2; a – basal layer; b – spinous layer; v – connective tissue scar. Paint G-E.10x10.

As a result, the massive degranulation of fat cells in this area and the exudative inflammatory process continue sharply, the response to secondary opportunistic infectious agents from the outside continues, and the inflammatory landscape in the damaged area worsens. In this experiment, 2 groups of rats suffer from secondary alteration due to alloxan-induced diabetes and stress factors that cause high emotional arousal.

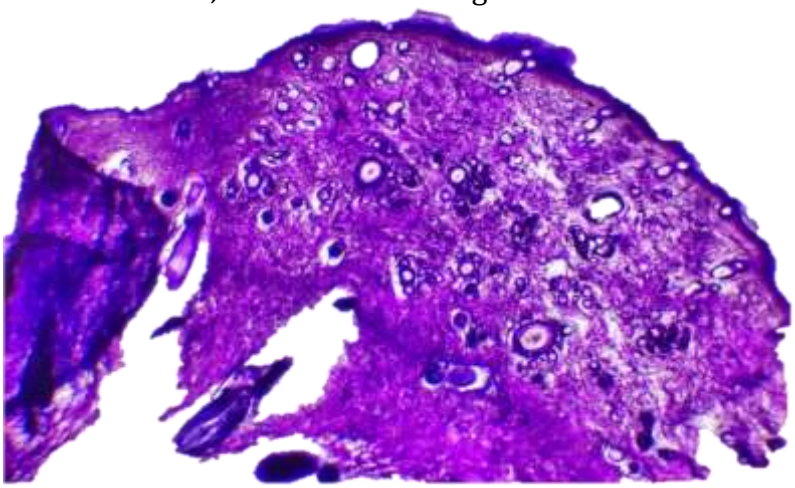

Figure 2. Group 2. Rat skin tissue. The histioarchitectonics of the dermis is unchanged, hematoma foci are not detected between the hypodermis and muscle fascia. A small number of foci of schiff formations are identified. Schiff structures are identified at the perimeter of hair shafts and around the follicle. The size is 10x10. Paint Altsian blue.

Bombyx mori, which was used for the treatment of the damaged area of the experimental rats of group 2, was found to form a membranoid layer of the gauze composed of 50% fibroin and 50% cotton fiber. However, the uneven filling of the blood vessels in the damaged area, the development of tumors in the perivascular areas, the occurrence of many fibrinoid necrosis in the stroma, the expansion of the boundary of the demarcation line, the occurrence of many neutrophil infiltrations in the damaged areas of the epidermis and the basal layers of the dermis, the presence of a large number of degranulated fat cells around the inflammatory exudate. , the different distribution of fibroblast proliferation foci, it is determined that the majority of hyperregenerative foci around the wound are composed of fibroblasts. These changes indicate

that the response of the above neuroendocrine system has continued to be seen, and most often, T and V lymphocytes are less in the damaged area, plasma cells are 3-5 in the 200x field of view, interstitial swelling in the stroma of the damaged area, thickening of fibrous structures, defragmentation and increased fibroid structures are determined.

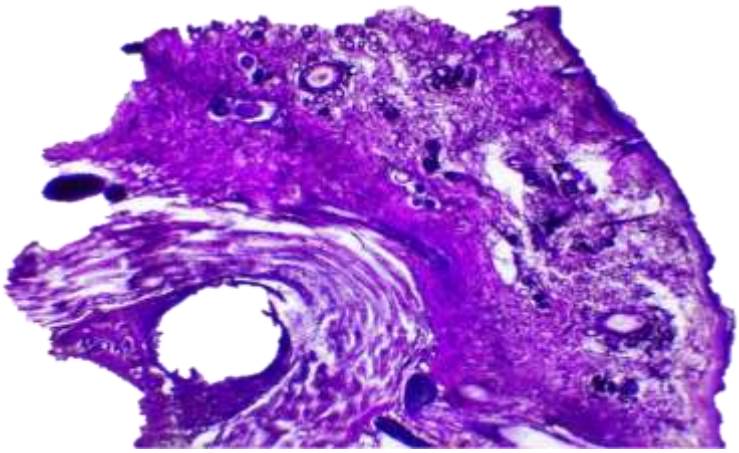

Figure 3. Group 2. Rat skin tissue. In the dermis, Schiff-positive structures with a homogeneous foci are identified without histioarchitectonic changes in the hypodermis. Under the hypodermis, cellulose fragments and weakly formed Schiff positive structures are detected. The size is 20x10. Paint Altsian blue.

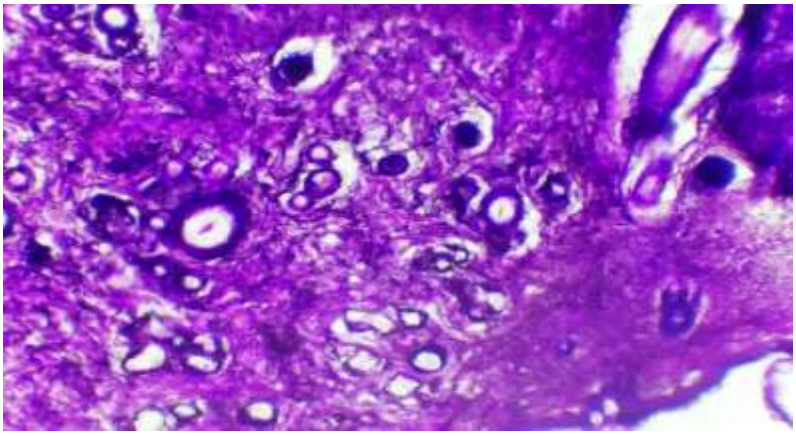

Figure 4. Group 2. Rat skin tissue. The histioarchitectonics of the dermis is unchanged, hematoma foci are not detected. The size is 10x10. Paint Altsian blue.

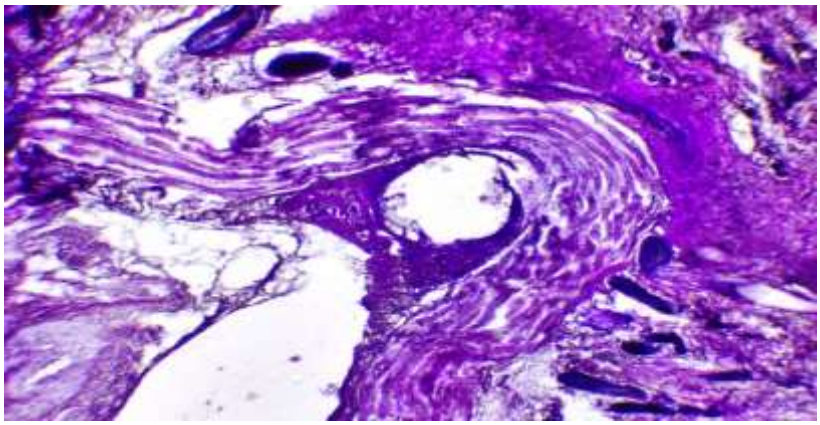

Figure 5. Group 2. Beneath the rat skin tissue, arc-shaped cellulose cell fragments are identified. The size is 10x10. Paint Altsian blue.

In the intermediate and damaged areas, the increase of many acidic mucopolysaccharides, the process of cytolysis, and the disorganization of fibrous structures were visible. It is determined that these

structures are developed further away from the areas where the membranoid layer is formed by Bombyx mori gauze, which is composed of 50% fibroin and 50% cotton fibers. This morphological substrate means that the effect of gauze composed mainly of Bombyx mori 50% fibroin and 50% cotton fiber is positive on the direct contact surfaces, but on the non-contact surfaces, the damage and inflammation processes develop sharply.

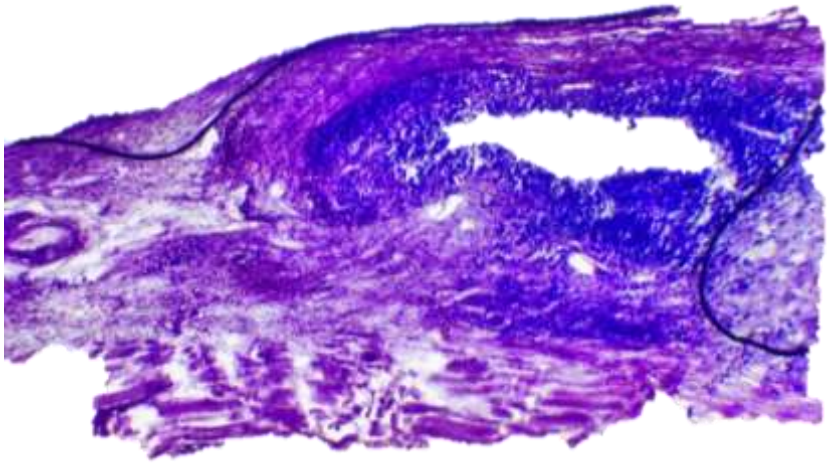

Figure 6. Group 2. Rat skin tissue. Under the hypodermis, Schiff positive is detected in dark blue. Residues of hemostatic fluff are detected. The size is 20x10. Paint Altsian blue.

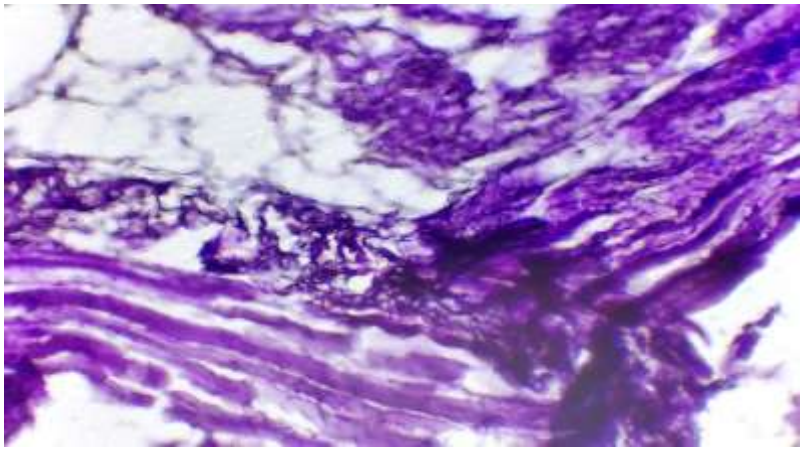

Figure 7. Group 2. Hemostatic fluff fragments and Schiff-positive structures are detected under the rat skin. Hematoma foci are not identified. The size is 40x10. Paint Altsian blue.

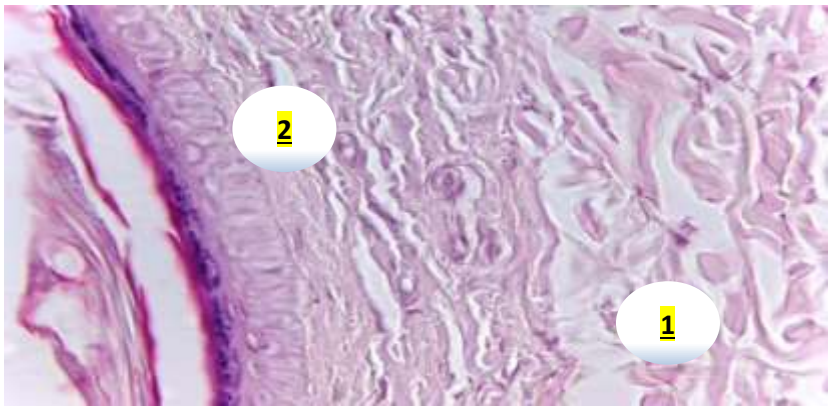

Figure 8. Group 2. The skin of white rats called QD (diabetes mellitus) through Alloxan in the preparation. Beneath the dermis, varying degrees of uneven interstitial swelling (1), foci of coagulation necrosis around the subcutaneous muscle layer, and defragmented muscle bundles are detected (2). Paint G-E.10x10

Summary:

1.Gauze made of 50% fibroin and 50% cotton fibers, used to stimulate regeneration of wounds in low emotional rats in group 1, Bombyx mori accelerates the regeneration process. Regeneration of hair and wool roots lost in the skin, increased recapillarization and angiogenesis processes in the vessels around the base of hair follicles were found.

2.The use of Bombyx mori 50% fibroin and 50% cotton fiber gauze used in the treatment to form a membranoid layer in the damaged area proves the high efficiency of the treatment and the rapid development of the reparative regeneration process based on the data obtained from the micrographs.

3.It is determined that the therapeutic efficiency of Bombyx mori 50% fibroin and 50% cotton fiber used in the treatment of trophic wounds in highly emotionally affected experimental rats in group 2 continued to be relatively positive.

4."Bombyx mori" fibroin bandage fluff has been studied to prove its clinical effectiveness, based on morphological tests, it allows to use it in medical practice with a high positive effect on the healing of trophic wounds (of various genesis: damaged skin covering, burn disease, infectious, etc.).

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