ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# The Efficacy of the Drug "Angiogen" in the Treatment of Wounds in Rats.

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**ABSTRACT**

The purpose of the research was to assess the effect of the drug "Angiogen" on fast and complete repair of microcirculation and soft tissue injuries. In the experiment, white rats were used, which were wounded with a linear incision in the skin and subcutaneous tissue. Sterile physiological sodium chloride solution was administered subcutaneously to the rats in the control group once a day for 6 days. Angiogen was administered to the rats in the experimental group according to the identical scheme. The studies have shown that the drug "Angiogen" accelerates the healing time, prevents from the development of inflammatory cell infiltration and destruction of tissues. The effect is caused by improved blood supply due to an increase in the number of newly formed vessels in the area of the injury.

**Keywords:** Wound, rat, angiogen.

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ISSN: 0975-8585

## INTRODUCTION

At present, much attention is paid to the development of the medicines that could optimize the processes of reparative regeneration acting directly on the lesion without adversely affecting the organism as a whole [1,2,3]. Treatment of wounds involves the elimination of the consequences that have developed as a result of the injury to cells and tissues. So far, the efforts of pharmacologists and surgeons have been aimed at eliminating these effects rather than complications of the wound process [4,5]. This fact caused the development of the new drug "Angiogen" and conducting the studies to assess its effect in preclinical trials. The purpose of the research was to assess the effect of the drug "Angiogen" on fast and complete repair of microcirculation and soft tissue injuries.

## MATERIALS AND METHODS

In the experiment, 18 nonlinear white male rats were used with a live weight of 300-350 g. Animal surgery was performed in a state of sedation caused by intramuscular injection of a 2% solution of xylazine hydrochloride (Xilanit, LLC"Nita-Pharm", Russia) at a dose of 0.2 ml/100g of live weight. Local anesthesia was not performed. Observing the rules of aseptic and antiseptic, the wound process was performed by layer-by-layer incision of skin and subcutaneous tissue (4.5-5 cm long) in the lumbo sacral area in all the rats. The wound was sewed up with interrupted sutures (4-5) with polyacrylamide fibres No.0.

The animals were divided into 2 groups. The animals in the control group (n = 9) were injected subcutaneously with sterile 0.9% physiological sodium chloride solution once a day for 6 days at a dose of 0.1 ml/100 g of live weight into two points at a distance of 1 cm from the edge in the proximal and distal parts of the wound immediately after the surgery. The experimental group (n = 9) received the drug "Angiogen" subcutaneously at a dose of 0.1ml/100g of live weight according to the identical scheme. In both groups, external treatment of wounds was not performed.

To assess the effect of the drug "Angiogen" on regeneration processes, clinical and morphological methods of research were used. During the clinical study, the general state of the operated animals was examined, as well as the signs of inflammation, the rate and degree of epithelialization of the wound on $3^{d}$, $5^{th}$, $7^{th}$ and $10^{th}$ days.

For the morphological studies, 3 animals from each group (n=6) were withdrawn from the experiment on $3^{d}$, $7^{th}$ and $10^{th}$ days. Samples of skin, subcutaneous tissue and muscles were taken from three places - the proximal and distal edges and the middle part of the wound. The samples were taken perpendicularly to the wound line with the intact skin areas. Preparation of tissue specimen was carried out according to a standard procedure. The resulting sections were stained with hematoxylin and eosin according to Van Gieson [6,7].

When studying the obtained samples, the area of blood vessels was determined as a percentage from the total area of the histologic section. At least 20-40 visual fields were examined on each sample. The calculation was carried out using a morphometric grid [8,9,10].

The statistical processing of the data was carried out using the SPSS v.13 package. The Student's t-test was used.

## RESULTS AND DISCUSSION

The studies have shown that the postoperative period in the animals of both groups was without complications. Operative wounds, in most cases, healed by primary intention. In 3 rats of the control group, the wounds healed by secondary intention because of the diastase of the edges of the wound that occupied 20% to 50% of the length (Table 1). In the experimental group, there was no allergic reaction to the drug "Angiogen".

ISSN: 0975-8585

**Table 1: Dynamics of operative wound healing**

| Group of animals | The length of operative wound | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | After operation | | 3$^{d}$ day | | 5$^{th}$day | | 7$^{th}$day | |
| | cm | % | cm | % | cm | % | cm | % |
| control | 4.78 ±0.08 | 100 | 4.67 ±0.23 | 97.10 ±4.90 | 3.83 ±0.27 | 80.20 ±5.80 | 2.50 ±0.31 | 52.30 ±6.60 |
| experimental | 4.94 ±0.15 | 100 | 4.80 ±0.23 | 97.00 3±3.30 | 3.83 ±0.42 | 77.50 ±8.50 | 1.40 ±0.63 | 28.70 ±12.90 |

Epithelialization of the operative wounds occurred faster in the animals of the experimental group. In 50% of the animals, the wounds were completely covered with epithelium on 7th day, whereas the wounds in the control rats were 2-4 cm on that day, and epithelialization of the operative wounds was not completed. Complete closure of the wounds with epithelium occurred on 10th day in the control group and on 8th day – in the experimental group. When studying tissue specimen, it was found that the wound healing in the middle part differed slightly from that in the proximal and distal sections, whereas in the latter it was almost identical, which allowed us to combine them into one group ("Edges of the wound").

In the control group of animals, leukocyte infiltration was partially replaced by lymphocyte and macrophage infiltration in the middle part of the wound, strands of fibroblasts and slit-like cavities appeared, some of which were lined with endothelium on the third day. Vessels of a sinusoidal type were formed, which, unlike the granulation tissue, had various sizes without a vertical orientation (Figure 1).

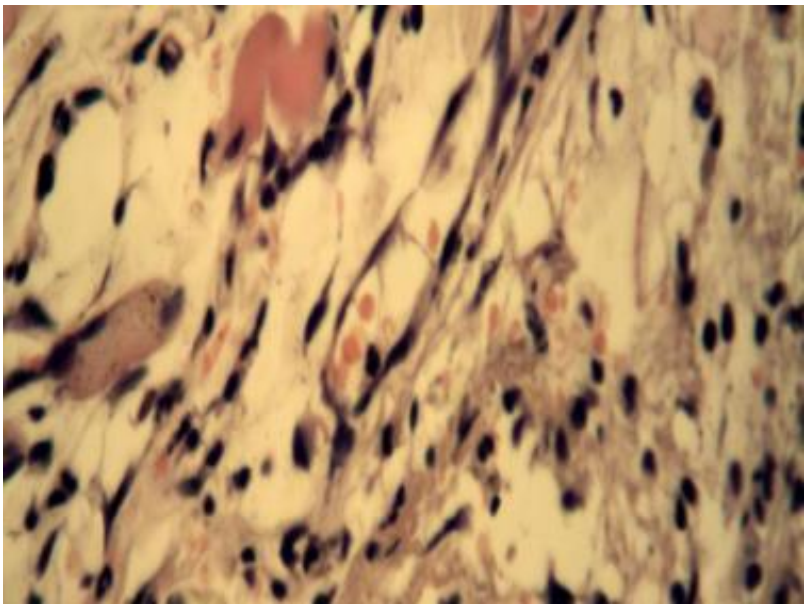

**Fig 1: Control group on3$^{d}$ day. Middle part of the wound. Strands of fibroblasts and slit-like cavities lined with endothelium. Stained with hematoxylin and eosin.x400.**

Unlike the middle part, the epithelialization at the edges of the wound occurred on that day due to healthy tissues adjacent to the wound. Epithelium, located at the edges of the wound, lost its differentiation, its cells were shifted toward the wound. There was “crawling” of the epithelium on the leukocyte necrotic layer or on the granulation tissue if it presents (Figure 2).

ISSN: 0975-8585

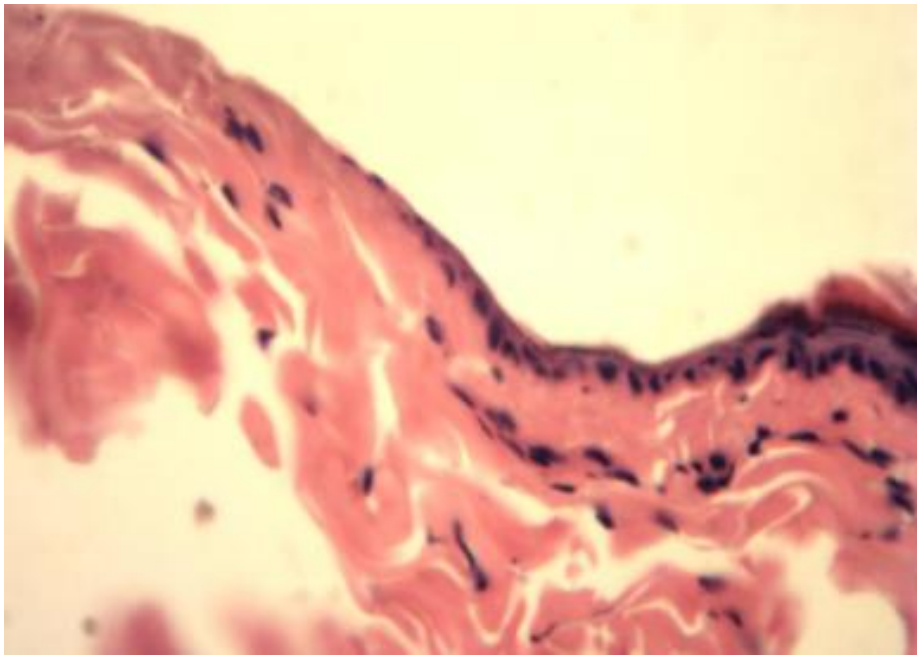

**Fig 2: Control group on 3$^{d}$day. Edges of the wound."Crawling" of the epithelium onto the wound surface. Stained with hematoxylin and eosin.x400.**

In addition, other changes were found in the nearby areas. So, there was a vascular congestion and vasodilation, edema, sometimes with sloughing, neutrophilic or lymphohisteocytic infiltration. Complications, which are characteristic of the wound healing in the middle part, as a rule, were absent.

It should be noted that the primary tension in this group was not always performed according to the classical scheme. Sometimes it manifested itself in the formation of small areas of granulation tissue with vertical vascular loops. Severe complications occurred rarer: pronounced neutrophilic infiltration of the wound, leukocyte necrotic masses with tissue destruction. At the same time, inflammatory cell infiltration and necrotic changes were also observed in the subjacent soft tissues. In one animal from the group, extensive necrosis of the edges of the wound was found, and there were no signs of epithelialization.

On the seventh day, dead tissue elements dissipated, the lymphocyte and macrophage infiltration was absent or was negligible. The cavity of the wound was filled with a young connective tissue. Vascular slits and vessels of sinusoidal type, formed earlier, were lined with endothelium, some of them contained erythrocytes. Most of them were transformed into veins and arteries, and the smaller part was transformed into capillaries (Figure 3).

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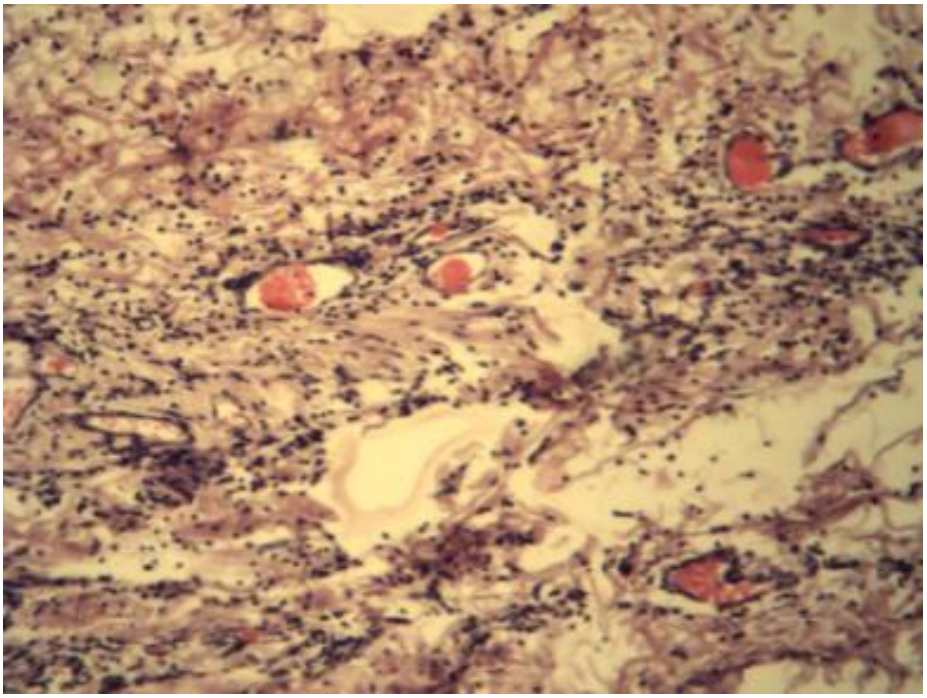

**Fig 3: Control group on 7th day. Middle part of the wound. Formed arteries, veins and capillaries. Stained with hematoxylin and eosin.x200.**

The wound was covered with one layer of epithelial cells. In most animals, the epithelium was flat, multilayer with differentiated cells, but with fuzzy layers. The process of keratinization was not observed. In some cases, small areas of granulation tissue and lymphohisteocytic infiltrates were seen in the wound, which were found both in the dermis and in the subjacent soft tissues.

On the same day, the pattern at the edges of the wound was similar to that in the middle part of the wound, but with more pronounced epithelialization. The formed epithelium, in most cases, was multilayer with signs of differentiation and well-defined layers. The wound completely healed on 10thday. Formed connective tissue filled the whole wound including the dermis and subjacent tissues (Figure 4).

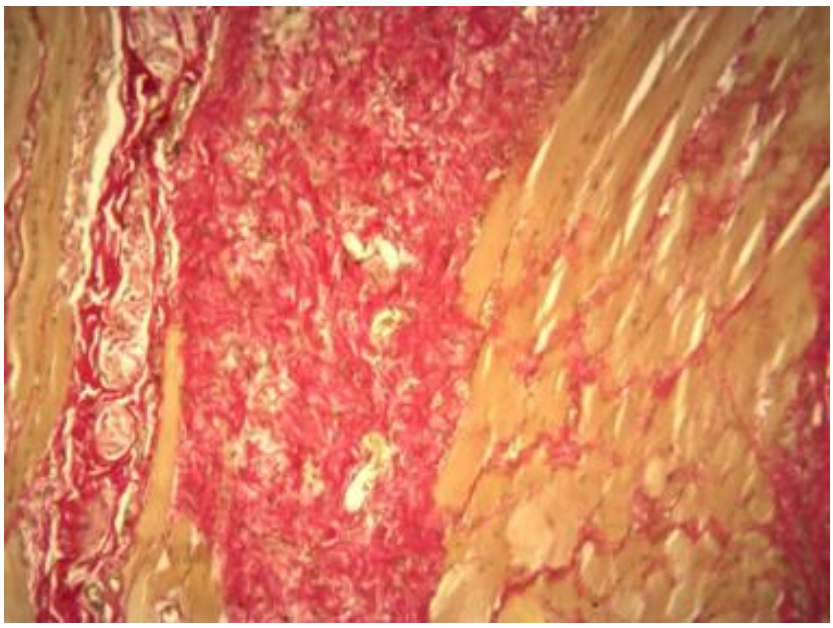

**Fig 4: Control group on 10thday.Middle part of the wound. Connective tissue that fills the operative wound. Van Gieson stain. x200.**

ISSN: 0975-8585

Multilayer flat keratinized epithelium with well-defined layers and differentiated cells covered the whole surface. Hair follicles in the area of the wound were not restored on that day.

Along the edges, there was complete wound healing with epithelialization, connective tissue formation along the entire length of the wound and skin regeneration. Hair follicles were found only in the skin of nearby areas (Figure 5).

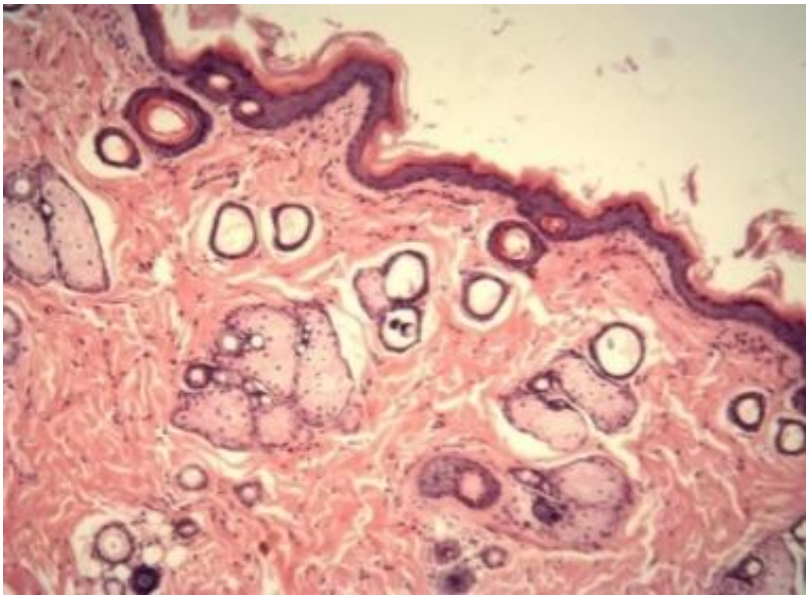

**Fig 5: Control group on 10$^{th}$ day. Edges of the wound. Normal skin of the area adjacent to the wound. Stained with hematoxylin and eosin.x200.**

On the third day, the middle part of the wound in the experimental group was healing in the same way as in the control group, but there were some peculiarities. Thus, lymphocyte and macrophage infiltration almost completely replaced neutrophil infiltration. In the fibers of connective tissue, far more new vessels were formed (Figure 6).

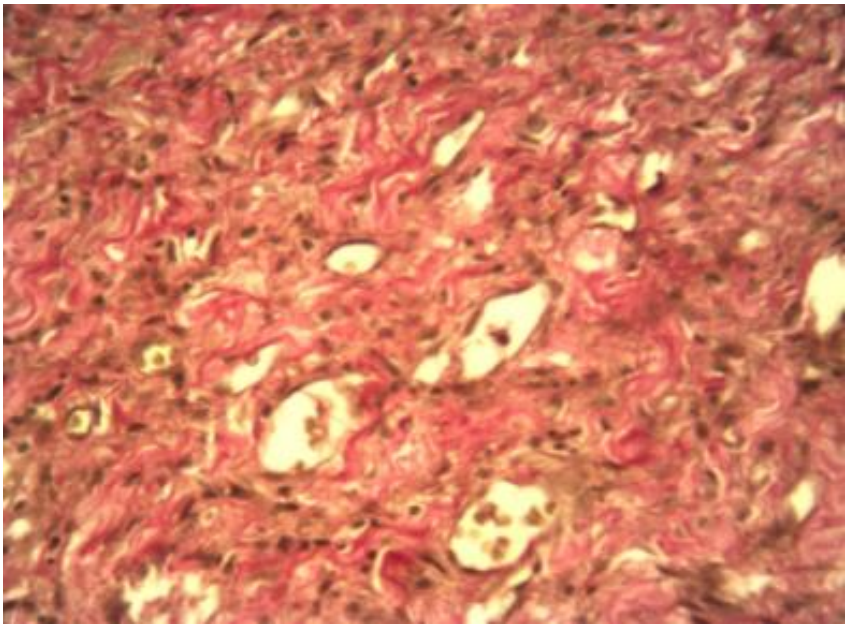

**Fig 6: Experimental group on 3$^{d}$ day. Middle part of the wound. Newly formed vessels in connective tissue. Van Gieson stain. x400.**

ISSN: 0975-8585

In addition, there were no necrotic changes and inflammatory reaction in the subjacent tissues, epithelialization was not observed.

On the seventh day, there was no inflammatory cell infiltration in the wound filled with a young connective tissue and many blood vessels filled with erythrocytes were found there, unlike the control group. Those vessels were partially transformed into arteries and veins which occupied an area larger than in the control group. In the epithelia, all the layers with differentiated cells were more clearly defined, but without any signs of keratinization (Figure 7).

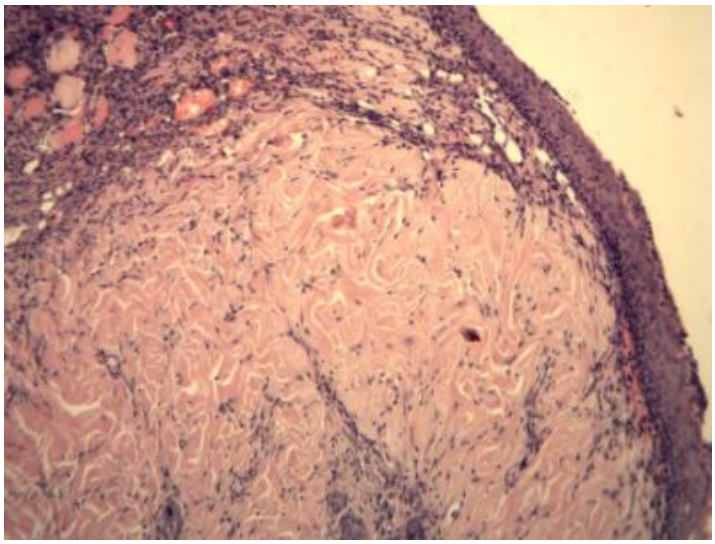

**Fig 7: Experimental group on 7$^{th}$ day. Middle part of the wound. Epithelium without signs of keratinization with well-defined layers and differentiated cells. Stained with hematoxylin and eosin.x200.**

On the tenth day, the histologic pattern in the middle part of the wound was identical to that in the control group – the skin was completely restored except for the appendages, but the lympho histeocytic infiltration of the subepithelial area was absent or insignificant (Fig. 8).

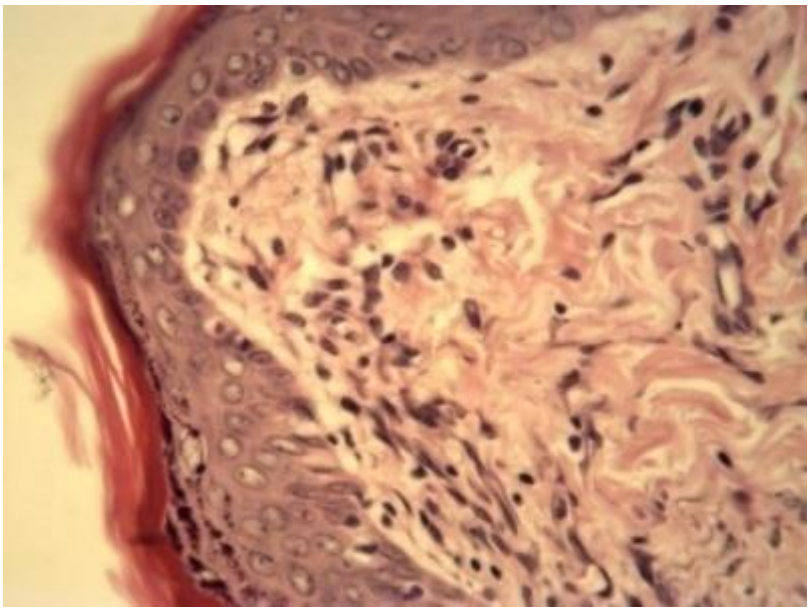

**Fig 8: Experimental group on 10$^{th}$ day. Middle part of the wound. Small lymphohisteocytic infiltration of the sub-epithelial area. Stained with hematoxylin and eosin.x400.**

On the third day, there was no edema in the tissues located near the wound, and the histologic pattern of the skin there was standard (Figure 9).

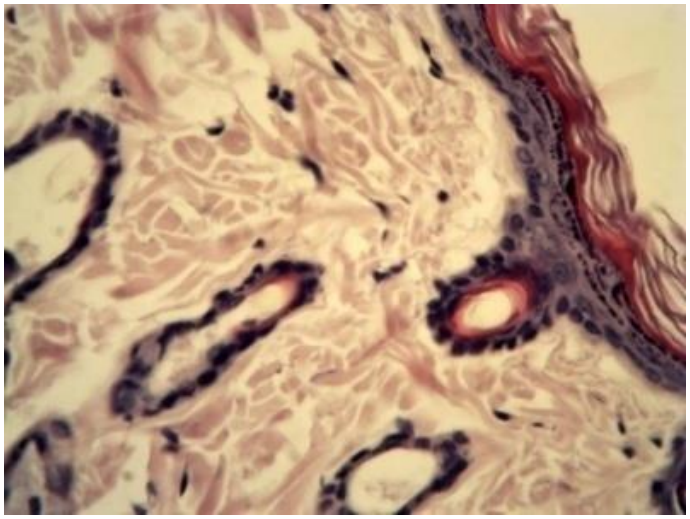

**Fig 9: Experimental group on 3$^{d}$ day. Edges of the wound. Normal histologic pattern of the skin in nearby wound areas. Stained with hematoxylin and eosin.x400.**

In addition, there was significant vasodilation and vascular congestion in the dermis, as well as in subjacent tissues. Epithelialization was similar to that in the control group.

In comparison with the control group, the microvasculature reaction was observed at the edges of the wound on the seventh day. Thus, against the background of epithelialization and the formation of a connective tissue in the wound slit, a large number of formed venous and arterial vessels both newly formed and penetrating into the wound from the side of nearby healthy tissues were observed. On the tenth day, there was no difference with the control group.

When assessing the morphometric parameters of the area of blood vessels along the edges of the wound, it was found that angiogenesis had a similar dynamics in both groups.

An increase in the total area of blood vessels (by about 30%) was noted from 3$^{d}$ to 7$^{th}$ days, and by10$^{th}$ day– a significant decrease (Table 2).

**Table 2: Dynamics of the area of blood vessels (%)**

| | Middle part of the wound | | Edges of the wound | |
|---|---|---|---|---|
| | Control group | Experimental group | Control group | Experimental group |
| 3$^{d}$ day | 5.47±0.41 | 6.09±0.39 | 7.30±0.40 | 8.55±0.64 |
| 7$^{th}$day | 7.19±0.45 | 10.53±1.01 | 9.68±0.73 | 11.73±1.20 |
| 10$^{th}$ day | 2.83±0.29 | 3.57±0.22 | 3.08±0.53 | 4.04±0.22 |

However, the total area of the vessels in the experimental samples was more than in the control ones throughout the experiment, but the results were not reliable.

When making an intra group comparison, a significant increase in the area of vessels was observed on 7$^{th}$ day of the experiment in relation to 3$^{d}$ day in both groups (in the control group p = 0.011, in the experimental group p = 0.010). The reliable dynamics of decrease in angiogenesis activity on 10$^{th}$day was also identical in both groups in relation to7$^{th}$ day (p = 0.001).

On 7th day of the experiment, the area of the vessels in the middle part of the wound in the experimental group was significantly higher than in the control group (p = 0.013). When making an intragroup comparison, the dynamics was the same as when examining the edges of the wound.

Thus, the increase in the area of blood vessels grew mainly due to an increase in their number and vascular congestion which was well expressed in the animals of the experimental group.

## CONCLUSIONS

1) The drug "Angiogen" activates microcirculation in surrounding tissues, improves blood supply, stimulates the formation of new vessels.
2) Enhanced vascularization reduces the alterative processes in the area of damage and the formation of inflammatory cell infiltration, due to this the process of formation of connective tissue is activated, epithelialization is stimulated and the time of wound healing is shortened.

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