Open Access Maced J Med Sci **electronic publication ahead of print,**
published on March 27, 2019 as https://doi.org/10.3889/oamjms.2019.220

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences.
https://doi.org/10.3889/oamjms.2019.220
eISSN: 1857-9655
Basic Science

The Study of the Wound Healing Activity of the Gel with a Comprehensive Therapeutic Effect

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Citation: Brkich LL, Nedorubov AA, Pyatigorskaya NV, Brkich GE, Odintsova ES. The Study of the Wound Healing Activity of the Gel with a Comprehensive Therapeutic Effect. Open Access Maced J Med Sci. https://doi.org/10.3889/oamjms.2019.220

Keywords: Chitosan-miramistin-hemopexin complex; Development of wound healing drugs; Models of linear and planar wounds; Wound treatment gel

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Received: 16-Jan-2019; **Revised:** 17-Mar-2019; **Accepted:** 18-Mar-2019; **Online first:** 27-Mar-2019

Funding: This research was supported by the "Russian Academic Excellence Project 5-100

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: The study was aimed at researching the specific wound healing activity of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origins on a model of a planar infected wound.

METHODS: The model of septic wounds in rats as per the method of P.I. Tolstykh was used during the study of the specific activity of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers for the treatment of infected wounds. The infection was performed with the *S. aureus* and *E. coli* strains. The study lasted 18 days, and during this period no full scarring occurred. The wound diameter was chosen as the effectiveness criterion. The planimetric method was used to assess the course of the wound process in experimental animals.

RESULTS: The obtained data prove the specific action of the drug with a comprehensive therapeutic action based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origins. The study has shown that bacterially infected wounds healed worse than noninfected ones. Both types of wounds — infected and uninfected ones — healed faster when applying the test drug or Levomekol ointment.

CONCLUSION: On the model of a planar infected wound, the developed drug with a comprehensive therapeutic action has shown better wound healing effect compared with the Levomekol reference drug.

Introduction

The drug with a comprehensive therapeutic action based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origin is a translucent gel based on hydroxypropyl cellulose and polyacrylamide, with the inclusion of chitosan, hemopexin, miramistin and lidocaine hydrochloride as pharmaceutical agents (Brkich, Pyatigorskaya, 2017) [1].

The drug for the treatment of infected wounds has four types of pharmacological effects, namely necrolytic, antimicrobial, wound healing and anaesthetic ones.

The necrolytic effect is ensured by hemopexin, providing a lysing effect on septic substrates and cleansing the wound surface from purple-necrotic masses. When applied to tissue, hemopexin splits fibrinous formations and necrotic tissue structures, and blood clots. It also thins exudate and vicious secret. Himopsin restores microcirculation in the wound walls, improves metabolic processes, which is clinically manifested by a decrease in local inflammation.

Chitosan is a carrier of the enzyme complex. It provides a prolonged therapeutic effect of himopsin and has a wound healing effect.

The antimicrobial action is ensured by the

presence of Miramistin antiseptic (benzyl dimethyl [3-(myristoylamino) propyl] ammonium chloride monohydrate). The compound has a pronounced bactericidal action against aerobic and anaerobic bacteria, gram-positive (Staphylococcus, Streptococcus, Bacillus subtilis, Bacillus anthracoides) and gram-negative organisms (Shigella, Pseudomonas aeruginosa, Escherichia coli, Salmonella), as well as against hospital strains multiresistant to antibiotics. Miramistin provides antimicrobial and fungicidal action, enhances the functional activity of immune cells, while stimulating local (nonspecific) immunity, accelerates the wound healing process, reduces the resistance of pathogenic microorganisms to antibiotic therapy, and activates protective reactions at the application site through the activation of the absorbing and digestive functions of phagocytes.

The analgesic effect is due to the presence of lidocaine anaesthetic, which has a local anaesthetic effect, blocks potential-dependent sodium channels, thereby preventing the generation of impulses in the endings of sensory nerves and conduction of impulses along nerve fibres. The anaesthetic action of lidocaine is 2 – 6 times stronger than that of novocaine and procaine. When applied locally, it dilates blood vessels and has no irritating local action.

The study was aimed at researching the specific wound healing activity of the drug with a comprehensive therapeutic effect on a model of a planar infected wound.

Material and Methods

The drug is a gel for external use, containing per 100 g: himopsin – 0.2 g, miramistin – 0.05 g, chitosan – 2 g, lidocaine – 0.1 g, polyacrylamide – 0.1 g, hydroxypropylmethylcellulose – 2 g, glycerin – 5.0 g, and water – up to 100 g.

A group of 45 male rats of the Wistar line, weighing 200 – 240 g, (Stolbovaya branch of the FSBUN NCBMT FMBA of Russia) were used in the study.

The bacterial strains of the American Type Culture Collection (ATCC) obtained from the ACM (All-Russian Collection of Microorganisms) of Moscow were used in the experiment. The species composition was represented by the following microorganisms: *Escherichia coli* (ATCC 25922); *Staphylococcus aureus* (ATCC 6538-R).

The antibacterial effect of the studied objects was studied by the "wells" method. Bacteria were cultivated in L-broth at 37°C for 20 hours. Then, the number of cells per 1 ml of the initial suspension was determined using the Koch method. For further research, dilutions were used, providing the medium contamination on a Petri dish of 10^4 and 10^6 CFU/ml, which corresponded to the initial dissemination of the wound.

From the obtained dilutions, previously thoroughly mixed, bacteria were sown on the surface of the agar plate in a Petri dish with a sterile pipette in the amount of 0.1 ml. The volume of the applied suspension was distributed over the surface of the medium with a sterile spatula.

The septic wound was modelled for the animals under anaesthesia under sterile conditions as per the P.I. Tolstykh method (1976) [2]. For this purpose, the skin with subcutaneous tissue 25 mm in diameter was dissected into on the antiseptic-treated back section shaved from wool. A gauze plug containing 1.2 x 10^9 microbial bodies of the *S. aureus* daily culture or 2.6 x 10^9 *E. coli* was introduced into the resulting wound, and the wound was sutured. On the following day (24 hours), after modelling, the abscess with all the characteristic signs of inflammation was formed in all animals. After the stitches' removal, the upper skin flap was removed, the gauze plug was removed, and the purulence was evacuated. The area of the original wound was determined by applying a contour to a transparent film.

Treatment of the wound with the test drug or Levomekol reference product was started 24 hours after the septic wound modelling. The drugs were applied for 18 days. During the treatment, the diameter of the wound was measured every three days. Planimetric method was used to assess the course of the wound process in experimental animals.

Results

The animals were divided into groups, in accordance with the doses of the injected substance (Table 1).

Table 1: Groups of animals

No.	Infection type	Drug	Number of animals	Drug dose, g/kg
1.	*S. aureus*	Gel, 0.2 g/200 g	3♂	1
2.	*E. coli*		3♂	
3.	*S. aureus*	Gel, 0.1 g/200 g	3♂	5
4.	*E. coli*		3♂	
5.	*S. aureus*	Levomekol, 0.2 g/200 g	3♂	1
6.	*E. coli*		3♂	
7.	*S. aureus*	Levomekol, 1 g/200 g	3♂	5
8.	*E. coli*		3♂	
9.	*S. aureus*	Without drug	3♂	-
10.	*E. coli*		3♂	
11.	Without infection	Gel, 0.2 g/200 g	3♂	1
12.		Gel, 1 g/200 g	3♂	5
13.		Levomekol, 0.2 g/200 g	3♂	1
14.		Levomekol, 1 g/200 g	3♂	5 g/kg
15.		Without drug	3♂	-

After modelling a septic wound, rats in all groups of the test drug, the Levomekol reference

drug, and the wounds without drugs were sluggish in the first two days of the study. The wound at the beginning of the study is presented in Figure 1.

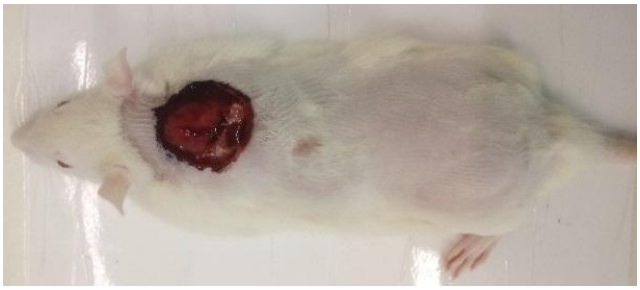

Figure 1: Wound at the beginning of the study

The wound diameter during the treatment was measured planimetrically every three days. The data are shown in Table 2.

Table 2: The result of measuring the size of the wound during the study

No.	Infection type	Drug	Wound size, cm						
			Days of infection						
			1	3	6	9	12	15	18
1.	*S. aureus*	Test drug, 0.2 g/200 g	2.7	2.5	2.1	2.0	1.2	1.0	0.5
			2.6	2.5	2.0	1.8	1.0	0.7	0.3
			2.5	2.3	2.1	1.9	1.4	1.1	0.7
		Average:	*2.6*	*2.4*	*2.06*	*1.9*	*1.2*	*0.93*	*0.5*
2.	*E. coli*	Test drug, 0.2 g/200 g	2.3	2.3	2.0	1.9	1.3	1.1	0.6
			2.5	2.5	2.3	2.2	1.2	1.0	0.5
			2.3	2.3	2.0	1.8	0.9	0.7	0.3
		Average:	*2.36*	*2.36*	*2.1*	*1.96*	*1.16*	*0.93*	*0.46*
3.	*S. aureus*	Test drug, 1 g/200 g	2.6	2.6	2.1	2	1.3	1	0.7
			2.6	2.5	2.1	1.8	1.1	1.1	0.3
			2.7	2.4	2	1.8	1.3	1	0.6
		Average:	*2.63*	*2.5*	*2.07*	*1.87*	*1.23*	*1.03*	*0.53*
4.	*E. coli*	Test drug	2.4	2.2	2	1.9	1.4	1	0.6
		1 g/200 g	2.4	2.4	2.2	2.1	1.2	1	0.6
			2.3	2.3	2.1	2	0.9	0.8	0.3
		Average:	*2.37*	*2.3*	*4.9*	*2.0*	*1.17*	*0.93*	*0.5*
5.	*S. aureus*	Levomekol, 0.2 g/200 g	2.8	2.6	2.4	2.0	1.4	1.1	0.9
			2.8	2.6	2.3	2.2	1.2	1.0	0.8
			2.3	2.2	2.1	2.0	1.3	1.1	0.7
		Average:	*2.63*	*2.5*	*2.27*	*2.07*	*1.3*	*1.07*	*0.8*
6.	*E. coli*	Levomekol, 0.2 g/200 g	2.5	2.3	2.3	2.0	1.6	1.2	1.0
			2.5	2.5	2.4	2.2	1.5	1.1	0.7
			2.6	2.5	2.4	2.2	1.4	1.1	0.6
		Average:	*2.53*	*2.43*	*5.57*	*2.13*	*1.5*	*1.13*	*0.77*
7.	*S. aureus*	Levomekol, 1 g/200 g	2.7	2.5	2.2	2.0	1.3	1.1	0.8
			2.6	2.6	2.4	2.1	1.3	1.1	0.7
			2.8	2.3	2.2	2.1	1.3	1.0	0.8
		Average:	*2.7*	*2.46*	*2.27*	*2.07*	*1.3*	*1.07*	*0.76*
8.	*E. coli*	Levomekol, 1 g/200 g	2.5	2.3	2.3	2.0	1.4	1.1	0.9
			2.6	2.4	2.4	2.1	1.5	1.1	0.8
			2.6	2.4	2.4	2.1	1.5	1.1	0.6
		Average:	*2.57*	*2.36*	*2.36*	*2.07*	*1.47*	*1.1*	*0.8*
9.	*S. aureus*	Without drugs	2.5	2.5	2.3	2.0	1.8	1.2	1.1
			2.6	2.5	2.4	2.3	2.0	1.6	1.2
			2.6	2.6	2.5	2.4	1.5	1.3	1.0
		Average:	*2.57*	*2.53*	*2.4*	*2.23*	*1.77*	*1.37*	*1.1*
10.	*E. coli*	Without drugs	2.6	2.5	2.5	2.2	1.8	1.6	1.2
			2.6	2.6	2.4	2.3	2	1.5	1
			2.7	2.8	2.5	2.3	1.7	1.5	1.1
		Average:	*2.63*	*2.63*	*2.47*	*2.27*	*1.83*	*1.53*	*1.1*
11.	Without infection	Test drug, 0.2 g/200 g	2.4	2.4	2.2	2.0	1.3	1.2	0.8
			2.7	2.6	2.2	2.0	1.0	0.9	0.6
			2.8	2.7	2.4	1.8	1.2	1.0	0.7
		Average:	*2.6*	*2.57*	*2.27*	*1.93*	*1.17*	*1.03*	*0.7*
12.		Test drug, 1 g/200 g	2.5	2.5	2.3	2.0	1.3	1.1	0.8
			2.7	2.6	2.3	2.0	1.3	1.0	0.7
			2.6	2.6	2.4	1.9	1.3	1.0	0.6
		Average:	*2.6*	*2.57*	*2.33*	*1.97*	*1.3*	*1.03*	*0.6*
13.		Levomekol, 0.2 g/200 g	2.6	2.6	2.4	1.8	1.4	1.2	1.1
			2.8	2.7	2.6	2.0	1.3	1.1	0.7
			2.7	2.7	2.2	1.4	1.2	1.0	0.6
		Average:	*2.7*	*2.67*	*2.4*	*1.73*	*1.3*	*1.1*	*0.8*
14.		Levomekol, 1 g/200 g	2.7	2.6	2.4	1.9	1.4	1.1	0.7
			2.7	2.6	2.4	1.9	1.4	1.1	0.9
			2.5	2.5	2.3	2.0	1.3	1.0	0.8
		Average:	*2.63*	*2.57*	*2.37*	*1.87*	*1.7*	*1.07*	*0.7*
15.		Without drugs	2.8	2.7	2.4	2.0	1.4	1.2	1.0
			2.7	2.7	2.6	2.3	1.8	1.5	1.2
			2.8	2.8	2.6	1.6	1.4	1.2	0.7
		Average:	*2.77*	*2.73*	*2.53*	*1.97*	*1.53*	*1.3*	*0.97*

Discussion

Levomekol was used as a reference drug. In the international practice, it has been known since the 70s as "Chloramphenicol + Methyluracil", and is a combined drug for local administration, having an anti-inflammatory effect. It is also active against gram-positive and gram-negative microbes (staphylococci, Pseudomonas bacilli and e-coli), penetrates tissues without damaging biological membranes and stimulates regeneration processes (Azuma, 2015; Dai, 2011) [3], [4]. Levomekol is widely used in surgical practice for any type of tissue damage, mainly infected ones, with mixed microflora in the first purulo-necrotic phase of the wound process (Brkich et al., 2018; Shipovskaia, Zudina, Fomina, 2015) [5], [6].

The model of septic wounds in rats as per the P.I. Tolstykh method was used during the study of the specific activity of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers for the treatment of infected wounds (Tolstykh et al., 2013) [2]. The infection was performed with the *S. aureus* and *E. coli strains.* The study lasted 18 days, and during this period no full scarring occurred. The wound diameter was chosen as the effectiveness criterion.

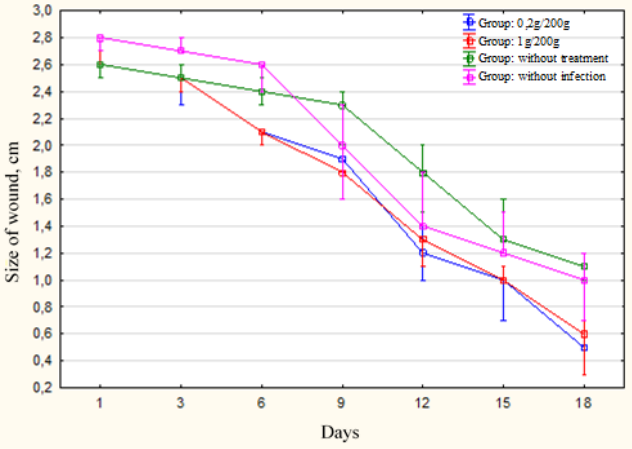

Figure 2: The wound healing dynamics in the Test Drug group in the case of S. Aureus infection

The bacterially infected wounds healed worse than the uninfected ones. On the 18th day of the study, the average diameter of the wounds infected with *S. aureus* (group 9) or *E. coli.* (group 10) was 1.1 cm, and in group 15 – 0.97 cm.

The healing rate of an uninfected and untreated wound was 0.1 cm/day (Table 2). The average diameter of the wounds without infection to which the preparations were applied on the first day of the study was 2.66 cm (groups 11 – 15). The rate of wound healing without infection after the application of the test drug was 0.105 cm/day for a dose of 0.2 g/200 g (group 11), and 0.111 for a dose of 1 g/200 g (group 12). The rate of wound healing with the

application of Levomekol was 0.105 cm/day for a dose of 0.2 g/200 g (group 13), and 0.107 cm/day for a dose of 0.2 g/200 g (group 14). The rate of healing of the uninfected wounds when applying a larger amount of the test or the reference drug was slightly higher. In general, the rate of healing of the uninfected wounds when applying the test drug was 1.11 times greater than the healing of the uninfected untreated wounds, and 1.07 times greater for the reference drug.

The size of the wounds on day 1 of the study after the infection with *S. aureus* in five groups of animals (1, 3, 5, 7, 9) averaged 2.63 cm. The dynamics of healing are presented in Figure 2.

The size of the wound on the 18th day of the study without the use of drugs was 1.1 cm. On the 18th day of the experiment, after applying the test drug to the wounds, the wound diameter decreased to 0.5 cm (dose of 0.2 g/200 g of the live weight) and 0.6 cm (dose of 1 g/200 g of the live weight). The wound healing rate was 0.117 cm/day for groups 1 and 3, which suggests that increasing the drug dose by more than 0.2 g per 200 g of the live weight lacked greater healing effect on wounds infected with *S. aureus.* For group 9 (healing with infection, without applying gels), the wound healing rate was 0.082 cm/day. The use of the studied gel increases the wound healing rate 1.43 times compared to the group of animals with the infected wounds, on which the gel was not applied.

The size of the wounds on day 1 of the study after the infection with *E. coli* in five groups of animals (2, 4, 6, 8, 10) averaged 2.63 cm. The dynamics of healing are presented in Figure 3.

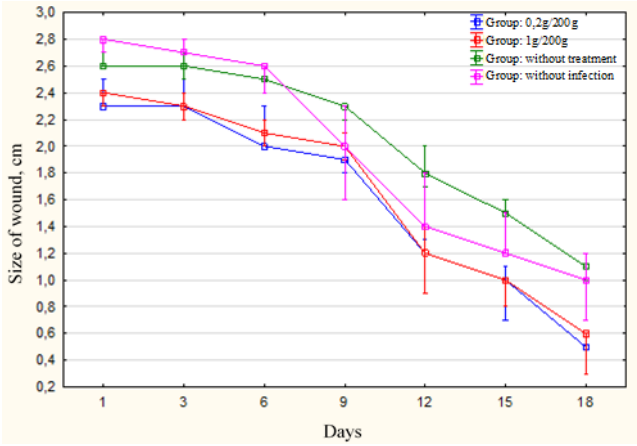

Figure 3: The wound healing dynamics in the Test Drug group in the case of E. coli infection

The size of the wound on the 18th day of the study without the use of drugs was 1.1 cm, as in case of infection with *E. coli* (healing rate – 0.077 cm/day). On the 18th day of the experiment, after applying the test drug to the wounds, the wound diameter decreased to 0.46 cm (dose of 0.2 g/200 g of the live weight) and 0.5 cm (dose of 1 g/200 g of the live weight). The wound healing rate was 0.105 cm/day for group 2 and 0.104 for group 4, which suggests that increasing the drug dose by more than 0.2 g per 200 g of the live weight lacked greater healing effect on the wounds infected with *E. coli.* The use of the studied gel increases the wound healing rate 1.36 times compared to the group of animals with the infected wounds, on which the gel was not applied.

After applying the Levomekol reference test drug to the wounds infected with *S. aureus,* on the 18th day of the experiment, the wound diameter decreased to 0.8 cm (dose of 0.2 g/200 g of the live weight) and 0.76 cm (dose of 1 g/200 g of the live weight). The data are presented in Figure 4.

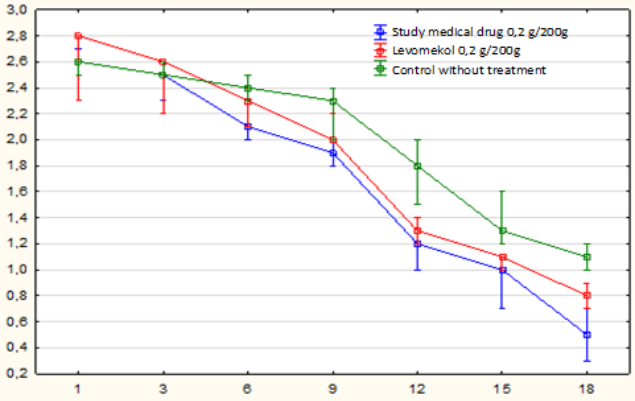

Figure 4: The wound healing rate in S. aureus infection

The wound healing rate was 0.102 cm/day for group 5 and 0.104 for group 7, which suggests that increasing the drug dose by more than 0.2 g per 200 g of the live weight lacked greater healing effect on the wounds. The use of Levomekol increases the healing rate of the wounds infected with *S. aureus* 1.27 times compared to the group of animals with the infected wounds to which the gel was not applied.

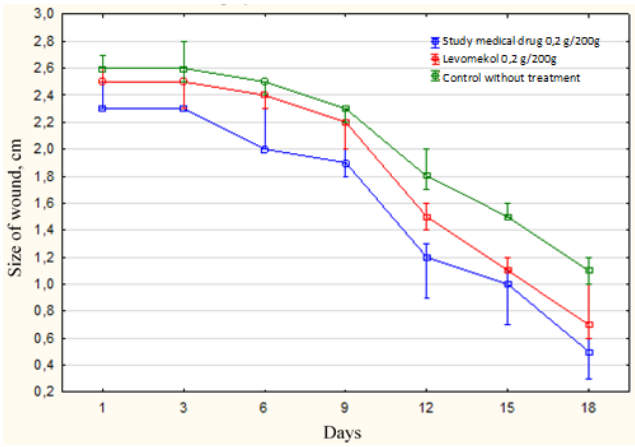

Figure 5: The wound healing rate in E. coli infection

In case of the wound infection with *S. aureus*, the healing rate in the group with the test drug was higher than in the group with the reference Levomekol drug by 1.2 mm/day in diameter. The wound healing rate in the group with the test drug was statistically significantly higher than that in the group of animals

with the infected and untreated wounds (group 9).

After applying the Levomekol reference test drug to the wounds infected with *S. aureus,* on the 18th day of the experiment, the wound diameter decreased to 0.8 cm (dose of 0.2 g/200 g of the live weight) and 0.76 cm (dose of 1 g/200 g of the live weight.). The data are presented in Figure 5.

The wound healing rate for groups 6 and 8 was 0.098 cm/day. The use of Levomekol increased the healing rate of the wounds infected with E. coli 1.27 times, as well as of the wounds infected with *S. aureus*, compared to the group of animals with the infected wounds, on which the gel was not applied, indicating the same effectiveness of Levomekol on this model of septic wounds.

In case of the wound infection with *E. coli*, the healing rate in the group with the test drug was higher than that in the group with the reference Levomekol drug by 1.2 mm/day in diameter. The wound healing rate in the group with the test drug was statistically significantly higher than that in the group of animals with the infected and untreated wounds (group 10).

Discussion

The obtained data prove the specific action of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origins. The study has shown that the bacterially infected wounds healed worse than the noninfected ones. Both types of wounds — infected and uninfected ones — healed faster when applying the test drug or Levomekol ointment. When infected with *S. aureus* and *E. coli*, the studied drug showed the best result in wound healing compared with Levomekol. Increasing the dose of drugs more than 0.2 g per 200 g of the live weight lacked greater healing effect both in *S. aureus* and in *E. coli* infection.

On a model of a planar infected wound, the developed drug with comprehensive therapeutic effect has shown better wound healing effect compared with the Levomekol reference drug.

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