



Research Paper

Creation of the wound healing pharmaceutical composition

Accepted 6th July, 2017

ABSTRACT

Designed wound healing pharmaceutical composition "Sedanil" was based on the amount of polypeptides and oils derived from black cumin seeds of *Nigella sativa*. In order to achieve maximum therapeutic effect of the treatment of wound and inflammatory processes, optimal concentrations of active ingredients were used. The positive effect of ointment developed and its on the course of wound healing help to reduce the clearance of wounds of necrotic tissue and earlier emergence of high-quality granulation and epithelialization of the wound defect top.

Yuilia Oshchepkova* and A.S. Sadykov

Institute of Bioorganic Chemistry, Uzbek
Academy of Sciences, Tashkent,
Uzbekistan.

*Corresponding author. E-mail:
joshepkova05@rambler.ru.

Keywords: Black cumin seeds *Nigella sativa*, pharmaceutical composition "Sedanil", the sum of polypeptides, Actovegin, Levomekol.

INTRODUCTION

Rational treatment of wounds is one of the most acute and complex problems of modern medical practice. To date, none of the methods of treating wounds with the use of medicines is universal, reliable and fully satisfying the clinicians. Etiology and pathogenesis of the wound process make it necessary to differentiate the approach to the creation of dosage forms for local treatment of wound pathology. Despite the abundance of medicines on the pharmaceutical market for local treatment of wound and inflammatory processes, it must be noted that the desired degree of therapeutic effect has not yet been achieved.

The principle of selecting drugs that are effective in this or that phase of the wound process is practically worked out and has not been revised for a long time. Medicines used in the phase of inflammation should have antimicrobial, analgesic, dehydrating and necrolytic effects. The drugs used in the phases of regeneration and reorganization of the epithelial cicatrix should have other properties: stimulate regenerative processes, promote granulation growth and accelerate epithelialization, protect granulation tissue from secondary infection and suppress the growth of microflora wound in the wound. As practice shows, the creation of medicinal forms that have similar properties is an actual problem. In an integrated approach to solving this problem, one of the main places is occupied by local treatment using ointments, suppositories, film coatings and aerosols, etc.

The aim of this work is to select the concentration of the active components (the sum of polypeptides and oil obtained from the seeds of the black cumin *Nigella sativa* (Oshchepkova et al., 2009; Rogozhin et al., 2011; Patent of the Republic of Uzbekistan, 2012).and excipients in order to create an effective drug for the treatment of wound and purulent-inflammatory processes.

MATERIALS AND METHODS

To achieve the goal of the study, the following medicinal and auxiliary substances were used: cetyl alcohol $C_{16}H_{33}OH$, emulsifier T₁, the sum of polypeptides isolated from the seeds of black cumin *N. sativa*, oil from the seeds of black cumin obtained by cold pressing, vitamins A and E, sorbic acid, ointment Actovegin Manufactured by "Nycomed Austria GmbH", Austria and Levomekol ointment manufactured by Nizhpharm, Russia.

Experimental study of the effectiveness of the developed pharmaceutical ointment composition was carried out on non-native white male rats weighing 200 to 250 g on models of thermal burn and mechanical wounds were used.

For the experiment, the animals were divided into 5 groups of 5 each:

- Control group (without treatment);

- Animals treated with the basis of the pharmaceutical composition;
- Animals treated with a composition containing the base and the sum of polypeptides obtained by acid extraction of defatted seeds;
- Animals treated with the standard of comparison with ointment Levomekol;
- Animals treated with the etiquette of the comparison with Actovegin ointment.

Planar skin-muscle wounds were applied on the experimental animals according to the generally accepted procedure. The coat was shaved and the site of operation treated with 70% alcohol solution (Petrakov, 2001). The operation was performed under local infiltration anesthesia (Veremey et al., 2001). Using a scalpel and tweezers, cut out full-thickness flaps of 1.5×1.5 cm.

The burns caused by an anesthetized rats (sodium etaminal with an intraperitoneal injection at a dose of 40 mg/kg), in which a cylinder (6.5×1.5 cm) was tightly applied to the shaved area located on the hind femur in which the boiling water was poured for 10 s. Under such conditions, a second degree burn occurred. One hour after the wounds and burns were applied to the wound, the ointment was applied to the maximum possible volume of 50 mg. Inspection and treatment of wounds and burns were performed daily until complete healing.

Clinical signs of the course of the wound process were assessed by the following parameters: relief of skin hyperemia, edema, granulation, granulation of the entire wound, initiation of marginal epithelization and complete epithelization of wounds. The measurements were performed on days 3, 7, 14, 20 and 30 from the start of treatment.

The area of the wound was determined and the percent reduction determined for the day in relation to the area calculated in the previous study, as well as, the percentage of wound area reduction over the entire treatment period. The obtained results were processed by the variational statistics method taking into account the Student's criterion.

RESULTS AND DISCUSSION

Early operative wound closure is impossible without the stage of drug treatment of the wound, taking into account the wound process phase. In such situations, it is important to select a drug that is highly active against the main pathogen (both aerobic and anaerobic components of the purulent process), which has analgesic action, prolonged osmotic activity or, conversely, properties aimed at preventing the drying of the wound surface and to stimulate granulation growth, which promote formation of the epithelium.

Based on the data on the antimicrobial activity, the sum

of polypeptides and oil obtained from the seeds of the black cumin *N. sativa* of the family *Ranunculaceae*, against strains were pathogenic to the human body (Oshepkova et al., 2015; 2012). A pharmaceutical wound healing composition based on them was developed.

In developing the formulation and technology, ointments were based on the modern requirements of complex (analgesic, anti-inflammatory, wound-healing and antimicrobial) effects on the wound with a low-component formulation.

The optimal carrier for ointments should ensure non-traumatic application to damaged areas, uniformity of distribution, exhibit long-lasting and strong osmotic activity in phase I wound process, create favorable conditions for wound regeneration in Phase II and above all, high bioavailability of LV. When choosing a base-forming component, the ability of diphylic bases to incorporate both fat and water-soluble substances was taken into account. As a high-molecular aliphatic alcohol, cetyl alcohol is chosen and as a surfactant providing release and absorption of the drug, the T_1 emulsifier.

In order to select the optimal concentration of active substances - the sum of polypeptides and oil from the seeds of the black cumin *N. sativa*, 6 compositions were studied (Table 1).

An experimental study of the effectiveness of ointment compositions was carried out on models of thermal burn and mechanical wounds. Every 10 days, animals were weighed and after 3-7-14-20 and 30 days, the wound area, the thickness of the skin fold and the body temperature near the burn was measured. The obtained results were processed by the variational statistics method taking into account the Student's criterion.

Experiments have shown that the condition of animals after a burn is severe. The burned part of the thigh and the paw are swollen. The skin of the burnt surface is waxy, hot and temperature 40.5°C . Animals were inactive. In the control group, the swelling increased the next day, decreased by 3 days, the burn wound was covered with a thick layer of the scab and the skin temperature dropped to 39°C .

Under the influence of treatment with an ointment containing 0.02 and 0.05 g of the sum of the polypeptides, on day 2 the general condition and behavior of the animals improved as compared to the untreated control.

At day 3, the wound area of the group in the untreated group decreased insignificantly, on average by 7.7% compared to the outcome. The thickness of the skin fold was 0.95 mm. The application of the base + oil helped reduce the burn wound by 28.6% ($P < 0.05$), while the thickness of the skin fold decreased to 0.9 mm. On 3 to 4 days in the treated rats, burn wounds were covered with a thin crust that fell off on days 5 to 6 in a composition containing 0.02 g of the sum of polypeptides, treated with a composition containing 0.05 g of the sum of polypeptides, while crumbling occurred on 7 to 8 days. The area of burn

Table 1: Compositions.

Number of compositions	Diphylic bases			Active substances		
	Cetyl alcohol (g)	Emulsifier (g)	Vitamins A, E, sorbic acid (ml)	Sum of polypeptides (g)	Oil (ml)	Purified water (ml)
1	33	3	to 0,01	0,01	-	up to 100,0
2	33	3	to 0,01	0,02	-	up to 100,0
3	33	3	to 0,01	0,05	-	up to 100,0
4	33	3	to 0,01	-	0,1	up to 100,0
5	33	3	to 0,01	-	0,2	up to 100,0
6	33	3	to 0,01	-	0,5	up to 100,0

Table 2: Selection of the optimal concentration of peptide and oils developed in the composition.

Use of the drug	Home rejection crusts	End rejection crusts
Control	27 day (1 from 5)	30 day (5 from 5)
Basis	13 day (1 from 5)	16 day (5 from 5)
Basis+ Sum of polypeptides 0.01 g	8 day (1 from 5)	11 day (5 from 5)
Basis+Sum of polypeptides 0.02 g	6 day (1 from 5)	9 day (5 from 5)
Basis+Sum of polypeptides 0.05 g	7 day (1 from 5)	10 day (5 from 5)
Basis+Oil 0.1 ml	8 day (1 from 5)	11 day (5 from 5)
Basis+Oil 0.2 ml	7 day (1 from 5)	9 day (5 from 5)
Basis+ Oil 0.5 ml	7 day (1 from 5)	10 day (5 from 5)
Levomekol	17 day (1 from 5)	20 day (5 from 5)
Actovegin	18 day (1 from 5)	20 day (5 from 5)

wounds in these groups decreased by 30.8 and 33.3% ($P < 0.01$) with respect to the outcome.

Treatment with "Levomekol" ointment and "Actovegin" cream (reference preparations) led to a reduction in the area of burn wounds by 32.3 and 37.5%, respectively, with respect to the outcome ($P < 0.01$).

The general condition and behavior of the experimental rats did not practically differ from the intact. In all groups, burn wounds were clean without suppuration. On the 7th day of treatment, the area of burn wounds decreased in the untreated group by 21.5%, in the second group - by 58.7, in the third - by 66.8 and in the fourth by 66% with respect to the outcome. Under the influence of Levomekol, the area of the burn wound was reduced by 35.4% and under the influence of Actovegin by 50% in relation to the outcome.

On the 14th to 15th day of treatment of burn wounds composition with 0.02 g of the sum of polypeptides observed wound healing, and in the group treated with a composition containing 0.05 g of the sum of polypeptides, the wound area decreased by 66.9% compared to the outcome. The area of wounds after treatment with Levomekol decreased by 41.5% as compared with the initial and under the influence of Actovegin by 43.1% in relation to the outcome.

By the 20th day of the study, in the groups treated with the composition containing 0.02 and 0.05 g of the sum of the polypeptides, there was complete healing and in the

groups with Levomekol and Actovegin, traces of the burn were still observed. In the control group (untreated), the area of burn wounds decreased by 41% compared to the outcome ($P < 0.01$).

After 30 days in the untreated control group, some rats still had traces of burn, while in the treated with the composition containing the sum of the polypeptides, the place of the burn was covered with wool. Tables 2 and 3 show the obtained data on the effect of the test compositions on the healing rate of thermal burns and mechanical wounds in rats, the criterion of which was the rate of size reduction and the rate of rejection of the wound crust and the dynamics of the state of the area of burn wounds.

The experiments allowed selecting the optimum concentrations of the sum of polypeptides - 0.02 g and oil - 0.2 ml, at which the greatest therapeutic effect of the developed composition is achieved. Based on the results of the data obtained, a composition containing an active principle 0.02 g of the sum of polypeptides from the seeds of the black cumin *N. sativa* and 0.2 ml of the black cumin seed oil obtained by cold pressing was created.

An experimental study of the effectiveness of a pharmaceutical composition called "Sedanil" was carried out (Table 4). Tables 5 and 6 show the obtained data on the effect of the test composition on the rate of healing of thermal burns and mechanical wounds in rats, the criterion

Table 3: Effect of composition on the healing speed of the burn wound

Days of experience/ Used drug	Basis	Basis + Sum of polypeptides 0.02 g	Basis + Sum of polypeptides 0.05 g	Basis + Sum of polypeptides 0.01 g	Levomekol
Initial wound size in mm ² (day 3)	48.0	34.0	37.0	25.0	37.0
5 day	36.0	24.0	28.0	22.0	30.0
6 day	34.0	20.0	24.0	20.0	24.0
7 day	28.0	16.0	22.0	18.0	22.0
8 day	22.0	12.0	16.0	14.0	18.0
9 day	19.0	6.0	11.0	9.0	16.0
10 day	16.0	3.0	8.0	6.0	12.0
11 day	8.0	0	5.0	4.0	8.0
12 day	3.4	-	0.3	0.5	4.3
13 day	0.3	-	0	0	2.2
14 day	0	-	-	-	-

Table 4: The composition of the composition in the following component ratio, g/ml

Diphylic bases			Active substances		Purified water
Cetyl alcohol (g)	Emulsifier (g)	Vitamin A, E, sorbic acid (ml)	Sum of polypeptides (g)	Oil (ml)	(ml)
33	3	to 0,01	0,02 r	0,2	up to 100,0

Table 5: Effect of composition on the rate of rejection of the wound crust

Use of the drug	Home rejection crusts	End rejection crusts
Control	27 day (1 from 5)	30 day (5 from 5)
Basis	13 day (1 from 5)	16 day (5 from 5)
Basis+Sum of polypeptides 0.02 g + oil 0.2 ml	5 day (2 from 5)	8 day (5 from 5)
Levomekol	17 day (1 from 5)	20 day (5 from 5)
Actovegin	18 day (1 from 5)	20 day (5 from 5)

Table 6: The dynamics of the state of the area of burn wounds in rats treated with the Sedanil formulation and comparators (M ± m; n = 5).

Surface wound area (mm ²)	3 day	5 day	7 day	10 day	14 day
	325.0±15.3				
Control	300.0±11.5	255.0±10.7	225.0±12.5	135.0±13.0	60.0±3.5
Basis + Sum of polypeptides 0.02 g + oil 0.2 ml	200.0±9.5	108.0±9.8	57.0±7.5 (in individual healing)	Healing	-
Levomekol	203.0±10.7	192.4±9.4	175.0±13.0	95.0±7.5	Healing
Actovegin	220.0±11.3	210.0±10.5	196.0±9.8	109.0±10.0	66.5±6.0

of which was the rate of size reduction and the rate of rejection of the wound crust and the dynamics of the state of the area of burn wounds.

In animal experiments, it was established that when the sum of polypeptides and oil is added together, the synergistic effect and the presence of pronounced wound healing, anti-burn and anti-inflammatory efficacy of the

developed ointment, as well as, the absence of local irritating effect are manifested. The data of pharmacological studies indicate that the developed ointment is not inferior to drugs manufactured by industry and in some parameters exceeds the efficiency of the "Levomekol" and "Actovegin" ointments.

When carrying out pharmacological studies it was

established that in the treatment of linear wounds in animals, the ointment promotes the acceleration of healing of burn wounds in animals expressed in the earlier appearance of granulations and the beginning of epithelization of the wound defect with complete closure of the wound defect with epithelium, devoid of signs of inflammatory hyperplasia.

Conclusions

The composition and technology of the wound healing pharmaceutical ointment "Sedanil" containing the sum of polypeptides in combination with the oil from the seeds of the black seed *N. sativa* on a diphylic basis was experimentally substantiated and developed.

In the experiments *in vivo* on models of mechanical wounds and thermal burns wound healing effect was studied and high efficiency established in the treatment of wounds in the first phase of the inflammatory process. The effectiveness of the ointment "Sedanil" is not inferior to the ointments "Levomekol" and "Actovegin" and has a more pronounced wound-healing effect, which is expressed in the reduction of the healing time and epithelization in the wound defect zone.

REFERENCES

- Oshchepkova Yu I, Veshkurova ON, Rogozhin EA, Musolyamov AKh, Smirnov AN, Odintsova TI, Egorov SA, Grishin EV, Salikhov ShI (2009). Isolation of the lipid-transporting protein Ns-LTP1 from seeds of the garden fennel flower (*Nigella sativa*). 35(3): 315–319.
- Rogozhin EA, Oshchepkova YI, Odintsova TI, Khadeeva NV, Veshkurova ON, Egorov TA, Grishin EV, Salikhov SI (). Novel antifungal defensins from *Nigella sativa* L. seeds. Plant Physiol Biochem. 49(2):131-137
- Patent of the Republic of Uzbekistan No. IAP 04614 (2012).
- Petrakov KA (2001). Operative surgery with topographic anatomy of animals/Kolos, Moscow. pp. 83.
- Veremey EI, Vlasenko VM, Eliseev AN (2001). Operative surgery with the basics of topographic anatomy. Harvest, Minsk.
- Oshchepkova YI, Veshkurova ON, Salikhov SI (2012). 3rd International Symposium on Edible Plant Resources and the Bioactive Ingredients, Urumchi, China, (2012), pp. 93-94.

Cite this article as:

Oshchepkova Y and Sadykov AS (2017). Creation of the wound healing pharmaceutical composition. Med. Med. Sci. 3(3): 013-017.

Submit your article at
<http://www.academiapublishing.org/mms>