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Investigation of the regenerative and analgesic effects of a novel anhydrous gel "Hypericum-Derm" for wounds treatment

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Key words: anhydrous gel, wound healing, analgesic activity, regenerative properties, topical formulation

According to data provided by the Office of the UN High Commissioner for Human Rights, more than 40 thousand people became victims of the military conflict from April 2014 to the end of 2020, more than 4 thousand military personnel were killed, more than 12 thousand were injured [1]. There is a clear gradation in the types of injuries: up to 60% are mine-explosive, 20–22% are combined, 10-13% are burns. These data indicate the relevance of the problem of wound and burn treatment for the healthcare system, both in the civil and military spheres, as general state tasks [2].

In modern literature, a wound is defined as a violation of the integrity of the skin or mucous membranes caused by mechanical action and usually accompanied by damage to deeper tissues or organs [3]. During the wound healing process, three phases are usually distinguished: Phase I is the inflammatory phase, characterized by the release of the wound from necrotic tissue and foreign bodies; Phase II is the proliferation or regeneration phase; Phase III is the maturation or remodeling phase, characterized by wound closure and final scar formation [4].

At the Department of General Chemistry of NUPh, assistants Maslov A. Yu. and Komisarenko N. A. under the supervision of Professor Kolesnik S. V. developed an anhydrous gel "Hypericum-Derm" consisting of α-arbutin (0.05%), clotrimazole (0.005%), lidocaine hydrochloride (2.0%), St. John's wort extract (4.0% by dry residue) and hawthorn leaf and flower extract (3.0% by dry residue). The anhydrous gel was obtained based on Levomekol technologies; polyethyleneglycol 400:1500 (8:2) was chosen as the basis for the anhydrous gel. This basis was chosen because in the first phase of the wound it is necessary to clean the wound from necrotic tissue and reduce the inflammatory reaction. This task is easily accomplished by the PEG base due to its high osmotic strength (336%) [5].

The next key issue is the composition of combinations of active pharmaceutical ingredients that will have an antimicrobial effect against of bacteria and fungi, as well as help suppress inflammation and inactivate free radicals. To solve this problem, we turned to the experience of Soviet pharmacists; in the 60s of the 20th century, the drug "Novoimanin" was developed and introduced in the USSR [6]. This drug was used in the form of a solution for the treatment of burns, purulent-inflammatory diseases of wounds infected with gram-positive strains. The active component of "Novoimanin"

is an extract of St. John's wort, the main biologically active substances of the extract are flavonoid derivatives (rutin, hyperoside, quercetin), and anthracene derivatives (hypericin). Many studies have described that St. John's wort extract has anti-inflammatory, antimicrobial, antioxidant, anticancer and analgesic effects [7], therefore, St. John's wort extract is a suitable component for creating anhydrous gel. Since we have a task to obtain anhydrous gel that can inhibit "superbugs", we selected an important component such as α-arbutin to solve this problem. In our previous studies [8, 9], it was shown that α-arbutin disrupts biofilm formation by resistant bacteria by affecting the LasI quorum sensing system.

To prevent the formation of a polymicrobial biofilm between bacteria and fungi, we selected an antifungal drug – clotrimazole. Polymicrobial biofilm is the main reason for non-healing in chronic wounds, especially in burns. To prevent the occurrence of symbiosis between bacteria and fungi, an antifungal agent should be used with the prescribed therapy of broad-spectrum antibacterial drugs.

To enhance anti-inflammatory and antioxidant activity, we included hawthorn leaf and flower extract in the composition, since the extract contains such active compounds as vitexin and isovitexin. In available studies, journals indexed in Scopus and Web of Science, it was shown that these compounds have high cardioprotective, antimicrobial, wound-healing, antiinflammatory, antioxidant, and neuroprotective effects [10].

The last and important component of our anhydrous gel is lidocaine hydrochloride. This component plays not only the role of a local anesthetic, but primarily as a compound that will suppress and prevent the formation of bacterial film [11].

The aim of the study was to investigate regenerative and analgesic effects of a novel anhydrous gel "Hypericum-Derm" for treatment of wounds for I and II phase.

Material and methods. The study was performed on 30 male albino rats (284.1 ± 5.32) g and on white non-linear male mice weighing 20–24 g. 28 mice housed in ventilated conditions with standard feed and free access to food and water. The experiment was conducted in compliance with the requirements of the "European Convention on the protection of vertebrate animals used in experiments and other scientific purposes" [12, 13] and approved by bioethic comisson of the National University of Pharmacy (protocol No. 7 01.11.2024).

The wound-healing effect of anhydrous gel "Hypericum-Derm" was studied on a model of a full-layer stencil wound. The wound plane was recreated on a previously depilated area of skin in anesthetized rats (thiopental, 40 mg/kg). For this purpose, the skin was cut using surgical scissors, tweezers and a stencil. In the framework of humane treatment of animals in our experiment, stencil wounds were made with a size of 1x1 cm^2 (100 mm^2). The skin and instruments were treated with 96% ethyl alcohol solution. After surgery, the wound was treated with 3% hydrogen peroxide solution. On the second day after modeling stencil wounds, the animals were randomized into groups according to the size of the wound area and treatment began.

The main indicators of the wound healing effect of the drugs were the area of stencil wounds (S, mm^2), the speed of healing and the percentage of

rats with healed wounds compared to the control group. The effectiveness of the drugs was studied in dynamics on the 1st, 4th, 7th, 10th, 13th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd and 24th day of treatment. Observations were carried out until the wounds were completely healed.

The area was measured according to the method of L. N. Popova [14], applying a transparent stencil to the wound and calculating the wound area (in mm^2). The wound healing coefficient (V) was calculated by the formula:

$V = (S_{max} - S_{invest}) / S_{max}$,

where: S_{max} – maximum wound area (1st day of treatment), mm^2; S_{invest} – wound area on the day of investigation, mm^2.

All animals were divided into 5 groups. The first group was a control pathology (the animals' stencil wound was treated with a 0.9% NaCl solution), the second group was a polyethylene glycol base 400/1500 (8:2) without active pharmaceutical ingredients, the third group was "Levomekol" ointment (Pharmaceutical Factory "Viola", series number LMK-03-250722-01-UA) at a dose of 1.0 g per 1 cm^2, the fourth group was "Wundehil" ointment (LLC "Scientific and Production Pharmaceutical Company "AIM", series number WUN-05-250825-01-UA) at a dose of 1.0 g per 1 cm^2, the fifth group was anhydrous gel "Hypericum-Derm" at a dose of 1.0 g per 1 cm^2. The treatment regimen was as follows: "Hypericum-Derm", "Levomekol" and "Wundehil" was applied to the wound surface in the amount of 1.0 g per 1 cm^2 once daily until complete wound healing.

To measure the pain threshold, the hot plate test was chosen, which allows investigating the analgesic activity of the experimental gels under thermal stimulation of the limbs of white mice [15]. During thermal stimulation, a hot plate was used, which was heated to 55 °C (Hot plate meter, Columbus Instruments, USA). The analgesic effect of the gels was determined by the ability to change the pain threshold of experimental animals to the corresponding stimuli. The analgesic activity of the gels in the hot plate test was measured as the time (s) the mice spent on the hot plate before the appearance of a defensive reflex – withdrawal from the surface and licking the limbs. In the experiments were used 4 groups of animals (7 mice each): 1 group – control group, animals without the use of gel; 2 group – animals, to whom polyethylene glycol gel 400/1500 (8 : 2) base was applied; 3 group – animals, which were applied the comparison drug – 2% lidocaine hydrochloride gel; 4 group – animals with application "Hypericum-Derm" gel. For the "hot plate" test gels were applied 20 min before the corresponding irritation to all limbs of the mice.

The experimental data were processed using the Statistica 8 software package using the values of the arithmetic mean (M) and the standard deviation of the arithmetic mean (m). Each value is presented as M ± m. The results were analyzed using Mann-Whitney test and differences were considered significant at $p < 0.05$.

The research was carried out within the framework of the topic "Development of anhydrous gel based on phenolic compounds for the treatment of purulent wounds caused by antibiotic-resistant *Pseudomonas aeruginosa*" of the list of scientific studies of the Ministry of Health of Ukraine, carried out at the expense of the state budget of Ukraine No. 0124U002080.

Results and discussion. Wound healing of anhydrous gel "Hypericum-Derm" in rats was assessed visually and by planimetric indicators. On the 5th day, the formation of a zone of necrosis was observed at the edges and in the subcutaneous layer of the wound. The wounds were covered with necrotic exudate, which during further processes dried up and formed scabs. The wound edges in the control group were more hyperemic than in the experimental groups. The control group also had more pronounced inflammation and large exudate. Analysis of planimetric indicators in Table 1 shows that on the 5th day of treatment, the wound area decreased in the group with anhydrous gel "Hypericum-Derm" by 21%, in the comparison groups ointment "Levomekol" and "Wundehil" by 16%, and ointment with a polyethylene glycol base by 24%. The healing rate of the studied anhydrous gel was 1.27, 1.62 and 2.0 times higher than that of the ointments "Levomekol", "Wundehil" and the control pathology group, respectively.

The characteristic active pathological granulation process began on the 8th day in the group with the polyethylene glycol-based gel, "Levomekol" and the anhydrous gel "Hypericum-Derm". The healing rate in the group with the anhydrous gel "Hypericum-Derm" was 50, 14, 2 and 46% higher than in the control pathology group, the polyethylene glycol-based gel, ointment "Levomekol" and ointment "Wundehil" respectively.

Complete wound healing with anhydrous "Hypericum-Derm" gel occurred on day 12, with "Levomekol" ointment – on day 15, with polyethylene glycol-based gel and "Wundehil" ointment – on day 15, and in the group with control pathology on day 18. Therefore, based on the above facts, it can be concluded that the developed composition of the anhydrous gel provides wound healing activity and has a positive effect on the speed of healing of the stencil wound.

To determine the analgesic activity, a model of pain reactions was used, which is connected with the activation of TRP receptors. According to the results shown in Table 2, it was established that the investigated anhydrous gel "Hypericum-Derm" reliably increases the latent period of the manifestation of pain on a hot surface by 3 times relative to the control values. The difference in the latent period was significant in the group of the investigated anhydrous gel "Hypericum-Derm" and 2% lidocaine hydrochloride. In the experimental group with polyethylene glycol base, a 2-fold increase in the latent period was observed compared to the control group.

Analgesic effect of "Hypericum-Derm" anhydrous gel is justified by the presence of lidocaine hydrochloride and its effect on pain receptors. Also, during the research, it was established that the polyethylene glycol base itself has a local anesthetic effect. In our opinion, this is related to the fact that the melting temperature of the polyethylene glycol base is 250 °C, and the temperature of the plate itself was 50 °C, so the polyethylene glycol base contributed to slowing down the action of the thermal stimulus on the pain receptors of the skin. Also, we did not observe the summation of the effect of polyethylene glycol base and lidocaine hydrochloride according to the results of the study.

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Table 1

Dynamics of planimetric indicators in rats with stencil wounds during treatment with the studied drugs, (M ± m)

Day of experiment	Parameters	Control patology	Polyethylene glycol gel 400/1500 (8 : 2) base	Levomekol	Wundehil	Hypericum-Derm
Output data	S_{begin}, mm^2	87.0 ± 4.35	102.0 ± 5.10	113.61 ± 5.67	85.0 ± 4.26	81.0 ± 4.05
5 days	S, mm^2	77.01 ± 4.59	76.50 ± 4.59	95.40 ± 4.77*	72.21 ± 3.60	63.60 ± 1.06*, **, #, &
	V	0.12	0.24	0.16	0.16	0.21
	v, mm^2/day	2.10	5.0	3.64	2.86	4.64
8 days	S, mm^2	43.50 ± 3.93	57.0 ± 5.13*	29.01 ± 2.61*, **	35.91 ± 3.24**	12.75 ± 0.50*, **, #, &
	V	0.50	0.81	0.74	0.58	0.84
	v, mm^2/day	8.25	14.50	16.60	9.08	16.95
12 days	S, mm^2	30.0 ± 4.50	15.0 ± 0.70*	8.49 ± 3.0*, **	13.29 ± 5.04*	0.0 ± 0.0*, **, #, &
	V	0.65	0.94	0.92	0.84	1.0
	v, mm^2/day	3.38	3.50	5.13	5.65	1.42
15 days	S, мм^2	13.74 ± 4.80	6.0 ± 3.00*	0.0 ± 0.0*, **	6.0 ± 3.0*	–
	V	0.84	0.94	–	0.93	–
	v, mm^2/day	1.81	1.0	–	0.81	–
18 days	S, мм^2	5.85	0.0 ± 0.0*	–	0.0 ± 0.0*	–
	V	0.93	–	–	–	–
	v, mm^2/day	0.88	–	–	–	–
21 days	S, мм^2	0.0 ± 0.0	–	–	–	–
	V	–	–	–	–	–
	v, mm^2/day	–	–	–	–	–

*Note. *Values are significant relative to the control pathology group, $p < 0.05$, **values are significant relative to the comparison drug polyethylene glycol base, # values are significant relative to the comparison drug "Levomekol", &values are significant relative to the comparison drug "Wundehil", S (mm^2) – area of stencil wounds, V – coefficient of stencil wound healing, (standard units), v – wound healing rate (mm^2/day).*

Table 2

Analgesic activity of the gels according to the "hot plate" test, M ± m

Experimental group	Latent period of pain reaction, s
1. Control	4.9 ± 0.3
2. Polyethylene glycol gel 400/1500 (8 : 2) base	8.7 ± 0.4*
3. 2% Lidocaine hydrochloride gel	13.3 ± 0.7*, **
4. Anhydrous gel "Hypericum-Derm"	12.0 ± 0.6*, **

*Примітка. *Values are significant relative to the control pathology group, $p < 0.05$, **values are significant relative to the comparison drug polyethylene glycol gel base, # values are significant relative to the comparison drug 2% Lidocaine hydrochloride gel.*

Conclusions

1. The wound-healing and analgesic effect of "Hypericum-Derm" anhydrous gel was investigated.
2. On the model of stencil wounds, it was confirmed that "Hypericum-Derm" anhydrous gel promotes complete healing of the wound on the 12th day, compared to the control group only on the 21st day.
3. On the "hot plate" model, it was established that "Hypericum-Derm" anhydrous gel has a local anesthetic effect, which is due to the effect on TRP receptors of the skin.

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O. Yu. Maslov, M. A. Komisarenko, O. V. Haltseva, S. V. Kolisnyk, L. V. Derymedvid

Investigation of the regenerative and analgesic effects of a novel anhydrous gel "Hypericum-Derm" for wounds treatment

Purulent wound is one of the thorniest, difficult and urgent issues in clinical practice. Due to the start of full-scale military actions in Ukraine in 2022, the number of purulent-necrotic wounds has increased several times, almost 40% of all wounds are purulent-necrotic. Purulent-inflammatory wounds are very acute and often lead to generalized infections, the development of sepsis and also the death of patients. Thus, the development of new anti-inflammatory and antioxidant drugs in the form of soft dosage forms is relevant today.

The aim of the study was to investigate regenerative and analgesic effects of a novel anhydrous gel "Hypericum-Derm" for treatment of wounds for I and II phase.

The wound-healing effect of anhydrous gel "Hypericum-Derm" was studied on a model of a full-layer stencil wound. The first group of rats was a control pathology (the animals' stencil wound was treated with a hydrogen peroxide solution), the second group was a polyethylene glycol gel 400/1500 (8:2) base without active pharmaceutical ingredients, the third group was "Levomekol" ointment, the fourth group was "Wundehil" ointment, the fifth group was anhydrous gel "Hypericum-Derm". To measure the pain threshold, the hot plate test was chosen. In the experiments were used 4 groups of animals (7 mice each): 1 group – control group, animals without the use of gel; 2 group – animals, to whom polyethylene glycol gel 400/1500 (8 : 2) base was applied; 3 group – animals, which were applied the comparison drug –2% lidocaine hydrochloride gel; 4 group – animals with application "Hypericum-Derm" gel. For the "hot plate" test gels were applied 20 min before the corresponding irritation to all limbs of the mice.

The wound healing rate when using the anhydrous gel "Hypericum-Derm" was 55 and 51% higher on days 5 and 8 compared to the control group. Complete wound healing was on day 12 when using the anhydrous gel "Hypericum-Derm", and in the case of control pathology on day 21. It was established that the investigated anhydrous gel "Hypericum-Derm" reliably increases the latent period of the manifestation of pain on a hot surface by 3 times relative to the control values.

It was established that anhydrous gel "Hypericum-Derm" possessed wound-healing and analgesic effects.

Key words: anhydrous gel, wound healing, analgesic activity, regenerative properties, topical formulation

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Дослідження регенеративної та аналгезуючої дії нового безводного гелю «Гіперикум-Дерм» для лікування гнійних ран

Гнійні рани є одними з найгостріших, складних та актуальних питань у клінічній практиці. У зв'язку з початком повномасштабних воєнних дій в Україні в 2022 році кількість гнійно-некротичних ран зросла в кілька разів, майже 40 % усіх ран є гнійно-некротичними. Гнійно-запальні рани протікають дуже гостро та часто призводять до генералізованих інфекцій, розвитку сепсису, а також смерті пацієнтів. Таким чином, розробка нових протизапальних та антиоксидантних препаратів у вигляді м'яких лікарських форм є актуальною сьогодні.

Мета дослідження – вивчення регенеративної та аналгезуючої дії нового безводного гелю «Гіперикум-Дерм» для лікування ран I та II фази.

Ранозагоювальний ефект безводного гелю «Гіперикум-Дерм» вивчали на моделі повношарової трафаретної рани. Перша група була контрольною патологією (трафаретну рану тварин обробляли розчином перекису водню), друга група – поліетиленгліколевий гель 400/1500 (8 : 2) без активних фармацевтичних інгредієнтів, третя група – мазь «Левомеколь», четверта група – мазь «Вундехіл»,

п'ята група – безводний гель «Гіперикум-Дерм». Для вимірювання больового порога було обрано тест «гаряча пластина»: 1 група – контрольна група, тварини без застосування гелю; 2 група – тварини, яким наносили поліетиленгліколевий гель 400/1500 (8 : 2) основу; 3 група – тварини, яким застосовували препарат порівняння – 2 % гель лідокаїну гідрохлориду; 4 група – тварини, яким наносили гель «Гіперикум-Дерм». У тесті «гаряча пластина» зазначені гелі наносили на всі кінцівки мишей за 20 хв до подразнення.

Швидкість загоєння ран при використанні безводного гелю «Гіперикум-Дерм» була на 55 і 51 % вищою на 5 та 8 дні відповідно порівняно з контрольною групою. Повне загоєння рани відбулося на 12 день у разі використання безводного гелю «Гіперикум-Дерм», а у випадку контрольної патології – на 21 день. Встановлено, що досліджуваний безводний гель «Гіперикум-Дерм» достовірно збільшує латентний період прояву болю на гарячій поверхні в 3 рази відносно контрольних значень.

Таким чином, встановлено, що безводний гель «Гіперикум-Дерм» має ранозагоювальну та анальгезуючу дію.

Ключові слова: безводний гель, загоєння ран, аналгезуюча активність, регенеративні властивості, місцева форма препарату

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