ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

UDC 615.276:582.632.1 https://doi.org/10.24959/nphj.18.2221

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The study of the wound healing effect of the ointment from the meal of black alder leaves (*Alnus Glutinosa*) on the model of planar wounds

Aim. To study the specific wound healing effect of a new medicinal form – an ointment from the meal of black alder leaves (*Alnus glutinosa*) on the model of planar wounds.

Materials and methods. The wound healing effect of the new ointment from the meal of black alder leaves was tested on the model of a full-thickness skin cut planar wound in rats with planimetric and morphological parameters compared to the reference drug Wundehyl.

Results and discussion. It was found that the wound healing activity of the ointment from the meal of black alder leaves was 1.84-4.76 times higher than Wundehyl ointment by its rate of wound healing on days 9-15 of application. The morphological analysis showed an intensive epithelialization and development of the granulation tissue when treating with the new ointment.

Conclusions. The new ointment from the meal of black alder leaves showed the best wound healing effect in relation to the control group and exceeded the reference drug by 3.33 times, accelerating healing of wounds by 3-4 days. The test dosage form can be considered as a promising drug for the treatment of wounds.

Key words: planar wound; wound healing effect; ointment from the meal of black alder leaves; Wundehyl

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Визначення ранозагоювальної дії мазі зі шроту листя вільхи клейкої на моделі площинної рани

Мета – дослідити специфічну ранозагоювальну дію нової лікарської форми – мазі зі шроту листя вільхи клейкої на моделі площинної рани.

Матеріали та методи. Ранозагоювальну дію нової мазі зі шроту листя Вільхи клейкої здійснювали на моделі повношарової вирізаної площинної рани шкіри у щурів із планіметричними та морфологічними показниками в порівнянні з референт-препаратом «Вундехіл».

Результати та їх обговорення. Встановлено, що ранозагоювальна активність мазі зі шроту листя вільхи клейкої була більш ефективною по швидкості загоєння рани на 9-15 добу у порівнянні з препаратом порівняння «Вундехіл» в 1,84-4,76 рази. При морфологічному аналізі спостерігалися інтенсивніша епітелізація та розвиток грануляційної тканини під час лікування новою маззю.

Висновки. Нова мазь зі шроту листя вільхи клейкої показала інтенсивну ранозагоювальну дію відносно контрольної групи та перевищила препарат порівняння в 3,33 рази, пришвидшивши загоєння ран на 3-4 доби. Досліджувана лікарська форма може розглядатися як перспективний засіб для лікування ран.

Ключові слова: площинна рана; ранозагоювальна дія; мазь зі шроту листя вільхи клейкої; Вундехіл

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Определение ранозаживляющего действия мази из шрота листьев ольхи клейкой на модели плоскостной раны

Цель – исследовать специфическое ранозаживляющее действие новой лекарственной формы – мази из шрота листьев ольхи клейкой на модели плоскостной раны.

Материалы и методы. Ранозаживляющее действие новой мази из шрота листьев ольхи клейкой осуществляли на модели полнослойной вырезной плоскостной раны кожи у крыс с планиметрическими и морфологическими показателями по сравнению с препаратом «Вундехил».

Результаты и их обсуждение. Установлено, что ранозаживляющая активность мази из шрота листьев ольхи клейкой была выше по скорости заживления раны на 9-15 суток относительно препарата сравнения «Вундехил» в 1,84-4,76 раза. При морфологическом анализе наблюдались интенсивная эпителизация и развитие грануляционной ткани при лечении новой мазью.

Выводы. Новая мазь из шрота листьев ольхи клейкой показала интенсивное ранозаживляющее действие относительно контрольной группы и превысила препарат сравнения в 3,33 раза, ускорив заживление ран на 3-4 суток. Исследуемая лекарственная форма может рассматриваться как перспективное средство для лечения ран.

Ключевые слова: плоскостная рана; ранозаживляющее действие; мазь из шрота листьев ольхи клейкой; Вундехил

Development of modern society is characterized by the growing burden of negative anthropogenic factors on a person. Not the last place belongs to mechanical damage of skin and tissues of different etiology, the increase of the number of surgical complications, allergic diseases. Therefore, the problem of healing and treating skin lesions of various genesis is rather relevant. One of the most common methods for treating skin lesions is the use of medicines for topical application in the form of ointments.

The aim of our work was to study the wound healing activity of an ointment from the meal of black alder (*Alnus glutinosa*) leaves (OMBAL) on the model of a full-thickness skin cut planar wound in rats.

Materials and methods

The object of the study was a soft dosage form – OMBAL containing galactoarabinose glucoside, flavonoids and phenolic compounds; it was developed at the Department of Industrial Technology of Drugs of the National University of Pharmacy.

The model of a planar wound was reproduced on 30 white nonlinear rats weighing 180-200 g. Animals were divided into three groups: group I – control pathology, group II – wounds treated with OMBAL, group III – wounds treated with Wundehyl ointment [1]. On the shaved areas of the skin of the experimental animals treated with 5 % alcoholic solution of iodine under barbamyl anesthesia in aseptic conditions the fragments of the skin with a diameter of 7-9 mm were cut in the region between shoulders in accordance with the methodical recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [2-5]. The wounds remained open. After surgery, once a day, the wound was treated with OMBAL and Wundehyl ointments in the dose of 0.5 g/kg [6]. The planimetric and morphological parameters of the wound healing effect of the ointment samples were studied on this model.

The area of wounds was measured immediately after their reproduction every three days till their complete healing. To estimate the dynamics of reduction of the wound surface area the method of L. N. Popova was used [7-10].

The wound area was determined using a transparent film, which wound contours were marked on in appropriate observation periods, followed by its stacking onto a profile paper for calculation. The conclusion about the speed of the wound healing (V) was made depending on the dynamics of healing, which was determined by the formula:

$$V = \frac{(S_o - S_t) \cdot 100\ \%}{S_t},$$

where: V – is the speed of wound healing, s.u (standard units); S_o – is the area of the wound in the previous measurement, mm^2; S_t – is the area of the wound in the current measurement, mm^2.

The experimental data obtained were processed by the method of variation statistics; to obtain statistical conclusions the Student's criterion at the accepted level of significance $p < 0.05$ was used. For statistical data processing the "Statistica 6.0" standard software [11-12] was used.

Under barbamyl anesthesia the animals were also taken out of the experiment on day 3, 6, 9 and 15, carving the wound that healed along with the tissues from the sides and bottom of the wound for microscopical examination [13-15]. The sections were stained with hematoxylin and eosin, as well as picrofuchsin by the method of Van Gieson [16].

Results and discussion

The use of OMBAL and the reference drug (Wundehyl ointment) showed the following results of accelerating the reduction of wound defects (Table), stimulating epithelialization and developing the granulation tissue of the animals treated on the model of a full-thickness skin cut planar wound.

Observations of the healing process indicated that the following day after the beginning of the experiment the wounds in rats of all groups (I-III) were dry, and the edges were slightly swollen. Later, the area of the wounds in rats began to decrease, in particular in animals without treatment (group I) the decrease of the area of the wound surface was slower than in the treated ones (groups II-III).

In six days the area of wounds treated by OMBAL decreased by 29.95 mm^2, while untreated wounds reduced by 16.55 mm^2. In 9-15 days, the rate of wound healing under the effect of OMBAL exceeded the control pathology by 7.4, 7.1, 9.4 times, and the rate of healing of wounds treated with Wundehyl ointment in the same time interval – by 4.02, 1.49, 2.8 times, respectively (compared to OMBAL – by 1.84, 4.76 and 3.4 times, respectively, an average by 3.33 times).

The comparative analysis of wound healing showed that the experimental ointment had the following results: day 12 – 20 %, day 15 – 60 %, day 18 – 100 %, while of Wundehyl ointment healed 40 % on day 15 and the complete healing (100 %) was achieved on day 21.

The morphological study of the group of rats (I-III) on day 3, 6, 9 and 15 affected the changes in power, the rate of appearance and the degree of maturity of the granulation tissue, the epithelium distinctiveness, and differentiation of the epithelial layer.

In the control pathology group (I) the wound defect was filled with the fibroid mass and infiltrated with polynuclear leucocytes on day 3 of the experiment. In the connective tissue layers there was an increase of the cellular composition (low differentiated fibroblasts). In the epidermis there were proliferative processes (formation hypertrophy, cell dystrophy), as well as a moderate mitotic activity of basal epidermocytes.

On day 6 of the experiment the wound surface was filled with inflammatory infiltrates (neutrophilic lymphocytes), and there was also the fibrinoid mass. Epithelial cells had the direction of growth simply under

Table

Parameters of the wound healing process of the rat skin when using OMBAL and Wundehyl ointment in the dose of 0.5 g/kg, n = 10

The period of the experiment, day	Parameter	Control	OMBAL	Wundehyl ointment
1 (initial data)	s_0, mm^2	52.14 ± 0.32	52.57 ± 0.41	53.32 ± 0.45
3	s_t, mm^2	45.36 ± 0.45	38.52 ± 0.51	44.22 ± 0.39
	V, s.u.	14.95	36.74	20.58
6	s_t, mm^2	35.59 ± 0.37	22.62 ± 0.43*/**	30.64 ± 0,42
	V, s.u.	46.5	132.4	74.02
9	s_t, mm^2	25.3 ± 0.47	13.11 ± 0.53*/**	20.25 ± 0.48
	V, s.u.	40.67	300.99	163.31
12	s_t, mm^2	16.58 ± 0.35	3.24 ± 0.47*/**	12.71 ± 0.36*
	V, s.u.	214.48	1522.53	319,51
	The share of animals with scars, %	0	20	0
15	s_t, mm^2	9.05 ± 0.35	1.15 ± 0.47*/**	3.77 ± 0.32*
	V, s.u.	476.13	4471.3	1314.32
	The share of animals with scars, %	0	60	40
18	s_t, mm^2	5.12 ± 0.45	0	3.2 ± 0.9*
	V, s.u.	1118.36	–	1566.25
	The share of animals with scars, %%	40	100	80
21	s_t, mm^2	2.65 ± 0.75	–	0
	V, s.u.	1867.55	–	–
	The share of animals with scars, %	80	100	100

Notes: S_t – is the area of a full-thickness skin cut planar wound, mm^2; *V* – is the speed of wound healing, s.u; * – the deviation is reliable in relation to the control pathology, $p < 0.05$; ** – the deviation is reliable in relation to the reference drug, $p < 0.05$; *n* – the number of animals in the experimental groups.

the scab, and the direction of the collagen matrix of the dermis was also remained.

On day 9 the fibrinoid mass in the surface areas of the defect was noticed and there was filling with the granulation tissue of immature nature up to ⅓ volume. From the edges of the newly formed tissue the initial zones of fiber formation were observed. The epithelialization of the surface of the defect remained at the same level.

On day 15 of the experiment the complete and incomplete healing of the defect was observed. The granulation tissue had a more mature character than the previous one. The epithelium was multilayered.

In rats treated with OMBAL (Group II) cellular and vascular proliferation was observed on day 3 of the experiment. In the defect zone there was the significant content of fibroblast cells characterizing immature granulation. The epithelialization of the wound defect was unclear.

On day 6 of the treatment there was a decrease in the size of the wound defect that was filled with a newly formed tissue. Along the edges of the wound there was a significant proliferation of fibroblasts. Epithelialization was markedly activated.

On day 9 of the treatment filling of the wound defect with ⅔ volume of the granulation tissue was observed. The newly formed tissue on the epithelized sites had a character of a fibrous scarring. Due to regeneration, the epithelium was thickened with a clear differentiation of layers, corresponding to the maturation rate of the granulation tissue.

On day 15 of the experiment the wound defect was healed completely and a small fibrous scar covered with thickened epidermis was observed.

Animals treated with Wundehyl ointment passed the same stages of the healing of the wound surface, but some time later (3-4 days).

CONCLUSIONS

As a result of the study of the specific effect of OMBAL on the model of a full-thickness skin cut planar wound in rats the conclusion can be made that the new wound healing dosage form OMBAL:

- accelerates the skin regeneration (by the planimetric parameters of the rate of reduction of the area of injury) 3.3 times quicker compared to the reference drug – Wundehyl ointment;
- intensifies the reparative processes of the skin cover (by the morphological parameters of appearance and development of the granulation tissue, its differentiation into the connective tissue, the rate of epithelialization), as well as accelerates healing of lesions of the skin by 3-4 days compared to Wundehyl, which is the known drug for the treatment of wounds and ulcerous lesions of the skin.

Conflict of Interests: authors have no conflict of interests to declare.

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Надійшла до редакції 20.05.2018 р.