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FLAXSEED OIL APPLICATION FOR WOUND HEALING IN WHITE RATS: EFFICACY AND HEMATOLOGICAL PROFILE

Actuality. Using of vegetable oils is considered as a promising therapeutical strategy in the treatment of skin wounds, what is explained by their potential effects on the different phases of the wound-healing process due to their antibacterial, antiinflammatory, and antioxidative properties as well as ability to promote the set of the cell processes like proliferation, increase of collagen synthesis, stimulation of the dermal reconstruction, restoration of the skins lipid barrier function etc. The study aimed to analyze and to study of inflammatory processes of different genesis. Here in we present the experimental results of Flaxseed oil wound healing activity evaluation on planar wounds in rats and studying of hematological profile of wound process with and without treatment, and comparing with reference hippophae rhamnoides fruit oil.

The aim – to evaluate the wound healing efficacy of flaxseed oil in a rats' flat wound model.

Materials and methods. The experiment was performed on 55 white male rats weighing 180–220 g. The wounds (size ~ 20×20 mm (400 mm²) and a depth ~ 2 mm) were formed by striking the metal rod of an appropriate diameter in the rat's lower back (the planar trafaret wounds model). Measurement of wound surface (S), wound contraction (W_c %) and speed of wound healing (V_h) was performed. Level of hemoglobin and blood cells (erythrocytes, platelets, leukocytes) were counted by automatic cell counter ABS-Micros 60-OT; white blood cell morphology was done after staining by Romanowsky-Giemsa.

Results. Here in we present the experimental results of Flaxseed oil wound healing activity evaluation on planar wounds in rats and studying of hematological profile of wound process with and without treatment. Obtained experimental data show a positive dynamic in changes of the red blood system (erythrocytes and hemoglobin levels), platelets level, and level of leukocytes and their morphology of the experimental animals.

Conclusions. The positive impact on the processes and speed of wound healing was noted, as well as the normalization of indicators of general clinical blood analysis under using flax oil in rats with planar trafaret wounds. Results of the study give grounds to talk about the good therapeutic effect in treating wounds with flaxseed oil which is equal/comparable with the activity and effects of hippophae rhamnoides fruit oil.

Key words: planar wounds, flaxseed oil, hematological profile, in vivo.

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ЗАСТОСУВАННЯ ОЛІЇ НАСІННЯ ЛЬОНУ ДЛЯ ЗАГОЮВАННЯ РАН БІЛИХ ЩУРІВ: ЕФЕКТИВНІСТЬ ТА ГЕМАТОЛОГІЧНИЙ ПРОФІЛЬ

Актуальність. Використання рослинних олій вважається перспективною терапевтичною стратегією у лікуванні ран шкіри, що пояснюється їх потенційним впливом на різні фази процесу загоєння ран завдяки їх антибактеріальним, проти-запальним та антиоксидантним властивостям, а також здатності стимулювати такі клітинні процеси, як проліферація, підвищення синтезу колагену, стимуляція репарації шкіри, відновлення бар'єрної функції шкіри, впливом на її ліпідний шар та ін. Дослідження спрямоване на аналіз та вивчення запальних процесів різного генезу. У цій роботі ми представляємо результати експериментального дослідження ефективності лляної олії у загоєнні площинних ран у щурів та вивчення гематологічного профілю процесу загоєння ран із застосуванням лікування та без нього, а також порівняння результатів із референтним засобом – олією плодів обліпихи.

Мета дослідження – оцінити ранозагоювальну ефективність олії насіння льону на моделі площинних ран у щурів.

Матеріали та методи. Експеримент проводили на 55 білих щурах-самцях масою 180–220 г. Рани (розміром ~ 20×20 мм (400 мм²) і глибиною ~ 2 мм) утворювали шляхом удару металевим стрижнем відповідного діаметру по нижній частині спини щура (модель площинних трафаретних ран). Проводили вимірювання поверхні рани (S), зменшення рани (Ws %) та швидкості загоєння рани (Vk). Рівень гемоглобіну та клітин крові (еритроцитів, тромбоцитів, лейкоцитів) підраховували автоматичним лічильником клітин АБС-Мікрос 60-ОТ; морфологію лейкоцитів вивчали після фарбування за Романовським-Гімза.

Результати дослідження. Результати експериментального дослідження представляють оцінку ранозагоювальної активності лляної олії на площинних ранах у щурів та вивчення гематологічного профілю ранового процесу з лікуванням та без нього. Отримані експериментальні дані свідчать про позитивну динаміку змін еритроцитів (рівнів еритроцитів та гемоглобіну), рівня тромбоцитів, а також рівня лейкоцитів та їх морфології у експериментальних тварин.

Висновки. Відзначено позитивний вплив на процеси та швидкість загоєння ран та нормалізацію показників загального аналізу крові у разі застосування лляної олії у щурів із площинними трафаретними ранами. Результати дослідження дають підстави говорити про достатній терапевтичний ефект лляної олії у лікуванні ран, який дорівнює/прирівнюється до дії олії плодів обліпихи.

Ключові слова: площинні рани, лляна олія, гематологічний профіль, *in vivo*.

Actuality. Wound healing is a complex process aimed at restoring the extracellular matrix and a functionally active coating with or without scar formation (Flanagan, 2000; Li 2007). The issues of wounds healing pathogenesis are studied intensively, currently (Demidova-Rice, 2012; Young, 2011). The treatment results of patients with non-healing wounds are improved a lot thanks using in practice of the obtained scientific data in this area in recent years (Murphy PS, 2012; Da LC, 2017). The optimal conditions creation for the fullest tissue regeneration is one of the main tasks in the various genesis skin wounds treatment (Lepow, 2011). Numerous research has been conducted in medicine and pharmacy field for search of effective pharmacological agents purposefully affecting the completeness of skin regeneration. Various pharmacological groups of substances: proteolytic enzymes, anabolic hormones, synthetic antiseptics, adsorbents and other active molecules that stimulate metabolic processes have been studied (Norman, 2016).

The most convenient and promising for treatment skin defects are soft dosage forms as creams, gels and oils (Qi C, 2018). The main advantages of these drugs are uniform distribution of the active substance and ease of use (Pereira, 2016; Sood, 2014). The plants oils as a natural sources of useful biologically active components that show a diverse spectrum of health-promoting properties are very interesting for wound treatment (Rekik, 2016; Sabale, 2012). Flaxseed oil (*Linum usitatissimum* L), also known as linseed oil or flax oil, is characterized by a high content of unsaturated fatty acids (in%): 44–61% alpha-linolenic (Omega-3), 15–30% linoleic (Omega-6), 13–29% oleic (Omega-9) and vitamin E (Bernacchia, 2014; Lewinska, 2015). Numerous studies demonstrated potential ability of unsaturated fatty acids (especially Omega-3 and Omega-6) (Hankenson, 2000; Lania, 2019) to affect epidermal wound healing because of their influence on proinflammatory cytokine levels and other cell molecules that are important for the healing process (Farahpour, 2011; Soleimani, 2017).

Aim – to evaluate the wound healing efficacy of flaxseed oil in a rats flat wound model.

Material and methods. *Animals.* The experiment was performed on 55 white male rats weighing 180–220 g. All animals used for this study were kept in standard cages and maintained under controlled laboratory conditions of temperature (22±3°C), humidity and 12 hours day–12 hours night and had free access to food (standard pellet diet) and water ad libitum. The animals were treated humanely throughout the study period, experiments were conducted in accordance with the EU Directive 2010/63 / EU on the protection of animals used for

scientific purposes. The experiment design and study protocol were approved by the Ethics Committee of the Danylo Halytsky Lviv National Medical University, protocol No. 8, May 15, 2018.

The planar trafaret wounds model. All the animals were shaved, cleaned and anaesthetized with 65 mg/kg BW ketamine and 15 mg/kg BW xylazine administered intraperitoneally. The wounds (size ~ 20×20 mm (400 mm²) and a depth ~ 2 mm) were formed by striking the metal rod of an appropriate diameter in the rat's lower back. The animals were randomly divided into 4 groups: Group I (intact control group, n=10) without wounds and without treatment; Group II (pathology model, n=15) with wounds and without treatment; Group III (flaxseed oil treatment, experimental group, n=15) with wounds and treatment with flaxseed oil; Group IV (hippocampal fruit oil treatment, experimental group, n=15) with wounds and with hippocampal fruit oil treatment. After 24 h of the wound formation, the animals of III and IV groups were treated topically once a day throughout 20 days with flaxseed oil treatment and hippocampal fruit oil were applied with A thin layer of oil was applied on the surface of the wound and its contour in a dose of 20 mg/cm², which is sufficient for the complete absorption and wetting of the surface of the wound. On the 21st day, all the animals were sacrificed under anesthesia.

Measurement of wound surface (S), wound contraction (W_c %) and speed of wound healing (V_k). The progressive changes in wounds area were monitored on 1, 5, 10, 15 and 20 days by a digital camera (Canon Power Shot, Japan). The images were then transferred to a computer where they were processed in the GIMP2 free software. The Fiji (ImageJ®, NIH, USA) (Schindelin J, 2012) free software was used for measurement of wound surface size (S) in mm². The wound contraction (W_c) was calculated using the following equation (expressed as a percentage) (Shivhare Y, 2010):

$$\%W_c = \frac{S_{day1} - S_{dayN}}{S_{day1}} * 100\%, \text{ where} \quad (1)$$

W_c represents the wound contraction, S_{day1} – wound surface size at the 1st day of experiment and S_{dayN} the specific day wound size.

The speed of wound healing (V_k) was calculated using the following equation:

$$\%V_k = \frac{S_{day1} - S_{dayN}}{S_{dayN}} * 100\%, \text{ where} \quad (2)$$

V_k represents speed of wound healing, S_{day1} – wound surface size at the 1st day of experiment and S_{dayN} the specific day wound size.

Hematological Studies. Sampling blood into vacuum tubes with solution Na₂EDTA from the lateral tail vein of

the rats was carried out for hematological studies in the 1, 5, 10 and 20 day of experiment (Lee, 2015). Level of hemoglobin and blood cells (erythrocytes, plates, leukocytes) were counted by automatic cell counter ABS-Micros 60-OT (Horiba Medical, Montpellier, France) in certified clinical laboratory of Danylo Halytsky Lviv National Medical University. White blood cell morphology was done (after staining by Romanowsky-Giemsa) by an experienced clinical laboratory specialist.

Statistical Analysis. All data were processed using the statistical package Statistica 10.0 (Statsoft/Dell, Tulsa, OK, USA). The descriptive statistics of the data in tables include mean \pm standard error of the mean (SEM) or mean \pm standard deviation. Significance was assessed by using the one-way ANOVA followed by *t*-test. Values were considered statistically significant when *P* value is less than 0.05.

Research results. After modeling of stencil wounds in the area of 403,7–411,2 mm² (Table 1) in animals developed inflammation of the skin, characterized by hyperemia and swelling of the edges of the wound, which acquired a roller-shaped form (Sorg, 2017). In day 1, the wounds of all groups were covered by a dehydrated wound crust or scab. Subsequently, the wound gradually covered with thick brown crusts. In the treated group of animals (III and IV), the macroscopic signs were less pronounced: hyperemia and swelling decreased rapidly, the exudate did not separate already in 5 days of treatment, and in the “pathology model” (group II) the wound surface was dry only in 9 days.

Animals with flaxseed oil and hippophaerhamnoides fruit oil treatment were more active and had better appetite. The crusts removal, under which there was noticeable granulation tissue, was observed in animals treated with flaxseed oil (group III) and hippophaerhamnoides fruit oil (group IV) already in 8–9 days. For untreated animals (group II) the same results were observed only in 12 days. The complete healing based on the macroscopic closure of the wound and restoration of the epithelial cover occurred in 17 days with the flaxseed oil treatment (group III) and in 18 days for the animals treated with the reference drug (group IV). For the untreated group, the wound persisted

even after 20 days showing a red scaly surface with no epidermal coverage and the full (100%) epithelization of wounds was observed only in 24 days.

The wound surface (*S*, mm²) actively have already decreased almost twice in 5 days in groups treated with plants oil in comparison with 1 day of experiment and with untreated group as well ($P \leq 0.001$) (table 2). The similar tendency was observed for the 10 and 15 day of experiment for all groups ($P \leq 0.001$).

The rate of wound contraction (*Wc*, %) in untreated control rats was 29,1% to 62,8% from day 5 to day 10 and 85,7% to 91,2% from day 15 to day 20, while complete epithelization and healing were observed on day 24. The percent rate of wound contraction in rats, treated with hippophaerhamnoides fruit oil (in a dose of 20 mg/cm²), was statistically significant from 46,2% on day 5 to 67,3% on day 10 and 91,3% to 100% from day 15 to day 20, respectively, while flaxseed oil (in a dose of 20 mg/cm²) treated rats showed statistically significant increase in wound contraction, from 46,2% on day 5 to 69,6% on day 10 and 92.1% to 100% from day 15 to day 20 respectively. The analysis of speed of wound healing (*Vk*) showed that the best statistically significant results were in the flaxseed oil treated rats' group.

The morphological blood analysis is a recognized informative test that reflects the general condition of animals and allows evaluating the reactivity of the organism. The study of the red blood system of animals showed that the level of erythrocytes in the 1st day after wound formation was statistically significantly decreased in experimental animals groups II (at 17%) and III, IV (at ~11% in each one) in comparison with intact animals but was within the physiological norms (Table 3).

A similar pattern was observed for hemoglobin level which was 25% less in the group II, 19% less in the group III and 21% less in the group IV on the 1st day compared to the group I animals. This can be explained by blood loss and related processes during the wound formation. However, starting from 5th day in groups III and IV there was an increase in red blood cells and hemoglobin levels in comparison with untreated group II. So, the erythrocytes levels on the 5th day in the groups III, IV

Table 1

Wound healing activity in dynamic (values were expressed as mean \pm SD)

Groups/ Parameters	Pathology model, n=15	Flaxseed oil treatment, n=15	Hippophaerhamnoides fruit oil treatment, n=15
The disappearance of inflammation symptoms, day	11,2 \pm 0,9	8,8 \pm 0,6	9,4 \pm 0,4
Granulation, day	11,9 \pm 1,7	8,7 \pm 0,7	9,1 \pm 1,3
The beginning of the wound edges epithelialization, day	18,2 \pm 1,8	13,2 \pm 1,5	14,8 \pm 1,3
The full epithelialization, day	23,4 \pm 1,1	16,6 \pm 1,8	18,1 \pm 1,4

Table 2

Wound healing activity parameters (values were expressed as mean±SD)

Groups/ Parameters	Pathology model, n=15	Flaxseed oil treatment, n=15	Hippophaerhamnoides fruit oil treatment, n=15
1 day			
S_r, mm^2	411,2±14,6	403,7±17,4	408,5±13,4
5 day			
S_r, mm^2	291,3±10,8	217,0±14,5 [#]	220,2±12,4 [#]
V_k	0,41±0,09	0,86±0,11 [#]	0,86±0,10 [#]
$W_r, \%$	29,1±1,9	46,2±1,8 [#]	46,2±1,4 [#]
10 day			
S_r, mm^2	153,0±6,2	122,4±5,4 [#]	133,9±4,9*
V_k	1,69±0,13	2,28±0,18*	2,06±0,12*
$W_r, \%$	62,8±2,9	69,6±1,1*	67,3±2,8
15 day			
S_r, mm^2	58,7±3,4	31,7±2,3 [#]	35,7±2,8 [#]
V_k	6,01±0,53	11,70±0,94 [#]	10,52±0,89 [#]
$W_r, \%$	85,7±1,7	92,1±2,2*	91,3±2,1*
20 day			
S_r, mm^2	36,2±4,4	0,0±0,0	0,0±0,0
V_k	10,35±0,76	---	---
$W_r, \%$	91,2±2,7	100,0	100,0

* $p < 0,05$; [#] $p < 0,001$ as compared with pathology model group in the appropriate day

were 5% higher and on the 10th day 10% higher compared to the first day of experiment and almost reached the level of intact control (group I). The hemoglobin level also changed in a similar way and its normalization was observed approximately in the region of the 12th day of the experiment. Whereas in group II, the increase in the levels of erythrocytes and hemoglobin was not so rapid and normalization was observed only in 20 days of the experiment. The platelets level was significantly higher compared to intact control in all experimental animals from the 1st day and remained high throughout the experiment. It should be noted that the platelets level in groups III and IV was statistically significantly higher by 35% on day 1 as compared with group II, but already only by 24% and 9% on days 5 and 10, respectively. Such changes can be explained by the termination of blood loss and wound healing dynamic (Chicharro-Alcántara, 2018). The white blood cells play a major role in the specific and non-specific protection of the body from external and internal impacts, as well as in typical pathological processes. The total number of leukocytes was statistically significantly increased on the 1st day after wounding by 51% in group II, by 37% in group III and by 39% in group IV compared with intact group I. But already on 5th experiment day there was a general tendency to decrease the leukocytes level in all experimental groups. The levels in groups 3 and 4 were only on 20.5 and 21.5% exceeding the intact control value, while in group 2 it was 41.1%, in comparison with intact

animals. On the 10th and 20th day of the experiment the number of white blood cells in rats continued to decrease and exceeded the values in group I only by 11,6 % and 7,0% (group III) and 13,2% and 10,0% (group IV) respectively. Whereas in group II, the number of leukocytes was 24,3% and 11,3% higher than that of group I at similar time points. This circumstance gives grounds to talk about the best therapeutic effect in treating wounds with flaxseed and hippophaerhamnoides fruit oils. The increase in the number of neutrophils and decrease in the number of lymphocytes was observed in II, III and IV animals' groups by white blood cell morphology analysis at 1st day after the application of model wounds. During the experiment in group II, the number of stab neutrophils exceeded the level in the group of intact animals by 100% in the 1st day, by 77% on day 5, by 32.5% on 10 day and only on day 20 it became equal to the value of intact control group I. In animals III and IV groups treated with flaxseed and hippophaerhamnoides fruit oils, the number of stab neutrophils exceeded the level in the group of intact animals by 60 and 65% on 1 day, by 50 and 45% on day 5, by 16% on day 10, but already on day 20 the level of stab neutrophils was 14% and 10% less than in group I. A similar tendency was observed for the segment neutrophils level and its number in group II exceeded the level in the intact animals' group by 48% on 1 day, by 15.6% on day 5, by 12.5% on day 10 and approached the level of intact animals only on day 20. At the same time, in groups III and IV treated with oils,

Table 3

Hematological profile of male rats with wounds treated with flaxseed oil and hippophaerhamnoides fruit oil

rGoups/ Days	Blood parameters									
	RBC, M/ μ L	HGB, g/dL	PLT, K/ μ L	WBC, K/ μ L	EOS (%)	BAS (%)	StLEU (%)	SegLEU (%)	LYMPH (%)	MON (%)
<i>1 day</i>										
IC	7,82 \pm 0,23	14,91 \pm 0,38	053 \pm 64	8,96 \pm 1,63	2,3 \pm 0,6	1,0 \pm 0,2	2,3 \pm 0,6	23,0 \pm 1,7	66,7 \pm 1,6	4,7 \pm 0,5
PM	6,52 \pm 0,18 [#]	11,2 \pm 0,25 [#]	1284 \pm 73 [*]	13,57 \pm 2,49	3,3 \pm 0,5	1,2 \pm 0,3	3,8 \pm 0,4 [#]	34,2 \pm 1,6 [#]	54,2 \pm 3,4 [*]	3 \pm 0,4 [*]
FO	6,98 \pm 0,17 [*]	12,10 \pm 0,27 [#]	1734 \pm 89 [#]	12,35 \pm 2,08	2,9 \pm 0,6	1,3 \pm 0,3	3,7 \pm 0,3 [#]	31,3 \pm 1,8 [*]	57,5 \pm 2,8 [*]	3,3 \pm 0,5
HO	7,03 \pm 0,15 [*]	11,92 \pm 0,21 [#]	1793 \pm 94 [#]	12,48 \pm 2,15	3,0 \pm 0,6	1,3 \pm 0,3	3,8 \pm 0,4 [#]	30,9 \pm 1,7 [*]	57,8 \pm 3,1 [*]	2 \pm 0,5 [*]
<i>5 day</i>										
IC	7,87 \pm 0,29	15,31 \pm 0,41	987 \pm 53	9,08 \pm 1,79	2,0 \pm 0,5	1,0 \pm 0,2	2,2 \pm 0,7	24,3 \pm 1,3	65,5 \pm 1,9	4,6 \pm 0,6
PM	6,86 \pm 0,19 [*]	12,07 \pm 0,28 [#]	1521 \pm 66 [#]	12,75 \pm 2,07	3,7 \pm 0,7	1,3 \pm 0,2	3,9 \pm 0,4 [*]	28,1 \pm 1,5	60,3 \pm 2,9	2,7 \pm 0,5 [*]
FO	7,23 \pm 0,20	13,34 \pm 0,30 [#]	1891 \pm 90 [#]	10,91 \pm 1,79	3,1 \pm 0,5	1,3 \pm 0,3	3,3 \pm 0,3	24,6 \pm 1,4	63,9 \pm 1,9	3,8 \pm 0,6
HO	7,31 \pm 0,21	13,82 \pm 0,27 [*]	1874 \pm 87 [#]	11,03 \pm 1,93	3,1 \pm 0,6	1,3 \pm 0,2	3,2 \pm 0,4	25,5 \pm 1,8	63,1 \pm 2,2	3,8 \pm 0,4
<i>10 day</i>										
IC	7,84 \pm 0,27	14,40 \pm 0,40	1011 \pm 67	9,03 \pm 1,58	2,2 \pm 0,5	1,0 \pm 0,3	2,5 \pm 0,5	23,9 \pm 1,5	65,9 \pm 1,8	4,5 \pm 0,5
PM	7,36 \pm 0,29	12,31 \pm 0,27 [#]	1543 \pm 57 [#]	11,23 \pm 1,51	3,5 \pm 0,5	1,2 \pm 0,2	3,3 \pm 0,4	26,9 \pm 1,7	62,0 \pm 1,9	3,0 \pm 0,5 [*]
FO	7,63 \pm 0,31	13,92 \pm 0,29	1687 \pm 64 [#]	10,08 \pm 1,12	2,7 \pm 0,4	1,1 \pm 0,2	2,9 \pm 0,3	21,7 \pm 1,4	67,7 \pm 1,6	3,9 \pm 0,5
HO	7,71 \pm 0,34	14,03 \pm 0,27	1653 \pm 70 [#]	10,23 \pm 1,20	2,8 \pm 0,5	1,2 \pm 0,2	2,9 \pm 0,4	21,3 \pm 1,8	67,9 \pm 1,5	3,9 \pm 0,5
<i>20 day</i>										
IC	7,80 \pm 0,25	14,30 \pm 0,33	1028 \pm 63	8,94 \pm 1,53	2,1 \pm 0,6	1,1 \pm 0,3	2,9 \pm 0,6	22,6 \pm 1,2	66,4 \pm 1,7	4,9 \pm 0,6
PM	7,75 \pm 0,33	13,20 \pm 0,27	1243 \pm 50 [*]	10,14 \pm 1,79	3,1 \pm 0,5	1,1 \pm 0,1	2,9 \pm 0,4	23,2 \pm 1,3	65,3 \pm 1,6	3,4 \pm 0,5 [*]
FO	7,87 \pm 0,28	14,24 \pm 0,28	1153 \pm 59	9,56 \pm 1,42	2,4 \pm 0,5	1,0 \pm 0,1	2,5 \pm 0,4	22,9 \pm 1,3	67,0 \pm 1,5	4,2 \pm 0,6
HO	7,90 \pm 0,34	14,12 \pm 0,28	1091 \pm 65	9,86 \pm 1,53	2,3 \pm 0,4	1,1 \pm 0,2	2,6 \pm 0,5	22,5 \pm 1,4	67,4 \pm 1,6	4,1 \pm 0,5
LNMU										
Male	6,3 – 8,6	11,8-16,1	537-1455	5,7-13,9	0,3-4,0	0-1,3	1,2-5,3	17,1-40,5	48,7-78,1	1,0-6,5
Vivarim										

Values were expressed as mean \pm SD, * $p < 0,05$; # $p < 0,001$ as compared with intact control group (IC) in the appropriate day; IC – intact control (n=10); PM – pathology model (n=15); FO – flaxseed oil treatment (n=15); HO – hippophaerhamnoides fruit oil treatment (n=15), LNMU – Lviv National Medical University; RBC: red blood cells; HGB: hemoglobin; PLT: platelet counts; WBC: white blood cells; EOS: eosinophils; BAS: basophils; StLEU: stab leukocytes; SegLEU: segmental leukocytes; LYMPH: lymphocytes; MON: monocytes.

the segment neutrophils level exceeded the level in the intact animals group by 35 and 34% on day 1, but was already at the level of intact animals on day 5, but was below the level of intact animals by 10,0 and 12,0% on day 10 and was again at the intact animals level on day 20. The neutrophils level decreases on the 10th day of wounding and may indicate the end of the inflammatory process. Data analysis of the lymphocytes level revealed their decrease in all experimental groups of animals in comparison with the group I at the 1st day after wound formation. The dynamics of changes in the lymphocytes level is characterized by a decrease by 23,0, 14,0 and 13,4% in groups II, III and IV, respectively, on the 1st day compared to the intact animals. But already on day 5, level is normalized in groups III and IV and retains a value equal to the intact control until the experiment end. Whereas in the II group without treatment the lymphocyte level reached normal values only after 10 days of study. It should also be noted a slight increase in the level of eosinophils and a more significant decrease in

the level of monocytes, which was observed in animals II, III and IV groups throughout the experiment.

Natural wound healing is a complex physiological process and, in general, without any external interventions depends on a variety of factors that occur sequentially in a particular order (Nayak, 2006). However, in a real life, many factors, such as bacterial infections, humidity etc., can delay and complicate wound healing without additional treatment/therapy (Albayrak, 2013). Using of vegetable oils is considered as a promising therapeutical strategy in the treatment of skin wounds, what is explained by their potential effects on the different phases of the wound-healing process due to their antibacterial, anti-inflammatory, and antioxidative properties as well as ability to promote the set of the cell processes like proliferation, increase of collagen synthesis, stimulation of the dermal reconstruction, restoration of the skin's lipid barrier function etc. (Poljšak, 2020). Due to obtained until nowadays theoretical and experimental data the fatty-acid components of vegetable oils play a

key role in the wound-healing process, and polyunsaturated fatty acids such as linoleic acid is the main object of focus regarding this issue. Flaxseed oil is a known rich source of essential fatty acids with high content of α -linolenic acid which level depending on the seeds growing places and a row of the other factors is about 60% (Bardaa, 2016).

Visually observed changes on the rats' skin under treatment conditions by both plant oils used in the study were similar to those reported in the work (Bardaa, 2016). Whereas herein reported data were mainly focused on the evaluation of the dynamics of the wound healing process under treatment with flaxseed oil by using simple routine tests like blood analysis. Obtained experimental data provided in the "Results" section shows a positive dynamic in changes of the red blood system (erythrocytes and hemoglobin levels), platelets level, and level of leukocytes and their morphology in the experimental animals under flaxseed oil treatment, which effect turned out to be equal/comparable to the activity of hippophaerhamnoides fruit oil (Sławińska, 2022).

Above-mentioned blood changes can be explained by an increase in the leukocyte formation in the organs

of the hematopoiesis that occurs during infection and necrosis of tissues. On the other hand, also it is possible that leukocytes are mobilized from the basal pool to the circulatory, which occurs during stress with the release of adrenaline and glucocorticoids, in the case of severe emotions, pain, overheating, overcooling, in the case of the action of endotoxins of microorganisms, as a result of redistribution of blood in case of shock or collapse.

Conclusions

An experimental in vivo study of the healing effects of topically administered flaxseed oil in white rats with the planar trafaret wounds model was performed. Flaxseed oil components possess valuable healing properties and its use in a dose of 20 mg/cm² demonstrates potent and excellent healing activity which was equivalent and comparable with hippophaerhamnoides fruit oil effect in the same dose. The positive impact of flax oil flaxseed oil in rats with planar trafaret wounds on the processes and speed of wound healing was revealed, as well as its positive influence on the indicators of the complete blood count.

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Authors' contribution:

Pinyazhko O.R. – idea, research design, writing the article, conclusions;

Havrylyuk I.M. – collection and analysis of literature;

Antoniv O.I. – participation in writing the article;

Stepaniuk N.H. – annotations, aim, conclusions;

Semenenko S.I. – participation in writing the article.

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