

Study of Anti-Inflammatory Activity of Creams with Sapropel Extracts

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Abstract

Sapropel being a unique natural organic product, which due to multicomponent composition, has a wide range of pharmacological activity. The resources of sapropel in Ukraine comprise approximately 74.5 million tonnes. Taking into consideration significant natural resources of sapropel in Ukraine as a promising raw material for preparing effective medicines products, sapropel was used as an active ingredient for the development of the cream composition. The anti-inflammatory activity of creams with sapropel extracts on the model of acute photodynamic skin inflammation in animals (UV erythema) was investigated. It is established that the creams with sapropel extracts have expressed anti-inflammatory properties, reduce the intensity of acute photodynamic inflammation and cut down the treatment duration. The anti-inflammatory activity of the studied medicinal products largely depends on the concentration of the active substance.

Key words: Sapropel extracts, anti-inflammatory activity, UV erythema, comparator product.

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INTRODUCTION

Sapropel is bottom sediment of freshwater lakes which consists of aqueous solutions of mineral salts, low molecular organic compounds, vitamins, enzymes, residues of plant and animal origin and complex organic substances that give sapropel a gel-like consistency [1, 2].

Humic substances are the main group of water-soluble biologically active substances in sapropel. They have an expressed biological activity, inhibit the development of malignant tumors, have antiviral, and wound healing and anti-inflammatory activity [3-5].

Sapropel contains a lipid complex, which is represented by glycerides, saturated and unsaturated organic acids, phospholipids (lecithins, cephalins), and sterols. Carotenoids, xanthophylls, chlorophylls were found in the lipid fraction of sapropel [6].

The obtained results of the antiinflammatory and reparative activity studies of of sapropel extracts from sapropel of Prybych deposit on the model of thermal inflammation of rats show the ability to accelerate the granulation processes and epithelialization of tissues and the presence of anti-

inflammatory (anti-burn) action of sapropel extracts. More expressed antiinflammatory activity had the oil sapropel extract [4].

The objective of our work was to determine the presence of photoprotective activity of creams with sapropel extracts and justify pharmacological concentration of them in the cream composition.

MATERIALS AND METHODS

Materials

The objects of the study were the samples of creams with aqueous sapropel extract (SE) in the amount of 5 and 10%, creams containing both: aqueous SE in the amount of 5 and 10%, and oil SE in the amount of 15%, and the comparator product - "Panthenol" ointment, "Hemofarm AD", Serbia. Test samples were storage at the temperature 4°C.

The aqueous and oil SEs were obtained from sapropel Prybych deposit, located at Volyn region, Ukraine [7]. Sapropel treated with 0.1 N alkali solutions and used Cavitation at a speed of 3000 rot/min for 60 minutes, at 50 - 60° C to obtain a homogeneous mixture. The resulting extract was evaporated to 1:10 of basal volume. Stressed and by lemon acid adjusted to pH 7.0 [8, 9].

Two-phase extraction was used to obtain the oil SE. Dehydrated spropel (by processing native spropel with 95% ethyl alcohol in a ratio of 1: 2) is extracted with 90% ethyl alcohol in a ratio of 1:5 at the temperature of 50 ° C for 1 hour in a reflux flask. The obtained extract was concentrated under vacuum to 1:3 from the initial volume and then extracted with corn oil at the temperature of 50-60 ° C with stirring for 2-3 hours.

In accordance with investigated organoleptic and physical and chemical properties of cream prototypes with SE, was selected the emulsifying base containing corn oil, emulsifier no. 1, cetylstearyl alcohol (CSA) and purified water [10]. As the results of the carried out experimental investigations, the effect of the SE on physicochemical and rheological properties of its emulsion base has been determined. Increasing the concentration of SE in the emulsion basis results in an increase of pH value and a decrease in the viscosity of the cream. The incorporation of SE into an emulsion base in a concentration of up to 20% retains structural and mechanical properties of the base and, therefore, does not require any further correction of the composition [11]. The technological parameters of cream production with the spropel extract were substantiated and the technological scheme for its production was worked out [9].

The comparator product- "Pantenol" ointment made by "Hemofarm AD" (Serbia) contains the active ingredient- dexpanthenol in concentration of 50 mg / g, and according to the manufacturer's annotation, it is used for rapid healing of skin and mucous membranes of various origin injuries: scratches, thermal and sun burns, aseptic postoperative wounds, bullous and blister dermatitis, skin grafts.

Methods

Sampling

Researches on animals were conducted taking into account the "General Ethical Principles of Animal Experiments" (Ukraine, 2001), Law of Ukraine "On the Protection of Animals from Cruel Treatment" dated February 21, 2006, which was confirmed by the protocol of the Bioethical Examination Commission (No. 65 dated 8.11. 2017), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine No 249 dated March 1, 2012 on the "The order of holding animal investigations, animal experiments by scientific institutions " and in accordance with the provisions of the Directive of the European Parliament [12-14].

In preclinical studies were used experimental animals grown in the vivarium of the central research laboratory of the NPhU, Kharkiv (certified by the Ministry of Health of Ukraine (MHU), the certificate

No. 58/15 dated 12.08.2015, valid until 07.12.2019), which is equipped in accordance with current sanitary and hygienic standards. Animal researches were conducted on the basis of the problem laboratory of morphofunctional researches at the NPhU, Kharkiv (Accreditation Certificate of the National Accreditation Agency of Ukraine No. 2H1422 dated 07.09.2017, valid until 06.09.2022).

Method of ultraviolet (UV) erythema of skin inflammation in rats

Investigation of anti-inflammatory activity of cream samples with spropel extract was conducted on the model of ultraviolet (UV) erythema in rats [18].

The acute photodynamic skin injury in rats was caused by the irradiator Promin "ZEMI" with a mercury-quartz lamp of the DRT 125-1 type (the range of ultraviolet radiation is 230-400 nm).

Experimental animals were kept in standard sanitary conditions: during the experiment the animals were in the vivarium at a temperature of 19-24 ° C, at humidity no more than 50%, in a natural light regime "day-night", in plastic cages, and in a balanced diet (Health guide for the care and use of laboratory animals [15-17]). Before the experiment, the animals were acclimatized in the room for testing within 7 days.

The studies were conducted on clinically healthy white, non-breeding rats aged 3-4 months (females) weighing 180-220 g, which were kept in standard vivarium conditions, in a standard diet [15,17,18].

Experimental animals were divided into five groups, 6 animals in each (n = 6):

I – control pathology (CP) - untreated animals with ultraviolet erythema;

II – comparison group - animals with ultraviolet erythema (UV erythema) treated with the comparator product - "Panthenol" ointment ("Hemofarm AD", Serbia).

III – animals with UV erythema treated with the medicinal product - cream with aqueous SE of 10% and oil SE of 15 %;

IV – animals with UV erythema treated with the medicinal product - cream with aqueous SE of 5%;

V – animals with UV erythema treated with the medicinal product - cream with aqueous and oil SEs of 5% and 15 % respectively.

The day before the experiment, the animal wool was removed on the left side. Before irradiation, the animals were narcoded with barbamil (0.8 ml of an

1% aqueous solution of barbamyil per 100 g of animal mass), the depilated skin area was covered with a wide plaster with an opening of 1 cm². The irradiator was located at a distance of 10 cm from the animal; the exposure time was 60 seconds.

Preparations were applied to the skin surface at a conditional therapeutic dose of 25 mg / cm² immediately after irradiation, and then daily from day 1 to day 7 of the experiment [18].

The anti-inflammatory activity of the studied preparations was evaluated for 7 days by the skinfold

thickness, which was measured with a caliper in mm, and the condition of the skin.

The degree of inflammation on the skin of experimental animals was evaluated in points: 0 - the absence of erythema; 1 - weak erythema (pink tone), 2 - moderately expressed erythema (pinkish-red tone), fine skin peeling; 3 - expressed erythema (red tone), large-scale skin peeling, and spot hemorrhage; 4 - dramatically expressed erythema (bright red tone), local necrosis and hemorrhages [19].

Anti-inflammatory activity of drugs (AIA, %) determined by the formula:

$$AIA, \% = 100\% - \frac{II_{EG}}{II_{CP}}$$

Where II_{EG} - the intensity of inflammatory reaction on the animal skin of the experimental groups (EG), points

II_{CP} – the intensity of inflammatory reaction on the animal skin of the control pathology group (CP), points.

All experimental animals were cured for a different time period.

Statistical analysis

The statistical analysis of the obtained results was carried out by the variation statistics method using Student's t-criterion and using the personal computer and Microsoft Excel software with the definition of indices of the average arithmetic means (M), errors of the average arithmetic means (m); the probable difference between the comparison groups. The difference between the average arithmetic means was considered statistically significant at: * p < 0.05 [20].

RESULTS AND DISCUSSION

According to the data literature, sapropels contain vitamins, macro- and microelements, amino acids, humic substances, estrogen-like compounds, carbohydrates, fats, enzymes, antibiotics, which are associated with a wide range of their pharmacological activity [2].

The research results of the inflammatory process intensity on the skin, the anti-inflammatory activity of the investigational preparations, and the skinfold thickness of rats are presented in Tables 1-3.

According to the obtained data, the macroscopic signs of photodynamic skin inflammation developed gradually and in the control pathology group (CP) reached the maximum intensity on the 2nd-3rd day after irradiation (Table 1). Inflammation of the skin was characterized by swelling tissues, hyperemia, hemorrhages, and the development of hemorrhagic crusts with ulcers. Complete restoration of the skin, in accordance with the indicators of the inflammatory reaction intensity and the skinfold thickness, in the CP group was observed on the 14th day after irradiation

Table-1: Intensity of inflammatory reaction on the skin of rats on the model of acute photodynamic inflammation, points (M±m, n=6)

Group	Observation time, days					
	1	2	3	4	6	7
I	1,00±0,00	3,50±0,22	3,50±0,22	3,33±0,21	3,00±0,00	0,83±0,17
II	0,50±0,22*	2,67±0,21*	2,50±0,22*	1,83±0,17*	1,50±0,22*	1,33±0,21*
III	0,83±0,17	2,33±0,21*	2,17±0,31*	1,50±0,22*	1,33±0,21*	1,17±0,17*
IV	0,83±0,17	3,67±0,21#	3,67±0,21#	3,33±0,21#	3,17±0,17#	2,67±0,21#
V	0,83±0,17	2,50±0,22*	2,33±0,33*	1,67±0,21*	1,50±0,22*	1,33±0,21*

Notes: * – the difference is statistically significant with respect to the values of group I (CP), p<0,05; # – the difference is statistically significant with respect to the values of group II (comparison group), p<0,05.

On the background of gel treatment with sapropel extract on the 2-7th days of the experiment, there was a significant decrease in the intensity of

photodynamic skin inflammation of experimental animals, which was characterized by a decrease in

edema and hyperemia, and a decrease in the skinfold

thickness relative to the CP group (Table 2).

Table-2: The thickness of the skin fold in rats on the model of acute photodynamic skin inflammation, mm (M ± m, n=6)

Group	Observation time, days					
	Initial data	1	2	3	7	14
I	2,07±0,06	2,27±0,06	2,75±0,09	2,79±0,08	2,57±0,09	2,20±0,06
II	2,05±0,06	2,15±0,05	2,47±0,06*	2,43±0,09*	2,23±0,06*	2,12±0,06
III	2,03±0,03	2,20±0,05	2,48±0,07*	2,50±0,09*	2,32±0,06*	2,13±0,07
IV	2,05±0,03	2,23±0,05	2,65±0,06#	2,68±0,04#	2,50±0,05#	2,17±0,08
V	2,00±0,05	2,18±0,06	2,52±0,03*	2,50±0,04*	2,30±0,04*	2,10±0,04

Notes: * – the difference is statistically significant with respect to the values of group I (CP), $p < 0,05$. # – the difference is statistically significant with respect to the values of group II (comparison group), $p < 0,05$.

So, on the 2nd day after ultraviolet irradiation in groups III and V, the decrease in the intensity of skin injury was observed in comparison with CP to 33,33 and 28,57% respectively.

It should be noted that the anti-inflammatory activity of creams in groups III and V was at the level of the comparison drug, whereas in group IV it was significantly inferior to the reference drug (Table 3).

Table-3: Anti-inflammatory activity of the studied preparations on the model of acute photodynamic inflammation of the skin in rats, % (n=6)

Group	Observation time, days					
	1	2	3	4	6	7
II	50,00*	23,81*	28,57*	45,00*	50,00*	52,94*
III	16,67	33,33*	38,10*	55,00*	55,56*	58,82*
IV	-	-	-	-	-	-
V	16,67	28,57*	33,33*	50,00*	50,00*	52,94*

Notes: * – the difference is statistically significant with respect to the values of group I (CP), $p < 0,05$;

The decrease in the intensity of the inflammatory reaction, which we observed in groups III and V, was accompanied by a significant decrease in the skinfold thickness relative to the CP group by 9.8 and 8.4% respectively (Table 2).

On the 3rd day after ultraviolet irradiation, the anti-inflammatory activity of medicinal preparations with sapropel extract in groups III and V was 38.10 and 33.33% respectively (Table 3). The decrease in the intensity of the inflammatory response in groups III and V, was accompanied by a further decrease in the skinfold thickness in relation to the CP group by 10,04 and 10,04% respectively (Table 2).

On the 6th day after UV irradiation, in groups III and V, the anti-inflammatory activity of drugs with sapropel extracts was 55.56 and 50.00% respectively (table 3).

Similarly, on the 7th day after UV irradiation, in groups III and V a decrease of the inflammatory response relative to CP by 58.82 and 52.94%, respectively was observed (Table 3).

CONCLUSIONS

Medicinal products containing sapropel extract have expressed anti-inflammatory properties, can

reduce the intensity of acute photodynamic inflammation and cut down the treatment time.

The anti-inflammatory activity of the studied medicinal products depends largely on the concentration of the active substance. According to the increase in the effectiveness of the anti-inflammatory action the test samples can be arranged in the following order: group IV, group V and group III (the cream with aqueous and oil SEs in the amount of 10% and 15% respectively).

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