

# Study of Acute Toxicity and Specific Pharmacological Activity of “Acreya-N” Cream for Treatment of Skin Dryness

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## Abstract

**Introduction.** In the scientific work was presented data of literary sources, which show that the treatment of diseases that lead to skin dryness is the most common and serious pathology among dermatological diseases, which today tends to increase its frequency and remains an urgent problem of modern medicine. It has been proven that the prospect of using compounds of natural origin, in particular, the standardized substance of bee products - powder of honey for effective treatment of this pathology.

**Materials and methods.** The experimental part presents the results of preclinical biological tests for the study of acute toxicity and the specific pharmacological activity of Acreya-N cream for the treatment of dry skin. Laboratory, pharmacological, physiological, toxicological, instrumental methods of research and methods of mathematical statistics were used in this work.

**Results and discussion.** According to the results of the conducted pharmacological studies on the acute toxicity of the developed medicinal product, some toxicological parameters were identified, which indicate that the test samples of the developed Acreya-N cream for the treatment of dry skin, when applied to the skin in rats, are classified into grade VI toxicity - "Relatively harmless substances", and they do not exhibit local irritation on the skin of experimental rabbits.

In the model of skin inflammation in guinea pigs caused by exposure to ultraviolet light, the expressed anti-inflammatory and antipyretic activity of the Acreya-H test cream for dry skin in comparison with the reference drug Panthenol was established.

**Key words:** acute toxicity, anti-inflammatory activity, cream for dry skin, local irritation effect.

## INTRODUCTION

Nowadays the problem of skin xerosis treatment is a topical issue of modern medicine [1, 5]. This pathology often manifests itself as a sensation of skin absorption, its redness, itching and peeling to the formation of cracks accompanied by pain, in connection with which it loses elasticity and becomes more sensitive to allergic manifestations [3]. Therefore, the creation of medicines for local use, which combine active pharmaceutical ingredients of anti-inflammatory, antispastic, reparative, softening and moisturizing action aimed at eliminating pathomorphological disorders of the skin, is one of the main tasks of dermatology, the solution of which is leading place occupy a comprehensive approach to treatment using soft medical forms - ointments, gels, creams, etc. [7, 15].

Given the need to eliminate the effects of inflammation associated with skin dryness, it is expedient to combine, in the form of complex medicinal preparations, compounds of natural origin, in particular products of beekeeping, which exhibit a wide range of pharmacological activity [1, 16, 17], and substances that suppress and eliminate the symptoms of xerosis [7]. In this regard, the spectrum of pharmacological action of api-products determines their prospects for use in medicinal forms for the local treatment of skin xerosis.

In order to expand the range of drugs which are manufactured in Ukraine for treatment of pathologies associated with dry skin, we have developed the composition and technology of the combined pharmaceutical formulation in the form of a cream under the conventional name "Acreya-N" on the basis of the standardized substance of bee products - powdered honey (TC U 10.8-39834691-001:2015) [14], allantoin, almond seed oils, macadamia oils, and also adjuvants that are widely used in medical practice [11].

The purpose of this work was to conduct preclinical biological studies to research acute toxicity and specific

pharmacological activity of the "Acreya-N" cream that was developed for the treatment of skin xerosis.

## MATERIALS AND METHODS

Laboratory, pharmacological, physiological, toxicological, instrumental methods of research and methods of mathematical statistics were used in this work.

In accordance with the Methodological Recommendations SEC MOH of Ukraine [8] when conducting preclinical pharmacological studies of a new perspective medicinal product, a mandatory fragment is the determination of the degree of its toxicity, which makes it possible to assess its harmlessness and the prospects for further introduction into production. It is believed that the greater the effectiveness and harmlessness of the drug, the wider its possible using in medical practice [4, 8]. In addition, the study of acute toxicity is a step aimed at obtaining information on the safety / danger of a pharmacological agent for health in conditions of short-term use in overdose. Conducting this type of research allows obtaining the necessary information to determine the level of toxicity of the pharmacological drug (FD) and to identify the organs and systems - the targets of potential toxic effects of the medicinal product.

The study of the acute toxicity of the developed Acreya-N cream for the treatment of dry skin was performed on the model of skin inflammation of rats caused by UV irradiation in the case of their application on the skin. Experimental tests were carried out in sexually mature rats of both sexes aged 3-4 months, obtained from the vivarium of the Central scientific-research laboratory of the National University of Pharmacy, which is equipped in accordance with existing sanitary and hygienic norms [2, 6, 10].

The research was conducted in compliance with the rules of the National "General Ethical Principles of Animal Experiments" (Ukraine, 2001), which comply with the provisions of the "European Convention for the Protection of Vertebrate

Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [2, 6].

Before the experiment had begun, the animals were acclimatized in the room for testing within 7 days. In the 24 hours prior to the testing of the investigational medicinal product, animals were wiped off. In accordance with the methodological recommendations [6, 18] the area of application of means for the application of the skin should be not less than 10% of the total area of the body of the experimental animal. Cream for dry skin was applied at the maximum dose of class VI toxicity - 22,6 g / kg. Animal access to water and food was free. To exclude the possibility of influencing the test sample on the results of the study of the basis of the cream, it was studied separately, using as a negative control in a similar dose - 22.6 g / kg. The number of test specimen and its bases were calculated relative to the weight of the animal's body and applied with a spatula carefully rubbing into the skin. Experiment results are given in Table 1.

### RESULTS AND DISCUSSION

According to the methodological recommendations during the whole period of the experiment, daily observations were made of the general physiological state. Evaluated the condition of the wool and mucous membranes of animals, observed their behavior, consumption of food and water, and also recorded their survival. The observation period for animals was 14 days after the application of the test specimens. The body mass dynamics was studied at 3rd, 7th and 14th day of the experiment [6, 18].

At the end of the observation period, rats were depleted by overdose of inhaled anesthesia and an autopsy and a macroscopic examination of their internal organs were performed. The results of the acute toxicity study of Acrea-N Cream for dry skin at the application of the skin are given in Table 2.

After the application of test-examples on the skin at a maximum dose of 22.6 g / kg, no signs of intoxication in animals were observed: the animals were clean, active, had a normal appetite, responded to sound and light irritants, the processes of urination and defecation were normal, breathing and the court

were not observed. Also, reflex excitability was preserved in all animals.

According to the methodological recommendations for the study of acute toxicity, an estimate was made for the increase in the body weight of animals, the results of which are given in Table 3.

The experimental data obtained were processed by the method of variation statistics (the arithmetic mean and standard error were calculated) [9]. For comparison of normal distribution, one-factor ANOVA analysis and the Newman-Keuls criterion for multiple comparisons were used, for nonparametric data, the Kruskal-Wallis (ANOVA) and the Mann-Whitney Criterion. The verification of the normal distribution of the actual data was performed using Leven's test [13]. Differences between groups were considered statistically significant at  $p < 0,05$ .

Statistical processing of the data was carried out using a program package Statistica 6.0 [9, 13].

Determination of the body weight of the experimental rats (Table 3) showed that the application of the cream for dry skin at the maximum dose did not mainly affect the growth of body weight, indicating the absence of toxic properties in test samples that could disrupt the whole-pathogenic processes of the mammalian organism.

At the end of the observation period (14 days), animals were eradicated, an autopsy and a macroscopic examination of the internal organs of the chest cavity were performed, the results of which indicated that the heart remained in a state of normal configuration and normal size, the lungs remained pale pink, filling the entire pleural cavity, without adhesions between pleura leaves. Larger lymph nodes were not enlarged.

The placement of organs was anatomically correct without third-party content in the peritoneal cavity. The liver was evenly reddish-brown color, the capsule remained not tense, the lateral edge of its particles not rounded. The pancreas was pale pinkish-yellow, similar to fatty tissue, without signs of multiple sclerosis and fatty necrosis. The spleen remained elastic, full-blooded, of red-cherry color.

**Table 1 Results of an experiment on the study of acute toxicity of a cream for dry skin at the skin application to male rats**

Experimental groups	Drug-path	Type/animal sex	Number of animals	Dose, g / kg
Intact control	–	–	–	–
Negative control (base of the cream)	cutaneous	rats / males	6	22,6
Cream "Acreya-N" for dry skin			6	22,6

**Table 2 Results of the analysis of the survival of white sexually mature male-rats after skin-application of the cream "Acreya-N" for dry skin**

Animal groups	Animal sex	Drug-path	Dose, g / kg	Number of dead animals / total number of animals in group
Intact control	males	–	–	0/6
Negative control (base of the cream)	males	cutaneous	22,6	0/6
Cream "Acreya-N" for dry skin	males	cutaneous	22,6	0/6

**Table 3 Dynamics of the mass of animals (g) in the study of acute toxicity of the cream "Acreya-N" for dry skin after skin-application to male rats, n=6 (M±m)**

Animal groups	The term of observation			
	Output data	3-d day	7-th day	14-th day
Intact control	200±4	210±3	217±5*	240±4*
Negative control (base of the cream)	200±3	212±3*	219±2*	240±3*
Cream "Acreya-N" for dry skin	205±2	211±3	218±3*	238±4*

Dispersion analysis (ANOVA RM) and the Newman-Keuls criterion;

\* – the differences are statistically significant for the output data,  $p < 0,05$ .

n – number of animals in the group.

The capsule of the kidneys is easily removed, on the section of the organ can be traced dense with the preservation of the drawing layers. Adrenal glands are remained without signs. The retroperitoneal lymph nodes were not enlarged. The mucous membrane of the glandular part of the stomach was characterized by a characteristic relief of folds, normal color, without hemorrhages, edema and erosive damages.

Thus, the results of the macroscopic examination of the internal organs of rats, which applied to the skin Acreya-N cream for dry skin, indicate that there is no toxic effect of the test sample on the general-pathogenic processes of the rats. The mean daily dose of the dry skin cream for skin application is more than 22.6 g / kg ( $LD_{50} > 22.6$  g / kg, Table 4).

**Table 4 Mid-lethal doses of cream "Acreya-N" for dry skin and its base during applying on the skin by white non-breeding male rats**

Experimental group	$LD_{50}$ , g / kg
Negative control (cream base)	>22,6
Cream for dry skin	>22,6

Therefore, the obtained results has showed that the application of the **Acreya-N** test cream for dry skin does not lead to the death of animals and macroscopically apparent pathological changes in the internal organs of rats. In accordance with the classification of substances for toxicity developed cream for dry skin belongs to the VI class toxicity - relatively harmless substances.

In the preclinical study of pharmacological studies, investigations of the acne-sensitive cream of **Acreya-N** for dry skin were also conducted with a single application of rabbits. The test of acute irritant action of the cream for dry skin was performed on 6 non-breeding white rabbits-males and females with a body weight of 2.0-2.5 kg [12]. To determine the irritant effect of the test sample, 3 parts of the skin (with hair cut out 24 hours before the start of the experiment without any pathological signs) were used in each animal with an area of 6 cm<sup>2</sup> each:

- 1 – intact site (intact control);
- 2 – the area on which the cream for dry skin was applied;
- 3 – the area on which the basis of a cream for dry skin was applied.

Each animal was used as its own control. The test specimens under study were applied at a dose of 0.5 g per animal and fixed on the skin using a 12-layer gauze napkin, which was secured with a surgical tape. The intact area of the skin was covered with a surgical napkin. The exposure period was 24 hours.

After the end of the exposure period, the animal's skin was released from the napkin and washed with water. Registration of the potentially irritant action was carried out three times: 1 hour after removal of the gauze wipes with the test samples (25 hours after the start of the exposure, "1"), 48 ("2") and 72 hours ("3") after the start of the exposure. For each animal, the presence of erythema, scapular, edema and evaluation of skin reaction in balls according to the appropriate scale were determined. [6]. The total degree of irritation (TDI) for each rabbit was calculated by the sum of points ("1" + "2" + "3"). Then the index of direct skin

irritation (IDI) was calculated according to the formula:  $IDI = \frac{TDI}{3} \times n$ , where

- 3 - number of observations;
- n - the number of animals.

Finally, the degree of irritation of the tested test samples was estimated by the size of the IDI as follows:

- 0,0 – irritation is absent;
- 0,0 – 0,5 – irritation that can be neglected;
- 0,5 – 2,0 – moderate irritation;
- 2,0 – 5,0 – average irritation;
- 5,0 – 8,0 – strong irritation.

The results of the experiment are presented in the table 5.

According to the results of the study of local irritation of the cream "Acreya-N" for dry skin with one-time application to rabbit skin (Table 5), it was found that 24-hour exposure of a cream for dry skin and its base in a dose of 0.5 ml per animal to the skin of rabbits does not lead to any manifestations of local irritation (erythematous changes, edema, signs of necrosis).

Thus, the results of the conducted research indicate that there is no evidence of local inflammatory action of a cream for single-application application in test samples.

Investigation of anti-inflammatory and antipyretic effect of Acreya-N cream for dry skin was performed on the model of inflammation of guinea pigs skin caused by UV irradiation. Experiments were carried out on 25 guinea pigs of light suit. Animals in the area on the right side in the size of 5 × 5 cm<sup>2</sup> removed the wool, fixed with elastic bandages to the table, and on a nude section of the hand was laid stencil sized 3 × 3 cm<sup>2</sup>. At a distance of 15 cm, irradiation was performed with UV light using a 250 W quartz lamp for 1 minute [6].

The severity of erythema or degree of skin damage was assessed on a points scale:

- 0 point – absence of visible signs of erythema or skin damage;
- 0,1-0,5 point – weak erythema;
- 1 point – pronounced erythema;
- 2 points – slight skin damage;
- 3 бали – significant skin damage (peptic ulcer).

The investigated drugs were applied after 1 hour after erythema simulation and continued to be applied until complete wound healing (26 days). Anti-inflammatory activity was assessed by reducing the severity of inflammation and the level of leukocytes in the blood, which are markers of the inflammatory process.

According to the results of the study of anti-inflammatory and antipyretic effects of Acreya-N cream for dry skin on a model of ultraviolet erythema in guinea-pigs, it was found that, according to the data obtained, irradiation with ultraviolet light led to a distinct animal skin burn. In place of irradiation, guinea-pigs formed wounds with crust and erythema surrounding tissues. The severity of skin damage as a whole was estimated at 2.8 points (table 6), according to the results of which it was found that for 15 days the severity of inflammation in the animal group of positive control remained at one level. Subsequently, spontaneous healing of wounds was observed, as evidenced by a reduction in wound area and a decrease in the severity of inflammation and skin damage.

**Table 5 Skin reactivation of rabbits for a 24-hour cream dry skin exposure, in balls**

Group	Term of research						IDI
	Erythema			Edema			
	25 h.	48 h.	72 h.	25 h.	48 h.	72 h.	
Intact control	0	0	0	0	0	0	0
Cream "Acreya-N" for dry skin	0	0	0	0	0	0	0
Cream base for dry skin	0	0	0	0	0	0	0

**Table 6 Effect of Acreya-N cream for dry skin on the dynamics of skin inflammation in guinea pigs caused by UV irradiation, n=5-6 (M (min; max))**

Animal groups	Severity of inflammation, points					
	Initial data	5-th day	10-th day	15-th day	20-th day	25-th day
Positive control	2,8 (2; 3)	2,8 (2; 3)	2,8 (2; 3)	2,7 (2; 3)	1,8 (1; 2)	1,3 (0; 2)
Reference drug Panthenol	2,8 (2; 3)	2,2 (0,5; 3)	2,0 (0; 3)	1,3 (0; 2,5)*	0,6 (0; 1)*	0,2 (0; 0,5)*
Cream "Acreya-N" for dry skin	2,8 (2; 3)	3 (3; 3)	2,0 (1; 3)	1,2 (0; 2)*	0,6 (0; 2)*	0,2 (0; 1)*

1. Variance analysis (ANOVA RM) and Newman-Keuls criterion;

2. \* - the differences are statistically significant for positive control, p <0,05;

3. n - number of animals in the group.

**Table 7 Influence of "Acreya-N" cream for dry skin on the level of leukocytes in blood of guinea pigs in case of inflammation of the skin caused UV irradiation, n=5-6 (M (min; max))**

Animal groups	Severity of inflammation, points			
	5-th day	10-th day	20-th day	25-th day
Intact control	8,55 (7,75; 9,75)	8,45 (7; 9)	8,4 (7,5; 9,5)	8,6 (7,3; 9,5)
Positive control	16,4 * (14,5; 19,75)	14,33 * (12,25; 15,5)	14,1 * (11,25; 15,75)	12,3 * (10,5; 14,0)
Reference drug Panthenol	11,9 ** (7,5; 17,75)	10,8 ** (8,5; 15,0)	10,25 ** (6; 16,25)	7,85 ** (6,25; 9,25)
Cream "Acreya-N" for dry skin	11,3 ** (8,25; 14,75)	9,6 ** (8,75; 11,25)	9,6 ** (8,5; 11,0)	9,15 ** (7,5; 11,5)

1. Variance analysis (ANOVA RM) and Newman-Keuls criterion;

2. \* - the differences are statistically significant for intact control, p <0,05;

3. \*\* - the differences are statistically significant for positive control, p <0,05;

4. n - number of animals in the group.

Increasing the content of leukocytes in blood of guinea pigs by 2 times in 5-th day of the experiment indicated an active inflammatory process caused by UV radiation (table 7).

It should also be noted that the statistically significant increase in the content of leukocytes in the blood of untreated animals was maintained throughout the entire period of observation (table 7).

The application of the Acreya-N test cream for dry skin contributed to lowering the severity of inflammation and more rapid healing of wounds. At 5-10 days of the experiment recorded decrease in the severity of inflammation, but not reliable (table 6). At day 15, the severity of inflammation under the influence of the investigated means was statistically significantly lower compared with the positive control group. At the rate of healing of wounds caused by UV irradiation, Acreya-N cream for dry skin was not inferior to the reference drug Panthenol. The recovery of leukocyte contents in guinea pigs blood under the influence of the test substance confirmed the effectiveness of the developed dry skin cream on this model (table 7).

Thus, under the condition of UV irradiation, was established the effectiveness of Acreya-N cream which was developed for dry skin at the level of the reference drug Panthenol.

#### CONCLUSIONS

1. A study was conducted of acute toxicity and specific pharmacological activity of Acreya-N cream for the treatment of dry skin.
2. Determination of some toxicological parameters showed that the "Acreya-N" cream for dry skin during applying to the skin, belongs to the VI class of toxicity - "Relatively harmless substances", but it does not show local irritation on the skin of rabbits.
3. In the model of skin inflammation in guinea pigs caused by UV irradiation, the expressed anti-inflammatory properties of

Acreya-N cream for dry skin were established in comparison with the reference drug Panthenol.

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