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Studying "Propolis-Gel" pharmacological activity

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Abstract
The results of conducted preclinical studies of "Propolis" gel based on Propolis Phenolic hydrophobic medication (PPHM) and azelaic acid (KA) had been enlighted in present article.
Pharmacological studies included the screening of finding the optimal composition of the gel by antiexudative action, choosing the optimal amount of gel, the membrane-stabilizing effect of the gel and research of the toxicological characteristics of the "Propolis" gel.
It has been found that the gel contains PPHM 2,0% and AA, and possesses a maximum antiexudative effect. The optimum amount of the "Propolis" gel for skin application is equal to 12.5 mg/cm2. It has been proved that the "Propolis" gel has a pronounced membrane-protective effect, and it is its main distinction fromthe comparing medication – the "Scinoren" gel 15%. As for acute toxicity parameters the "Propolis" gel refers to a class of practically non-toxic substances.

Keywords: gel, Propolis phenolic hydrophobic medication (PPHM), anti-inflammatory action, membrane-stabilizing action, acute toxicity.

1. Introduction
The prevalence of skin diseases largely determine the scope of dermatological care provision. One of the features of the current state of medical services in the national dermatology is a prevalence of certain skin diseases, for treating of which patients seek for specialized medical care. Such syndrom as acne is one of the most common pathologies in dermatology and cosmetology of present time [6].
According to medical statistics data, 85% of people aged 12 to 24 years are suffering from acne eruption, the number of the aforementioned pathologies is being reduced in the age groups 25-34 and 35-44 years, the incidence in these groups is 8% and 3% respectively [9, 14].
Present problem is important today due not only to high morbidity, recurrent or severe course, but also to the difficulties in the treatment and secondary prevention of this skin pathology. Unlike many dermatoses, the acne mainly affects the face, which is one of the main and major parts of interpersonal and social communication [10]. According to modern concepts, the pathogenesis of acne is closely associated to the hypersecretion of sebum and increased proliferative activity in the area of the hair follicle, intensive and excessive colonization of the skin and appendages of Rropionibacterium acnes (P. acnes), which causes the development of inflammatory reactions, besides the impact of hyperandrogenism on the follicular keratinization had been established as well [13, 15]. Genetic characteristics of the patient tend to be an important factor in the development of acne. Probability of incidence of acne for adolescents in case both parents had it, equals to 50%. It was established that the heavier the extent of the disease, the more often the genetic correlation with the presence of the disease in close relatives is being observed. [16] Many acne researchers put this disease in a direct dependence on the way of nutrition and the state of the gastrointestinal tract of the patient. It was found that more than in more than 50% of cases the developement of acne inducing gastritis and an existing dysbiosis, in 30% of cases – other intestinal pathologies.
The main aspect of the pathogenesis of this disease is an imbalance of «lipid peroxidation/antioxidant protection" [8]. Granulocytes and macrophages represent the most powerful source of free radicals as well as primary and secondary products of their transformations. Reactive oxygen species (ROS) produced by neutrophils, are also involved in the damage and destruction in the follicle wallside in case of acne disease. It has been established that accumulation of products of lipids free radical oxidation in the skin causes damage to the membranes both of individual cells and the entire body, causing dysfunction of immune cells and other organs [11]. Despite numerous clinical and laboratory research, much of the pathogenesis of this disease remains unexplored. As a result there is a lack of effective treatment and prevention approaches.

All abovementioned facts make finding and creating new effective treatment or prevention of disease urgent and actual.

Currently, according to the of the Ministry of Health of Ukraine State Expert Center (MOHUSEC), the State Register of Medicinal Products of Ukraine, namely the group D10 "for the treatment of acne" (Anatomical Therapeutic Chemical Classification System) includes 25 medicines,4 of which are the medicines of systemic action and 21 are topical medicines. As for the medicines of local action, 38% of them contain antibiotics, 23% are based on sulfur or zinc, 20% contain adapalene and 19% (4 medications) based on azelaic acid. They are "Skinoren" Gel 15% and 20% (Bayer Helthcare manufacturing S.R.L, Italy), "Azohel" 15% (JSC "Fitofarm", Ukraine) and "Acnestol" (JSC "Kievmedpreparat", Ukraine). Analyzing these data, it should be noted that the pharmaceutical market is practically deprived of complex preparations for acne treatment, which would be focused on the pathogenesis of the disease, but not just on decreasing the symptoms. Besides the quantity of locally produced medicines is too low.

Thus the development of new effective drugs of the complex formula that would provide polymodal pharmacological effect, and would be able to influence the pathogenetic links of acne is a topical issue in modern medicine and pharmacy.

One of the most promising substances in this aspect is Propolis phenolic hydrophobic medication (PPHM) and azelaic acid (AA). PPHM is a standardized natural substance – a light brown powder, which is obtained with the help of unique technology from propolis – bees waste product [7]. It has antimicrobial, anti-inflammatory, and antipruritic and analgetising properties that promote the growth of granulation, accelerate of regeneration and epithelialization processes on the wounded surface, and stimulate metabolic processes.AA is a limiting dibasic carboxylic acid. The AA therapeutic efficacy for acne treatment is determined by its antimicrobial action and a direct influence on follicular hyperkeratosis; a significant reduction in colonization density of Propionibacterium acnes and a significant reduction in fraction of free fatty acids in the skin surface lipids, besides the AA inhibits the proliferation of keratinocytes and normalizes the disturbance of terminal differentiation of the epidermis in the formation of acne.

The goal of this study is to conduct experimental research aimed at finding the optimal composition of complex drug based on PPHM in the form of gel for the treatment of inflammatory dermatological diseases, for which there have been found a wide spectrum of antibacterial activities in previous studies [1, 7] as well as pharmacological studies of a new gel.

2. Materials and methods of research

As PPHM substance is the main component of the gel, having distinct antioxidant properties, the screening tests aimed at search of the optimal composition of the new gel have been performed on models formalin inflammation in the mechanism of which, along with the launch of a cascade of pro-inflammatory mediators and cytokines, the leading action is the activation of free radical oxidation that causes damage to membrane proteins [3].

The test sample of the "Propolis" gel with different concentrations of the PPHM substance have been explored: test sample №1 – PPHM 1,5%, dissolved in propylene glycol and AA 8%; Test sample №2 – PPHM 2,0%, dissolved in propylene glycol and AA 8%; Test sample №3 – PPHM 3,0%, dissolved in propylene glycol and AA 8%.

The experiments had been conducted on animals weighing 210-250. The following groups of animals have been used: 1-st group of rats – control pathology, 2nd to 4th groups – the "Propolis" had been aplied on animals' paws, various concentrations of PPHM had been used – 1 5%, 2% and 3%, 5th group of animals – the comparator "Skinoren" gel 15% had been applied (production: Bayer Helsker Manufakturinh SRL, Italy, 1 g contains 0.15 g AA). The investigated products and the comparator was applied for 40 minutes in case of modeling local inflammation and for 30 minutes after the administration of formalin. Swelling of the foot for rats caused the introduction of 0.1 ml of 2% formalin solution under the aponeurotic plate of the right paw of rats [3]. The development of edema had been observed for 6 hours. Paw thickness had been measured by mechanical oncometer both before the introduction of formalin (raw data) and through 0.5, 1, 2, 3 and 6 hours. Growth in size of paw was calculated by the difference in paw thickness after administration of formalin and the output one.

Anti-inflammatory activity (AIA) of the "Propolis" gel and the comparator product was determined by the ability to reduce the swelling of the legs of experimental animals compared with the control animals and expressed in%.

The next step of the study pharmacological compositions of the leader according to the antiexudative activity test sample №2, containing PPHM 2,0%, which had been dissolved in propylene glycol and AA 8% (the "Propolis" gel) was the selection of the optimal amount of gel.

To determine the optimum amount of gel "Propolis" for skin application, the test sample have been applied to the skin area of 4 cm2 (2x2 cm) and was rubbed into the skin of the animal for 30 seconds. The evaluation was performed visually according to the view of the skin. The optimal amount of gel had been considered the one, after the rubbing of which the skin remained bright and sufficiently hydrated. If the skin remained dry, not damp – the amount of gel had considered to be insufficient; if the skin had a gloss then the number of gel had been considered to be excessive. [3] Determining the optimal number of test performed on a sample of white male rats. 24 hours before applying the "Propolis" gel on animals, on the right side there have been shaved an area of 4 cm2 (2x2 cm) where the gel was applied in an amount of 40 mg for the 1st group of animals, 50 mg – for 2nd group of animals, and 60 mg for 3rd group of animals. Testing of each amount of the gel had been conducted on three animals.

A prerequisite for manifestation of anti-inflammatory and cytoprotective effect of the drug is the presence of membrane-protective effect of the "Propolis" gel, which was studied in a model of spontaneous erythrocyte hemolysis by Jager F.C. [1]. As the comparator the "Skinoren" Gel 15% had been chosen.

The studies of acute toxicity of the "Propolis" gel had been performed on mature rats of both sexes aged 3-4 months, grown in the vivarium of CSRL pharmacy, equipped in accordance with existing sanitary standards and by the methods recommended by the Ministry of Health of Ukraine [5].

The statistical analysis of the results of research had been conducted by means of generally accepted in pharmacology methods by determining the arithmetic values (X) and standard error (SE), the reliability of differences was determined by Student's t-test at a level of at least 95% (p-0,05) [4]. Summary data for the study of acute toxicity of "Propolis" gel had been expressed as the mean and its standard error (mean ± St. er).

For statistical conclusions when comparing samples relative variables used single-factor analysis of variance and Daneth criterion. Differences between control and experimental groups were considered statistically significant at condition of $p<0.05$. Statistical data processing was performed using standard statistical software package «Statistica v.6,0».

All studies have been conducted in compliance with the rules of bioethics – the humane treatment of animals in accordance with the provisions of the European Convention for the protection of laboratory animals (c. Strasbourg, 1986) [5].

3. Results and its Discussion

It is known that in the inflammation pathogenesis of various origins the significant role belongs to free radicals, which possess highly reactive ability. Today, there is evidence of intensification of free radical oxidation processes (FRO) in patients with acne disese. The largest number of primary free radicals and secondary products of their transformation is generated by granulocytes and macrophages both resident and migrating into the skin. ROS produced by neutrophils, are involved in the damage and destruction of the wall of the follicle in acne [8]. Therefore, one of the most promising approaches to the treatment of such diseases, along with the means of distinct inflammatory effect is the use of compounds which exhibit antioxidant properties. As the main component of the gel is a substance PPHM, which shows distinct antioxidant properties, the study of anti-inflammatory effect of investigational product had been carried out on the model of formalin inflammation in the mechanism of developing of which, along with the launch of a cascade of pro-inflammatory mediators and cytokines, the leading place had been taken by the activation of free radical oxidation that had caused the damage to membrane proteins [11, 12]. Results of the study are presented in Table 1.

It has been established that in the control group of animals with pathology the introduction of formalin induced the paw edema development, which was most pronounced on 3th to 4th hour of the experiment. Therapeutic and prophylactic application of "Propolis" gel containing various concentrations of PPHM had helped to reduce severity of rats' paws edema. The investigational agent had showed the significant antiexudative action that, by severity, did not differ from comparison drug, the "Skinoren" gel.

The "Propolis" gel containing PPHM at concentrations of 1.5% and 2% had shown almost the same activity. With increasing concentration of PPHM to 3% the antiexudative action of "Propolis" gel were reducing, but also remained at the level of the drug comparison, the "Skinoren" gel.

So, according to the expressiveness of antiexudative action the studied medications can be arranged in ascending order as follows: "Propolis" gel 3% (AIA – 39%), the comparator, "Skinoren" gel (AIA – 43%), "Propolis" gel 1.5% (AIA – 56%) and "Propolis" gel 2% (AIA – 57%).

Thus, the results of the study found a marked antiexudative effect of the "Propolis" gel. The greatest activity is defined while the application of PPHM medium containing a concentration of 2%, which makes the feasibility of further studies of the "Propolis".

Table1: The impact of "Propolis" gel with different concentrations of PPHM on the development of formalin paw edema in rats (2% formalin solution of 0.1 ml/ animal) in comparison to "Skinoren" gel, n = 6

Gropus of animals	Term of observation					AIA for 6 h., %
	0,5 h.	1 h.	2 h.	3 h.	6 h.	
Control pathology	11,2±1,9	13,5±1,4	12,2±1,6	10,0±1,4	9,6±1,4	–
“Propolis” gel 1,5%	5,0±0,7*	7,5±0,8*	5,8±0,8*	3,5±0,7*	3,7±0,6*	56
“Propolis” gel 2%	5,0±1,2*	8,7±1,2*	5,5±1,0*	2,8±0,8*	3,2±0,7*	57
“Propolis” gel 3%	7,5±0,9*	9,3±1,6*	8,2±1,8*	5,0±0,89*	5,0±0,93*	39
“Skinoren” gel 15%	6,2±1,0*	10,2±1,2	7,3±0,8*	4,8±0,3*	4,5±0,2*	43

Notes: 1. * − differences are reliable as to data of positive control, $p<0,05$;
2. n – quantity of animals in each group.
3. AIA – anti-inflammatory activity

The next stage of research was to establish the optimum amount of "Propolis" (Table 2). According to the data, the optimal number for application of Propolis to the skin is 50 mg/4 cm2, which converted to 1 cm2 is equal to 12.5 mg.

Table 2: Results of the study of optimum amount of "Propolis" gel for application to the skin of animals

Sample test	Condition of the skin within 30 seconds after application of the "Propolis" gel.		
	40 mg/4 cm^2	50 mg/4 cm^2	60 mg/4 cm^2
“Propolis” gel	skin remains dry	skin is hydrated enough	skin is hydrated excessively

In order to establish membrane-protecting effect of the "Propolis" gel at a dose of 12.5 mg per 1 cm2, the mentioned amount had been applied to the sides of shaven parts of skin on bodies of male rats (4 sq. cm) for 7 days. After 40 minutes after the last application the study of possible membrane-protecting effect had been conducted of the new gel models of spontaneous erythrocyte hemolysis by Jager F.C. The results are shown in Table 3.

Table 3: Membrane-protecting effect of “Propolis” gel on the model of spontaneous erythrocyte hemolysis by Jager F.C., (n=8)

№	Terms of the experiment	The degree of erythrocyte hemolysis, %	Membrane-stabilizing activity, %	P
1	Intact control	28,2 ± 1,48	-	-
2	“Propolis” gel	19,5 ± 1,31	30,8	$p_{2-1}<0,01$ $P_{2-3}<0,05$
3	“Skinoren” gel	24,5 ± 1,72	15,1	

For animals of intact control group, the degree of hemolysis of red blood cells caused by activation of oxygen peroxide process of membrane lipids, is 28,2 ± 1,48%.

The "Skinoren" gel possess 15%, on condition if the previous 7-days application, has a little membrane-protective effect, that has no statistically significance with the index of intact control.

Unlike the reference medication, the new gel – Propolis "Propolis" significantly reduces the degree of hemolysis of erythrocytes in 1.4 times ($p<0$, 01) related to intact control group, indicating the presence of a large membrane-stabilizing activity.

Membrane–protective effect of "Propolis" gel is probably being realized due to the introduction of PPHM to the new gel, as this substance contains significant amount of polyphenols, such as catechins, flavones, flavonols, anthocyanins and more. It is known that membrane-protective effect of polyphenolsexists due to their ability to reduce destructive peroxidation processes as well as to neutralize ROS and prevent damage to the cell membranes.

Thus, according to the results of the experiment considerable advantages of the new medication – the "Propolis" gel in comparison to its famous drug reference – the "Skinoren" 15% gel have been installed.

The neccessary condition of pre-clinical research of new drugs is the study of their toxicological profile. The results of study of the level of toxicity of "Propolis" gel in cutaneous application are presented in Table 4.

After application of the maximum dose (15,000 mg / kg) on the skin, no signs of toxicity in animals were observed, animals were neat, active, had a normal appetite, responded to sound and light stimuli, the process of urination and bowel movements were normal, no respiratory failure or spasms were observed. Reflex excitability of all animals was normal.

According to the guidelines for the study of acute toxicity, the studies of increase in body weight have been made, results can be seen in Table 5 and 6.

After application of the maximum dose (15,000 mg/kg) on the skin, no signs of toxicity in animals were observed, animals were neat, active, had a normal appetite, responded to sound and light stimuli, the process of urination and bowel movements were normal, no respiratory failure or spasms were observed. Reflex excitability of all animals was normal.

Table 4: Mortality of white outbred mature rats of both sexes after applying the "Propolis" gel.

Groups of animals	Sex of animal	Dose, mg/kg	Quantity of dead animals/ total quantity of animals in the group
Negative control group (gel base)	male	15000	0/6
	female		0/6
“Propolis” gel	male	15000	0/6
	female		0/6

According to the guidelines for the study of acute toxicity, the studies of increase in body weight have been made; results can be seen in Table 5 and 6.

Table 5: The dynamics of the animal weight (g) in the study of acute toxicity of "Propolis" gel in male rats cutaneous application, n=6 (M±m)

Groups of animals	Term of observation			
	Output data	3 day	7 days	14 days
Negative control group (gel base)	212,5±4,8	211,7±5,1	215,0±5,5	220,0±3,7
“Propolis” gel	210±4,3	211,6±3,3	214,2±3,0	222,5±3,1

Table 6: The dynamics of the animal weight (g) in the study of acute toxicity of "Propolis" gel in female rats cutaneous application, n=6 (M±m).

Groups of animals	Term of observation			
	Output data	3 day	7 days	14 days
Negative control group (gel base)	203±4,6	205±3,4	206,7±3,3	215,0±3,4
“Propolis” gel	205,8±4,0	205,0±4,3	206,7±3,3	215,0±3,4

Note: Dispersed analysis (ANOVA RM) and Danethe criterion

Determination of the body weight of experimental rats showed that applying the gel "Propolis" in its maximum dose had no effect on body weight gain, indicating the absence of toxic properties of the topical agent that could dramatically affect body processes of mammals.

After the observation period (14 days) the animals have been mortified and an autopsy and macroscopic examination of internal organs had been implemented. In the study of the skin, mucous membranes orifices no signs of intoxication manifestations and pathological processes found. The size, color, texture, and location of the internal organs of experimental animals did not differ from the bodies of intact animals and do not go beyond the physiological norm.

The results of the study found that average lethal doses of "Propolis" gel in cutaneous application excess 15,000 mg / kg (LD50> 15000 mg / kg Table. 7).

Thus, the analysis of the results leads to the conclusion that the parameters of acute toxicity of the "Propolis" gel refer to the toxicity class V – practically nontoxic substances.

Table 7: Average lethal doses of the "Propolis" gel and the basis of the gel when applied to cutaneous white outbred rats of both sexes.

Study group	LD_{50}, mg/kg
Male	
Negative control (gel base)	>15000
The “Propolis” Gel	>15000
Female	
Negative control (gel base)	>15000
The “Propolis” Gel	>15000

4. Conclusion

1. The results of the pharmacological study had found that in terms of antiexudative action the "Propolis" gel which contains PPHM 2,0% dissolved in propylene glycol and AA,causes the maximal activity.
2. It has been found that the optimum amount of gel for skin application "Propolis" is 12.5 mg / cm2.
3. It has been proved that the "Propolis" gel possesses a high membrane-protective effect in comparison to "Skinoren" gel.
4. Acute toxicity parameters refer the "Propolis" gel to the V class of toxicity - practically nontoxic substances.

5. References

1. Bobro SG, Tikhonov OI. Experimental study of antimicrobial activity of gel based on PPHM and its combinations with azelaic acid and benzoyl peroxide. Pharmaceutical Journal. 2014, 36-40.
2. The State Register of medicines Ukraine. Electronic resource: http://www.drlz.com.ua/ Search date 21th April, 2016.
3. Stephanova OV, Avicene K. Preclinical research of medication: methodic research, recommended by/ under edition of, 2001, 528.
4. Lapach SN, Chubenko AV, Babich PN. Statistic methods in medical and biological research using Excel, Morion, 2000, 319.
5. Liapunova NA. Zagoria VA, Georgievsky VP, Bezuglaya EP. The Appropriate practice of production of medications. Under the edition of Kyiv Morion, 1999, 508-519.
6. Taran AA, Tsepkolenko VO. Epidemiological characteristics of skin disease under the results of provision of aesthetic medical care. Clinical immunology

Allergology Infectology. 2010; 8(37):70-73.
7. Propolis phenolic hydrophobic medication (PPHM) – PC № UA/4505/01, Ministry of Health of Ukraine legislation act, № 337 d/d 7th, 2011.
8. Mills OH, Criscito MC, Schlesinger TE. Addressing Free Radical Oxidation in Acne Vulgaris. J Clin Aesthet Dermatol. 2016; 9(1):25-30.
9. Çerman AA, Aktaş E, Altunay İK. Dietary glycemic factors, insulin resistance, and adiponectin levels in acne vulgaris. J Am Acad Dermatol. 2016; 6:190-196.
10. Hanai S, Sato T, Takeda K. Drug-induced lupus caused by long term minocycline treatment for acne vulgaris. Arerugi. 2015, 64(9):1269-73.
11. Kircik L.H. Advances in the Understanding of the Pathogenesis of Inflammatory Acne. J Drugs Dermatol. 2016; 15(1):7-10.
12. Leyden J. How does our increased understanding of the role of inflammation and innate immunity in acneimpact treatment approaches, J Dermatolog Treat. 2016; 27(1):1-3.
13. Marcinkiewicz M, Majewski S. The role of antimicrobial peptides in chronic inflammatory skin diseases Postepy Dermatol Alergol. 2016; 33(1):6-12.
14. Mc Carty M. Evaluation and Management of Refractory Acne Vulgaris in Adolescent and Adult Men Dermatol Clin. 2016; 4(2):203-206.
15. Brănişteanu DE, Cotrutz CE, Luca MC. Morphopathological stigmata in acne fulminans. Rom J Morphol Embryol. 2015; 56(3):1185-1190.
16. Toyoda M, Morohashi M. Pathogenesis of acne. Med Electron Microsc. 2001; 34(1):29-40.