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STUDY OF THE EFFECTIVENESS OF SAPROPEL INFLUENCE ON EXPERIMENTAL DERMATOSIS

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Abstract

In white rats with a reproduced model of dermatosis, the possibility of a corrective action of sapropel was investigated. The work used morphological, immunological, and statistical methods. After a course of applications with sapropel, the disappearance of manifestations of inflammation in the skin itself and activation of the restoration of the epidermis were microscopically established. When analyzing hematological parameters, it was established that the percentage contribution of lymphocytes, eosinophils, neutrophils, and monocytes was restored. There was also a restoration of phagocytosis and normalization of the cellular and humoral links of the immune system - that is, a moderate limitation of the development of the pathological process and a decrease in the degree of allergization of the body. The data obtained proved the effectiveness of using sapropel in rats with experimental dermatosis.

Keywords: *dermatosis, structural condition of the skin, hematological parameters, sapropel*

Introduction

In recent years, the problem of inflammatory-allergic skin diseases has become increasingly important, which is explained by unfavorable ecology and causes a significant number of patients with this pathology. Particular attention is drawn to atopic dermatitis, which today is an important medical and social problem in Ukraine and throughout the world [1, 2]. The authors point out the relationship of the clinical manifestations of this disease in different species of mammals; both in animals and in humans, atopic diseases are becoming more common, and there are significant similarities in terms of immunological aberrations and propensity for allergic sensitization [3, 4]. Atopic dermatosis (AD) is a multifactorial chronic inflammatory-allergic skin disease resulting from complex interactions between genetic and environmental factors [5]. Although the pathogenesis of AD is not fully understood, numerous studies show that dysfunction of the skin barrier and impaired immune regulation make a significant contribution to the pathobiology of AD. Keratins, filaggrin, intercellular proteins, and transglutaminases are the leading proteins responsible for the function of the epidermis, and their defects promote the penetration of microbes and allergens into the skin [6]. The dysfunction of the skin barrier against the background of immune dysregulation (including activation of type 2 immune responses) leads to disruption of the epidermal barrier. It is considered the first step in the development of AD [7, 8].

Various drugs are widely used in the treatment of AD; however, on the one hand, they are not always effective (due to the complexity of the pathogenesis), and on the other hand, along with their positive effect, complications develop - suppression of the functions of adaptive systems, the occurrence of allergic reactions, etc. [9, 10, 11]. Therefore, at certain stages of therapy and rehabilitation of patients, it is advisable to use natural, non-drug methods [12, 13, 14]. Our attention was attracted by sapropel - one of the representatives of natural medicinal resources. Due to the presence of a significant content of biologically active substances, macro and microelements are successfully used in balneology and cosmetology [15]. Sapropels are natural organo-mineral formations, deposits of freshwater reservoirs, formed from dead plant and animal organisms, mineral substances of biochemical and chemical origin, and mineral components. Various conditions for the formation of bottom sediments largely determine the qualitative composition of sapropels. The complex chemical and biological structure of sapropel explain its multifunctional effect on the body.

The biological activity of sapropel is determined by its humic acids, fulvic acids, and heratomelinic acids, various vitamins, and microorganisms that secrete antibiotics [16]. In previous studies, we proved the safety and biological activity of sapropel and its processed products (sapropastes), which justified the feasibility of researching on their use as medicines [17, 18, 19].

Based on the preceding, this work aims to assess the effectiveness of the use of sapropel in experimental dermatosis.

Methods

Experimental studies were carried out on 30 clinically healthy white rats - males at the age of 8 months, weighing 200-220 grams. The experimental animals were kept in conditions following the standard rules for the ordering, equipment, and maintenance of experimental biological clinics (vivariums), the rules for the care of experimental animals and work with them established by the Directive of the European Parliament and of the Council (2010/63 / EU) [20] and the order of the Ministry Health of Ukraine (06.04.2012, No. 249) "On approval of the Procedure for conducting experiments and experiments on animals by scientific institutions [21].

Following the study's objectives, all rats were divided into three groups, ten animals each. Group 1 consisted of intact animals (control group). A model of dermatosis in rats of groups 2 and 3 was reproduced by successive applications of xylene first (from 5 to 7 seconds, the skin should turn red), and then paraformaldehyde (from 5 to 7 seconds) on the shaved area of the skin below the rib area measuring 20.0 mm × 20 0 mm. In the 2nd group, rats with a dermatosis model were not subjected to further treatment. The 3rd group of rats, on the 4th day of the experiment (after reproduction of the

pathology), began to carry out a course of 6 applications with sapropel every other day (20 minutes each).

To assess the structural and functional changes in the skin of animals, we carried out morphological studies t. For which we removed a piece with an area of 1 cm^2. The piece was passed through alcohols of increasing concentration and poured into celloidin. Histological sections were prepared and stained with hematoxylin-eosin. The obtained sections were used for microscopic studies of structural changes in the skin.

Hematological studies determined the reaction from the peripheral blood to the development of the pathological process based on the change in the number of leukocytes and the ratio of the elements of the blood formula. The state of the immune system was assessed by changes in its cellular and humoral links. The response from the cellular link of the immune defense was assessed by determining the number of total T-lymphocytes and their subpopulations: TR and TS - lymphocytes. The activity of the phagocytic process was evaluated by determining the number of active phagocytes, their absorbing function - phagocytic index (PI), a metabolic function in the nitro-blue tetrazolium test (NBT-test) - spontaneous and stimulated. The study of the state of the humoral link of immunity was carried out by determining the content of circulating immune complexes (CIC) and the range of heterophilic antibodies (HA). HA - natural antibodies of hemolysins and heterophilic agglutinins, their content was determined by the passive hemagglutination reaction using sheep erythrocytes. The presence of HA is an indicator of the degree of immunological maturity of the organism and the normal functioning of the immune system [22]. The determination of the CEC content was carried out by the precipitation method in solutions of the field of ethylene glycol 3.5% and 7% with a molecular weight of 6000 Daltons. Animals from the experiment were taken out under ether anesthesia to obtain biological material.

Statistical processing of the data obtained in a series of experiments was carried out using the program for biomedical research Statistica 5.0. When processing statistical material, significant shifts were considered those that were within the confidence limits according to Student's tables $p < 0.05$.

The study used sapropel peloids from Lake Pribich (Volyn region, Ukraine). According to their physicochemical characteristics, sapropels are silt algae, homogeneous in composition, dark green in color; they belong to low-ash organic sapropels. The concentration of organic matter is 29%. An essential characteristic of peloids from a balneological point of view is a large mass fraction of moisture and stickiness and shear stress, which are pretty high in the sapropels of Lake Pribich. The mass fraction of moisture is more than 96%, and the bias voltage is 610.96 Pa (Pascal).

Results

On the 12^{th} day of the experiment, the following results were obtained. Macroscopically, in rats of group 2, the skin is reddened, edematous, with scabs. Microscopic studies of the skin of rats with dermatitis have established the following. The skin itself is represented by swollen fibrous fibers and bundles of normal myocytes, which are arranged irregularly. There is lymphoid infiltration in the skin.

The vessels are full-blooded, the papillae of the skin itself are smoothed. In the epidermis, the basal layer is represented by randomly located cells with rounded nuclei of various sizes. In the spinous layer, which is visually challenging to separate, there are sparse cells with oval light-colored nuclei. The granular and keratin layers do not differ; they look like a thin homogeneous, somewhat swollen layer. From above, the affected area is covered with homogeneous eosinophilic plates.

The data of hematological parameters of rats with a model of dermatosis are shown in Table 1. A significant redistribution of blood corpuscles characterizes the reaction of peripheral blood. There is a significant increase in neutrophils by 35% and eosinophils by 142% (which indicates the development of an allergic process) against the background of a significant decrease in lymphocytes and monocytes by 11% and 26%, respectively. The number of leukocytes and the level of erythrocyte sedimentation rate did not change reliably.

The study of the state of the immune system in rats with a model of dermatosis showed suppression of the parameters characterizing the cellular link of immunity (Table 1). The total

percentage of T-lymphocytes significantly decreased by 14% ($p < 0.01$); in addition, inhibition of phagocytosis was observed.

The phagocytic index and indices of the absorbing function and the spontaneous NBT test, which characterizes the metabolic function, are significantly lower than in the rats of the 1st group. On the part of the indicators of the humoral link of the immune response, an increase in the content of HA by 46% ($p < 0.01$) and CEC by 34% ($p < 0.01$) was found.

In rats of group 3, macroscopically, the skin was characterized by a pale pink color and the absence of scabs. In the skin itself and around the hair sheaths, the accumulation of lymphocytes is undetectable. There are small clusters of lymphocytes under the epidermis. The papillae of the skin itself are broad, not too deep. Epidermis: The basal layer is represented by disordered cells with succulent nuclei. All other layers are scattered, not readable; a uniform layer replace them. Individual cells with oval-shaped nuclei are randomly located, which turn pale towards the surface. Thus, the disappearance of inflammation in the skin itself and the activation of the restoration of the epidermis were established.

In the study of hematological parameters in rats of the third group, it was found that the use of sapropel moderately limits the development of the pathological process and reduces the degree of allergization of the body, as evidenced by the restoration of the percentage of lymphocytes, eosinophils, neutrophils, and monocytes. The normalization of the processes of phagocytosis was established - the indices of the absorption capacity and metabolic function (spontaneous NBT-test) of active phagocytes of peripheral blood increase and reach the level of reference values.

The percentage of total T-lymphocytes (an indicator of the cellular link of immune defense) slightly increases but remains significantly reduced compared to group 1 of rats. On the part of the indicators of the humoral link, a decrease in the content of CEC is observed. Still, their content slightly exceeds the data of group 1 and a significant reduction in the range of heterophilic antibodies, even lower than the corresponding indicator of the control group. The established changes indicate the limitation of the development of inflammatory processes in the body of animals.

Conclusions

1. The development of experimental dermatosis in rats is accompanied by changes in the immune status: a significant redistribution of blood corpuscles, suppression of the cellular link of immunity and activation of the humoral component; dedifferentiation of the layers of the epidermis, lymphoid infiltration of the dermis, hyperemia of the blood vessels.

2. The use of sapropel has a positive effect on the state of the peripheral blood and the immune system of rats with dermatosis, as evidenced by the normalization of the peripheral blood composition (disappearance of redistribution of blood corpuscles), restoration of phagocytosis processes, almost complete restoration of the percentage of total T-lymphocytes, the content of heterophilic antibodies and circulating immune complexes.

3. The revealed signs of restoration of the structural and functional organization of the skin itself, with a weak repair of the organization of the epidermis, against the background of the restoration of most parameters of the immune system and blood cells, indicate the presence of a curative effect that sapropel has on the course of experimental dermatosis.

The data obtained substantiate the practicality of using sapropaste (a product obtained from the sapropel of Lake Pribich) in the study of its medicinal properties in rats with a model of dermatosis.

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The authors declare that there are no conflicts of interest.

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Table 1. Dynamics of hematological parameters in rats, M± m

Indexes	1st group	2nd group	3rd group
Leukocytes, 10^9/l	5,59 ± 0,21	5,44 ± 0,70	5,84 ± 0,17
Lymphocytes,%	81,36 ± 0,93	74,18 ± 0,86*	78,40 ± 1,45
Neutrophils,%	12,82 ± 0,41	17,38 ± 1,15*	14,05 ± 1,03
Eosinophils,%	2,34 ± 0,17	5,68 ± 1,29*	2,19 ± 0,22
Monocytes,%	3,69 ± 0,33	2,74 ± 0,26*	3,32 ± 0,13
ESR, mm / h	1,54 ± 0,08	1,70 ± 0,12	1,40 ± 0,19
T-lymphocytes total,%	47,36 ± 0,55	40,64 ± 1,07*	44,83 ± 1,39
Phagocytosis, number of active phagocytes,%	39,90 ± 0,51	39,45 ± 0,48	40,75 ± 0,63
Phagocytic index	2,16 ± 0,02	1,90 ± 0,04*	2,09 ± 0,06
HCT test, mg: Spontaneous stimulated	 0,039± 0,001 0,090± 0,002	 0,035 ± 0,001* 0,085 ± 0,002*	 0,038 ± 0,001 0,089 ± 0,002
CEC, mg/ml	5,70 ± 0,20	7,66 ± 0,37*	6,99 ± 0,17*
HA, c.u.	5,87 ± 0,33	8,63 ± 0,95*	4,15 ± 0,23*

Note. Reliability is calculated between comparisons of 1st group and 2nd group and 1st group and 3rd group.
* - significant changes (p <0,05).