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APPLICATION OF BLUE CLAY IN CORRECTION OF EXPERIMENTAL ARTHROSIS

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Abstract

The results of complex studies of the effect of blue (Cambrian) clay on the condition of the rats' body with experimental osteoarthritis are presented. At the 1st stage of the research it was determined that blue clay belongs to the type of glauconite, the chemical composition of the aqueous extract of clay belonging to the sulfate-hydrocarbonate calcium-sodium type with a mineralization of 0.16 g / dm^3. its physico-chemical parameters were established: the mass fraction of moisture is 39.40%, the value of volume weight is 1.70, the value of clogging with silicate particles with a diameter of more than 0.25 10^{-3} m, the shear stress of clay is 404.61 PA, stickiness is 1249.68 Pa. Clay's specific heat is (2.16 kJ / (kgK)) and absorption capacity - adsorption coefficient – is (0.92). The concentration of hydrogen ions is 7.70 units pH, redox potential Eh is (+ 210 mV). In stage 2, a model of osteoarthritis of the knee joint was reproduced in two groups of white rats (n = 10 in each group). Rats of the last group at the background of the development of the pathological model received a course of applications with blue clay on the damaged limb. It is determined that the clay has a corrective effect primarily on the structural and functional organization of the joint and cartilage as a significant reduction in the knee joints inflammation, the disappearance of dystrophic manifestations and improved reparative processes in cartilage took place. At the same time, there are signs of recovery of metabolic processes: catalase activity and malonic dialdehyde content, energy supply of transmembrane transport (activity of Mg^{2+}-Ca^{2+}-ATP and Mg^{2+}-Na^{+} / K^{+}-ATP) are normalized as well as intensity of lipid metabolism.

The improvement of the peripheral blood condition was determined: the indicators of white blood and the indicators of the state of nonspecific phagocytosis were restored against the background of maintaining the indicators of red blood within the norm. Significant positive changes are observed on the part of the body's immune response: the content of circulating immune complexes and heterophilic antibodies is restored. Restoration of phagocytosis, i. e. normalization of blood active phagocytes and indexes of their metabolic functions took place.

Conclusion. The blue glauconite clay meets the requirements for therapeutic peloids in physicochemical parameters, biological activity and justifies the possibility of their use in balneological practice and restorative medicine.

Keywords: *experimental arthrosis, metabolic and hematological parameters, blue glauconite clay*

Introduction

An important problem of modern medicine that needs to be solved is destructive-degenerative diseases of the musculoskeletal system, including arthrosis (osteoarthritis) and gonarthrosis, which are becoming more common around the world [1]. Osteoarthritis is the most common pathology of the joints and therefore their treatment is of great practical significance. Osteoarthritis affects from 10% to 15% of the world's population. With age, the risk of osteoarthritis increases significantly [2]. 22% of women and 15% of men over 50 y.o. have not only radiological but also clinical signs of osteoarthritis. According to researchers, radiological symptoms of osteoarthritis are observed in 87% of women and 83% of men aged 55 - 64 y.o., with a significant proportion of asymptomatic osteoarthritis [3]. In Ukraine musculoskeletal disorders are the most common phenomenon among the socially active and able-bodied part of the population [4].

A tendency of post-traumatic osteoarthritis spread in working age persons, including those who go in for sports [5] has been noted.

According to WHO prognosis, gonarthrosis by 2030 will be the fourth leading cause of disability in women and the eighth - in men. That is, the problem of effective treatment of such patients becomes relevant not only in the medical and social but also in the economic aspect [6].

Modern medical treatment of osteoarthritis involves the use of corticosteroids and analgesics, which relieves debilitating and painful syndromes and improves reparative processes in cartilage. New generation drugs that are being implemented in treatment are more effective and less likely to cause side effects, which are inherent in corticosteroids, but do not completely eliminate them [7].

To influence all the main links of the pathological process and correct systemic disorders it is necessary to expand significantly the range of prescribed drugs, which may result in a steady increase of the undesirable accumulation of side effects and toxic lesions [8].

In order to prevent the development of drug intoxications and allergies and maintain the effectiveness of treatment, natural healing resources (NHR) are widely used in modern medicine, the healing properties of which have been proven by experimental and clinical studies [9].

In this aspect clays of various physicochemical composition attract attention. In recent decades interest in the use of clay is growing. This is due to their biological activity and therapeutic properties and ability to affect tissue regeneration due to the peculiarities of physical and chemical composition [10]. Blue clay (glauconite) contains almost all minerals and elements that the human body needs, namely: silica, phosphate, iron, calcium, magnesium, potassium, etc., in a form that is easily absorbed by the body [11].

The purpose: to determine the physical and chemical composition of blue clay and evaluate its external application impact to the body of rats with a model of osteoarthritis.

Materials and methods

The blue clay of the village of Satanivka (Horodok district, Khmelnytsky region, Ukraine) was studied. In determining the physical and chemical characteristics of blue clay, the methods regulated by the Passport of the Standardization Center accredited by the National Accreditation Agency of Ukraine [12] and Metrological characteristics of analytical methods were used. The methods mentioned are given in form 5 of the Passport of the Ukrainian State Center for Standardization and Quality Control of preformed means and in the relevant regulatory documentation [13].

The following data were clarified:

- values of mass fraction of moisture, volume weight, stickiness, shear stress, clogging with particles with a diameter of more than 0.25 10^{-3} m, specific heat capacity;
- complete chemical analysis of colloidal dispersions according to Schukarev scheme;
- granulometric analysis of clay and clay frame by the method of Robinson-Hrychuk;
- the concentration of calcium, magnesium, chloride, hydrocarbonate and carbonate ions in the clay solution was determined by titration;
- the content of sulfates was determined by gravimetric method.

34 adult white female Wistar outbred breeding rats with a body weight of 170.0 g - 190.0 g were used in the experiment. The animals were in the experimental biological clinic (vivarium) of the State

Institution "Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of the Ministry of Health of Ukraine " (Odessa) with a constant food, drinking and light regime. Animal studies have been conducted in accordance with existing legal documents and guidelines, their nutrition and manipulation with them were carried out in accordance with the provisions of the European Convention on the Protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986).

Rats were ranked into 3 groups. The first group, control, consisted of 14 intact animals. The second group (n =10) consisted of animals with a model of osteoarthritis. The third group (n =10) consisted of animals with a model of osteoarthritis, which from the 7th day of the experiment received a course of blue clay applications. The data obtained in groups 2 and 3 were compared with the corresponding indicators of control group. Methods and techniques used in the research were approved by the orders of the Ministry of Health of Ukraine [14, 15].

The model of osteoarthritis was reproduced by three injections of 0.1 ml of dexamethasone into the rat knee joint at a rate of 1.6 mg / kg of animal body weight. Dexamethasone solution was administered once a day. Verification of the model was performed on the basis of daily measurement of joint volume, temperature, morphological changes of the joint in 16 days. The course of treatment consisted of 5 procedures with one day interval and began from the first day of pathology (seventh day from the beginning of the experiment). Around the area of the rat's knee joint an area of approximately 2.0 x 2.0 cm^2 was shaved. A piece of polyethylene film with an aqueous suspension of clay (in a ratio of 1: 1.5) at a temperature of 39 - 40 °C was applied to the shaved area. The duration of one procedure was 20 minutes After application the skin surface was washed off with warm water. The biological material was selected in 16 - 18 hours after the last application. Upon completion of the study, the animals were removed from the experiment under ether anesthesia. Changes in the body's major systems, namely metabolism, peripheral blood and immune system were evaluated. Structural changes in the knee joint were determined microscopically.

The structural and functional organization of the knee joints were determined morphologically for this a piece of the joint was removed, passed through alcohols of increasing concentration and poured into celloidin. Histological sections were stained with hematoxylin-eosin. Microscopic studies of structural changes were performed on the sections obtained.

The content of urea, creatinine, total protein and protein fractions, and seromucoids in serum was determined by biochemical methods. The state of the transmembrane energy-dependent transport system was also studied by the activity of Mg^{2+}-Ca^{2+}-ATPase and Mg^{2+}-Na^{+} / K^{+}-ATPase; state of antioxidant system (AOS) was determined by catalase activity; state of lipid peroxidation (LPO) - by the content of malonic dialdehyde (MDA).

Hematological studies determined the response of the peripheral blood to the development of the corresponding process, which was assessed by changes of leukocytes number, the ratio of the blood formula elements. The state of the immune system was assessed by changes in its cellular and humoral links. The response of the cellular immune defense was assessed by determining the number of total T lymphocytes and their subpopulations: TGF and TPF lymphocytes. The activity of the phagocytic process was assessed by determining the number of active phagocytes, their absorption function - phagocytic index (PHI), metabolic function in the HCT test (spontaneous and stimulated). The response of the humoral immune system was assessed by determining the content of heterophile antibodies (HA), antibodies to liver, kidney, and circulating immune complexes (CIC).

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 ± standard error of the mean (SEM) or mean ± standard deviation. Significance was assessed using the one-way ANOVA followed by t-test. Values were considered statistically significant when P value is less than 0.05.

Results

Clay is blue, odorless hydrogen sulfide. The main physicochemical properties of clays are presented in Table. 1. The clay's pH is 7.70 units. i. e. there is a tendency to alkaline reaction. Positive values of Eh

(+ 210 mV) indicate that oxidative processes predominate in clays. Values of mass fraction of moisture (39.40%) are within the limits allowed for **sludge p**eloid systems (25-75%). The value of the bulk density, which is expressed by the "disorder" of the sludge grains, is 1.70. The shear stress of dewatered sludge depends on the molecular attraction forces that occur between water molecules and sludge particles, on the one hand, and water molecules and the surface of the body in contact with sludge, on the other. The value of the shear stress of the clay is 404.61 PA. Plastic-viscous properties of clay are determined by the stickiness, the value of which in the test sample is 1249.68 PA. Clays are characterized by low values of clogging with silicate particles with a diameter of more than 0.25 10^{-3} m (0.60%). The content of hydrogen sulfide and C_{org} is not determined. Clay samples are characterized by high values of specific heat (2.16 kJ / (kgK)) and high absorption capacity, which is reflected in the high value of the adsorption coefficient (0.92). Thus, the blue clays under study meet the requirements for therapeutic peloids by their physicochemical properties.

The basic chemical composition of the aqueous extract of clay are presented in Table 2. In terms of chemical composition, the aqueous extract of blue clay belongs to the sulfate-hydrocarbonate calcium-sodium type with mineralization of 0.16 g / dm^3. The composition of the liquid part of the clay corresponds to the intensity of the processes that take place in it. The nature of the interaction between solid and liquid phases, as well as the content of organic matter in its composition is of great importance.

Between the solid and liquid phases of clays, the content of the individual components of the phases in their environment a dynamic equilibrium establishes.

A complete chemical analysis of colloidal dispersions according to Schukarev scheme was performed (Table 3). The solution of blue clay is a liquid phase of clay and consists mainly of salts dissolved in water. The amount of dissolved salts is 0.16%, the bulk of which is represented by bicarbonate ions (0.83%), sulfate ions (0.029%), sodium and potassium (0.025%) and calcium (0.017%). The crystalline part is represented by a clay frame (79.26%), the bulk of which consists of silicate particles with a diameter (0.10 - 0.01) 10^{-3} m - 58.24%. There are no particles with a diameter of more than 0.25 · 10^{-3} m. The fine part of blue clay or its hydrophilic colloidal complex (17.98%) includes silicate particles with a diameter of less than 0.001 10^{-3} m (6.20%) and substances dissolved in 10% HCl (11.78%), including: Al_2O_3 (8.01%); Fe_2O_3 (3.52%); SiO_2 (0.15%); P_2O_5 (0.06%); MnO (0.04%).

It should be noted that the hydrophilic colloidal complex of the blue clay (17.98%) under study is much larger than in the silt sulfide peloids used in the sanatorium practice of Ukraine: Lake Velyke (Berdyansk resort, Zaporozhye region) - 4%, Lake Gopri, Kherson region) - 5%, Kuyalnytsky estuary (Kuyalnyk resort, Odessa region) from 9% to 15%.

Clay deposits are the result of the accumulation of substances in a state of fine crushing, when the growing surface can cause the appearance of pronounced surface properties or the decomposition of the substance into simple molecules. Great importance is attached (along with the thermal factor) to the particle size distribution of sediments, considering it the main in their therapeutic use. The most important indicators of peloid quality (their high water holding capacity and due to its plastic-viscous, adsorption and thermal properties) are largely related to the particle size distribution of sediments, i. e. their dispersion - the higher it is, the more developed is the surface of the phase separation, the higher is physical and chemical activity and hydrophilicity. Nanosized particles are the most active part of the solid phase. The main properties of the system of nanosized colloidal particles are determined by their molecular interaction. Highly dispersed particles, having a large surface area, actively interact with each other and with the clay solution. With a large contact surface, such processes as dissolution, absorption and exchange acquire a large quantitative scope. Due to this, the largest particles of sediments are the least active, because their surfaces are covered with films of mineral and organic colloids, which, in fact, are the carriers of their activity. The source of colloidal and silt particles formation are primary and secondary minerals, as well as decomposition products of plants, etc.

The relationship between nanosized sludge particles is established by the forces of molecular gravity both between the particles themselves and between the particles and water molecules. With a sufficient mass fraction of moisture, the particles bind to each other through the water molecules that surround them, and can move under the influence of external forces without disturbing the integrity of the whole mass of clay, i. e. clays will have the ability to significant plastic deformation. Thus, the presence of nanosized particles and water in clays determines one of their most important properties - plasticity. The results of particle size analysis of clay and clay frame are presented in Table. 4. According to research results, the number of particles with a diameter of more than $0.25 \bullet 10^{-3}$ m in the blue clay is within normal limits and equals to 0.6%. Coarse fractions of the granulometric composition of blue clay, represented by particles with a diameter of $(0.25 - 0.10) \bullet 10^{-3}$ m and $(0.10 - 0.01) \bullet 10^{-3}$ m, are in greater quantities than the particles of the pellet fraction with particles sized $(0.01 - 0.001) 10^{-3}$ m and less than $0.001 \bullet 10^{-3}$ m - 70.10 and 29.30%, respectively. Coarse fractions are much more present in the sediment skeleton than pellets. The pellet fraction of the skeleton is 23.00%, and the siltstone fraction is 62.46%. The content of more valuable in balneological terms particles with a diameter less than $0.001 \bullet 10^{-3}$ m in the clays under study is 6.80%.

The arthrosis model in the 2nd group of animals was accompanied by a knee joint increase from (0.75 ± 0.01) cm in diameter in healthy rats to (0.93 ± 0.02) cm in rats with pathology on the 16th day of the experiment. Palpation of the joint showed an increase in its temperature and soreness. Rats moved around the cage without leaning (caressing) the sore limb.

At microscopic research the fibrous capsule of a joint is edematous, there are fibroblasts with juicy nuclei, and lymphoid infiltration is present. The articular space looks like a common slit. There are areas of edema in epithelium. Substance of cartilage is homogeneous, the cells of the surface layer have flat, elongated nuclei, distributed unevenly. The papillae of the cartilage are smoothed. Closer to the cartilage, the nuclei become more rounded. Hyaline cartilage of articular surfaces is of unequal thickness throughout the sample.

The main substance of cartilage is swollen in places. Chondrocytes have poorly stained cytoplasm, enlarged light-colored nuclei, which are also located throughout the mass of cartilage. Chondrocyte nests are with edematous fuzzy capsule. Chondrocytes with pycnosis of the nucleus are present. In the interbeam spaces of the cartilaginous zone of the bone are very dense clusters of lymphoid elements and erythrocytes. That is, there are structural signs of formation and development of dystrophy and inflammation in the joints.

Biochemical parameters of osteoarthritis rats are shown in Table 5. The inhibition of the activity of the antioxidant system was established, as evidenced by a decrease in catalase activity by 15% ($p < 0.01$). Against this background, the activity of prooxidant processes increased significantly - MDA content increased by 50% ($p < 0.05$). That is, the balance of LP / AO system is disturbed. The content of creatinine and urea is significantly increased - 18% and 42%, respectively ($p < 0.01$), which indicates the development of endogenous intoxication. Significant decrease in the content of total protein ($p < 0.05$) and albumin ($p < 0.05$), increase in α1, α2, γ - globulins ($p < 0.05$) against the background of increased content of seromucoids ($p < 0.01$) indicates the development of an inflammation in the joints of rats. The activity of energy supply system enzymes of transmembrane transport is significantly reduced - the activity of Mg^{2+}-Ca^{2+}-ATP and Mg^{2+}-Na^{+}/K^{+}-ATP decreases by 23% ($p < 0.05$) and by 53% ($p < 0.01$), respectively.

Data of the osteoarthritis rats peripheral blood are shown in Table 6. The response of peripheral blood was characterized by negative changes: limited white blood cell count ($p < 0.001$) and a significant increase in ESR ($p < 0.001$), redistribution of blood cells - a significant increase the percentage of neutrophils by 184% ($p < 0.001$) and a decrease in lymphocyte content by 31% ($p < 0.01$), a significant increase of monocytes by 32%; increase of acidophiles (eosinophils in humans) by 20%. Other indicators do not undergo significant changes in comparison with the control group.

Data characterizing the state of the immune system in the osteoarthritis rats are shown in Table 7. There was a significant decrease of T lymphocytes ($p < 0{,}001$). Inhibition of the functional activity of the phagocytic process was also observed. The number of active peripheral blood phagocytes, their absorption activity and metabolic function in the osteoarthritis rats was significantly lower than in intact animals ($p < 0.001$). There was a significant increase in the content of CIC by 14% ($p < 0.005$), an increase in the level of heterophilic antibodies by 20% ($p < 0.001$); the content of antibodies to the joints tissues increases by 200%, which indicates the development of the inflammation.

The studies of blue clay applications completed, a reduction in knee volume to 0.81 ± 0.03 cm in diameter was observed. Palpation of the joint did not show any pain or fever. During visual observations, it was found that the animals did not caress the injured limb, moved around the cage calmly.

Morphological studies of the knee joint determined that its capsule tissues are dense, normal in appearance. In the joint, the gap between the bones is thin. The surface of the cartilage is smooth, the thickness of the cartilage is the same throughout the joint. The papillae of cartilage are shallow into the bone, but they are evenly spaced, quite often. The cartilage substance is dense, its color is uniform. Chondroblasts are mainly located in the basal layers of cartilage, in the surface layers they are single. The nuclei of chondroblasts are enlarged, light-colored, the cytolemma is thickened, basophilic. Thus, upon completion of the course of external use of blue clay applications, the manifestations of knee joint inflammation, manifestations of dystrophy have significantly reduced while reparative processes in cartilage improved.

The use of blue clay significantly reduces lipooxidation (Table 5). The content of MDA is significantly decreased, but does not reach the level of control indicators ($p < 0{,}05$). At the same time significantly increases the level of catalase activity, but also does not reach the control level. The activity of Mg^{2+}-Ca^{2+}-ATP ($p < 0.05$) - one of the energy metabolism enzymes is restored, but the activity of Mg^{2+}-Na^{+} / K^{+}-ATP is not completely reproduced (does not reach control values). Normalization of the content of most protein fractions ($p > 0.5$) and seromucoids is observed, which indicates a limitation of the inflammatory process.

At the same time, some indicators of metabolism retain the shifts characteristic of the osteoarthritis. The level of total protein, creatinine and urea content (indicators of the detoxification system) is not completely restored (below the control data) - they remain at the level of rats with uncorrected arthrosis ($p < 0.05$).

Therefore, the course of applications of blue clay moderately limits the metabolic disorders that are characteristic of osteoarthritis.

Course external application of blue clay has a significant anti-inflammatory effect on the experimental animals under the conditions of osteoarthritis simulation of (Table 6). This is evidenced by the restoration of ESR, normalization of leukocytes number; limiting the redistribution of blood cells - reducing the number of neutrophils by 40% ($p < 0.001$) and increasing the average percentage of lymphocytes by 30% ($p < 0.001$) relative to the control group. The number of monocytes and acidophiles also recovered. Red blood cell counts (Hb content, erythrocyte count and CI) in osteoarthritis rats under the influence of the course external use of blue clay remain within normal limits.

At the end of the course of applications with blue clay in the development of simulated osteoarthritis a restorative effect on the immune system (Table 7) in rats established. Indicators of humoral immune defense in animals of the experimental group correspond to those of the control group (CIC level and content of heterophilic antibodies). On the part of cellular indicators, phagocytosis is restored, as evidenced by the normalization of the number of active peripheral blood phagocytes and indicators of their metabolic function (spontaneous and stimulated HCT test).

Conclusions

1. Applications with blue glauconite clay significantly reduce the structural manifestations of inflammation of the joints and dystrophic processes in them, while improving the reparative processes in cartilage.

2. Improvement of metabolic processes is determined: the state of LP / AOS, energy supply of transmembrane transport and intensity of protein metabolism are approaching the norm (level 1 of animals), but the intensity of decomposition of nitrogen-containing compounds remains high.

3. Peripheral blood condition significantly improves: white blood cells normalize, indicators of

nonspecific phagocytosis restore at the background of maintaining within the normal range of red blood cells.

4. There are significant positive changes in the indicators of cellular and humoral parts of the immune system.

5. Glauconite blue clay on the main indicators meets the requirements for therapeutic peloids which is confirmed by the data of physical - and - chemical research.

6. The data obtained allow to conclude that glauconite blue clay has corrective properties, is effective in its external application against the background of the development of experimental osteoarthritis and can be recommended for further clinical trials.

Conflict of interest

The authors declsre that there are no conflicts of interest.

References

1. Spitaels D, Mamouris P, Vaes B, Miek S, Frank L, Rosella H, Patrik V. Epidemiology of knee osteoarthritis in general practice: a registry-based study. BMJ Open. 2020;10:e031734. DOI: 10.1136/bmjopen-2019-031734.
2. Allen KD, Thoma LM. Golightly YM. Epidemiology of osteoarthritis. Osteoarthritis and Cartilage. 2021. DOI: https://doi.org/10.1016/j.joca.2021.04.020.
3. Guilak F. Biomechanical factors in osteoarthritis. Best Pract Res Clin Rheumatol. 2011 Dec;25(6):815-23. DOI: 10.1016/j.berh.2011.11.013.
4. Efremenkova LN. Influence of the complex treatment with the inclusion of cryomagnetotherapy, diadynamotherapy and physical trainings on the course of osteoarthosis with metabolic syndrome. Medical Rehabilitation, Balneology, Physiotherapy. 2014;2:16-19. (in Russian).
5. Kosareva MA, Mikhailov IN, Tishkov NV. Modern principles and approaches to gonarthrosis treatment. Modern Problems of Science and Education. 2018.;6. https://www.science-education.ru/ru/article/view?id=28292. (in Russian).
6. Shramko YI, Zhmurova TA. Modern approaches for the course of medical rehabilitation of physical culture in elderly patients with gonarthrosis. Pedagogy of Physical Culture and Sporte. 2013;17(9):96-100. doi:10.6084/m9.figshare.751564.
7. Chen TM, Chen YH, Sun HS, Tsai SJ. Fibroblast growth factors: Potential novel targets for regenerative therapy of osteoarthritis. Chin J Physiol. 2019 Jan-Feb;62(1):2-10. doi: 10.4103/CJP.CJP_11_19.
8. Zagorodniy N.V., Karpovich N.I., Skvortsov D.V., Damaj Ali I., Akhpashev A.A. Clinical-biomechanical basis of intra-articular injection therapy in patients with knee osteoarthrosis. Clinical Practice. 2015;1-2:35-41. (in Russian). https://cyberleninka.ru/article/n/kliniko-biomehanicheskoe-obosnovanie-vnutrisustavnoy-inektsionnoy-terapii-patsientov-s-gonartrozom-1.
9. Nasibullin BA, Gushcha SG, Oleshko AYa, Zmievsky AV, Savitskyi IV. Corrective influence 2.5 % suspension of glauconitic clay on the structural and metabolic manifestations of stress-induced endogenous intoxication. Balneo Research Journal. 2015;6(3):143–146. DOI:10.12680/balneo.2015.1096
10. Gozhenko AI, Badiuk NS, Nasibullin BA, Gushcha SG, Gozhenko OA et al. The role of macronutrients in the implementation of the corrective effect of low-mineralized water in experimental metabolic syndrome. Rocz Panstw Zakl Hig. 2020;71(4):423-430. DOI: 10.32394/rpzh.2020.0136.
11. Alekseenko NA, Gushcha SG, Zmiievsky AV. Effect of bentonite clays on the development of chronic alcohol intoxication in the experiment. Environment & Health. 2014;1:17-21. https://cyberleninka.ru/article/n/vpliv-bentonitovih-glin-na-rozvitok-hronichnoyi-alkogolnoyi-intoksikatsiyi-v-eksperimenti. (in Ukrainian).
12. Atestat akredytatsiyi Natsional'noho ahent·stva z akredytatsiyi Ukrayiny na vidpovidnist' do vymoh Derzhstandart Ukrayiny ISO/IEC 17025:2017. № 20386 vid 09.06.2020 r. Accreditation certificate of the National Accreditation Agency of Ukraine in accordance with the requirements of Gosstandart of Ukraine ISO / IEC 17025: 2017. № 20386 dated 09.06.2020http://kurort.gov.ua/images/doc/At_

NAAU.jpg. (in Ukrainian).
13. Derzhstandart Ukrayiny 7942:2015 Yakist' gruntu. Vyznachennya zol'nosti torfu i torfovoho gruntu. State Standard of Ukraine 7942: 2015 Soil quality. Determination of ash content of peat and peat soil.http://shop.uas.org.ua/ua/katalog-normativnih-dokumentiv/13-zakhyst-dovkillya-ta-zdorovya-bezpeka/jakist-gruntu-viznachennja-zol-nosti-torfu-i-torfovogo-gruntu.html. (in Ukrainian).
14. On approval of the recommendations of the research methods of biological effects of natural medical resources and preformed medicines: of Ministry of Health of Ukraine № 692, from 28.09.09. Kiev: 2009. http://old.moz.gov.ua/ua/portal/dn_20090928_692.html. (in Ukrainian).
15. About the statement of the Order of realization of a medico-biological assessment of quality and value of natural medical resources, definition of methods of their use: the order from 02.06.2003 № 243 of the Ministry of Health of Ukraine https://zakon.rada.gov.ua/laws/show/z0752-03#Text. (in Ukrainian).

Table 1. Physical-and-chemical features of blue clay, dilution 1 : 0,5

pH, units pH	Eh, mB	mass fraction of moisture, %	Volume weight	Bias voltage, Pa	Stickiness, Pa	Particle clogging D > $0,25 \cdot 10^{-3}$ m	Specific heat capacity, kJ / (kg × K)	Content of H_2S, %	Corg$_г$, % on air-dry clay	Adsorption coefficient
7,70	+210	39,40	1,70	404,61	1249,68	0,6	2,16	-	-	0,92

Table 2. Macro composition of aqueous extract of blue clay 100.0 g of clay + 1000.0 ml H2O, g / dm^3

pH, од. pH	CO_3^{2-}	HCO_3^-	SO_4^{2-}	Cl^-	$Na^+ + K^+$	Ca^{2+}	Mg^{2+}	Total mineralization
8,05	-	0,0834	0,0285	0,0047	0,0253	0,0167	0,0020	0,160

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Table 3. Complete chemical analysis of blue clay according to Schukarev's scheme,%

Clay components, %	
Liquid phase	
Clay solution, including water	
Dissolved salts	0,16
$Na^{+} + K^{+}$	0,025
Ca^{+}	0,017
Mg^{2+}	0,002
SO_4^{2-}	0,029
Cl^{-}	0,005
CO_3^{2-}	Traces
HCO_3-	0,083
Solid phase	
I crystal part	81,86
1 Calcium-magnesium skeleton,	
Including $Ca\ SO_4 \times 2\ H_2O$	—
$Ca_3(PO_4)_2$	—
CaO	2,27
MgO	0,33
2 Clay skeleton (silicate particles $D > 0{,}001 \cdot 10^{-3}$)	79,26
Silicate particles $D > 0{,}25 \cdot 10^{-3}$ m	0
Silicate particles D $(0{,}25—0{,}10) \cdot 10^{-3}$ m	4,22
Silicate particles D $(0{,}10—0{,}01) \cdot 10^{-3}$ m	58,24
Silicate particles D $(0{,}010—0{,}001) \cdot 10^{-3}$ m	16,80
II Hydrophilic colloidal complex	17,98
1. silicate particles $D < 0{,}001 \cdot 10^{-3}$ м	6,20
2. substances soluble in 10 % HCl, incl..:	11,78
SiO_2	0,15
Al_2O_3	8,01
Fe_2O_3	3,52
FeO	
MnO	0,04
P_2O_5	0,06
3. Hydrotroilitis	—
4. Organic substances, incl. $Corg_{r.}$	—
5. Absorbed ions	—

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Table 4. Granulometric content of blue clay and skeleton of clay, %

Ø of fractions, 10-3 m	More than 0,25	0,25-0,10	0,10-0,01	0,01-0,001	Less than 0,001	Total sum
Clay	0,6	4,79	65,31	22,5	6,80	100,00
Skeleton of clay	0	4,22	58,24	16,80	6,20	85,46

Table 5. The effect of blue clay applications on biochemical parameters in rats with experimental osteoarthritis

Indications	G1	G 2		G 3	
	$(M_1 \pm m_1)$	$(M_2 \pm m_2)$	%	$(M_3 \pm m_3)$	%
POL (MDA), nmol/(min·mg)	5,94 ± 0,21	8,94 ± 0,78*	150	7,10 ± 0,26**	119
AOS (Catalase), %	76,71 ± 1,52	65,11 ± 1,68*	85	65,04 ± 1,96**	65
Creatinin, mkmol/l	47,80 ± 0,63	57,11 ± 1,70*	119	52,87 ± 1,79**	111
Urea, mmol/l	2,80 ± 0,27	3,99 ± 0,26*	142	5,50 ± 0,20	196
Mg^{2+}- Ca^{2+}-ATP, mg P/g of tissue	9,11 ± 0,29	7,12 ± 0,15*	78	8,26 ± 0,32**	90
Mg^{2+}-Na^{+}/K^{+}-ATP, mg P/g of tissue	6,40 ± 0,62	2,98 ± 0,19*	46	3,80 ± 0,15	59
Albumin, g/l	68,70 ± 2,74	61,50 ± 0,93*	89	58,24 ± 1,10**	84
α-1 Globulin, g/l	25,80 ± 1,9	11,00 ± 0,32*	42	20,65 ± 0,52	80
α-2 Globulin,g/l	8,28 ± 1,06	12,80 ± 0,80*	154	6,11 ± 0,56	73
β- Globulin, g/l	10,70 ± 2,20	15,75 ± 0,75*	147	11,00 ± 0,80	103
γ- Globulin, g/l	11,80 ± 1,79	8,00 ± 0,71*	68	12,13 ± 0,36	102
Seromucoids	11,10 ± 0,73	14,00 ± 0,41*	126	8,35 ± 0,22**	75
Albumin, g/l	0,200 ± 0,009	0,307 ± 0,007*	153	0,244 ± 0,008**	122

Notes: * — significant changes ($p < 0.05$) were calculated between groups 1 and 2;
** - significant changes ($p < 0.05$) were calculated between 1 and 3 groups;
100% of the data of the control group of animals were accepted.

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Table 6. The effect of external use of blue clay on the total blood parameters in rats with experimental osteoarthritis, (M ± m)

Indicators	G1	G2		G 3	
	(M_1 ± m_1)	(M_2 ± m_2)	%	(M_3 ± m_3)	%
Leukocytes, 10^9/l	5,51 ± 0,22	3,90 ± 0,18*	70	5,83 ± 0,19	101
ESR, mm/h	1,54 ± 0,08	1,95 ± 0,12*	126	1,65 ± 0,13	107
Neutrophils, %	12,79 ± 0,64	36,44 ± 0,67*	284	21,7 ± 1,16**	169
Acidophiles, %	2,25 ± 0,12	2,70 ± 0,13*	120	2,01 ± 0,21	89
Monocytes, %	3,72 ± 0,21	4,90 ± 0,43*	132	2,80 ± 0,29**	75
Lymphocytes, %	81,20 ± 0,80	56,00 ± 0,89*	69	73,60 ± 1,26**	90
Hb, g/l	157,08 ± 4,73	162,4 ± 1,81	103	146,80 ± 1,90	93
Erythrocytes, 10^{12}/l	4,01 ± 0,09	3,98 ± 0,14	99	3,93 ± 0,07	98
CI, U/L	1,09 ± 0,01	1,08 ± 0,04	99	1,12 ± 0,01	103

Notes: * - significant changes (p <0.05) are calculated between 1 and 2 groups;
** - significant changes (p <0.05) were calculated between 1 and 3 groups;
100% of the data of the control group of animals were accepted.

Table 7. The effect of external use of blue clay on the immunological parameters of the blood in rats with experimental osteoarthritis, (M ± m)

Indicators	G 1	**G 2**		**G 3**	
	(M_1 ± m_1)	(M_2 ± m_2)	%	(M_3 ± m_3)	%
T- lymphocytes, total, %	47,23 ± 0,6	34,64 ± 0,8*	73	41,30 ± 1,24**	87
Phagocytosis, number of active phagocytes,%	39,91 ± 0,56	38,47 ± 0,51*	96	38,75 ± 0,83	97
Phagocytic index	2,10 ± 0,04	1,80 ± 0,05*	85	1,93 ± 0,04**	92
HCT-test, mg/ml: spontaneous stimulated	 0,040 ± 0,001 0,090 ± 0,002	 0,032 ± 0,001* 0,058 ± 0,001*	 80 64	 0,041 ± 0,001 0,082 ± 0,003	 100 91
Heterophilic antibodies, U/L	6,04 ± 0,83	7,27 ± 1,66*	120	5,25 ± 0,68	87
CIC, mg/ml	5,70 ± 0,20	6,50 ± 0,06*	114	5,37 ± 0,11	94