Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Special Issue-**2** **Year 2023** Page **4768:4776***

EXPERIMENTAL USE OF GLAUCONITE IN THE TREATMENT OF TROPHIC ULCERS IN WHITE RATS

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Article History

Received: 08 Sept 2023

Revised: 29 Oct 2023

Accepted: 05 Nov 2023

ABSTRACT

Background: The escalating prevalence of chronic non-healing trophic ulcers (TUs) in dermatological practice necessitates innovative therapeutic approaches. This study explores the potential of glauconite, a natural sorbent rich in hydrous aluminosilicate clay minerals, in treating experimentally induced TU in rats. The

etiological factors for TU include endocrinopathy, vascular diseases, and neurological disorders, posing a significant challenge for conventional treatments.
Methods: Twenty outbred rats were subjected to a TU model by applying 70% acetic acid. The animals were divided into control (Group I) and treatment (Group II) groups, with the latter receiving glauconite powder. Clinical and histological examinations, including microscopic analyses, were conducted at various intervals to evaluate the therapeutic efficacy of glauconite.
Results: Glauconite application exhibited notable effects, accelerating lesion resorption, epithelization, and fibroblast activation. Histological observations revealed improved tissue regeneration, reduced inflammation, and enhanced cellular reactions in glauconite-treated rats. Comparative analysis indicated statistically significant differences in crust development, epithelization, and purulent-inflammatory resorption compared to the control group.
Conclusion: Glauconite, a hydrous aluminosilicate clay mineral, demonstrated promising therapeutic potential in the experimental treatment of trophic ulcers. The accelerated healing process, characterized by enhanced cellular reactions and reduced inflammation, suggests the feasibility of glauconite as a novel therapeutic agent for dermatological applications, offering an alternative approach to conventional treatments for chronic skin lesions.
KEYWORDS: ulcerative pyoderma, glauconitis, pathomorphology.

INTRODUCTION

Recently, in dermatological practice, chronic, persisting, non-healing ulcerative lesions of the skin and subcutaneous tissue have become increasingly common, creating an acute problem in choosing optimal therapy. The etiological factors for developing ulcerative skin lesions are endocrinopathy (primarily diabetes mellitus and obesity), vascular diseases, and neurological disorders.

Among ulcerative lesions, a special place is occupied by the problem of treating trophic ulcers (TU), which are characterized by defects of the skin or mucous membrane that occur after rejection of necrotic tissue, a low tendency to heal and a tendency to recur [6, 7].

In the treatment of ulcerative skin lesions, several methods are used: invasive and conservative ones. Changes in the skin caused by vascular disorders and exposure to pathogenic microorganisms lead to disruption of the barrier protective and regulatory functions of the skin and become the focus of the formation of tissue toxic metabolites, which lead to dysfunction of homeostatic processes and contribute to the aggravation of adverse changes in the body [3].

Traditional methods of treating TU of venous etiology are characterized by complexity and high cost, implying a combination of pharmacotherapy and local therapy. However, these methods, as a rule, could be more effective. The search for new methods of treating trophic ulcers represents a pressing problem in dermatological practice.

In order to find new methods for healing ulcerative skin lesions, glauconite powder from a deposit located in the Tashkent region, Uzbekistan, has been developed.

Glauconite is a group of clay minerals, hydrous iron aluminosilicates, formed at the final stage of sedimentogenesis in diagenesis as a result of co-coagulation of Fe, Al, and Si gels with their subsequent interaction with sea and silt waters containing K and Mg, a highly mineralized natural finely dispersed mixture of green colour from a natural deposit located in Uzbekistan. It contains 15 types of rare (glauconite, silica, zeolite, illite, montmorillonite, clinoptillonite, muscovite, orthoclase, albite, anorthite, biotite, maghemite, goethite, magnetite, etc.) 100% natural ion-exchange minerals - ionites, humic and silicic acid in a form accessible to the body, фтв siliceous water.

The research aimed to evaluate the effectiveness of glauconite in the treatment of experimentally induced ulcerative skin lesions.

MATERIALS AND METHODS

The experiments were performed on 20 white outbred rats weighing 200-250 g on the trophic ulcer model. Ulcerative skin lesions in rats were caused by applying a single subcutaneous dose of 0.7 ml 70% acetic acid solution on the inner surface of the back area. [4].

To evaluate the effectiveness of glauconite, the animals were divided into groups: group I (control) – rats with ulcerative lesions without treatment, group II (treatment) – rats with ulcerative lesions, which received glauconite powder.
All animals underwent clinical and histological examinations.

Clinical evaluation of effectiveness treatment was carried out based on visual observation of the course of the wound process, paying particular attention to the presence of purulent-inflammatory phenomena, the nature of granulation, and the timing of epithelization . The dynamics of ulcer healing were assessed by measuring its maximum size, which was determined by applying a sterile transparent film to the wound. Then, the outline of the lesion was traced with a marker, transferred to a sheet of linear graph paper, and the area of the ulcerative surface was calculated.

To conduct morphological studies biopsy samples were fixed in 10% neutral formalin solution for 24 hours. After fixation, the solution was passed through alcohols of increasing concentration in a Leika histoprocessor and embedded in paraffin. 4-5 μm thick sections were prepared from the resulting blocks, which, after deparaffinization and rehydration, were stained with hematoxylin and eosin. Microscopic studies were conducted using an “Eclipse E200” light microscope manufactured by Nikon (Japan) with a magnification of x180 and x400 times.

Statistical processing of the data obtained was done with the help of the Student's test using the Microsoft Office Excel and Biostatistics 4.03 programs. The criterion for statistical significance was $p<0.05$.

RESULTS

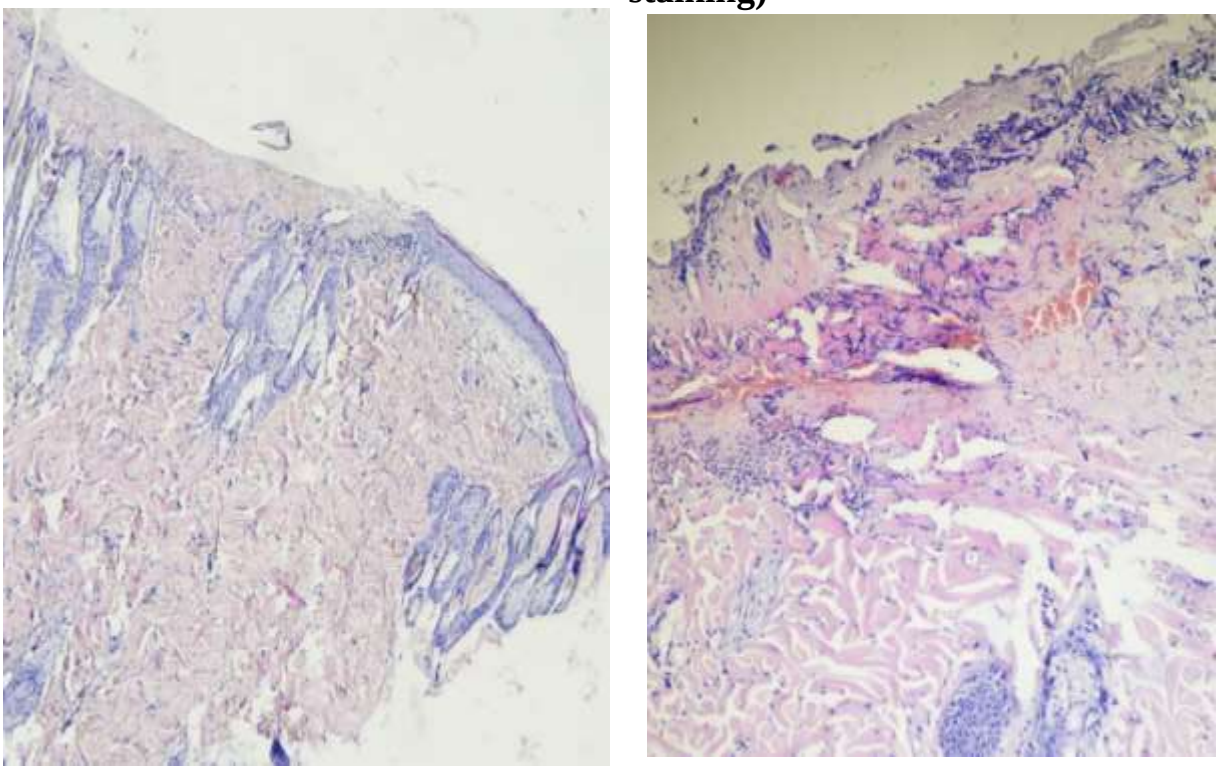
After administration of the toxin, the TU animal model was characterized by forming an ulcerative lesion averaging 1.5x2 and 2x3 cm in diameter. On the ^{second} day of the experimental study, the appearance of serous crusts in ulcerative lesions was noted in 8 out of 10 rats. On the ^{third} day of the study, the lesion was characterized by severe hyperemia and infiltration with exudative-purulent discharge. On the 4^{th} day of observation, rats of group II received glauconite powder applied to the lesions of the entire pathological lesion once a day.

Pathomorphological study on the skin of laboratory rats after experimentally induced trophic ulcer on the ^{second} day in the course of treatment using glauconite powder demonstrated the absence of the stratum corneum on the lesion, destruction and necrotic changes in the epidermis: the granular layer of the epidermis was in a state of destruction, its nuclei were destroyed, the boundaries between cells were not visible; in the spinous and basal layers, vacuolar degeneration and spongiosis were pronounced. There was no epidermis in some areas. In some places, the dermis was covered with a serous-hemorrhagic crust. In the papillary layer of the dermis, there is pronounced edema, the vessels are dilated, and around them, there are perivascular lymphohistiocytic infiltrates containing large numbers of predominantly granulocytic leukocytes and fibroblasts. Foci of extravasation are also observed. Collagen fibers are swollen, loosened, and frayed. Skin appendages:

Hair follicles are in a state of destruction and necrosis; a similar lymphohistiocytic infiltration is also observed around them. (Fig.1)

Thus, analysis of morphological changes in the skin one day after chemical exposure to acid (using the Falco method) indicates a trophic ulcer.

Fig. 1. Histological picture of experimentally induced trophic ulcer (HE staining)



Glauconite in the form of sterilized powder was applied to the lesion once a day for 10 days to 10 white rats of the main group of animals.

Clinical observations in animals of the main group showed that the use of glauconite powder contributed to the resorption of lesions, characterized by a decrease in serous-inflammatory processes, swelling, and infiltration on an average of 4.4 ± 1.02 days of observation.

On days 6-7, epithelization, crusts, and a decrease in the size of the lesions was noted.

Pathomorphological study on the skin of laboratory rats after experimentally induced trophic ulcer on the ^{seventh} day of treatment using glauconite powder demonstrated the absence of stratum corneum in spots and orthokeratosis in spots. In some preparations, a peeling crust is visible and areas of growth of epithelial cells are observed under them. In one of the animals, a layer of squamous epithelial cells, located under the serous-hemorrhagic crust and separated from it by a layer of infiltrate, is clearly visualized. In the preserved areas of the epidermis, the latter is loosely bonded to the dermis; a layer of loosely arranged collagen fibers, imitating the papillary layer of the skin, is defined subepithelially. The

epidermis is less acanthotic, the granular layer is thinned, and vacuolar degeneration and spongiosis remain in the spinous layer but are less pronounced than in the control groups. The boundary of the basal layer has been mostly restored; in some areas, exocytosis and vacuolization of cells are observed. In the papillary dermis, swelling and local fibrosis of collagen fibers are observed. Inflammatory infiltration is somewhat less pronounced: the number of lymphocytes has decreased; cellular response from mononuclear cells and fibroblasts is enhanced; the histiocytic reaction increased compared to the control group. The dilation and blood filling of the vessels are preserved. (Fig.2)

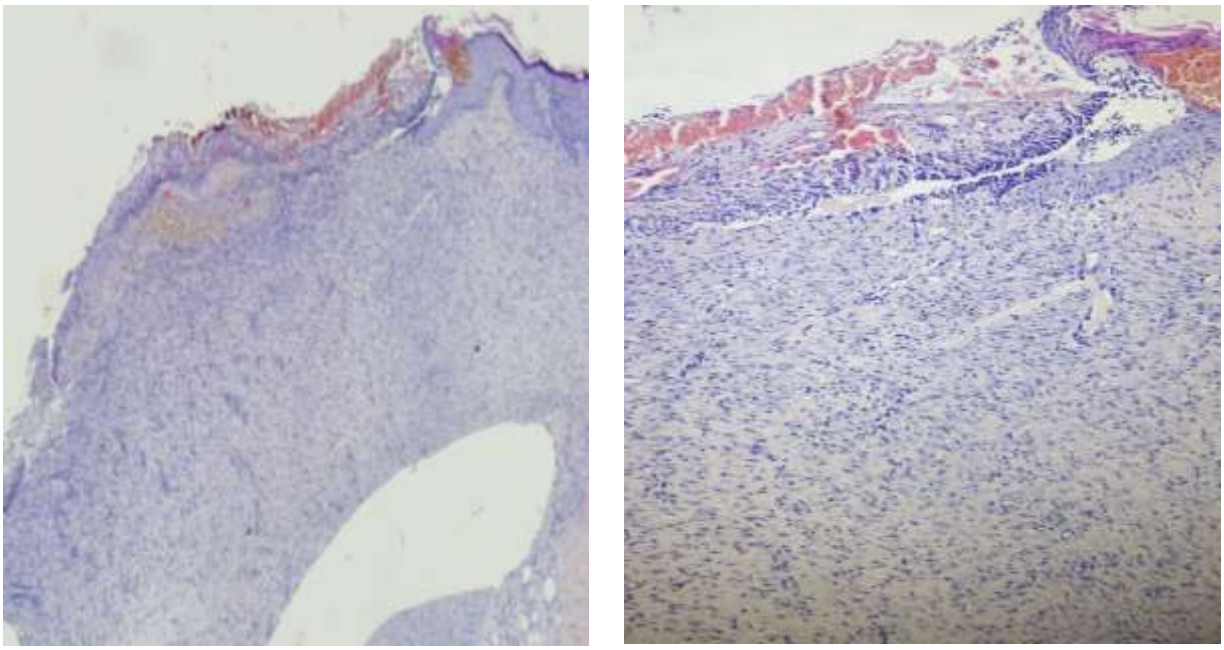


Fig. 2 Histological picture of an experimentally induced trophic ulcer on the 7th day of treatment in the course of using glauconite powder. (HE staining)

Pathomorphological study on the skin of laboratory rats after an experimentally induced trophic ulcer on the 14th day in the course of treatment using glauconite powder demonstrated the restoration of the stratum corneum on the lesion and ortho-parakeratosis observed in spots. In the epidermis, all layers and structures are completely restored. Granular and spinous layers are with no pathological phenomena. The basal layer is within normal limits, the basement membrane is thickened in some areas. Slight swelling remains in the dermis, congestion of the dermis and hypodermis vessels and swelling are observed, which may indicate inflammation that has developed during healing; inflammatory infiltration around vessels and collagen fibers decreased significantly. An enhanced cellular reaction of mononuclear cells and fibroblasts remains. Collagen fibers are swollen, in spots, due to inflammation, there are fibrotic changes. Integumentary structures are practically absent; there are single remains of hair follicles in the deep layers of the dermis.

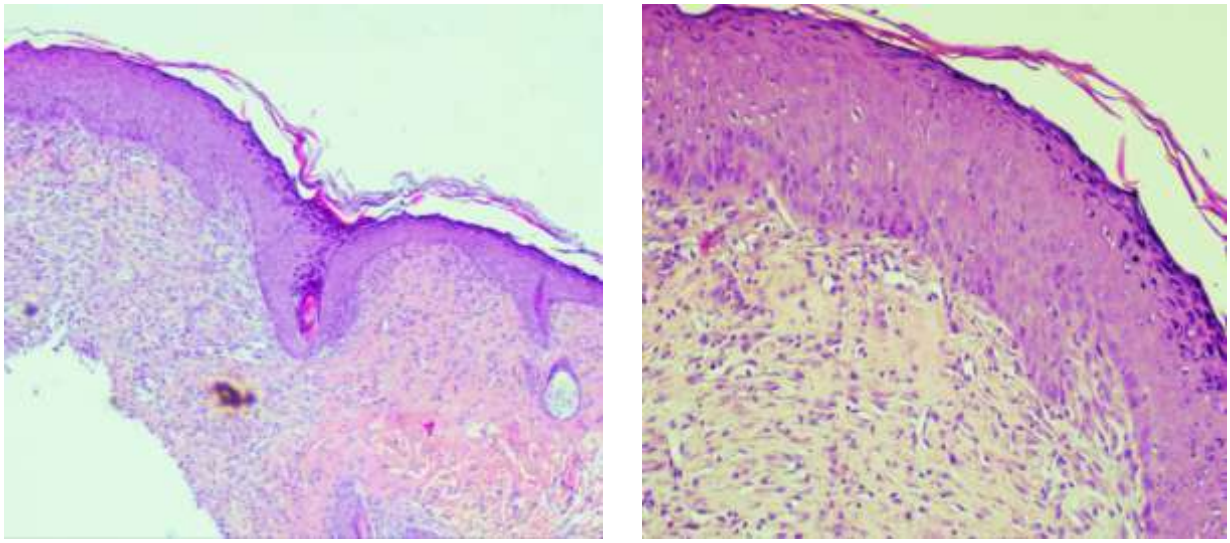


Fig. 3 Histological picture of experimentally induced trophic ulcer on the 14th day of treatment in the course of using glauconite powder. (HE staining)

Table 1

Comparative characteristics of epithelization of ulcerative lesions in the course of using glaucanite (in days)

Group	Crusts development	Epithelialization			Resorption of the purulent-inflammatory process
		Marginal	Central	Full	
Group I (control) N =10	19.8±0.4	24.3±0.4		30.3±0.8	20.1±0.1
Group II N =10	3.8±0.1*	3.9±0.1*	5.4±0.3	17.6±0.8*	13.9±0.2*

Note: * - reliability indicator in relation to the control group (P <0.05).

Healing of trophic ulcers was achieved in all experimental animals, while the healing in animals of group II was on day 17.6±0.8 on average, while in the control group (I) it was on day 30.3±0.8 of observation. The results obtained were statistically significant. (P <0.05).

Thus, our studies have shown that the use of glauconite in the treatment of experimentally induced TU is characterized by resorption of the skin pathological process, the phenomenon of epithelization and activation of fibroblasts in the

dermis and promotes pronounced epithelization of ulcerative lesions on the skin in research animals.

CONCLUSIONS

1. Glauconite is a group of clay minerals, hydrous aluminosilicates of iron, belongs to the group of hydromicas, and it is a natural sorbent formed at the final stage of sedimentogenesis in diagenesis, as a result of coagulation of Fe, Al and Si gels with their subsequent interaction with sea and silt waters containing K and Mg.
2. The use of glauconite in powder form in the experimental treatment of trophic ulcers in white rats enhances the cellular reaction of mononuclear cells and fibroblasts in the lesion and thereby facilitates cell regeneration and resorption of lesions.

References

1. Nicholas MN, Yeung J. Current Status and Future of Skin Substitutes for Chronic Wound Healing. Med Surg. 2017 Jan/Feb;21(1):23-30. doi: [10.1177/1203475416664037](https://doi.org/10.1177/1203475416664037). Epub 2016 Aug 20. Review. PMID: [27530398](https://pubmed.ncbi.nlm.nih.gov/27530398/).

2. Wagner DO, Zinoviev EV, Krylov KM, et al. Clinical Experience of Allogeneic Fibroblasts Application in Patients with Extensive Skin Burns. Vestnik Severo-Zapadnogo Gosudarstvennogo Meditsinskogo Universiteta im. I.I. Mechnikova. 2018;10(3):65-72.

3. Mavlyanova SZ, Ismagilov AI, Mirzakulova SN. Detoxifying Therapeutic Action of Natural Mineral Glauconite in Patients with Atopic Dermatitis. Zhurnal "Terapevt" [Therapist Journal]. 2023;4(191):24-32. ISSN 2075-0277.

4. Mavlyanova SZ, Makhsudov M, Mirzakulova SN, Jaffarov KH. A New Method of Detoxification of the Body with External Application of Glauconite in Patients with Allergic Skin Diseases. Dermatovenerology and Aesthetic Medicine. 2023;1(57):18-24.

5. Mavlyanova SZ, Mirzakulova SN, Jaffarov KH. Detoxifying Ability of Activated Glauconite in Patients with Allergic Skin Diseases. HIV Nursing. 2023;23(3):724-727.

6. Potekaev NN, Frigo NV, Michenko AV, Lvov AN, Panteleev AA, Kitaeva NV. Chronic, Prolonged Non-Healing Ulcers and Skin Wounds. Klinicheskaya Dermatologiya i Venerologiya. 2018;17(6).

7. Falko OV, Shevchenko NA, Prokopyuk VY, Roenko AA, Prokopyuk OS. Comparative Analysis of Methods for Modeling Trophic Ulcers of the Limbs in Mice. Experimental Surgery. 2017:561-567.

8. Sedov VM, Andreev DY, Smirnova TD, et al. Cellular Therapy in the Treatment of Trophic Ulcers of the Lower Extremities. Vestnik Khirurgii. 2006;2:90-94.
9. Method for the Treatment of Burns of the Second Degree by Applying Glauconite Powder. RU2760838C1.
10. Tamrazova OB. Prolonged Non-Healing Ulcers of the Lower Extremities: Pathogenetic Substantiation of Therapy Selection Tactics. Dissertation for the Degree of Doctor of Medical Sciences. Moscow. 2013.
11. Tolstikh PI, Tamrazova OB, Pavlenko VV, et al. Prolonged Non-Healing Wounds and Ulcers (Pathogenesis, Clinic, Treatment). Moscow: Dipak; 2009.
12. Tukhtaev FM, Sultanov DD, Shaymonov AK, et al. Current State of the Problem of Managing Patients with Post-Thrombotic Disease of the Lower Extremities (Literature Review). Vestnik Rossiyskikh Universitetov. Matematika. 2017;2.
13. Urazmetova MD, Khajibaev AM, Mirkamalova LI, et al. Blood Indicators in Rats with Burn Disease during Transplantation of Cultivated Allofibroblasts. Zhurnal Teoreticheskoy i Klinicheskoy Meditsiny. 2006;2:34-37.
14. Fayazov AD, Khajibaev AM, Urazmetova MD, Kamilov UR. Application of Cellular Technologies in the Surgical Treatment of Severe Burns. Actual Issues of Surgery: Proceedings of the XV Congress of Surgeons of the Republic of Belarus. 2014:77-78.
15. Khrebtova OM. Biotesting of Glauconite on Infusoria. Vestnik Baltiyskogo Federal'nogo Universiteta im. I. Kanta. 2016;2:73-76.
16. Volkov MY, Kalilets AA. Enterosorbent and Method of Its Production. Russian Federation Patent RU2545711C1. Published April 10, 2015.