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***V. O. Gelmboldt*[1]** *http://orcid.org/0000-0001-8492-964X*
***L. M. Khromagina*[2]** *https://orcid.org/0000-0002-8340-7329*
***O. V. Nikitin*[1]** *http://orcid.org/0000-0002-2173-0796*
***N. S. Fizor*[1]** *https://orcid.org/0000-0003-2204-9879*

BIOLOGICAL ACTIVITY OF 3-HYDROXYMETHYLPYRIDINIUM HEXAFLUOROSILICATE MONOHYDRATE AND 4-HYDROXYMETHYLPYRIDINIUM HEXAFLUOROSILICATE AS CANDIDATES FOR ANTICARIES AGENTS

[1] Odessa National Medical University, Odesa, Ukraine,
[2] State Institution "Institute of Stomatology and Maxillofacial Surgery of the National Academy of Medical Sciences of Ukraine", Odesa, Ukraine

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V. O. Gelmboldt[1], L. M. Khromagina[2], O. V. Nikitin[1], N. S. Fizor[1]

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[2] State Institution "Institute of Stomatology and Maxillofacial Surgery of the National Academy of Medical Sciences of Ukraine", Odesa, Ukraine

The aim of the study. Determination of the biological activity of new compounds – 3-hydroxymethylpyridinium hexafluorosilicate monohydrate **(I)** and 4-hydroxymethylpyridinium hexafluorosilicate **(II)**, which we synthesized earlier.

Materials and methods. The experiments were carried out on 42 white male Wistar rats, divided into 7 equal groups; working groups received Stefan's cariogenic diet. Fluorine-containing salts were used in the composition of gels, with dose of fluorine of 1.00 mg/kg. All rats (except Sundays) had applications of gels, covering the teeth and gums. Reference drugs – sodium fluoride and $(NH_4)_2SiF_6$.

Results and discussion. The caries-preventive efficacy (CPE) of fluorine-containing compounds was calculated. The activity of acid and alkaline phosphatases was determined in the incisor pulp homogenate, and the levels of malondialdehyde, elastase, and lysozyme were determined in the homogenate of the oral mucosa. Alanine aminotransferase activity was determined in blood serum. The CPE value for **II** is 41.5%, which is 1.4 times greater than NaF. A significant positive effect of fluoride agents on the biochemical parameters of the dental pulp and oral mucosa of rats (mineralizing index, elastase, urease and lysozyme activities) that received a cariogenic diet was established. Hexafluorosilicates more effectively normalize biochemical parameters compared to the action of sodium fluoride in the absence of hepatotoxic effects for all the studied compounds.

Key words: pyridinium hexafluorosilicates, caries-preventive efficacy, mineralizing index, activity of alanine aminotransferase, level of malondialdehyde.

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В. О. Гельмбольдт[1], Л. М. Хромагіна[2], О. В. Нікітін[1], Н. С. Фізор[1]

БІОЛОГІЧНА АКТИВНІСТЬ 3-ГІДРОКСИМЕТИЛПІРИДИНІЮ ГЕКСАФТОРСИЛІКАТУ МОНОГІДРАТУ ТА 4-ГІДРОКСИМЕТИЛПІРИДИНІЮ ГЕКСАФТОРОСИЛІКАТУ ЯК КАНДИДАТІВ У ПРОТИКАРІЄСНІ АГЕНТИ

[1] Одеський національний медичний університет, Одеса, Україна,
[2] ДУ «Інститут стоматології та щелепно-лицевої хірургії Національної академії медичних наук України», Одеса, Україна

Об'єктами дослідження є дві нові сполуки – 3-гідроксиметилпіридинію **(I)** і 4-гідроксиметилпіридинію **(II)** гексафторсилікати. Досліди проводили на білих щурах-самцях Вістар; робочі групи отримали карієсогенний раціон Стефана. У складі гелів використовували фторвмісні солі з дозою фтору 1,00 мг/кг. Препарати порівняння – фторид натрію та $(NH_4)_2SiF_6$. Значення карієспрофілактичної ефективності (КПЕ) для **II** становить 41,5%, що в 1,4 раза більше, ніж NaF. Встановлено достовірний позитивний вплив фторвмісних препаратів на біохімічні показники пульпи зуба та слизової оболонки порожнини рота щурів. Гексафторсилікати більш ефективно нормалізують біохімічні показники порівняно з дією натрію фториду за відсутності гепатотоксичної дії для всіх досліджуваних сполук.

Ключові слова: піридинові гексафторсилікати, карієспрофілактична ефективність, індекс мінералізації, активність аланінамінотрансферази, рівень малонового діальдегіду.

Introduction. Caries – progressive damage to tooth tissues – is one of the most common chronic diseases, and this pathology is a problem for health care systems in most countries of the world [1]. In the existing schemes for the treatment and prevention of caries, the undisputed leader is fluoride preparations, mainly inorganic fluorides, as well as fluorides with organic ammonium cations, such as aminofluoride and fluorinol – 3-hydroxymethylpyridinium fluoride [2]. The latter drugs have certain advantages over inorganic analogues. Thus, according to in vitro experiments [2], the rate of adsorption and the amount of fluoride ions absorbed by the surface of tooth enamel or synthetic apatites when using fluorinol significantly exceeds the similar effect of sodium fluoride, which indicates the effectiveness of fluorinol as a remineralizing agent. In recent years, ammonium hexafluorosilicates (AHFS) have been actively studied as potential anti-caries agents [3], the use of which is accompanied by the effect of prolonged occlusion of dentinal tubules by calcium fluoride deposits. Of special interest are AHFS with biologically active cations: in this case, there is a potential opportunity to enhance the caries-preventive effect of the fluorine-containing anion due to the positive contribution of the pharmacological action of the cation (for example, bactericidal, anti-inflammatory) [3]. As evidenced by the results [4; 5], AHFS with substituted pyridinium cations, which are well soluble in water [3], relatively low-toxic and characterized by a wide spectrum of biological activity of pyridine ligands. Recently, we demonstrated [6] the possibilities of qualitative identification of a series of AHFS with pyridinium cations. In the course of studying this group of AHFS, this publication presents the results of determining the biological activity of 3-hydroxymethylpyridinium hexafluorosilicate monohydrate and 4-hydroxymethylpyridinium hexafluorosilicate as potential anti-caries agents.

The aim of the work is to study caries preventive efficacy of 3-hydroxymethylpyridinium hexafluorosilicate monohydrate **(I)** and 4-hydroxymethylpyridinium hexafluorosilicate **(II)** in an experiment on rats fed a cariogenic diet.

Materials and methods. 3-Hydroxymethylpyridinium hexafluorosilicate monohydrate $(3\text{-}HOCH_2C_5H_4NH)_2[SiF_6]$ H_2O and 4-hydroxymethylpyridinium hexafluorosilicate $(4\text{-}HOCH_2C_5H_4NH)_2[SiF_6]$ were synthesized and identified earlier [6; 7]. Ammonium hexafluorosilicate and sodium fluoride are commercial reagents of analytical grade ("Reakhim"). Fluorine-containing salts were used in the composition of phytogels based on the sodium salt of carboxymethyl cellulose [8]. The concentration of substances in the gel corresponds to a dose of fluorine 1.00 mg/kg.

Animal experiments were carried out in compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the Law of Ukraine "On the Protection of Animals from Cruelty" (Ukraine, 2006). The experiments were carried out on 42 white Wistar male rats (1.5 months old, body weight 67–70 g), divided into 7 equal groups. Groups 2–7 received Stefan's Cariogenic Diet (CGD) [9].

All rats in control groups 2–7 were given daily (except Sundays) applications of phytogels at a dose of 0.3 ml per rat, covering teeth and gums. After application, the animals were not fed or watered for one hour. Animals were euthanized on the 36th day of the experiment using thiopental anesthesia (20 mg/kg) by total bloodletting from the heart. Then the pulp was removed from the incisors, in the homogenate of which the activity of acid (AcP) and alkaline phosphatases (AlP) was determined [10]. The level of malondialdehyde (MDA) [10], elastase [10] and lysozyme [10] was determined in the homogenate of the oral mucosa. Blood serum was obtained, in which the activity of alanine aminotransferase (ALT) was determined [11].

The mineralizing index (MI) was calculated based on the ratio of alkaline and acid phosphatase [12]. The pulp was extracted from the incisors and the number and depth of carious lesions of the teeth were counted [13]. Caries preventive efficiency (CPE) was calculated according to the presented formula (1):

$$CPE = [(A–B)/A] \times 100\%, \quad (1)$$

where A is the average number of carious lesions per rat treated with CGD, B is the average number of carious lesions per rat treated with CGD and fluoride-containing drugs.

All research results were subjected to statistical processing, arithmetic mean (M) and error (±m) were calculated. Comparisons by groups of indicators were performed using Student's t-test [14].

Table 1

Caries-prophylactic effectiveness of fluorine-containing compounds

No. i/o	Groups	Number of lesions	Depth of lesions	CPE, %
1.	Intact	6.4±0.3	7.0±0.3	–
2.	CGD + gel-placebo	8.2±0.4 $p<0.002$	8.5±0.4 $p<0.02$	–
3.	CGD + gel-NaF	5.8±0.4 $p>0.4$; $p_1<0.002$	6.4±0.6 $p>0.4$; $p_1<0.01$	29.3
4.	CGD + gel-$(NH_4)_2SiF_6$	5.6±0.7 $p>0.4$; $p_1<0.02$	6.9±0.2 $p>0.8$ $p_1<0.002$	31.7
5.	CGD + gel-**I**	5.6±0.8 $p>0.4$; $p_1<0.02$	6.6±0.4 $p>0.4$ $p_1<0.002$	31.7
6.	CGD + gel-**II**	4.8±0.6 $p<0.01$; $p_1<0.001$	5.6±0.5 $p<0.02$; $p_1<0.01$	41.5

Note: p – compared to group 1; p_1 – compared to group 2.

Results and discussion

The table 1 and Fig. 1 show the results of the determination of dental caries and the calculated CPE values of the studied fluorine-containing salts.

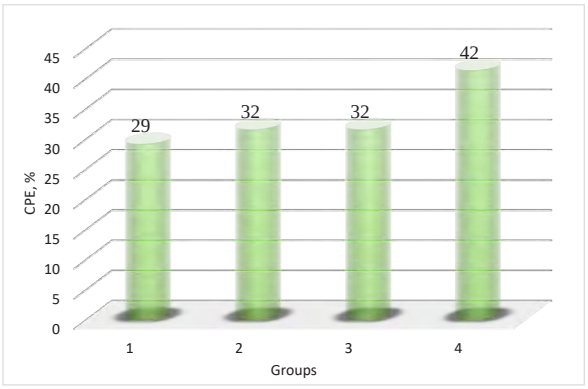

Fig. 1. CPE of fluoride preparations. Note: 1 – CGD + gel-NaF; 2 – CGD + gel-$(NH_4)_2SiF_6$; 3 – CGD + gel-I; CGD + gel-II

Table 1 demonstrates that the use of CGD significantly increases the incidence of dental caries. On the contrary, all fluoride preparations investigated in the experiment show a caries-prophylactic effect. The maximum CPE value was found for compound **II**, which 1.4 times exceeds the value for sodium fluoride. However, the use of gel with compound **I** leads to slight increase in CPE relative to sodium fluoride (29.3% and 31.7%, respectively).

The results of determining the activity of acid and alkaline phosphatases and the calculated indicators of MI of the dental pulp in rats that received CGD under the influence of fluorine-containing compounds are shown in table 2 and Fig. 2.

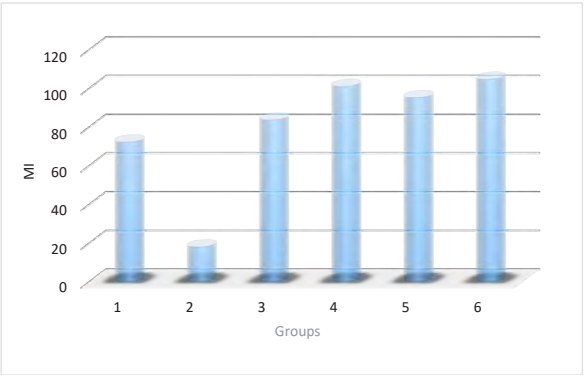

Fig. 2. The effect of fluorine-containing compounds on MI pulp in rats treated with CGD. Note: 1 – intact; 2 – CGD + gel-placebo; 3 – CGD + gel-NaF; 4 – CGD + gel-$(NH_4)_2SiF_6$; 5 – CGD + gel-I; 6 – CGD + gel-II

As a result of the action of CGD, there is a significant decrease in the activity of alkaline phosphatase and an increase in the activity of AcP; at the same time, the value of MI decreases 4 times. When using gels with fluoride preparations, an effective increase in AlP activity and a decrease in AcP activity were recorded, so that all relevant MI values exceeded this indicator for the group of intact animals; all studied AHFS show a more significant increase in MI compared to the similar effect of sodium fluoride. The maximum value of MI was recorded when using a gel with complex **II**, which 1.25 times exceeds that for sodium fluoride.

The table 3 shows the results of determining the level of inflammatory markers (elastase activity and MDA level) in the homogenate of the oral mucosa and the "hepatic"

Table 2

The effect of fluorine-containing compounds on the activity of phosphatase and MI of dental pulp in rats receiving a cariogenic diet

№ i/o	Groups	AlP, μ-kat/kg	AcP, μ-kat/kg	MI
1.	Intact	26.30±0.10	36.22±0.18	72.61±4.82
2.	CGD + gel-placebo	12.60±0.09 p<0.002	68.32±0.28 p<0.001	18.44±1.05 p<0.001
3.	CGD + gel-NaF	22.30±0.09 p<0.002 p_1<0.02	26.53±0.18 p<0.001 p_1<0.001	84.06±5.86 p>0.2 p_1<0.001
4.	CGD + gel-$(NH_4)_2SiF_6$	25.00±0.08 p>0.3 p_1<0.001 p_2<0.02	24.65±0.16 p<0.001 p_1<0.001 p_2<0.001	101.42±5.84 p<0.002 p_1<0.001 p_2<0.05
5.	CGD + gel-**I**	26.90±0.10 p>0.7 p_1<0.001 p_2<0.002	28.15±0.12 p<0.001 p_1<0.001 p_2<0.002	95.56±6.52 p<0.01 p_1<0.001 p_2>0.2
6.	CGD + gel-**II**	25.50±0.08 p>0.5 p_1<0.001 p_2<0.02	24.25±0.20 p<0.001 p_1<0.001 p_2<0.002	105.15±7.53 p<0.002 p_1<0.001 p_2<0.05

Notes: p – In comparison with gr. 1; p_1 – In comparison with gr. 2; p_2 – In comparison with gr. 3.

marker (ALT activity) in the blood serum of rats treated with CGD and fluorine-containing salts.

It is known that the degree of lipid peroxidation (LPO) in biological objects can be evaluated by the level of MDA [15]. Under the influence of CGD, a significant increase in the level of inflammatory markers is observed: in particular, the content of MDA, one of the main products of lipids, almost 2 times increases compared to the norm. The use of gel applications with fluorine-containing preparations was accompanied by a significant decrease of this indicator to values close to the norm, and all studied AHFS more effectively normalized the level of elastase compared to sodium fluoride (Fig. 3). As for the results of determining the activity of ALT (Table 3), which is a sensitive marker of hepatotoxicity of drugs [16], the obtained data indicate the absence of hepatotoxic effects for all the studied compounds.

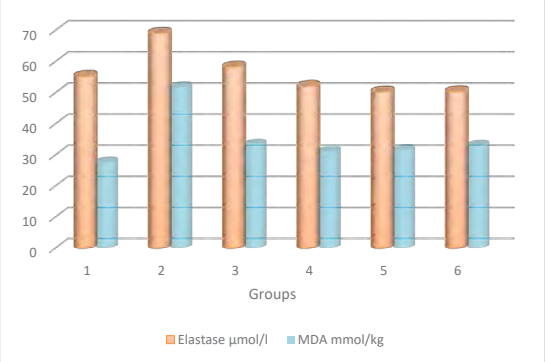

Fig. 3. Effect of fluorine-containing salts on the level of inflammatory markers in rats fed a cariogenic diet. Note: 1 – intact; 2 – CGD + gel-placebo; 3 – CGD + gel-NaF; 4 – CGD + gel-$(NH_4)_2SiF_6$; 5 – CGD + gel-I; 6 – CGD + gel-II

The results of determining the activity of urease and lysozyme in the homogenate of the mucous membrane of the oral cavity of rats that received CGD are presented in table 4. As you know, lysozyme activity is an indicator of the state of the antimicrobial systems of the microorganism [13], and urease activity indirectly characterizes the degree of microbial contamination of the oral cavity [13]. According to the data in table 4, a significant increase in urease activity is observed in rats treated with CGD, which indicates an increase in microbial contamination of the oral cavity. All investigated fluorine-containing compounds effectively reduce urease activity, and the maximum effect is achieved when compound **II** is used.

In contrast, lysozyme activity in the oral cavity is significantly reduced in rats treated with CGD and increased after the use of gels with fluorine-containing drugs. At the same time, all AHFS increase the activity of lysozyme to a greater extent compared to the similar effect of sodium fluoride.

Judging by the data [4, 5] and the results of this work, AHFS with substituted pyridinium cations in an experiment on rats have a higher CPE compared to sodium fluoride and in some cases $(NH_4)_2SiF_6$. In particular, the CPE value of 4-carboxymethylpyridinium, 3-carboxyethylpyridinium salts of the composition $[X\text{-}HO(O)C(CH_2)_nC_5H_4NH]_2SiF_6$ (X = 4, n = 1, **III**; X = 3, n = 2, **IV**) and 4-hydroxymethylpyridinium $(4\text{-}HOCH_2C_5H_4NH)_2SiF_6$, **I**, 5, 1.75 and 1.4 times higher than for sodium fluoride, respectively [4; 5]. In addition, as stated in [4], the presence of pharmacophores of anti-inflammatory activity in the cations of salts **III** and **IV** – residues of acetic and propionic acids can be accompanied by the realization of the corresponding pharmacological effect and indirectly lead to an increase in the anti-caries effect of compounds **III** and **IV**. However, to confirm the presence of anti-inflammatory activity in an experiment on the carrageenan model of inflammation for a series of compounds $[X\text{-}HO(O)CCH_2C_5H_4NH]_2SiF_6$

Table 3

The effect of fluorine-containing salts on the level of inflammatory markers (oral mucosa homogenate) and ALT activity (blood serum) in rats fed a cariogenic diet

№ i/o	Groups	Elastase activity, μ-kat/kg	MDA level, mmol/kg	ALT activity, μ-cat/l
1.	Intact	55.38±3.62	27.74±1.10	0.254±0.005
2.	CGD + gel-placebo	69.19±2.83 p<0.002	51.92±2.32 p<0.001	0.316±0.002 p<0.001
3.	CGD + gel-NaF	58.33±3.3 p>0.8 p_1<0.02	33.46±1.22 p<0.001 p_1<0.001	0.421±0.007 p<0.001 p_1<0.001
4.	CGD + gel-$(NH_4)_2SiF_6$	52.14±1.45 p>0.8 p_1<0.001 p_2>0.1	31.25±1.56 p>0.1 p_1<0.001 p_2>0.2	0.360±0.009 p<0.001 p_1<0.001 p_2<0.001
5.	CGD + gel-**I**	50.33±2.0 p>0.8 p_1<0.001 p_2<0.05	31.73±1.48 p<0.05 p_1<0.001 p_2>0.4	0.313±0.010 p<0.001 p_1>0.6 p_2<0.001
6.	CGD + gel-**II**	50.43±2.60 p>0.8 p_1<0.001 p_2>0.1	33.12±1.24 p<0.02 p_1<0.001 p_2>0.8	0.329±0.008 p<0.001 p_1>0.2 p_2<0.001

Notes: p – In comparison with gr. 1; p_1 – In comparison with gr. 2; p_2 – In comparison with gr. 3.

Table 4

The effect of fluorine-containing salts on the activity of urease and lysozyme in the homogenate of the mucous oral cavity of rats receiving a cariogenic diet

№ i/o	Groups	Urease activity, μ-kat/kg	Lysozyme activity, units/kg
1.	Intact	0.215±0.009	99±4
2.	CGD + gel-placebo	0.618±0.012 p<0.001	31±1 p<0.001
3.	CGD + gel-NaF	0.327±0.009 p<0.001 p_1<0.001	67±3 p<0.001 p_1<0.001
4.	CGD + gel-$(NH_4)_2SiF_6$	0.281±0.008 p<0.001 p_1<0.001 p_2<0.002	88±3 p<0.05 p_1<0.001 p_2<0.001
5.	CGD + gel-**I**	0.280±0.008 p<0.001 p_1<0.001 p_2<0.002	88±4 p<0.05 p_1<0.001 p_2<0.002
6.	CGD + gel-**II**	0.268±0.007 p<0.001 p_1<0.001 p_2<0.002	87±4 p<0.05 p_1<0.001 p_2<0.002

Notes: p – In comparison with gr. 1; p_1 – In comparison with gr. 2; p_2 – In comparison with gr. 3.

(X = 2, 3, 4) did not succeed. In the context of evaluating the prospects for the use of compounds **I** and **II** as anti-caries agents, it should be emphasized that these complexes do not have a hepatotoxic effect. Note that the absence of manifestations of hepatotoxic activity was also previously established for hexafluorosilicates of other substituted pyridinium cations – 2-, 3-, 4-carboxymethylpyridinium [4], 2-, 3-, 4-carboxyethylpyridinium [5].

Along with the caries-prophylactic effect, the effect of the fluorine-containing salts studied in the work, including salts **I** and **II**, is accompanied by a significant improvement in the biochemical parameters of the dental pulp and the homogenate of the oral mucosa of rats treated with CGD. In addition, the normalization of MI indicators, elastase, urease and lysozyme activity when using gels of hexafluorosilicates $(NH_4)_2SiF_6$, **I** and **II** is more effective compared to the action of sodium fluoride.

Conclusions. Thus, salts **I** and **II** in the composition of phytogels in the conditions of the model of experimental caries in rats demonstrate a caries-prophylactic effect in the absence of manifestations of hepatotoxicity. Salt **I** and $(NH_4)_2SiF_6$ are similar in their caries prevention parameters and slightly different from sodium fluoride, while complex **II** shows a more pronounced anti-caries effect: CPE **II** 1.4 times exceeds that of sodium fluoride. The investigated hexafluorosilicates **I** and **II** more effectively normalize the biochemical parameters of the dental pulp and the homogenate of the mucous membrane of the oral cavity of rats compared to sodium fluoride; according to some indicators, the use of complex **II** leads to the best results. The previously published [3] characteristics of solubility and hydrolytic instability of salt **II** and the results of this work make it possible to consider 4-hydroxymethylpyridinium hexafluorosilicate a promising object for further in-depth study as an anti-caries agent.

BIBLIOGRAPHY

1. Pitts NB, Zero DT, Marsh PD. et al. Dental caries. *Nat. Rev. Dis. Primers*. 2017; 3: 17030. https://doi.org/10.1038/nrdp.2017.30.
2. Sharkov N. Effects of nicomethanol hydrofluoride on dental enamel and synthetic apatites: a role for anti-caries protection. *Eur. J. Paediatr. Dent*. 2017; 18: 411–418. https://doi.org/10.1007/s40368-017-0314-8.
3. Gelmboldt VO, Kravtsov VCh, Fonari MS. Ammonium hexafluoridosilicates: Synthesis, structures, properties, applications. *J. Fluor. Chem*. 2019; 221: 91–102. https://doi.org/10.1016/j.jfluchem.2019.04.005.
4. Gelmboldt VO, Anisimov VYu, Shyshkin IO, Fonari MS, Kravtsov VCh. Synthesis, crystal structures, properties and caries prevention efficiency of 2-, 3-, 4-carboxymethylpyridinium hexafluorosilicates. *J. Fluor. Chem*. 2018; 205: 15–21. https://doi.org/10.1016/j.jfluchem.2017.11.004.
5. Gelmboldt VO, Lytvynchuk IV, Shyshkin IO, Khromagina LN, Kravtsov VCh, Fonari MS. *Bis*(2-,3-,4-carboxyethylpyridinium) hexafluorosilicates as potential caries prophylactic agents. *Arch. Pharm.* 2022; 335(7): e2200074. https://doi.org/10.1002/ardp.202200074.
6. Shyshkin IO, Nikitin OV, Gelmboldt VO. Identification of ammonium hexafluorosilicates using chemical methods of analysis. *Odesa Med. J*. 2023; 4(185): 94–98. (in Ukrainian). https://doi.org/10.32782/2226-2008-2023-4-18.
7. Gelmboldt VO, Shyshkin IO, Anisimov VYu, Fonari MS, Kravtsov VCh. *Bis*(3-hydroxymethylpyridinium) hexafluorosilicate monohydrate as a new potential anticaries agent: Synthesis, crystal structure and pharmacological properties. *J. Fluor. Chem.* 2020; 235: 109547. https://doi.org/10.1016/j.jfluchem.2020.109547.

8. Lepskiy VV, Anisimov VYu, Prodan OV, Gelmboldt VO. Experimental evaluation of caries preventive efficiency “onium” hexafluorosilicates. *Visnyk stomatologii*. 2015; 2: 10–13 (in Russian).
9. Stephan RM, Harris MR. Location of experimental caries on different tooth surfaces in the Norway rat, in: R.F. Sognnaes (Ed.), Advances in Experimental Caries Research. Washington: American Association for the Advancement of Science, 1955; 47–65.
10. Makarenko OA, Khromagina LM, Khodakov IV, Maikova HV, Mudryk LM, Kika VV, Mogilevska TV. Methods of researching the state of intestines and bones in laboratory rats. Directory. Odesa: publisher S.L. Nazarchuk, 2022; 81 p. (in Ukrainian).
11. Goryachkovsky AM. Clinical biochemistry in laboratory diagnostics. Odessa: Ecology, 2005; 616 p. (in Russian).
12. Levitsky AP, Makarenko OA, Khodakov IV, Zelenina YuV. Enzymatic method of the estimation of bone tissue state. *Odesa Med. J*. 2006; 3: 17–21 (in Ukrainian).
13. Levitsky AP. Therapeutic and prophylactic dental elixirs: a tutorial. Odessa: KP OGT, 2010; 258 p. (in Russian).
14. Prylutskyi YuI, Ilchenko OV, Tsymbalyuk OV, Kosterin SO. Statistical methods in biology: tutorial. Kyiv: Nauk. dumka, 2017. 216 p.
15. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell. Longev.* 2014. Article ID 360438. http://dx.doi.org/10.1155/2014/360438.
16. Kobayashi A, Suzuki Yu, Sugai S. Specificity of transaminase activities in the prediction of drug-induced hepatotoxicity. *J. Toxicol. Sci.* 2020; 45(9): 515–537. https://doi.org/10.2131/jts.45.515.

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Електронна адреса для листування vgelmboldt@te.net.ua