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COMPARATIVE EVALUATION OF THE EFFECTIVENESS OF NATURAL SILICON MINERAL WATERS AND THEIR ARTIFICIAL ANALOGICS ON THE CURRENT EXPERIMENTAL PATIOLOGY OF SLEEVE-SURFACE TRACT

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Abstract

The article presents data on the effect of silicon-containing mineral waters (MW) and their artificial analogues with the corresponding content of metasilicic acid on the state of the stomach wall in the modeling of gastritis in white rats. It was established that the use of MW with different content of metasilicic acid has a unidirectional, positive effect on the course of gastritis. MW with greater mineralization, molarity and high content of methacrylic acid completely eliminate inflammation and moderately stimulate reparative processes.

In the application of MW with less mineralization, molarity and lower content of metasilicic acid, there were definitive inflammation manifestations, but the manifestations of reparative processes were more pronounced. The same trend was observed with the use of artificial analogues - model solutions on distilled water with the corresponding content of metasilicic acid. It is determined the same restorative in the direction, but much weaker corrective effect than the corresponding MW. The important component of the corrective action of these silicon MW when used in animals with gastritis is determined by the

peculiarities of the macro component composition and the proportion of the main macro components - Na +, K +, Mg^{2+} , Ca^{2+} , HCO_3 , Cl^- , SO_4^{2-} and, to a lesser extent, silicon content.

Keywords: silicon-containing mineral waters, gastrointestinal tract, metasilicic acid

Introduction. Among the diseases of the gastrointestinal tract, chronic gastritis occupies one of the leading places, have a significant distribution in the population of people and a steady tendency to increase. According to various authors, they suffer from 30 to 40% of the world's population [1 - 6]. In this case, in developed countries of the world there is a tendency to decrease the incidence [1].

However, despite the wide arsenal of modern diagnostic and medicinal products used for diagnosis and treatment of chronic gastritis, a high incidence of complications is maintained, which predisposes the development of relapses and resistance to therapy [7, 8, 9].

According to modern concepts, chronic gastritis is a process that is progressive in nature, which begins with superficial, localised inflammatory infiltrations leading to gradual atrophy of the proper glands of the mucous membrane with possible intestinal metaplasia. Exponents of chronic gastritis include: infiltration from lymphoidal and plasmatic cells, and atrophy of the glands of the body and the antrum (with or without intestinal metaplasia). The activity of the inflammatory process is evidenced by the presence of eosinophilic and neutrophilic granulocytes, which may generate micro-abscesses in the lumina of glandular tubes. The above changes sometimes coexist with oedema of the stroma, minute extravasations, or shallow erosions, as well as dysplasia (generally low degree) of epithelial cells. Massive inflammatory infiltrations along with other lesions may lead to disturbances in the architectonics of gastric mucosa.

Also, the pathogenesis of gastritis and ulcer formation are associated with a violation of the blood supply to the stomach wall (vascular thrombosis, embolism), with subsequent atrophic changes in the mucous membrane (ischemic damage). It was shown that in the zone of ulcerous damage sclerotic lesions of terminal arterioles and their obliteration, the spread of veins and capillaries, and microcirculation violations are revealed.

Clinical studies have shown an increase in the incidence of gastritis and ulcers in patients with definite stenosis of the abdominal aorta and with specific changes in the submucosal vessel vessels characteristic of arterial hypertension and diabetes mellitus, which have an effect on the state of the protective mucosal barrier [11]. In addition, there are also

other factors that are pathogenetic mechanisms of gastritis - these are chronic psychoemotional stresses, infectious diseases, poisoning, etc. [12-16].

The complexity of the pathogenesis of gastritis and the inadequate effectiveness of their treatment initiate the search for new adequate methods, their comprehensive substantiation, which becomes relevant to theoretical and experimental medicine [17-20].

In the complex treatment of diseases of the gastrointestinal tract, medicinal mineral waters (MW) of various physico-chemical composition, including silicon MW of the average mineralization of the Transcarpathia [21- 25] are used. Most specialists and doctors believe that the healing properties of MW are due only to the macro component composition - a significant content of hydrocarbon and other ions.

At the same time, the question of the biological role of silicon and its therapeutic abilities, especially in the CF, is practically open. Our previous studies were devoted to this problem [26, 27]. However, experimental work on the study of the physiological role of silicon, including information on the use of silicon MW of weak mineralization (up to 1 g/dm³) in experimental stomach pathologies, on the basis of which it would be possible to recommend the use of such MW in clinical practice is extremely small.

Taking into account the above, the purpose of the work is to investigate the effect of silicon mineral waters of various macro- and microstructures and molar and model artificial solutions with the corresponding content of metasilicic acid on the course of experimental gastritis in white rats.

Materials and methods of research. The work was performed on white rats, females with body weight 180-200 g. The studies were conducted in accordance with the rules for the maintenance and operation of experimental animals, established by the relevant institutions [28, 29, 30].

A model of chronic gastritis was triggered by the introduction of potassium permanganate into the stomach. The choice of this model is due to the fibrinating effect of potassium permanganate on the vessels of the stomach wall. The pathology was reproduced two days in a row, introduction into the stomach of the animal with a soft probe with a metal olivine solution of potassium permanganate in a dose of 200 mg per 1 kg. For a rat with a body weight of 200.0 g, a solution was prepared: 40 mg of potassium permanganate was dissolved in 2 ml of distilled water [31]. Starting from the 3rd to the 9th day of the experiment, the rats were given in the mode of internal metered drinking in the amount of 1% of body weight, 1 time per day, corresponding MW. Animals were divided into 9 groups:

One control group was intact rats.

Group 2 - animals with gastritis, which received a standing water intake water.

Group 3 - animals with gastritis receiving silicon weakly mineralized hydrocarbonate sodium-magnesium-calcium water "Regina" (sources of Zhytniki village of Murovano-Kurilovets district of Vinnytsia region). The content of hydrocarbonates - 0,3904 g/l; chloride-ion content - 0,0107 g/l; sulfate content - 0.0193 g/l; the content of sodium ions and potassium ions - 0,0207 g/l; calcium ion content 0.0820 g/l; the content of magnesium ions - 0,0255 g/l; content of methacrylic acid - 37 mg/l; the molarity is - 10.2 mmol/l; total mineralization - 0,55 g/l.

Group 4 - animals with gastritis received a course of model solution with a concentration of methacrylic acid - 37 mg/l (analogue of Regent's MB).

Group 5 - animals with gastritis received weakly mineralized hydrocarbonate sulfate sodium water (SAR N_{2} 3766 of Zinkiv city of Poltava region). The content of hydrocarbonates - 0,3440 g/l; chloride-ion content - 0,0400 g/l; sulfate content - 0,0600 g/l; the content of ions of sodium and potassium - 0,1610 g/l; content of calcium ions 0,0550 g/l; the content of magnesium ions - 0.0150 g/l; the content of methacrylic acid is 50 mg/l, the molarity is 18.3 mmol/l; total mineralization - 0,61 g/l.

Group 6 - gastric animals received a course of a model solution with a concentration of metasilic acid - 50 mg / 1 (analogue of the MW of the South of the city N_{2} 3766 of the city of Zinkiv).

Group 7 - animals with gastritis received silicon mineralized bicarbonate sodium water (well number 1-GG village Barvinok Uzhgorod district of the Transcarpathian region). The content of hydrocarbonates - 1,8544 g/l; chloride ion content - 0,2165 g/l; sulfate content - 0.0177 g/l; the content of ions of sodium and potassium - 0,07051 g/l; content of calcium ions 0,0580 g/l; The content of magnesium ions is 0.0401 g/l. The content of metasilic acid is - 226 mg/l; the molarity is - 50,6 mmol/l, the total mineralization - 2,89 g/l.

Group 8 - animals with gastritis received a course of model solution with a concentration of methacrylic acid - 226 mg / 1 (analogous to MB of the village Barvinok).

As a kind of control in determining the therapeutic properties that provide the macrocomponent to the MH, mineral water was used in the study with a higher total mineralization, higher content of anions and cations, and without the content of metasilicic acid.

Group 9 - gastric animals received a course of low-mineralized sodium chloride without specific components and compounds (well No. 1-GG of Lebedivka village of the Tatarbunary district of the Odessa region). The content of hydrocarbonates is 0.6220 g/l; chloride-ion content - 2.130 g/l; sulfate content - 0.0031 g/l; the content of sodium ions and

potassium ions - 1,390 g/l; content of calcium ions of 0.007 g/l; the content of magnesium ions - 0.0080 g/l; molarity - 72,2 mmol/l and total mineralization - 4,30 g/l.

At the end of the experiment, the animals were withdrawn from the experiment under ether anesthesia.

Morphological studies on histological preparations studied changes in the structure of the tissues of the stomach wall, which removed the lobules of the stomach, which fixed 4% paraformaldehyde, spent everywhere spirits of high strength and filled with alloids. From the blocks, sections were made that stained hematoxylin-eosin. The methodical techniques and techniques used in the studies are presented and published in the methodological guidelines [32]. The statistical processing of the data was carried out using programs of medical and biological researches Statistica and Exel. Relevant changes were those that were within the validity of Student tables <0,05.

Results and discussion

Morphological studies of the stomach in 2 groups of rats with a gastritis model have been found that on the first day of the pathology development macroscopic mucus thickened, swollen, folds enlarged, rough. Mucus in reddish-brown color. On the 3rd day of the experiment, macroscopically, she is the same. At the 5th day of gastritis modeling the folds approach the usual ones in height, the color of the mucus is paleer, the mucus is visually coarse. At the end of the experiment (7 days) - mucous in pale brown color, the folds of the mucus remain slightly enlarged.

Microscopically, for 1-3 days of the experiment, the submucosa plate of swollen, densely packed fibers was infiltrated by lymphoid juicy elements. The mucous membrane is thickened due to an increase in the diameter of the tubular glands and edema of the interstitial layers of fibers and there is diffuse massive infiltration with the lymphoid elements of the latter. Epithelial cells of the glands are enlarged, their cytoplasm is swollen, light colored, medium sized nuclei, juicy. In the outflow ducts, glassy cells, which are full of mucus, have been sharply increased. On the 5th day of gastritis modeling in the submucosal plate fibers are densely packed, swollen, diffuse infiltration with lymphocytes is preserved.

In the mucous membrane of the gland tubular form of enlarged diameter, the location of epithelial cells is disordered, due to this lumen of the glands is not read. The cytoplasm of their light is colored, in the nuclei we see granular-fibrous chromatin pattern, interstitial layers are widespread, their fibers are quite dense. In the mucous membrane diffuse enough massive lymphoid infiltration. At the 7th day of the experiment in the submucosal plate, along with edema of the fibers and lymphoid infiltration, vascular spasm and fibrosis of their walls are observed. In the mucous membrane, whose width is normal, the interstitial layers are widespread due to coagulation of fibers and moderate lymphoid infiltration.

The location of epithelial cells in most glands is well-organized, their cytoplasm is weakly basophilic, and medium-sized nuclei are closer to the basement membrane. Goblet cells of the ducts are quite large, filled with mucus.

Morphological studies of the gastric wall in the rats of the 3 rats using Regina's MW were established: in the stomach, in the macroscopic study, differences from the norm are observed only in the form of the average intensity of the cinnamon color of the mucosa. Microscopically, the difference from the norm is only in the disordered location of epithelial cells of the glands of the mucous membrane (in some glands, they are sometimes located in two rows).

An addition, there is a small lymphocytic infiltration at the edge of the mucosa and submucosal plate, as well as a significant increase in the content of mucus in gonococcal cells. That is, there are remnants of inflammation in the wall of the stomach.

Structural-functional changes in the stomach of rats in group 4 have the same orientation as in the application of natural water, but they had a different certainty. The macroscopic mucosal stomach is pale-colored, the folds are defined, the surface is clean, shiny. Microscopically - submucous plastic is slightly diffused due to edema, there is a slight lymphoid infiltration.

The same infiltration is observed in the interstitial layers of the mucous membrane. The glands of the mucous are of the usual tubular form, the epitheliocytes in them are disordered, their cytoplasm is dark eosinophilic, the nuclei are swollen. It should be noted that interstitial layers are slightly watered. Gonadal cells of the outflow ducts with moderate mucus content.

Thus, under the influence of a model solution of methacrylic acid with a concentration of 37 mg/l (analogue of Regent's CF), a lower positive effect was determined than under the influence of natural MW, namely, a tendency for improvement - the certainty of dystrophic changes was preserved.

In rats, 5 groups, who received a course on chronic gastritis with a MW cure. No. 3766 establishes the following. In the wall of the stomach, the submucosa plate is formed by bundles of fibrous fibers, the fibers themselves are dense, but the beams can be separated, that is, it is swollen. Tubular tube glands. In a significant number of glands, the location of epithelial cells is disordered, cells themselves with a normal cytoplasm and somewhat enlarged juicy nuclei. Interstitial layers are widespread due to edema.

That is, there are remnants of the inflammatory process - swelling and increased reparative processes in the epithelium of the glands. This MW has a somewhat higher restorative effect than the natural microbial microcrystalline cellulose "Regina" due to the slightly higher content of the silica and sulfate and hydrocarbon ions.

In rats of the 6 groups that used the model solution (analogue of MW of the United Kingdom No. 3766), submucous plastic was slightly overshadowed by edema, and insignificant lymphoid infiltration was observed. The same infiltration is determined in the interstitial layers of the mucous membrane.

The glands of the mucous are of the usual tubular form, the epitheliocytes in them are disordered, their cytoplasm is dark eosinophilic, the nuclei are swollen. Interstitial layers are slightly watered. Gonadal cells of the outflow ducts with moderate mucus content.

Consequently, under the influence of a model solution of metasilic acid with a concentration of 50 mg/l (analogue of MW SV No. 3766), a greater tendency was observed for improvement than in groups of 4 rats, against the background of remnants of degenerative changes.

When used in rats, 7 groups of animals with gastritis of silicon MW well number 1-SG with the highest molar content and the content of metasilicic acid at the end of the course are macroscopically established - mucous stomach is moist, shiny, pale brown (residues of potassium permanganate), the folds are rare and not very high. In a microscopic study, the layered organization of the wall of the stomach is unaltered. Submucosal plate formed by densely located fibrous fibers, visually distinguishing individual beams is very difficult. There is a small number of fibroblasts with dark extracted nuclei and single lymphocytes.

In the mucous membrane of the gland of the usual tubular form, the epithelium is located in one layer, the epitheliocytes are juicidly colored, their nuclei are of medium size. In the excretory ducts of the glands there are enlarged goblet cells rich in mucus. Interstitial layers are somewhat widespread due to edema, lymphoid infiltration is not determined, only in some layers, there are single lymphocytes. Consequently, only the ultimate manifestations of inflammation in the mucous membrane are observed.

In morphological studies in 8 groups of rats with a gastritis model, which received a model solution of metasilicic acid at a concentration of 226 mg/l, macroscopically determined - mucous stomach of pale-cinnamon color, shiny, without any deposits, folds small but stable. Microscopically, the submucosa plate is slightly swollen, the fibers of the fibers are visually separated. There is a diffuse very weak lymphoid infiltration in the mucous membrane of the gland of the tubular structure, the epithelium in them is ordered.

The cytoplasm is homogeneous, weakly basophilic, juicy nuclei, and somewhat increased interstitial layers are common due to lymphoid infiltration. Gonadal cells of the ductus arterios significantly increased due to the accumulation of mucus. Consequently, the use of a model solution (an analogue of BV SV No. 1-SG) does not completely eliminate inflammatory changes in the mucous membrane. That is, it has a lower restorative effect than natural MH. In rats, 9 groups that used chloride-sodium CF (without silicon) found that macroscopically, the mucous membrane of the stomach was brown. The folds are a bit rolled up.

Microscopically, the submucosal plate of lymphoid infiltration is not defined. In the mucous membranes of the usual tubular form, the epithelial cells are swollen, the glands themselves are enlarged. Goblet cells with increased mucus content. Consequences of gastritis are practically not observed. That is, sodium chloride MW with the highest molecular weight exhibits significant corrective ability when used in rats with gastritis.

CONCLUSIONS

1. It was established that MW with greater osmolarity and the content of the biologically active component (metasilicate acid) have a more definite corrective effect on the course of experimental gastritis - completely eliminate the manifestations of inflammation and moderately stimulate reparative processes.

2. It has been determined that MW with less molar content and lower content of metasilicic acid have a weaker corrective effect, the final manifestations of inflammation remain, but the manifestations of reparative processes are more definite than under the influence of silicon MW with higher molarity.

3. Moderate corrective properties of artificial model solutions, the effectiveness of which depends on the concentration of metasilicic acid, has been established.

Thus, it can be considered proven that metasilicic acid exhibits biological activity both in the model solution and in the composition of MW when used in rats with gastritis. It should be noted that natural MW is more effective. A significant component of the corrective action of these silicon MWs determines the features of the complex macro- and microcomponent composition and the molarity (proportion of the major anions and cations).

Reference

1. <u>Sipponen</u> P, <u>Maaroos</u> HI. Chronic gastritis. Scand J Gastroenterol. 2015; 50(6): 657–667. doi: 10.3109/00365521.2015.1019918.

2. Suzuki H, Mor H. Different Pathophysiology of Gastritis between East and West? An Asian Perspective. Inflamm Intest Dis. 2016; 1:.123–128. 3. Dragomeretskaya NV, Zabolotnaya IB, Izhza AV, Shevchenko NA, Kalinichemko NV. Early rehabilitation of the patients presenting with digestive organ diseases based at a spa and health resort: the 30 year experience and prospects for further development. Physiotherapy, balneotherapy, rehabilitation. 2013; 3: 19–22.

4. Du Y, Bai Y, Xie P, Fang J, Wang X, Hou X, Tian D, Wang C [et al]. Chronic gastritis in China: a national multi-center survey. BMC Gastroenterology. 2014; 14: 21. doi: 10.1186/1471-230X-14-21.

5. Report of the Kyoto international consensus on gastritis associated with the Helicobacter pillory. Modern Gastrornterology. 2016; 1 (87): 35–53.

6. Telaranta-Keerie A, Kara R, Paloheimo L, Härkönen M, Sipponen P. Prevalence of undiagnosed advanced atrophic corpus gastritis in Finland: an observational study among 4,256 volunteers without specific complaints. Scand J Gastroenterol. 2010;45:1036–41.

7. Bilash SM. Morphometric characteristics of cardia gastric wall of intact rats with, acute gastritis, when using the drug "Plateks placenta" and their combined action. World of medicine and Biology. 2012; (3): 7-10.

8. Dorofeev AE, Rudenko NN. Treatment of gastropathy associated with taking nonsteroidal anti-inflammatory drugs. The place of omeprazole (according to the materials of the conferences Gastrosphere 1.0 and 2.0). Journal of Gastroenterology. 2017; 51 (1). http://www.mif-ua.com/archive/issue-34568/.

9. Filippov YuA., Zak MYu. The modern paradigm of chronic gastritis associated with biliary reflux. Modern Gastrornterology. 2018; 2(100): 95–103.

Muszyński J, Ziółkowski B, Kotarski P, Niegowski A, Górnicka B, Bogdańska M, Ehrmann-Jóśko A, Zemlak M, Młynarczyk-Bonikowska B, Siemińska J. Gastritis – facts and doubts. Prz Gastroenterol. 2016; 11(4): 286–295. doi: 10.5114/pg.2016.57793.

11. Fedulova LE, Sorokina AA. Correlation of experimental gastritis with hematological indices of blood. Eurasian Union of Scientists, biological sciences. 2015; 7(16): 117–120.

12. Nasibullin BA, Gushcha SG,Yaroshenko NA, Oleswhko AYa, Baholdina EI, Kalinichenкo MV. Complex assessment of parameter changes of homeostasis in rats with experimental gastrites. J. Clin. Exp. Med. Res. 2017; 5(1): 713–719.

13. Zub LO, Klugin VO, Vivsiynik VV. Pathogenetic substantiation of some mechanisms of occurrence of erosive-ulcerative stomach lesions in patients with chronic kidney disease of II-III stages. Galician Medical Journal. 2009;16(1): 38–39.

14. <u>Luo</u> J, <u>Wang</u> T, <u>Liang</u> S, <u>Hu</u> X, <u>Li</u> W, <u>Jin</u> F. Experimental gastritis leads to anxiety- and depression-like behaviors in female but not male rats. Behav. Brain Funct. 2013; 9(46). Retrieved from: https://www.ncbi.nlm.nih.gov/pms/articles/PMC3878489/.

15. <u>Ruggiero P</u>. Helicobacter pylori and inflammation. <u>Curr Pharm. Des.</u> 2010; 16(38): 4225–4236.

16. Basir HRG, Ghobakhlou MM, Akbari P, Dehghan A, Seif MAR. Correlation between the Intensity of Helicobacter pylori Colonization and Severity of Gastritis. Gastroenterology Research and Practice. 2017; Retrieved from: https://doi.org/10.1155/2017/8320496.

17. <u>Larauche</u> M, <u>Anton</u> P, <u>Garcia-Villar</u> R, Theodorou V, Frexinos J, Buéno L, Fioramonti J. Protective effect of dietary nitrate on experimental gastritis in rats. <u>Br</u>. J. Nutr. 2003; 89(6): 777–788.

18. <u>Bhardwaj</u> V, <u>Bhardwaj</u> R, Krishna<u>R</u>BV, <u>Sharma</u> PL. Investigation of Gastroprotective Potential of Grape Seed Proanthocyanidin Exract in Experimental Models of Gastric Ulcer, in Wistar Rats. J Gastrointest Dig Syst. 2018; 8(2): 561. doi: <u>10.4172/2</u>161-069X,1000561.

19. Stefanov AV. Preclinical studies of drugs: Methodic recommendations / Kiev: Avicena, 2002: 567 p.

20. <u>Painsipp</u> E. <u>Wultsch</u> T. <u>Shahbazian</u> A, Edelsbrunner M, Kreissl MC, Schirbel A, Bock E, Pabst MA, Thoeringer CK, Huber H.P, Holzer P. Experimental gastritis in mice enhances anxiety in a gender-related manner. Neuroscience. 2007; 150(3): 522–536.

21. Komleva NE, Maryanovsky AA, Danilov AN, Agasarov LG. The novel approaches to the rehabilitation of the patients presenting with gastroesophagel reflux disease and co morbid pathology. Questions of balneology, physiotherapy and physical therapy. 2017; 2: 20–23.

22. Zolotareva TA, Babov KD, Nasibullin BA [et al]. Medical rehabilitation. – Kiev: KIM, 2012: – 496 p.

23. Alekseenko NA, Babov KD, Gushcha SG [et al]. New Niniv mineral water resort of Morshyn: monograph / By red. Babov KD, Nikipelova EM, Tokaria I.M. – Drohobych: Kolo, 2012: 148 p.

24. Lemko IS, Feksishgasi BM, Kirtich LP [et al]. Microelement composition of mineral waters and medical-geographical regionalization of Zakarpatiy. Medical hydrology and rehabilitation. 2005; 3(2): 4–13.

25. Mineral waters of Ukraine / Ed. By Kolesnik E.O., Ba6ov K.D. – Kiev: Kupriyanova, 2005, 576 p.

26. Gushcha SG, Nasibullin BA, Plakida AL, Trubka IA, Volyanskaya VS, Kalinichenko NV, Balashova IV. Comprehensive assessment of functional changes in the organism of healthy rats in external and internal use of silicone malomineralized mineral water. European Journal of Clinical and Biomedical Sciences. 2018; 4(1): 1–5. doi: 10.11648/j.ejcbs.20180401.11

27. Gushcha SG, Nasibullin BA, Plakida AL, Kalinichenko VN, Tikhokhod LV, Mohylevska TV. Pathogenetic and sanogenetic mechanisms of the influence of mineral waters (siliconed and with increased organic substances) of different osmularity on the exposure of toxic nephritis Journal of Biotechnology and Bioengineering. 2018; 2(2):.7–12.

28. Kozhemyakin YuN, Khromov OS, Boldyreva NE, Dobrelya NV, Sayfetdinova GA. Scientific and practical recommendations for the maintenance of laboratory animals and work with them: monograph. – Kiev: Interservice, 2017: 182 p.

29. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Text with EEA relevance), Official Journal. 2010; 276: 0033–0079.

30. Order of Ministry of Education and Science, Youth and Sport of Ukraine No. 249 dated 01.03.2012. Official Journal of Ukraine dated 06.04.2012, article 942, № 24, 82 p., code of the act 60909/2012.

31. Nasibullin BA, Gushcha SG, Babov KD, Trubka IA, Oleshko AYa, Baholdina EI. Guidance on the reproduction of experimental models of common nosological forms and their verification. – Odesa: POLIGRAF, 2018: 82 p.

32. On approval of the recommendations of the research methods of biological effects of natural medical resources and preformed medicines: MOH of Ukraine № 692, from 28.09.09. Kiev: 2009.